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Frequency Distribution of the Microbial Isolates in Major Nosocomial Infections Groups

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Abstract

This second part presents the results of the survey of the microbial isolates frequency distribution by infection sites (principal NI classification groups) in the country throughout the period 1999-2011. The results refer to the overall percentage distribution of the isolates in major NI classification groups (VAP, LRTIs, SSIs, sepsis, UTIs) on the basis of the official registration data of the Computerized Information System-Nosocomial Infections (CIS-NI).

The objective was to emphasize the tendencies in the isolation rate of the most frequent nosocomial pathogens for the period studied attempting to propose links for binding the surveillance of NI microbiological diagnostic with the special surveillance of patients undergoing risk procedures as well as a link for monitoring of the drug-resistance.

The generalized CIS-NI database for the total country is extrapolated. The predominance of the ten most frequently isolated microbial species is presented in the form of percent from the total number of isolates in the microbiologically confirmed cases for the corresponding infections group (infection site), and for the corresponding year of the period indicated.

The microbial characteristics presented mark out in broad outlines the involvement of the most common microbial agents in important for the clinical practice nosocomial infections. The isolates assigned as NI causative agents refer predominantly to strains of *S. aureus* and *E. coli* (of substantial importance in SSIs), *S. aureus* (sepsis), *E. coli* (UTIs), a number of opportunistic bacteria as *Pseudomonas* spp., *Acinetobacter* spp., *Klebsiella* spp., *enterococci*, other *Enterobacteriaceae* as *Enterobacter* spp. and *Serratia* spp., *Proteus* spp. (VAP, LRTIs, sepsis). These species in comparatively constant or in separate years in an increasing percentage are invariably present in the visualized by years microbiological spectrum of the discussed infections groups. The presented microbiologic characteristics emphasizes the necessity of strict implementation of the NI prevention and control measures endorsed, updated guidelines including, in the risk clinical practices as operative/resuscitation procedures and manipulations, installation of vascular devices, urinary catheters, etc., and the care respectively for the patients, undergoing such procedures as preoperative, postoperative care, or attendance in the course of other clinical treatment.

Schemes for assessment of the antibacterial resistance based on NHSN pattern are proposed for approbation as well as adapted from external sources e-files intended for supervision of the observance of the correct hospital practices for care of the patients on mechanical ventilation and/or vascular catheter.

Keywords: Nosocomial infections; Computerized information system-nosocomial infections; Ventilator-associated pneumonia; Lower respiratory tract infections; Pulmonary infections; Surgical site infections; Urinary tract infections; Sepsis; Central line-associated bloodstream infection; Catheter-associated urinary tract infection; Coagulase-negative *Staphylococci*

Introduction

The microbiological diagnosis, comprising the relevant antimicrobial sensitivity testing of the isolates in nosocomial infections is of basic importance for the proper treatment of the patients, the latter relying in a large number of urgent cases on rapid tests. The microbiological tests are of particular importance in the treatment of infections caused by multi-resistant bacteria. Due to emerging or increasing resistance of hospital strains selected in the hospital environment, the exact microbial diagnosis and the complementary antibiogram are the primary precondition for the treatment of infections caused by drug-resistant bacteria. The information system in the country exerts a continued surveillance of nosocomial infections microbiological characteristics. The indices engage frequency distribution (percentage of isolates) by infection sites, and by infection sites and types of wards. Along the first line we dispose of the overall data for the country about the total percentage of the identified to species and subspecies isolates in each infection site, the data considered indicative of the common isolation level, attributable to nosocomial infections by years.

Objective

This second part presents the results of the survey of the microbial isolates frequency distribution by infection sites (principal NI classification groups) in the country throughout the period 1999-2011. The results refer to the overall percentage distribution of the isolates in major NI classification groups (VAP, LRTIs, SSIs, sepsis, UTIs) on the basis of the official registration data of the Computerized

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Information System-Nosocomial Infections (CIS-NI). The objective was to emphasize the tendencies in the incidence rates (in the first part of the study) and the isolation rate of the most frequent nosocomial pathogens (second part) for the period studied attempting to propose links for binding the surveillance of NI microbiological diagnostic with the special surveillance of patients undergoing risk procedures as well as a link for monitoring of the drug-resistance.

Materials and Methods

The bacterial etiology is discussed of the principal NI groups, subject to surveillance in the country as VAP, LRTIs (pulmonary infections), SSIs, UTIs, sepsis, in the course of a comparatively long period from 1999 to 2011. The generalized CIS-NI database for the total country is extrapolated. The predominance of the ten most frequently isolated microbial species is presented in the form of percent from the total number of isolates in the microbiologically confirmed cases for the corresponding infections group (infection site), and for the corresponding year of the period indicated. The importance of some subspecies is specifically emphacised. Although the comparisons by years of the most frequent isolates in each of the above-mentioned infection sites utilize only percentages as a statistical criterion, this frequency distribution still gives an idea of the microbial characteristics of important nosocomial infections, analyzing the changes in the thus extrapolated isolation level of the leading causative agents.

Results

Frequency distribution of the microbial isolates by infection sites

Ventilator-associated pneumonia (VAP): Table 1 indicates the ten most frequently isolated microbial species isolated from tracheal apirate samples of patients registered with Ventilator-Associated Pneumonia (VAP). The data refer only to 2011, the year of VAP introduction as a separate category into the surveillance system. Practically these are the data, originating grow the critical care units in the country, attending for patients on mechanical ventilation, i.e. the units classified, according to the latest update of the wards nomenclature as Anesthesiology & Reanimation (A&R), and Intensive Therapy (IT).

VAP	
2011	
Acinetobacter spp	28.6
Acinetobacter baumannii	14.7
Pseudomonas spp	26.9
P. aeruginosa	24.1
Klebsiella spp	15.3
K. pneumoniae	15.1
E. coli	5.2
Enterobacter spp	5.1
Enterobacter cloacae	2.4
Enterobacter aerogenes	1.0
S. aureus	5.0
Candida spp	3.9
C. albicans	1.8
Other *Candida* spp	2.0
Proteus spp	3.0
P. mirabilis	2.8
Coagulase-negative *Staphylococci* (CoNS)	2.2
Enterococcus spp	2.0
E. faecalis	1.0

Table 1: The ten most frequent pathogens of VAP 2011 (% of the microbiologically confirmed cases-CIS-NI data).

On the basis of our data of greatest importance for pulmonary infection in intubated patients are opportunistic (conditionally pathogenic bacteria) of high potential for survival in the environment at minimum requirements for humidity, and of proved multiple drug resistance of the hospital strains of these species by a number of foreign and our studies [1-5], *P. aeruginosa*-27%, *Acinetobacter baumannii*-29%, *K. pneumoniae*-15%. A comparison with 2006-2007 NHSN* data indicates the same species ranking among the most common pathogens, e.g., *P. aeruginosa*-16%, *Acinetobacter baumannii*-8%, *K. pneumoniae* -7% [6]. One of the differences is that *S. aureus* accounts for the highest percentage of the isolates according to NHSN data (24.4%), in our country it is isolated in 5% of VAP microbiologically confirmed cases. Both systems, however, submit data about the isolation of intestinal flora-facultative anaerobes as: coliforms (sanitary-indicative microorganisms, used for testing of environmental samples for fecal contamination-pneumonia. In our (of 5% each species-CIS-NI data). The two subspecies of the latter-*E. aerogenes* and *E. cloacae* have been proved as causative agents of sepsis and VAP-immunocompromized patients and patients on mechanical ventilation [1,4,7], Proteus spp. (3% in our country), *Enterococci* (2%-CIS-NI, 1,3%-NHSN), of which *E. faecalis* (1%, NHSN-0,4%), fungi-Candida spp. [2,3,7,9].

LRTIs (Pulmonary infections)

Brief introduction to the problem: Nosocomial bacterial pneumonia is often a complication subsequent to operative procedure. By reason of that NHSN reports it separately as postoperative pneumonia-"post procedure pneumonia". In our system the International Disease Classification nosologic group of the pulmonary infections is adopted, including pneumonia and borderline conditions as bronchiolitis and bronchitis, no differentiation, however, made strictly for the cases of postoperative pneumonia.

By analogy with VAP cases (patients on prolonged mechanical ventilation), the patients undergoing operative intervention, critically ill patients and immunocompromized due to other reasons, are supposedly especially susceptible to a pulmonary infection. Nosocomial infections may occur as a result of primary and much more rarely secondary bacteremia, the latter occurring mainly following skin infections [8].

The follow up of LRTIs bacterial etiology for the prolonged period studied (1999-2011) demonstrates to a high extent similarity with the indicated for VAP (Table 2). Main causative agents are the above-mentioned opportunistic bacteria, according to 2011 data: *P. aeruginosa* (19%), *Acinetobacter* spp. (17%), *K. pneumoniae* (14%), responsible as a total for a half of all the registered cases of nosocomial pneumonia. *S. aureus* isolates maintain a comparatively stable percentage in the course of years-between 8 and 13%. Fungi (*Candida* spp.) and fecal microorganisms (*E. coli*, *Enterobacter* spp. and *Enterococcus* spp.) are isolated as comparatively common pathogens.

The isolated in VAP or postoperative pneumonia strains of conditionally pathogenic flora, normally commensal bacteria of the upper respiratory tract (*K. pneumoniae*) or the environment (*P. aeruginosa, Acinetobacter* spp.), are treated as hospital strains. The isolation of such strains, belonging to species known for their multiresistance, may pose serious problems to the treatment of the infections, and implies on the strict compliance with the clinical protocols for operating rooms/resusctation/surgery as regards the specified NI control measures for the operative/resuscitation procedures

***LRTIs* (Pulmonary infections)**									
1999		**2000**		**2001**		**2002**		**2003**	
P. aeruginosa	22.0	*P. aeruginosa*	25.0	*P. aeruginosa*	28.8	*P. aeruginosa*	23.2	*P. aeruginosa*	26.3
Acinetobacter spp	16.0	*Acinetobacter* spp	17.0	*S. aureus*	12.7	*Acinetobacter* spp	12.4	*Acinetobacter* spp	13.1
S. aureus	13.0	*K. pneumoniae*	11.3	*Acinetobacter* spp	12.7	*S. aureus*	11.9	*K. pneumoniae*	10.2
K. pneumoniae	9.9	*S. aureus*	10.8	*K. pneumoniae*	11.9	*K. pneumoniae*	11.4	*S. aureus*	8.9
E. coli	5.7	Viruses	6.0	*Candida* spp	6.1	Viruses	9.7	*Viruses*	9.2
Viruses	5.4	*P. mirabilis*	3.8	*E. coli*	4.5	*Candida* spp	5.5	*E. coli*	5.9
Serratia spp	3.9	*E. coli*	3.8	Viruses	3.7	*E. coli*	5.4	*Str. pneumoniae*	4.0
Str. pneumoniae	3.8	*Str. pneumoniae*	3.8	*P. mirabilis*	3.4	*Str. pneumoniae*	2.8	*Candida* spp	3.2
P. mirabilis	3.4	*Candida* spp.	3.1	*Enterobacter* spp	3.2	*P. mirabilis*	2.0	*P. mirabilis*	2.7
Candida spp	2.9	*Enterobacter* spp	3.0	*Str. pneumoniae*	3.1	*Serratia* spp	1.8	*Serratia* spp	2.2

***LRTIs* (Pulmonary infections)**									
2004		**2005**		**2006**		**2007**		**2008**	
P. aeruginosa	25.9	*P. aeruginosa*	27.7	*P. aeruginosa*	23.8	*P. aeruginosa*	23.2	*Acinetobacter* spp	20.1
Acinetobacter spp	19.7	*Acinetobacter* spp	21.3	*Acinetobacter* spp	19.1	*Acinetobacter* spp	18.0	*P. aeruginosa*	19.5
S. aureus	9.85	*S. aureus*	9.3	*K. pneumoniae*	12.4	*K. pneumoniae*	9.6	*K. pneumoniae*	11.8
K. pneumoniae	7.98	*K. pneumoniae*	8.9	*S. aureus*	8.4	*S. aureus*	9.1	*S. aureus*	7.5
E. coli	6.68	*E. coli*	4.1	*E. coli*	5.5	*Candida* spp	6.1	*E. coli*	6.7
Viruses	4.64	*Candida* spp	4.1	*Candida spp.*	4.6	*E. coli*	5.6	*Candida* spp	6.3
Enterobacter spp	3.42	*Serratia* spp	4.0	*Enterobacter* spp	4.1	*Enterobacter* spp	4.0	*Enterobacter* spp	5.5
Str. pneumoniae	3.17	*Enterobacter* spp	3.1	*P. mirabilis*	3.3	*Serratia* spp	3.2	*Enterococcus* spp	2.3
Candida spp	3.17	*P. mirabilis*	2.7	*Enterococcus* spp	2.2	*P. mirabilis*	1.9	*Serratia* spp	2.0
Serratia spp	2.85	*Str. pneumoniae*	1.3	*Str. pneumoniae*	2.0	*Str. pneumoniae*	1.4	*P. mirabilis*	1.9
						Streptococcus spp	2.6	*Streptococcus* spp	2.8
						Str. pneumoniae	1.4	*Str. pneumoniae*	0.9
						Enterococcus spp	1.0	CoNS	3.3
						CoNS	4.2		

***LRTIs* (Pulmonary infections)**					
2009		**2010**		**2011**	
Acinetobacter spp	19.4	*P. aeruginosa*	19.8	*P. aeruginosa*	19.3
P. aeruginosa	19.0	*Acinetobacter* spp	19.7	*Acinetobacter* spp	16.6
K. pneumoniae	11.5	*K. pneumoniae*	15.2	*K. pneumoniae*	14.0
S. aureus	9.0	*S. aureus*	8.4	*S. aureus*	9.8
Candida spp	7.2	*E. coli*	5.7	*Candida* spp	7.0
Enterobacter spp	5.4	*Enterobacter* spp	5.3	*E. coli*	4.9
E. coli	4.2	*Candida* spp	5.0	*Enterobacter* spp	4.7
Str. pneumoniae	2.6	P. mirabilis	2.7	*Str. pneumoniae*	3.2
Serratia spp	2.2	*Str. pneumoniae*	1.8	*P. mirabilis*	2.1
P. mirabilis	2.0	*Enterococcus* spp	1.7	*Serratia* spp	2.0
Streptococcus spp	4.9	*Streptococcus* spp	3.2	*Streptococcus spp*	6.6
Enterococcus spp	1.7	*Str. pneumoniae*	1.8	*Str. pneumoniae*	3.2
CoNS	3.2	*Serratia* spp	1.4	*Enterococcus* spp	1.2
		CoNS	3.0	CoNS	3.6

Table 2: The ten most frequent pathogens of lrtis (pulmonary infections). 1999-2011 (% of the microbiologically confirmed cases).

and postoperative care, for example the requirements for reproccesing of reusable resuscitation/anesthesia equipment etc.

Surgical Site Infections (SSIs)

Brief introduction to the problem: Surgical Site Infections (SSIs) are frequent complications, diminishing the effect of the operative procedure, in certain cases to a fatal outcome from the intervention, at the same time prolonging the hospital stay and increasing the treatment costs because of additional antibiotic coverage. The surveillance of these infections takes into account the impact of the type of the operative procedure, i.e. the routine surgical technique applied, over the incidence and etiology of infections. For the present our system reports the overall incidence rates and etiology (percent of the causative agents) by infection site and hospital wards. A more precise surveillance system submits the NHSN, former NNISS component respectively. The SSIs module takes into account risk factors as surgical wound class, duration of operation and ASA score, the latter evaluating patients' physical status prior to the surgical intervention in five subcategories.

Another criterion, for the present interpreted only clinically, is the anatomical localization of the infection and the classification of SSIs accordingly to incisional, including superficial and deep incisional, and organ/space or organ/cavity type. An eventual categorization according

to this definition should engage precised criteria applying to the special surgery interventions, e.g, comments proposed for the elective colon surgery by DE Fry [9].

All above-mentioned factors (type of procedure, incision respectively) influence not only the morbidity (the incidence of infections), but their etiology as well. On the one hand, the normal skin flora (resident flora), ensuring a natural defence against pathogenic microorganisms should be considered, i.e. Gram positive cocci: *Micrococcus*, *S. saprophyticus*, *S. epidermidis*; Gram positive bacilli: *Corynebacterium*, *Propionibacterium*. On the other hand, there should be taken into account the most frequent contaminants of the digestive tract, the multiplication of which in case of impaired integrity of the tissues and certain conditions (presence of blood, pus), is able to lead to infection development, i.e.: Gram (+) cocci as *Streptococci, Enterococci, Staphylococci*; Gram (-) bacilli as enteric bacteria: *E. coli, Klebsiella* spp., *Enterobacter* spp.; non-fermentative Gram negative bacilli as *Pseudomonas* spp., *Acinetobacter* spp.; anaerobes including cocci: *Streptococci, Peptostreptococci,* and *Bacilli*: *Bacteroides, Clostridium, Fusobacterium*. The complex interpretation of SSIs microbiological results requires considerable experience in the testing, e.g., in some cases coagulase-negative *Staphylococci* (CoNS) isolates, normally commensal skin bacteria, may be consudered etiologic agents.

In general, SSIs etiologic structure is determined by bacteria, widely distributed and easily multiplying in the environment. At the conditions of the human organism they colonize the skin, the respiratory, gastrointestinal, genitourinary tract with special affinity to damaged and/or immunocompromised (of weakened immunity) tissues. In a number of SSIs hospital isolates, especially opportunistic bacteria, is found high-level resistance pattern. The therapeutic practices should dispose of international institutions' consultative manuals (Guidelines and User Manuals) on antibiotics usage as the yearly updated CLSI (NCCLS)** standards for antibiotic sensitivity, and recommendations for clinical approach to the initial choice of antimicrobial therapy, for e.g., Sanford Guide [10,11]. A special accent in the preventive measures against SSIs is put on the antibiotic prophylaxis [12]. Improvements as regards the choice of the correct moment for initial/single dose application, appropriate antibiotic and application scheme of short duration precised, defined more distinctly the value of this technique for postoperative wound infections rates reduction (Nichols RL) [13].

****Clinical and Laboratory Standards Institute (CLSI), till 2005 known as National Committee on Clinical Laboratory Standards (NCCLS)**

The basic etiological role of *S. aureus* is admitted by the studies on SSIs etiology, e.g the 2006-2007 NHSN data-30% of the cases [6]. The pathogen is identified in our country approximately ¼ of the cases its 21-26%, in another ¼ of the cases (20-23%). *E. coli* is isolated, the latter supposedly due to contamination during the operative procedure or in the process of wound care afterwards (Table 3).

The next by order isolates are *P. aeruginosa* strains (our data 8-12%, NHSN-6%) and *enterococci* [6]. *Acinetobacter* spp. are isolated in 6-8%, *Klebsiella* spp. in increasing percentage from 4-5% in the beginning of the period 1999-2011 to 8% for the last year of the period. Among the most frequent isolates in SSIs are also other bacteria of intestinal flora as *Enterobacter* spp.-2-4%, *P. mirabilis* in a decreasing percentage from 7 to 4%, in separate years of the period-*Serratia* spp. in 2-1%. In the last two years a total of five cases of anaerobic infection have been registered -2 cases of *Cl. perfringens*, 2-*Clostridium* non-specified in 2010, and 1-*Cl. difficile* in 2011. *Candida* spp. isolation level ranges between 1 and 2%. For the last two years of the studied period coagulase-negative staphylococci are implicated as causative agents in 6-7%, as of 2006-2007 NHSN data indicate a double percentage-14% [6].

Sepsis

Brief introduction to the problem: Under the nosologic category "sepsis" our system reports a total number of the cases with clinical evidence of bacteremia in hospitalized patients, acquired postoperatively including and/or related to procedures of mechanical ventilation, vascular catheterization, etc. A lot of studies on nosocomial septic complications, including trials on biofilms have demonstrated the underlying role of the catheter contamination. The foreign surveillance systems operate with effective criteria for calculating the incidence rates per 1000 patient-days with a device, as regards, particularly, the vascular catheters-per 1000 patient-days with a central line [14,15,17-19]. The infections associated with a peripheral catheter for the present are exempt from calculation of this specific rate. The Central Line-Associated Bloodstream Infection (CLABIs) are subject to special supervision at the basis of considering the complex catheterization technique with penetration into large vessels, associated with high risk for bacteremia. A calculation per 1000 patient-days permits the comparison of the results for the so formed cohorts of patients with a central line.

In view of the severity of the infection, and the possibility of fatal outcome, the treatment of the nosocomial septicemia presents a serious problem. The prevention and control srategy envisages special supervision over the observance of the requirements for procedures of insertion, maintenance and removal of a vascular catheter.

The etiology of these infections is determined by the same, the so called "nosocomial pathogens", of concern in the foreign studies. The cases of staphylococcal sepsis comprise the greatest part of the reported cases of sepsis in the country. It refers particularly to *S. aureus,* isolated in 12-26% yearly in the course of the studied period, as well as coagulase-negative *Staphylococci* (CoNS), of which predominantly *S. epidermidis* isolates (23-27% in the course of the years), the other CoNS of rising percentage recently. Studies have demonstrated that CoNS invade the vascular endotel at the catheter insertion site, forming biofilms and multiplying in the blood stream to the extent of clinically manifested sepsis. Their role in the etiology of vascular device-associated sepsis was confirmed by transmissible electron microscopy of the biofilm along the catheter surface [16]. The 2006-2007 NHSN data indicate CoNS as the most common causative agent of CLABSI-34% [6].

According to CIS-NI data for 1999-2011 the percentage of *K. pneumoniae* isolates has increased two-fold-from 6 to 15-14% for the last two years. The isolates in CLABSI-NHSN are about 5% [6]. Taking into account that *K. pneumoniae* colonizes predominantly the respiratory tract, septic complications in pulmonary infections are possible. *Acinetobacter* spp. and *Pseudomonas* spp. determine 6-14% of the cases. Studies of nosocomial infections and outbreaks in our country in neonatal wards, surgeries, cases of sepsis including, have confirmed the multiresistance of *A. baumannii* and *P. aeruginosa* hospital strains [20-23]. These subspecies have been isolated in septicemias of nosocomial origin in the country in 4-6% of the isolates from notified cases of sepsis, in CLABSI (NHSN) in 2-3%. According to K. Todar [24] data *Pseudomonas* spp. is responsible for about 25% of all acquired Gram negative bacteremias in the hospital.

Recently the *Enterococci*, and especially multiresistant strains or vancomycin resistant enterococci emerge as one of the causative agents of bacteremias in surgical patients and intravascular catheter related

SSIs									
1999		2000		2001		2002		2003	
S. aureus	25.4	*S. aureus*	26.5	*S. aureus*	26.2	*E. coli*	22.7	*S. aureus*	24.1
E. coli	21.6	*E. coli*	22.0	*E. coli*	21.3	*S. aureus*	21.2	*E. coli*	23.1
P. aeruginosa	11.4	*P. aeruginosa*	9.9	*P. aeruginosa*	12.0	*P. aeruginosa*	11.9	*P. aeruginosa*	10.7
P. mirabilis	7.1	*Enterococcus* spp	6.8	*Enterococcus* spp	6.5	*Enterococcus* spp	7.9	*Enterococcus* spp	7.8
Acinetobacter spp	5.7	*P. mirabilis*	6.8	*Acinetobacter* spp	5.7	*Acinetobacter* spp	5.4	*Acinetobacter* spp	5.0
Enterococcus spp	5.7	*Klebsiella* spp	5.2	*P. mirabilis*	5.7	*Klebsiella* spp	5.2	*Klebsiella* spp	5.0
Klebsiella spp	5.2	*Acinetobacter* spp	4.7	*Klebsiella* spp	4.1	*P. mirabilis*	5.0	*P. mirabilis*	4.8
Enterobacter spp	3.0	*Enterobacter* spp	2.5	*Enterobacter* spp	3.7	*Enterobacter* spp	4.3	*Enterobacter* spp	4.3
Serratia spp	2.3	*Serratia* spp	2.4	*Streptococcus* spp	2.4	*Streptococcus* spp	2.3	*Streptococcus* spp	1.7
Streptococcus spp	1.5	*Streptococcus* spp	1.7	*Serratia* spp	2.3	*Candida* spp	1.4	*Serratia* spp	1.3

SSIs									
2004		2005		2006		2007		2008	
E. coli	23.5	*E. coli*	21.4	*E. coli*	22.7	*E. coli*	22.7	*E. coli*	20.5
S. aureus	21.0	*S. aureus*	21.2	*S. aureus*	19.2	*S. aureus*	19.7	*S. aureus*	19.7
P. aeruginosa	10.0	*P. aeruginosa*	9.7	*P. aeruginosa*	10.1	*P. aeruginosa*	9.9	*P. aeruginosa*	10.0
Enterococcus spp	8.5	*Enterococcus* spp	8.6	*Enterococcus* spp	9.0	*Enterococcus* spp	9.6	*Enterococcus* spp	9.4
Acinetobacter spp	6.3	*Acinetobacter* spp	7.1	*Acinetobacter* spp	6.4	*Acinetobacter* spp	7.1	*Acinetobacter* spp	7.1
Klebsiella spp	5.9	*P. mirabilis*	6.0	*Klebsiella* spp	6.2	*Klebsiella* spp	4.8	*Klebsiella* spp	5.4
P. mirabilis	5.5	*Enterobacter* spp	4.7	*P. mirabilis*	6.2	*P. mirabilis*	4.6	*P. mirabilis*	4.6
Enterobacter spp	4.4	*Klebsiella* spp	3.9	*Streptococcus* spp	3.7	*Enterobacter* spp	3.9	*Enterobacter* spp	4.0
Streptococcus spp	1.8	*Streptococcus* spp	3.0	*Enterobacter* spp	3.5	*Streptococcus* spp	2.9	*Streptococcus* spp	3.1
Candida spp	1.3	*Candida* spp	1.4	*Candida* spp	1.4	*Candida* spp	1.8	*Candida* spp	1.0

SSIs					
2009		2010		2011	
E. coli	21.0	*E. coli*	21.4	*S. aureus*	22.6
S. aureus	19.4	*S. aureus*	19.9	*E. coli*	20.7
Enterococcus spp	10.3	*P. aeruginosa*	9.8	*Enterococcus* spp	8.3
P. aeruginosa	8.2	*Enterococcus* spp	8.1	*P. aeruginosa*	8.2
Acinetobacter spp	6.4	*Klebsiella* spp	7.4	*Klebsiella* spp	7.6
Klebsiella spp	5.8	*Acinetobacter* spp	5.6	*Acinetobacter* spp	7.6
P. mirabilis	5.7	*P. mirabilis*	5.3	P. mirabilis	4.4
Enterobacter spp	4.9	*Enterobacter* spp	4.6	*Enterobacter* spp	3.9
Streptococcus spp	3.0	*Streptococcus* spp	2.6	*Streptococcus* spp	1.8
Candida spp	1.0	*Serratia* spp	1.0	*Serratia* spp	1.2
				Candida spp	1.2
		Candida spp	0.8		
		CoNS	6.1	CoNS	7.3
		Clostridium spp	0.1	*Clostridium* spp	0.03

Table 3: The ten most frequent pathogens of ssis. 1999-2011 (% of the microbiologically confirmed cases).

bacteremias [25-27]. As NHSN data indicate, these species are isolated in the second place in CLABSI incidence ranking: *E. faecalis* -5%, *E. faecium* 8%, non-differentiated-2%, i.e. a total of 15% of the cases; according to CIS-NI data correspondingly: *E. faecalis* 2-3%, *Enterococci* as a total-5-6% [6]. Other isolates of intestinal flora - *Serratia* spp. and *Enterobacter* spp., mostly the clinically important *S. marcescens* and *E. cloacae* of proved etiological role in NI outbreaks in the country, comprise 3-6% of the isolates. The *Candida* spp. strains from blood cultures of hospitalized patients account for 2-5% in our country, in CLABSI 11, 8% of the isolates (NHSH data). Streptococcal sepsis was confirmed in 2% of the cases in 2010, 2011 respectively, 1 case of anaerobic sepsis (*Cl. perfringens*) was registered in 2010 (Table 4).

Urinary tract infections (UTIs)

Brief introduction to the problem: References data indicate that the urinary tract infections amount to 40-50% of all NI [28,29]. Underlying the role of catheterization procedures (an indwelling or an intermittent urinary catheter) and/or interventions (diagnostic procedures, eg., cystoscopies, operative interventions) over the urinary tract. Considering the particular risk related to the indwelling catheter, urosepsis respectively, NHSN was the first to introduce the exact index of calculating the incidence rate per 1000 urinary catheter-days.

The UTIs etiologic structure demonstrates conditionally pathogenic species, in the cases of urinary catheterization (indwelling urethral catheterization particularly) these are the catheter contaminants. Recent studies draw attention to a trend for selection of multiresisrtant hospital strains, problematic in terms of the clinical therapy [28,29]. The prevention of the acute infections and the chronic course is dependent on the observance of the requirements for urinary catheter care, i.e. insertion of the catheter, duration of the catheterization and maintenance of the urinary catheter, aseptic technique, sterile equipment

Sepsis									
1999		**2000**		**2001**		**2002**		**2003**	
S. aureus	21.6	*S. aureus*	22.7	*S. aureus*	21.9	*S. aureus*	25.7	*S. aureus*	21.9
S. epidermidis	12.1	*P. aeruginosa*	14.5	*S. epidermidis*	12.4	*E. coli*	10.5	*Klebsiella* spp	11.2
Serratia spp	8.8	*S. epidermidis*	13.1	*P. aeruginosa*	12.0	*S. epidermidis*	8.8	*Acinetobacter* spp	10.2
E. coli	8.1	*Klebsiella* spp	10.7	*Acinetobacter* spp	9.6	*Acinetobacter* spp	8.8	*P. aeruginosa*	9.7
P. aeruginosa	6.6	*Acinetobacter* spp	7.0	*Klebsiella* spp	9.2	*P. aeruginosa*	7.4	*E. coli*	9.7
Acinetobacter spp	6.2	*E. coli*	6.1	*E. coli*	8.1	*Klebsiella* spp	7.1	*S. epidermidis*	8.1
Klebsiella spp	6.2	*Enterobacter* spp	5.1	*Serratia* spp	6.2	*Serratia* spp	5.0	*Enterobacter* spp	4.2
Enterobacter spp	4.2	*Serratia* spp	4.7	*Candida* spp	3.4	*Enterobacter* spp	4.3	*Enterococcus* spp	3.9
Viruses	4.0	*Enterococcus* spp	4.0	*Enterobacter* spp	2.6	*Candida* spp	3.6	*Serratia* spp	2.9
Enterococcus spp	3.8	*Streptococcus* spp	1.9	*Streptococcus* spp	2.1	*Enterococcus* spp	2.4	*Streptococcus* spp	1.8

Sepsis									
2004		**2005**		**2006**		**2007**		**2008**	
S. epidermidis	14.8	Other CoNS	15.1	*S. aureus*	16.4	*S. aureus*	16.8	*S. aureus*	17.4
S. aureus	13.1	*Acinetobacter* spp	13.3	*S. epidermidis*	14.7	*S. epidermidis*	16.5	*S. epidermidis*	14.8
Acinetobacter spp	13.9	*S. epidermidis*	12.3	*Klebsiella* spp	12.0	*Acinetobacter* spp	12.2	Other CoNS	9.9
E. coli	8.9	*S. aureus*	10.9	*E. coli*	11.3	*P. aeruginosa*	7.6	*Klebsiella* spp	9.2
Klebsiella spp	8.3	*Serratia* spp	9.5	*Acinetobacter* spp	9.4	*Enterococcus* spp	7.5	*Acinetobacter* spp	7.5
Enterobacter spp	7.0	*Klebsiella* spp	8.3	Other CoNS	8.1	*E. coli*	7.3	*E. coli*	7.5
P. aeruginosa	6.5	*E. coli*	7.5	*Enterococcus* spp	6.4	Other CoNS	6.1	*Enterococcus* spp	6.6
Enterococcus spp	4.8	*P. aeruginosa*	6.0	*P. aeruginosa*	6.0	*Klebsiella* spp	5.8	*P. aeruginosa*	6.4
Candida spp	4.8	*Enterobacter* spp	4.6	*Serratia* spp	3.6	*Candida* spp	5.3	*Serratia* spp	4.0
Serratia spp	3.9	*Candida* spp	3.8	*Candida* spp	3.2	*Enterobacter* spp	3.7	*Enterobacter* spp	4.0
						Serratia spp	3.5	*Candida* spp	2.4

Sepsis					
2009		**2010**		**2011**	
S. aureus	16.6	*S. aureus*	17.4	CoNS	24.6
S. epidermidis	14.2	*S. epidermidis*	15.5	*Klebsiella* spp	14.1
Klebsiella spp	11.0	*Klebsiella* spp	15.1	*K. pneumoniae*	13.7
E. coli	10.0	*K. pneumoniae*	14.6	*S. aureus*	12.2
Other CoNS	9.1	Other CoNS	11.0	*Acinetobacter* spp	8.9
Acinetobacter spp	7.7	*E. coli*	7.3	*A. baumannii*	5.9
Enterococcus spp	5.0	*Acinetobacter* spp	7.0	*Pseudomonas* spp	6.5
Candida spp	3.8	*A. baumannii*	3.6	*P. aeruginosa*	5.7
Serratia spp	3.8	*Pseudomonas* spp	5.8	*E. coli*	6.0
Enterobacter spp	3.5	*P. aeruginosa*	3.7	*Enterococcus* spp	5.9
		Enterococcus spp	5.2	*E. faecalis*	3.2
P. aeruginosa	3.2	*E. faecalis*	2.1	*Serratia* spp	5.7
		E. faecium	1.3	*S. marcescens*	5.7
		Enterobacter spp	3.3	*Candida* spp	5.3
		E. cloacae	2.2	*C. albicans*	1.6
		Candida spp	2.7	*Enterobacter* spp	3.6
		C. albicans	0.7	*E. cloacae*	1.5
		Serratia spp	2.5	*Streptococcus* spp	2.0
		S. marcescens	2.4		
		Streptococcus spp	2.2		

¶CoNS-S. epidermidis. included

Table 4: The ten most frequent pathogens of sepsis. 1999-2011 (% of the microbiologically confirmed cases).

and closed drainage system including. The choice of antimicrobials for treatment is strictly according to the uroculture antibiogram results in consultation with the microbiologist [29].

Comparing the CIS-NI data about UTIs isolates with the NHSN data specified for the catheter-associated urinary tract infection (CAUTI), the predominance of *E. coli* (intestinal microflora) is evident [6]. For the whole period studied (1999-2011) the *E. coli* strains invariably comprise about one third (31-34%) of the isolates, according to 2006-2007 NHSN data-21% (Table 5) [6]. With increasing incidence are isolated *Enterococci*-from 3% in the beginning of the period to 15% in 2011, *E. faecalis* as a predominant serotype 7-11% (2010-2011). *Klebsiella* spp. percentage has raised two-fold-from 8 to 15%, predominantly *K.*

UTIs									
1999		**2000**		**2001**		**2002**		**2003**	
EE. coli	31.9	*E. coli*	32.7	*E. coli*	30.4	*E. coli*	32.5	*E. coli*	33.6
P. aeruginosa	23.7	*P. aeruginosa*	21.0	*P. aeruginosa*	21.9	*P. aeruginosa*	18.6	*P. aeruginosa*	19.6
P. mirabilis	9.5	*P. mirabilis*	9.1	*Klebsiella* spp	9.8	*Klebsiella* spp	9.2	*Klebsiella* spp	8.7
Klebsiella spp	7.8	*Candida* spp	6.7	*P. mirabilis*	7.9	*P. mirabilis*	7.1	*P. mirabilis*	6.1
Candida spp	4.7	*Klebsiella* spp	4.5	*Candida* spp	6.0	*Enterobacter* spp	5.0	*Enterococcus* spp	5.0
S. aureus	3.7	*Citrobacter* spp	3.9	*S. aureus*	4.3	*Candida* spp	5.0	*Candida* spp	4.6
Acinetobacter spp	3.4	*S. aureus*	3.9	*Enterobacter* spp	3.2	*S. aureus*	4.9	*S. aureus*	4.5
Enterobacter spp	3.1	*Enterobacter* spp	3.9	*Acinetobacter* spp	3.0	*Enterococcus* spp	3.7	*Enterobacter* spp	3.7
Serratia spp	2.7	*Acinetobacter* spp	3.3	*Citrobacter* spp	2.9	*Acinetobacter* spp	3.0	*Acinetobacter* spp	3.7
Citrobacter spp	2.5	*Enterococcus* spp	3.2	*Enterococcus* spp	2.7	*Citrobacter* spp	2.9	*Citrobacter* spp	2.8

UTIs									
2004		**2005**		**2006**		**2007**		**2008**	
E. coli	30.5	*E. coli*	32.5	*E. coli*	30.7	*E. coli*	31.0	*E. coli*	31.9
P. aeruginosa	19.0	*P. aeruginosa*	15.7	*P. aeruginosa*	12.6	*P. aeruginosa*	12.1	*Klebsiella* spp	13.7
Klebsiella spp	11.1	*Klebsiella* spp	10.3	*Klebsiella* spp	11.8	*Enterococcus* spp	11.7	*Enterococcus* spp	12.3
P. mirabilis	7.4	*Enterococcus* spp	10.0	*Enterococcus* spp	11.7	*Klebsiella* spp	11.4	*P. aeruginosa*	10.0
Enterococcus spp	6.3	*P. mirabilis*	6.4	*Candida* spp	5.6	*Candida* spp	6.5	*Candida* spp	7.3
Candida spp	4.9	*Candida* spp	5.6	*P. mirabilis*	5.1	*P. mirabilis*	5.7	*P. mirabilis*	5.1
Enterobacter spp	3.6	*S. aureus*	3.7	*Enterobacter* spp	4.4	*Enterobacter* spp	5.2	*Enterobacter* spp	4.3
Acinetobacter spp	3.5	*Acinetobacter* spp	3.5	*S. aureus*	4.0	*S. aureus*	4.2	*S. aureus*	3.1
Citrobacter spp	3.1	*Enterobacter* spp	3.2	*Acinetobacter* spp	3.6	*Acinetobacter* spp	2.6	*Acinetobacter* spp	2.8
S.aureus	2.8	*Citrobacter* spp	2.3	*Serratia* spp	1.8	*Serratia* spp	1.1	*Serratia* spp	1.8

UTIs					
2009		**2010**		**2011**	
E. coli	31.4	*E. coli*	31.6	*E. coli*	28.1
Klebsiella spp	15.0	*Klebsiella* spp	15.3	*Klebsiella* spp	15.9
Enterococcus spp	12.3	*K. pneumoniae*	14.1	*K. pneumoniae*	14.8
Candida spp	8.5	*Enterococcus* spp	12.3	*Enterococcus* spp	15.4
P. aeruginosa	7.8	*E. faecalis*	7.4	*E. faecalis*	10.6
P. mirabilis	5.5	*E. faecium*	2.2	*Candida* spp	8.8
Enterobacter spp	4.8	*Candida* spp	8.1	*C. albicans*	5.0
S.aureus	2.7	*C. albicans*	4.8	*Pseudomonas* spp	7.7
Acinetobacter spp	2.7	*Pseudomonas* spp	8.0	*P. aeruginosa*	7.3
Serratia spp	1.5	*P. aeruginosa*	7.4	*Proteus* spp	7.4
		Proteus spp	7.6	*P. mirabilis*	6.9
		P. mirabilis	6.5	*Enterobacter* spp	3.8
		Enterobacter spp	3.6	*E. cloacae*	1.9
		E. cloacae	2.2	*Acinetobacter* spp	3.0
		S. aureus	2.9	*A. baumannii*	2.1
		Acinetobacter spp	2.4	*S. aureus*	3.0
		A. baumannii	1.7	*Serratia* spp	1.4
		Serratia spp	1.3	*S. marcescens*	1.4
		S. marcescens	1.1		
				CoNS	2.3
		CoNS	2.9		

CoNS-S. epidermidis. included

Table 5: The ten most frequent pathogens of UTIs. 1999-2011 (% of the microbiologically confirmed cases).

pneumoniae, for the last two years in the second place by isolation rate. As a matter of comparison according to the cited NHSN data [6], *K. pneumoniae* isolates rank fifth in UTIs (7.7%). *K. oxytoca* isolates are less important as NI pathogens in our country, NHSN report the subtype among the ten most frequent isolates in NI, ranking tenth in UTIs [6]. In the last years there has been an increase in the fungal infections to the extent of ranking fourth by isolation rate, NHSH-in a second place, an inadequate antibitic treatment presumed as a reason of concern.

Pseudomonas spp. is among the main isolates in the nosocomial UTIs. However, there has been a decrease in *P. aeruginosa* percentage from 19-24% in the beginning of the studied period to 8% in 2010-2011 years. On the account of other species, which gain a higher place in the rank order of isolates, the NHSN data report a similar percent (10% of CAUTI isolates [6].

In the course of the period 1999-2011 *Proteus* . A comparison with 2006-2007 NHSN* data (7-8%) and other belonging to the normal gastrointestinal microflora bacteria [*Enterobacter* spp. (4%), *Serratia* spp. (2%)] have been yearly isolated. The *P. mirabilis* serotype has been isolated in 6-7%, *S. marcescens*-in 1% (2010, 2011). Both serotypes are of proved clinical significance for complicated infections of the upper urinary tract and outbreaks [27,28]. *Acinetobacter* spp. isolates account for 3%, of which *A. baumannii* strains-2%, NHSN indicates a similar percent-1.2% [6].

Discussion

The frequency distribution of the most common isolates in the principal NI groups, analized in the study attempts to present the problematic microorganisms for each infection site within a follow up of a 13-year period. These results may be considered comprehensive for the country with respect to the current requirements of reporting of the isolates in infections, clinically confirmed as nosocomial ones. CIS-NI provides a possibility for separate studies of the frequency distribution of the isolates by infection sites and wards, using the same statistical criterion as percent distribution. The exact determination of an isolation level of a microbial species in terms of statistical evaluation would require the forming of special cohorts of patients, and consideration, depending on the objective of the study, of certain specific microbiological criteria, e.g., Colony-Forming Unit (CFU), specific limits of antimicrobial sensitivity variation etc.

At a next stage of updating of our reporting system should be considered a sub categorization of the patients in the critical care units on the basis of installed device, providing the possibility for introduction of incidence rates and level of the microbial isolates for the device-associated infections (VAP, CAUTIs, UTIs) per 1000 patient days.

The microbial characteristics presented marks out in broad outlines the involvement of the most common microbial agents in important for the clinical practice nosocomial infections, namely VAP, LRTIs, SSIs, sepsis, UTIs. The results are to a high extent consistent with the reference studies on the distribution of the nosocomial pathogens for each of these infection sites. The isolates assigned as NI causative agents refer predominantly to strains of *S. aureus* and *E. coli* (of substantial importance in SSIs), *S. aureus* (sepsis), *E. coli* (UTIs), a number of opportunistic bacteria as *Pseudomonas* spp., *Acinetobacter* spp., *Klebsiella* spp., *Enterococci*, other Enterobacteriaceae as *Enterobacter* spp. and *Serratia* spp., *Proteus* spp. (VAP, LRTIs, sepsis). These species in comparatively constant or in separate years in an increasing percentage are invariably present in the visualized by years microbiological spectrum of the discussed infections groups. The presented microbiologic characteristics emphasizes the necessity of strict implementation of the NI prevention and control measures endorsed, updated guidelines including, in the risk clinical practices as operative/resuscitation procedures and manipulations, installation of vascular devices, urinary catheters, etc., and the care respectively for the patients, undergoing such procedures as preoperative, postoperative care, or attendance in the course of other clinical treatment. Some hospital strains of these microbial species may manifest resistance of different level to the ordinarily used antibacterials, or multi-drug resistance, as a number of studies, in the country including, have stated. It is considered that feed-back information to the wards about the resistance of their isolates would contribute to précising the antibiotics prescriptions. Several specific programs for monitoring of the antibacterial resistance of the hospital isolates have been functionating in intervals in a few of our university hopitals, of questanable benefit, however, for the clinical practice. The assessment of the antibacterial resistance should respond to the following tasks:

Antimicrobial-resistant pathogen
Methicillin-resistant *Staphylococcus aureus* (MRSA)
Methicillin- resistant CoNS
Vancomycin-resistant *Enterococcus* spp
Ciprofloxacin/ofloxacin-resistant *Pseudomonas aeruginosa*
Levofloxacin-resistant *P. aeruginosa*
Imipenem-resistant *P. aeruginosa*
Ceftazidime-resistant *P. aeruginosa*
Piperacillin-resistant *P. aeruginosa*
Cef3-resistant *Enterobacter* spp
Carbapenem-resistant *Enterobacter* spp
Cef3-resistant *Klebsiella pneumoniae*
Cef3-resistant *Eschericia coli*
Quinolone-resistant *E. coli*
Penicillin-resistant *Pneumococci*
Cefotaxime/ceftriaxone-resistant *Pneumococci*

MRSA: Methicillin-resistant *Staphylococcus aureus*; *CoNS*: Coagulase-negative *Staphylococci*; Cef3: Ceftazidim, cefotaxime or ceftriaxone; Quinolone; Ciprofloxacin; Ofloxacin or levofloxacin; Carbapenem, imipenem or meropenem.

Table 6: Antimicrobial resistance to specific antibiotics accepted as a standard for resistance Combination of pathogen and antimicrobial agent (relevant class. group) accepted as a standard for resistance[***].

-Promotion of the provision of additional extended tests for hospital strains identification and antibiotic sensitivity testing;

-Workout of periodic analyses of the antimicrobial resistance intended for the specific wards as a guide in the choice of antimicrobials.

On the basis of NHSN patterns could be reported:

The antimicrobial resistance rates of a given microbial species by types of infections and wards (number, percent of the resistant strains and rates per 1000 patients treated), the antimicrobial resistance to specific antibiotics being accepted as a standard for resistance in the form of combinations of pathogen/antimicrobial agent (relevant class, group) (Table 6) [29].

For each antimicrobial agent/pathogen combination the resistance rates are calculated as:

Number of resistant isolates x 100

Number of isolates tested

Number of resistant isolates x 1000

Patients treated

Conclusion

In view of adopting criteria for evaluation of NI supervision of high-risk patients on mechanical ventilation and/or vascular catheter, two types of electronic version file-cards are proposed for approbation in the hospital settings. The files allow the calculation of the indices on the basis of patient-days with a device, i.e. ventilator-days, central line/periphral catheter-days; they visualize, thus documenting the observance of the correct hospital practices for care of these patients [30,31].

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