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Prevalence study on carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in Czech hospitals – results from Czech Part of European Survey on Carbapenemase--Producing Enterobacteriaceae (EuSCAPE)

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ABSTRACT

Objective: One of the most important threats of current medicine is the spread of multiresistant Gram-negative bacteria. We report here data from a six-month prevalence study on carbapenemase-producing *K. pneumoniae* and *E. coli* performed in Czech hospitals participating on European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE).
Methods: Ten hospitals covering all regions of the Czech Republic were selected. During the study period (1st November 2013 to 30th April 2014), first ten carbapenem non-susceptible isolates of *K. pneumoniae* or *E. coli* isolated from non-surveillance specimens (i.e., blood, lower respiratory tract secretions, urine, puncture fluids, and wound secretions) of single successive patients were collected. Successive carbapenem-susceptible isolates of the same species were also preserved as controls. Susceptibility to 15 antibiotics was determined using EUCAST recommendations. Carbapenemase activity was detected by MALDI-TOF MS meropenem hydrolysis assay. Positive isolates were subjected for molecular typing (multi-locus sequence typing, identification of carbapenemase gene).
Results: During the study period, thirty non-susceptible isolates (*K. pneumoniae* n = 28, *E. coli* n = 2) were identified in 5 hospitals. Only two of them were confirmed to be carbapenemase producers. A NDM-1-producing *K. pneumoniae* ST11 was recovered from a patient, transferred from Ukraine, being injured during a Maidan revolution. The second isolate, an OXA-48-producing K. pneumoniae, belonging to ST101, was recovered from a patient admitted to a hospital for an ischemic stroke.
Conclusions: This study again confirmed that the Czech Republic still belongs to the countries with low prevalence of carbapenemase-producing Enterobacteriaceae (CPE). Cases of CPE are usually restricted to an import from high-prevalence countries or countries with unknown epidemiological situation.

KEYWORDS

carbapenemase – CPE – MALDI-TOF – susceptibility – resistance – *Enterobacteriaceae*

SOUHRN

Hrabák J., Študentová V., Jakubů V., Adámková V., Dvořáková L., Balejová M., Bergerová T., Chmelařová E., Ježek P., Kabelíková P., Kolář M., Paterová P., Tejkalová R., Papagiannitsis C. C., Žemličková H.: Prevalenční studie kmenů *Escherichia coli* a *Klebsiella pneumoniae* produkujících karbapenemázu v českých nemocnicích – výsledky české části studie EuSCAPE (European Survey on Carbapenemase-Producing Enterobacteriaceae)
Cíl: Jedním z faktorů ohrožujících současnou medicínu je šíření multirezistentních gramnegativních bakterií. V článku jsou shrnuty výsledky 6měsíční prevalenční studie zaměřené na kmeny *K. pneumoniae* a *E. coli* provedené v českých nemocnicích v rámci projektu EuSCAPE (European Survey on Carbapenemase-Producing Bacteria).

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Metodika: Do studie bylo zapojeno 10 nemocnic ze všech krajů České republiky. Během sledovaného období (1. listopad 2013 až 30. duben 2014) bylo shromažďováno deset prvozáchytů *K. pneumoniae* a *E. coli* necitlivých ke karbapenemům z klinických vzorků (krev, dolní cesty dýchací, moč, punktát, stěry z ran). Zároveň byl deponován první citlivý izolát zachycený od téhož pacienta a vybraného bakteriálního druhu. Byla stanovena citlivost k 15 různým antibiotikům podle metodiky EUCAST. Karbapenemázy byly identifikovány metodou MALDI-TOF MS hydrolýzy meropenemu. Kmeny produkující karbapenemázy byly podrobeny multilokusové sekvenační typizaci a byly identifikovány geny karpapenemáz.
Výsledky: Během sledovaného období bylo shromážděno 30 izolátů (*K. pneumoniae* n = 28, *E. coli* n = 2) necitlivých ke karbapenemům. U pacienta přeloženého z Ukrajiny po zranění během revoluce na Majdanu byl identifikován sekvenční typ (ST) 11 *K. pneumoniae* produkující karbapenemázu NDM-1. Druhý izolát produkující karbapenemázu produkoval enzym OXA-48. Jednalo se o kmen K. pneumoniae ST101 identifikovaný u pacienta přijatého z komunity.
Závěr: Tato prevalenční studie opět potvrdila nízkou prevalenci enterobakterií produkujících karbapenemázy (CPE) v České republice. Zároveň opět ukazuje na riziko spojené s importem CPE ze zemí s vysokou prevalencí nebo neznámou epidemiologickou situací..

KEYWORDS

karbapenemázy – CPE – MALDI-TOF – citlivost – rezistence – *Enterobacteriaceae*

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INTRODUCTION

One of the most important antibiotic resistance mechanisms in *Enterobacteriaceae* is production of carbapenemases. Those enzymes are mostly able to hydrolyse almost all β-lactams, including carbapenems. Their genes are usually encoded on mobile genetic elements, (e.g., plasmids) encoding other resistance determinants such as aminoglycoside-modifying enzymes, gyrase/topoisomerase-protecting proteins (Qnr) etc. Genes of metallo-β-lactamases, a diverse group of carbapenemases, are commonly found as gene cassettes of integrons resulting in multiresistant phenotype of the strain. Some carbapenemase genes are closely connected to epidemiologically successful clones (e.g., KPC-producing *Klebsiella pneumoniae* ST258/ST512) or epidemiologically successful plasmids with high conjugation potential (e.g., blaOXA-48-type connected with IncL/M plasmids) [1, 2, 3].

In the Czech Republic, occurrence of carbapenemase-producing *Enterobacteriaceae* (CPE) has been reported low, restricted on cases identified in patients repatriated from countries with high CPE prevalence (e.g., Greece, Italy, Sri Lanka) and sporadic outbreaks [4, 5, 6]. In some European countries, however, epidemiological situation seems to be alarming (e.g., Greece and Italy) with more than 30% carbapenem-resistant *K. pneumoniae* invasive isolates reported annually to the EARS-Net database [7]. In patients infected by a CPE, treatment options are usually limited to only few choices (e.g., colistin, combination therapy) with unpredicted outcome [8].

In April of 2010, the first meeting funded by European Centre for Disease Prevention and Control (ECDC) held in Netherlands' National Institute for Public Health and the Environment (RIVM). As a result from that workshop, the first position article describing epidemiological situation in participated countries was published [9]. Followed that initiative, European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) was established. The Czech Republic belongs to the members, that have participated on EuSCAPE activities since its establishment in 2010.

Based on national data, a surveillance article describing the progress in the spread of CPEs in Europe was

Table 1. Number of isolates included in the study collected from each hospital and characteristics of the region/hospitalFigure 1. Distributions of hospitals participated on EuSCAPE project in the Czech Republic

Tabulka 1. Počet izolátů zahrnutých ve studii identifikovaných v jednotlivých nemocnicích včetně charakteristiky spádové oblasti

Hospital	Estimated catchment population	Number of beds	Admissions per year (2013)	Average bed occupancy (2013) [%]	Average length of stay per admission (2013)	Number of non-susceptible isolates[1]	Number of CPEs (type)
České Budějovice Hospital	637 000	1 175	52 792	71,9	6,10	1	0
General University Hospital in Prague	1 247 000	1 539	50 907	74,8	5,10	9	0
Masaryk's Hospital in Ústí nad Labem	828 000	2 840	127 672	66,9	4,97	0	-
Regional Hospital in Příbram	250 000	255	18 659	70,5	6,20	0	-
St. Anna University Hospital	1 167 000	913	27 230	77,7	8,63	0	-
University Hospital in Hradec Kralove	554 000	1 500	40 957	73,0	8,50	5	0
University Hospital in Motol	1 247 000	2 316	76 000	75,0	6,10	10	1 (NDM-1)
University Hospital in Olomouc	639 000	1 184	47 593	74,5	6,10	0	-
University Hospital in Ostrava	1 231 000	1 187	45 872	78,1	6,50	0	-
University Hospital in Plzeň	572 000	1 689	63 136	74,0	7,48	5	1 (OXA-48)

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Figure 1. Distributions of hospitals participated on EuSCAPE project in the Czech Republic

Obr. 1. Distribuce nemocnic zúčastněných v projektu EuSCAPE v České republice

published in collaboration with national EuSCAPE representatives in 2013 [7]. The data suggest that except few countries with high rate of KPC- and VIM-producing isolates and silent spread of OXA-48-type carbapenemases, the situation seems to be stabilized. As reported by most European countries (including the Czech Republic), national guidelines for detection, verification and for management of colonized/infected patient are available. We report here data from a prevalence study on carbapenemase-producing *K. pneumoniae* and *Escherichia coli* performed in 10 Czech hospitals between 1st November 2013 and 30th April 2014. Similar study was performed in all European countries participating on EuSCAPE project (n = 39).

MATERIALS AND METHODS

Study Design

Ten hospitals (see Table 1, Figure 1) covering all regions were selected. During the study period (1st November 2013 to 30th April 2014), first ten carbapenem non-susceptible isolates of *K. pneumoniae* or *E. coli* recovered from single successive patients had been collected. Selected clinically-significant isolates were included in the study (i.e., blood, lower respiratory tract secretions, urine, puncture fluids, and wound secretions). No surveillance or screening isolates were included (e.g., stool, upper respiratory tract). Non-susceptibility was defined according to the epidemiological cut-off value (ECCOF) of *K. pneumoniae* of at least one carbapenem (i.e., ertapenem MIC > 0,064 mg/l, meropenem MIC > 0,125 mg/l, imipenem MIC >1 mg/l, or inhibition zone diameter [IZ] for ertapenem < 29 mm, meropenem < 25 mm, and imipenem < 24 mm respectively). Carbapenem-susceptible isolates (a wild-type) of the same species and the same patient identified were also preserved as carbapenem-susceptible control group.

Species Identification and Susceptibility Testing

Each isolate sent to the Department of Microbiology, Faculty of Medicine in Plzen, Charles University in Prague was re-identified using MALDI-TOF mass spectrometry (MS), MALDI Biotyper version 3.1 (Bruker Daltonik GmbH, Bremen, Germany). Susceptibility to ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cefepime, aztreonam, ciprofloxacin, gentamicin, tobramycin, co-trimoxazole, piperacillin/tazobactam was determined by disk diffusion method and interpreted using EUCAST recommendations [10]. Minimal inhibitory concentrations (MICs) of ertapenem, meropenem, imipenem, colistin and fosfomycin, were examined using E-test (bioMérieux) and categorized according to the EUCAST criteria as well [10].

Detection of β-Lactamases

Carbapenemase activity was detected using MALDI-TOF MS meropenem hydrolysis assay [11, 12] with recently described modification [13]. In that modification, reaction buffer is supplemented by ammonium bicarbonate to enhance sensitivity against OXA-48-type carbapenemases [13]. Carbapenemases were preliminary identified using an inhibitor-based phenotypic method [14]. Precise identification was performed by PCR amplification followed by sequencing of respective carbapenemase gene [14].

Typing of the Isolates

Carbapenemase-producing isolates were subjected for multi-locus sequence typing (MLST) as previously described [15]. Sequence types (STs) were assigned at the *K. pneumoniae* MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).

RESULTS

During the study period, thirty-nine isolates were collected (*Klebsiella pneumoniae* n = 37, *Escherichia coli* n = 2). Thirty, including both *E. coli* isolates, were non-susceptible to at least one carbapenem. Seven isolates were collected as negative controls. Carbapenem non-susceptible isolates were identified in 5 of 10 hospitals included in the study. All of those five hospitals were tertiary-care university settings providing a specialized care (including hematooncology wards). Description of the hospitals is summarized in Table 1.

Among the non-susceptible isolates, only two *K. pneumoniae* isolates were confirmed as carbapenemase producers. In the University Hospital in Motol, *K. pneumoniae* ST11 producing NDM-1 metallo-β-lactamase was recovered from a wound of 51 years old man transferred from Ukraine being injured during Maidan revolution. Based on the Czech National Guideline [16], the patient was isolated immediately after the admission to the hospital and screened for the presence of CPE. Therefore, no transmission to other patients occurred.

The second isolate, a ST101 *K. pneumoniae* producing OXA-48 carbapenemase was recovered from a bronchoalveolar lavage of a 39 years old man hospitalized for an ischemic stroke. The isolate was identified immediately after patient's admission (hospitalization in ICU). The patient died on the 5th day of hospitalization. Because of an isolation of that patient immediately after identification of carbapenem non-susceptible isolate and screening of the ICU patients for 4 weeks according to the Czech National Guideline [16], no spread of the OXA-48-producing isolate has been observed.

Resistance of the other carbapenem-non-susceptible isolates (n = 28) was caused by an alteration of a cell-wall permeability together with a production of β-lactamases with no activity against carbapenems (competitive inhibition).

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DISCUSSION

Occurrence of CPEs in Europe varied from sporadic occurrence (e.g., Denmark, Latvia, Lithuania, Slovenia) to the countries with endemic situation (e.g., Greece, Italy) [7]. The majority of European countries, including the Czech Republic, are categorized as regions with single/sporadic hospital outbreaks [7]. Since 2010, when a complex European study on CPEs was performed [9], in almost all countries an increase of CPEs occurrence has been observed. Only in Israel, which was included in the study as well, the situation changed from an epidemiological stage "endemic" to "inter-regional spread" [7].

This study again confirmed that the Czech Republic still belongs to the countries with low CPE prevalence. During the study period, we identified only two carbapenemase-producing *K. pneumoniae* isolates. In 2011, only three hospital out-breaks (VIM-1-producing *K. pneumoniae* ST11, KPC-3-producing *K. pneumoniae* ST512, KPC-2-producing *K. pneumoniae* ST258) and two sporadic cases (VIM-4--producing *K. pneumoniae,* KPC-2-producing *K. pneumoniae* ST258) were observed [5]. In period that followed, occurrence of CPEs in the Czech Republic remains the same, restricted to few hospital outbreaks and sporadic imported cases [6, 17]. In Czech Republic, current epidemiological situation may be probably caused by strict surveillance (included a selective screening and isolation procedures) in patients repatriated from foreign countries or those in a risk with a contact with patient infected/colonized by CPE as it is recommended by Czech Ministry of Health [16]. Interestingly, the OXA-48-producing *K. pneumoniae* identified at the University Hospital in Plzen was recovered from a community patient. This finding suggests a potential unnoticed spread of this β-lactamase in community as described in other countries [2, 18]. Another remarkable finding was that the NDM-1-producing *K. pneumoniae* was recovered from an Ukrainian patient. To our knowledge, this is the first description of carbapenemase-producing *Enterobacteriaceae* in Ukraine. Unfortunately, there is no epidemiological data focusing on resistance in this European country. As many people from Ukraine works in EU countries, this may represent a potential risk for public health as it has also been described for other serious pathogens (HIV, Mycobacterium tuberculosis) [19, 20].

Those findings, however, have demonstrated the necessity of the detection of carbapenemase-producing bacteria in routine microbiological laboratories. Even if the epidemiological situation in the Czech Republic seems to be optimistic, unnoticed spread of CPEs may represent serious problem for public health. Based on EU level, surveillance recommendations have been introduced in many European countries as well as a Risk Assessment has been published by European Centre for Disease Prevention and Control based on EuSCAPE project results [21].

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