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SHORT COMMUNICATION

Probable Interspecies Transfer of the $bla_{\text{VIM-4}}$ Gene between *Enterobacter cloacae* and *Klebsiella pneumoniae* in a Single Infant Patient

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Abstract

We report the interspecies transfer of the $bla_{\text{VIM-4}}$ gene in MBL-producing *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates from a newborn patient who had received meropenem therapy. We show evidence that gene $bla_{\text{VIM-4}}$ was transmitted as a part of the class-1 integron on a ca. ~90 kb conjugative plasmid. High homology of nucleotide sequence was observed between the integron found in VIM-4 producing *E. cloacae* and *K. pneumoniae* strains tested and class-1 integrons previously reporteded in *Pseudomonas aeruginosa* from Hungary and Poland. This finding may suggest *P. aeruginosa* as a potential source of acquired VIM-4 in *Enterobacteriaceae.*

Key words: *Enterobacteriaceae*, *Enterobacter cloacae*, *Klebsiella pneumonia*, MBL, VIM-4

Carbapenemase-producing bacteria are an increasing international public health threat (Canton *et al.*, 2012). Metallo-β-lactamases (MBLs), which confer resistance to carbapenems have been detected worldwide in Gram-negative non-fermenting bacteria. More recently, MBLs became frequently reported among *Enterobacteriaceae*, in which these enzymes are encoded by genes located in transferable plasmids (Vatopoulos, 2008). Besides NDM (New Delhi MBL), the VIM-type (Verona integron-encoded MBLs) enzymes are the most important MBLs for epidemiological dissemination and clinical relevance. After the first detection of plasmid acquired VIM-4 in *Pseudomonas aeruginosa* in Greece in 2001 (Pournaras *et al.*, 2002), VIM enzymes have been soon reported in many countries, including Poland, where VIM-2 and VIM-4 producing *Pseudomonas* spp. were observed (Fiett *et al.*, 2006; Patzer *et al.*, 2009). To the best of our knowledge, the first case of VIM-1 group producing *Klebsiella pneumoniae* in Poland was reported in 2010 by Sękowska from a 61 year-old patient admitted at a Teaching Hospital in Bydgoszcz, Poland (Sękowska *et al.*, 2010a, 2010b). Until now, VIM-1, VIM-4 and VIM-35-producing *Enterobacteriaceae* were detected in our country (Castenheira *et al.*, 2014).

Herein, we report VIM-4-producing *Enterobacter cloacae* and *K. pneumoniae* strains isolated in 2010 from newborn patient. We also show evidence arguing for the interspecies transmission of a conjugative plasmid carrying the $bla_{\text{VIM-4}}$ gene located as a part of class 1 integron.

A one month-old male-newborn, that had been hospitalised in an tertiary maternity hospital was admitted to Intensive Care Unit (ICU) of a secondary children hospital in Warsaw, Poland on March 18, 2010 due to prematurity with extremely low birth weight and necrotising enterocolitis. During his stay in the ICU (till July 15, 2010), the newborn-patient received a broad-range antimicrobial-regimen, including meropenem. On 16 and 21 July, 2010, when the patient was at a surgery ward, *E. cloacae* and *K. pneumoniae* isolates with reduced susceptibility to ertapenem were collected. The both isolates were found to produce MBL-type carbapenemase (Table I) as shown by use of the phenotypic

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Table I
Characteristics of VIM-4 producing clinical isolates of *E. cloacae* and *K. pneumoniae* together with the respective *E. coli* electrotransformants.

Strain	Species	Specimen	MBL[b]	MIC[a] (mg l^{-1}) of:											
				CTX	CAZ	FEP	ATM	ETP	IPM	MEM	GEN	AMK	CIP	CST	TGC
ECV	*E. cloacae* (16.07.2010)	wound	+	>256	48	32	64	0.75	0.5	0.19	64	16	1.5	0.5	4
KPV	*K. pneumoniae* (21.07.2010)	stool	+	24	6	1.5	0.047	0.75	0.75	0.38	1	16	0.125	0.38	3
ET	*E. coli* DH5α	NA	–	0.023	0.25	0.016	0.032	0.004	0.125	0.016	0.125	0.25	0.023	0.094	0.38
ET-pEc90	*E. coli* DH5α	NA	+	12	6	0.38	0.047	0.125	0.75	0.38	0.5	12	0.012	0.125	0.38
ET-pKp90	*E. coli* DH5α	NA	+	16	4	0.38	0.032	0.19	0.75	0.125	0.25	6	0.016	0.125	0.38

[a] Abbreviations: AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; CTX, cefotaxime; FEP, cefepime; ETP, ertapenem; GEN, gentamicin; IPM, imipenem; MEM, meropenem; TGC – tigecycline. NA – not applicable.
[b] MBLs were detected as described previously (Franklin *et al.*, 2006; Tato *et al.*, 2010).

Table II
Primers used for PCR mapping and sequencing of *bla*$_{VIM}$ genes and their environment

Primer	Sequence 5'-3'	Reference
VIM-F	ATCATGGCTATTGCGAGTCC	In this study
VIM-R	ACGACTGAGCGATTTGTGTG	
5'-CS	GGCATCCAAGCACAAGC	Zhao *et al.* (2001)
3'-CS	AAGCAGACTTGACTGAT	
VIMA	CAACTCATCACCATCACGGACAAT	In this study
VIMB	GGGGAGCCGAGTGGTGAGTATC	In this study

tests described previously by Franklin *et al.* (2006) and Tato *et al.* (2010). Detection of the *bla*$_{VIM}$ gene and characterization of its environment were performed using conventional PCR mapping and DNA-sequencing with the primers listed in Table II.

The *bla*$_{VIM}$ gene was located on *ca.* ~90kb plasmids named: pEc90 and pKp90, as shown by DNA/DNA Southern-blot with VIM-probe. The Southern-blot was conducted as previously described (Wardak *et al.*, 2007). Both plasmids were separately transferred to *Escherichia coli* DH5α by electro-transformation (Zacharczuk *et al.*, 2011), and MICs of the both transformants were determined (Table II). Restriction endonuclease *Pst*I profiles of the pEc90 and pKp90 from the transformants were indistinguishable. DNA sequencing of the *bla*$_{VIM}$ genes from pEc90 and pKp90 revealed VIM-4 variant. The ability for conjugational transfer of the both plasmids was exhibited, using a conventional liquid-medium mating-test with rifampicin-resistant *E. coli* CSH26 recipient strain. PCR mapping and DNA-sequencing (Zhao *et al.*, 2001) shown the *bla*$_{VIM-4}$ gene was located as a part of the class 1 integron in pEc90 and pKp90. The class-1 integron found in the both plasmids carried two resistance gene-cassettes, where in the first position was the *aac(6')-Ib* gene (also named *aac*A4) followed by *bla*$_{VIM-4}$. Subsequent DNA-sequence analysis using blast-n (NCBI) revealed that the class-1 integron reported herein has 100% homology to integron found in *P. aeruginosa* (access. no. GU181265) from Hungary. Moreover, the class-1 integron reported herein in carbapenemase non-susceptible isolates of *E. cloacae* and *K. pneumoniae*, was probably closely related to the integron from *P. aeruginosa* (access. no. AJ585042) from Poland. The only difference were two point-mutations (T1670G and C1671T).

In conclusion, the evidence collected in our laboratory may strongly argue for the lateral transfer of the *bla*$_{VIM-4}$ gene between *E. cloacae* and *K. pneumoniae* isolates form the same patient. Similar findings were reported in 2002, by Luzzaro and colleagues (Luzzaro *et al.*, 2004), who recovered VIM-4 producing strains of *E. cloacae* and *K. pneumoniae* from a 72-year-old patient that had received 4-weeks of imipenem therapy. Moreover, the nucleotide sequence of the class 1 integron that carries the *bla*$_{VIM-4}$ gene in the both *Enterobacteriaceae* strains described in this paper may suggest *P. aeruginosa* as a potential source of VIM-4.

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