SHORT COMMUNICATION

Probable Interspecies Transfer of the \( \text{bla}_{\text{VIM-4}} \) Gene between \textit{Enterobacter cloacae} and \textit{Klebsiella pneumoniae} in a Single Infant Patient

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Submitted 17 February 2015, revised 28 April 2015, accepted 28 April 2015

Abstract

We report the interspecies transfer of the \( \text{bla}_{\text{VIM-4}} \) gene in MBL-producing \textit{Enterobacter cloacae} and \textit{Klebsiella pneumoniae} isolates from a newborn patient who had received meropenem therapy. We show evidence that gene \( \text{bla}_{\text{VIM-4}} \) 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 \textit{E. cloacae} and \textit{K. pneumoniae} strains tested and class-1 integrons previously reported in \textit{Pseudomonas aeruginosa} from Hungary and Poland. This finding may suggest \textit{P. aeruginosa} as a potential source of acquired VIM-4 in \textit{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-\( \beta \)-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 \textit{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 \textit{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 \textit{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 \textit{Enterobacter cloacae} and \textit{K. pneumoniae} strains isolated in 2010 from newborn patient. We also show evidence arguing for the interspecies transmission of a conjugative plasmid carrying the \( \text{bla}_{\text{VIM-4}} \) 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, \textit{E. cloacae} and \textit{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
tests described previously by Franklin et al. (2006) and Tato et al. (2010). Detection of the blaVIM gene and characterization of its environment were performed using conventional PCR mapping and DNA-sequencing with the primers listed in Table II.

The blaVIM 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 DH5a by electro-transformation (Zacharczuk et al., 2011), and MICs of the both transformants were determined (Table II). Restriction endonuclease PstI profiles of the pEc90 and pKp90 from the transformants were indistinguishable. DNA sequencing of the blaVIM 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 blaVIM-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 aacA4) followed by blaVIM-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 blaVIM-4 gene between E. cloacae and K. pneumoniae isolates from 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 blaVIM-4 gene in the both Enterobacteriaceae strains described in this paper may suggest P. aeruginosa as a potential source of VIM-4.

Acknowledgements
This research was supported by internal-grant from NIZP-PZH (no. 3/EM/2015).
Literature


