EUROPEAN CONFERENCE

Antibiotic Resistance and Infection Control (ARIC)

17th November, 2011

ABSTRACT BOOK

Kaunas, 2011

LITHUANIA

This publication presents the abstracts of scientific works
of European Conference Antibiotic Resistance and
Infection Control (ARIC),
which was held on 17th November, 2011
in Kaunas, LITHUANIA.

Congress Organizers

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RIZIKOS VERTINIMO INSTITUTAS

National Food and
Veterinary Risk Assessment
Institute

Lithuanian Medical Students
Association

CONTENT

WELCOME MESSAGE

Dear collegues,

Recently, the World Health Organization (WHO) identified antimicrobial resistance as one of three greatest threats to human health, jeopardizing patient safety and public health. The problem of antibiotic resistance worldwide, is one of the foremost issues that we face in the coming decades. We strongly believe that there is an urgent requirement for a debate within Europe on how to comprehensively address the problems of antimicrobial resistance, with the goal to implement a coordinated a European approach.

Lithuania needs a new attitude towards the infection control as the present efforts are not efficient enough. Unmotivated use of antibiotics should be precluded that has already reached dangerous extent.

It is both related with excessive administration of antibiotics, irrational antibiotic therapy, food processing by means of antibacterial preparation and present application of modern diagnostic technologies in which the agent is identified too slowly by opening the door to "blind" use of a wide range of antibiotics. It causes formation of antibiotic-resistant strains of microorganisms.

In the EU, the treatment of infections caused by antibiotic-resistant bacteria costs 1.5 milliard euros every year. Outbreaks of those diseases cause dangerous social and economic losses. The infections caused by antibiotic-resistant bacteria prolong inefficient antibiotic therapy and period of hospitalization, increase morbidity and mortality. For example, the treatment of drug-resistant tuberculosis costs Lithuanian taxpayers about 10 million Litas every year.

Without efficient antibiotics, such modern methods of treatment as operations, oncologic chemotherapy and intensive care become impossible. By reaching for rational use of antibiotics, programmes of hospital infection management should be implemented in hospitals in order to perform the observation of developing resistance of microbes in real time and take measures to preclude the development of resistance of those microbes.

New reliable and fast diagnostic technologies are also necessary which involve the detection of resistant strains of microorganisms among patients getting to the emergency room and doctors and make conditions to preclude the expansion of resistant microorganisms.

For that purpose, laboratories should be provided with new, reliable and fast diagnostic technologies increasing the efficiency of the infection control and enabling to cut down the expenses on treatment. It should be the underlying purpose of the national Health Programme.

Yours sincerely,

Assoc. Prof. Dr. Saulius Čaplinskas
Conference chair,
Director of Center of Communicable
Diseases and AIDS,
Mykolas Romeris University

Assoc. Prof. Dr. Astra Vitkauskienė
Conference co-chair,
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GENERAL INFORMATION

Congress Venue
Business leaders centre (BLC) conference hall Elipsė. K.Donelaičio g. 62, LT-44248 Kaunas.
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Congress website
http://www.antibiotic-awareness.eu

Language
The official languages of the Congress are English and Lithuanian

Registration Desk
The registration desk will be open during the following hours:
Thursday, November 17, 2011   8:00 AM – 18:00 PM

Certificate of Attendance
Certificates of attendance will be available for all participants at the registration desk. The certificate of participation will be issued to those who are properly registered.

Poster Presentations
Posters will be displayed in the conference hall Elipsė. Posters should be installed on the numbered places. Conference staff will provide technical means to install posters.

Lunch, Coffee and Tea
During the conference session breaks coffee and lunch will be served free of charge to participants of conference.

Liability and Insurance
The Congress Secretariat and Organizers cannot accept responsibility whatsoever for injury or damage involving persons and property either during or indirectly arising from the congress. Participants are advised to make their own arrangements with respect to personal health and travel insurance.

SCIENTIFIC PROGRAMME

Antibiotic Resistance and Infection Control(ARIC)

9:00–9:30	**Video welcome message of WHO (TBC), official welcome messages**
	Lithuania – active member of the European Antibiotic Awareness Day! Assoc. Prof. S. Čaplinskas, Center of Communicable Diseases and AIDS, Mykolas Romeris University, Vilnius, Lithuania
9:35–9:50	**Streptococcus pneumoniae infection acquired in community: threat of antibiotic resistance or a myth?** Prof. R. Sakalauskas. Department of Pulmonology and Immunology, Lithuanian University of Health Sciences, Kaunas, Lithuania
9:55–10:10	**Etiology of complicated infections and problem of antibiotic resistance at the dawn of 21st Century** Assoc. Prof. Dr. A. Vitkauskienė. Department of Laboratory Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania
10:15–10:30	**Attitudes and stereotypes in Lithuanian population: antibiotics and treatment of infectious diseases** Dr. R. Ališauskienė and S. Novikas. Joint Venture of Lithuania and Great Britain “Baltic Surveys” /The Gallup Organization, Vilnius, Lithuania
10:30–11:00	**Coffee break**
11:00–11:15	**Antimicrobial resistance in Staphylococcus aureus: development, possibilities of prevention** Prof. A. Pavilonis, Ž. Maželienė. Microbiology Department, Lithuanian University of Health Sciences, Kaunas, Lithuania
11:20–11:35	**Prevalence of residues of Veterinary Medicinal Products and other substances in live animals and animal origin products in the Republic of Lithuania** Assoc. Prof. Dr. P. Mačiulskis. National Food and Veterinary Risk Assessment Institute, Vilnius, Lithuania
11:40–11:55	**Antimicrobial resistance of the clinically important bacteria monitoring at the Republic of Belarus** Prof. L. P. Titov. Research & Practical Centre for Epidemiology & Microbiology, Minsk, Belarus
12:00–12:15	**Standard infection control precautions. Sterilisation of medical devices and opportunities in Lithuanian hospitals** Dr. R. Budginaitė. Centre for Communicable Diseases and AIDS, Vilnius, Lithuania

12:20–12:35	**Lab organization for surveillance of Hospital Acquired Infections (HAI) and emerging resistance** Dr. E. Montrucchio. ID/AST European Application and Scientific Manager Diagnostic Systems, BD Biosciences, Italy
12:40–12:55	**Threat of the post-antibiotic era: do we need to change our prescribing habits?** Assoc. Prof. Dr. J. Gulbinovič, Dr. G. Barcys. State Medicines Control Agency, Vilnius, Lithuania
13:00–14:00	**Lunch**
14:00–14:15	**Use of antiseptic (octenidine chloride and phenoxyethanol) in multiresistant germ caused wound infection treatment** Dr. L. Pilipaityte. Hospital of Lithuanian University of Health Sciences Kauno Klinikos, Kaunas, Lithuania
14:20–14:35	**Early diagnostics and treatment strategy of sepsis and septic shock** Prof. D. Adukauskienė. Intensive Care Clinic, Lithuanian University of Health Sciences, Kaunas, Lithuania
14:40–14:55	**Innovative molecular diagnostic approaches for fast screening from blood cultures** Dr. P. Castan. Sanchinarro Hospital North Madrid, Spain
15:00–15:15	**Presepsin – new diagnostic marker for sepsis** Dr. A. Eidukaitė, MD, PhD. Vilnius University Children‘s Hospital, Vilnius, Lithuania
15:20–15:35	**Drug resistant tuberculosis in Lithuania: prevalence and reasons, cost and perspective** Dr. K. Miškinis. National Health Insurance Fund under the Ministry of Health, Vilnius, Lithuania
15:40–15:55	**Detection of mutations associated with drug resistance in Mycobacterium tuberculosis strains in Saint Petersburg and Leningrad oblast (Russia)** Prof. A. Kozlov. Biomedical Centre, Saint Petersburg University, Russia
15:55–16:25	**Coffee break**
16:25–16:40	**MDR-TB: early diagnostics and treatment** Dr. A. Cīrule. Infectology Centre of Latvia, Ryga, Latvia
16:45–17:00	**The HIV-I resistance mutations revealed at patients on HAART in Belarus** Prof. V. Eremin. Clinical Virology Department, Research & Practical Centre for Epidemiology & Microbiology, Minsk, Belarus
17:05–17:20	**Antiviral drugs resistance: impact on treatment strategies for hepatitis B and C** Dr. M. Puoti. Infectious Diseases Department, AO Ospedale Niguarda Cà Granda, Milan, Italy
17:25–17:45	**Discussion**
8:00–18:00	**Poster session**

Introductory lecture 00

LITHUANIA – ACTIVE MEMBER OF THE EUROPEAN ANTIBIOTIC AWARENESS DAY!

Assoc. Prof. Dr. Saulius Čaplinskas
Center of Communicable Diseases and AIDS,
Mykolas Romeris University, Vilnius, Lithuania

Infections with antibiotic-resistant bacteria frequently lead to a delay in appropriate antibiotic therapy resulting in increased morbidity and mortality, as well as increased length of hospital stay.

Without effective antibiotics, modern medical treatments such as operations, cancer chemotherapy and intensive care are no longer possible.

The European Antibiotic Awareness Day, the US's Get Smart About Antibiotics Week and the Canadian Antibiotic Awareness Week are being launched simultaneously during the week of 18 November in an effort to show global solidarity in addressing antibiotic resistance through promotion of prudent antibiotic use.

Risk assessment produced by ECDC that evaluated the risk to the citizens of Europe, of the spread of CPE through patient transfer between healthcare facilities, with special emphasis on cross-border transfer. Patient transfer between hospitals and in particular between countries, is a risk factor for the spread of bacteria that are resistant to last-line antibiotics.

Resistance to last resort antibiotics such as the carbapenems is increasing in Enterobacteriaceae bacteria commonly responsible for hospital infections such as Klebsiella pneumoniae.

An example of carbapenemase-producing Enterobacteriaceae (CPE) is New Delhi metallo-beta-lactamase (NDM)-producing Enterobacteriaceae. These epidemic bacteria are resistant to last-line antibiotics, such as the carbapenems. The emergence of human carriage of NDM has been described in Europe. The NDM enzyme was first detected in Escherichia coli and Klebsiella pneumoniae isolates from a patient repatriated to Sweden after being treated in a hospital in New Delhi, India in 2008.

The European Antibiotic Awareness Day is a European health initiative which aims to provide a platform and support for national campaigns about prudent use of antibiotics.

9:35–9:50

STREPTOCOCCUS PNEUMONIAE INFECTION ACQUIRED IN COMMUNITY: THREAT OF ANTIBIOTIC RESISTANCE OR A MYTH?

Prof. Raimundas Sakalauskas
Department of Pulmonology and Immunology,
Lithuanian University of Health Sciences, Kaunas, Lithuania

Streptococcus pneumoniae (*S. pneumoniae*) still remains the most common etiologic agent of community-associated infections – pneumonia, meningitis, otitis media, influencing significant morbidity and mortality rates worldwide. Penicillin administration and pneumococcal vaccine saved many people's lives, but development and spread of penicillin-resistant *S. pneumoniae* strains became a new challenge. According to International Alexander project's and other researches data, development of resistance to macrolids and other antibiotic classes, determined the multidrug resistance phenomenon of this pathogen, detected in 15–30 percent of all isolated pneumococcal strains in different geographical regions. Additionally, there are published data, showing increase of resistance of pneumococcal strains, isolated in nosocomial infection cases. *S. pneumoniae* resistance development reasons and serotypic dependence of resistant strains in different countries is ambiguity, monitoring of such strains from point of vaccine composition revise is especially actual. However, particularly actual the empirical treatment guidelines of diseases, caused by pneumococci, raising the question of national and international agreement of diagnostics and treatment, corresponding the circulating pathogen features. According to valid community-associated infection treatment recommendations, it is suggested to choose one or two agents from four antimicrobial agent classes. There are published some cases of treatment failure, administering inactive drugs against resistant pneumococcus isolated, but till now only the virulence of this pathogen (as well as patient's resistance and criteria of illness singularity) is proven factor, having certain correlation with lethality.

In context of Lithuania *S. pneumoniae* resistance is not yet actual, multiyear observation findings reflects low-level resistance of isolated strains in comparison. It is advisable to observe the dynamics of pathogen resistance and, according to obtained data, to choose recommended treatment drugs in priority order.

9:55–10:10

ETIOLOGY OF COMPLICATED INFECTIONS AND PROBLEM OF ANTIBIOTIC RESISTANCE AT THE DAWN OF 21ST CENTURY

Assoc. Prof. Dr. Astra Vitkauskienė
Department of Laboratory Medicine,
Lithuanian University of Health Sciences, Kaunas, Lithuania

In hospitals broad spectrum antibiotics are usually used widely with purpose to affect all possible pathogens, especially in critical ill patients. But physicians can't feel concern even administering broad spectrum antibiotics, because the number of antibiotic resistant strains is increasing. It does not always succeed to make the correct diagnosis on the first illness day, so often antibiotics are administered even if there is no real basis. This is the reason for selection of resistant strains and increase of health care costs.

Extended spectrum beta lactamases (ESBL) producing Enterobacteriaceae, multidrug resistant (MDR) *Pseudomonas aeruginosa* (*P. aeruginosa*), Acinetobacter spp., meticillin resistant *S. aureus* (MRSA) and vankomycin resistant *Enterococcus* spp. (VRE) are major antimicrobial resistance problems in XXI century. ESBL-producing Enterobacteriaceae are resistant not only to cephalosporins, but may be also resistant to aminoglycosides, tetracyclines, chloramphenicol, and/or sulfonamides; ESBL-producing *K. pneumoniae* strains are more likely to be resistant to fluoroquinolones than their non-ESBL-producing counterparts. ESBL-producing strains have a greater pathogenic potential than non-ESBL-producing strains. The acquisition of an ESBL plasmid appeared to up regulate the phenotypic expression of one or more genes, resulting in greater invasion ability.

P. aeruginosa remains an important nosocomial pathogen with relatively high overall resistance to antimicrobial agents, and the resistance level is increasing. According to our study data, changes in the resistance rates of *P. aeruginosa* strains isolated from patients treated in intensive care units of the largest university hospital showed an increase in the resistance rates to ciprofloxacin (+24%, $P<0.001$) and ceftazidime (+8.3%, $P<0.05$) over 5 years period. In 2003, there were 66.7% of *P. aeruginosa* strains sensitive to all antibiotics tested, and this percentage decreased to 47.5% in 2008 ($P<0.05$). During the study, a significant increase in the median MICs for ciprofloxacin and amikacin was observed ($P<0.001$); however, no significant change was documented for ceftazidime. The increasing resistance of *P.aeruginosa* to reserve antibiotics of carbapenem group was observed as well.

In other study we found that previous administration of carbapenems and fluoroquinolones were significant risk factors for carbapenem resistant *Acinetobacter* spp. infection. In multivariate analysis, previous use of carbapenems (odds ratio (OR) 10.1; 95% confidence interval (CI) 1.16-87.2) and fluoroquinolones (OD 3.6; 95% CI 1.13-11.51) were independently associated with carbapenem resistance.

Molecular tests should be used to confirm different phenotypes of antimicrobial resistance as a basis for infection control measures. There are many different technologies available for molecular testing, but they are time consuming, expensive, need well trained staff. It is very important to establish reference laboratories in hospital laboratory system, which would be fully equipped with knew, reliable, fast diagnostic technologies to warrant early diagnostic of infectious diseases, detection of antimicrobial resistance mechanisms to avoid unnecessary consumption of broad spectrum antibiotics and collect resistant pathogens banks for further researches of antibacterial resistance genes. Accumulated data would be used to prepare antibacterial treatment guidelines, it is impossible to use algorithms prepared by other countries, as rates of antibacterial resistance is different.

10:15–10:30

ATTITUDES AND STEREOTYPES IN LITHUANIAN POPULATION: ANTIBIOTICS AND TREATMENT OF INFECTIOUS DISEASES

Dr. Rasa458Ališauskienė and Saulius Novikas
“Baltic Surveys Ltd.”/“Baltijos tyrimai” / The Gallup Organization,
Vilnius, Lithuania

“Baltic Surveys” conducts Public opinion surveys and Household Panel project of Lithuanian population to learn about the usage and attitudes on antibiotics.

Household panel data shows that expenses of Lithuanian households on antibiotics makes 4% of all their total expenses on medicines (Medical Household Panel, Baltic Surveys).

In October 2011, 40% of adult Lithuanians reported the usage of antibiotics in last 12 month (for comparison, Eurobarometer data from 2009 reported 37% share). Women, middle-aged and older people use antibiotics more often. 67% of the users bought only prescribed antibiotics and 11% were given them at the hospital (in 2009 – 86% combined). More 10% has some leftover antibiotics at home (5% in 2009) and 5% (8% in 2009) managed to buy them without prescription.

77% of Lithuanians usually use antibiotics only if prescribed by the doctor, 6% decide themselves, 4% follow advice of relatives, friends, etc. and 8% said it varies.

Only 37% of all adults said that they would not use antibiotics without medical consultation – these are more often men, people under 30 years of age, people with higher incomes and residents of bigger cities.

Lithuanians most often use antibiotics without consulting the medical professional in case of bronchitis, flu, urinal infection, otitis, sinusitis, fever, rhinitis, sore throat and cough.

The most important sources of information about antibiotics are doctors (84%), pharmacists (35%), friends and relatives (22%). Internet, TV or press is important for less than 10% of population looking for the information on this issue.

The survey results also illustrate the attitudes and stereotypes of Lithuanians regarding usage and effectiveness as well as dangers of usage of antibiotics.

11:00–11:15

ANTIMICROBIAL RESISTANCE IN *STAPHYLOCOCCUS AUREUS*: DEVELOPMENT, POSSIBILITIES OF PREVENTION

Prof. Alvydas Pavilonis, Žaneta Maželienė
Microbiology Department, Lithuanian University of Health Sciences,
Kaunas, Lithuania

Staphylococcus aureus is the leading cause of bacterial infections in developed countries and produces a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia. *Staphylococcus aureus* is an important pathogen causing a wide range of infections in the hospital and community setting. Although *Staphylococcus aureus* infections were historically treatable with common antibiotics, emergence of drug-resistant organisms is now a major concern. Methicillin-resistant *Staphylococcus aureus* (MRSA) was endemic in hospitals by the late 1960s, but it appeared rapidly and unexpectedly in communities in the 1990s and is now prevalent worldwide. *Staphylococcus aureus* has a strong adaptive capacity and thus acquired various types of resistance to antistaphylococcal agents. More than 90% of isolates produce a penicillinase. Oxacillin remains active against these strains, but hospital associated *Staphylococcus aureus* and more recently community acquired *Staphylococcus aureus* have developed crossed resistance between methicillin (MRSA), oxacillin and other beta-lactams by production of a penicillin binding protein (PBP) with low affinity for beta-lactams, PBP2a. By contrast, community acquired MRSA (CA-MRSA) are only resistant to kanamycin, fusidic acid and tetracycline, in addition to methicillin.

As an aggressive pathogen, *Staphylococcus aureus* poses a significant public health threat and is becoming increasingly resistant to currently available antibiotics, including vancomycin, the drug of last resort for gram-positive bacterial infections. *Staphylococcus aureus* with intermediate levels of resistance to vancomycin - vancomycin-intermediate *Staphylococcus aureus* (VISA) was first identified in 1996.

Multidrug-resistant *Staphylococcus aureus* is an example of a bacterium for which the role of exposure to aerosolized organisms in disease transmission should be more closely evaluated. An international survey of infections due to *Staphylococcus* species resulted in the finding that *Staphylococcus aureus* was the most prevalent cause of hospital- and community-acquired bloodstream, skin and soft tissue, and lower respiratory infection. In the hospital setting, the most common mode of transmission of resistant *Staphylococcus aureus* is close contact with infected persons or with health-care workers with contaminated hands or clothing. Although drug-resistant *Staphylococcus aureus* has historically been a significant problem only in hospitals, the urgent need for further study of the ambient airborne concentrations and the role of airborne transmission of this organism in non-hospital environments is demonstrated by the increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the community.

Bacteria are great evolvers for many reasons. For example, their short generation times and large population sizes boost the rate at which they can evolve. In addition, one quirk of bacterial genetics is particularly salient to the evolution of antibiotic resistance:

- Evolution with vertical transmission,
- Evolution with horizontal transfer,
- Horizontal transfer can speed up the evolution of antibiotic resistance,
- Horizontal transfer aids the evolution of multiply-resistant strains.

In 2008, 33 countries reported result of 31819 *Staphylococcus aureus* isolates to EARSS, of which 21 were identified as MRSA. The incidence of MRSA, particularly skin and soft-tissue infections, is increasing in both academic and rural hospitals, ranking it one of our top stories of the year. In response, the CDC has developed a 12-step interventional program. The 12-step program includes four components: prevent infection, diagnose and treat infection effectively, use antimicrobials wisely and prevent transmission.

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11:20–11:35

PREVALENCE OF RESIDUES OF VETERINARY MEDICINAL PRODUCTS AND OTHER SUBSTANCES IN LIVE ANIMALS AND ANIMAL ORIGIN PRODUCTS IN THE REPUBLIC OF LITHUANIA

Assoc. Prof. Dr. Petras Mačiulskis

The National Food and Veterinary Risk Assessment Institute,

Vilnius, Lithuania

Maximum residue limits of the veterinary medicinal products and certain substances in live animals and animal origin products are established in accordance with generally recognized principles of safety assessment, taking into account toxicological risks, environmental contamination, as well as the microbiological and pharmacological effects of residues in order to protect public health (EC, 2009, 2010). Council Directive *96/23/*EC lays down measures to monitor certain substances and residues thereof, mainly veterinary medicinal products, in live animals and animal products (EC, 1996). Additionally, Commission Decision *97/747/*EC lays down levels and frequencies of sampling for certain animal products (EC, 1997). Number of samples for monitoring of residues in live animals and animal origin food products are calculated according number of animals slaughtered and quantity of animal origin food products made. Analysis showed that 3441 samples of live animals and animal origin food products were tested in 2010 compared to 3949 samples in 2008 and 3432 samples in 2009 in Lithuania. There were 0.25% samples of live animals in 2010 non-compliant to legal requirements compared to 0.4% samples in 2008 and 0.5% samples in 2009 in Lithuania. Investigation of animal origin food product samples displayed that 0.34% samples in 2010 in comparison to 0.67% samples in 2008 and 0.43% samples in 2009 were non-compliant to legal requirements in Lithuania. Aggregated analysis showed that antimicrobial substances in 0.22% of animal samples and in 0.3% of animal origin food product samples, non steroidal anti-inflammatory drugs in 0.07% of animal samples and chemical substances in 0.09% of animal samples, and dioxins, furans and dioxin type PCB substances in 0.16% of animal origin food product samples were noncompliant to legal requirements in 2008–2010 in Lithuania (NFVRAI, 2010, 2011).

Key words: veterinary medicinal products, other contaminants, residue monitoring, residue prevalence, food safety

References:

1. EC (European Commission), 1996. Council Directive *96/23/*EC, on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives *85/358/*EEC and *86/469/*EEC and Decisions *89/187/*EEC and *91/664/*EEC. OJ L 125, 23/05/1996, p.10–32.

2. Commission Decision *97/747/*EC 27 October 1997 fixing the levels and frequencies of sampling provided for by Council Directive EC (European Commission), 1996. 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products. OJ L 303, 6.11.1997, p. 12–15.

3. EC (European Commission), 2009. Regulation of the European Parliament and of the Council (EC) No 470/2009 of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive *2001/82/*EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council. OJ L 152, 16.06.2009, p. 11.

12:00–12:15

STANDARD INFECTION CONTROL PRECAUTIONS. STERILISATION OF MEDICAL DEVICES AND OPPORTUNITIES IN LITHUANIAN HOSPITALS

Dr. Ramutė Budginaitė

Centre for Communicable Diseases and AIDS, Vilnius, Lithuania

To prevent transmission of microorganisms including those resistant to antimicrobials in the personal health care institutions, the standard infection control measures are applied in all patients. That are protective measures (personal staff protection measures, hand hygiene, cleaning, disinfection, sterilisation of medical devices and environment, etc.) against infections transmitted by direct contact with non-intact skin, mucous membrane with blood or other body fluids, secrets, excreta or contaminated objects. Standard and excess infection control measures (patient isolation, individual nursing means, excess environment cleaning and disinfection) are applied in cases of infection or colonisation with antimicrobial-resistant microorganisms (MRSA, VRE, Clostridium difficile, carbanemase producing enterobacteria).

Sterilisation of medical devices is significant standard infection control measure. Probability of sterility of the medical devises depend on elimination of device microbiological contamination (treatment process) prior to sterilisation (cleaning/disinfection procedures) and efficiency of the sterilisation process. Treatment and sterilisation process of the multiple-use medical devices includes several stages and procedures: purchase of appropriate devices, cleaning and disinfection, of devices, packing, sterilisation, appropriate transportation, storage, use. Devices should be treated by acknowledged ways and means, and process quality should be controlled. All this requires effective management system of the treatment process in the single structure (organisation) with competent staff qualified in the field of medical device treatment procedures.

To assess organisation of medical device treatment and sterilisation process, technologies applied, means maintained and their demand, a survey of Lithuanian hospitals (including 90 hospitals) was conducted. The survey revealed the following:

1. Majority of medical devices in Lithuanian hospitals are treated (cleaned, disinfected) in a non-centralised way (in departments and places of medical device use). Only 12% of hospitals maintain centralised departments for disinfection and sterilisation.
2. 40% of hospitals providing surgical treatment do manually wash the instruments.
3. Current new technologies and potential of equipment are not availed.
4. Provision and purchase of the services of medical device cleaning, disinfection, sterilisation is not popular.
5. Organisation of medical device treatment and sterilisation in each single hospital is rather expensive and prevent from implementation of requirements of all best practices.

Treatment and sterilisation services of medical devices must be centralised in the Lithuanian hospitals to create effective, economical, safe, and reliable system for treatment of medical devices and means that meets European standards. This would precondition use of modern cleaning, disinfection and sterilisation equipment, ways and means that meets European Union and Lithuanian standards, quality improvement in using, cleaning, disinfection and sterilisation processes, and reduction of respective costs. This proves expediency for development of the programme project for renovation of sterilisation rooms.

12:20–12:35

LAB ORGANIZATION FOR SURVEILLANCE OF HOSPITAL ACQUIRED INFECTIONS (HAI) AND EMERGING RESISTANCE

Dr. E. Montrucchio
ID/AST European Application and Scientific Manager Diagnostic Systems,
BD Biosciences, Italy

The organization of the laboratory should reflect the need to respond to those that are the cornerstones of the surveillance of Health Associated Infections (HAI): in particular early detection and communication for alert organisms, indirect surveillance of infections, detection of epidemic outbreaks, surveillance of increased resistance to antimicrobials. Standard microbiological techniques to perform diagnostic tests, automated systems and computer systems for the early determination of pathogens causing HAI, and real-time communication of HAI events to wards and Infection Control, are actually provided to the laboratory by companies such as BD, that since so many years are working in the field of Microbiology.

12:40–12:55

THREAT OF THE POST-ANTIBIOTIC ERA: DO WE NEED TO CHANGE OUR PRESCRIBING HABITS?

Assoc. Prof. Dr. Jolanta Gulbinovič[1,2], Gintautas Barcys[1]
[1] *State Medicines Control agency*, *Vilnius, Lithuania*
[2] *Vilnius University, Vilnius, Lithuania*

Over the last decades the development of antimicrobial resistance has surpassed the development of new antibacterial drugs. In addition, large companies have withdrawn from this field despite a growing clinical need. More and more cases of infections caused by MDR resistant microorganisms are reported in medical literature. For some of them there is no curable agent any more. There is a threat that we are approaching the post-antibiotic era again.

It has been recognized generally that bacterial resistance is an unavoidable consequence of antimicrobial drug use, and that it correlates with the overall use of antimicrobial drugs in a defined setting. Factors that promote the emergence of resistance include frequent use of broad-spectrum antimicrobial agents, prolonged use of antimicrobial agents, more frequent use of invasive devices and procedures, large numbers of patients with complex medical problems in small areas within a hospital, and the presence of patients who require prolonged hospitalisation and often harbour antibiotic resistant bacteria. It is also accepted that rational use of antimicrobial drugs and appropriate infection control measures could slow down the development and spread of resistance.

Monitoring of antimicrobial use and knowledge of prescription habits are some of the strategies recommended by the EU Microbial Threat Conference in Copenhagen 1998 and by WHO to combat the antimicrobial resistance. A number of studies on antibacterial drug use in hospitals and outpatient settings have been performed in Lithuania and other countries (e.g. J. Gulbinovic et al. Microbial Drug Resistance 2001;7(4): 383-389; V. Vlahovic-Palčevski et al. Clin Microbiol Infect 2007;13: 277-283; U. Dumpis et al. Int J Clin Pharmacol Ther 2007;45(10): 568-76, K. Garuolienė et al. Bendrosios praktikos gydytojas 2011) to evaluate and benchmark the patterns of antibacterial drug use and to envisage possible strategies for rational antibiotics use in order to preserve them for the future.

14:00–14:15

USE OF ANTISEPTIC (OCTENIDINE CHLORIDE AND PHENOXYETHANOL) IN MULTIRESISTANT GERM CAUSED WOUND INFECTION TREATMENT

Loreta Pilipaitytė, Domantas Rainys

Hospital of Lithuanian University of Health Sciences Kauno Klinikos, Kaunas, Lithuania

Wound infection is associated with delayed healing, extended hospital stay, increased costs also present challenges for clinicians managing it. Recent guidelines have suggested that topical dressings with antiseptics may help reduce wound bioburden.

The aim of this study, was to evaluate the antimicrobial effectiveness of Octenidine Dihydrochloride and Phenoxyethanol in multiresistant germ caused wound infection.

A review was conducted of clinical outcomes for patients who had been treated because of wound infection caused by Methicillin-resistant *Staphylococcus aureus*, Multidrug-resistant *Pseudomonas aeruginosa*, Multidrug-resistant *Acinetobacter* spp. Trying to overcome the problem topical antimicrobial therapy was applied. The patients were washed with Octenidine wash lotion and wounds were dressed with Octenidine Dihydrochloride every day.

In all cases clinical signs of infection disappeared, the results of microbiological assessment were negative. All wounds became clean with healthy granulation tissue or epithelized.

Conclusion: Octenidine Dihydrochloride was effective antiseptic agent in the treatment of the multiresistant bacteria-contaminated wounds. Octenidine Dihydrochloride can be considered as a treatment choice because of its beneficial ability to prevent from bacterial invasion into the deep tissue and to cause systemic infection.

14:20–14:35

EARLY DIAGNOSTICS AND TREATMENT STRATEGY OF SEPSIS AND SEPTIC SHOCK

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Sepsis is an invasion of microorganisms or/and their toxins into the blood stream together with the host response to this invasion. Any infection may be complicated by sepsis. Normally, microorganisms are recognized by their specific cell wall products (endotoxins), egzotoxins, viral RNA, bacterial DNA. The host uses specific receptors (Toll-like receptors) to sense the presence of mentioned molecular patterns of microorganisms. Hyperinflammation, procoagulant state, endothelial dysfunction, myocardial depression, microcirculatory dysfunction leads to multiorgan dysfunction due to tissue hypoxia and cytokine induced apoptosis. **Severe sepsis** defines an acute organ dysfunction secondary to infection, **septic shock** – hypotension not reversed with fluid resuscitation. Sepsis, severe sepsis and septic shock similar to acute myocardial ischaemic attack and acute brain attack needs the speed and appropriateness of therapy administered in hours after syndrom develops. This influences outcome.

Diagnostic sepsis criteria include infection, documented or suspected, and some of general variables (fever >38.3^{o}C, core temperature <36^{o}C, heart rate >90/min.etc.), inflammatory (leucocytosis >12,000μL^{-1} , leucopenia <4000 μL^{-1} , normal WBC count with >10% immature forms etc.), hemodynamic (arterial hypotension SBP <90 mmHg or SBP decrease >40 mmHg etc.), organ dysfunction variables (arterial hypoxemia PaO_2 /FiO_2 <300, urine output <0.5 mg/kg /hr, thrombocytopenia <100,000 μL^{-1} etc.), tissue perfusion (decreased capillary refill, hyperlactatemia) variables. Severe sepsis presents with organ failure, septic shock – with multiorgan failure in face of ineffective fluid therapy to correct hypotension.

Recommendation to obtain at least two blood cultures before antibiotic administration with at least one drawn percutaneously and one drawn through each vascular access device (of >48 hrs in situ). Cultures of other sites as wounds, respiratory secretions, urine, cerebrospinal fluid etc. should also be obtained if not associated with significant delay in antibiotic administration. To confirm any source of infection imaging studies must be performed promptly. Any patient with septic shock requires **monitoring** with an arterial catheter to enable reliable and continuous assessment of arterial pressure. The catheter also facilitates blood sampling especially for blood gas analysis. The value of placing a pulmonary artery catheter in the absence of randomised controlled trials is likely to be of use particularly in patients with concomitant cardiopulmonary disease.Blood lactate level has a slow rate of change so it cannot be used to guide a treatment.

Management of sepsis consists of antibiotic therapy, infection source control, hypovolemia correction. Management of severe sepsis and septic shock consisits of bundles of recommendations or suggestions as initial resuscitation, antibiotic therapy, source identification and control, fluid therapy, vasopressors, inotropic therapy, steroids, blood products administration, mechanical ventilation of sepsis-induced ALI/ARDS, glucose control, renal replacement, bicarbonate therapy, deep vein thrombosis and stress ulcers prophylaxis according to evidence-based guidelines. The treatment of fever is controversial. Increased body temperature increases oxygen requirements, but the increased cellular metabolism may present part of the host natural defence.

Animal studies suggested that the release of heat shock proteins may have important protective effects. Nutritional support in such patient with septic shock must be provided by enteral route after hemodynamic stability achieved with careful control of blood glucose levels. Organ support is provided for individual organ dysfunction. Starting of mechanical ventilation can be related with worsening of arterial hypotension, and it can suggest a presence of hypovolemia responsible for a reduction in venous return due to increased intrathoracic pressure. Limited tidal volume useful for hemodynamic reasons and decreases inflammatory reaction. Exessive sedation of patient with ventilatory support can compromise hemodynamic stability and prolong duration of mechanical ICU stay. Sepsis is leading cause of acute renal failure in the ICU. The main prophylaxis of it is to maintain adequate volemia and renal perfusion. Continuous venovenous hemofiltration with or without hemodialysis are preferrable in purpose to control fluid balance.

In summary, sepsis may progress to severe sepsis and septic shock, but clinically it is difficult to predict which patient is going to develop septic shock and when as it can present abruptly without previuos signs of sepsis. It is necessary to use evidence-based diagnostic and management guidelines to improve sepsis outcomes.

14:40–14:55

INNOVATIVE MOLECULAR DIAGNOSTIC APPROACHES FOR FAST SCREENING FROM BLOOD CULTURES

Dr. Pablo Castan

Sanchinarro Hospital North Madrid, Spain

Sepsis is a systemic inflammatory response which occurs in response to microbial infection and is mostly due to the presence of micro-organisms in the blood, urine, skin, lymph or other tissues. In recent times a number of patients are developing this condition as a result of three main factors: a rise in the number of organ transplants and other surgical procedures that require suppressing the patient's immune system; the greater number of elderly people in the population and the overuse of antibiotics to treat infectious illnesses, resulting in the development of drug-resistant bacteria. Conventionally, identifying the causative agent of sepsis is a time-consuming process, necessitating culturing infected samples with selective media. The protocol implemented on our lab using the BlackLight® Sepsis Kit assists in the rapid identification of bacteria by providing a standardized PCR reaction to amplify three regions of the 16S gene to provide reliable high-quality template for Pyrosequencing®. This protocol has been designed to fully characterise sepsis no matter where bacteria can gain entry to the body causing tissue contamination. Common sites of testing include blood and blood cultures, the genitourinary tract and other in-development applications such as the liver and its bile ducts, the gastrointestinal tract, and the lungs.

15:00–15:15

PRESEPSIN – NEW DIAGNOSTIC MARKER FOR SEPSIS

Dr. Audronė Eidukaitė, MD, PhD
Vilnius University Children‘s Hospital, Vilnius, Lithuania

Sepsis is a leading cause of death in critically ill patients despite the use of modern antibiotics and resuscitation therapies. Several biochemical markers have been proposed over the past years to manage critically ill patients with acute inflammation and sepsis. The state of the art in diagnosing and monitoring severe sepsis and septic shock consists of the measurement of plasma C-reactive protein (CRP) and procalcitonin (PCT) at the onset and in the course of the disease. CRP and PCT in combination are clinically significant in diagnosing and monitoring septic; however, CRP and PCT have a very limited value for risk stratification and in predicting outcome.

Novel sepsis marker Presepsin is a fragment of CD14, the lipopolysaccharide-binding protein (LPS–LBP) complex receptor, and it is produced in response to bacterial infections. CD14 is present in macrophage, monocyte, and granulocyte cells and their cell membranes, and is said to be responsible for intracellular transduction of endotoxin signals. Its soluble fraction is present in blood and is thought to be produced in association with infections. It is called the soluble CD14 subtype (sCD14-ST) or Presepsin.

The levels of Presepsin were significantly higher in septic patients than in patients with SIRS (systemic inflammatory response syndrome) or apparently healthy individuals. Presepsin levels were elevated earlier than IL-6 and D-dimer along with occurrence of blood bacteria in animal model. Presepsin demonstrated a strong relationship with disease severity and outcome. Presepsin values were related to the course of the disease. In contrast to PCT, Presepsin enabled more reliable prognosis and risk prediction of 30-day mortality already at admission.

Preliminary decision cutoffs

Presepsin (pg/ml)	**Diagnosis**	**Clinical consequences based on study data**
<200	Exclusion of sepsis	No blood culture necessary
≥300	Systemic infection (sepsis) possible	Further diagnostic measures including blood culture
≥500	Moderate risk of progression of systemic infection (severe sepsis)	Early goal directed therapy after taking blood cultures
≥1000	High risk of progression of systemic infection (severe sepsis/septic shock).	Consideration of adjunctive therapy

In summary, Presepsin appeared to be a more accurate diagnostic marker for differentiation between sepsis grades and prediction of mortality risk which could be improved by combination of Presepsin and clinical scores. The use of Presepsin may improve the management of sepsis patients.

Using the PATHFAST analyzer Presepsin concentration can be determined within 17 minutes from whole blood samples. PATHFAST Presepsin is a chemiluminescent enzyme immunoassay (CLEIA) for the quantitative measurement of Presepsin concentration.

15:20–15:35

DRUG RESISTANT TUBERCULOSIS IN LITHUANIA: PREVALENCE AND REASONS, COST AND PERSPECTIVES

Dr. Kęstutis Miškinis

National Health Insurance Fund under Ministry of Health

Drug resistant tuberculosis is a consequence of inefficient national tuberculosis programme. The main reasons are incorrect technical actions of different origin and/or patients' incompliance, mainly related to social and economic reasons. The most adverse phenomenon of resistance is multi-drug-resistant tuberculosis (MDR TB), when resistance is defined at least to two principal anti-tuberculosis drugs – rifampin and isoniazid. 113 to 128 new MDR TB cases are registered in Lithuania every year (from 2008 to 2010), while in 2010 the total number of registered MDR TB cases was 310, and the growth trend in the last decade has continued. Lithuania is among 10 countries in the world heavily hit by MDR TB. The treatment of drug resistant tuberculosis, especially MDR TB, is very expensive. It cost Lithuanian taxpayers from 9.32 to 10.54 million litas (1euro = 3.4528 litas) annually during 2008–2010. Investigation of reasons why MDR TB is increasing shows that the source of new MDR TB is very much dependent of poor treatment results of new smear positive TB cases. In 1996–2009 cure rate was from 65 to 82%, which does not reach World Health Organization (WHO) target to cure 85% and more. All first-attempt failures to cure are treated repeatedly, and that is much more expensive than initial treatment.

To make the tuberculosis programme more efficient and decrease figures of MDR TB as well as the financial burden to the country, first and foremost the high cure rate nominated by WHO must be achieved. In addition, ambitious goals must be envisaged and specific tasks should be set in the new 2011-2020 Lithuanian Health Programme (tuberculosis part). As a result, if tasks are accomplished, the MDR TB burden would decline significantly.

15:40–15:55

DETECTION OF MUTATIONS ASSOCIATED WITH DRUG RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS* STRAINS IN ST. PETERSBURG AND LENINGRAD OBLAST (RUSSIA)

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Biomedical Centre, Saint Petersburg University, Russia

Introduction. Spread of tuberculosis (TB) in many countries of the world became a natural consequence not only of such factors as social and economic recession and spread of HIV infection. It is also a result of biological changes of the pathogen – generation of *Mycobacterium tuberculosis* complex strains resistant to many drugs. Nowadays, spread of TB caused by MDR and XDR *M. tuberculosis* strains grows rapidly.

Antituberculous drugs resistance significantly reduces the success of TB treatment. Thus, rapid detection of drug-resistance is essential for correct anti-tuberculosis therapy. That is why it is important to use molecular-genetic methods of drug-resistance detection based on analysis of point-mutations, which appear to be molecular markers of *M. tuberculosis* resistance. Mutations associated with drug resistance were described for all first-line and for some second-line anti-TB drugs. However, the frequency of these drug-resistance mutations in *Mycobacterium tuberculosis* strains may vary in different geographical regions.

Aim. To evaluate the frequency of mutations associated with *M. tuberculosis* drug resistance to Isoniazid, Rifampin and Ofloxacin in St. Petersburg and Leningrad oblast.

Results. To analyze the frequency of mutations in *katG* gene 45 Isoniazid-resistant *M. tuberculosis* cultures were selected in a random way in St.Petersburg TB Center. DNA-sequencing of *katG* gene fragment showed that 93% of Isoniazid-resistant strains have mutation S315T.

To evaluate the frequency of mutations in *rpoB* gene for MTB strains circulated in St. Petersburg, we selected randomly 37 Rifampin-resistant isolates. Mutations in codons 531, 516, 522 and 526 of *rpoB* gene were detected for 88% of analysed isolates.

Point mutations in *gyrA* and *gyrB* genes were analyzed for 30 Ofloxacin-resistant *M. tuberculosis* isolates spread in St.Petersburg. Mutations in *gyrA* gene were detected in 57% of Ofloxacin resistant isolates. There were no mutations within sequenced region of *gyrB* gene for all 30 Ofloxacin-resistant MTB isolates. Thus, 43% of studied Ofloxacin-resistant strains did not have mutations neither within *gyrA* gene, nor in *gyrB* gene.

Thirty three Ofloxacin-resistant *M. tuberculosis* isolates and were revealed in Leningrad oblast. Results of DNA sequencing showed that 69.7% of Ofloxacin-resistant *M. tuberculosis* isolates had mutations within short region of *gyrA* gene. Ten isolates (30.3%) did not have mutations associated with Ofloxacin-resistance. There were no mutations associated with Ofloxacin-resistance found among susceptible *M. tuberculosis* isolates.

Conclusions. Detection of mutations within short regions of *katG* and *rpoB* genes allows to detect about 90% of Isoniazid- and Rifampin-resistant *M. tuberculosis* strains in St. Petersburg, respectively. Detection of mutations in *gyrA* gene allows to detect at least 50% of Ofloxacin-resistant MTB strains in St. Petersburg. Detection of point mutations in *gyrA* short region allows to discover about 70% of Ofloxacin-resistant *M. tuberculosis* isolates in Leningrad oblast.

16:45–17:00

THE HIV-I RESISTANCE MUTATIONS REVEALED AT PATIENTS ON HAART IN BELARUS

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Resume. In article the results of researches spent in 2007-2011 on HIV resistance mutations definition at patients, being on HAART are presented. Of 35 patients 24 were adults and 11 children. As a result of the researches have been shown adult patients have mutations in position K103N (41,2 %), defining HIV high level resistance to NNRTIs: nevirapine (NVP), delavirdine (DLV), both efavirenz (EFV), and M184V (38.2 %) which is at the bottom of occurrence of high level resistance to NRTI: lamivudine (3TC) and emtricitabine (FTC), and also of low level resistance to didanosine (ddI) and abacavir (ABC). At children is more often resistance mutations in position M184V (36 %) have been revealed.

Materials and methods. Plasma of HIV-infected patients, EIA, Western blot, RT-PCR, PCR, sequencing, commercial database «ViroSeq HIV-1 genotyping system software v2.6 analysis» (Abbott, the USA), software products «Sequencing Analysis Software v.5.1.1», «BioEdit», «SeqScape», «Mega 4.1»

Results. Of 24 patients (adults), 13 were men (middle age of 35.9 years) and 11 female (middle age of 31.5 years). 16 patients were from different areas of the Minsk region, on 2 persons from the Gomel, Vitebsk areas and Minsk, and 1 – from the Brest region. 22 (91.7%) the person were carriers of HIV-1 subtype A and 2 (8.3%) have been infected by the CRF06_cpx for the first time described by us in territory of Belarus. In 14 (41.2 %) cases K103N mutation defining high level of resistance to NNRTI: NVP, DLV, and EFV was defined. At 13 (38.2%) patients the mutation in M184V position which is at the bottom of occurrence of high level resistance to NRTIs: 3TC and FTC. In 4 (11.8%) cases we have found out G190S mutation with which occurrence of high level resistance mutations to NNRTIs: NVP and EFV. In two cases have defined a mutation in Y188L position which conducts to occurrence of HIV-I high level resistance to NNTRIs NVP and EFV. At 7 patients the combinations of K103N+M184V mutations defining high level resistance to NNRTIs and NRTIs have been revealed. From 11 patients − children born to an HIV-infected mothers in 9 (36%) cases we revealed mutation in position M184V, defining high level resistance to NRTIs. In one case the mutation in position K103S, leading to occurrence of high level resistance to NVP and intermediate resistance to DLV and EFV has been revealed. At two children mutation at position Y188L which conducts to occurrence of high level resistance to NNRTIs NVP and EFV have been revealed.

Conclusion. The conducted researches have show that for successful APT inclusion in treatment schemes a new drugs for treatment of patients at which come to light multiresistant HIV strains is necessary. 25 sequences of genes gag-pol are registered in the International database GenBank. Researches are executed with financial support of Global Fund, the project of the international technical help «Preventive maintenance and treatment HIV/AIDS in Belarus».

Abstract P01

THE ASSESSMENT OF PUBLIC'S KNOWLEDGE OF ANTIBIOTIC USE IN LITHUANIA

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Background. Antimicrobial resistance is a serious public health problem worldwide. Irrational use of antibiotics increases antimicrobial resistance. Reasons of irrational antibiotic use can be lack of knowledge of these preparations and high rate of self-medication with antibiotics.

Purpose. The main objective of this study was to assess public's knowledge of antibiotic use in different society groups.

Methods. The anonymous survey method was performed according to original questionnaire containing 37 questions. The questionnaire was distributed to clients of chosen pharmacies in different cities of Lithuania. 1209 adults completed the questionnaire. A group of doctors and nurses from different hospitals of Lithuania was constituted and involved in this study. A descriptive and comparative statistical data analysis was processed with SPSS Statistics 17.0.

Findings. More than half of respondents' (52.9%) knowledge of antibiotics was poor. Almost half of responders (42.7%) incorrectly identified antibiotics as being effective in treating viral infections. Knowledge of antibiotics was associated to the age, education level, occupation field, frequency of antibiotic use and frequency of visits to family doctors. 36.0% of respondents claimed that they have brought antibiotics without prescription at least once. 19.8% of respondents who have children claimed, that they give antibiotics to children according to their own knowledge or advices of family members.

Conclusion. Knowledge of antibiotics in Lithuanian society is insufficient. More information about antibiotic use should be given by physicians, while prescribing these preparations. Self–medication with antibiotics is a serious problem in Lithuania and considerable attention should be noticed.

STAPHYLOCOCCUS AUREUS: PREVALENCE IN COMMUNITY AND HOSPITAL, RESISTANCE TO ANTIBIOTICS

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Background. *Staphylococcus aureus* is one of the most common causes of endemic and epidemic infections. Colonizing strains are as endogenous reservoirs for overt infections or may spread to other patients. Multidrug-resistant strains of staphylococci have been reported with increasing frequency worldwide.

Purpose. The aim of this study was to confirm the carriage rates of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in community and hospital patient and susceptibility of *Staphylococcus aureus* to antimicrobial agents.

Methods. We investigated 243 preschool- and 300 school-aged, 531 university/colledge students and 277 hospital patients. *Staphylococcus aureus* identification and antibiotic susceptibility was tested manually according to NCCLS (National Committee for Clinical Laboratory Standards) and EARSS (European Antimicrobial Resistance Surveillance System) recommendations. *Staphylococcus aureus* identification was made by Plasmacoagulase and DNA'ase tests. Isolated *S. aureus* strains resistance to antibiotics has been compared by determination of minimal inhibitory concentration (MIC) using "Sensititre" plates (TREK Diagnostic System). The results were evaluated statistically using SPSS 12.0 program. Differences were considered significant if the P value was below 0.05.

Findings. 711 *Staphylococcus aureus* strains were detected: 252 *Staphylococcus aureus* strains from preschool- and school-aged pupils, 290 *Staphylococcus aureus* strains from students and 169 strains from hospital patients. The carriage rate of *Staphylococcus aureus* in community was 54.6% and in hospital patients was 61.0% ($p>0.05$). 25 (5.45%) strains out of all isolated *Staphylococcus aureus* were resistant to methicillin: 12 (4.13%) MRSA strains were isolated from community and 13 (7.69%) MRSA strains were isolated from hospital patients ($p<0.001$). 212 *Staphylococcus aureus* strains were investigated from clinical material resistant to antibiotics. Methicillin susceptive *Staphylococcus aureus* strains were most resistant to penicillin (91.60%) and ampicillin (65.9%). *Staphylococcus aureus* clinical strains were sensitive to rifampicin (98.2%) and vancomycin (100%) and resistant to penicillin (97.2%), ampicillin (96.3%), clarithromycin (45.8%), erythromycin (38%) and tetracycline (27.8%). MRSA strains were sensitive to vancomycin (100%), daptomycin (100%), clindamycin (82.7%), rifampicin (97.3%).

Conclusion. Well-designed community-based and clinical studies with adequate risk factor analysis are required to further elucidate the epidemiology of *Staphylococcus aureus* and MRSA. Surveillance of MRSA provides relevant information on the extent of the MRSA epidemic, identifies priorities for infection control and the need for adjustments in antimicrobial drug policy.

IN VITRO ANTIMICROBIAL ACTIVITY OF EXTENDED-SPECTRUM BETA-LACTAMASES-PRODUCING *KLEBSIELLA PNEUMONIAE* IN HOSPITAL OF LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

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Background. Infections caused by extended- spectrum β-lactamases-producing (ESBL-producing) *Klebsiella pneumoniae* (*K. pneumoniae*) isolates are a major concern for clinicians, since the choice of drugs appropriate for treatment is limited. The study of antibiotic resistance prevalence for appropriate antibiotic choice is needed.

Purpose. The aim of our study was to evaluate *in vitro* activity of ertapenem, amikacin, ciprofloxacin against ESBL-producing *K. pneumoniae* strains.

Methods. We included all *K. pneumoniae* strains, isolated from patient's lower respiratory tract samples, hospitalized in Hospital of Lithuanian University of Health Sciences during 2009 January and 2011 May and showing resistance to cefotaxime and/or ceftazidime by disk diffusion method. In vitro activity of ertapenem, amikacin, ciprofloxacin was determined by minimal inhibitory concentrations (MIC), which were performed by E-test method. MIC's were evaluated using breakpoints defined by Clinical Laboratory Standard Institute.

Findings. Total 167 *K. pneumoniae* strains were included in this study. In vitro susceptibility of ESBL-producing *K. pneumoniae* were found for ertapenem 96.4%, for amikacin 98.2%, for ciprofloxacine 48.5% respectively. MIC50 and MIC90 values of ertapenem were 0.094 µg/ml and 0.5 µg/ml, of amikacin were 3µg/ml and 8µg/ml and of ciprofloxacine were 12µg/ml and 32µg/ml. MIC50 and MIC90 values of ertapenem amikacin were found in susceptible limits, whereas MIC50 and MIC90 values of ciprofloxacine were found in resistance breakpoints.

Conclusion. We observed that ertapenem and amikacin activity was no affected in ESBL-producing *K. pneumoniae* strains, but ciprofloxacin activity was affected. Our discovered elevated MIC's of ertapenem makes us worry about carbapenem resistance in the future.

CONFIRMATION OF *ESCHERICHIA COLI* AND DIFFERENTIATION OF THE MOST IMPORTANT MUTATIONS IN THE BETA-LACTAMASE GENES TEM, SHV AND CTX-M BY ESBL ASSAY

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Background. Prevalence of extended spectrum β-lactamases (ESBL) production in *Escherichia coli* strains has been increasing worldwide. Rapid identification of bacteria, detection and evaluation of these enzymes is important as *E. coli* clinical isolates excel high frequency of CTX-M, TEM and SHV.

Purpose. The aim of this study was to confirm *E. coli* strains, and to evaluate the prevalence and frequency of extended β-lactamases-CTX-M, TEM, SHV, wild- type TEM and SHV β-lactamases in *E. coli* clinical isolates at Hospital of Lithuanian University of Health Sciences Kaunas Clinics.

Methods. We evaluated the molecular diagnostic ESBL assay (GenID, Straβberg, Germany) that employs amplified DNA fragments to confirm *E. coli* strains, and discriminates CTX-M, TEM and SHV and wild-type TEM and SHV-genes. 30 clinical samples were isolated from blood (n=4), urine (n=15), trachea (n=1), bronchus (n=2), stomach (n=3), suspected site of infection (n=1), wound (n=4). Total DNAs were extracted using Qiagen DNA mini kit. Internal positive control is detected in the reverse hybridisation assay so it is not necessary to analyze the PCR products by gel electrophoresis. Susceptibility of isolates for 16 antibiotics were analysed by using Confirmatory MIC plates (ESB1F, Sensititre).

Findings. ESBL assay allowed to confirm *E. coli* strains, and to detect fastly TEM, SHV and CTX-M ESBL and non ESBL TEM, SHV (wild-type) genes in clinical samples. The most prevalent of ESBLs found in *E. coli* was CTX-M (n=30/25). TEM AS 104 E (wild) was detected in 19 *E. coli* clinical isolates when TEM AS 164 R (wild) and TEM AS 238 G (wild) were found equally in isolates (n=18). TEM AS 104 K (ESBL) and TEM AS 238 S (ESBL) were detected in one *E. coli* strain (no CTX-M gene detected). None of TEM AS 164 S (ESBL), TEM AS 164 H (ESBL), SHV AS 179 D (wild), SHV AS 179 A (ESBL), SHV AS 179 G (ESBL), SHV AS 179 N (ESBL) and SHV AS 238/240 (wild) were detected. All isolates were resistant to cephalothin and susceptable to imipenem, piperacillin/tazobactam, ceftazidime, ceftazidime/clavulanic acid, cefotaxime/clavulanic acid. 24 *E. coli* strains expressed resistance to cefazolin and cefpodoxime, 7 – to ceftriaxone, 13 – to gentamicin, 25 – to ampicillin, 5 – to cefotaxime.

Conclusion. The ESBL assay is a quick way to confirm *E. coli* strains and to distinguish TEM, SHV and CTX-M ESBL genes and non ESBL TEM, SHV (wild-type) genes in clinical samples. The majority of *E. coli* expressed multidrug resistance.

EVALUATION OF *SALMONELLA ENTERICA* RESISTANCE TO ANTIMICROBIAL AGENTS

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Background. Rapid increase in the incidence of antibiotic resistance and the emergence of multiresistant strains of *Salmonella* isolated from animals and humans has been observed in the world for two decades, especially in developing countries.

Purpose. The aim of this study was to investigate the emergence of antibiotic resistant *Salmonella enterica* that spread to humans by consumption of contaminated food and direct contact.

Methods. 125 antibiotic resistant strains of *Salmonella enterica* were isolated and identified. *Salmonella enterica* resistance to 9 antimicrobial classes to 27 antibiotics has been compared by determination of minimal inhibitory concentration (MIC) using “Sensititre” plates (TREK Diagnostic System). The results were evaluated statistically using SPSS 12.0 program. Differences were considered significant if the P value was below 0.05.

Findings. *Salmonella enterica* was the most resistant to penicillins: 88% strains showed resistance to ampicillin, 74.4% – to piperacillin, 57.2% – to mezlocillin. All investigated salmonella strains were susceptible only to 3rd generation cephalosporin cefotaxime. High resistance of isolated strains was observed to combinations with beta-lactamase inhibitors: ampicillin/sulbactam – 52%, ticarcillin/clavulanic acid – 60.8% and piperacillin/tazobactam – 15.2%. Resistance to aminoglycosides was 1.6%–6.4%, to quinolones – 3.9%–4.0%. We found that 32.3% of investigated *Salmonella enterica* strains showed multiresistance to antimicrobial agents: 18.3% strains were resistant to three classes of antibiotics, 7.5% – to four classes of antibiotics, and 6.5% of isolated salmonella were resistant to five or six antimicrobial classes. Resistance patterns were similar among strains from all hospitals and differences were not statistically significant.

Conclusion. The presented data suggest that resistance of *Salmonella enterica* isolated from humans in Lithuanian hospitals related to high resistance of strains to penicillin class antimicrobial agents and its combinations with beta-lactamase inhibitors and more than 30% of resistant strains showed multiresistance to antimicrobial preparations.

COMPARISON OF SOURCE OF INFECTION AND ANTIBIOTIC RESISTANCE IN CARBAPENEM-RESISTANT AND CARBAPENEM-SENSITIVE *ACINETOBACTER* SPP.

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Background. *Acinetobacter* is important nosocomial pathogen with increasing carbapenem resistance, it is alarming, because of limited choice of treatment.

Purpose. To compare source of infection and resistance to antibiotics in carbapenem- resistant and carbapenem-sensitive *Acinetobacter* spp. strains.

Methods. A retrospective data analysis was performed of patients, treated in Hospital of Lithuanian University of Health Sciences. Identification and antimicrobial susceptibilty of isolated *Acinetobacter* spp. strains were performed by Phoenix ID system (Becton Dickinson, USA), results were interpreted according to Clinical and Laboratory Standards Institute guidelines.

Findings. Total 99 *Acinetobacter* spp. strains were isolated, 40.4% of isolates were resistant to carbapenems. We found source of infection in patients with carbapenem-resistant versus carbapenem-sensitive *Acinetobacter* spp. strains: respiratory tract 67.5% (n=27) and 59.3% (n=35), p=0.27, sterile body side 16.9% (n=10), 2.5% (n=1), p=0.022, wounds 10.2% (n=6), 17.5% (n=7), p=0.22, urinary tract 3.4% (n=2), 7.5% (n=3), p=0.32, catheters 5.1% (n=3), 2.5% (n=1), p=0.47. Detected resistance rates to other antibiotics in carbapenem-resistant *Acinetobacter* spp. strains and carbapenem-sensitive: piperacillin-tazobactam (100% n=10, 91.5% n=54 p=0.07), ceftazidime (100% n=40, 81.4% n=48, p=0.002), cefepime (65.0% n=26, 37.3% n=22, p=0.004), ciprofloxacin (95% n=38, 79.7% n=47, p=0.03), gentamicin (95% n=38, 76.3% n=45, p=0.011).

Conclusion. Predominant source of infection in carbapenem-resistant and carbapenem- sensitive *Acinetobacter* spp. isolates was lower respiratory tract. Carbapenem-sensitive *Acinetobacter* spp. isolates were more frequently isolated from sterile body side. The resistance rates to other group antibiotics of carbapenem-resistant *Acinetobacter* spp. are higher than carbapenem-sensitive *Acinetobacter* spp. strains.

THE SUSCEPTIBILITY CHANGES OF YEASTS TO ANTIFUNGAL AGENTS IN HOSPITAL OF LITHUANIAN UNIVERSITY OF HEALTH SCIENCES KAUNO CLINICS DURING 5-YEAR PERIOD

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Background. In recent years, yeast infections have increased significantly worldwide. Yeast species have various degrees of susceptibility to the frequently used antifungal agents. Studies demonstrate that antifungal resistance has increased.

Purpose. The aim of this study was to determine the changes of frequency and susceptibility to antifungal agents of yeast species isolated from specimens of patients in 2005 and 2010, who were treated in Hospital of Lithuanian University of Health Sciences Kauno Clinics.

Methods. Study included 142 yeasts species isolated in 2005 and 306 yeast species isolated in 2010. Yeast species were isolated from various clinical specimens. All yeasts were cultivated on Sbouraud's dextrose agar and identified using either CHROM agar or API 20C AUX system. The minimum inhibitory concentrations to fluconazole and itraconazole were determined by the ATB FUNGUS 2 agar micro dilution test.

Findings. Most common yeast isolated in 2005 were *Candida albicans* (65.5%, n=93) followed by *C. parapsilosis* (8.5%, n=12), *C. kefyr* (6.3%, n=9), *C. glabrata* (4.9%, n=7) and ect.; in 2010 – *C. albicans* (64.4%, n=198) followed by *C. glabrata* (7.2%, n=22), *C. krusei* (5.6%, n=17), *C. tropicalis* (5.6%, n=17) and etc. (distribution is presented in figure). The *C. albicans* resistance to fluconazole decreased from 15.1% to 1.5% between 2005 and 2010 year, p<0.001. *C. krusei* resistance in vitro to fluconazole increased from 33.3% in 2005 to 88.2% in 2010, p=0.02. Non-albicans Candida species resistance to fluconazole from 4.1% in 2005 to 16.5% in 2010, p=0.03. *C. albicans* resistance to itraconazole decreased from 25.3% in 2005 to 4.1% in 2010, p<0.001. *In vitro C. krusei* resistance to itraconazole not significantly decreased, 50% and 17.6% respectively, p=0.12. Non-albicans Candida species resistance to itraconazole in 2005 and 2010 was the similar (20.4% and 14.7%, respectively p=0.37).

Conclusion. *C. albicans* remains the most frequently isolated yeast in Hospital of Lithuanian University of Health Sciences Kauno Clinics. During 5-year period *C. albicans* resistance to fluconazole and itraconazole decreased. *C. krusei* and non-albicans Candida species resistance to fluconazole increased while to itraconazole was without any changes.

MUTATIONS IN *GYRA* GENE DETECTED AMONG OFLOXACIN-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* ISOLATES IN LENINGRAD OBLAST IN 2011

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Aim. The main goal of the study was a determination of mutation frequency in *gyrA* gene, associated with ofloxacin (OFL) resistance.

Methods and materials. Thirty three OFL-resistant *Mycobacterium tuberculosis* isolates and 11 susceptible isolates were revealed in Leningrad oblast in winter 2011. Mycobacterial DNA was extracted from bacterial colonies grown on solid media by heat killing at 95°C during 1 hour. Supernatant was used for *gyrA* QRDR amplification. PCR products were sequenced using Sanger method.

Results. Results of DNA sequencing showed that 23 of 33 (69.7%) OFL-resistant *M. tuberculosis* isolates had mutations within *gyrA* QRDR. Point mutations leading to following amino acids substitutions were detected: Asp94Gly (6 isolates); Asp94Asn (3 isolates); Asp94Ala (3 isolates); Asp94Tyr (1 isolate); Ala90Val (6 isolates); Ser91Pro (1 isolate); Ser91Leu (1 isolae) and Gly88Cys (2 isolates). Ten isolates (30.3%) did not have mutations associated with OFL-resistance. There were no mutations associated with OFL-resistance found among susceptible *M. tuberculosis* isolates.

Conclusions. Detection of point mutations in *gyrA* QRDR allows to discover about 70% of OFL-resistant *M. tuberculosis* isolates. Mutations leading to Asp94 substitution were the most common mutations associated with OFL-resistance in studied population of *M. tuberculosis* strains.

DRUG RESISTANT TUBERCULOSIS IN LITHUANIA: PREVALENCE AND REASONS, COST AND PERSPECTIVE

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Background. Drug resistant tuberculosis is a consequence of inefficient national tuberculosis programme. The main reasons are incorrect technical actions of different origin and/or patients' incompliance, mainly related to social and economic reasons.

Purpose. The most adverse phenomenon of resistance is multi-drug-resistant tuberculosis (MDR TB), when resistance is defined at least to two principal anti-tuberculosis drugs − rifampin and isoniazid.

Methods. 113 to 128 new MDR TB cases are registered in Lithuania every year (from 2008 to 2010), while in 2010 the total number of registered MDR TB cases was 310, and the growth trend in the last decade has continued. Lithuania is among 10 countries in the world heavily hit by MDR TB. The treatment of drug resistant tuberculosis, especially MDR TB, is very expensive. It cost Lithuanian taxpayers from 9.32 to 10.54 million Litas (1 Euro = 3.4528 Litas) annually during 2008−2010.

Findings. Investigation of reasons why MDR TB is increasing shows that the source of new MDR TB is very much dependent of poor treatment results of new smear positive TB cases. In 1996−2009 cure rate was from 65 to 82%, which does not reach World Health Organization (WHO) target to cure 85% and more. All first-attempt failures to cure are treated repeatedly, and that is much more expensive than initial treatment. To make the tuberculosis programme more efficient and decrease figures of MDR TB as well as the financial burden to the country, first and foremost the high cure rate nominated by WHO must be achieved.

Conclusion. In addition, ambitious goals must be envisaged and specific tasks should be set in the new 2011−2020 Lithuanian Health Programme (tuberculosis part). As a result, if tasks are accomplished, the MDR TB burden would decline significantly.

THE ROLE OF HOUSEFLIES (*MUSCA DOMESTICA*) IN SPREADING ANTIBIOTIC RESISTANT BACTERIA

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Background. There are many studies which demonstrate that houseflies can transmit foodborne pathogens. But only a few investigate whether houseflies can successfully transmit opportunistic pathogens and antibiotic resistant bacteria in particular.

Purpose. The aim of the study was to investigate carriage by the houseflies (*Musca domestica*) of antibiotic multi-resistant bacteria that are potentially pathogenic to humans in different environments of Lithuania.

Methods. 250 houseflies (*M. domestica*) was captured with sterile cotton net from residential, school premise, hospital premise, place nearby sewage and also ecological clean, forest surrounded areas. Bacteria from homogenized houseflies were isolated and identified till species using standard microbiological procedures. For bacterial isolates antimicrobial susceptibility testing we used Trek Diagnostic System ,,SENSITITRE'' plates. For genetic analysis of beta-lactamases producing strains we did Polymerase Chain Reaction (PCR) with *in house* primers.

Findings. The tested areas were located at different distances from hospitals. The residential house was located 9 km and the school 1.5 km from the nearest hospital. As a control group we have chosen a forest surrounded village, about 35 km from the nearest hospital. Our study did not establish a high possibility of houseflies to transfer pathogenic bacteria from heavy contaminated areas to residential premises and school. Samples collected in the residential premises were colonized with normal skin and environment flora. Houseflies from the village surrounded by forest were colonized with normal environment flora. The low level of enteric bacteria in houseflies makes gastrointestinal infections spreading by flies not as common as in other countries. We isolated very resistant *Klebsiella pneumoniae* strains from the houseflies collected in the hospital premises. Bacteria strains were multi-resistant to many antibiotics (aminoglycosides, cephalosporins, fluoroquinolones). Genetic examination of these bacteria showed the *ctx-M-1* and *oxa-1* family genes that are the most common in clinical samples.

Conclusion. Our studies show that the most of the hospital houseflies are colonized with *S. aureus*, *K. pneumonia*. Isolated bacteria from hospitals flies were resistant to most antibiotics used for treatment. Genetic examination of antibiotic multi resistant *K. pneumonia* shows that they come from hospitals environment, not from farms.

IMPACT OF TIME TO ANTIBIOTIC THERAPY INITIATION ON MORTALITY IN PATIENTS WITH SEVERE SEPSIS AND SEPTIC SHOCK

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Objectives. To estimate mortality rate and impact of time to antibiotic therapy initiation on mortality in patients with severe sepsis (SS) and septic shock (SSh), awareness of physicians of early antibiotic therapy importance for outcome.

Material and methods. Retrospective analysis of 134 cases of patients with SS and SSh hospitalized at Klaipeda regional hospital in Lithuania during period of 2008–2009. 59 (44 percent) of cases were enrolled into the study due to clear time of antibiotic administration registered in case histories. Time from diagnosis of SS and SSh to administration of antibiotics was analysed and ranked to cutoffs: ≤1 hr, 1–3 hr, 3–6 hr, >6 hr. Statistical significance was defined as $p<0.05$. Statistical analyses were performed using SPSS statistics 17.0.

Results. In 75 (56 percent) case histories physicians did not record the exact time of start of antibiotic therapy. SS was diagnosed in 54.2 percent (n=32), SSh in 45.8 percent (n=27). 30.5 percent (n=18) of patients has died. Microbiological tests were obtained in 88.1 percent (n=52). 49.2 percent (n=29) were female, 50.8 percent (n=30) – male. Genders did not differ in age (p=0.1), morbidity rate of SS and SSh (p=0.5), and mortality rate (p=0.2), mean age 60.4 yrs (SD 17.1). 55.9 percent (n=33) of patients received antibiotics during the 1st hour from recognition of SS and SSh, 18.2 percent (n=6) of them has died; 20.3 percent (n=12) during 1–3 hr, 33.3 percent (n=4) has died; 13.6 percent (n=8) during 3–6 hr, 50 percent (n=4) has died; 6.8 percent (n=4) after 6 hr, 50 percent (n=2) has died; 3.4 percent (n=2) did not receive antibiotics, none of them survived. 44.1 percent (n=26) received antibiotics later than 1 hr, 46.1 percent (n=12) has died. Mortality rate of 18.2 percent (n=6) was estimated if antibiotic therapy was initiated within 1st hr (n=33), 46.2 percent (n=12) if antibiotics were administered after 1 hr (n=26) (p=0.02).

Conclusions. Mortality rate of 30.5 percent was estimated. Mortality rate of 18.2 percent was lower only in patients with antibiotic therapy initiated within the first hour from recognition of severe sepsis and septic shock in comparison with later administration. Physicians did not present awareness of importance of early antibiotic therapy in severe sepsis and septic shock as exact time of start of antibiotic therapy was not recorded in 56 percent of case histories.

STUDY OF OCCUPATIONAL RISK FACTORS OF HEPATITIS B AND C INFECTION IN MEDICAL WORKERS (HCWs)

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Background. Healthcare workers are one of the risk groups for infection with blood-born hepatitis B and C (HBV and HCV). Infection risk is extremely high in the healthcare staff performing invasive medical procedures and exposed to potentionally contaminated biological material.

Methods. In 2010 the Lithuaniain Centre for Communicable Diseases and AIDS in cooperation with Departments of Cardiothoracic Surgery and Intensive Therapy and Reanimation of the Vilnius University Santariškes Clinics conducted research of occupational risk factors of HBV and HCV. HCWs of these Departments were voluntarily tested on biological markers of HIV, hepatitis B and C: HIV antibodies, anti-HCV, HBsAg, anti-HBc, anti-HBs. 169 medical workers enrolled into this research were also questioned by applying anonymous questionnaire including 18 questions (socio-demographic data, occupational involvement in performing invasive procedures, history of HIV, HBV, HCV, VHB testing; vaccination, etc.). Data were analysed using SPSS programme, version 13.0.

Results. Study cohort consisted of totally 169 HCWs (25 male, 144 female) of the Departments of Cardiothoracic Surgery and Intensive Therapy and Reanimation of the Vilnius University Santariškes Clinics. Age moda – 46, mediana – 42 years. Study involved 75.1% of nurses and their assistants, 24.9% – reanimatologists, cardiologists, and cardiothoracic surgeons. Median work experience – 17 years.

75.1% (n=127) have indicated previous injuries with blood or other body fluids contaminated instruments while performing invasive procedure, 19.5% had no injuries, and others did not know. During entire work experience more than 10 times were injured 24.2% of respondents, from 3 up to 10 times – 33.1% of respondents. Have you ever been tested for HCV? – 26.6% (45) reported yes, 59.2% (100) – no, 14.2% (24) haven't know. Majority (89%, n=40) knew result of their HCV testing, 5 did not. 41.4% (70) respondents reported being ever tested on HbsAg, 47.3% (80) were not tested, 11.2% (19) did not know. 56.2% (95) had previous test on HBV, 36.7% (62) did not, 7.15% (12) did not know. 7.7% (13) reported vaccination on HBV and were aware of their anti-HBs level, 49.1% (83) did not know. 29% (49) reported ever being tested on HIV, 67.5% (114) were not tested, 3.6% (6) did not know. All respondents tested on HIV were aware of the testing result. Revealed biological markers: 3.6% (6) anti-HCV(+), 95.3% (161) anti-HCV(-), 1.2% (2) anti-HCV (+/-); 130(76.9%) anti-HBc (-), 37 (21.9%) anti-HBc(+), 2 (1.2%) anti-HBc (+/-); no case of HIV1/2 Ab (+) and HBsAg(+). Sufficient (protective) immunity (in case of anti-HBs titre >10mTV/ml) was identified in 82 (48.52%) persons, others (n=87) did not have protective immunity. N=29 had anti-HBc(+) and anti-HBs markers. 35 out of 95 respondents insisted having previous HBV vaccination, though, anti-HBs markers in their blood were not found.

Conclusions. Majority of HCWs were exposed to occupational risk factors: two thirds had history of injury with blood or other body fluids contaminated instruments while performing invasive procedures; during their work experience almost 23% were injured more than 10 times, from 3 up to 10 times – one third. More than a half of respondents were never tested on HCV, 47.3% and 67.5% were never tested on HbsAg and HIV respectively. 56.2% reported being vaccinated of HBV, 36.7% were not vaccinated, and 7.15% did not know. A very few (n=13) HBV vaccinated HCWs were aware of their anti-HBs level/titre.

However, in approximately half (48.5%) of respondents protective HBV immunity was confirmed (post-infection or post-vaccination). Majority anti-HCV and anti-HBc were negative. No cases of HBsAg(+) and HIV1/2Ab (+) were found. 3.6% anti-HCV(+), 21.9% anti-HBc(+). Biological markers proved that majority of respondents were not infected with HBV or HCV but occupational risk factors due to frequency of injuries remained. Awareness of respondents on protective HBV post-vaccination immunity was insufficient.

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A NEW UNIQUE RECOMBINANT HIV TYPE 1 ISOLATED IN BELARUS

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Introduction. By November 1, 2011, 12,751 cases of HIV (136 per 100,000 population) were registered in Belarus. Of these, 992 are new HIV cases registered within the 10 months of 2011. More than 62% of all HIV cases involve young people 15–29 years of age. Beginning in 2005 the main route of HIV transmission has been sexual (75% of all HIV cases registered in 2011). For the period from 1987 to November 1, 2011, 1922 children were born to HIV-infected mothers, including 143 born in 2011. Of all HIV-exposed children 198 (10%) were confirmed to be HIV positive and eight HIV-positive children died (www.aids.by).

Materials and methods. EIA, Western blot, RT-PCR, sequencing, phylogenetic analysis.

Results and discussion. In April 2010 we have performed resistance tests of plasma sample obtained from patient Mos, 6 years old girl, born to an HIV-infected mother. Results have not revealed any HIV-1 resistance. However, the phylogenetic analysis of the DNA fragment of patient Mos had shown that sample has been clustered with HIV-1 subtype A on gene pol, but was different from other analyzed samples, subtype A consensus IDU-A and reference sequences (AF004885). Mos isolate is the most similar to AF413987 from Ukraine (subtype A), the p-distance was 0.066. This data confirmed relation of this isolate to subtype A in pol gene. The comparison of sequences from gag gene p17/p24 region of Mos isolate with reference sequences HIV-1 of subtype A demonstrates that average p-distance was 0.129, and with reference sequences of subtype B was 0.075. Average p-distance on gag gene (Mos isolate) with CRF03_AB (AF414006.1, Belarus and AF193276.1 CRF03_AB KAL153) was 0.121 if compared with 0.013 p-distance between reference sequences. Thus, on the basis of gag gene p17/p24 region analysis (Mos isolate) has been inferred to HIV-1 subtype B. The analysis of Mos isolate sequences on V3 loop gp120 gene env region HIV-1 has shown that average p-distance with reference isolates subtype B was 0.323, and with A subtype was 0.155. Average p-distance sequence of Mos isolate with reference isolates AF414006.1 and AF193276.1 (CRF-03_AB) was 0.308. Thus, on V3 loop gp120 region of env gene the isolate was identified as HIV-1 subtype A.

Conclusion. Thus, it has been shown that Mos isolate is a unique recombinant form, but differs in genome structure from the one described earlier CRF03_ AB ($A^{gag}B^{pol}B^{env}$). The new recombinant HIV-1 has the following structure: B^{gag}B-A-B-$A^{pol}A^{env}$. Sequences of new HIV-1 unique recombinant in gag, pol and env genes were submitted to EMBL/Genbank/DDBJ under accession numbers: FR775442.1, FN995656.1, FR775443.1.

HIV/AIDS MOLECULAR EPIDEMIOLOGY IN BELARUS, 2008−2011

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Introduction. HIV/AIDS epidemic in Belarus develops dynamically. More than 3000 newer HIV-infection cases have been revealed in the country during last three years. New cases are connected as with citizens constantly living in Belarus, and virus delivery from neighboring countries, first of all from Russia.

Materials and methods. EIA, Western blot, RT-PCR, sequencing, phylogenetic analysis, Sequencing analysis, BioEdit, SeqScape, MEGA4.1 software.

Results. HIV-1 subtype A is still dominating among HIV/AIDS patients in the territory of Belarus. Of analysed 139 cases 125 (89.9%) have been belong to subtype A. Proceeds, though it is considerable to a lesser degree, subtype B circulation 4 (2%) cases. The separate cases of HIV-1 subtypes C and G (0.7%) are revealed. The quantity of HIV-1 recombinant forms cases, for example CRF02_AG, CRF06_cpx, which have been brought on territory of the country from the Russia increased. CRF03_AB has been described earlier on territories of Belarus, however two new cases in the Grodno region most likely are connected with new virus drifts from the Lithuania, and CRF03_AB from the Minsk and Mogilyov areas have been brought in republic earlier from Kaliningrad city and now there is their circulation among HIV/AIDS patients. At last, we described absolutely new HIV-1 recombinant form received as result recombinant events between subtype B (Georgia) and A (Ukraine). HIV-1 subtypes strengthening heterogeneity is observed now in population of HIV/AIDS patients in comparison with flash earlier described in Svetlogosk. Average p-distances between viruses from regions were equal 0.039-0.044 that specifies on long time circulation virus and drifts of new variants of subtype A from Ukraine and Russia. Low p-distance values (0.034) in the Grodno region specify on more later epidemic process development in the region. From presented data it is visible that the contribution of subtype A to epidemic remains considerable, but decreases in comparison with 1996–2004 years basically at the expense inclusion of recombinant forms in territory of Belarus.

Conclusion. Sequences of all 139 HIV-1 isolates in pol gene were submitted to EMBL/GenBank/DDBJ.

MODEST DEVIATIONS FROM OPTIMAL ADHERENCE TO ANTIRETROVIRAL THERAPY PROMOTE RESIDUAL HIV-1 REPLICATION IN THE ABSENCE OF VIROLOGICAL REBOUND IN PLASMA

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Background. Modern antiretroviral therapy (ART) is assumed to allow a certain degree of nonadherence while still maintaining complete suppression of viral replication ("forgiveness"), as virological suppression, measured by the commercial plasma viral load assays, is common at adherence levels >55−70% [1]. Yet, it is unknown whether HIV-1 replication is completely suppressed at these levels of adherence. Here we investigated whether modest non-adherence to ART influences levels of HIV-1 RNA and DNA in peripheral blood mononuclear cells (PBMC).

Materials and methods. Levels of HIV-1 unspliced RNA (usRNA) and viral DNA were quantified by seminested real-time PCR [2−4] in PBMC of 40 HIV-infected patients who had been on successful ART for a median of 3.8 years before the start of the study and with good immune reconstitution (median baseline CD4+ count, 620 cells/mm3). For every patient, three longitudinal samples, taken with 3−4 month intervals, were analyzed. One-week mean adherence to ART (percentage of prescribed doses taken) prior to the sampling moments was measured electronically.

Results. Adherence never fell below 70% in any patient, and concurrent plasma viral loads of 109/120 (91%) PBMC samples were undetectable (<50 cop/ml); for 10/11 remaining samples they were <100 cop/ml. Longitudinally, 23 patients were constantly 100% adherent, eight demonstrated improving adherence in time, and nine ("poor adherers") showed decreasing, variable, or constantly <100% adherence. Notwithstanding the lack of virological rebound in any of the patients, poor adherence, but not optimal or improving adherence, caused a significant longitudinal increase in usRNA levels (P=0.006). Remarkably, the change in adherence patterns from optimal through improving to poor was paralleled by a gradual increase in the corresponding viral RNA trends. Significant differences between the poor adherers and the remaining patients were observed in time-weighted changes from baseline (P=0.0006) and regression slopes (P=0.009) of usRNA, but not of viral DNA. These effects were independent of the therapy regimen or the time of virological suppression. Same effects were observed in patients with constantly undetectable plasma viremia (n=30).

Conclusions. As ART only blocks the infection of new cells, but not viral RNA transcription in infected cells, the observed effect of decreased ART pressure (resulting from decreased adherence) on HIV RNA levels in PBMC strongly suggests new replication cycles despite ART, and not simply enhanced HIV-1 transcription in cells infected prior therapy initiation.

Our results represent the first evidence indicating that constant optimal adherence to modern ART may be necessary to stop all HIV replication.

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