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Identification of *Pseudomonas aeruginosa* Clinical Strains Harboring the bla_{VIM-2} Metallo-β-Lactamase Gene in Akita Prefecture, Japan

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Gram-negative bacilli producing metallo-β-lactamases (MBLs) show resistance to carbapenems, such as imipenem, which are often used to treat infections caused by Gram-negative bacteria that are resistant to other β-lactam antibiotics. Therefore, the emergence of MBL-producing bacteria is becoming a severe therapeutic problem. Two carbapenem-hydrolyzing MBLs, IMP and VIM, have been reported (1). An IMP-type MBL, IMP-1, was identified in *Pseudomonas aeruginosa* in Japan (2) and variants of the IMP-type MBL have subsequently been identified in Asian countries (3,4), and in European countries (5). Strains producing the VIM-type MBLs, on the other hand, were originally identified in European countries (6,7). Thereafter, VIM-3, an MBL closely related to VIM-2, was identified in Taiwan (8), indicating that the strains producing VIM-type MBLs have disseminated in Europe and Asia, but there have been few reports describing VIM-type MBL-producing bacteria in Japan. We have previously reported that four *P. aeruginosa* clinical strains isolated in one hospital in Akita Prefecture, Japan, harbored a class 1 integron containing *ORF1*, a gene of unknown function, bla_{VIM-2}, and *aacA4* gene cassettes, suggesting that the class 1 integron spread horizontally among these four strains (9). In this report, we describe the antimicrobial resistance and pulsed-field gel electrophoresis (PFGE) patterns of bla_{VIM-2} gene-positive *P. aeruginosa* clinical strains isolated in Akita.

Gram-negative clinical isolates were screened for MBL production by a disk-diffusion test using commercially available disks containing sodium mercaptoacetate, as

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Table 1. MIC's of *P. aeruginosa* strains harboring bla_{VIM-2} gene isolated in Akita Prefecture

Strain No.	Isolation date	Source	MIC (μg/ml)						
			PIPC	CAZ	IPM	MEPM	GM	CPFX	TFLE
Mβ-2	2001.9.21	sputum	32	>64	>16	>16	>16	0.25	≦0.25
Mβ-6	2002.1.9	sputum	32	64	>16	>16	>16	1	0.5
Mβ-7	2002.1.30	pus	32	>64	>16	16	>16	1	0.5
Mβ-9	2002.6.14	sputum	64	64	>16	16	>16	0.25	≦0.25
Mβ-20	2003.4.17	sputum	16	32	>16	16	8	≦0.12	≦0.25
Mβ-33	2003.7.17	sputum	16	32	4	2	2	≦0.12	≦0.25
Mβ-35	2003.7.30	sputum	16	64	4	2	1	≦0.12	≦0.25
Mβ-40	2003.10.8	sputum	16	32	>16	16	8	≦0.12	≦0.25

PIPC, piperacillin; CAZ, ceftazidime; IPM, imipenem; MEPM, meropenem; GM, gentamicin; CPFX, ciprofloxacin; TFLX, tosufloxacin.

described previously (9). The presence of bla_{IMP} or bla_{VIM} genes was confirmed by PCR in the screening-positive isolates using consensus primer pairs for bla_{IMP} (IMP S: 5′-AAA GAT ACT GAA AAG TTA GT-3′ and IMP AS: 5′-TCY CCA AYT TCA CTR TGA CT-3′, amplicon size: 446 bp), and for bla_{VIM}, (VIM S: 5′-CCG ATG GTG TTT GGT CGC AT-3′, and VIM AS: 5′-GAA TGC GCA GCA CCA GGA-T-3′, amplicon size: 391 bp), respectively, as described previously (9). The VIM gene-type was determined by direct sequencing as described previously (9), or by PCR using bla_{VIM} type-specific primer pairs for bla_{VIM-1} (VIM-1A: 5′-TAG TAG TTT ATT GGT CTA CA-3′ and VIM-1B: 5′-TGT GCT TTG ACA ACG TTC GC-3′, amplicon size: 762 bp), bla_{VIM-2} (VIM-2A: 5′-ATG TTC AAA CTT TTG AGT AAG-3′ and VIM-2B: 5′-CTC AAC GAC TGA GCG ATT TG-3′, amplicon size: 798 bp), and bla_{VIM-3} (VIM-3S: 5′-TTG GTC GCA TAT CGC AAC GA-3′ and VIM-3AS: 5′-AGA GTG CGT GGG AAT CTC GC-3′, amplicon size: 304 bp), respectively. The minimum inhibitory concentrations (MICs) of the isolates were determined by the broth microdilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (10,11) using a commercially available plate, Dryplate "Eiken" DP-25 (Eiken Kagaku Co., Tokyo, Japan). PFGE was performed using *Spe*I as described previously (9).

From September 2001 to October 2003, 42 isolates were shown to be positive for MBL production using the disk diffusion-screening test in the clinical laboratories of several hospitals in Akita. Among the 42 isolates, PCR using the consensus primer pairs revealed eight strains positive for the bla_{VIM} gene and 24 strains positive for the bla_{IMP} gene (data not shown). The remaining ten strains were negative for both bla_{IMP} and bla_{VIM}, and the mechanism involved in the resistance of the ten strains has not been further examined. The bla_{VIM} gene detected in the eight strains was bla_{VIM-2} (data not shown). Isolation date, source, and the antibiotic susceptibility patterns of the eight bla_{VIM-2} gene-harboring strains are summarized in Table 1. These eight strains were isolated in one hospital. Strains Mβ-20 and Mβ-40 were isolated from the same patient. All these strains were sensitive to piperacillin, ciprofloxacin, and tosufloxacin. Strains except Mβ-33 and Mβ-35 were resistant to imipenem and meropenem according to the NCCLS resistance breakpoint of ≥16 μg/ml. Strains Mβ-2, Mβ-6, Mβ-7, and Mβ-9 which were previously shown to harbor the *aacA4* gene (9) were resistant to gentamicin. Only strain Mβ-9 was resistant to ceftazidime. The *Spe*I PFGE patterns of the eight strains are shown in Fig. 1. Strains Mβ-2, Mβ-6, Mβ-7, and Mβ-9 showed similar PFGE patterns with only a few band differences, indicating that they belong to a closely related clone, as described previously (9). Strains Mβ-20 and Mβ-40 which were isolated from identical patient showed quite similar PFGE patterns with a minor one band difference, and the pattern was markedly different from those of strains Mβ-2, Mβ-6, Mβ-7, and Mβ-9. The PFGE patterns of strains Mβ-33 and Mβ-35 were different from those of other strains, indicating that four independent clones of bla_{VIM-2} gene-harboring *P. aeruginosa*, Mβ-2 to Mβ-9, Mβ-20 and Mβ-40, Mβ-33, and Mβ-35, have emerged in one hospital in Akita.

VIM-type MBL-producing strains were first reported only in European countries (6,7). Recently, Yan et al. isolated *P. aeruginosa* strain-producing the VIM-3 type MBL, a novel variant of the VIM-2 type, in Taiwan (8) and pointed out that the VIM-2-related MBLs are the most prevalent MBLs in Taiwan. VIM-2-producing *P. aeruginosa*, *Pseudomonas putida* (12), *Serratia marcescens* (13), and *Acinetobacter baumannii* (14) have also been reported in Korea. We have previously shown that *P. aeruginosa* strains that harbor the bla_{VIM-2} gene were also present in Akita (9), and VIM-2-producing *P. aeruginosa* strains have been increasingly

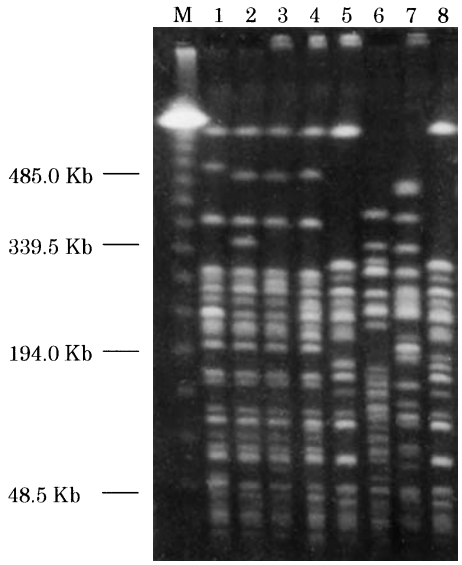

Fig. 1. PFGE patterns of the *Spe*I-digested chromosomal DNA fragments from bla_{VIM-2}-harboring *P. aeruginosa* isolates. Lanes: M, lambda molecular weight ladder; 1, Mβ-2; 2, Mβ-6; 3, Mβ-7; 4, Mβ-9; 5, Mβ-20; 6, Mβ-33; 7, Mβ-35; 8, Mβ-40.

isolated in hospitals in Japan, even though there have been few reports. Our present results provide molecular epidemiological evidence that bla_{VIM-2} gene-harboring *P. aeruginosa* strains belonging to independent clones have emerged in one hospital in Akita. Strains Mβ-33 and Mβ-35 were imipenem- and meropenem-sensitive, even though they were positive for the bla_{VIM-2} gene. The reason for this discrepancy is unclear, but low VIM-2 enzyme expression or altered membrane permeability of these strains against imipenem and meropenem is a possible candidate for this mechanism, which should be further elucidated.

Bacterial strains harboring the bla_{VIM-2} gene are a severe therapeutic problem, but their emergence in Japan still has not been well demonstrated. Our present results along with previous one pose the possibility that these strains have already emerged in Japan as in the other Asian countries, and that this possibility should be further elucidated.

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