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Clonal Distribution and Its Association With the Carbapenem Resistance Mechanisms of Carbapenem-Non-Susceptible *Pseudomonas aeruginosa* Isolates From Korean Hospitals

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Background: Carbapenem resistance in *Pseudomonas aeruginosa* is a serious global health problem. We investigated the clonal distribution and its association with the carbapenem resistance mechanisms of carbapenem-non-susceptible *P. aeruginosa* isolates from three Korean hospitals.

Methods: A total of 155 carbapenem-non-susceptible *P. aeruginosa* isolates collected between 2011 and 2019 were analyzed for sequence types (STs), antimicrobial susceptibility, and carbapenem resistance mechanisms, including carbapenemase production, the presence of resistance genes, OprD mutations, and the hyperproduction of AmpC β-lactamase.

Results: Sixty STs were identified in carbapenem-non-susceptible *P. aeruginosa* isolates. Two high-risk clones, ST235 (N = 41) and ST111 (N = 20), were predominant; however, sporadic STs were more prevalent than high-risk clones. The resistance rate to amikacin was the lowest (49.7%), whereas that to piperacillin was the highest (92.3%). Of the 155 carbapenem-non-susceptible isolates, 43 (27.7%) produced carbapenemases. Three metallo-β-lactamase (MBL) genes, bla_{IMP-6} (N = 38), bla_{VIM-2} (N = 3), and bla_{NDM-1} (N = 2), were detected. bla_{IMP-6} was detected in clonal complex 235 isolates. Two ST773 isolates carried bla_{NDM-1} and *rmtB*. Frameshift mutations in *oprD* were identified in all isolates tested, regardless of the presence of MBL genes. Hyperproduction of AmpC was detected in MBL gene–negative isolates.

Conclusions: Frameshift mutations in *oprD* combined with MBL production or hyperproduction of AmpC are responsible for carbapenem resistance in *P. aeruginosa*. Further attention is required to curb the emergence and spread of new carbapenem-resistant *P. aeruginosa* clones.

Key Words: AmpC, Carbapenem resistance, Metallo-β-lactamase, OprD, *Pseudomonas aeruginosa*, Sequence type

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INTRODUCTION

Pseudomonas aeruginosa is an important nosocomial pathogen that causes various opportunistic infections, including pneumonia and urinary tract, bloodstream, and surgical wound infections, primarily in immunocompromised or severely ill patients [1, 2]. This microorganism is notorious for its ability to develop antimicrobial resistance [3, 4]. Multidrug-resistant (MDR) or extensively drug-resistant (XDR) *P. aeruginosa* strains significantly increase morbidity and mortality rates in infected patients [4-6]. The WHO has listed carbapenem-resistant *P. aeruginosa* (CRPA) as a critical priority pathogen for the development of new antimicrobials [7].

Carbapenems are commonly used to treat MDR *P. aeruginosa* infections, which has resulted in the prevalence of CRPA [4]. Carbapenem resistance in *P. aeruginosa* is attributed to intrinsic resistance mechanisms, including reduced membrane permeability, overexpression of efflux pumps, or hyperproduction of derepressed AmpC β-lactamase, or the acquisition of diverse genes encoding carbapenem-hydrolyzing enzymes [8-12]. Ambler class B metallo-β-lactamases (MBLs) confer a high level of resistance to carbapenems and are highly associated with global epidemic clones [6]. Hyperproduction of chromosomal AmpC β-lactamases is another important mechanism of resistance to cephalosporins and carbapenems [13]. Inducible AmpC by certain β-lactams and mutations in the regulatory components of AmpC leads to stable hyperproduction of AmpC. The outer membrane porin OprD allows the entrance of carbapenems into the periplasmic space in *P. aeruginosa* [14, 15]. Loss or alteration of OprD by mutation leads to carbapenem resistance.

The dissemination of global epidemic clones is responsible for the worldwide prevalence of MDR *P. aeruginosa* isolates. Based on prevalence, global spread, association with MDR and XDR profiles, and horizontally acquired β-lactamases, such as extended-spectrum β-lactamases and carbapenemases, del Barrio-Tofiño *et al*. [6] suggested top 10 *P. aeruginosa* high-risk clones globally, including sequence type (ST) 111, ST175, ST233, ST235, ST244, ST277, ST298, ST308, ST357, and ST654. Among them, ST235 was the most widespread and carried over 60 different β-lactamase gene variants [16, 17]. The prevalence of ST235 *P. aeruginosa* carrying $bla_{\text{IMP-6}}$ or $bla_{\text{VIM-2}}$ in Korea has been reported [18-20]. We investigated the clonal distribution and its association with carbapenem resistance mechanisms of carbapenem-non-susceptible *P. aeruginosa* isolates from three hospitals located in southern Korea to obtain a detailed perspective on the molecular epidemiology of carbapenem resistance in *P. aeruginosa*. This study demonstrated that diverse clones of *P. aeruginosa*, including global high-risk clones, are circulating in Korean hospitals. Carbapenem resistance in Korean *P. aeruginosa* isolates is mediated by frameshift mutations in *oprD* combined with MBL production or *ampC* overexpression.

MATERIALS AND METHODS

Bacterial strains

A total of 155 carbapenem-non-susceptible *P. aeruginosa* isolates from three Korean hospitals, Kyungpook National University Hospital (KNUH) located in Daegu (N = 50), Gyeongsang National University Hospital (GNUH) located in Jinju (N = 45), and Jeonbuk National University Hospital (JNUH) located in Jeonju (N = 60), collected between 2011 and 2019, were used in this study (Supplemental Data Table S1). The isolates were obtained from three regional branches of the National Culture Collection for Pathogens (NCCP), KNUH-NCCP, GNUH-NCCP, and JNUH-NCCP, which collect and deposit representative bacterial pathogen resources from associated hospitals. Of the 345 carbapenem-non-susceptible *P. aeruginosa* isolates deposited in KNUH-NCCP (N = 111), GNUH-NCCP (N = 89), and JNUH-NCCP (N = 145), 155 (44.9%) isolates were randomly selected based on the isolation year and date, source clinical samples, antimicrobial susceptibility profiles, and patient clinical information. The isolates were obtained in 2011 (N = 10 of 24 isolates), 2012 (N = 24 of 54), 2013 (N = 28 of 59), 2014 (N = 8 of 19), 2015 (N = 14 of 27), 2016 (N = 10 of 25), 2017 (N = 21 of 47), 2018 (N = 24 of 51), and 2019 (N = 16 of 39). Carbapenem-non-susceptible *P. aeruginosa* isolates were obtained from blood (N = 50 of 101 isolates), sputum and respiratory tract (N = 43 of 99), urine (N = 28 of 61), ascites and bile juice (N = 14 of 31), wound and pus (N = 13 of 30), cerebrospinal fluid and other body fluids (N = 3 of 10), and other samples (N = 4 of 13). *P. aeruginosa* was identified using the VITEK 2 system (bioMérieux, Marcy-l'Étoile, France), matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany), and 16S rDNA gene sequencing (Macrogen, Seoul, Korea). This study was approved by the Institutional Review Board of KNUH, Daegu, Korea (IRB No. 2018-11-023-005).

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the *P. aeruginosa* isolates was determined using the VITEK 2 system with an AST-N225 card

(bioMérieux), according to CLSI guidelines [21]. Twelve antimicrobial agents were tested: aminoglycosides (amikacin, gentamicin, and tobramycin), antipseudomonal penicillins (piperacillin), antipseudomonal penicillins/β-lactamase inhibitors (piperacillin/tazobactam), antipseudomonal cephalosporins (cefepime and ceftazidime), carbapenems (imipenem and meropenem), monobactam (aztreonam), fluoroquinolones (ciprofloxacin), and polymyxins (colistin). The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of three antimicrobial agents, including imipenem, meropenem, and colistin. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains. MDR or XDR phenotypes were determined according to the criteria suggested by Magiorakos *et al*. [22].

Screening of carbapenemase production and resistance gene amplification

The Rapidec Carba NP test (bioMérieux) was used to screen for carbapenemase production in imipenem- or meropenem-non-susceptible *P. aeruginosa* isolates [23]. PCR and subsequent sequencing were used to detect antimicrobial resistance genes and *oprD* mutations. Briefly, bacteria were grown in lysogeny broth (LB) overnight, washed with phosphate-buffered saline, and resuspended in sterilized distilled water. The bacteria were boiled for 10 min to prepare template DNA for PCR. Carbapenemase-producing isolates were found to carry carbapenemase genes such as bla_{GES}, bla_{KPC}, bla_{IMP}, bla_{NDM}, bla_{SPM}, bla_{VIM}, $bla_{OXA\text{-}48}$, and $bla_{OXA\text{-}58}$ [15, 24-26]. Aminoglycoside-resistant isolates amplified 16S rRNA methyltransferase genes *armA* and *rmtB* [27]. Full-length bla_{IMP}, bla_{NDM}, and bla_{VIM} were amplified by PCR and sequenced to subtype the genes [28, 29]. Full-length *oprD* was amplified and sequenced to identify mutations [24]. oprD nucleotide sequences were compared with those in *P. aeruginosa* PAO1 (accession No. NC_002516.2) using the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLAST). The primers used for PCR and sequencing are listed in Supplemental Data Table S2.

RNA isolation and quantitative real-time PCR (qPCR)

Bacteria were grown in LB at 37°C under shaking overnight and then diluted to 1:100 in fresh LB. Bacterial cultures were grown until the optical density at 600 nm reached 1.0. Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Random hexamer primers and TOPscript reverse transcriptase (Enzynomics, Daejeon, Korea) were used to synthesize cDNA. qPCRs were run using TOPreal qPCR 2X PreMIX (SYBR Green with high ROX) (Enzynomics) in a StepOnePlus Real-time PCR System (Applied Biosystems, Foster City, CA, USA) according to the manufacturers' instructions. *ampC* expression levels were normalized to the levels of the housekeeping gene, *rpsL* [15, 30], and fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method [31]. Gene expression levels in clinical isolates were compared with those in *P. aeruginosa* PAO1. The specific primers for *ampC* and *rpsL* are listed in Supplemental Data Table S2. The assay was performed three times independently.

ST analysis

Seven genes (*acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA*, and *trpE*) were PCR-amplified and sequenced to determine the STs. The allelic numbers of each gene and STs were determined using a multilocus sequence typing database for *P. aeruginosa* (https://pubmlst.org/organisms/pseudomonas-aeruginosa).

RESULTS

Clonal distribution of *P. aeruginosa* isolates

A total of 155 carbapenem-non-susceptible *P. aeruginosa* isolates were classified into 60 STs, including eight unassigned STs (Supplemental Data Table S1). Three STs were prevalent, ST235 (N=41), ST111 (N=20), and ST245 (N=10), accounting for 45.8% of the isolates. ST111 and ST235 isolates were detected in the three hospitals, whereas ST245 isolates were detected only in one hospital (JNUH) (Fig. 1A). ST111 isolates were detected throughout the study period, whereas ST245 isolates were detected only between 2011 and 2013 (Fig. 1B). Eight carbapenem-non-susceptible *P. aeruginosa* isolates belonging to unassigned STs were detected after 2017. Of the three single locus variants of ST235, ST622, ST660, and ST1015, ST622 (N=6) and ST1015 (N=4) isolates were detected between 2018 and 2019.

Antimicrobial susceptibility of *P. aeruginosa* isolates

MDR or XDR phenotypes were present in 20 (12.9%) and 124 (80.0%) of the 155 carbapenem-non-susceptible *P. aeruginosa* isolates, respectively (Supplemental Data Table S1). Carbapenem-non-susceptible *P. aeruginosa* isolates exhibited the highest resistance rate to piperacillin (92.3%), and the lowest to amikacin (49.7%) (Table 1). Resistance rates to amikacin, gentamicin, tobramycin, piperacillin, piperacillin/tazobactam, cefepime, and ciprofloxacin were higher in ST235 than in other STs. Colistin resistance was not observed. Resistance rates to

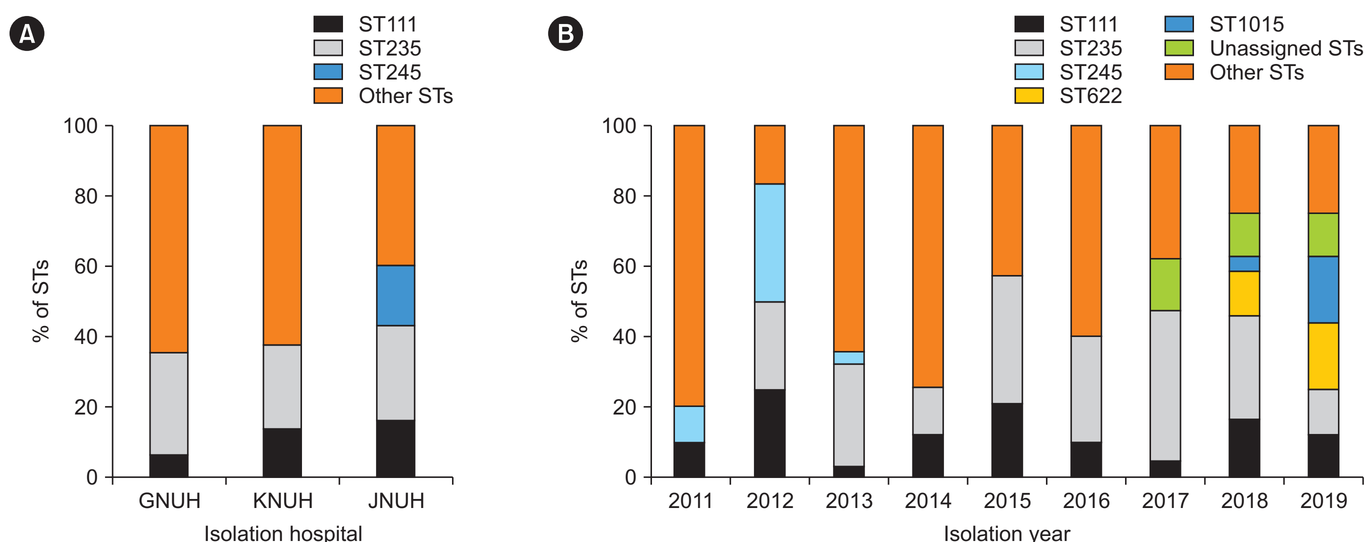

Fig. 1. Distribution of STs in carbapenem-non-susceptible *Pseudomonas aeruginosa* isolates collected in three Korean hospitals between 2011 and 2019. (A) STs of *P. aeruginosa* isolates according to the isolation hospital. (B) STs of *P. aeruginosa* isolates according to the isolation year.

Abbreviations: ST, sequence type; GNUH, Gyeongsang National University Hospital; KNUH, Kyungpook National University Hospital; JNUH, Jeonbuk National University Hospital.

Table 1. Antimicrobial susceptibility of carbapenem-non-susceptible *Pseudomonas aeruginosa* isolates collected from three Korean hospitals between 2011 and 2019

Antimicrobial agent	Non-susceptible isolates, N (%)				
	ST111 (N = 20)	ST235 (N = 41)	ST245 (N = 10)	Other STs (N = 84)	Total (N = 155)
Amikacin	8 (40.0)	39 (95.1)*	2 (20.0)	28 (33.3)	77 (49.7)
Gentamicin	17 (85.0)	40 (97.6)*	8 (80.0)	49 (58.3)	114 (73.5)
Tobramycin	16 (80.0)	40 (97.6)*	2 (20.0)	31 (36.9)	89 (57.4)
Piperacillin	18 (90.0)	40 (97.6)*	9 (90.0)	76 (90.5)	143 (92.3)
Piperacillin/tazobactam	18 (90.0)	39 (95.1)*	9 (90.0)	75 (89.3)	141 (91.0)
Cefepime	11 (55.0)	38 (92.7)*	7 (70.0)	65 (77.4)	121 (78.1)
Ceftazidime	13 (65.0)	36 (87.8)	9 (90.0)*	66 (78.6)	124 (80.0)
Imipenem	20 (100)*	41 (100)*	10 (100)*	82 (97.6)	153 (98.7)
Meropenem	18 (90.0)	41 (100)*	10 (100)*	76 (90.5)	145 (93.5)
Aztreonam	15 (75.0)*	29 (70.7)	7 (70.0)	63 (75.0)*	114 (73.5)
Ciprofloxacin	18 (90.0)	40 (97.6)*	9 (90.0)	62 (73.8)	129 (83.2)

*The highest resistance rate among the STs.

the tested antimicrobial agents were similar among the hospitals (Table 2).

Detection of MBL and 16S rRNA methyltransferase genes in *P. aeruginosa* isolates

The Rapidec Carba NP test revealed that 43 (27.7%) of the 155 carbapenem-non-susceptible *P. aeruginosa* isolates produced carbapenemases (Supplemental Data Table S1). Sequencing of PCR amplicons revealed that bla_{IMP-6}, bla_{VIM-2}, and bla_{NDM-1} were present in 38, 3, and 2 isolates, respectively. All bla_{IMP-6} genes were detected in clonal complex (CC) 235 isolates, including ST235 (N = 35), ST622 (N = 1), ST660 (N = 1), and ST1015 (N = 1). bla_{VIM-2} was detected in ST235 (N = 2) and ST463 (N = 1) isolates. bla_{NDM-1} was detected in ST773 isolates. The bla_{IMP-6}-

Table 2. Antimicrobial susceptibility of carbapenem-non-susceptible *Pseudomonas aeruginosa* isolates according to isolation hospital

Antimicrobial agent	Non-susceptible isolates, N (%)			
	KNUH (N = 50)	GNUH (N = 45)	JNUH (N = 60)	Total (N = 155)
Amikacin	27 (54.0)*	24 (53.3)	26 (43.3)	77 (49.7)
Gentamicin	37 (74.0)	31 (68.9)	46 (76.7)*	114 (73.5)
Tobramycin	32 (64.0)*	23 (51.1)	34 (56.7)	89 (57.4)
Piperacillin	45 (90.0)	42 (93.3)*	56 (93.3)*	143 (92.3)
Piperacillin/tazobactam	45 (90.0)	40 (88.9)	56 (93.3)*	141 (91.0)
Cefepime	39 (78.0)	37 (82.2)*	45 (75.0)	121 (78.1)
Ceftazidime	40 (80.0)	34 (75.6)	50 (83.3)*	124 (80.0)
Imipenem	50 (100)*	43 (95.6)	60 (100)*	153 (98.7)
Meropenem	48 (96.0)*	42 (93.3)	55 (91.7)	145 (93.5)
Aztreonam	40 (80.0)*	30 (66.7)	44 (73.3)	114 (73.5)
Ciprofloxacin	38 (76.0)	38 (84.4)	53 (88.3)*	129 (83.2)

*The highest resistance rate among the three hospitals.
Abbreviations: KNUH, Kyungpook National University Hospital; GNUH, Gyeongsang National University Hospital; JNUH, Jeonbuk National University Hospital.

and bla_{NDM-1}-carrying *P. aeruginosa* isolates exhibited high-level resistance to meropenem (>256 μg/mL). Seventy isolates were non-susceptible to the three aminoglycosides tested. Among them, two ST773 isolates carried the 16S rRNA methyltransferase gene, *rmtB*.

oprD mutations and AmpC hyperproduction in CRPA isolates

Nineteen CRPA isolates were selected on the basis of their STs to determine whether *oprD* mutations and AmpC hyperproduction were responsible for their resistance to carbapenems. All isolates tested carried frameshift mutations in *oprD*, resulting in the formation of a premature stop codon (Table 3). In contrast to the 443 amino acids found in OprD of *P. aeruginosa* PAO1, the predicted number of amino acids of OprD in the isolates tested was 0–441. Hyperproduction of AmpC (i.e., ≥10-fold higher expression than in *P. aeruginosa* PAO1) was detected in six isolates belonging to ST244 (N = 1), ST245 (N = 3), ST622 (N = 1), and ST1154 (N = 1). The AmpC-hyperproducing isolates did not carry MBL genes.

DISCUSSION

High-risk *P. aeruginosa* clones are characterized by global spread, MDR/XDR phenotypes, and association with the production of extended-spectrum β-lactamases and carbapenemases [6]. ST111 (N = 20), ST235 (N = 41), ST244 (N = 6), ST357 (N = 3), and ST654 (N = 1) were identified in this study, accounting for 32.9% of the isolates of the 10 high-risk clones. The wide spread of ST111, ST235, and ST244 in Korean hospitals, including the study sites, has been previously reported [18, 19, 32, 33]. ST245 was detected in Korea between 2006 and 2012 [32, 34] but not after 2014. After 2018, single locus variants of ST235, including *aroE* (ST622) and *nuoD* (ST1015) variants, were more prevalent than ST235. After 2017, eight unassigned STs that carried all *aroE* variations in the existing STs emerged (Supplemental Data Table S1). Our findings indicate that temporospatial clonal changes, primarily because of *aroE* variations, occur in Korean carbapenem-non-susceptible *P. aeruginosa* isolates.

CC235 *P. aeruginosa* isolates are notorious for being highly resistant to antimicrobial agents, including carbapenems [6]. In comparison to isolates of other STs, ST235 isolates showed higher resistance rates to aminoglycosides, piperacillin, piperacillin/tazobactam, cefepime, and ciprofloxacin. The therapeutic options for treating *P. aeruginosa* infections are limited by the prevalence of the carbapenem-resistant ST235 clone.

Carbapenem resistance in *P. aeruginosa* has been rapidly increasing via the clonal spread of high-risk clones carrying carbapenemase genes. In *P. aeruginosa* high-risk clones, two MBL genes, bla_{IMP} and bla_{VIM}, are widely distributed [6, 35]. The most frequent carbapenemase gene detected in Korean *P. aeruginosa* isolates was bla_{IMP-6}, followed by bla_{VIM-2} [33, 36]. The presence of bla_{IMP-1}, bla_{IMP-6}, and bla_{VIM-2} in Korean ST235 isolates has

Table 3. Mutations in *oprD*, carbapenemase genes, expression of *ampC*, and carbapenem MICs in carbapenem-non-susceptible *Pseudomonas aeruginosa* isolates

No. isolate	ST	Frameshift mutation in *oprD* (predicted amino acids [N]*)	Carbapenemase gene	Expression of *ampC*†	MIC (μg/mL)	
					Imipenem	Meropenem
KBN10P02278	111	+ (0)	-	2.0	32	16
KBN12P02599	111	+ (241)	-	0.8	16	4
KBN06P05035	111	+ (441)	-	1.2	8	0.5
KBN06P06750	111	+ (414)	-	1.6	128	16
KBN10P06128	235	+ (224)	bla_{VIM-2}	1.2	256	128
KBN10P06311	235	+ (93)	bla_{IMP-6}	1.3	16	>256
KBN12P04564	235	+ (93)	bla_{IMP-6}	5.7	8	>256
KBN06P03632	235	+ (224)	bla_{VIM-2}	4.5	128	64
KBN06P03793	235	+ (93)	bla_{IMP-6}	1.4	64	>256
KBN10P04040	244	+ (235)	-	17.2†	16	16
KBN06P05117	244	+ (355)	-	9.5	64	16
KBN06P02912	245	+ (344)	-	32.9†	32	16
KBN06P03210	245	+ (344)	-	32.8†	32	8
KBN06P02359	245	+ (344)	-	37.5†	64	16
KBN12P06040	357	+ (52)	-	0.2	32	32
KBN10P06125	622	+ (354)	-	14.7†	16	64
KBN06P06881	622	+ (93)	bla_{IMP-6}	0.5	64	>256
KBN12P06387	1015	+ (235)	-	1.1	16	64
KBN10P03382	1154	+ (224)	-	10.2†	64	16

*Nucleotide sequences of *oprD* in clinical isolates were compared with those of *P. aeruginosa* PAO1. OprD of *P. aeruginosa* PAO1 has 443 amino acids.
†Overexpression of *ampC* in clinical isolates.
Abbreviations: ST, sequence type; MIC, minimum inhibitory concentration.

been reported [18, 20]. In Korean ST298 and ST357 isolates, bla_{VIM-2} was also detected [18, 33]. All bla_{NDM-1} genes have been identified in ST773 isolates from Korean hospitals [20, 37, 38]. In recent years, bla_{NDM-1}-carrying ST773 isolates were increasingly prevalent in Korea, mostly in hospitals located in Daejeon city and Gyeongsang-do [20, 37]. Interestingly, most bla_{NDM-1}-carrying ST773 isolates originated from urine samples [20, 38]. In this study, two bla_{NDM-1}- and *rmtB*-carrying ST773 *P. aeruginosa* isolates were identified in sputum and blood samples collected at GNUH, located in Gyeongsang-do. The bla_{IMP-6}-carrying CC235 isolates from Korean hospitals harbored identical or similar class 1 integrons carrying bla_{IMP-6} as a gene cassette [18, 36, 39], indicating clonal spread rather than horizontal gene transfer. Of the three bla_{VIM-2}-carrying isolates identified in this study, two were ST235 and one was ST463. bla_{VIM-2} can also be inserted as a gene cassette in class 1 integrons [36]. As ST463 is not clonally related to ST235 and ST357, it is possible that the ST463 isolate acquired bla_{VIM-2} via horizontal gene transfer. This should be confirmed by genetic analysis surrounding bla_{VIM-2}. Our results demonstrated that the prevalence of MBL-producing *P. aeruginosa* in Korea is caused by the clonal spread of CC235 carrying bla_{IMP-6}, and the emergence and spread of new clones carrying bla_{VIM-2} or bla_{NDM-1} should be carefully monitored.

Of the 155 carbapenem-non-susceptible isolates, 43 produced MBLs, whereas the remaining 112 isolates may possess other mechanisms of carbapenem resistance, such as membrane permeability alterations, efflux pump overexpression, or AmpC β-lactamase de-repression. Interestingly, all CRPA isolates tested carried frameshift mutations in *oprD*, resulting in no or truncated protein production. A frameshift or substitutional mutation in *oprD* resulting in no or very low production in the outer membrane was found in 21 of 22 carbapenem-resistant bloodstream *P. aeruginosa* isolates from KNUH, in line with previous study results [19]. Overexpression of efflux pump genes, including *mexB* for MexAB-OprM, *mexD* for MexCD-OprJ, *mexF* for MexEF-OprN, and *mexY* for MexXY-OprM, was not observed in a

previous study [19], indicating that carbapenem resistance is not associated with overexpression of efflux pumps. Overexpression of *ampC* was observed in one ST308 isolate [19]. In this study, six of 19 isolates overexpressed *ampC*. AmpC hyperproduction was observed in MBL gene–negative isolates. It is generally responsible for resistance to cephalosporins and carbapenems [13], but one AmpC-hyperproducing ST245 isolate was susceptible to cephalosporins. Low membrane permeability is more closely associated with imipenem resistance than with meropenem resistance, whereas overexpression of efflux pumps is involved in reduced susceptibility to meropenem [15]. We previously demonstrated that $bla_{IMP\text{-}6}$-carrying *P. aeruginosa* isolates exhibited higher meropenem MICs than imipenem MICs [19]. In this study, nine of the 13 carbapenem-resistant isolates without MBL genes showed higher MICs for imipenem than for meropenem. All *P. aeruginosa* isolates carrying $bla_{IMP\text{-}6}$ exhibited higher meropenem MICs than imipenem MICs. Two $bla_{NDM\text{-}1}$-carrying *P. aeruginosa* isolates exhibited high-level resistance to imipenem and meropenem (≥256 μg/mL). In light of earlier findings, the present study suggests that a combination of frameshift mutations in *oprD* and the production of MBLs or hyperproduction of AmpC is responsible for carbapenem resistance in Korean *P. aeruginosa* isolates.

One limitation of this study is the small sample size. Although we carefully selected 155 from 345 carbapenem-non-susceptible *P. aeruginosa* isolates collected in three NCCPs, the limited sample size may have influenced the clonal distribution of *P. aeruginosa* isolates. Another limitation is that recent isolates were not included; therefore, our results cannot reflect the clonal changes and carbapenem resistance mechanisms of circulating *P. aeruginosa* isolates from Korean hospitals. The intrinsic carbapenem resistance mechanisms of *P. aeruginosa* were investigated in 13 isolates selected from the 112 MBL-negative isolates, which may have resulted in underestimation of the intrinsic carbapenem resistance mechanisms. However, the results obtained in this study, including *oprD* mutations and *ampC* overexpression, were in line with those from a previous study [19]. This emphasizes the importance of intrinsic carbapenem resistance mechanisms in Korean *P. aeruginosa* isolates.

In conclusion, numerous clonally diverse carbapenem-non-susceptible *P. aeruginosa* strains are circulating in Korean hospitals. The most common high-risk *P. aeruginosa* clone during the study period was ST235; however, single locus variants of ST235 caused by *aroE* or *nuoD* variations have recently emerged. Carbapenem resistance in Korean *P. aeruginosa* isolates is caused by low drug permeability because of the mutational inactivation of OprD combined with MBL production or AmpC hyperproduction. The spread of MBL-producing clones, particularly the $bla_{NDM\text{-}1}$-carrying ST773, and clonal changes in *P. aeruginosa* isolates should be carefully monitored.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2023.0369

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None.

AUTHOR CONTRIBUTIONS

Kim YK and Lee JC conceptualized the study. Kwon KT, Kim YK, and Lee JC contributed to data curation. Kim N, Ko SY, Park SY, Kim SY, and Lee DE contributed to methodology. Kwon KT acquired funding. Kim N and Lee JC wrote the original manuscript draft. Kim N, Kim YK, and Lee JC edited and reviewed the manuscript. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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