Nosocomial infection by VIM-2 metallo-\(\beta\)-lactamase-producing *Pseudomonas putida*


Clinical Microbiology Unit, University Hospital Complex of Santiago de Compostela, C/ Travesía Coupuna s/n, 15706 Santiago de Compostela, Spain

Nosocomial infections caused by multidrug-resistant and carbapenem-resistant *Pseudomonas putida* isolates have been reported occasionally in severely ill or immunocompromised patients. Here we report the microbiological characteristics of what are believed to be the two first carbapenem-resistant VIM metallo-\(\beta\)-lactamase (MBL)-producing *P. putida* strains in Spain, which were isolated from patients at the University Hospital Complex of Santiago de Compostela.

Both patients were immunocompromised with severe underlying diseases and had been hospitalized for more than 15 days. One of them had previously been treated with a broad-spectrum therapy. Antimicrobial susceptibility testing showed that both strains were resistant to piperacillin/tazobactam, ceftazidime, cefepime, imipenem, gentamicin, tobramycin, aztreonam, trimethoprim/sulfamethoxazole and ciprofloxacin, but sensitive to amikacin and colistin.

For both isolates PCR and sequencing was positive for the *bla*\(^{VIM-2}\) gene. Fingerprinting analysis revealed these were two different strains. One patient recovered clinically and one died; no direct link could be established between the isolation of *P. putida* and death. Our data expose the emergence of multidrug-resistant *P. putida* VIM-2 MBL, probably arising by independent horizontal transfer of resistance genes. So, although *P. putida* is not frequently isolated, it may survive easily in the hospital setting and occasionally cause difficult-to-treat nosocomial infections in severely ill patients.

**Introduction**

The emergence of carbapenemases, such as acquired metallo-\(\beta\)-lactamas (MBLs) and other \(\beta\)-lactamas affecting carbapenems, is becoming a therapeutic challenge (Cornaglia et al., 2007) because these enzymes confer a high level of resistance to most \(\beta\)-lactams, including carbapenems, with the exception of aztreonam (Walsh et al., 2005). Among the MBLs acquired by *Pseudomonas putida*, IMP-1 was reported by Senda and colleagues in Japan in 1996 (Senda et al., 1996), and later reported in Taiwan and Japan (Yomoda et al., 2003). IMP-12 was the first IMP MBL described in *P. putida* in Europe (Docquier et al., 2003). VIM-1 in *P. putida* was first reported in Europe (Lombari et al., 2002), and VIM-2 in *P. putida* was first reported in Taiwan, Korea, Japan, France and Argentina between 2002 and 2007 (Lee et al., 2002; Poirel et al., 2006; Almuzara et al., 2007). Recently, VIM-5 MBL-producing *P. putida* has been described in Turkey (Poirel et al., 2009). Nosocomial infections caused by multidrug-resistant and carbapenem-resistant *P. putida* isolates have been occasionally reported in severely ill or immunocompromised patients frequently hospitalized in intensive care units (Almuzara et al., 2007). Here we report the microbiological characteristics of what are believed to be the first two carbapenem-resistant VIM-2 MBLs *P. putida* strains identified in Spain, which were isolated from patients at the University Hospital Complex of Santiago de Compostela (in the north-west of Spain).

**Case reports**

**Case 1**

A 71-year-old female with a history of hypertension, insulin-dependent diabetes mellitus, stroke, chronic obstructive pulmonary disease, psychiatric and lymphoproliferative syndrome was admitted to the University Hospital Complex of Santiago de Compostela for exacerbation of dyspnoea. The patient had been treated with meropenem during previous hospital admissions. Examination revealed congestive heart failure, possibly aggravated by an infectious process. Empirical treatment
was started with ceftriaxone (1 g daily). On hospital day 10, carbapenem-resistant *P. putida* were recovered from blood cultures. After a daily single dose of amikacin (1 g daily), the patient’s disease progression was favourable and she was discharged. Nevertheless, due to her underlying disease she was readmitted a month later and died.

**Case 2**

A 71-year-old male with a urostomy (due to bladder cancer) was admitted to our hospital because of abdominal pain and cellulitis at the stoma. The patient was treated with cloxacillin, gentamicin and metronidazole in conjunction with surgery. The outcome was favourable. After 3 days, carbapenem-resistant *P. putida* was isolated from a urine control culture (>100 000 c.f.u. ml⁻¹). The patient was treated with amikacin (1 g daily) plus ceftriaxone (1 g daily) and, again, his clinical progression was good. From the clinical and microbiological point of view, the inclusion of ceftriaxone was not justified in the treatment of this patient resulting, in this case, in inappropriate medical treatment.

**Results and Discussion**

Identification to the species level of the isolates from the two patients was achieved using the Vitek 2 system (bioMérieux). Antimicrobial susceptibility testing was assayed by Vitek 2 and Etest (bioMérieux), and showed that these strains were multidrug resistant, and only sensitive to amikacin and colistin. The MICs obtained are presented in Table 1. The carbapenemase screening tests were positive using a modified Hodge test (Lee *et al.*, 2001). An MBL-production assay was positive both by double-disc synergy test and MBL double-sided Etest (imipenem/imipenem-EDTA). Molecular confirmation was completed by PCR targeting *bla*<sub>IMP</sub>, *bla*<sub>VIM-1</sub> and *bla*<sub>VIM-2</sub> genes (Yan *et al.*, 2001). A PCR product of 865 bp was obtained from both strains when *bla*<sub>VIM-2</sub> primers were used. The amplicons were purified and sequenced with the primers described by Yan *et al.* (2001). Sequencing results showed that the products encoded VIM-2 MBL.

Strain typing of the isolates was performed by automated repetitive-sequence-based PCR using the DiversiLab system (bioMérieux). The results for both isolates identified as VIM-2 can be seen in Fig. 1 and show that these are two genetically unrelated strains, which is consistent with the epidemiological data. Although the *P. putida* isolates were obtained only 3 weeks apart, these patients were admitted in wards located in different buildings of the hospital complex. Summarizing, our results document the emergence of multidrug resistant VIM-2-MBL-producing *P. putida* strains. Since, until now, VIM-2-MBL-producing bacteria had not been isolated in our hospital, it is likely that these two clones arose independently and resistance genes could be transferred between them. We emphasize that *P. putida*, although infrequently isolated from clinical samples, may survive in the hospital setting and occasionally cause difficult-to-treat nosocomial infections in severely ill patients.

**Table 1. Susceptibility profile of the carbapenem-resistant *P. putida***

<table>
<thead>
<tr>
<th>Strain</th>
<th>Imipenem</th>
<th>Meropenem</th>
<th>Ceftazidime</th>
<th>Cefepime</th>
<th>Aztreonam</th>
<th>Piperacillin/ Ciprofloxacin</th>
<th>Gentamicin</th>
<th>Tobramycin</th>
<th>Amikacin</th>
<th>Trimethoprim/ sulfamethoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>16</td>
<td>16</td>
<td>12</td>
<td>24</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>48</td>
<td>4</td>
</tr>
<tr>
<td>Patient 2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>64</td>
<td>192</td>
<td>12</td>
<td>&gt;256</td>
<td>32</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Fig. 1.** Repetitive-sequence-based PCR of carbapenem-resistant *P. putida* from the two patients studied.
References


