Original Article
http://dx.doi.org/10.3947/ic.2015.47.1.33
Infect Chemother 2015;47(1):33-40
ISSN 2093-2340 (Print) · ISSN 2092-6448 (Online)

Infection & Chemotherapy

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

Jun Sung Hong[1], Jung Ok Kim[2], Hyukmin Lee[3], Il Kwon Bae[4], Seok Hoon Jeong[2], and Kyungwon Lee[2]

[1]Brain Korea 21 PLUS Project for Medical Science, Yonsei University, [2]Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul; [3]Department of Laboratory Medicine, Kwandong University College of Medicine, Goyang; [4]Department of Dental Hygiene, Silla University, Busan, Korea

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.

Received: February 13, 2015 **Revised:** February 23, 2015 **Accepted:** February 26, 2015
Corresponding Author : Seok Hoon Jeong, MD, PhD
Department of Laboratory Medicine, Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 211 Eonju-ro, Gangnam-gu, Seoul 135-720, Korea
Tel: +82-2-2019-3532, Fax: +82-2-2057-8926
E-mail: kscpjsh@yuhs.ac

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-

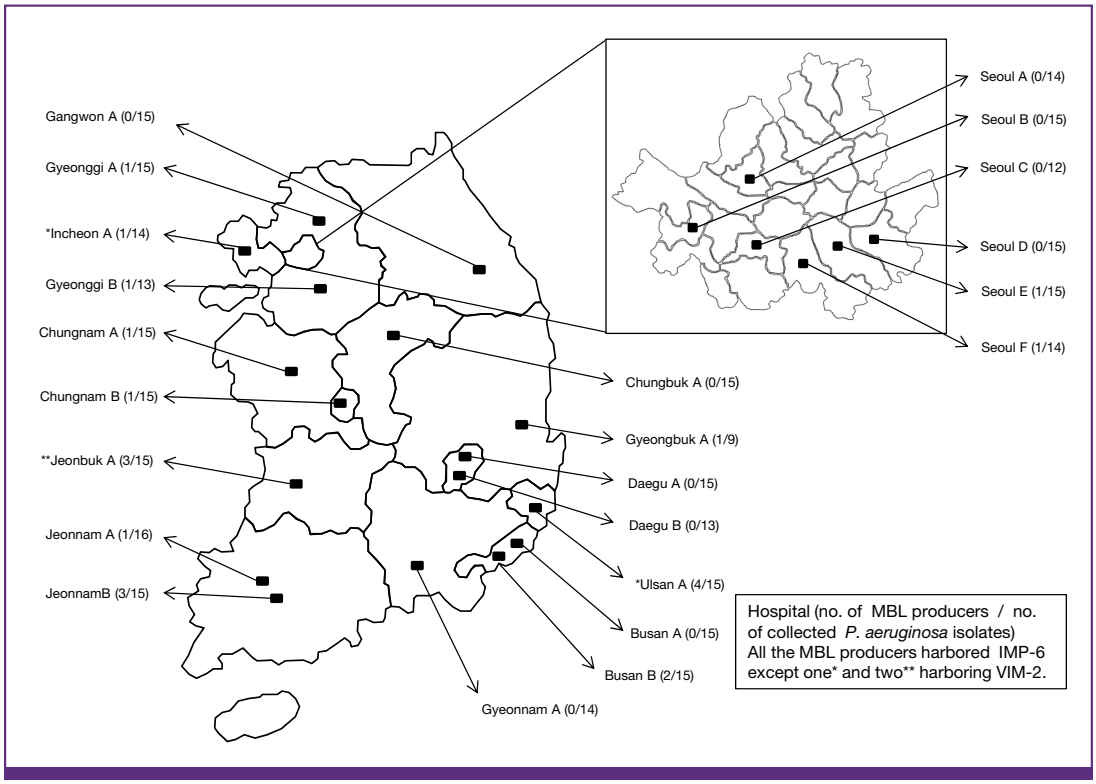

Figure 1. Map showing the locations of the participating hospitals in this study.
MBL, metallo-β-lactamase; IMP, imipenemase; VIM, Verona imipenemase.

Table 1. Antimicrobial susceptibilities of *Pseudomonas aeruginosa* clinical isolates by 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 (0)	120 (100)	0 (0)	24 (100)	0 (0)	96 (100)	0 (0)	78 (100)	0 (0)	11 (100)	0 (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)	230 (69.9)	73 (22.2)
FEP	87 (72.5)	15 (12.5)	16 (66.7)	3 (12.5)	56 (58.3)	30 (31.3)	60 (77.0)	9 (11.5)	7 (63.6)	3 (27.3)	226 (68.7)	60 (18.2)
IPM	81 (67.5)	38 (31.7)	18 (75.0)	5 (20.8)	63 (65.6)	31 (32.3)	62 (79.5)	16 (20.5)	6 (54.5)	4 (36.4)	230 (69.9)	94 (28.6)
MEM	84 (70.0)	27 (22.5)	20 (83.3)	3 (12.5)	64 (66.6)	30 (31.3)	62 (79.5)	13 (16.7)	6 (54.5)	3 (27.3)	236 (71.7)	76 (23.1)
AMK	111 (92.5)	8 (6.7)	23 (95.8)	1 (4.2)	69 (71.9)	27 (28.1)	72 (92.3)	5 (6.4)	10 (90.9)	1 (9.1)	285 (86.6)	42 (12.8)
GEN	103 (85.9)	13 (10.8)	21 (87.5)	3 (12.5)	65 (67.7)	28 (29.2)	67 (85.9)	11 (14.1)	10 (90.9)	1 (9.1)	266 (80.9)	56 (17.0)
TOB	104 (86.6)	14 (11.7)	20 (83.3)	3 (12.5)	68 (70.8)	28 (29.2)	65 (83.3)	12 (15.4)	9 (81.8)	2 (18.2)	266 (80.9)	59 (17.9)
TMP/SMT	0 (0)	120 (100)	0 (0)	24 (100)	0 (0)	96 (100)	0 (0)	78 (100)	0 (0)	11 (100)	0 (0)	329 (100)
CIP	78 (65.0)	38 (31.7)	15 (62.5)	8 (33.3)	53 (55.2)	41 (42.7)	51 (65.4)	24 (30.8)	9 (81.8)	2 (18.2)	206 (62.6)	113 (34.3)
TET	2 (1.7)	116 (96.6)	0 (0)	24 (100)	1 (1.0)	95 (99.0)	0 (0)	78 (100)	0 (0)	11 (100)	3 (0.9)	324 (98.5)
CST	120 (100)	0 (0)	24 (100)	0 (0)	96 (100)	0 (0)	78 (100)	0 (0)	11 (100)	0 (0)	329 (100)	0 (0)

S, susceptible; R, resistant; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; TIM, ticarcillin-clavulanic acid; ATM, aztreonam; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; TMP/SMT, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin; TET, tetracycline; CST, colistin.

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	GTCACTGTATCGCCGTCTAGTTC	909	This study
KPC-R		TGGTGGGCCAATAGATGATT		
GES-F	GES-type	CGCTTCATTCACGCACTATT	855	20
GES-R		GTCCGTGCTCAGGATGAGTT		
IMP-1F	IMP-1-type	AAGGCGTTTATGTTCATACTTCG	605	This study
IMP-1R		TTTAACCGCCTGCTCTAATGTAA		
VIM-2F	VIM-2-type	ATCATGGCTATTGCGAGTCC	749	21
VIM-2R		ACGACTGAGCGATTTGTGTG		
NDM-F	NDM-type	GCCCAATATTATGCACCCGG	738	This study
NDM-R		CTCATCACGATCATGCTGGC		
OXA-48F	OXA-48-type	GATTATCGGAATGCCTGCGG	845	20
OXA-48R		CTACAAGCGCATCGAGCATCA		

KPC, *klebsiella pneumoniae* carbapenemase; F, forward; R, reverse; GES, greece extended-spectrum β-lactamase; IMP, imipenemase; VIM, Verona imipenemase; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase.

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 3. Antimicrobial susceptibilities of *Pseudomonas aeruginosa* clinical isolates

	No. of 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)
Cefepime	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)

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)

*Xba*I-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 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	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT
CSU13	235	bla_{IMP-6}	>32	>32	AMC, ATM, FEP, CAZ, CIP, TZP, TET, TIM, TMP/SMT
UUS6	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
UUS7	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
UUS15	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
CMSEO8	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT
CNU7	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT
SCHGM7	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
CMEUI9	235	bla_{IMP-6}	>32	>32	AMC, ATM, FEP, CIP, TET, TMP/SMT
CMDAE14	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
BUPAIK7	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
BUPAIK15	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT
JNU2	235	bla_{IMP-6}	>32	>32	AMK, AMC, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
JNU3	235	bla_{IMP-6}	>32	>32	AMK, AMC, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
JNU13	235	bla_{IMP-6}	>32	>32	AMK, AMC, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT
JBNU1	235	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
BC3	463	bla_{IMP-6}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TET, TIM, TOB, TMP/SMT
JBNU5	235	bla_{VIM-2}	>32	>32	AMK, AMC, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
JBNU9	235	bla_{VIM-2}	>32	>32	AMK, AMC, ATM, FEP, CAZ, CIP, GEN, TZP, TET, TIM, TOB, TMP/SMT
CMIN8	235	bla_{VIM-2}	>32	>32	AMC, FEP, CAZ, TET, TIM, TMP/SMT
UUS4	309	bla_{VIM-2}	>32	2	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.

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 bla_{IMP-6} and the bla_{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) *Xba*I-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 multidrug 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).

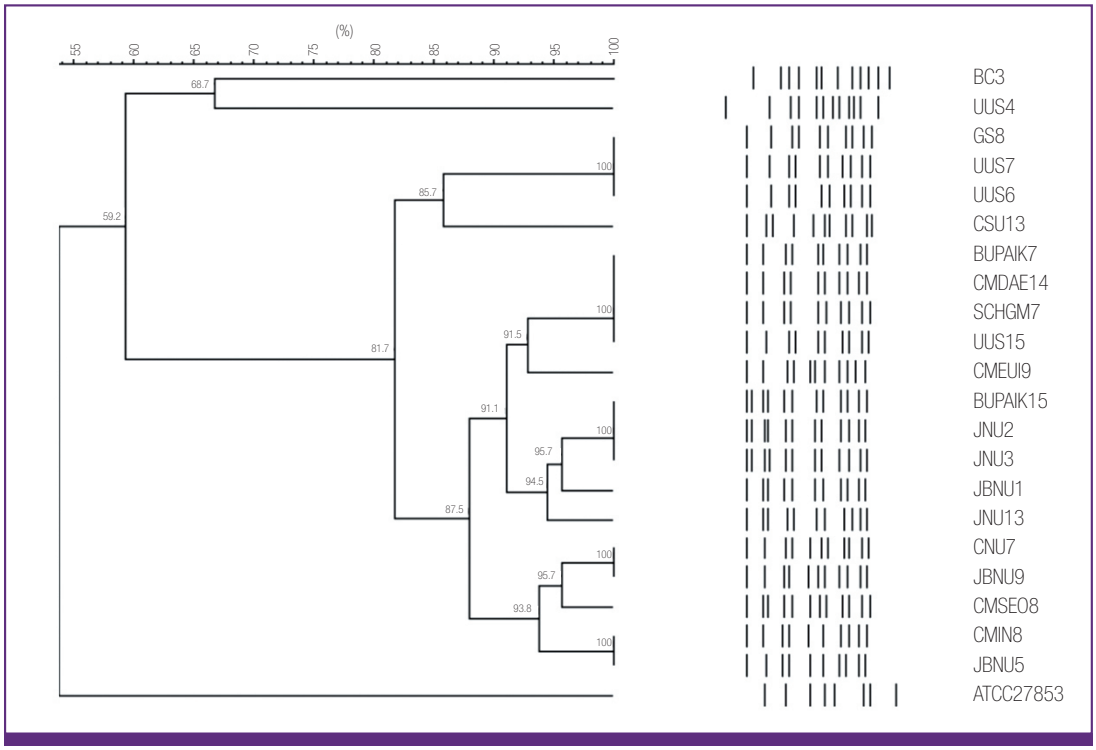

Figure 2. *XbaI*-macrorestriction patterns of metallo-β-lactamase-producing *Pseudomonas aeruginosa* clinical isolates.

Discussion

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, ST591, 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.

Acknowledgement

This study was supported by a grant (2013-E4405-01) by the Korean Center for Disease Control and Prevention, Republic of Korea.

ORCID

Jun Sung Hong http://orcid.org/0000-0003-4280-6187
Jung Ok Kim http://orcid.org/0000-0002-4136-1537
Hyukmin Lee http://orcid.org/0000-0002-8523-4126
Il Kwon Bae http://orcid.org/0000-0003-1633-3240
Seok Hoon Jeong http://orcid.org/0000-0001-9290-897X
Kyungwon Lee http://orcid.org/0000-0003-3788-2134

References

1. Diene SM, Rolain JM. Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* species. Clin Microbiol Infect 2014;20:831-8.
2. Yong D, Shin HB, Kim YK, Cho J, Lee WG, Ha GY, Choi TY, Jeong SH, Lee K, Chong Y, KONSAR group. Increase in the prevalence of carbapenem-resistant *Acinetobacter* isolates and ampicillin-resistant non-typhoidal *Salmonella* species in Korea: a KONSAR study conducted in 2011. Infect Chemother 2014;46:84-93.
3. Jeong SJ, Yoon SS, Bae IK, Jeong SH, Kim JM, Lee K. Risk factors for mortality in patients with bloodstream infections caused by cabapenem-resistant *Pseudomonas aeruginosa*: clinical impact of bacterial virulence and strain on outcome. Diagn Microbiol Infect Dis 2014;80:130-5.
4. Poirel L, Nordmann P, Lagrutta E, Cleary T, Munoz-Price LS. Emergence of KPC-producing *Psedomonas aeruginosa* in the United States. Antimicrob Agents Chemother 2010;54:3072.
5. Wang C, Cai P, Chang D, Mi Z. A *Pseudomonas aeruginosa* isolate producing the GES-5 extended-spectrum beta-lactamase. J Antimicrob Chemother 2006;57:1261–2.
6. Lee K, Lim JB, Yum JH, Yong D, Chong Y, Kim JM, Livermore DM. *bla*$_{VIM-2}$ cassette-containing novel intergrons in metallo-β-lactamase-producing *Pseudomonas aeruginosa* and *Pseudomonas putida* isolates disseminated in a Korean hospital. Antimicrob Agents Chemother 2002;46:1053-8.
7. Jovcic B, Lepsanovic Z, Suljagic V, Rackov G, Begovic J, Topisirovic L, Kojic M. Emergence of NDM-1 metallo-β-lactamase in *Pseudomonas aeruginosa* clinical isolates from Serbia. Antimicrob Agents Chemother 2011;55:3923-31.
8. Potron A, Poirel L, Nordmann P. Plasmid-mediated transfer of the *bla*$_{NDM-1}$ gene in Gram-negative rods. FEMS Microbiol Lett 2011;324:111-6.
9. Yezil S, Shibl AM, Memish ZA. The molecular basis of β-lactamase production in Gram-negative bacteria from Saudi Arabia. J Med Microbiol 2015;64:127-36.
10. Martins AF, Zavascki AP, Gaspareto PB, Barth AL. Dissemination of *Pseudomonas aeruginosa* producing SPM-1-like and IMP-1-like metallo-β-lactamases in hospitals from Southern Brazil. Infection 2007;35:457-60.
11. Sevillano E, Gallego L, García-Lobo JM. First detection of the OXA-40 carbapenemase in *P. aeruginosa* isolates, located on a plasmid also found in *A. baumannii*. Pathol Biol (Paris) 2009;57:493-5.
12. El Garch F, Bogaerts P, Bebrone C, Galleni M, Glupczynski Y. OXA-198, an acquired carbapenem-hydrolyzing class D β-lactamase from *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2011;55:4828-33.
13. Seok Y, Bae IK, Jeong SH, Kim SH, Lee H, Lee K. Dissemination of IMP-6 metallo-β-lactamses-producing *Pseudomonas aeruginosa* sequence type 235 in Korea. J Antimicrob Chemother 2011;66:2791-6.
14. Curran B, Jonas D, Grudmann H, Pitt T, Dowson CG. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J Clin Microbiol 2004;42:5644-9.
15. Samuelsen O, Toleman MA, Sundsfjord A, Rydberg J, Leegaard TM, Walder M, Lia A, Ranheim TE, Rajendra Y, Hermansen NO, Walsh TR, Giske CG. Molecular epidemiology of metallo-β-lactamase-producing *Pseuomonas aeruginosa* isolates from Norway and Sweden shows import of international clones and local clonal expansion. Antimicrob Agents Chemother 2010;54:346-52.
16. Castanheira M, Deshpande LM, Costello A, Davies TA, Jones RN. Epidemiology and carbapenem resistance mechanisms of carbapenem-non-susceptible *Pseuomonas aeruginosa* collected during 2009-11 in 14 European and Mediterranean countries. J Antimicrob Chemother 2014;69:1804-14.
17. Kitao T, Tada T, Tanaka M, Narahara K, Shimojima M, Shimada K, Miyoshi-Akiyama T, Kirikae T. Emergence of a novel multidrug-resistant *Pseudomonas aeruginosa* strain producing IMP-type metallo-β-lactamases and AAA(6`)-lae in Japan. Int J Antimicrob Agents 2012;39:518-21.
18. Kim MJ, Bae IK, Jeong SH, Kim SH, Song JH, Choi JY, Yoon

SS, Thamlikitkul V, Hsueh PR, Yasin RM, Lalitha MK, Lee K. Dissemination of metallo-β-lactamse-producing *Pseudomonas aeruginosa* of sequence type 235 in Asian countries. J Antimicrob Chemother 2013;68:2820-4.
19. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twenty-fourth informational supplement (M100-S24). Wayne, PA, USA: CLSI; 2014.
20. Bae IK, Jang SJ, Kim J, Jeong SH, Cho B, Lee K. Interspecies dissemination of the *bla* gene encoding PER-1 extended-spectrum β-lactamase. Antimicrob Agents Chemother 2011;55:1305-7.
21. Tam VH, Chang KT, Abdelraouf K, Brioso CG, Ameka M, McCaskey LA, Weston JS, Caeiro JP, Garey KW. Prevalence, resistance mechanisms, and susceptibility of multidrug-resistant bloodstream isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2010;54:1160-4.
22. Bae IK, Suh B, Jeong SH, Wang KK, Kim YR, Yong D, Lee K. Molecular epidemiology of *Pseudomonas aeruginosa* clinical isolates from Korea producing β-lactamases with extended-spectrum activity. Diagn Microbiol Infect Dis 2014;79:373-7.
23. Chen Y, Sun M, Wang M, Lu Y, Yan Z. Dissemination of IMP-6 producing *Pseudomonas aeruginos*a ST244 in multiple cities in China. Eur J Clin Microbiol Infect Dis 2014;33:1181-7.