Emergence of VIM-2 metallo-\(\beta\)-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-\(\beta\)-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 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*\(_{\text{VIM}}\) PCR amplicons revealed that all belonged to the *bla*\(_{\text{VIM}}\) type of MBL. Nucleotide sequences of those five *bla*\(_{\text{VIM}}\) genes shared, within sequenced areas, 99 % identity with *bla*\(_{\text{VIM}}\) genes from *P. aeruginosa* strains RA-02000709 and VMH-106, which are located on a class I integron. Multiple alignment of partial *bla*\(_{\text{VIM}}\) nucleotide sequences from our isolates showed that they were identical within overlapping regions. Strains carrying *bla*\(_{\text{VIM}}\) 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.
by ERIC2 rep-PCR and PFGE. Bearing in mind that we identified three clonal groups (PFGE) among five VIM-2-producing isolates, and that \( \text{bla}_{\text{VIM-2}} \) genes were identical in these isolates, it could be inferred that the prevalence of \( \text{bla}_{\text{VIM-2}} \) is a consequence of horizontal gene transfer rather than clonal dissemination.

The study demonstrated a low prevalence of MBL-producing \( \text{P. aeruginosa} \) 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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**Table 1. Relevant characteristics of the five VIM-2 MBL-producing \( \text{P. aeruginosa} \) isolates**

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


