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# Class 1 Integron Containing Metallo-β-Lactamase Gene *bla*$_{VIM-2}$ in *Pseudomonas aeruginosa* Clinical Strains Isolated in Japan

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**Four *bla*$_{VIM-2}$ gene-harboring *Pseudomonas aeruginosa* strains were identified. These strains possessed a class 1 integron harboring *ORF1*, *bla*$_{VIM-2}$, and *aacA4* gene cassettes. The transposon-mediated horizontal spread of the *bla*$_{VIM-2}$ gene among these strains was suggested, which increases the threat that the *bla*$_{VIM-2}$ gene will disseminate among diverse genera of bacteria.**

The emergence of metallo-β-lactamase (MBL)-producing bacilli that are resistant to carbapenems is becoming a severe therapeutic problem (9). Two types of MBLs, IMP and VIM, have been reported (13). IMP-1 was identified in *Pseudomonas aeruginosa* in Japan in 1991 (15). Strains producing IMP-type MBLs have also been reported in Hong Kong (3), Taiwan (17), and Italy (12). Strains producing VIM-type MBLs were originally reported in European countries. VIM-1 was identified in *P. aeruginosa* in Italy in 1999 (7), and VIM-2 was identified in France (10). Thereafter, VIM-3 was identified in Taiwan (16). However, there have been few reports describing VIM-type-MBL-producing bacteria in Japan. The genes of both IMP- and VIM-type MBLs (*bla*$_{IMP}$ and *bla*$_{VIM}$, respectively) are often encoded on mobile gene cassettes inserted into class 1 integrons (1, 7, 10). The class 1 integrons are genetic elements capable of integrating gene cassettes by a site-specific recombination mechanism (4). Gene cassettes are mobile units composed of a gene, most often an antibiotic resistance gene, and a recombination site, the 59-base element (4). Integrons are sometimes found as a part of transposons (4), which is probably the reason that they are found in many different genetic locations. In this work, we report on the characterization of the *bla*$_{VIM-2}$ gene cassette-harboring class 1 integron identified in the *P. aeruginosa* clinical strains isolated in one hospital in Akita Prefecture, Japan.

Clinical isolates were screened for MBL production by a disk diffusion test (2) modified for use with disks containing 3 mg of sodium mercaptoacetate. An increase of more than 5 mm in

TABLE 1. Primers used for detection of MBL genes and genes comprising the *bla*$_{VIM-2}$ gene-containing integron and for sequencing of the *bla*$_{VIM-2}$ gene containing integron

| Primer | Positions[a] | Sequence (5′ to 3′) | Strand | Target gene | Accession no. | Amplicon size (bp) |
|---|---|---|---|---|---|---|
| VIM S[b] | 1400–1419 | CCG ATG GTG TTT GGT CGC AT | + | | | |
| VIM AS[b] | 1772–1790 | GAA TGC GCA GCA CCA GGA T | − | *bla*$_{VIM}$ | Y18050 | 391 |
| IMP S | 661–680 | AAA GAT ACT GAA AAG TTA GT | + | | | |
| IMP AS | 1087–1106 | TCY CCA AYT TCA CTR TGA CT | − | *bla*$_{IMP}$ | S71932 | 446 |
| ORF1 S | 519–539 | ATG ATT ACC GGC ATC AAT CAC | + | | | |
| ORF1 AS | 900–917 | TCA GCT CCA CAC CAG CCC | − | *ORF1* | AY294333 | 399 |
| AACA4 S | 1975–1994 | ATG ACT GAG CAT GAC CTT GC | + | | | |
| AACA4 AS | 2474–2493 | TTA GGC ATC ACT GCG TGT TC | − | *aacA4* | AY294333 | 519 |
| INT5/CSBH[c] | 758–779 | AGC TAG ATC CTT CTA GAA AAC CGA GGA TGC | + | *IntI1* | AF191564 | —[e] |
| INT3/CSJY2ER[d] | 2408–2431 | AGC TAA AAT TGC GAT GCC ATA ACC GAT TAT GAC | − | *qacEΔ1* | AF191564 | — |
| VIMseq S | 1286–1305 | TGA CCG CGT CTG TCA TGG CT | + | *bla*$_{VIM-2}$ | Y18050 | — |
| VIMseq AS | 1880–1899 | CAG ATC GGC ATC GGC CAC GT | − | *bla*$_{VIM-2}$ | Y18050 | — |
| INT1 S | 321–338 | GAA CGC AGC GGT GGT AAC | + | Noncoding region | AY294333 | — |
| INT/5CS2 | 521–544 | GAT TAC CGG CAT CAA TCA CAT CAC | + | *ORF1* | AY294333 | — |
| VIMS2 | 1665–1687 | CGT GGC CGA TGC CGA TCT GGC TG | + | *bla*$_{VIM-2}$ | AY294333 | — |

[a] Positions given in nucleotides.
[b] Also employed for sequencing.
[c] *Bam*HI recognition site is added to the 5′ end.
[d] *Eco*RI recognition site is added to the 5′ end.
[e] —, sequence primer.

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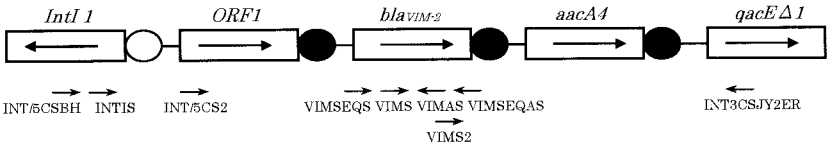


FIG. 1. Schematic structure (not to scale) of the approximately 3-kb integron of *P. aeruginosa* strain Mβ-7, containing the $bla_{VIM-2}$ gene cassette. The gene cassettes are boxed. Arrows indicate transcriptional orientation. The *attI1* recombination site is represented by a white circle, and the 59-base elements are indicated by black circles. Primers used for sequencing are shown under the integron structure.

the diameter of the inhibition zone around the ceftazidime disk (30 μg) in the presence of the sodium mercaptoacetate disk indicated a screening test positive for MBL production. The $bla_{IMP}$ and $bla_{VIM}$ genes were detected by PCR by using the consensus primer pairs IMP S and IMP AS for the $bla_{IMP}$ genes and VIM S and VIM AS for the $bla_{VIM}$ genes (Table 1). The $bla_{VIM}$ gene was typed by direct sequencing by using the VIMseq S and VIMseq AS primers (Table 1). Four $bla_{VIM-2}$ gene-positive *P. aeruginosa* strains, Mβ-2, Mβ-6, Mβ-7, and Mβ-9, were employed in this study. Two fragments, INT5CS-VIM AS and VIM S-INT3CS, were amplified from strain Mβ-7 by PCR with two primer sets, INT5/CSBH and VIM AS and VIM S and INT3/CSJY2ER, and sequenced by using the primers listed in Table 1, as described previously (18). The annealing sites of these primers are shown schematically in Fig. 1. Pulsed-field gel electrophoresis (PFGE) was performed as described by Speijer et al. (13) by using *Spe*I. The chromosomal DNA fragments were analyzed by Southern blot hybridization as described previously (18) by using the $bla_{VIM-2}$ DNA probe, which was prepared by PCR by using the VIMseq S and VIMseq AS primers (Table 1). INT5CS-VIM AS and VIM S-INT3CS fragments amplified from strains Mβ-2, Mβ-6, and Mβ-9 were analyzed by Southern blot hybridization for the presence of the *ORF1*, $bla_{VIM-2}$, and *aacA4* genes. The *ORF1* DNA probe and the *aacA4* DNA probe were prepared by PCR by using the ORF1 S and ORF1 AS primers and the AACA4 S and AACA4 AS primers (Table 1), respectively.

From September 2001 to October 2002, 16 isolates tested positive for MBL production by the disk diffusion screening test. Five strains, Mβ-3, Mβ-4, Mβ-5, Mβ-8, and Mβ-12, were positive for the $bla_{IMP}$ gene, and four *P. aeruginosa* isolates, Mβ-2, Mβ-6, Mβ-7, and Mβ-9, were positive for the $bla_{VIM-2}$ gene. Sequence analysis of the integron from strain Mβ-7 revealed a class 1 integron structure. As shown in Fig. 1, this class 1 integron contained three gene cassettes. The first cassette contained a 399-bp open reading frame of unknown function, *ORF1*. The second cassette contained the $bla_{VIM-2}$ gene, and the third cassette contained the *aacA4* gene, which encodes aminoglycoside acetyltransferase. Southern blot hybridization analysis of the INT5CS-VIM AS and VIM S-INT3CS fragments amplified from strains Mβ-2, -6, and -9 revealed that all of these strains also contained the integron harboring the *ORF1*, $bla_{VIM-2}$, and *aacA4* genes (data not shown). *Spe*I PFGE patterns for the four isolates differed only within three bands, indicating that these isolates are closely related (Fig. 2A). The Southern blot analysis of the *Spe*I-digested chromosomal PFGE fragments revealed that the $bla_{VIM-2}$ gene is located on an approximately 60-kb fragment in Mβ-2, a 280-kb fragment in Mβ-7, and a 240-kb fragment in Mβ-9 but on no fragment in Mβ-6 (Fig. 2B).

We have shown in this study that *P. aeruginosa* strains harboring the $bla_{VIM-2}$ gene have been disseminated in one hospital in Akita Prefecture, Japan, confirming that $bla_{VIM-2}$ gene-harboring strains are now widespread in eastern Asian countries. Earlier studies reported that $bla_{VIM-1}$ is located on the chromosome of *P. aeruginosa* strain VR-143/97 (7) but that $bla_{VIM-2}$ is located on an approximately 45-kb plasmid (10) or on the fragments of *Xba*I-digested genomic DNA (8). In this study, the $bla_{VIM-2}$ gene was found in various genetic locations, which suggests the horizontal spread of the $bla_{VIM-2}$ gene among these four *P. aeruginosa* strains. The precise mechanism by which the $bla_{VIM-2}$ gene achieved a horizontal spread among these four strains is unclear. Our results demonstrate that the four $bla_{VIM-2}$ gene-positive *P. aeruginosa* isolates harbored integrons of the same size and containing three genes, *ORF1*, $bla_{VIM-2}$, and *aacA4*, which suggests the horizontal spread of the integron itself, rather than of the $bla_{VIM-2}$ gene-containing gene cassette among different integrons. Although integrons themselves are not mobile, several class 1 integrons have been found in Tn*21* and Tn*21*-related transposons (4, 5, 14), which enables the integrons to be transposed. These findings raise the possibility that the class 1 integron described in this study is also part of a transposon. The structures of several

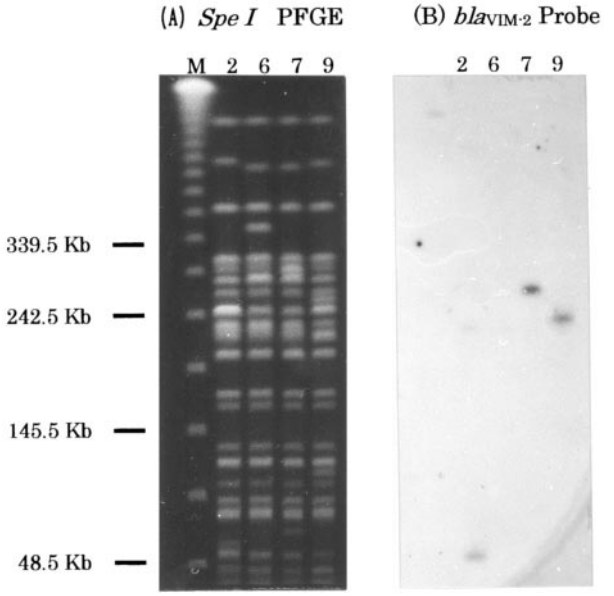


FIG. 2. Southern hybridization analysis of the *Spe*I-digested chromosomal DNA fragments from Mβ-2, -6, -7, and -9. PFGE patterns (A) and results of Southern hybridization using a $bla_{VIM-2}$ DNA probe (B) are shown. Lanes: M, lambda molecular weight ladder; 2, Mβ-2; 6, Mβ-6; 7, Mβ-7; 9, Mβ-9.

*bla*$_{VIM-2}$ gene-containing integrons (6, 8, 10, 11), including the integron identified in strain Mβ-7, are unique, indicating that the *bla*$_{VIM-2}$ gene cassette has disseminated among various integrons worldwide. Moreover, our present results suggest the possibility that the *bla*$_{VIM-2}$ gene cassette-harboring integron is associated with a transposon, which increases the threat that the *bla*$_{VIM-2}$ gene will disseminate among diverse genera of bacteria.

**Nucleotide sequence accession numbers.** Sequences for the *bla*$_{VIM-2}$ genes from isolates Mβ-2, Mβ-6, Mβ-7, and Mβ-9 were submitted to GenBank under accession no. AY242981 to AY242984. The sequence for the integron from strain Mβ-7 was submitted under accession no. AY294333.

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