Sirs,

Organisms with acquired metallo-β-lactamases (MBLs) have been identified worldwide in numerous species, including Pseudomonas aeruginosa. VIM and IMP types are the most prevalent MBLs, being encoded by gene cassettes inside class 1 integrons. IMP-13, initially detected in clinical isolates of P. aeruginosa from Italy, has become a widespread carbapenem resistance determinant, being involved in relatively large outbreaks in Italy. IMP-13 has also been occasionally detected in P. aeruginosa isolates from Austria and Romania. Recently, IMP-13-producing isolates were found in Argentina that belonged to the same clonal sequence type (ST), i.e. ST621, as the Italian isolates. In these isolates, the blaIMP-13 gene was found to be carried in different class 1 integron structures (InPSG, In88 and In89), usually located on the chromosome or, less frequently, on plasmids.

From November 2006 to September 2008, four multidrug-resistant (MDR) P. aeruginosa isolates with reduced susceptibility to carbapenems (being susceptible only to colistin) were isolated from clinical samples of four patients hospitalized in three Belgian hospitals (two in the Brussels area and one located 70 km south of Brussels) (Table 1). Despite multiple investigations, no other carbapenem-resistant P. aeruginosa isolates were recovered from other patients hospitalized in the same wards before or concomitantly to the infected patients, and no overlap in hospital stay could explain cross-transmission between patients. Furthermore, none of these patients had recently travelled or had been hospitalized abroad, suggesting that these isolates were acquired in Belgium.

Two of these isolates (P1 and P2) displayed only slightly decreased susceptibility to imipenem and meropenem (MICs of 4 mg/L) and were categorized as susceptible according to CLSI breakpoints (according to current breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST), P1 and P2 isolates would be classified as susceptible to imipenem (susceptible breakpoint is ≤4 mg/L) and intermediately resistant to meropenem (susceptible breakpoint is ≤2 mg/L), while isolates P3 and P4 displayed high-level resistance to carbapenems (Table 1). The Etest, performed according to the manufacturer’s recommendations, showed a reduction in the imipenem MICs in the presence of EDTA for all of the MDR isolates (Table 1). However, the extent of MIC reduction (2- to 4-fold) was insufficient to indicate the production of an MBL, for isolates P1 and P2, according to the manufacturer’s recommendations.

Enzyme assays revealed the presence of EDTA-inhibitable carbapenemase activity in crude extracts of the four P. aeruginosa isolates. Analytical isoelectric focusing revealed the presence of two β-lactamase bands with pIs of 8.0 and of 8.2 (data not shown).

PCR experiments followed by sequencing identified β-lactamase genes encoding the carbapenemase IMP-13 and the chromosome-encoded AmpC and OXA-50. Characterization of the variable regions of the class 1 integrons carrying the blaIMP-13 cassette from the Belgian P. aeruginosa isolates revealed structures identical to those previously found in the Italian epidemic clone, harbouring integron InPSG, which was also the most common integron platform among Italian isolates, and in isolates from Romania and Austria. These isolates carried the blaIMP-13 and aacA4 cassettes. Moreover, PCR mapping showed that InPSG was inserted into a Tn5051-like transposon as described for the InPSG-harbouring Italian isolates, suggesting a clonal origin among these genetic structures.

In order to investigate the genomic relatedness of the P. aeruginosa isolates, a novel semi-automated rep-PCR technique (Diversilab, bioMerieux) was carried out. This technique revealed that all four Belgian isolates exhibited banding patterns that were identical to the Italian reference clone AV65 (data not shown), suggesting a clonal relatedness between the Belgian isolates and the Italian epidemic clone.

By multilocus sequence typing analysis the AV65 isolate and all the Belgian isolates were identified as belonging to ST621 and by serotyping all were identified as serotype O4. Interestingly, the same ST was also reported for the IMP-13-producing isolates from Austria and Romania. Altogether, these results revealed a common genetic lineage for the IMP-13-producing P. aeruginosa isolates circulating worldwide.

Efforts aimed at purifying plasmids carrying blaIMP-13 or transferring the resistance determinant by conjugation or transformation were unsuccessful. Southern blot hybridization, using a blaIMP-13 probe and a 16S rRNA probe after I-Ceu I digestion and PFGE separation, showed that the blaIMP-13 gene was chromosomally located, since the bands that hybridized with the blaIMP-13 probe also hybridized with the 16S rRNA probe (data not shown).

The overall genomic relatedness of the IMP-13-producing isolates and the fact that the blaIMP-13 gene was inserted in a conserved genetic context point to a common ancestry for the IMP-13-producing strains disseminated in South America, Italy, Romania, Austria and now in Belgium, thus underscoring the role of the ST621 clonal lineage as a highly successful P. aeruginosa epidemic clone.

The fact that the MIC values of carbapenem drugs for two of the four isolates were in the susceptibility range is worrisome, since this may lead to failure of detection, thus...
Table 1. Epidemiological data and carbapenem MICs (in mg/L)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Date of isolation</th>
<th>Hospital (city)</th>
<th>Gender, age (years)</th>
<th>Underlying condition(s)</th>
<th>Ward</th>
<th>Diagnosis</th>
<th>Culture</th>
<th>Therapy</th>
<th>Outcome</th>
<th>MIC IPM</th>
<th>MIC MEM</th>
<th>MIC IPM/IMEM + EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>11/2006</td>
<td>A (Brussels)</td>
<td>male, 73</td>
<td>outpatient</td>
<td>ICU</td>
<td>UTI</td>
<td>urine</td>
<td>ciprofloxacin</td>
<td>cured</td>
<td>4¹</td>
<td>3⁰</td>
<td>40/0.5</td>
</tr>
<tr>
<td>P2</td>
<td>12/2006</td>
<td>B (Brussels)</td>
<td>male, 67</td>
<td>pyelonephritis, septic shock</td>
<td>ICU</td>
<td>bacteroabscess</td>
<td>pus</td>
<td>cefuroxime, ciprofloxacin/vancomycin/clavulanic acid</td>
<td>deceased</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32/2</td>
</tr>
<tr>
<td>P3</td>
<td>03/2007</td>
<td>B (Brussels)</td>
<td>male, 62</td>
<td>liver cirrhosis, hip prosthesis</td>
<td>ICU</td>
<td>mediastinitis</td>
<td>ETA</td>
<td>piperacillin/tazobactam/amikacin/vancomycin</td>
<td>deceased</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32/2</td>
</tr>
<tr>
<td>P4</td>
<td>08/2008</td>
<td>C (Mont-Godinne)</td>
<td>female, 59</td>
<td>cardiac surgery</td>
<td>ICU</td>
<td>UTI</td>
<td>urine</td>
<td>imipenem, meropenem</td>
<td>cured</td>
<td>4d</td>
<td>3d</td>
<td>4/1</td>
</tr>
</tbody>
</table>

IPM, imipenem; MEM, meropenem; ICU, intensive care unit; BAL, bronchoalveolar lavage; ETA, endotracheal aspirate; UTI, urinary tract infection.

²All four cases occurred as sporadic cases in a non-outbreak setting due to MDR P. aeruginosa so that there was not a high degree of suspicion of a resistant isolate in any of these cases. Treatment choice was empirical depending on the infection site. Antibiotic therapy could not be adapted according to microbiological results since they were only available after the death of three patients.

³Three of the patients died rapidly and it was not clear in any of these cases whether the fatal outcome could be attributed to infection rather than to underlying diseases and co-morbidities.

⁴Patient had been hospitalized in Brussels for abdominal surgery 4 months before isolation of the IMP-13-producing P. aeruginosa isolate.

⁵MIC of 4 mg/L (without heeding the squatter colonies for which repeated MIC was 32 mg/L for both imipenem and meropenem.

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