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bla$_{VIM-2}$ and *bla*$_{VIM-7}$ Carbapenemase-Producing *Pseudomonas aeruginosa* Isolates Detected in a Tertiary Care Medical Center in the United States: Report from the MYSTIC Program$^{\nabla}$

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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*$_{VIM}$ primers, and sequence analysis revealed *bla*$_{VIM-7}$ and *bla*$_{VIM-2}$. The isolates had distinct ribotype and pulsed-field gel electorphoresis 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. 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 nonfermentative gram-negative bacilli 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 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 to 2006) as part of the Meropenem Yearly Susceptibility Test Information Collection 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 criteria (2). Screening for metallo-β-lactamase was performed by the disk approximation test using a modification of the procedure described by Arakawa et al. (1). *Acinetobacter baumannii* 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′ and 3′ conserved sequence 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

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

Antimicrobial agent	MIC (μg/ml) for isolate no. (yr):		
	7-406 (2001)	4623 (2003)	1-1852 (2004)
Imipenem	>8	>8	>8
Meropenem	>8	>8	>8
Piperacillin-tazobactam	>64	>64	>64
Aztreonam	>16	16	>16
Cefepime	>16	16	>16
Amikacin	>32	>32	>32
Ciprofloxacin	>2	>2	>2
Polymyxin B	2	≤1	2

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TABLE 2. Summary of molecular epidemiologic investigations of metallo-β-lactamase-producing *P. aeruginosa* isolated from MDACC

Isolate no.	Yr	Disk approximation test result with[a]:					VIM PCR result	Ribotype/PFGE[b] pattern	Carbapenemase(s)
		IMI + EDTA	MEM + EDTA	IMI + 2-MPA	MEM + 2-MPA	CAZ + EDTA			
7-406	2001	+	−	−	−	−	+	105.528.6/A	VIM-7, OXA-45
4623	2003	−	+	−	−	+	+	258.151.2/B	VIM-7
1-1852	2004	+	+	+	+	−	+	258.231.1/C	VIM-2

[a] Various substrate-inhibitor combination interactions described. IMI, imipenem; MEM, meropenem; CAZ, ceftazidime; 2-MPA, 2-mercaptopropionic acid.
[b] PFGE, pulse-field gel electrophoresis.

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 the recorded responses of all three strains were phenotypically different (Table 2). Generic bla_{VIM} primers yielded PCR products in both new cases. Sequencing results revealed bla_{VIM-7} 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_{OXA-45} was identified in the index strain (not on the integron carrying bla_{VIM-7}) but was not present in strain 4623 (Table 2). Sequencing of the PCR amplicon obtained from strain 1-1852 (case 3) revealed bla_{VIM-2}, which has many key amino acid variations compared to bla_{VIM-7}, ruling out the possibility of simple evolution from the bla_{VIM-7} gene pool that had previously been identified at MDACC. The VIM-7-producing strain in this study (strain 4623) and the index strain (strain 7-406) showed different ribotypes as well as pulsed-field gel electrophoresis 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, cephamycins, and carbapenems but not monobactams (3). The VIM enzymes of this group are usually carried on mobile gene cassettes inserted into the class 1 integron, located chromosomally on a resistant plasmid. The VIM type of enzymes (VIM-1 to -11) were initially described for *P. aeruginosa* and *Acinetobacter* spp. and subsequently for *Serratia marcescens*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Klebsiella pneumoniae*, and *E. coli*, predominantly in Asia and Europe (3, 8). Analysis of molecular and epidemiological characterization combined with patient demographics leads to the following assumptions regarding the emergence of these VIM-type metallo-β-lactamases at MDACC. (i) The VIM-7-producing index case appears to have arisen independently in the United States, possibly under pressure of carbapenem usage rather than by dissemination from Europe or Asia. (ii) bla_{VIM-7} reemerged 2 years later in a clonally unrelated strain. Considering that the integron sequences of the strains isolated in this case and in the index case were identical, a horizontal transfer of the entire bla_{VIM-7}-containing integron probably occurred. (iii) 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_{VIM-2} may be present, as it is widespread in Eastern Europe and Asia (4). However, bla_{VIM-2} could also have been acquired in the United States. Although metallo-β-lactamases generally do not hydrolyze aztreonam, all 3 strains were nonsusceptible to the monobactam, suggesting another mechanism of resistance (11).

Metallo-β-lactamase-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.

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