Characteristics of Metallo-β-Lactamase-Producing
Pseudomonas aeruginosa in Korea

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Background: The aim of this study was to investigate the molecular epidemiological characteristics of metallo-β-lactamase (MBL)-producing Pseudomonas aeruginosa clinical isolates in Korea.

Materials and Methods: Three hundred and twenty nine P. aeruginosa clinical isolates were collected from 23 general hospitals in Korea from March to June 2014. Species were identified by matrix-assited laser desorption/ionization-time of flight and 16S rRNA sequencing. Antimicrobial susceptibility was determined by disk diffusion methods. Further, minimum inhibitory concentrations of carbapenems were determined by Etest. Polymerase chain reaction and sequencing were performed to identify genes encoding MBLs. Multi-locus sequence typing and pulsed-field gel electrophoresis were performed to determine epidemiological characteristics of MBL-producing P. aeruginosa isolates.

Results: Of the 329 isolates, 229 (69.6%) were susceptible to the carbapenems tested, including imipenem and meropenem; while 100 (30.4%) were non-susceptible to more than one of the carbapenems. Genes encoding imipenemase-6 (IMP-6) and Verona imipenemase-2 (VIM-2) MBLs were identified in 21 (6.4%) isolates (n = 17 and 4, respectively). All MBL-producing isolates showed multi-drug resistant phenotype, and a majority (n = 19) of the isolates were identified as sequence type 235 (ST235). The remaining isolates (n = 2) were identified as ST309 and ST463.

Conclusion: P. aeruginosa ST235 might play an important role in dissemination of MBL genes in Korea.

Key Words: Pseudomonas aeruginosa; metallo-β-lactamase; VIM-2 metallo-β-lactamase; International clone; Multi-locus sequence typing

Introduction

Carbapenems have widely been used as the mainstay for the treatment of severe infections caused by Pseudomonas aeruginosa. This is because they can easily permeate through the porins on the outer membrane of these microorganisms.
Moreover, they have a high affinity for penicillin-binding proteins and a structure resistant to hydrolytic activities of most β-lactamases [1]. Unfortunately, a carbapenem-resistant _P. aeruginosa_ (CRPA) has now emerged and is disseminating worldwide. According to a survey conducted in Korea in 2011, imipenem (a carbapenem) resistance rate of _P. aeruginosa_ was 22% among 15,032 clinical isolates [2]. In fact, CRPA is considered a significant clinical threat because CRPA clinical isolates usually exhibit co-resistance to other classes of antimicrobial agents. Thus, there remain only a few alternatives for the treatment of systemic infections caused by carbapenem-resistant microorganisms [3].

Production of carbapenemase is the most important mechanism in _P. aeruginosa_ for acquiring carbapenem resistance. Diverse kinds of carbapenemases have been identified in _P. aeruginosa_, including KPC and GES variants of class A; IMP-, VIM-, SPM-, and NDM-type metallo-β-lactamases (MBLs) of class B; OXA-40 and OXA-198 enzymes of class D [4-12]. MBL-producing _P. aeruginosa_ (MPPA) has repeatedly been identified in Korea, since the first report for VIM-2 MPPA clinical isolates in 2002 [6]. A study in 2009 reported that IMP-6 was the dominant MBL (7.8%, 30/386) in _P. aeruginosa_ clinical isolates collected in Korea followed by VIM-2 (0.3%, 1/386) [13].

A multi-locus sequence typing (MLST) scheme for _P. aeruginosa_ proposed by Curran et al. [14] has facilitated comparisons of epidemiological characteristics of strains from different hospitals or countries. Earlier studies reported that two international _P. aeruginosa_ clonal complexes (CCs), CC111 and CC235, played a major role in dissemination of MBL genes worldwide [15, 16]. However, only a few studies have performed MLST experiments on MPPA in Asian countries. _P. aeruginosa_ sequence type 235 (ST235) and ST357 producing IMP-1 MBL were identified in Japan [17]. _P. aeruginosa_ ST235 producing IMP-6 or VIM-2 were also identified in Korea [13]. Furthermore, a recent study identified MPPA isolates of ST235 from Malaysia, Thailand, Sri Lanka, and Korea; ST773 from India, and ST298 from Taiwan [18].

The present study was conducted to investigate the molecular epidemiological characteristics of MPPA clinical isolates in Korea.

### Materials and Methods

**1. Bacterial strains and susceptibility testing**

Non-duplicate _P. aeruginosa_ clinical isolates (n = 329) were collected from 23 hospitals across 15 cities in Korea (Fig. 1) from March to June, 2014. The isolates were recovered from respiratory specimens (n = 120), urine (n = 96), pus (n = 78), blood (n = 24), and others (n = 11) (Table 1). Species identifi-
cation was performed using the Bruker MALDI Biotyper (Bruker, Billerica, MA, USA) and 16S rRNA gene sequencing.

Antimicrobial susceptibilities were tested by disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. Antimicrobial agents tested were amoxicillin-clavulanate, piperacillin-tazobactam, ticarcillin-clavulanate, aztreonam, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin, tobramycin, trimethoprim-sulfamethoxazole, ciprofloxacin, tetracycline, and colistin. Minimum inhibitory concentrations (MICs) of imipenem

Table 1: Antimicrobial susceptibilities of Pseudomonas aeruginosa clinical isolates by specimen

| Specimen | No. of isolates (%) | Respiratory (n = 120) | Blood (n = 24) | Urine (n = 96) | Pus (n = 78) | Other (n = 11) | Total (n = 329) |
|----------|---------------------|----------------------|----------------|-------------|-------------|-------------|-----------------|
|          | S | R | S | R | S | R | S | R | S | R | S | R |
| AMC      | 0 | 120 | (100) | 0 | 24 | (100) | 0 | 96 | (100) | 0 | 78 | (100) | 0 | 11 | (100) | 0 | 329 | (100) |
| TZP      | 101 | 84.2 | 19 | 15.8 | 20 | 83.3 | 4 | 16.7 | 72 | 75.0 | 22 | 22.9 | 64 | 82.1 | 14 | 17.9 | 9 | 81.8 | 2 | 18.2 | 266 | 80.9 | 61 | 18.5 |
| TIM      | 98 | 81.7 | 22 | 18.3 | 21 | 87.5 | 3 | 12.5 | 66 | 68.7 | 30 | 31.3 | 60 | 76.9 | 18 | 23.1 | 8 | 72.7 | 3 | 27.3 | 253 | 76.9 | 76 | 23.1 |
| ATM      | 75 | 62.5 | 29 | 24.2 | 12 | 50.0 | 8 | 33.3 | 54 | 56.2 | 26 | 27.1 | 48 | 61.5 | 19 | 24.4 | 8 | 72.7 | 3 | 27.3 | 197 | 59.9 | 85 | 25.8 |
| CAZ      | 87 | 72.5 | 21 | 17.5 | 18 | 75.0 | 4 | 16.7 | 62 | 64.6 | 28 | 29.2 | 56 | 71.8 | 16 | 20.5 | 7 | 63.6 | 4 | 36.4 | 203 | 69.9 | 73 | 22.2 |
| AMC      | 0 | 0 | 120 | (100) | 0 | 24 | (100) | 0 | 96 | (100) | 0 | 78 | (100) | 0 | 11 | (100) | 0 | 329 | (100) |
| TZP      | 101 | 84.2 | 19 | 15.8 | 20 | 83.3 | 4 | 16.7 | 72 | 75.0 | 22 | 22.9 | 64 | 82.1 | 14 | 17.9 | 9 | 81.8 | 2 | 18.2 | 266 | 80.9 | 61 | 18.5 |
| TIM      | 98 | 81.7 | 22 | 18.3 | 21 | 87.5 | 3 | 12.5 | 66 | 68.7 | 30 | 31.3 | 60 | 76.9 | 18 | 23.1 | 8 | 72.7 | 3 | 27.3 | 253 | 76.9 | 76 | 23.1 |
| ATM      | 75 | 62.5 | 29 | 24.2 | 12 | 50.0 | 8 | 33.3 | 54 | 56.2 | 26 | 27.1 | 48 | 61.5 | 19 | 24.4 | 8 | 72.7 | 3 | 27.3 | 197 | 59.9 | 85 | 25.8 |

Table 2: Nucleotide sequences of primers used in this study

| Primer name | Target gene | Nucleotide sequence (5’ to 3’) | Product size (bp) | Reference |
|-------------|-------------|--------------------------------|-------------------|-----------|
| KPC-F       | KPC-type    | GTCACTGTTATCGCGCTCTAAGTGCCTTGTTGGCCAATAGATGATT | 909 | This study |
| KPC-R       |             | TGGTGGCCAATAGATGATT            | 855 | 20 |
| GES-F       | GES-type    | CCGCTTACCTCGCGACTAATT          | 855 | 20 |
| GES-R       |             | GTCGCTGTCGAGGATGAGT            | 855 | 20 |
| IMP-1F      | IMP-1-type  | AAGGGGTTATGTCATCATTTGGAGCAGATT | 605 | This study |
| IMP-1R      |             | TTTAACGCCCTGCATTAA             | 749 | 21 |
| VIM-2F      | VIM-2-type  | ATCATGCTATGGCGAGGATT          | 738 | This study |
| VIM-2R      |             | GACGAAGCGATATTTGATT          | 845 | 20 |
| NDM-F       | NDM-type    | GCCCGTATATGTCACCGGCGG         | 845 | 20 |
| NDM-R       |             | CTGATATGTCACCGGCGG          | 845 | 20 |

KPC, Klebsiella pneumoniae carbapenemase; F, forward; R, reverse; GES, Greek extended-spectrum β-lactamase; IMP, imipenemase; VIM, Verona imipenemase; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase.
and meropenem were determined by Etest on Mueller-Hinton agar (Becton, Dickinson and Company, Sparks, MD, USA) according to manufacturer’s instruction.

2. Characterization of carbapenemase genes

Carbapenemase genes were detected by PCR using primers as previously described (for genes encoding KPC, VIM-2-, and OXA-48-type carbapenemases) [20, 21] and those designed in this study (for genes encoding GES-, IMP-1-, and NDM-type carbapenemases) (Table 2). Templates for PCR amplification from clinical isolates were whole cell lysates, and amplified products were subjected to direct sequencing. Both strands of the PCR product were sequenced twice with an automatic sequencer (model 3730xl; Applied Biosystems, Weiterstadt, Germany). Experimentally determined nucleotide sequences were compared to sequence databases using BLAST (http://blast.ncbi.nlm.nih.gov/).

3. Multi-locus sequence typing (MLST)

PCR and sequencing for 7 housekeeping genes (acsA, aroE, guaA, mutL, nuoD, ppsA, and trpE) were performed as described previously [14]. Experimentally determined nucleotide sequences of both strands were compared to pre-existing sequences in the MLST database to assign allelic numbers and STs (http://pubmlst.org/paeruginosa).

4. Pulse-field gel electrophoresis (PFGE)

XbaI-digested genomic DNA was prepared and DNA fragments were separated for 20 h at 6 V/cm at 11°C using the CHEF-DRII System (Bio-Rad, Hercules, CA, USA) with initial and final pulse times of 0.5 s and 30 s, respectively [13]. A lambda ladder (Bio-Rad) was used as DNA size marker. Band patterns were analyzed with UVIband/Map software (UVItech Ltd, Cambridge, UK) to generate a dendrogram based on the unweighted pair group method using arithmetic averages from the Dice coefficient.

Results

1. Antimicrobial susceptibilities of P. aeruginosa clinical isolates

Out of the 329 P. aeruginosa clinical isolates, 229 (69.6%) were found to be susceptible to the carbapenems tested, including imipenem and meropenem; while 100 (30.4%) were

| Table 3. Antimicrobial susceptibilities of Pseudomonas aeruginosa clinical isolates |
|----------------------------------------|---------------------|---------------------|---------------------|
|                                      | Carbapenem-susceptible (n = 229) | Carbapenem-non-susceptible (n = 100) | Total (n = 329) |
|                                      | Susceptible | Intermediate | Resistant | Susceptible | Intermediate | Resistant | Susceptible | Intermediate | Resistant |
| Amoxicillin-clavulanate               | 0 (0)       | 0 (0)        | 229 (100) | 0 (0)       | 0 (0)        | 100 (100) | 0 (0)       | 0 (0)        | 329 (100) |
| Piperacillin-tazobactam               | 215 (93.9)  | 0 (0)        | 14 (6.1)  | 51 (51)     | 2 (2)        | 47 (47)   | 266 (80.9) | 2 (0.6)      | 61 (18.5)  |
| Ticarcillin-clavulanate               | 208 (90.8)  | 0 (0)        | 21 (9.2)  | 45 (45)     | 0 (0)        | 55 (55)   | 253 (76.9) | 0 (0)        | 76 (23.1)  |
| Aztreonam                             | 174 (76.0)  | 24 (10.5)    | 31 (13.5) | 23 (23)     | 23 (23)      | 54 (54)   | 197 (59.9) | 47 (14.3)    | 85 (25.8)  |
| Ceftazidime                           | 193 (84.3)  | 15 (6.5)     | 21 (9.2)  | 37 (37)     | 11 (11)      | 52 (52)   | 230 (69.9) | 26 (7.9)     | 73 (22.2)  |
| Ceferpine                             | 194 (84.7)  | 26 (11.4)    | 9 (3.9)   | 32 (32)     | 17 (17)      | 51 (51)   | 226 (68.7) | 43 (13.1)    | 60 (18.2)  |
| Imipenem                              | 229 (100)   | 0 (0)        | 0 (0)     | 1 (1)       | 5 (5)        | 94 (94)   | 230 (69.9) | 5 (1.5)      | 94 (28.6)  |
| Meropenem                             | 229 (100)   | 0 (0)        | 0 (0)     | 7 (7)       | 17 (17)      | 76 (76)   | 236 (71.7) | 17 (5.2)     | 76 (23.1)  |
| Amikacin                              | 222 (97.0)  | 1 (0.4)      | 6 (2.6)   | 63 (63)     | 1 (1)        | 36 (36)   | 285 (86.6) | 2 (0.6)      | 42 (12.8)  |
| Gentamicin                            | 209 (91.2)  | 6 (2.7)      | 14 (6.1)  | 57 (57)     | 1 (1)        | 42 (42)   | 266 (80.9) | 7 (2.1)      | 56 (17.0)  |
| Tobramycin                            | 211 (92.1)  | 2 (0.9)      | 16 (7.0)  | 55 (55)     | 2 (2)        | 43 (43)   | 266 (80.9) | 4 (1.2)      | 59 (17.9)  |
| Trimethoprim-sulfamethoxazole         | 0 (0)       | 0 (0)        | 229 (100) | 0 (0)       | 0 (0)        | 100 (100) | 0 (0)       | 0 (0)        | 329 (100) |
| Ciprofloxacin                         | 186 (81.2)  | 5 (2.2)      | 38 (16.6) | 20 (20)     | 5 (5)        | 75 (75)   | 206 (62.6) | 10 (3.1)     | 113 (34.3) |
| Tetracycline                          | 2 (0.9)     | 0 (0)        | 227 (99.1)| 1 (1)       | 2 (2)        | 97 (97)   | 3 (0.9)     | 2 (0.6)      | 324 (98.5) |
| Colistin                              | 229 (100)   | 0 (0)        | 0 (0)     | 100 (100)   | 0 (0)        | 0 (0)     | 329 (100)   | 0 (0)        | 0 (0)      |
non-susceptible to more than one of the carbapenems (Table 3). Further, 92 of these 100 isolates exhibited non-susceptibility to both imipenem and meropenem. However, 7 imipenem-resistant isolates exhibited susceptibility to meropenem while only a single meropenem isolate was susceptible to imipenem.

Compared with carbapenem-susceptible isolates, carbapenem-non-susceptible isolates exhibited higher resistance rates to ceftazidime (9.2% versus 52%), cefepime (3.9% versus 51%), amikacin (2.6% versus 36%), gentamicin (6.1% versus 42%), tobramycin (7% versus 43%), and ciprofloxacin (16.6% versus 75%). Additionally, the 329 isolates were susceptible to colistin. Antimicrobial susceptibility rates, by specimen, are described in Table 1.

### 2. Identification of MBL genes

PCR amplification and subsequent sequence analyses identified the \( \text{bla}_{\text{IMP-6}} \) and the \( \text{bla}_{\text{VIM-2}} \) in 5.2% (\( n = 17 \)) and 1.2% (\( n = 4 \)) carbapenem-non-susceptible isolates, respectively (Table 4).

None of the isolates showed positive results when amplified for the detection of genes encoding KPC-, GES-, NDM, and OXA-48-type carbapenemases.

### 3. Characteristics of MPPA clinical isolates

All MPPA clinical isolates were identified as ST235 (38-11-3-13-1-2-4), except one isolate (BC3) of ST463 (6-5-5-3-1-6-3) producing IMP-6 and another (UUS4) of ST309 (13-8-9-3-1-17-15) producing VIM-2 (Table 4). Moreover, all MPPA ST235 isolates exhibited similar (>80% similarity) XbaI-macrorestriction banding patterns by PFGE, while MPPA of ST463 and ST309 isolates displayed different (<70% similarity) patterns (Fig. 2). Further, all MPPA clinical isolates presented multi-drug resistant phenotype in addition to high MIC values (>32 mg/L) for both imipenem and meropenem. UUS4 was the only exception that showed a low MIC value (2 mg/L) for meropenem (Table 4).

### Table 4. Characteristics of *Pseudomonas aeruginosa* clinical isolates producing metallo-β-lactamase

| Isolate | ST  | MBL genotype | MIC (mg/L) | Co-resistant to: |
|---------|-----|--------------|------------|------------------|
|         |     |              | IPM | MEM |                  |
| GS8     | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| CSU13   | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMC, ATM, FEP, CAZ, CIP, TZP, TET, TIM, TOB, TMP/SMT |
| UUS6    | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT |
| UUS15   | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT |
| CMSEO8  | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| CNU7    | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| SCHGM7  | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT |
| CMEU19  | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| CMDAE14 | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT |
| BUPAIK7 | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| BUPAIK15| 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| JNU2    | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| JNU3    | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| JNU13   | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| JNII1   | 235 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| BC3     | 463 | \( \text{bla}_{\text{IMP-6}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| JNII5   | 235 | \( \text{bla}_{\text{VIM-2}} \) | >32 | >32 | AMK, AMC, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| JNII9   | 235 | \( \text{bla}_{\text{VIM-2}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| CMIN8   | 235 | \( \text{bla}_{\text{VIM-2}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |
| OUS4    | 309 | \( \text{bla}_{\text{VIM-2}} \) | >32 | >32 | AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT |

ST, sequence type; MBL, metallo-β-lactamase; MIC, minimum inhibitory concentration; IPM, imipenem; MEM, meropenem; AMK, amikacin; AMC, amoxicillin-clavulanic acid; ATM, aztreonam; FEP, cefepime; CAZ, ceftazidime; CIP, ciprofloxacin; GEN, gentamicin; TET, tetracycline; TIM, ticarcillin-clavulanic acid; TOB, tobramycin; TMP/SMT, trimethoprim-sulfamethoxazole; TZP, piperacillin-tazobactam.
A molecular epidemiological study in 2008, based on a survey of 205 non-duplicated *P. aeruginosa* clinical isolates, collected from 18 university hospitals across 8 provinces of Korea, identified them as 62 different STs. Of these, 47.8% isolates (n = 98) were identified as CC235. These, in turn, comprised of ST235 (n = 96) and two single-locus ST235 variants- ST1015 (n = 1) and ST1162 (n = 1). The remaining isolates (n = 107) were identified as 59 different STs, including ST111, ST170, ST244, ST59, ST641, ST708, ST773, ST829, ST983, ST1015, ST1154, ST1162, and ST1166, sharing alleles with ST235 at less than 5 out of the 7 loci [22].

We previously reported (based on a survey in 2009) that clonal dissemination of MPPA ST235 is the principal cause for the diffusion of IMP-6 and VIM-2 MBL genes in Korea [13]. Despite a 5-year interval, our present study shows results similar to the previous survey. Compared with the earlier report, prevalence of MPPA is slightly lower in this study- from 8.0% (31/386) to 6.4% (21/329). This is due to lower prevalence of IMP-6- from 7.8% (30/386) to 5.2% (17/329). Although the prevalence of VIM-2 increased marginally from 0.3% (1/386) to 1.2% (4/329), IMP-6 was still the dominant MBL type in *P. aeruginosa* in Korea. The antimicrobial susceptibility rates of *P. aeruginosa* clinical isolates, recovered from blood specimens, for imipenem (18/24, 75.0%) and meropenem (20/24, 83.3%) were higher compared to those recovered from specimens other than blood, including respiratory, urinary, and wound specimens, (212/305, 69.5%; and 216/305, 70.8%, respectively) (Table 1).

Interestingly, new MPPA clones emerged in our current study. In the previous survey, all the MPPA clinical isolates (n = 31) were identified as ST235. Although ST235 continues to be the dominant strain among MPPA clinical isolates in this study, we identified two isolates as different STs- ST309 and ST463. MPPA ST235 has been identified in many Asian countries, including Japan, Malaysia, Thailand, Sri Lanka, and Korea [17, 18]. However, there are other MPPA strains that have also been identified in Asian countries: ST357 in Japan, ST773 in India, and ST298 in Taiwan. Recently, dissemination of IMP-6 MPPA ST244 in China was reported [23]. There is no evidence though, whether MPPA ST309 and ST463 entered Korea from foreign countries, or the strains acquired the MBL gene by horizontal transfer in this country itself. Nevertheless, what is alarming is that diversification of MPPA strains in Korea might be a signal for further dissemination of MPPA in the country.

In conclusion, our study underscores the findings that IMP-6 MPPA ST235 has disseminated in Korea, and that new MPPA strains, ST309 and ST463, have surfaced in the country. Given the impending hazards of these phenomena, it is crucial to monitor changes in MBL types and MPPA strains through periodic surveys for MPPA.
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