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SHORT COMMUNICATION

KPC-2-producing *Klebsiella pneumoniae* ST11 in a Children's Hospital in Poland

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Abstract

Four *Klebsiella pneumoniae* isolates from children hospitalized over 10 months in an intensive care unit in a children's teaching hospital in Poland were analyzed. All of the isolates belonged to a single pulsotype and sequence type (ST) 11, and produced the KPC-2 carbapenemase and extended-spectrum β-lactamase (ESBL) CTX-M-15. They were resistant to a variety of antimicrobials, and their β-lactam resistance patterns were typical for KPC producers. This is one of few cases of identification of KPC (or carbapenemase)-producing *K. pneumoniae* in a pediatric center in Poland.

Key words: carbapenemase, epidemiology, KPC-2 producing *K. pneumoniae* ST11

In recent years resistance to carbapenems has become a matter of the highest concern in medicine of bacterial infections (Bush, 2010; Nordmann and Poirel, 2014). It has been associated largely with carbapenemase-producing *Enterobacteriaceae* which express various carbapenem-hydrolyzing enzymes, including *Klebsiella pneumoniae* carbapenemases (KPCs) (Munoz-Price *et al.*, 2013; Nordmann and Poirel, 2014). KPCs hydrolyze virtually all β-lactams of clinical use (Mehta *et al.*, 2015), and are produced by many species, predominantly by *K. pneumoniae*. Strains with KPCs disseminate rapidly and cause outbreaks; since the late 1990s these have spread in the United States, followed by Israel from 2005, and then worldwide (Munoz-Price *et al.*, 2013; Nordmann and Poirel, 2014). In large part, this has been due to clonal expansion of *K. pneumoniae* strains belonging to the sequence type (ST) 258 and related clones, forming the clonal group (CG) 258 (Chen *et al.*, 2014; Mathers *et al.*, 2015). bla_{KPC} genes, mainly bla_{KPC-2} or bla_{KPC-3}, are carried by Tn*4401*-like transposons of some structural polymorphism (Naas *et al.*, 2008; Baraniak *et al.*, 2015). Most KPC-producing bacteria also express other β-lactamases and contain genes conferring resistance to other antimicrobials, such as aminoglycosides, fluoroquinolones or co-trimoxazole (Nordmann and Poirel, 2014). Therefore, infections caused by multidrug-resistant KPC producers have scarce treatment options and are associated with high mortality rates (Tumbarello *et al.*, 2015).

KPC-2-producing *K. pneumoniae* ST258 emerged in Poland in 2008 (Baraniak *et al.*, 2009) and by the end of 2009 it caused a large outbreak in Warsaw and its region, Mazowieckie (Baraniak *et al.*, 2011). At the same time sporadic cases of other STs of *K. pneumoniae* with KPC-2, namely ST11 and ST23, were observed in Warsaw and Kielce, respectively. All bla_{KPC-2} genes identified were located within the Tn*4401*a transposon variant. In 2010–2014 still the most affected region was Mazowieckie but new KPC outbreaks occurred in four other areas: Świętokrzyskie, Lubelskie, Podlaskie and Śląskie (Baraniak *et al.*, 2017). The outbreak organisms were *K. pneumoniae* ST258 or ST512 and they produced KPC-3 encoded by Tn*4401*a or Tn*4401*b elements. According to the National Reference Centre for Susceptibility Testing (NRCST) data, since 2014 the situation has had a tendency to stabilize at a low prevalence level in most of the regions mentioned above (D. Żabicka, A. Baraniak, M. Gniadkowski, W. Hryniewicz, unpublished data).

In this study four KPC-positive *K. pneumoniae* isolates from children were analyzed. All patients were hospitalized between February and November

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2016 in an intensive care unit (ICU) in a large tertiary pediatric center in Kraków, and included chronologically: a 7-years-old female with aspiration pneumonia, a 2-months-old male with respiratory distress syndrome, a 9-months-old female with multiple organ failure, and a 6-years-old female with pneumonia. The *K. pneumoniae* isolates were recovered mainly from tracheal or bronchial aspirates; one isolate, 2214/16, was cultured from blood. The isolates were identified by the hospital microbiology laboratory, using the Phoenix system (BD Biosciences), and owing to carbapenem resistance these were sent to the NRCST in Warsaw for reference diagnostics and surveillance purposes. The NRCST has confirmed carbapenem resistance by the Carba NP test (Nordmann *et al.*, 2012), and the positive combined disk test with phenylboronic acid (Doi *et al.*, 2008) and PCR (Navon-Venezia *et al.*, 2006) revealed the KPC presence.

The isolates were typed using pulsed-field gel electrophoresis (PFGE) (Seifert *et al.*, 2005), and produced identical DNA banding patterns. The following multilocus sequence typing (MLST) (Diancourt *et al.*, 2005) classified them into ST11 (http://bigsdb.web.pasteur.fr/klebsiella). Amplicons containing the bla_{KPC} genes were digested by the *Rsa*I restriction enzyme (Thermo Scientific) which distinguishes the bla_{KPC-2}- and bla_{KPC-3}-like genes (Lopez *et al.*, 2011). All isolates carried the bla_{KPC-2}-like alleles, which turned out to be bla_{KPC-2} by sequencing performed for the representative isolate 2214/16. A PCR mapping assay that discerns various polymorphs of the Tn*4401* transposon (Naas *et al.*, 2008; Baraniak *et al.,* 2015) demonstrated the presence of the Tn*4401*a variant exclusively. Specific PCRs for major β-lactamase types (Baraniak *et al.*, 2011) allowed detecting additionally ESBLs of the CTX-M-1 group and TEM-like enzymes in all of the isolates, identified by sequencing as CTX-M-15 and TEM-1, respectively, in the isolate 2214/16. Susceptibility of the KPC-producing *K. pneumoniae* was tested by MIC Test Strips (Liofilchem®) and by the broth microdilution method in the case of colistin (http://eucast.org). The results were interpreted according to the EUCAST guidelines (http://eucast.org). The isolates showed multi-drug resistance patterns, with uniform resistance to all β-lactams, gentamicin and ciprofloxacin, susceptibility only to amikacin, tigecycline and colistin (Table I).

K. pneumoniae is a relatively frequent cause of nosocomial outbreaks, including those in neonatal or pediatric wards that are a matter of special concern (Paczosa and Mecsas, 2016). The constantly and rapidly increasing resistance of this pathogen remarkably magnifies the problem. The NRCST data indicates that the carbapenemase-producing multi-drug-resistant *K. pneumoniae* strains have been rarely observed in pediatric centers in Poland so far (D. Żabicka, A. Baraniak, M. Gniadkowski, W. Hryniewicz, unpublished data); therefore, the cases analyzed in this study might signalize the risk of their expansion into these environments. The high genetic relatedness of the four isolates suggests the epidemic character of the KPC infections in the ICU. However, the infection cases were separated in time from each other, and identification of each case was followed by implementation of enhanced infection control measures. It is possible that the outbreak was mediated by unidentified carrier(s) or a hidden environmental source, but the repeated introduction of the KPC-2-producing *K. pneumoniae* ST11 organism cannot be totally excluded either. Since November 2016 to the moment of writing this report (March 2017) no new KPC cases have been recorded in the hospital.

Interestingly, the isolates were not related to any of the outbreak or sporadic KPC-producing *K. pneumoniae* isolates ever studied in detail in Poland so far, predominantly representing various lineages of the CG258 group (Baraniak *et al.*, 2009; 2011; 2017). These comprised two KPC-3-positive ST512 isolates from another hospital in Kraków from 2012 (Baraniak *et al.*, 2017), as well as the only two "Polish" ST11 isolates with KPC-2 from 2009 from Warsaw (Baraniak *et al.*, 2011). ST11 is a truly pandemic *K. pneumoniae* clone, and an evolutionary precursor of ST258 (Chen *et al.*, 2014). It has been identified in many countries with a variety of resistance mechanisms, including diverse β-lactamases, and for example in Poland it has

Table I
Susceptibilities of the *K. pneumoniae* isolates obtained in the study.

Isolate	MIC[1] (μg/ml) of:																
	AMX	AMC	PIP	TZP	CAZ	CTX	FEP	ATM	IMP	MEM	ERT	AMK	GEN	CIP	TET	TGC	CST
2213/16	>256	>256	>256	>256	>256	>256	64	>256	>32	>32	16	4	32	>32	>256	1.5	0.25
2214/16	>256	>256	>256	>256	>256	>256	128	>256	>32	>32	>32	4	32	>32	>256	1.5	0.125
6465/16	>256	>256	>256	>256	>256	>256	128	>256	>32	>32	>32	4	32	>32	>256	1.5	1
6973/16	>256	>256	>256	>256	>256	>256	128	>256	16	>32	>32	4	32	>32	>256	1.5	0.125

[1]Abbreviations: AMC, amoxicillin-clavulanic acid; AMK, amikacin; AMP, ampicillin; AMX, amoxicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; CTX, cefotaxime; ERT, ertapenem; FEP, cefepime; GEN, gentamicin; IPM, imipenem; MEM, meropenem; PIP, piperacillin; TET, tetracycline; TGC, tigecycline; TZP, piperacillin-tazobactam.

been responsible for a spectacular on-going outbreak of New Delhi metallo-β-lactamase (NDM) producers (Baraniak *et al.*, 2016). KPC-producing *K. pneumoniae* ST11 has been identified in several countries, being the predominant KPC producer in China (Qi *et al.*, 2011; Cheng *et al.*, 2016; Hu *et al.*, 2016) and playing a significant role in Spain (Oteo *et al.*, 2016) or Brazil (Pereira *et al.*, 2013; Andrade *et al.*, 2014). The emergence of the KPC-2-producing ST11 in Kraków might be due to a new KPC introduction to Poland; considering the relatively low prevalence of KPCs in the country and especially in Kraków, the on-site transmission of a KPC-encoding plasmid from a CG258 member to ST11 seems to be less likely.

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