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bla$_{\text{VIM-7}}$, an Evolutionarily Distinct Metallo-β-Lactamase Gene in a *Pseudomonas aeruginosa* Isolate from the United States

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As part of the CANCER Antimicrobial Surveillance Program in North America, a *Pseudomonas aeruginosa* isolate, strain 07-406, was shown to possess a metallo-β-lactamase, designated VIM-7. *bla*$_{\text{VIM-7}}$ is located on a 24-kb plasmid which can be readily transferred into *Enterobacteriaceae* and other pseudomonads. This is the first report of a mobile metallo-β-lactamase gene, *bla*$_{\text{VIM-7}}$, being detected within the United States.

In 1999, a novel family of class B metallo-β-lactamases, the VIM family (VIM-1 to VIM-6 enzymes) of *Pseudomonas aeruginosa* and *Acinetobacter* spp. in Europe, was initially described (4, 5, 10) The VIM family was subsequently found in strains of *Serratia marcescens* and *Acinetobacter* spp. in Korea (VIM-2) (15) and *P. aeruginosa* (VIM-3), *Pseudomonas putida*, and *Pseudomonas stutzeri* spp. (VIM-2) in Taiwan (12, 14, 15). More recently, VIM variants have been found in *Escherichia coli* (VIM-1) (6) and *P. aeruginosa* (VIM-4; EMBL accession no. AY135661 and AF531419) (8) in Greece, in *Klebsiella pneumoniae* (VIM-5; EMBL accession no. AY144612) in Turkey, and in *P. putida* (VIM-6; EMBL accession no. AY165025) in Singapore. The *bla*$_{\text{VIM}}$ gene, like the *bla*$_{\text{IMP}}$ gene, is carried on mobile gene cassettes inserted into class 1 integrons and located chromosomally or on resident plasmids (3). The class 1 integrons are the most common mechanisms by which bacteria are able to move resistant gene cassettes from one bacterium to another. The process involves recombination between 59-bp elements on the gene cassette and *attI*1 sites on the integron (2).

P. aeruginosa, isolate 07-406, was cultured from a 58-year-old female with a history of autoimmune hepatitis that required a liver transplant in 1988. In May 2001, she was diagnosed with left-breast carcinoma. Six days after admission of the patient, a diffuse pneumonitis was noted, and an invasive pulmonary sample was taken by bronchoscopy that revealed *P. aeruginosa* pneumonia. The organism was resistant to all tested antimicrobials except polymyxin B (MIC, ≤2 μg/ml) by local tests. *P. aeruginosa* isolate 07-406 gave a positive result with the metallo-β-lactamase Etest strip, the imipenem MIC being reduced from 256 to 4 μg/ml in the presence of EDTA (11).

The genomic library was created by partial *Sau3*A restriction, cloned into pK18, and expressed in *E. coli* strain DH5α [*supE44* Δ*lacU169* (ϕ80*lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] as previously described (9). Recombinants were screened on agar containing ampicillin plus the serine β-lactamase inhibitor BRL42715 (GlaxoSmithKline), ensuring that any resistance detected was not mediated by a serine-type β-lactamase (1). Twelve colonies harboring identical inserts were isolated, and one colony demonstrating imipenemase activity (extinction coefficient, −7,000) inhibited by EDTA was chosen randomly for further study. *E. coli* DH5α carrying pMATVIM-7 conferred resistance to nearly all β-lactams except for aztreonam and the carbapenems; *P. aeruginosa* 07-406 was resistant to all β-lactams (Table 1). *bla*$_{\text{VIM-7}}$ is found on a 24-kb plasmid which can be readily transferred by electroporation into *Enterobacteriaceae* and other pseudomonads (data not shown).

The clone harbored the recombinant plasmid pMATVIM-7, containing a 1,540-bp insert that was sequenced on both strands. Primer sequences used for both sequencing the insert pMATVIM-7 and back probing are shown in Table 2. Sequence analysis of the pMATVIM-7 insert revealed the presence of a 795-bp open reading frame showing high homology with a number of previously cloned metallo-β-lactamases (Fig. 1). The phylogenetic tree (Fig. 2), which is based on a CLUSTAL W multiple alignment of the putative amino acid sequence of the β-lactamase from *P. aeruginosa* strain 07-406 with other metallo-β-lactamases, demonstrates that it clusters more closely to the VIM family than the IMP family of metallo-β-lactamases. VIM-7 has 77% amino acid identity to

TABLE 1. MICs of β-lactams for *P. aeruginosa* 07-406, *E. coli* DH5α, and *E. coli* DH5α containing pMATVIM-7

β-Lactam	MIC (μg/ml)		
	P. aeruginosa 07-406	*E. coli* DH5α	*E. coli* DH5α (pMATVIM-7)
Ceftazidime	>256	0.125	128
Cefotaxime	>256	0.125	128
Cefepime	>256	0.06	8
Cefoxitin	>256	2	4
Ceftriaxone	>256	0.125	32
Imipenem	>256	0.06	0.5
Meropenem	>256	0.06	0.125
Piperacillin	>256	0.5	32
Ampicillin	>256	1	256
Amoxicillin	>256	2	256
Oxacillin	>256	4	32
Aztreonam	>256	0.06	0.125

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TABLE 2. Primers used to sequence pMATVIM-7 and to back probe to the *P. aeruginosa* 07-406 chromosome to verify origins of clones

Primer name	Nucleotide sequence reading 5′→3′
M13F	GTAAAACGACGGCCAGTG
pK18R	GCAAGGCGATTAAGTTGG
PVIM-F	GCCGTGCGCGTACTGACTGTTTG
PVIM-R	CAAACAGTCAGTACGCGCACGGC
FPF	ATTCGCAGCTTTCTGGTTGG
FPR	TAGCCTTGAGCGCAGCGTTG

VIM-1, whereas VIM-1 to VIM-6 have 89 to 99% identity (4, 7, 13). These amino acid variations among VIM-7 and the other VIM-type enzymes cluster in the leader sequence but are also found throughout the mature protein. The amino acid changes within the mature protein are often changes involving functionally different residues, namely, Q48K, S61K, D64G, S192R, Y195F, N216D, E225K, and H219R. The most likely site for cleavage occurs between amino acid positions 26 and 27 (YSA-QP), which would leave a mature peptide of 25,392 Da with a pI of 5.71. This pI is in close agreement with the experimental pI of 6.6 (data not shown).

Analysis of the DNA sequence (with the Lasergene DNASTAR software package) of the pMATVIM-7 insert (Fig. 1) indicates that bla_{VIM-7} is preceded by a ribosome binding site (AGGAG) 6 bp upstream of the start codon. The presence of a conserved core site 13 bp upstream of the ribosome binding site, together with an inverse core site 7 bp after the stop codon and a 59-bp element, demonstrates that bla_{VIM-7} is harbored on a gene cassette. Additionally, an *attI*1 site identified immediately upstream of the gene cassette containing the promoter of the *intI* gene suggests that bla_{VIM-7}, like other metallo-β-lactamases, is harbored on an integron (Fig. 3).

The gene encoding the enzyme described in this paper, bla_{VIM-7}, like the bla_{VIM-1} and bla_{VIM-3} genes, is harbored on a gene cassette which gives it the potential to move readily from one genome to another (Fig. 3). The presence of an *attI*1 site upstream of bla_{VIM-7} also strongly suggests that the bla_{VIM-7} cassette is part of an integron, which characteristically consists of an integrase gene followed by a recombination site

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ttttgtacagtctatgcctcgggcatccaagcagcaagcgcgttacgccgtgggtcgaTGTTTGATGTTATG 72
                                  attI1
GAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAAACAAAgttatcgcagtcggcccccgaggagta 144
   blaVIM-7 -->
ttgatgtttcaaattcgcagctttctggttggtatcagtgcattcgtcatggccgtacttggatcagcagca 216
    M  F  Q  I  R  S  F  L  V  G  I  S  A  F  V  M  A  V  L  G  S  A  A
tattccgcacagcctggcggtgaatatccgacagtagatgacataccggtaggggaagttcggctgtacaag 288
 Y  S  A  Q  P  G  G  E  Y  P  T  V  D  D  I  P  V  G  E  V  R  L  Y  K
attggcgatggcgtttggtcgcatatcgcaactcagaaactcggtgacacggtgtactcgtctaatggactt 360
 I  G  D  G  V  W  S  H  I  A  T  Q  K  L  G  D  T  V  Y  S  S  N  G  L
atcgtccgcgatgctgatgagttgcttcttattgatacagcgtgggggcgaagaacacggtagcccttctc 432
 I  V  R  D  A  D  E  L  L  L  I  D  T  A  W  G  A  K  N  T  V  A  L  L
gcggagattgaaaagcaaattggacttccagtaacgcgctcaatttctacgcacttccatgacgatcgagtc 504
 A  E  I  E  K  Q  I  G  L  P  V  T  R  S  I  S  T  H  F  H  D  D  R  V
ggtggagttgatgtcctccgggcggctggagtggcaacgtacacctcacccttgacacgccagctggccgaa 576
 G  G  V  D  V  L  R  A  A  G  V  A  T  Y  T  S  P  L  T  R  Q  L  A  E
gcggcgggaaacgaggtgcctgcgcactctctaaaagcgctctcctctagtggagatgtggtgcgcttcggt 648
 A  A  G  N  E  V  P  A  H  S  L  K  A  L  S  S  S  G  D  V  V  R  F  G
cccgtagaggttttctatcctggtgctgcgcattcgggcgacaatcttgtggtatacgtgccggccgtgcgc 720
 P  V  E  V  F  Y  P  G  A  A  H  S  G  D  N  L  V  V  Y  V  P  A  V  R
gtactgtttggtggctgtgcagttcatgaggcgtcacgcgaatccgcgggtaatgttgccgatgccaatttg 792
 V  L  F  G  G  C  A  V  H  E  A  S  R  E  S  A  G  N  V  A  D  A  N  L
gcagaatggcctgctaccattaaacgaattcaacagcggtatccggaagcagaggtcgtcatccccggccac 864
 A  E  W  P  A  T  I  K  R  I  Q  Q  R  Y  P  E  A  E  V  V  I  P  G  H
ggtctaccgggcggtctggaattgctccaacacacaactaacgttgtcaaaacgcacaaagtacgcccggtg 936
 G  L  P  G  G  L  E  L  L  Q  H  T  T  N  V  V  K  T  H  K  V  R  P  V
gccgagtaacaaatgcggcataacaacgccaaggagggggacgcattttccactacgcccttcgggcttc   1008
 A  E  *
cctccaaaagcgcccctcatggcgggcgttatacggcaatatcagttcgcaagcagccgacagcggagcatc 1080

cgacggcgcttcaaatttgagcgtcaatgggccggcgtcctcaaattcggtgtgccttctgcaatcgtgcct
1152
agccgcgcattcgccgaaattatttcctccggtgcagcgtgcaacgtcccacggcagccgcataacccggcg
1224
ctcaacttgacccgcctgggcgggctgctcttgtgtgttactcatggtcttctccacgcccagtcgggcaag
1296
ttagctttgcgttaggtggcgcaaatgacactcctgatccgacccgtagaacaaagcgacgccgaatcatgg
1368
gagcacctacgtaacctttgtgggagggcgacgaccggcgttgttgcacaaacgcccccagtctgtaggct
1440
accgtgcccatgacgaagcctgctcccgcccaataccgtaccaccaactggaaagactacaacgctgcgctc
1512
aaggctagaggctcgctctcggtctggctggatc 1546
```

FIG. 1. Nucleotide sequence of the 1,546-bp insert of recombinant plasmid pMATVIM-7 containing the bla_{VIM-7} coding sequence. The start codon of bla_{VIM-7} is indicated with a horizontal arrow, and the stop codon is indicated with an asterisk. The deduced amino acid sequence is reported below the gene sequence. The conserved core and inverse core sites are enclosed in boxes, and the *attI*1 site is highlighted in italicized capital letters.

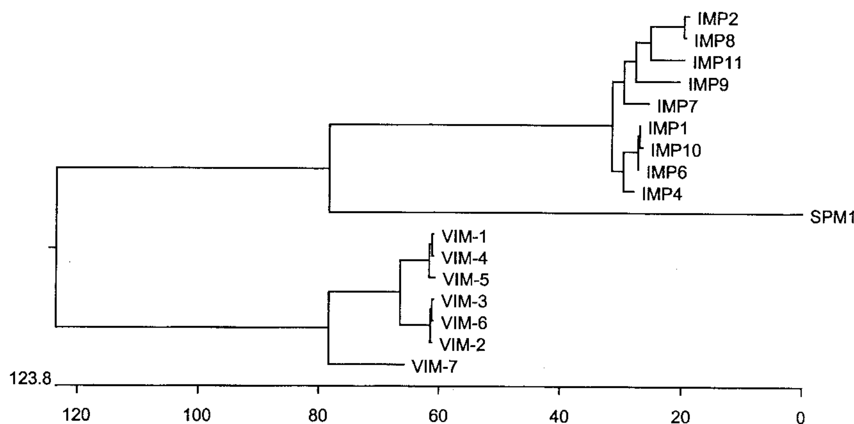

FIG. 2. Phylogenetic tree showing the relatedness of VIM-7 to other mobile metallo-β-lactamases. The phylogenetic tree is based on a CLUSTAL W alignment of a metallo-β-lactamase proteins generated by using the PAM250 MATRIX.

(*attI*1 site). The $bla_{\text{VIM-7}}$ genetic locus does differ in that no additional antimicrobial resistance genes were found directly downstream of $bla_{\text{VIM-7}}$. The 600 bp of DNA downstream of $bla_{\text{VIM-7}}$ in the recombinant plasmid pMATVIM-7 displays no homology with any antimicrobial resistance genes or the 3′ conserved sequence (CS) found in most class 1 integrons. The 3′ CS is a section of DNA conserved among many class 1 integrons, consisting of the remnants of a quaternary ammonium compound resistance gene, *qacEΔ1*, fused to a sulfonamide resistance gene, *su1*. It is possible that this section is present further downstream of the $bla_{\text{VIM-7}}$ gene that is not represented in the clone pMATVIM-7. However, the 3′ CS is not found in all class 1 integrons (e.g., Tn*402*) and is not required for mobility.

VIM-7, unlike the other enzymes of the VIM group, is markedly divergent (Fig. 2). VIM-7 shows 77% identity with VIM-1, whereas the other enzymes vary from each other by less than 11%. Many of the changes unique to VIM-7 were changes in amino acids possessing different functions and may well alter the biochemical properties of the enzyme. However, though divergent from other VIM-like proteins, VIM-7 shares even less homology with other metallo-β-lactamases and therefore may be considered part of the VIM family. These data and the genetic context of the $bla_{\text{VIM-7}}$ gene indicate that VIM-7 is only distantly related to the other VIM-like metallo-β-lactamases. It is therefore unlikely that VIM-7 has arrived on the North American continent via immediate dissemination from Europe or East Asia, where the other VIM enzymes have been re-

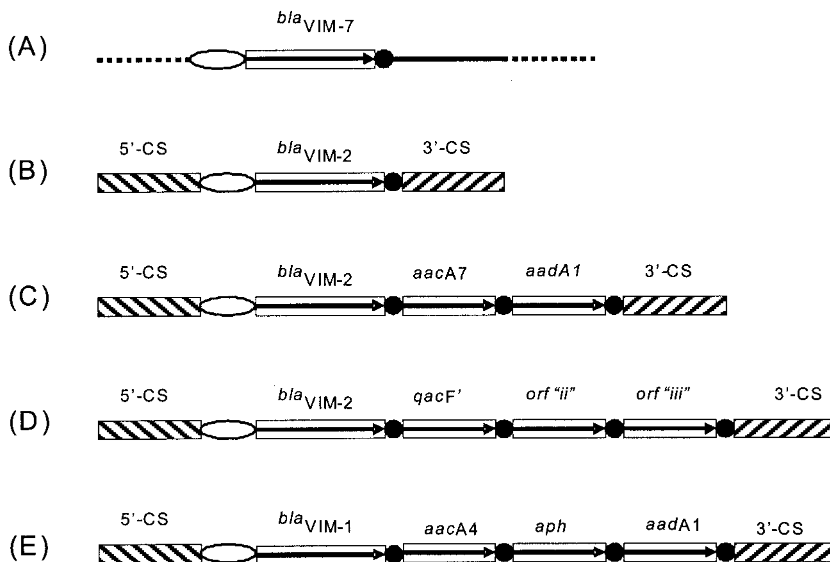

FIG. 3. Genetic context of VIM-7 and VIM metallo-β-lactamases. A schematic map of the recombinant plasmid PMATVIM-7 carrying $bla_{\text{VIM-7}}$ (accession no. AJ536835 [A]) is compared with examples of structures of $bla_{\text{VIM-2}}$-containing class 1 integrons isolated in France (accession no. AF191564 [B]) and Korea (accession no. AF369871 [C] and accession no. AY030343 [D]) and with the $bla_{\text{VIM-1}}$ integron isolated from Italy (accession AJ278514 [E]). Hatched rectangles indicate 5′ and 3′ CSs, black circles indicate 59-bp elements, and open ellipses indicate *attI*1 sites. Open reading frames of the various resistance genes are boxed, with an arrow indicating the direction of transcription. The solid line in pMATVIM-7 indicates the clones' insert from *P. aeruginosa* 07-406, and dotted lines indicate the cloning vector pK18.

ported. It is probable that VIM-7 has arisen independently within the United States, possibly by the therapeutic use of broad-spectrum β-lactams.

This is the first report of a mobile metallo-β-lactamase, VIM-7, being found in the bacterial population of the United States. bla_{VIM-7} has been shown to be highly mobile and can be expressed in *Enterobacteriaceae* as well as *Pseudomonas* spp. Given that these enzymes can hydrolyze all known classes of therapeutic β-lactams and that there is no clinically available metallo-β-lactamase inhibitor, mobile metallo-β-lactamases will pose a serious threat to broad-spectrum β-lactam therapies within the United States.

Nucleotide sequence accession number. The nucleotide sequence reported here has been registered with the EMBL database under accession no. AJ536835.

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