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, blaVIM-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-lactamas (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 blaIMP-1, blaVIM-2, and blaSIM-1-like genes were detected by PCR as previously described (1, 7, 11). The blaVIM-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 TTACTGCGCTTTAG-3’) and SHV-EXT-R (5’-TTATGGGCCTACCTTGG-3’) were used to detect and sequence the blaszln 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 blaVIM-2-like, but we also detected for the first time in Korea the presence of two isolates carrying blaIMP-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 blaVIM-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 blaIMP-1-like genes were present in Acinetobacter spp. (15), but since 2001, the proportion of isolates with blaVIM-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 blaVIM-2-like genes, while only 29% of them had blaIMP-1-like genes (Table 1). Two isolates of Acinetobacter baumannii with a novel MBL, SIM-1, in addition to seven previously...
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*<sub>VIM-2</sub>-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*<sub>VIM-2</sub> from these two isolates (GenBank accession no. DQ153217 and DQ153218) were identical to those of *bla*<sub>VIM-2</sub>-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*<sub>VIM-2</sub>-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*<sub>VIM-2</sub>-like gene was detected in a transconjugant, which explains the high-level resistance to the monocarbapenems.

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*<sub>VIM-2</sub>-like genes remain highly prevalent, while the incidence of *Acinetobacter* spp. positive for *bla*<sub>VIM-2</sub>-like genes has increased significantly. The spread of *bla*<sub>IMP-1</sub>-like genes to *P. aeruginosa* and *bla*<sub>VIM-2</sub>-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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### REFERENCES


