bla\textsubscript{VIM-2} and bla\textsubscript{VIM-7} Carbapenemase-Producing \textit{Pseudomonas aeruginosa} Detected in a Tertiary Care Medical Center in the United States: Report from the MYSTIC Program

Short running title: Carbapenemases in a USA Hospital

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Two *Pseudomonas aeruginosa* strains resistant to beta-lactams, fluoroquinolones, aminoglycosides, tetracyclines and carbapenems, and susceptible only to polymyxin B (MIC ≤ 2 µg/ml) were identified as part of the Meropenem Yearly Susceptibility Test Information Collection Program. Metallo-β-lactamase screening tests were positive, PCR yielded products with *bla*<sub>VIM</sub> primers, and sequence analysis revealed *bla*<sub>VIM-7</sub> and *bla*<sub>VIM-2</sub>. The isolates had distinct ribotype and PFGE patterns and appeared independently, remote in time and location, at the same cancer center.
Carbapenems are among the best choices for the treatment of infections caused by gram-negative bacilli (GNB). However, carbapenem resistance due to various mechanisms is being reported. Modifications in outer membrane permeability result in imipenem resistance, with low-grade meropenem resistance. Up-regulation of the efflux system likely affects meropenem and ertapenem more than imipenem. Hyperproduction of AmpC β-lactamases with these two resistance mechanisms can further reduce carbapenem potency (7). A fourth mechanism is the production of carbapenemases which hydrolyze many β-lactam antibiotics, including carbapenems (6). These enzymes have been detected among non-fermentative GNB as well as the Enterobacteriaceae (12).

*Pseudomonas aeruginosa* is a leading cause of nosocomial infections (9). It is a significant pathogen in cancer patients and identification of carbapenem–resistant strains is a concern. The first metallo-β-lactamase producing *P. aeruginosa* strain in the United States (USA) was reported from M. D. Anderson Cancer Center (MDACC) (10, 11). Here we report the characteristics and genetic relationships of two additional metallo-β-lactamase producing *P. aeruginosa* strains from the same center, compared to the index strain.

A total of 196 *P. aeruginosa* isolates were referred to JMI Laboratories (North Liberty, IA), from MDACC over a 7 year period (1999-2006) as part of the MYSTIC longitudinal surveillance program. Isolate identification was confirmed using standard biochemical tests and Vitek cards (bioMérieux, Hazelwood, MO). Antimicrobial susceptibility testing was performed using CLSI (formerly NCCLS) described microdilution methodology (2). *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 were used as quality control organisms for these experiments. Interpretations of susceptibilities for all agents tested were by CLSI (2006) criteria (2). Screening for metallo-β-lactamase was performed by the disc approximation test using a modification of the procedure described by
Arakawa et al, 2000 (1). *Acinetobacter baumanii* 54/97 (IMP-2 producer) was used as a positive control. Metallo-β-lactamase E-test strips (AB BIODISK, Solna, Sweden) were used to confirm the disk approximation test results. Isolates exhibiting a positive disk approximation test for metallo-β-lactamase were screened for IMP-and VIM-like genes using primers spanning the conserved sequences within the respective enzyme types. Metallo-β-lactamases and their genetic contexts were studied by sequencing the gene segment accomplished using 5' conserved sequence (CS) and 3' CS primers from the Class 1 integron as previously described. Sequences obtained were analyzed using NCBI BLAST search to determine the enzyme types.

Characteristics of the index case from 2001 have been previously described (10,11). Two additional cases at the same institution (MDACC) occurred remote in time and location (July 2003 and January 2004) from the index case and each other. The *P. aeruginosa* strains isolated from these two cases were further characterized in the present study. Strains from the index case, and case 3 (2004) were resistant to all β-lactams as well as the aminoglycosides and fluoroquinolones as summarized in Table 1. The isolates were susceptible to polymyxin B. All three strains exhibited positive disk approximation tests to one or more β-lactam substrates (imipenem, meropenem, or ceftazidime) and recorded responses of all three strains were phenotypically different (Table 2). Generic *bla*<sub>VIM</sub> primers yielded PCR products in both new cases. Sequencing results revealed *bla*<sub>VIM-7</sub> in the first position (5' end) of the integron amplified from strain 4623 (case 2), with the same genetic context as the index strain reported in 2001 (strain 7-406; Case 1) *bla*<sub>OXA-45</sub> was identified in the index strain (not on the integron carrying *bla*<sub>VIM-7</sub>), but was not present in strain 4623 (Table 2). Sequencing of PCR amplicon obtained from strain 1-1852 (Case 3) revealed *bla*<sub>VIM-2</sub> which has many key aminoacid variations when compared to *bla*<sub>VIM-7</sub>, ruling out the possibility of simple evolution from the *bla*<sub>VIM-7</sub> gene pool that had previously been identified at MDACC. The VIM-7 producing strain in this study (strain...
and the index strain (strain 7-406) showed different ribotypes, as well as PFGE patterns that were also distinct from the VIM-2 producing strains (1-1852).

In the past few years there have been several reports of metallo-β-lactamase producing *P. aeruginosa* isolates from Europe, Latin America, and Asia (3,4,5,12). This mechanism of carbapenem resistance remains uncommon in North America with only a few published reports so far (11). Metallo-β-lactamases have a broad spectrum of hydrolytic activity against amino-carboxyl- and ureidopenicillins, cephalosporins, cephamicins and carbapenems, but not monobactams (3). The VIM enzymes of this group are usually carried on mobile gene cassettes inserted into class 1 integron, located chromosomally on a resistant plasmid. The VIM-type of enzymes (VIM-1 to -11) were initially described in *P. aeruginosa* and *Acinetobacter* spp. and subsequently in *Serratia marcescens, Pseudomonas putida, Pseudomonas stutzeri, Klebsiella pneumoniae*, and *E. coli*, predominantly in Asia and Europe (3,8). Analysis of molecular and epidemiologically characterization combined with patient demographics leads to the following assumptions regarding the emergence of these VIM-type metallo-β-lactamase at MDACC: 1) the VIM-7 producing index case appears to have arisen independently in the USA, possibly under pressure of carbapenem usage rather than by dissemination from Europe or Asia. 2) *bla*<sub>VIM-7</sub> re-emerged two years later in a clonally unrelated strain. Considering the integron sequences of the strains isolated in this case and in the index case were identical, an horizontal transfer of the entire *bla*<sub>VIM-7</sub> containing integron probably occurred; and 3) the VIM-2 producing strain was isolated from a patient who may have been treated with carbapenems in Jordan. Although the carbapenemase gene pool in Jordan is not well known, *bla*<sub>VIM-2</sub> may be present as it is widespread in Eastern Europe and Asia (4). However, *bla*<sub>VIM-2</sub> could also have been acquired in the USA. Although metallo-β-lactamases generally do not hydrolyze aztreonam, all 3 strains were non-susceptible to the monobactam, suggesting another mechanism of resistance (11).
Metallo-β-lactamases producing strains pose a serious threat to patients mandating careful antibiotic stewardship and infection control programs. Additionally, the need for routine diagnostic smears for these enzymes (1,4,7) and the development of novel antimicrobial agents highly active against organisms producing these enzymes, is paramount.
References:


Table 1: Antimicrobial agent susceptibilities of metallo-β-lactamase producing *P. aeruginosa* isolates from MDACC

MIC in µg/ml for isolate number (year):

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Imipenem</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Meropenem</td>
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<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
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<td>&gt;64</td>
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<td>Aztreonam</td>
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<td>16</td>
<td>&gt;16</td>
</tr>
<tr>
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<td>&gt;16</td>
<td>16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Amikacin</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<td>&gt;2</td>
<td>&gt;2</td>
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<tr>
<td>Polymyxin B</td>
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Table 2. Summary of molecular epidemiologic investigations of metallo-\(\beta\)-lactamase-producing *P. aeruginosa* isolated from MDACC.

<table>
<thead>
<tr>
<th>Isolate #</th>
<th>Year</th>
<th>IMI+EDTA</th>
<th>MEM+EDTA</th>
<th>IMI+2-MPA</th>
<th>MEM+2-MPA</th>
<th>CAZ+EDTA</th>
<th>VIM</th>
<th>Ribotype/PFGE patterns</th>
<th>Carbapenemase</th>
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<tr>
<td>7-406</td>
<td>2001</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>105.528.6/A</td>
<td>VIM, OXA-45</td>
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<tr>
<td>4623</td>
<td>2003</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>258.151.2/B</td>
<td>VIM-7</td>
</tr>
<tr>
<td>1-1852</td>
<td>2004</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>258.231.1/C</td>
<td>VIM-2</td>
</tr>
</tbody>
</table>

a. Various substrate inhibitor combination interactions described. IMI = imipenem, MEM = meropenem, CAZ = ceftazidime, 2-MPA = 2-mercaptopropionic acid.