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Increasing Prevalence and Diversity of Metallo-β-Lactamases in *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacteriaceae* from Korea

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Among imipenem-nonsusceptible isolates, acquired metallo-β-lactamase genes were detected in 36 of 581 (6.2%) *Pseudomonas aeruginosa* isolates, 42 of 44 (95.4%) other *Pseudomonas* species, and 136 of 513 (26.5%) *Acinetobacter* species from 2003 to 2004 at a Korean hospital. Overall, bla_{VIM-2}-like genes were the most prevalent and were also detected in *Enterobacteriaceae*, including *Klebsiella pneumoniae*.

Carbapenems have been the β-lactam antibiotics used most successfully to evade bacterial resistance (8), but acquired carbapenem resistance due to the production of metallo-β-lactamases (MBLs) has been increasingly reported, particularly for *Pseudomonas aeruginosa* and *Acinetobacter* spp. (13).

In recent years, carbapenem resistance found in *P. aeruginosa* and *Acinetobacter* spp. has gradually increased in Korea, and a significant proportion of these carbapenem-resistant isolates have been shown to produce VIM-2- or IMP-1-type MBL (5). Among the members of the family *Enterobacteriaceae*, imipenem resistance has been found to be virtually nonexistent.

In 2003 and 2004, an increasing trend in the rates of carbapenem resistance in *P. aeruginosa* and *Acinetobacter* spp., along with a sudden increase in imipenem-resistant *Klebsiella pneumoniae* isolates, was observed at a Korean tertiary-care hospital. In this study, we determined the prevalence of MBL-producing isolates and identified the types of MBL in imipenem-nonsusceptible *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacteriaceae* isolates from that hospital during that period.

The study included randomly selected imipenem-nonsusceptible *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacteriaceae* isolates from clinical specimens collected in 2003 and 2004. Isolates were identified by conventional testing (4) or with an ATB 32 GN system (bioMerieux, Marcy-l'Etoile, France). A disk diffusion method (2) was used for routine susceptibility testing. MBL-producing isolates were screened with the modified-imipenem-disk Hodge test using MacConkey agar plates and with the double-disk synergy test using imipenem and EDTA-sodium mercaptoacetic acid disks (6). The bla_{IMP-1}-, bla_{VIM-2}-, and bla_{SIM-1}-like genes were detected by PCR as previously described (1, 7, 11). The bla_{VIM-2} gene-carrying integrons from two *K. pneumoniae* isolates were amplified and sequenced as previously described (12). A template prepared from a transconjugant (the recipient was azide-resistant *Escherichia coli* J53) and primers SHV-EXT-F (5′-TTCT TTACTCGCCTTTATCG-3′) and SHV-EXT-R (5′-TTTATG GCGTTACCTTTGAC-3′) were used to detect and sequence the bla_{SHV} allele originally carried in an aztreonam-resistant *K. pneumoniae* isolate.

In 2004, a notable increase in imipenem resistance rates was observed for *P. aeruginosa*, other *Pseudomonas* spp., and *Acinetobacter* spp. compared to those in 2003 (Table 1). Of the relatively small number of imipenem-resistant isolates of *Enterobacteriaceae* found from 2003 to 2004, the majority were *K. pneumoniae*, while the minority consisted of *Enterobacter cloacae* and *Serratia marcescens* (Table 1).

Among the imipenem-nonsusceptible isolates tested, 36 of 581 (6%) *P. aeruginosa* isolates and 42 of 44 (95%) other *Pseudomonas* spp. produced an acquired MBL (Table 1). In Korea, VIM-2 has been the only MBL type detected in *Pseudomonas* spp. since 1995 (5; K. Lee, unpublished results). In the present study, most MBL genes detected in *P. aeruginosa* were bla_{VIM-2}-like, but we also detected for the first time in Korea the presence of two isolates carrying bla_{IMP-1}-like genes. This may suggest dissemination from Japan, where IMP-type enzymes are the most prevalent MBLs in *Pseudomonas* spp. (10). Interestingly, all 42 MBL-producing isolates of other *Pseudomonas* spp. were found to be *Pseudomonas putida* (Table 1). In this study, the number of MBL-producing isolates of *P. aeruginosa* was surpassed by the number of *P. putida* isolates, which suggests that *P. putida* plays a significant role as a reservoir of MBL genes, even though it is a species that is rarely involved in infection (4). The MBL genes detected in all 42 *P. putida* isolates were bla_{VIM-2}-like.

Overall, 136 of the 513 (26.5%) imipenem-nonsusceptible *Acinetobacter* sp. isolates tested carried an acquired MBL determinant (Table 1). In 1998, only bla_{IMP-1}-like genes were present in *Acinetobacter* spp. (15), but since 2001, the proportion of isolates with bla_{VIM-2}-like genes has gradually increased (J. H. Yum, unpublished results). In the present study, 64% of the MBL-producing *Acinetobacter* isolates in 2004 had bla_{VIM-2}-like genes, while only 29% of them had bla_{IMP-1}-like genes (Table 1). Two isolates of *Acinetobacter baumannii* with a novel MBL, SIM-1, in addition to seven previously

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TABLE 1. Results of routine imipenem susceptibility testing and detection of MBL-producing gram-negative bacilli in 2003 and 2004 in Korea

Species	Yr	No. (%) of isolates		No. (%) of nonduplicate imipenem-nonsusceptible isolates					
		Total	Imipenem-resistant	Tested	Positive with:				
					Hodge test	Double-disk synergy test	bla_{VIM-2}-like gene[a]	bla_{IMP-1}-like gene[a]	bla_{SIM-1}-like gene[a]
P. aeruginosa	2003	2,913	816 (28)	243	27 (11)	16 (7)	16 (100)	0	0
	2004	3,057	1,009 (33)	338	38 (11)	20 (6)	18 (90)	2 (10)	0
Other *Pseudomonas* spp.	2003	144	79 (55)	14	14 (100)	14 (100)	14 (100)[b]	0	0
	2004	114	70 (61)	30	28 (93)	28 (93)	28 (100)[b]	0	0
Acinetobacter spp.	2003	1,765	282 (16)	194	65 (34)	51 (26)	27 (53)	21 (41)	3 (6)
	2004	1,529	459 (30)	319	222 (70)	85 (27)	54 (64)	25 (29)	6 (7)
K. pneumoniae	2003	1,819	36 (2)	22	2 (9)	2 (9)	2 (100)	0	0
	2004	2,006	39 (2)	22	2 (9)	0			
E. cloacae	2003	821	2 (<1)	1	1 (100)	1 (100)	1 (100)	0	0
	2004	773	1 (<1)	1	1 (100)	0			
S. marcescens	2003	536	1 (<1)	1	1 (100)	1 (100)	1 (100)	0	0
	2004	483	4 (<1)	4	1 (25)	0			
Total		15,960	2,797 (18)	1189	402 (34)	217 (18)	161 (74)	48 (22)	9 (4)

[a] Numbers in parentheses indicate the proportions of isolates among all MBL-producing isolates.
[b] All bla_{VIM-2}-like-gene-positive isolates were *P. putida*.

reported isolates, were also detected (7). It is important to note that many imipenem-nonsusceptible but MBL-negative isolates of *P. aeruginosa* and *Acinetobacter* spp. were detected in this study. These findings suggest a wide dissemination of isolates with other resistance mechanisms. Carbapenem-hydrolyzing OXA-23 β-lactamase was reported for *Acinetobacter* strains from another Korean hospital (3).

Only bla_{VIM-2}-like genes were detected in enterobacterial isolates (Table 1), and to the best of our knowledge, this is the first detection of similar determinants in *K. pneumoniae*. The structures of class 1 integrons carrying bla_{VIM-2} from these two isolates (GenBank accession no. DQ153217 and DQ153218) were identical to those of bla_{VIM-2}-containing integrons from a *Providencia rettgeri* isolate (GenBank accession no. AY887109), suggesting the dissemination of these integrons among *Enterobacteriaceae* by horizontal transfer.

The MICs of imipenem for VIM-4-producing *K. pneumoniae* and for VIM-2-producing *Citrobacter freundii* were reported to be low, 2 μg/ml and 1 μg/ml, respectively (9, 14). However, the imipenem MICs for the two VIM-2-producing *K. pneumoniae* isolates in our study, determined by an agar dilution method (2), were much higher, namely, 32 μg/ml and >128 μg/ml, suggesting the contribution of additional resistance mechanisms. Although MBLs do not hydrolyze aztreonam, the MIC of aztreonam for one of the bla_{VIM-2}-positive *K. pneumoniae* isolates (YMC 03/10/U1702) was significantly higher (128 μg/ml) than that for the other isolate (0.5 μg/ml). The double-disk synergy test was positive for the aztreonam-resistant isolate, and the bla_{SHV-12} gene was detected in a transconjugant, which explains the high-level resistance to the monobactam.

In this study, 90% of the MBL-producing isolates were from patients in a general ward or an intensive care unit, while 10% were from patients in an emergency room or another outpatient department (data not shown). The majority (82%) of MBL-producing isolates were from sputum and urine specimens, as reported in a previous study (5).

In conclusion, imipenem-nonsusceptible *P. aeruginosa* isolates carrying bla_{VIM-2}-like genes remain highly prevalent, while the incidence of *Acinetobacter* spp. positive for bla_{VIM-2}-like genes has increased significantly. The spread of bla_{IMP-1}-like genes to *P. aeruginosa* and bla_{VIM-2}-like genes to *Acinetobacter* spp. or even to *K. pneumoniae* is a cause for concern and warrants continuous surveillance to control the further spread of these resistance mechanisms.

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