Metallo- β -Lactamases in Clinical *Pseudomonas* Isolates in Taiwan and Identification of VIM-3, a Novel Variant of the VIM-2 Enzyme

JING-JOU YAN,¹ PO-REN HSUEH,² WEN-CHIEN KO,³ KWEN-TAY LUH,²
SHU-HUEI TSAI,¹ HSIU-MEI WU,⁴ AND JIUNN-JONG WU^{4*}

Department of Pathology, National Cheng Kung University Medical Center,¹ and Departments of Medicine³ and Medical Technology,⁴ National Cheng Kung University Medical College, Tainan, Department of Laboratory Medicine, National Taiwan University Hospital, Taipei,² Taiwan

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A total of 209 clinical isolates of *Pseudomonas* (193 *Pseudomonas aeruginosa*, 10 *P. putida*, 4 *P. stutzeri*, and 2 *P. fluorescens* isolates) with reduced susceptibilities to imipenem and/or ceftazidime were subjected to PCR assays with primers specific for *bla*_{IMP-1}, *bla*_{IMP-2}, *bla*_{VIM-1}, and *bla*_{VIM-2} and sequence analysis to identify the metallo- β -lactamases (MBLs) prevalent among these organisms in Taiwan; and 21 isolates gave positive results. Five isolates including two *P. putida* and three *P. stutzeri* isolates were found to carry *bla*_{IMP-1}, and six isolates including five *P. putida* and one *P. stutzeri* isolates harbored *bla*_{VIM-2}. The remaining 10 isolates were *P. aeruginosa*, and all were found to carry a novel variant of *bla*_{VIM-2}, designated *bla*_{VIM-3}. There are only two nucleotide differences between *bla*_{VIM-2} and *bla*_{VIM-3}, leading to two amino acid alterations. Our findings indicate that VIM-2 and its variant have become the most prevalent metalloenzymes in *Pseudomonas* in Taiwan. Southern hybridization with the *bla*_{VIM-2}, *bla*_{VIM-3}, and *bla*_{IMP-1}-specific probes revealed that only two VIM-2-producing *P. putida* isolates appeared to carry the MBL gene on plasmids. Pulsed-field gel electrophoresis showed that six VIM-3-producing *P. aeruginosa* isolates and two IMP-1-producing *P. stutzeri* isolates were genetically related, suggesting that the spread of these MBL genes in Taiwan could be due to clonal dissemination as well as genetic exchange between different clones.

Carbapenems are often used as antibiotics of last resort for the treatment of infections caused by gram-negative bacteria resistant to other β -lactam agents because of their broad spectra of activity and stability to hydrolysis by most β -lactamases including extended-spectrum β -lactamases (4, 12, 26, 27). The mechanisms of resistance to carbapenems in *Pseudomonas aeruginosa* often result from reduced levels of drug accumulation or increased levels of expression of pump efflux (8, 15, 17). The resistance is occasionally due to production of metallo- β -lactamases (MBLs), which can be either chromosomally encoded or plasmid mediated (8, 13, 22, 28, 32). IMP-1 was the first MBL identified in *P. aeruginosa* (28, 29). The enzyme has also been described in other nonfastidious, gram-negative nonfermenters and members of the family *Enterobacteriaceae* (9, 10, 20, 29). Several novel MBLs were identified recently, including VIM-1 from *P. aeruginosa* and IMP-2 from *Acinetobacter baumannii* in Italy (13, 25), VIM-2 from *P. aeruginosa* in France (22), and IMP-3 from *Shigella flexneri* in Japan (11). The spread of MBLs in gram-negative rods has been described in several other countries and is becoming an emerging threat (32; T. H. Koh, G. S. Babini, N. Woodford, L.-H. Sng, L. M. C. Hall, and D. M. Livermore, Letter, Lancet 353:2162, 1999; K. Lee, J. B. Lim, J. Yum, D. Yong, J. R. Choi, Y. Chong, J. M. Kim, and D. M. Livermore, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2003, p. 123, 2000). It re-

mains unknown whether these MBLs have appeared in Taiwan. Thus, the purpose of the present study was to determine the IMP- and VIM-type MBLs prevalent among clinical isolates of the genus *Pseudomonas* in Taiwan.

MATERIALS AND METHODS

Bacterial strains. The isolates studied were selected from clinical isolates of *Pseudomonas* spp. randomly collected between January 1997 and May 2000 at two university hospitals in Taiwan. Since susceptibilities to imipenem for IMP-1 producers covered a wide range (9, 10, 28, 29), all isolates that showed reduced susceptibilities to imipenem (inhibition zone diameter, <16 mm) and/or ceftazidime (inhibition zone diameter, <18 mm) on the basis of the results of the disk diffusion method (19) were examined. Thus, a total of 209 isolates, including 164 *Pseudomonas* isolates (154 *P. aeruginosa*, 4 *P. stutzeri*, 4 *P. putida*, and 2 *P. fluorescens* isolates) from the National Cheng Kung University Medical Center, a 900-bed teaching hospital in southern Taiwan, and 45 *Pseudomonas* isolates (39 *P. aeruginosa* and 6 *P. putida* isolates) from the National Taiwan University Hospital, a 1,800-bed medical center in northern Taiwan, were selected. All these isolates were recovered from different patients and were identified by conventional techniques (6) and/or with the API 20NE system (bioMérieux, Marcy l'Etoile, France).

Screening tests for detection of MBL producers. The disk diffusion test for the screening of MBL producers was performed as described by Arakawa et al. (1). 2-Mercaptopropionic acid and 100 mM EDTA were used as MBL inhibitors.

PCR amplification and DNA sequencing. Plasmids from clinical isolates were prepared by a rapid alkaline lysis procedure (31). PCR assays were performed to amplify the entire sequences of the *bla*_{IMP-1}, *bla*_{IMP-2}, *bla*_{VIM-1}, and *bla*_{VIM-2} genes with the oligonucleotide primers listed in Table 1. PCR conditions for all these genes were 3 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C; and finally, 7 min at 72°C. The amplicons were purified with PCR Clean Up Kits (Roche Molecular Biochemicals, Mannheim, Germany) and were sequenced on an ABI PRISM 377 sequencer analyzer (Applied Biosystems, Foster City, Calif.) with the primers listed in Table 1.

Analytical IEF. Crude preparations of β -lactamases were obtained by sonication (3) and were subjected to analytical isoelectric focusing (IEF) as described

* Corresponding author. Mailing address: Department of Medical Technology, National Cheng Kung University Medical College, No. 1 University Rd., Tainan, Taiwan 70101. Phone: 886-6-2353535, ext. 5775. Fax: 886-6-2363956. E-mail: jjwu@mail.ncku.edu.tw.

TABLE 1. Primers for PCR detection of MBL genes

Gene	Use	Primers (5' to 3')	Position ^a	Expected PCR product (bp)
<i>bla</i> _{IMP-1}	Amplification and sequencing	TGAGCAAGTTATCTGTATTC	2 to 21	740
	Sequencing	TTAGTTGCTTGGTTTTGATG	741 to 722	
		CGGGCCTGGATAAAAAACTT	465 to 446	
		GCCACAAATTCATTTAGCGG	394 to 413	
<i>bla</i> _{IMP-2}	Amplification	GGCAGTCGCCCTAAACAAA	−37 to −18	737
		TAGTTACTTGGCTGTGATGG	740 to 721	
<i>bla</i> _{VIM-1}	Amplification	TTATGGAGCAGCAACGATGT	−81 to −62	920
		CAAAAGTCCCCTCCAACGA	839 to 820	
<i>bla</i> _{VIM-2}	Amplification and sequencing	AAAGTTATGCCGCACTCACC	−34 to −15	865
		TGCAACTTCATGTTATGCCG	831 to 812	
	Sequencing	TCGACGGTGATGCGTACGTT	415 to 396	
		TTGATGTCCTTCGGGCGGCT	368 to 387	

^a Position number 1 for every MBL gene corresponds to the first base of the start codon.

previously (18, 33). β -Lactamase activity was detected by overlaying the gels with 0.5 mM nitrocefin (Oxoid, Basingstoke, United Kingdom) in 50 mM HEPES (pH 7.5) supplemented with 2 mM ZnCl₂ (25). Extracts from TEM-1-, TEM-10-, SHV-1-, CMY-1-, SHV-5-, CTX-M-3-, and CMY-2-producing strains were used as standards for pIs of 5.4, 5.6, 7.6, 8.0, 8.2, 8.4, and 9.0, respectively. In addition, the pI was also calculated by reference to a calibration curve constructed from the relative mobility of the IEF markers (Amersham Pharmacia Biotech, Hong Kong, China).

Transfer of resistance. Conjugation experiments were performed as described previously (23, 33) with streptomycin- and rifampin-resistant *Escherichia coli* C600 as the recipient (2). Tryptic soy agar plates supplemented with 500 μ g of streptomycin (Sigma Chemical Company, St. Louis, Mo.) per ml or 64 μ g of rifampin (Sigma) per ml and 10 μ g of ceftazidime (Glaxo Group Research Ltd., Greenford, United Kingdom) per ml were used to select the transconjugants. To determine the sensitivity of the assay, direct transfer of the plasmid-encoded *bla*_{OXA-10} gene from a clinical isolate of *P. aeruginosa* into *E. coli* C600 was attempted, and transconjugant selection was performed on tryptic soy agar plates containing 100 μ g of amoxicillin (SmithKline Beecham Pharmaceuticals, Surrey, United Kingdom) per ml and 64 μ g of rifampin per ml.

Hybridization assays. In order to confirm negative PCR results, colony hybridization was performed for all 209 isolates studies, as described elsewhere (7). The PCR products of *bla*_{VIM-2}, *bla*_{VIM-3}, and *bla*_{IMP-1} were labeled with [α -³²P]dCTP (Amersham Pharmacia Biotech) by the random priming technique with a commercial kit (GIBCO BRL, Life Technologies, Gaithersburg, Md.). The *bla*_{VIM-1}- and *bla*_{IMP-2}-specific probes were the PCR-generated amplicons from VIM-1-containing *P. aeruginosa* VR-143/97 (13) and IMP-2-containing *A. baumannii* AC-54/97 (25), respectively.

Plasmids from the clinical isolates harboring the MBL genes were digested with *Bst*EII (Roche Molecular Biochemicals) overnight. The digested DNA samples were electrophoresed on 0.8% agarose gels and subjected to Southern hybridization according to the original protocol (30) with the *bla*_{VIM-2}-, *bla*_{VIM-3}-, and *bla*_{IMP-1}-specific probes prepared as described above.

Susceptibility tests. The MICs of the antimicrobial agents were determined by the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (19). The antimicrobial agents and their sources are as follows: aztreonam, Bristol-Myers Squibb, New Brunswick, N.J.; ceftazidime, Glaxo Group Research Ltd.; cefotaxime, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.; imipenem, Merck Sharp & Dohme, West Point, Pa.; meropenem, Sumitomo Pharmaceuticals Ltd., Osaka, Japan; and piperacillin and tazobactam, Lederle Laboratories, Pearl River, N.Y.

PFGE analysis. Genomic DNAs prepared by the procedure of Piggot et al. (21) were digested overnight with 10 U of *Spe*I (New England Biolabs, Beverly, Mass.) and subjected to pulsed-field gel electrophoresis (PFGE) as described elsewhere (5, 33). The samples were electrophoresed with the Pulsaphor plus system (Amersham Pharmacia Biotech) at 200 V for 30 h, with pulse times ranging from 5 to 30 s.

Nucleotide sequence accession number. The nucleotide sequence of the novel MBL described in this paper has been assigned to the GenBank database under accession number AF300454.

RESULTS

Detection of MBL producers. Among the 209 *Pseudomonas* isolates tested, 21 (10 *P. aeruginosa*, 7 *P. putida*, and 4 *P. stutzeri* isolates) potentiated the inhibitory zones between ceftazidime disks and EDTA disks or 2-mercaptopropionic acid disks, suggesting production of MBLs, the activities of which were inhibited by EDTA and 2-mercaptopropionic acid, by these isolates.

PCR amplification and DNA sequencing. Of the 209 *Pseudomonas* isolates that were subjected to PCR assays, all 21 isolates positive by the screening test gave positive PCR results, while the remaining 188 isolates were negative. Sixteen isolates (10 *P. aeruginosa*, 5 *P. putida*, and 1 *P. stutzeri* isolates) were positive with the primers specific for *bla*_{VIM-2}, and 5 (2 *P. putida* and 3 *P. stutzeri* isolates) were positive with the primers specific for *bla*_{IMP-1}. Neither *bla*_{VIM-1} nor *bla*_{IMP-2} was amplified from any of the 209 isolates. The characteristics of these isolates are shown in Table 2. The sequence analysis revealed that 5 *P. putida* and 1 *P. stutzeri* isolates carried *bla*_{VIM-2} and that 2 *P. putida* and 3 *P. stutzeri* isolates harbored *bla*_{IMP-1}. All 10 *P. aeruginosa* isolates were found to carry a variant *bla*_{VIM-2}, designated *bla*_{VIM-3}. The novel MBL gene differs from *bla*_{VIM-2} by replacement of a C with an A and an A with a G at nucleotides 178 and 443 of the structural gene, respectively, which results in Gly-to-Lys and Asn-to-Ser changes at amino acid positions 32 and 120 (24), respectively, according to the numbering for *Bacillus cereus* β -lactamase II (24).

Hybridization assays and conjugation experiments. The results of colony hybridization assays were consistent with those obtained by PCR. All 10 VIM-3 and 6 VIM-2 producers that were recognized by PCR hybridized with both the *bla*_{VIM-3}- and the *bla*_{VIM-2}-specific probes, and the 5 IMP-1 producers hybridized with the *bla*_{IMP-1}-specific probe. All other isolates showed negative results with the probes specific for *bla*_{VIM-1}, *bla*_{VIM-2}, *bla*_{VIM-3}, *bla*_{IMP-1}, and *bla*_{IMP-2}.

Conjugation experiments failed to demonstrate transfer of carbapenem resistance from any of the isolates. Amoxicillin resistance was transferred from the control strain to *E. coli* C600 at a low frequency (10^{-8} to 10^{-10} per donor cell). Plasmid DNAs were isolated from three *P. aeruginosa* isolates

TABLE 2. Characteristics of the 21 *Pseudomonas* isolates carrying MBL genes

Species and isolate ^a	Source	Collection date (yr.mo)	pI(s) of β -lactamase(s)	MBL	Location of MBL genes ^b	MIC (μ ml) ^c							PFGE profile
						PIP	TZP	CTX	CAZ	ATM	IPM	MEM	
<i>P. aeruginosa</i>													
NTU-23/99	Urine	1999.11	5.1, 8.0	VIM-3	Chromosome	128	128	>256	>256	8	>32	8	A
NTU-26/99	Blood	1999.12	5.1, 5.6, 8.0	VIM-3	Chromosome	>256	64	>256	>256	1	>32	8	B
NTU-33/99	Tonsil	1999.12	5.1, 5.6, 8.3	VIM-3	Chromosome	256	128	>256	>256	1	>32	16	C
NTU-34/99	Urine	1999.12	5.1, 8.0	VIM-3	Chromosome	256	64	>256	>256	8	>32	8	A1
NTU-39/00	Urine	2000.1	5.1, 8.0	VIM-3	Chromosome	128	64	>256	>256	16	>32	>32	A2
NTU-43/00	Sputum	2000.1	5.1, 5.6, 8.3	VIM-3	Chromosome	256	64	>256	>256	16	16	4	D
NTU-79/00	Sputum	2000.4	5.1, 8.0	VIM-3	Chromosome	>256	>256	>256	>256	2	>32	>32	E
PB-59	Blood	1997.11	5.1, 8.0	VIM-3	Chromosome	256	64	>256	>256	8	>32	32	A3
PS-64/99	Sputum	1999.12	5.1, 8.0	VIM-3	Chromosome	256	32	>256	>256	0.5	32	>32	A4
PS-1205/00	Sputum	2000.5	5.1, 8.0	VIM-3	Chromosome	128	32	>256	>256	2	>32	8	A5
<i>P. putida</i>													
NTU-91/99	Urine	1999.4	5.0, 8.9	VIM-2	Plasmid	128	64	>256	>256	32	>32	>32	F
NTU-93/00	Urine	2000.3	5.0, 8.9	VIM-2	Plasmid	>256	>256	>256	64	32	>32	>32	G
PS-579/00	Urine	2000.2	5.0, 8.2	VIM-2	Chromosome	256	128	>256	128	8	>32	>32	H
PS-679/00	Urine	2000.2	5.0	VIM-2	Chromosome	256	256	>256	64	8	>32	>32	I
PS-693/00	Drainage tube	2000.2	5.0, 8.2	VIM-2	Chromosome	256	256	>256	64	64	>32	>32	J
NTU-92/99	Urine	1999.9	8.7	IMP-1	Chromosome	8	4	>256	>256	128	0.5	0.25	K
PB-166	Blood	1998.11	6.2, 8.7	IMP-1	Chromosome	64	32	>256	>256	64	16	>32	L
<i>P. stutzeri</i>													
PB-24	Blood	1997.5	8.7, 8.9	IMP-1	Chromosome	64	8	>256	>256	16	2	8	M
PB-101	Blood	1998.2	6.9, 8.7	IMP-1	Chromosome	64	32	>256	256	0.25	32	32	N
PB-207	Blood	1999.3	8.7, 8.9	IMP-1	Chromosome	64	8	>256	>256	1	1	2	M
PS-1153/00	Urine	2000.5	5.0	VIM-2	Chromosome	64	32	>256	64	16	>32	>32	O

^a Isolates with the prefix NTU were from the National Taiwan University Hospital, and those with the prefix PB or PS were from the National Cheng Kung University Medical Center.

^b Locations of the MBL genes were suggested by Southern hybridization of plasmid DNA preparations.

^c PIP, piperacillin; TZP, piperacillin-tazobactam; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; IPM, imipenem; MEM, meropenem.

(isolates NTU-39/00, PS-64/99, and PB-59) and three *P. putida* isolates (isolates NTU-91/99, NTU-93/00, and PB-166). For all three *P. aeruginosa* isolates the same restriction pattern was generated by *Bst*EII digestion. The restriction patterns of the three *P. putida* isolates were different, and the *bla*_{VIM-2}-specific probe hybridized to approximately 9-kb DNA fragments for two VIM-2 producers. The probes specific for *bla*_{VIM-2}, *bla*_{VIM-3}, and *bla*_{IMP-1} did hybridize significantly with contaminated chromosomal DNAs for the remaining four VIM-2 producers and all VIM-3 and IMP-1 producers, respectively. Therefore, the MBLs in most of these isolates seem to be chromosomally located.

Analytical IEF. The results of IEF are summarized in Table 2. Since VIM-type MBLs are acidic enzymes (13, 22) and all VIM-3-producing *P. aeruginosa* isolates contained one major band that focused at pH 5.1, the band of pI 5.1 was most likely contributed by VIM-3. The bands with pIs of 5.0 and 8.7 were consistent with those of VIM-2 and IMP-1, respectively (16, 22). The bands with pIs ranging from 8.0 to 8.9 were possibly contributed by AmpC-type β -lactamases of *Pseudomonas* (4).

Susceptibility tests. The drug susceptibilities of the 21 isolates carrying MBL genes were determined and are shown in Table 2. All these isolates were resistant to cefotaxime and ceftazidime. The susceptibilities of these isolates to aztreonam, piperacillin, and carbapenems appeared to be diverse.

PFGE analysis. PFGE revealed that six MBL-producing *P. aeruginosa* isolates were closely or possibly genetically related and that two IMP-1-producing *P. stutzeri* isolates were genetically indistinguishable (Table 2 and Fig. 1).

DISCUSSION

In the present study, 21 isolates of *Pseudomonas* spp. were found to produce MBLs. Only 5 (23.8%) of them carried IMP-1, while 16 (76.2%) isolates harbored the VIM-2-related enzymes. These data indicate that the VIM-2-related enzymes are the most prevalent MBLs among clinical isolates of *Pseudomonas* spp. in Taiwan. The countrywide spread of the IMP-1 enzyme in gram-negative bacilli was reported in Japan (9, 10, 28, 29). Shortly after VIM-1 and VIM-2 were identified in Europe (13, 22), VIM-type MBLs were also found in *Pseudomonas* and *Acinetobacter* spp. in Korea (Lee et al., 40th ICAAC). These findings together indicate that both IMP- and VIM-related MBLs are widespread in eastern Asian countries. The novel enzyme, VIM-3, identified in the present study had only two amino acid differences from VIM-2. Whether the amino acid changes lead to differences in the biochemical characteristics of these two MBLs needs further investigation.

Acquired MBL genes in *P. aeruginosa* are often carried on plasmids and are usually nontransferable by conjugation, at least to *E. coli* (10, 11, 13, 22). In the present study, the MBL genes in our isolates could not be transferred to *E. coli* by conjugation experiments. Southern hybridization showed that only two *P. putida* isolates carried the *bla*_{VIM-2} gene on plasmids. All other isolates seemed to carry these MBL genes on their chromosomes. In addition, clonal spread was suggested by PFGE for six *P. aeruginosa* and two *P. stutzeri* isolates (Fig. 1). Since the *bla*_{VIM} and *bla*_{IMP} genes were found to be on mobile gene cassettes inserted into integrons (11, 13, 22, 25),

FIG. 1. PFGE profiles of *Spe*I-digested genomic DNAs from 21 *Pseudomonas* isolates carrying IMP- or VIM-type MBL genes. (A) Lanes 1 to 9 and 11, VIM-3-producing *P. aeruginosa* isolates NTU-23/99, PS-1205/00, PB-59, NTU-34/99, NTU-39/00, PS-64/99, NTU-26/99, NTU-33/99, NTU-43/00, and NTU-79/00, respectively; lane 10, VIM-2-producing *P. putida* isolate PS-693/00; lane M, a bacteriophage lambda ladder. (B) Lanes 1, 4, 6, 9, and 10, IMP-1 producer isolates PB-207, NTU-92/99, PB-166, PB-101, and PB-24, respectively; lanes 2, 3, 5, 7, and 8, VIM-2 producers isolates PS-1153/00, NTU-91/99, NTU-93/99, PS-579/00, and PS-679/00, respectively; lane M, a bacteriophage lambda ladder.

our findings suggest that the spread of these MBL genes in Taiwan could be due to clonal dissemination as well as to movement of the resistance genes between different clones.

Consistent with the findings reported previously (9, 10, 28, 29), all our MBL-producing isolates were resistant to the broad-spectrum cepheims ceftazidime and cefotaxime, while their susceptibilities to carbapenems, ureidopenicillins, and aztreonam were diverse (Table 2). Expression of MBLs could be cryptic or could be suppressed in the strains demonstrating low-level carbapenem resistance (10). The different permeability coefficients of each β -lactam may play an important role in resistance to each β -lactam in gram-negative bacteria that produce metalloenzymes (15, 17). Active efflux also acts as a contributing factor to β -lactam resistance in *P. aeruginosa* (14). Therefore, the lower MICs of piperacillin for some isolates could be due to their higher permeability coefficients or less efficient efflux pumps. Aztreonam is notable for its relatively low MICs for MBL-producing strains (13, 20, 22, 25). Therefore, reduced susceptibilities to aztreonam in a few isolates could be due to the presence of alternative resistance mechanisms. Isolate NTU-92/99, for which carbapenem MICs were notably low, had a relatively slow growth rate, although it possessed the biochemical and morphologic features characteristic of *P. stutzeri*. The slow growth rate and the difficulty in making suspensions might be partially responsible for the low carbapenem MICs for the isolate.

P. putida and *P. stutzeri* are environmental organisms and rarely cause human diseases (6). *P. stutzeri* has been reported to cause outbreaks of pseudobacteremia by contaminating antiseptics used to prepare skin (6). The medical records of the patients at the National Cheng Kung University Medical Center were reviewed. All these isolates were considered to be from the hospital environment. The three *P. stutzeri* and one *P. putida* isolates from blood samples and the *P. putida* isolate recovered from a drainage tube were of no clinical significance.

They could originate from contaminated antiseptics, the patients' skin, blood culture bottles, or the drainage tube. The two *P. putida* and one *P. stutzeri* isolates from urine samples were all urinary catheterization related, and their clinical significance was not clear. Our findings suggest that the environmental organisms acquired the MBL genes under long-term selection pressure in the hospital environment.

Early detection of MBL-producing strains is important for clinicians for the selection of appropriate antimicrobial agents. PCR assays with primers specific for *bla*_{IMP-1} have been shown to be useful and reliable for detection of IMP-1 producers (29). Given that the numbers of metalloenzymes are increasing (11, 13, 22, 25), the use of multiple primers is needed. Since the PCR method is not cost-effective for use for daily testing in clinical laboratories, Arakawa et al. (1) have designed a simple disk diffusion test for screening for MBL-producing gram-negative rods; the test has a high degree of specificity and a high degree of sensitivity for the detection of IMP-1 producers. The results obtained by the screening test in the present study also correlated well with those obtained by the PCR method, indicating that the test is very useful for the detection not only of IMP-1-producing strains but also of VIM producers.

In conclusion, both IMP- and VIM-type MBLs have emerged in *Pseudomonas* spp. in Taiwan, and the VIM-2-related enzymes were the most prevalent types. The MBL genes were largely located on chromosomes, and their dissemination in Taiwan could be due to both mobilization of the resistance genes and clonal spread of the resistant strains.

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