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Original Article

Antimicrobial resistance of selected invasive bacteria in a tertiary care center: results of a prospective surveillance study

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Abstract

Introduction: We aimed to report the distribution and resistance patterns of eight invasive clinically relevant bacteria surveyed in the Clinical Center of Serbia (CCS) in Belgrade.

Methodology: A total of 477 clinical blood stream isolates of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecium*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. were collected in the period from January to December 2013. Antimicrobial susceptibility testing was performed using standard methods and interpreted using the Clinical and Laboratory Standards Institute (CLSI) breakpoint criteria.

Results: *Acinetobacter* spp. was the most prevalent bacteria encountered (37%), followed by *K. pneumoniae* (25.7%). Multidrug resistance was observed in 92.5% of all isolates. Out of 177 strains of *Acinetobacter* spp., 97.7% were resistant to fluoroquinolones and carbapenems. Resistance to aminoglycosides, fluoroquinolones, and third-generation cephalosporins was 97.1%, 95.4%, and 95.8% among *K. pneumoniae* and 21.4%, 21.7%, and 31% among *E. coli* isolates, respectively. In total, 65.1% of *K. pneumoniae* and 12.1% of *E. coli* isolates were determined to be extended-spectrum beta-lactamase (ESBL) positive. High-level aminoglycoside resistance of *E. faecalis* was 71.4%, and glycopeptide resistance of *E. faecium* was 95%. Out of 66 strains of *S. aureus*, 63.4% were methicillin resistant.

Conclusions: The majority of bloodstream isolates of clinically relevant bacteria in CCS were multidrug resistant. The biggest concerns are carbapenem-resistant *Acinetobacter* spp., *K. pneumoniae*, and *P. aeruginosa*; third-generation cephalosporin-resistant *E. coli*; vancomycin-resistant *E. faecium*; and methicillin-resistant *S. aureus*. Stricter measures of infection control and antibiotic use are needed.

Key words: bacteremia; antimicrobial resistance; multi-drug resistance; surveillance

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Introduction

Antimicrobial resistance (AMR) is a major public-health issue, since the multidrug-resistant (MDR), extensively drug-resistant (XDR), and even pan-drug resistant microorganisms (PDR) have emerged as an ever-increasing threat for both developed and less developed countries [1]. In the United States, on an annual basis, approximately two million people acquire infection caused by bacteria resistant to one or more antibiotics and at least 25,000 people die as a result of these infections [2]. In Europe, the same number of fatal outcomes occurs annually due to infections caused by selected antibiotic-resistant bacteria, which consequently accounts for EUR 15 billion extra healthcare costs and productivity losses [3].

One specific issue in everyday clinical practice is the empirical treatment of bacteremia acquired in hospitals and caused by MDR microorganisms, which is common especially in hospitals where PDR strains have already been found. In such cases, initiation of adequate antimicrobial therapy based on the most likely causative agent and its presumable susceptibility pattern in a particular medical setting is an essential step in treatment of bacteremia, and it is associated with better outcome and lower mortality [4,5].

Several bacterial species have emerged as particularly important causative agents of bacteremia because the non-susceptibility of their strains to the last-line antibiotics usually suggests a multi-resistant nature of the bacteria. As such, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumanii* were recognized as major threats, and, since 1998, have been subject to active surveillance and annual reporting to the European

Antimicrobial Resistance Surveillance Network (EARS-Net) in most European countries [6]. Serbia joined the CAESAR (Central Asian and Eastern European Surveillance of Antimicrobial Resistance) network in 2013, whose principal aim is to survey antimicrobial resistance in all countries of the region that are not members of the AMR surveillance network EARS-Net. However, while awaiting results of the national AMR surveillance network, representative data on antimicrobial resistance of major microorganisms causing bacteremia in the main university hospital are needed.

The purpose of this study was to estimate the frequency and distribution of seven invasive bacterial species, and to restate their antimicrobial resistance patterns. We also aimed to estimate the frequency of MDR clinical isolates among specified microorganisms.

Methodology

Study design

A laboratory-based surveillance study was conducted in the entire Clinical Center of Serbia (CCS) in the period from January to December 2013. The CCS is the largest tertiary healthcare institution in Serbia, comprising 28 departments. It serves the population of Central Serbia and performs nearly six million laboratory services annually. Three microbiological laboratory sections of CCS (emergency center, clinic for infectious and tropical diseases, and polyclinic) were prospectively collecting susceptibility data of invasive clinical isolates of *Staphylococcus aureus* (*S. aureus*), *Enterococcus faecalis* (*E. faecalis*), *Enterococcus faecium* (*E. faecium*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Acinetobacter* spp. obtained from blood cultures of adult patients hospitalized in different departments of the CCS. Only the first positive culture per patient was analyzed (primary isolate). Isolates were usually obtained in set of two aerobic and two anaerobic bottles and the first invasive isolate per culture was reported.

Data collection

Affiliated laboratories of the CCS were prospectively collecting microbiological data and forwarding them, along with patients' demographic information (standardized isolate record form). Collected data were gathered and checked for consistency with the agreed protocol. Data on patients' characteristics were age, sex, and the department in which they were staying at the onset of bacteremia (surgery, intensive care unit [ICU], internal medicine, or other). Microbiological data comprised antimicrobial susceptibility test results of specified invasive clinical isolates.

Microbiological assessment

In all laboratories, isolation and identification of bacterial strains were done following standard microbiological procedures. Antimicrobial susceptibility was estimated using the Kirby-Bauer disk diffusion method and a Vitek2 automated system (bioMérieux, Marcy-I'Etoile, France). The spectrum of antimicrobial drugs reported is in accordance with EARS-Net [6]. Zone diameter was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. Strains that showed intermediate susceptibility and resistance to the specific antibiotic were considered resistant. Strains were denoted as MDR if they showed non-susceptibility to at least one agent in three or more classes of antimicrobial drugs [8]. Methicillin resistance of *S. aureus* was determined using the Kirby-Bauer disk diffusion method with a 30 μg cefoxitin disk (Becton Dickinson, Sparks, USA). A zone size of ≥ 22 mm was considered sensitive and ≤ 21 mm was considered resistant. Suspected extended-spectrum beta lactamase (ESBL)-producing organisms within *E. coli* and *K. pneumoniae* isolates were confirmed using the double-disk synergy test described by Jarlier *et al*. or by an ESBL combination disks test (Becton Dickinson, Sparks, USA) [9]. Minimum inhibitory concentrations (MICs) were determined and results were interpreted according to CLSI clinical breakpoints.

Numbers and percentages were used to express the distribution of different bacterial isolates and their susceptibility patterns using SPSS software for Windows, version 20.0 (IBM, Armonk, USA).

Results

Over a one-year period, 477 primary blood cultures were obtained from the same number of adult patients hospitalized in clinics of the CCS in Belgrade. Of these, 300 (62.89%) cultures were isolated from men and 165 (34.59%) from women. In total, 278 (58.28%) were under 65 years of age, while 161 patients (33.75%) were 65 or older. The majority of patients were hospitalized in the surgery unit (46.49%) and ICU (35.37%), while 13.83% and 2.27% of them were inpatients of the internal medicine unit and other departments, respectively.

Table 1 shows the distribution of major bacteria isolated in CCS during the study period and the percentage of their MDR strains. Among selected bacterial species, *Acinetobacter* spp. was the most prevalent bacteria encountered (37.1%), followed by *K. pneumoniae* (25.7%), *S. aureus* (13.2%), *Enterococci* (11.5%), *E. coli* (6.5%), *P. aeruginosa* (5.2%), and *S. pneumoniae* (0.8%). In total, 92.5% of all blood isolates grew cultures resistant to three or more antimicrobial classes. The lowest percentages of MDR strains were in *S. pneumoniae* (33.3%) and *E. coli* (45.2%), while the highest MDR percentage was observed in *E. faecium* (100%).

The results of antimicrobial susceptibility testing of the selected invasive Gram-negative and Gram-positive bacteria listed alphabetically are shown in Tables 2 and 3. Out of 177 strains of *Acinetobacter* spp., 97.7% of isolates were resistant to fluoroquinolones (ciprofloxacin), as well as to carbapenems (imipenem and meropenem). Resistance to aminoglycoside antibiotics ranged from 73.4% to amikacin to 90.2% to tobramycin. Similarly, the great majority of *P. aeruginosa* isolates showed resistance to aminoglycosides (95.5% to gentamicin), carbapenems (80.0% to imipenem and 76.0% to meropenem), and fluoroquinolones (82.6% to ciprofloxacin). Slightly more than half the isolates (56.0%) were resistant to piperacillin-tazobactam. Resistance to fourth-generation cephalosporins was 66.7% to cefepime and 72.0% to ceftazidime. However, there was no

Table 1. Distribution of bacteria of public-health importance in 477 invasive blood culture isolates obtained in 2013 in the Clinical Centre of Serbia.

Microorganism	n (%)	n (%) of MDR strains
Acinetobacter spp.	177 (37.1)	174 (98.3)
Klebsiella pneumoniae[†]	120 (25.2)	116 (96.7)
Staphylococcus aureus	66 (13.8)	64 (97.0)
Enterococcus faecalis[¥]	14 (2.9)	12 (85.7)
Enterococcus faecium[¥]	41 (8.6)	41 (100)
Escherichia coli[§]	31 (6.5)	14 (45.2)
Pseudomonas aeruginosa	25 (5.2)	19 (76.0)
Streptococcus pneumoniae	3 (0.7)	1 (33.3)
Total	**477 (100)**	**441 (92.5)**

MDR: multidrug resistant; ESBL: extended-spectrum β-lactamase; [†]ESBL + *Klebsiella pneumoniae* was found in 23 out of 120 (19.2%) strains; [§]ESBL + *Escherichia coli* was found in 4 out of 31 (12.9%) strains; All ESBL + strains of *Klebsiella pneumoniae* and *Escherichia coli* were MDR; [¥]Only *Enterococcus faecalis* and *Enterococcus faecium* were identified within *Enterococcus* species.

Table 2. Antibiotic resistance of selected Gram-negative invasive isolates in the Clinical Centre of Serbia in 2013.

Bacteria		***Acinetobacter* spp.**	***P. aeruginosa***	***K. pneumoniae***	***E. coli***
Antimicrobial class	**Antimicrobial agent**	**(n = 177)**	**(n = 25)**	**(n = 120)**	**(n = 31)**
Aminoglycosides	Amikacin	130 (73.4)	17 (68.0)	82 (68.9)	6 (21.4)
	Gentamicin	139 (90.8)	19 (95.5)	102 (97.1)	9 (42.1)
Carbapenems	Imipenem	171 (97.7)	20 (80.0)	81 (67.5)	2 (6.5)
	Meropenem	167 (97.7)	19 (76.0)	69 (65.1)	1 (3.2)
Extended-spectrum cephalosporins	Ceftriaxone			113 (95.8)	9 (31.0)
	Ceftazidime	161 (99.4)	18 (72.0)		
	Cefepime	139 (96.5)	8 (66.7)		
Fluoroquinolones	Ciprofloxacin	171 (97.7)	19 (82.6)	104 (95.4)	5 (21.7)
Folate pathway inhibitors	SMX-TMP	116 (73.9)			
Non-extended spectrum cephalosporins	Cephalexin/cephalotin			46 (100.0)	12 (60.0)
Penicillins	Ampicillin				22 (78.6)
Penicillin + β-lactamase inhibitors	Amoxicillin-clavulanic acid			105 (95.5)	14 (46.7)
	Piperacillin-tazobactam	119 (98.3)	14 (56.0)		
	Ampicillin-sulbactam (ampisullcilin)	124 (82.7)		105 (95.5)	14 (46.7)
Polymyxins	Colistin	0 (0.0)	0 (0.0)		

R: resistant; SMX-TMP: sulfamethoxazole-trimethoprim.

Acinetobacter spp. nor *P. aeruginosa* isolate resistant to colistin. Combined resistance of these Gram-negative rods to carbapenems, fluoroquinolones, and aminoglycosides was observed in 81.4% strains of *Acinetobacter* spp. and 72% of *P. aeruginosa* isolates.

Strains of *K. pneumoniae* were highly resistant to all antimicrobial agents they were tested against. Moreover, almost all strains were resistant to key antimicrobial classes: aminoglycosides (97.1% to gentamicin), fluoroquinolones (95.4% to ciprofloxacin) and third-generation cephalosporins (95.8% to ceftriaxone). Combined resistance to these antimicrobial groups was 80%. The percentage of isolates resistant to carbapenems was very high and ranged from 65.1% to meropenem to 67.5% to imipenem. ESBL production was confirmed in 21 (17.5%) of *K. pneumoniae* isolates, and all of those were MDR.

E. coli was the least resistant to carbapenems (3.2% to meropenem and 6.5% to imipenem) and the most resistant to aminopenicillins (78.6% to ampicillin). Antimicrobial resistance to fluoroquinolones and aminoglycosides was similar (21.7% to ciprofloxacin and 21.4% to amikacin). One-third of *E. coli* isolates (31.0%) were resistant to the third-generation cephalosporin ceftriaxone. Four of the *E. coli* isolates (12.1%) were ascertained to be ESBL positive.

Resistance to high-level aminoglycosides (gentamicin and amikacin) was observed in 10 (71.4%) out of 14 of *E. faecalis* isolates, while 28.6% of strains were resistant to glycopeptides (vancomycin and teicoplanin). On the contrary, almost all isolates of *E. faecium* (95%) were resistant to glycopeptides and all other antibiotics they was tested against. All strains of both *E. faecalis* and *E. faecium* were susceptible to linezolid.

Out of 66 strains of *S. aureus*, 42 (63.6%) were methicillin-resistant (MRSA) while no vancomycin-resistant *S. aureus* (VRSA) was encountered. Non-susceptibility of MRSA isolates to other antibiotics ranged from 16.0% to trimethoprim-sulfamethoxazole and 100% to gentamicin. However, all MRSA isolates were susceptible to vancomycin and linezolid.

Out of three *S. pneumoniae* isolates obtained from the blood cultures during 2013, one isolate showed non-susceptibility to tetracycline, erythromycin, and clindamycin. Although classified as MDR, this isolate was susceptible to antimicrobial agents within other classes of antibiotics, namely to penicillins, third-generation cephalosporins, carbapenems, glycopeptides, fluoroquinolones, and ansamycins. One strain of *S. pneumoniae* was resistant to penicillin and the third one appeared to be susceptible to all the antibiotics it was tested against.

Table 3. Antibiotic susceptibility of invasive isolates of selected Gram-positive bacteria in the Clinical Centre of Serbia in 2013.

Bacteria		***E. faecalis***	***E. faecium***	***S. aureus***	***MRSA*[§]**	***S. pneumoniae***
Antimicrobial class	**Antimicrobial agent**	**(n = 14)**	**(n = 41)**	**(n = 66)**	**(n = 42)**	**(n = 3)**
Aminoglycosides	Gentamicin	10 (71.4)[†]	41 (100.0)[†]	20 (83.3)	18 (100)	
	Streptomycin	10 (71.4)[¥]	38 (95.0)[¥]			
Ansamycins	Rifampin/rifampicin			25 (37.9)	17 (40.5)	0 (0.0)
Anti-staphylococcal β-lactams	Methicillin/oxacillin			42 (63.6)		1 (100)
Extended-spectrum cephalosporins	Ceftriaxone					0 (0.0)
Fluoroquinolones	Ciprofloxacin	10 (90.9)	37 (97.3)	33 (57.9)	30 (83.3)	0 (0.0)
Folate pathway inhibitors	SMX-TMP			5 (15.2)	4 (16.0)	
Glycopeptides	Vancomycin	4 (28.6)	39 (95.1)	0 (0.0)	0 (0.0)	0 (0.0)
	Teicoplanin	4 (28.6)	39 (95.1)			
Lincosamides	Clindamycin			24 (47.1)	20 (58.8)	1 (0.33)
Macrolides	Erythromycin				20 (57.1)	1 (50.0)
Oxazolidolines	Linezolid	0 (0.0)	40 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Penicillins	Amoxicillin	8 (61.5)	40 (97.6)			
Phenicols	Chloramphenicol			11 (28.2)	10 (33.3)	0 (0.0)
Tetracycline	Tetracycline	8 (72.7)	35 (94.6)			1 (100.0)

R: resistant; SMX-TMP: sulfamethoxazole-trimethoprim; MRSA: methicillin-resistant *S. aureus;* [§]All MRSA strains are inclusive of *S. aureus* group (42/66; 63.6%); [†]High-level gentamicine; [¥]High-level streptomycin.

Discussion

A growing number of bacteremia is caused by highly resistant bacteria. Representative surveillance data on the resistance profiles of its major causative agents is of great importance to keep clinicians versed with the most adequate treatment and public-health officials with the extent of the AMR issue.

In our surveillance study conducted over a period of one year, *Acinetobacter* spp. and *K. pneumoniae* were the most common pathogens isolated from blood, accounting for more than half of all tested blood isolates, while *S. aureus*, *E. faecalis*, *E. faecium*, *E. coli*, *P. aeruginosa*, and *S. pneumoniae* together accounted for the other half. Other hospitals worldwide are facing increasing trends of Gram-negative bacteremia, which poses a great concern considering their persisting nature in the medical setting and diminished susceptibility to available antibiotics [10,11].

One of the major findings of this surveillance was the alarmingly high percentage of *K. pneumoniae* and other Gram-negative rods, *Acinetobacter* spp., and *P. aeruginosa* resistant to last-line antimicrobials (carbapenems) along with high resistance to three key groups of antibiotics (fluoroquinolones, third-generation cephalosporins, and aminoglycosides). In addition, more than two-thirds of all *K. pneumoniae* isolates were carbapenem resistant in our investigation. The most recent European Centre for Disease Prevention and Control (ECDC) data showed an increasing trend of carbapenem resistance of this rod in European countries, and 5 out of 24 countries reported a significantly increasing trend [6]. According to the last World health Organization report [12], resistance of *K. pneumoniae* invasive isolates causing bloodstream infections and meningitis to carbapenems varied from 0%–68% in the European region, 0%–54% in the Eastern Mediterranean region, and 0%–52% in the Southeast Asian region. The resistance to carbapenems is of particular concern because this group of antimicrobial agents is one of the rare antibiotics effective against infections caused by *K. pneumoniae*.

We found more than 70% of *Acinetobacter* spp. isolates resistant to each antibiotic and more than 90% resistant to aminoglycosides, fluoroquinolones, and carbapenems. This, to a great extent, fits the North to South/East gradient encountered in the EU region according to the latest EARS-Net AMR report, since Finland and Norway had zero, and Croatia, Romania, and Greece had more than 90% of *Acinetobacter* spp. isolates resistant to these antibiotics [6]. In the last three countries, combined resistance to aminoglycosides, fluoroquinolones, and carbapenems ranged from 74% to 86%, which is similar to the 81.4% that we observed. Similar rates of strains resistant to both key antibiotics and multiple antimicrobial classes were observed among the other major bacteria under surveillance. However, much higher resistance rates of *P. aeruginosa* and *K. pneumoniae* were noted in the CCS compared to the uppermost values in northern European countries, Romania, and Greece. The opposite was observed only for the resistance to fluoroquinolones within *E. coli*, which was much lower in CCS than in Cyprus (21.0% versus 51.9%), but similar to Croatia (20.2%), Czech Republic (20.8%), and the overall European population mean (22.5%).

E. coli is the most frequent cause of bloodstream infections in Europe and in the past few years evinced the largest annual increase, mostly due to expansion of MDR *E. coli* bacteremia [13]. In our study, one-third of *E. coli* isolates were resistant to third-generation cephalosporins and 21% were resistant to amikacin, which is slightly lower than the uppermost value in Europe reported by Bulgaria (39.6% and 32.1%, respectively) [6]. Although not common, resistance to carbapenems were also higher in the CCS (3.2%) compared to nearby European member states Bulgaria (2.8%) and Greece (1.4%). Promising data were obtained regarding fluoroquinolone resistance of *E. coli* in our hospital (21.7%), which was similar to the values reported by Austria (22%) and Germany (22.1%), but lower than the rate reported in 2007 from another clinical center in the northern part of Serbia (28.6%) [14]. This could be the reaction to extensive ciprofloxacin use in the period from 2003 to 2007 in Serbia and the favoring of third-generation cephalosporins over fluoroquinolones in the following years in the treatment of hospital-acquired *E. coli* bloodstream infections [15].

Several factors could have possibly contributed to such a high incidence of MDR Gram-negative bacteria found in this surveillance. Prolonged ICU or hospital stay, increased disease severity, frequent interventions, and admission of broad-spectrum antibiotics (especially third-generation cephalosporins, fluoroquinolones, and carbapenems) are well-known risk factors for acquiring MDR Gram-negative infections [16,17]. Although most of our patients were inpatients of surgery (46.5%) and ICU (35.4%) medical wards and were likely critically ill and exposed to several of these factors, consistent data on comorbidities, length of stay, and previous administration of antibiotics could not have been collected by the means of laboratory-based surveillance. Alternatively, usage of CLSI beyond 2009 or EUCAST guidelines in susceptibility testing can

result in higher rates of MDR Gram-negative strains due to higher clinical breakpoints (CBPs) for certain antibiotics compared to guidelines prior to CLSI 2010. Hombach *et al*. showed that 21% of *E. coli*, 22% of *K. pneumoniae*, and 12% of *P. aeruginosa* were classified as MDR due to CBP changes from CLSI 2009 to EUCAST [18]. This was mainly attributed to increased CBPs for cephalosporins and fluoroquinolones. Liu *et al.* observed a 10.5% increase in *Enterobacteriaceae* resistance rates to cefotaxime due to the CLSI CBP change from 2009 to 2010 guidelines, as well as 6.6% and 13.2% increase in resistance rates of *K. pneumoniae*, respectively [19]. However, the most likely causes of high MDR Gram-negative rates in this study are the overconsumption of antibiotics and the lack of both general and bacteria-specific healthcare strategies for prevention and control of hospital-acquired infections. Serbia is among the southern and eastern European countries with the highest rates of total antimicrobial consumption, and is second in utilization of first-generation cephalosporins, macrolides, and tetracyclines [20]. While an increase in invasive procedures and aggressive treatment in ICUs have led to the increased rates of Gram-negative bacteremia, an overuse of antibiotics in treatment of such bacteremia consequently increased resistance through the selective pressure of broad-spectrum antibiotics and selection of multi-resistant clones.

Other important findings of this surveillance were high rates of MRSA and vancomycin-resistant *E. faecium* (VRE). In our study, 63.6% of MRSA strains were encountered, similar to the uppermost percentage in Europe reported from Romania (64.5%) to the EARS-Net [6]. A recent study on MRSA nasal carriage in CCS showed that 7.7% of patients and 5.7% of healthcare workers (HCWs) were carriers of MRSA [21]. Moreover, 76.2% of all MRSA isolates carried SCCmec I or III and agr I or II genetic elements and were mainly recovered from patients and indistinguishable from those obtained from HCWs, indicating oligoclonal dissemination and HCW-patient exchange of hospital-acquired-MRSA strains. Implementation of adequate strategies to reduce transmission of MRSA strains in hospital settings is required, as it has been proven by countries which significantly reduced rates of HA-MRSA, including the United Kingdom [22], the Netherlands [23], and France [24].

With respect to glycopeptide susceptibility of *E. faecium*, we noted that 95.1% of strains were resistant to vancomycin and teicoplanin, which is six times higher than reported in 2007 (13%) [14]. Furthermore, in the period from 1997 to 2002 in Serbia, all *Enterococcus* strains were susceptible to vancomycin [25]. This is highly alarming given the fact that VRE in our study was susceptible only to linezolid, which, to a great extent, increases the difficulty and the cost of the treatment [26]. In contrast, countries of our region, Croatia, Slovenia, and Greece reported decreasing trends of VRE [6].

There were optimistic results only regarding *S. pneumoniae* since dual resistance to penicillin and macrolides were not found; one strain showed single penicillin-resistance and all strains were sensitive to ceftriaxone, rifampicin and chloramphenicol. This is lower than previously reported in Serbian hospitals and suggestive of penicillins remaining useful in treatment of streptococcal bacteremia [27,28].

Conclusions

To the best of our knowledge, this is the first study to survey antimicrobial resistance of seven major pathogens within the entire Clinical Center of Serbia. Worrisome results indicate a high level of resistance of *Acinetobacter* spp., *K. pneumoniae*, and *P. aeruginosa* to the key antibiotics (fluoroquinolones, third-generation cephalosporins, and aminoglycosides) and the last-line antibiotics (carbapenems). Data from this survey also demonstrated high percentages of MRSA and VRE in CCS resistant to other antibiotics as well as a high overall percentage of MDR strains. All these findings stress the necessity for urgent implementation of united measures for antibiotic consumption restriction and general and pathogen-specific measures for transmission interruption of resistant clones.

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References

1. Huttner A, Harbath S, Carlet J, Cosgrove S, Goossens H, Holmes A, Jarlier V, Voss A, Pittet D (2013) Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated Infections Forum. Antimicrob Resist Infect Control 2: 31.
2. Centers for Disease Control and Prevention (2014) Antibiotic resistance threats in the United States, 2013. Available: http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf. Accessed 15 October 2014.
3. European Centre for Disease Prevention and Control (2009) The bacterial challenge: time to react. Available: http://ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf. Accessed 22 March 2014.
4. Kim YJ, Yun YH, Kim YR, Park KG, Park YJ, Kang JY, Kim SI (2014) Risk factors for mortality in patients with

Pseudomonas aeruginosa bacteremia; retrospective study of impact of combination antimicrobial therapy. BMC Infect Dis 14: 161.
5. Asgeirsson, H, Kristjansson M, Kristinsson KG, Gudlaugsson O (2011) *Staphylococcus aureus* bacteremia--nationwide assessment of treatment adequacy and outcome. J Infect 62: 339-346.
6. European Centre for Disease Prevention and Control (2014) Antimicrobial resistance surveillance in Europe 2013. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Available: http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2013.pdf. Accessed 11 September 2015.
7. Clinical and Laboratory Standards Institute (2013) Performance standards for antimicrobial susceptibility testing, 20th informational supplement, M100-S23. Wayne, PA: CLSI.
8. Falagas ME, Karageorgopoulos DE (2008) Pandrug resistance (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) among Gram-negative bacilli: need for international harmonization in terminology. Clin Infect Dis 46: 1121-1122.
9. Jarlier V, Nicolas MH, Fournier G, Philippon A (1988) Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev Infect Dis 10: 867-878.
10. Dizbay M, Guzel Tunccan O, Karasahin O, Aktas F (2014) Emergence of carbapenem-resistant Klebsiella spp. infections in a Turkish university hospital: epidemiology and risk factors. J Infect Dev Ctries 8: 44-49. doi: 10.3855/jidc.3091.
11. European Centre for Disease Prevention and Control (2013) Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals 2011-2012. Available: http://ecdc.europa.eu/en/publications/Publications/healthcare-associated-infections-antimicrobial-use-PPS.pdf. Accessed 5 April 2014.
12. World Health Oraganization (2014) Antimicrobial resistance: Global Report on Surveillance 2014. Available: http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua=1. Accessed 5 April 2014.
13. de Kraker ME, Jarlier V, Monen JC, Heuer OE, van de Sande N, Grundmann H (2013) The changing epidemiology of bacteremias in Europe: trends from the European Antimicrobial Resistance Surveillance System. Clin Microbiol Infect 19: 860-868.
14. Kulauzov M, Medić D, Jovanović J, Mihajlović UM, Mikic SS, Sevic S (2008) Antimicrobial resistance of the bacteria isolated from blood cultures in 2007. Med Pregl 61: 21-26.
15. Velickovic-Radovanovic R, Petrović J, Kocić B, Antić S, Randelović G (2009) Correlation between antibiotic consumption and bacterial resistance as quality indicator of proper use of these drugs in inpatients. Vojnosanit Pregl 66: 307-312.
16. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y (2006) Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. Antimicrob Agents Chemother 50: 43-48.
17. Chan MC, Chiu SK, Hsueh PR, Wang NC, Wang CC, Fang TC (2014) Risk factors for healthcare-associated extensively drug-resistant *Acinetobacter baumannii* infections: a case-control study. PLoS One 9: e85973.
18. Hombach M, Wolfensberger A, Kuster SP, Bottger C (2013) Influence of clinical breakpoint changes from CLSI 2009 to EUCAST 2011 antimicrobial susceptibility testing guidelines on multidrug resistance rates of Gram-negative rods. J Clin Microbiol 51: 2385-2387.
19. Liu PY, Shi ZY, Tung KC, Shyu CL, Chan KW, Liu JW, Wu ZY, Kao CC, Huang YC, Lin CF (2014) Antimicrobial resistance to cefotaxime and ertapenem in Enterobacteriaceae: the effects of altering clinical breakpoints. J Infect Dev Ctries 8: 289-296. doi: 10.3855/jidc.3335.
20. Versporten A, Bolokhovets G, Ghazaryan L, Abilova V, Pyshnik G, Spasojevic T, Korinteli I, Raka L, Kambaralieva B, Cizmovic L, Carp A, Radonjic V, Maqsudova N, Celik HD, Payerl-Pal M, Pedersen HB, Sautenkova N, Goossens H (2014) Antibiotic use in eastern Europe: a cross-national database study in coordination with the WHO Regional Office for Europe. Lancet Infect Dis 14: 381-387.
21. Cirkovic I, Dukic S, Carevic B, Mazic N, Mioljevic V, Stepanovic S (2014) Methicillin-resistant *Staphylococcus aureus* nasal carriage among hospitalized patients and healthcare workers in the clinical center of Serbia. Arch Biol Sci 66: 87-92.
22. Rampling A, Wiseman S, Davis L, Hyett AP, Walbridge AN, Payne GC, Cornaby AJ (2011) Evidence that hospital hygiene is important in the control of methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 49: 109-116.
23. Wassenberg MW, de Wit GA, van Hout BA, Bonten MJ (2010) Quantifying cost-effectiveness of controlling nosocomial spread of antibiotic-resistant bacteria: the case of MRSA. PLoS One 5: e11562.
24. Chalfine A, Kitzis MD, Bezie Y, Benali A, Perniceni L, Nguyen JC, Dumay MF, Gonot J, Rejasse G, Goldstein F, Carlet J, Misset B (2012) Ten-year decrease of acquired methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia at a single institution: the result of a multifaceted program combining cross-transmission prevention and antimicrobial stewardship. Antimicrob Resist Infect Control 1: 18.
25. Mirovic V, Tomanovic B, Konstantinovic S (2004) The frequency of resistance to antibiotics of most frequently isolated bacteria from blood cultures during the period 1997-2002. Vojnosanit Pregl 61: 391-397.
26. Wilke MH (2010) Multiresistant bacteria and current therapy - the economical side of the story. Eur J Med Res 15: 571-576.
27. Jovanovic M, Stosovic B, Tosic T, Dulovic O (2004) Susceptibility of invasive strains of Streptococcus pneumoniae to antibiotics. Acta Infectologica Yugoslavica 8: 17-21.
28. Gajic I, Mijač V, Ranin L, Andjelković D, Radičević M, Opavski N (2013) Invasive isolates of *Streptococcus pneumoniae* in Serbia: antimicrobial susceptibility and serotypes. Srp Arh Celok Lek 141: 48-53.

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