ORIGINAL ARTICLES

UDC: 579.61::[615.281:615.015.8
https://doi.org/ 10.2298/VSP150917260T

Carbapenemase production in hospital isolates of multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli* in Serbia

Produkcija karbapenemaza kod bolničkih multirezistentnih sojeva *Klebsiella pneumoniae* i *Escherichia coli* u Srbiji

Anika Trudić*†, Zora Jelesić†‡, Mira Mihajlović-Ukropina†‡, Deana Medić†‡, Branka Zivlak†‡, Vera Gusman†‡, Milan Djilas‡

***Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia; University of Novi Sad, †Faculty of Medicine, Novi Sad, Serbia; ‡Institute for Public Health of Vojvodina, Novi Sad, Serbia**

Abstract

Background/Aim. Carbapenem resistance has escalated in medically important enterobacteria such as *Klebsiella pneumoniae* and *Escherichia coli* worldwide. Multidrug-resistant strains represent an important source of concern as effective therapeutic options of infections they cause are limited or none. There were no comprehensive studies considering the presence of carbapenemase production in enterobacteria in Serbia so far. The aim of the study was to determine carbapenemase production in hospital isolates of multidrug-resistant *K. pneumoniae* and *E. coli* in Serbia. **Methods.** Strains of *K. pneumoniae* and *E. coli* resistant to at least one carbapenem (imipenem, meropenem, ertapenem) were collected from November 2013 to May 2014. Isolates were obtained from clinical samples of patients treated in 14 hospitals in Serbia. Carbapenem resistance was confirmed using phenotypic tests and polymerase chain reaction (PCR) in National Reference Laboratory for Registration and Surveillance of Antimicrobial Resistance of Bacterial Strains in Novi Sad. **Results.** Of 129 collected strains, 121 (93.8%) were *K. pneumoniae* and 8 (6.2%) were *E. coli*. Seventy (54.3%) strains were obtained from urine, 26 (20.2%) from blood, 19 (14.7%) from wound secretions and 14 (10.9%) from lower respiratory tract secretions. Carbapenemase genes were detected in 58 (45%) isolates. The gene bla New Delhi-metallo-beta-lactamases (bla_{NDM}) was found in 33 (27.3%) *K. pneumoniae*, bla oxacillinases-48 (bla_{OXA-48}) in 10 (8.3%), bla *K. pneumonia* carbapenemase (bla_{KPC}) in 1 (0.8%), and 7 (5.4%) strains harbored both bla_{OXA-48} and bla_{NDM}. Seven *E. coli* harbored bla_{NDM} gene. **Conclusion.** In Serbia, the most common type of carbapenemase in both multidrug-resistant *K. pneumoniae* and *E. coli* is NDM. Co-production of OXA-48 and NDM was found in *K. pneumoniae*. To our knowledge, KPC production was detected for the first time in Serbia.

Keywords:
enterobacteriaceae; drug resistance, bacterial; carbapenems; cross infection; genome, bacterial; serbia; beta lactamases.

Apstrakt

Uvod/Cilj. Rezistencija na karbapeneme među medicinski značajnim enterobakterijama kao što su *Klebsiella pneumoniae* i *Escherichia coli* u porastu je širom sveta. Zabrinjavajuća je činjenica da su terapijske mogućnosti kod infekcija uzrokovanih multirezistentnim sojevima ograničene ili ih nema. Do sada nije rađena sveobuhvatnija studija o produkciji karbapenemaza kod enterobakterija u Srbiji. Cilj istraživanja bio je utvrđivanje produkcije karbapenemaza kod bolničkih multirezistentnih sojeva *K. pneumoniae* i *E. coli* u Srbiji. **Metode.** Izolati *K. pneumoniae* i *E. coli* rezistentni na najmanje jedan karbapenem (imipenem, meropenem, ertapenem) prikupljani su od novembra 2013. do maja 2014. Bakterijski sojevi izolovani su iz klinički značajnih uzoraka od bolesnika lečenih u 14 bolnica u Srbiji. Rezistencija na karbapeneme potvrđena je fenotipskim testom i reakcijom lančane polimerizacije u Nacionalnoj referentnoj laboratoriji za registrovanje i praćenje rezistencije bakterijskih sojeva na antimikrobna sredstva u Novom Sadu. **Rezultati.** Od ukupno 129 prikupljenih sojeva, bio je 121 (93,8%) izolat *K. pneumoniae* i 8 (6,2%) izolata *E. coli*. Iz urina je izolovano 70 (54,3%) sojeva, iz krvi 26 (20,2%), iz sekreta rana 19 (14,7%) i 14 (10,9%) iz sekreta donjeg respiratornog trakta. Geni koji kodiraju karbapenemaze su nađeni kod 58 (45%) izolata. Gen *bla New Delhi metallo-beta-lactamases* (bla_{NDM}), dokazan je kod 33 (27,3%) izolata *K. pneumoniae, bla oxacillinases-48* (*bla*OXA-48) kod 10 (8,3%), *bla K. pneumonia carbapenemase* (*bla*KPC) kod 1 (0,8%), a kod 7 (5,4%) izolata su istovremeno nađeni geni *bla*OXA-48 i *bla*NDM. Kod 7 izolata *E. coli* su detektovani *bla*NDM geni. **Zaključak.** Najčešći tip karbapenemaza u Srbiji kod multirezistentnih izolata *K. pneumoniae* i *E. coli* je NDM. Istovremena produkcija OXA-48 i NDM detektovana je kod izolata *K. pneumoniae*. Prema našem saznanju, prvi put je nađena produkcija KPC u Srbiji.

Ključne reči:
enterobacteriaceae; lekovi, rezistencija mikroorganizama; karbapenemi; infekcija, intrahospitalna; genom, bakterijski; srbija; beta-laktamaze.

Correspondence to: Anika Trudić, Institute for Pulmonary Diseases of Vojvodina, Center for Microbiology, Immunology and Virology; Put doktora Goldmana 4, 21 204 Sremska Kamenica, Serbia. Phone: +381(21)4805348. E-mail: anika.trudic@mf.uns.ac.rs

Introduction

Due to increased global transport, there is an increased exposure of people all around the world to diverse Gram-negative bacteria from gut flora, especially *Escherichia coli* and *Klebsiella spp*. Fecal carriage is recognized as the most important for spreading multidrug-resistant strains in the hospital environment [1]. Multidrug-resistant bacteria represent an important source of concern as effective therapeutic options of infections they cause are limited or none [2]. Carbapenems are often used for the treatment of nosocomial infections as the last line therapy [3]. In the last decade, carbapenem resistance has escalated in medically important bacteria [4, 5]. In Europe *Klebsiella pneumoniae* is the most frequently reported carbapenem-resistant enterobacteria [6]. Isolation of carbapenem-resistant *E. coli* is of concern, as it spreads more easily in the community. Also, the treatment of such community-acquired infections might become a challenge [7]. Two main mechanisms are responsible for carbapenem resistance, the first refers to carbapenem-hydrolyzing enzymes (carbapenemases) and the second is usually a combination of deficiency of porin expression and overexpression of beta-lactamases with weak affinity for carbapenems [7]. The most frequently isolated carbapenemases are *K. pneumoniae* carbapenemases (KPC), Verona integron-encoded metallo-beta-lactamases (VIM), imipenemases (IMP), New Delhi metallo-beta-lactamases (NDM) and oxacillinases-48 (OXA-48) [5]. Carbapenemase-encoding genes are usually located on self-conjugative plasmids often accompanied with other non-beta-lactam resistant determinants [8]. Acquisition of genetic material through horizontal transfer explains the urge for proper detection of carbapenemase-producing strains [7, 8]. Unfortunately, the detection of carbapenemase producer cannot rely only on the resistance profile routinely done in microbiology laboratory as their minimal inhibitory concentration (MIC) values may sometimes lay within the susceptibility range. Also, some strains may produce other enzymes and mechanisms responsible for lower resistance to carbapenems [8, 9]. Therefore, multidrug-resistant isolates with lower susceptibility to carbapenems should be tested for the presence of carbapenemase in order to prevent hospital outbreaks. To our knowledge, there were no comprehensive studies considering the presence and the occurrence of carbapenemase production in enterobacteria in Serbia so far.

The aim of the study was to determine carbapenemase production in hospital isolates of multidrug-resistant *K. pneumoniae* and *E. coli* in Serbia.

Methods

The study included 129 nonrepetitive multidrug-resistant strains of *K. pneumoniae* and *E. coli* isolated from a clinical specimen (urine, blood, wound secretion/swab and lower respiratory tract secretions: tracheal aspirate, broncho-aspirate, broncho-alveolar lavage) from November 2013 to May 2014. The strains were collected from microbiology laboratories in Clinical Center Serbia (Belgrade), Clinical Center "Zvezdara" (Belgrade), Clinical Center "Dragiša Mišović" (Belgrade), Institute for Public Health Čačak (Čačak), Institute for Public Health Kikinda (Kikinda), Clinical Center Kragujevac (Kragujevac), Institute for Public Health Kraljevo (Kraljevo), Institute for Pulmonary Diseases of Vojvodina (Novi Sad), Institute for Public Health of Vojvodina (Novi Sad), Institute for Public Health Niš (Niš). Estimated population coverage of the 14 hospitals involved was around 5 million. Collected strains were reported intermediate or resistant to at least one carbapenem (imipenem, meropenem, ertapenem) according to the Clinical and Laboratory Standards Institute (CLSI) [10].

The study was conducted at National Reference Laboratory for Registration and Surveillance of Antimicrobial Resistance of Bacterial Strains in the Institute for Public Health of Vojvodina, Novi Sad, Serbia. Identification of isolated strains was done using VITEK 2 Compact GN cards (BioMérieux, Marcy I Etoile, France). Antimicrobial susceptibility was determined using the disk diffusion method and/or using VITEK 2 AST-GN71 and AST-N240 cards according to CLSI. Susceptibility to fosfomycin was tested by E test strip (AB, Biodisk, Solna, Sweden). For the interpretation of tigecycline MICs, Food and Drug Administration (FDA) breakpoints for *Enterobacteriaceae* were used (susceptible $\leq$ 2 mg/L, intermediate 4 mg/L; resistant $\geq$ 8 mg/L). Phenotypic testing of extended-spectrum beta-lactamases (ESBL) production was done using combined disk tests (CDT) using cefotaxime disk and cefotaxime/clavulanic acid disk and ceftazidime and ceftazidime/clavulanic acid disk (Bio-Rad, France). Enhancement of the zone of inhibition more than 5 mm of the inhibitor-containing disc was considered to be a positive result. Phenotypic testing of carbapenemase production was done by double-disk synergy test (DDST) using tablets containing meropenem (10 μg), cloxacillin, dipicolinic acid, boronic acid (Rosco Diagnostica Neo-Sensitabs, Taastrup, Denmark) according to manufactures' instructions. Enhancement of the zone of inhibition in the area between meropenem disc and the inhibitor-containing disc was considered to be a positive result. Confirmation of carbapenemase production was done using polymerase chain reaction (PCR). PCR reaction of five genes was performed as two separate multiplex reactions and one simplex reaction with Mastercycler personal (Eppendorf, Hamburg, Germany). The first reaction included primers for bla_{NDM} [11] and bla_{KPC} [12] genes, the second included primers for bla_{OXA48} [13] and bla_{VIM} [14] genes. PCR cycling conditions for the first reaction were 1 cycle at 95 ºC for 5 min, 30 cycles of 95 ºC for 30 s, 60 ºC for 30 s, and 72 ºC for 60 s, followed by 1 cycle at 72 ºC for 3 min and holding stage at 4ºC. PCR cycling conditions for the second reaction were 1 cycle at 95 ºC for 5 min, 30 cycles of 95 ºC for 30 s, 58 ºC for 30 s, and 72 ºC for 60 s, followed by 1 cycle at 72 ºC for 3 min and holding stage at 4 ºC. Gene bla_{IMP} [14] was tested separately under following conditions: one cycle at 95ºC for 5 min, 30 cycles of 95 ºC for 30 s, 58 ºC for 30 s, and 72 ºC for 60 s, followed by 1 cycle at 72 ºC for 3 min and holding stage at 4ºC. All primers are shown in Table 1. The PCR-amplified products were analyzed by 2% agarose (MBG, Fischer Scientific, USA) gel electrophoresis and stained with

Table 1

Primers for carbapenemase genes

Primer name	Sequence 5'–3'
*bla*KPC Fw	ATGTCACTGTATCGCCGTCT
*bla*KPC Rw	TTTTCAGAGCCTTACTGCCC
*bla*VIM Fw	GATGGTGTTTGGTCGCATA
*bla*VIM Rw	CGAATGCGCAGCACCAG
*bla*NDM Fw	GGGCAGTCGCTTCCAACGGT
*bla*NDM Rw	GTAGTGCTCAGTGTCGGCAT
*bla*IMP Fw	GGAATAGAGTGGCTTAATTCTC
*bla*IMP Rw	CCAAACCACTACGTTATCT
*bla*OXA-48 Fw	TTGGTGGCATCGATTATCGG
*bla*OXA-48 Rw	GAGCACTTCTTTTGTGATGGC

KPC – *Klebsiella pneumoniae* carbapenemases; VIM – Verona-inkgron encoded metallo-beta-lactamases; NDM – New Delhi metallo-beta-lactamases; IMP – imipenemases; OXA-48 – oxacillinases-48.

ethidium bromide. Images were documented by a BioDocAnalyze system (Biometra, Germany).

The statistical analysis of the results was performed using the Statistical Package for the Social Sciences (SPSS), version 20. Results were expressed through the descriptive statistics, as simple frequencies and percentages. The χ^2 test was used for determination of statistically significant differences. The tested significance level was $\alpha = 0.05$.

Results

The study included 129 isolates of multidrug-resistant *K. pneumoniae* and *E. coli* isolated from a different clinical specimen of hospitalized patients. There were 121 isolates of *K. pneumoniae* and 8 isolates of *E. coli*. The patients from whom the isolates were obtained included 69 (53.5%) males and 59 (45.7%) females. The patients' mean age was 53 (SD ± 24) years. According to the location in hospital in the moment when the sample was taken 71 (55%) were collected from non-intensive care units and 58 (45%) were taken from intensive care units. The distribution of clinical specimen is shown in Table 2.

Table 2

Clinical specimen used for obtaining carbapenemase-producing isolates

Clinical specimen	n	%
Urine	70	54.2
Blood	26	20.2
Wound secretion/swab	19	14.7
Lower respiratory tract secretions	14	10.9
Total	129	100.0

The antimicrobial resistance patterns of collected isolates are presented in Table 3.

Susceptibility to carbapenems of *K. pneumoniae* and *E. coli* is shown separately in Table 4.

Using PCR carbapenemase genes were detected in 58 (45%) isolates. Gene bla_{NDM} was detected in 40 (31%) isolates, bla_{OXA-48} in 10 (7.8%), bla_{KPC} in 1 (0.8%) isolate. Seven (5.4%) tested strains were positive for both bla_{OXA-48} and bla_{NDM}. Genes bla_{VIM} and bla_{IMP} were not detected in tested isolates. Types of carbapenemase genes found in both *K. pneumoniae* and *E. coli* are shown in Table 5.

Table 3

Antimicrobial susceptibility of collected *Klebsiella pneumoniae* and *Escherichia coli* isolates

Tested antibiotic	S%	I%	R%
Ampicillin	0	0	100
Amoxicillin	0	0	100
Amoxicillin with clavulanate	0	0	100
Piperacillin	0	0	100
Cefazoline	0	0	100
Cefuroxime	0	0	100
Ceftriaxone	0	0	100
Ceftazidime	0	0	100
Ciprofloxacin	6.2	0	93.8
Levofloxacin	6.2	0	93.8
Cefepime	0	7	93
Cotrimoxazole	9.3	0	90.7
Piperacillin-tazobactam	0.8	8.5	90.7
Gentamicin	10.9	0	89.1
Amikacin	40.3	26.4	33.3
Tigecycline	82.9	5.4	10.9
Fosfomycin	90.7	0	9.3
Colistin	96.1	0	3.1

Table 4

Susceptibility of tested isolates to carbapenems

Antibiotics	*Klebsiella pneumonia* n (%)	*Escherichia coli* n (%)
Imipenem		
S	67 (55.4)	1 (12.5)
I	5 (4.1)	1 (12.5)
R	49 (40.5)	6 (75)
Meropenem		
S	47 (38.8)	21 (12.5)
I	16 (13.2)	0 (0)
R	58 (47.9)	7 (87.5)
Ertapenem		
S	0	0
I	13 (10.7)	1 (12.5)
R	108 (89.3)	7 (87.5)

S – sensitive; I – intermediate; R – resistant.

Phenotypic tests for ESBL producers and carbapenemase producers were performed in order to enlighten the beta-lactam resistance mechanism of carbapenem-resistant strains. The results are presented in Table 6.

All except one isolate with positive DDST suggested the presence of metallo-beta lactamases. Carbapenemase ge-

Table 5

Carbapenemase genes detected in *K. pneumoniae* and *E.coli*

Carbapenemase genes	*K. pneumoniae*	*E. coli*
	n (%)	n (%)
bla_{KPC}	1 (0.8)	0
bla_{NDM}	33 (27.3)	7 (87.5)
bla_{OXA-48}	10 (8.3)	0
bla_{OXA-48} and bla_{NDM}	7 (5.8)	0
No genes detected	70 (57.8)	1 (12.5)

KPC – *K. pneumoniae* carbopenemases; OXA-48 – oxacillinases; NDM – New Delhi metallo-beta-lactamases.

Table 6

Relation between phenotypic tests and carbapenemase genes detection

Phenotypic testing	Carbapenemase genes detected	No genes detected	Total
CDT for ESBL-P	0	39	39
DDST for CP	41 (89.1%)	5 (10.9%)	46
Negative	17 (38.6%)	27 (61.4%)	44
Total	58	71	129

***CDT for ESBL-P – combined disk test for extended beta-lactamase production; DDST for CP – double-disk synergy test for carbapenemase production.**

nes were detected in 41 (89.1%) DDST-positive isolates. Among strains negative for phenotypic testing 17 (38.6%) carried carbapenemase genes, bla_{OXA-48} was found in 10 and both bla_{OXA-48} and bla_{NDM} in 7 strains.

Among 58 isolates with carbapenemase-encoding genes, 52 (89.7%) were resistant to imipenem. All 58 isolates were resistant to meropenem and ertapenem (Figure 1).

Among 71 isolates without carbapenemase-encoding genes, 3 (4.2%) were resistant to imipenem and 7 (9.9%) were resistant to meropenem.

According to the hospital location in the moment of sampling 37 (63.8%) carbapenemase-producing isolates were collected from patients treated in intensive care units and 21 (29.6%) from other wards ($\chi^2 = 15.848$; $p = 0.007$).

Discussion

K. pneumoniae and *E. coli* are frequently responsible for numerous community and hospital acquired infections [15]. Although originally being human commensals susceptible to almost all antimicrobial agents, in last 15 years we witness a rapid dissemination of multidrug-resistant strains [16]. Resistance to carbapenems is usually a consequence of the acquisition of carbapenemase genes. Being mostly plasmid-encoded, carbapenemase-producing enterobacteria are often accompanied by other resistance genes [15]. The horizontal genetic transfer may cause rapid and extensive dissemination of multidrug-resistant carbapenemase-producing strains not only in healthcare facilities but also within the region or even across borders [17].

On the other hand, carbapenemase-producing enterobacteria are not necessarily clinically resistant to carbapenems thus representing a diagnostic challenge to routine laboratories. Usually, recommendations are either based on epidemiological cutoff values of minimal inhibitory concentrations or clinical breakpoints for carbapenems. It is advised that screening criteria may and should be adjusted depending on the epidemiological situation in a given ecological setting [16]. So far, there have been no comprehensive studies considering the presence and the occurrence of carbapenemase production in enterobacteria in Serbia. Their presence may be missed if different criteria are followed, especially by laboratories lacking the experience in interpreting and performing phenotypic tests.

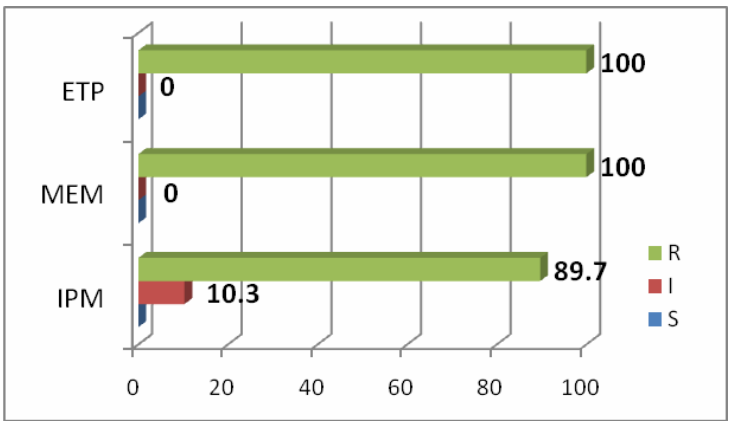

Fig. 1 – Susceptibility to carbapenems of the isolates with carbapenemase encoding genes.
ETP – ertapenem; MEM – meropenem; IPM – imipenem; R – resistant; I – intermediate; S – sensitive.

A total of 129 multidrug-resistant strains with lower susceptibility to routinely used carbapenems was collected in 6 months period from various hospitals in Serbia. *K. pneumoniae* and *E. coli* were isolated mostly from urine and less frequently from blood, wound secretion or swab and lower respiratory tract secretions. Resistance rates for 13 tested antimicrobial agents were very high. Good activity maintained for amikacin, fosfomycin, tigecycline, and colistin.

Collected strains were all intermediately susceptible or resistant to ertapenem. Ertapenem is considered the most sensitive indicator for carbapenemase production, though often with low specificity due to either production of ESBL or overproduction of AmpC beta-lactamases [18]. In our study, none of solely ertapenem intermediate or resistant isolates harbored carbapenemase-encoding genes nor were positive in phenotypic testing for carbapenemase production. All isolates with carbapenemase genes were resistant to meropenem, suggesting that meropenem susceptibility might be an indicator for carbapenemase production among *K. pneumoniae* and *E. coli* in Serbia.

After performing phenotypic testing, 46 strains were suspected for carbapenemase production and 41 carried tested carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}). Positive phenotypic test in 5 isolates that tested negative for carbapenemase genes indicated the presence of metallo-beta lactamase. Additional testing is needed to detect the type of metallo-beta lactamase.

Phenotypic tests for carbapenemase production failed to detect 17 isolates with carbapenemase genes. There are no specific inhibitors for OXA-48 carbapenemase commercially available, therefore 7 strains would be missed without molecular testing. Also, phenotypic tests for carbapenemase production are unreliable when two different mechanisms of carbapenemase production occur [19].

More isolates with carbapenemase production were found among samples collected in intensive care units compared to other wards. Together with various risk factors such as age of patients, underlying illness and co-morbid conditions of the host, the intensive care unit stay and carbapenem resistance are the most important predictors of increased mortality and treatment failure [16].

Carbapenemase genes were detected in 58 isolates. The most prevalent carbapenemase was NDM found in both *K. pneumoniae* and *E. coli*. NDM producing strains are found in 9 hospitals from 7 different cities in Serbia. NDM was first identified in 2008 from *K. pneumonia* in Sweden from a patient treated in India [20] and has been reported worldwide [4]. India is considered to be endemic although after the first comprehensive analyses a smaller percentage of cases were connected to Balkan countries [4]. However, the most Balkan countries were lacking data or were uncertain considering the occurrence of carbapenemases among enterobacteria [6]. In Serbia, NDM was detected for the first time in *Pseudomonas aeruginosa* in 2010 [21]. In 2011 NDM was detected for the first time in *K. pneumoniae* in Belgrade isolated from urine of a 7-month-old baby boy, though without any clinical signs of infection [22]. As for Bulgaria, an outbreak caused by NDM producing *E. coli* was reported [23], but also VIM and KPC producing *K. pneumoniae* were documented showing the diversity of circulating carbapenemases [24]. In Croatia, VIM producing enterobacteria were the most prevalent but NDM producing strains were isolated [25]. In Greece, KPC and VIM reached epidemic proportions [6, 26]. No available data were found considering Albania, Bosnia and Herzegovina and the Republic of Macedonia. NDM producing enterobacteria originated from Montenegro and Kosovo, Serbia was reported in Belgium [27]. Also, NDM and OXA-48 carbapenemase were found in carbapenem-resistant enterobacteria in the neighboring Romania [28]. In general, NDM did not reach such a wide distribution in Europe as KPC according to data from 2013 [6]. United Kingdom has been reporting more NDM isolates comparing to other European countries [17]. Although an outbreak caused by metallo-lactamase producing *Proteus mirabilis* (VIM, IMP) in surgical intensive care unit of Clinical Center Serbia, Belgrade was described [29], none of tested isolates carried bla_{VIM} nor bla_{IMP} genes.

OXA-48 was first detected in *K. pneumoniae* in Turkey followed with hospital outbreaks across the country [30]. Among European countries, OXA-48 was the most frequently detected in Belgium, France and Malta [6]. OXA-48 producing *K. pneumoniae* was reported in Slovenia [31], but no OXA-48 carbapenemase was found in a multicentre study in Croatia [25]. Recently, in Hungary, two isolates of *K. pneumoniae* harboring OXA-48-like carbapenemases were characterized [32]. In our study OXA-48 carbapenemase was found in 10 *K. pneumoniae* isolates obtained from 4 different healthcare facilities from 3 different cities (Belgrade, Niš and Kikinda).

Co-production of OXA-48 and NDM in *K. pneumoniae* is rarely reported. According to published data, both OXA-48 and NDM were found in *K. pneumoniae* isolates in Tunisia [33], Morocco [34] and Turkey [35]. An extensively drug-resistant isolate of *K. pneumoniae* with both OXA-48 and NDM obtained from a rectal swab of a patient transferred from the intensive care unit of a hospital located in Belgrade (Serbia) to Bern University Hospital in Switzerland was described [36]. Among collected isolates, both OXA-48 and NDM were found in 7 *K. pneumoniae* isolates. Isolates were obtained from patients hospitalized in 2 healthcare facilities in Belgrade from different clinical specimens (urine, wound secretion and blood).

The first KPC producing *K. pneumoniae* was identified in 1996 in the eastern part of the United States and has been spreading since [7]. KPC is the most frequently detected carbapenemase among *Enterobacteriaceae* in Europe particularly in Italy and Greece [6]. KPC harboring *K. pneumoniae* has been isolated in the neighboring Hungary [37] and Croatia [25]. In our study, KPC carbapenemase was detected in only one isolate of *K. pneumoniae* from the patient treated in the intensive care unit in the Institute for Pulmonary Diseases of Vojvodina near Novi Sad without previous hospitalization. As far as we know, KPC production was detected for the first time in Serbia.

Conclusion

To our knowledge, this is the most comprehensive national report on carbapenemase-producing enterobacteria in Serbia. NDM carbapenemase is the most prevalent among isolates of *K.*

pneumoniae and *E. coli.* Also, rarely described co-production of OXA-48 and NDM is found in *K. pneumoniae* isolates. KPC production is documented for the first time. Further characterization of detected genes as well as further detailed epidemiological studies are needed. It is of great importance to make a unique and precise guideline for routine microbiology laboratories in order to detect carbapenemase-producing strains adequately and to monitor the epidemiological situation on the national and international level.

Acknowledgement

The authors would like to express their deepest gratitude to Snežana Jovanović from Clinical Center Serbia (Belgrade), Ana Perućica, Clinical Center "Zvezdara" (Belgrade), Teodora Vitorović from Clinical Center "Dragiša Mišović" (Belgrade), Tatjana Bošković from Institute for Public Health Čačak (Čačak), Zorka Grbić from Institute for Public Health Kikinda (Kikinda), Sanja Zornić from Clinical Center Kragujevac (Kragujevac), Vesna Karanović from Institute for Public Health Kraljevo (Kraljevo), Anka Vukelić from Institute for Pulmonary Diseases of Vojvodina (Novi Sad), Branislava Kocić from Institute for Public Health Niš (Niš) for their contribution to the isolate collection. We gratefully thank Vesna Kukučka from Institute for Public Health (Novi Sad) for her valuable help in laboratory work.

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Received on September 17, 2015.
Revised on November 22, 2015.
Accepted on February 9, 2016.
Online First September, 2016.