

Emergence of VIM-2 metallo- β -lactamase-producing *Pseudomonas aeruginosa* isolates in a paediatric hospital in Serbia

Molecular detection and surveillance of the resistance genes harboured by *Pseudomonas aeruginosa* are becoming increasingly important in assessing and controlling spread and colonization in hospitals, and in guiding the antibiotic treatment of infections. Metallo- β -lactamase (MBL)-producing *P. aeruginosa* strains are slowly but steadily increasing within hospitals, causing outbreaks and/or hyperendemic situations in some centres, mostly in the Far East and the south of Europe (Queenan & Bush, 2007). The global dissemination of MBL-producing *P. aeruginosa* strains has also reached the Balkan region (Lepsanovic *et al.*, 2008; Sardelic *et al.*, 2003). The objective of our study was to detect and characterize *P. aeruginosa* isolates producing MBLs from the 400-bed paediatric tertiary care hospital Mother and Child Health Institute of Serbia 'Dr Vukan Cupic'.

During the 1-year period of sampling, 526 isolates of *P. aeruginosa* were collected from clinical specimens and routine surveillance cultures from humid hospital environments (taps, drains, incubators and ventilatory equipment) since the latter may serve as a reservoir of nosocomial pathogens, as has been recognized in numerous studies (Reuter *et al.*, 2002; Trautmann *et al.*, 2001).

Overall, 163 (31 %) of the 526 *P. aeruginosa* isolates from our hospital were non-susceptible to imipenem. Of those, 148 (90.8 %) had lower susceptibility to meropenem as well. Isolates resistant to carbapenems were most abundant in the paediatric and surgical intensive care units (51.5 %) and in the pulmonology unit

(23.9 %, mainly from cystic fibrosis patients). The vast majority of carbapenem-resistant isolates were sampled from the respiratory tract (76.6 %) and urine (6.1 %) while isolates from other sources each represented under 4 %. Six isolates from the hospital environment were resistant to carbapenems; these represented 46.2 % of all *P. aeruginosa* isolates collected from the hospital environment. Antibiotic susceptibility testing was carried out using the disc diffusion method, according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2009). The highest susceptibility of the 163 imipenem non-susceptible isolates was to polymyxin B and colistin (100 %), followed by levofloxacin (61.3 %), ciprofloxacin (49.7 %), aztreonam (42.3 %), amikacin (36.2 %), ceftazidime (25.2 %), piperacillin-tazobactam (21.5 %), cefepime (20.9 %), tobramycin (17.8 %), piperacillin (17.2 %), ticarcillin-clavulanic acid (17.2 %), gentamicin (15.3 %) and ticarcillin (12.9 %). Among the tested isolates, 29.4 % were pan-resistant since they were resistant to all antibiotics except polymyxins.

Out of the 163 imipenem non-susceptible isolates, 138 (84.7 %) had a positive combined disc test and 58 (35.6 %) had a positive MBL Etest. PCR assay for detection of MBL genes showed that 5/163 isolates (3.1 % of all imipenem-resistant isolates and 0.95 % of all *P. aeruginosa* isolates collected during the study period), all phenotypically MBL-positive in both tests, gave amplicons with primers for the *bla*_{VIM} gene (IMD12, IMD39, IMD58, IMD85 and IMD91) (Table 1). PCRs for *bla*_{IMP}, *bla*_{SPM}, *bla*_{GIM} and *bla*_{SIM} were negative. Contrary to some of the previous studies, our results revealed low specificity and positive predictive value of conventional phenotypic methods for detection of MBL-producers among

imipenem non-susceptible strains.

However, this phenomenon has previously been described for countries with a low prevalence of MBL-positive *P. aeruginosa* strains (Samuelsen *et al.*, 2008). Subsequent DNA sequencing of the *bla*_{VIM} PCR amplicons revealed that all belonged to the *bla*_{VIM-2} type of MBL. Nucleotide sequences of those five *bla*_{VIM-2} genes shared, within sequenced areas, 99 % identity with *bla*_{VIM-2} genes from *P. aeruginosa* strains RA-02000709 and VMH-106, which are located on a class I integron. Multiple alignment of partial *bla*_{VIM-2} nucleotide sequences from our isolates showed that they were identical within overlapping regions. Strains carrying *bla*_{VIM-2} exhibited high-level imipenem resistance as determined by Etest (MIC >32 mg l⁻¹). Antibiotic susceptibility testing for VIM-2-producing isolates revealed that, apart from the polymyxins (100 % susceptibility), levofloxacin and ciprofloxacin (80 % susceptibility) were the most active antimicrobial agents, while these strains were uniformly resistant to aminoglycosides. Interestingly, three of five VIM-2-producing isolates showed *in vitro* susceptibility to piperacillin, piperacillin-tazobactam and/or antipseudomonal cephalosporins, once again demonstrating the high phenotypic diversity observed in MBL-producers (Cornaglia *et al.*, 2007). In addition, four of the VIM-2-positive strains were susceptible to fluoroquinolones, which is not very common in MBL-producing isolates (Pitout *et al.*, 2005, 2008). Furthermore, one of the carbapenem non-susceptible isolates collected from the hospital environment was VIM-2-positive, and it could be assumed that these serve as a possible source of nosocomial infections and further dissemination, as already stated (Trautmann *et al.*, 2001). VIM-2-producing isolates belonged to three different genotype groups as determined

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of IMD12, IMD39, IMD58, IMD85 and IMD91 are FR695882-FR695886, respectively, and those for the *bla*_{VIM-2} gene sequences of IMD12, IMD39, IMD58, IMD85 and IMD91 are FR695887-FR695891, respectively.

Table 1. Relevant characteristics of the five VIM-2 MBL-producing *P. aeruginosa* isolates

Isolate	Date (day/month/year)	Hospital unit	Source	PFGE profile	<i>bla</i> gene
IMD12	25/11/2007	Neonatology ward	Water tap swab	I	<i>bla</i> _{VIM-2}
IMD39	23/1/2008	Paediatric intensive care unit	Endotracheal aspirate	II	<i>bla</i> _{VIM-2}
IMD58	19/3/2008	Surgical intensive care unit	Intra-abdominal swab	III	<i>bla</i> _{VIM-2}
IMD85	20/5/2008	Paediatric intensive care unit	Endotracheal tube swab	III	<i>bla</i> _{VIM-2}
IMD91	26/5/2008	Paediatric intensive care unit	Endotracheal aspirate	III	<i>bla</i> _{VIM-2}

by ERIC2 rep-PCR and PFGE. Bearing in mind that we identified three clonal groups (PFGE) among five VIM-2-producing isolates, and that *bla*_{VIM-2} genes were identical in these isolates, it could be inferred that the prevalence of *bla*_{VIM-2} is a consequence of horizontal gene transfer rather than clonal dissemination.

The study demonstrated a low prevalence of MBL-producing *P. aeruginosa* in our paediatric hospital. VIM-2 was the only MBL type detected, which is also the most widespread VIM-type MBL worldwide and probably the most common acquired MBL in the south of Europe.

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