

Brief Communication



Genetic Epidemiology and Antimicrobial Susceptibilities of Carbapenem-Resistant *Pseudomonas aeruginosa* Isolates from a Multicenter Study in Korea

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ABSTRACT

Owing to concern that carbapenemase-producing strains among carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) isolates is on the rise, we investigated the genetic epidemiology and antimicrobial susceptibilities of clinical CRPA isolates collected in four academic hospitals in Korea. Carbapenemase genes were detected in 46 of 63 CRPA isolates (73.0%) collected between 2021 and 2024, and *bla*_{NDM} ST773 was the most common genotype (27 isolates, 42.9%), followed by *bla*_{NDM} ST644 (9 isolates, 14.3%) and *bla*_{IMP} ST235 (7 isolates, 11.1%). Overall susceptibility to ceftazidime/avibactam was only 17.5%, and none of the carbapenemase-producing isolates were susceptible to it. All ST644 strains were also resistant to aztreonam.

Keywords: Carbapenemase; Carbapenem-resistant *Pseudomonas aeruginosa*; Epidemiology; Multilocus sequence typing

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Pseudomonas aeruginosa is a non-fermenting Gram-negative rod that causes a variety of diseases. Its tendency to become resistant to antimicrobials makes it challenging to treat [1]. Carbapenem-resistant *P. aeruginosa* (CRPA) is in the high-risk pathogen category in the World Health Organization priority pathogen list [2]. It is estimated that 38,100 deaths are attributable to CRPA globally [3]. The prevalence and type of carbapenemase gene varies by region. In a global study, carbapenemase genes were present in 22% (211/972) of CRPA isolates, ranging from 2% in the United States (US) to 69% in South and Central America [3]. *bla*_{KPC-2} was the most common carbapenemase gene in South and Central America and China, while *bla*_{NDM-1} was the most common in Australia and Singapore. In an analysis by the Korea Global Antimicrobial Resistance Surveillance System, carbapenemase genes were detected in 30.5% (33/108) of CRPA isolates, and *bla*_{IMP-6} was the most common carbapenemase, followed by *bla*_{NDM-1} [4]. However, recent reports on the genetic epidemiology of CRPA isolates in Korea have raised concerns about the spread of a high-risk clone harboring *bla*_{NDM-1} [5, 6]. Updating the genetic epidemiology of CRPA isolates is important for infection control and prevention and for selecting newer antibiotics such as ceftolozane/tazobactam and ceftazidime/avibactam for treating CRPA because these agents are not active on isolates carrying metallo-beta-lactamase. Hence, we have investigated the carbapenemase genes and antimicrobial susceptibilities of CRPA identified in Korea.

This multicenter study was conducted at four academic hospitals in Seoul, Korea (Hospital A, Hospital D), Bucheon (Hospital B), and Cheonan (Hospital C) between January 2021 and September 2024. To minimize disruption to the routine workflow of the hospital laboratories, we collected samples over a three-month period at each hospital, which was selected based on their operational capacity and cooperation. For the samples from Hospital A, however, we utilized pre-existing specimens that had been collected since 2021. CRPA was defined as *P. aeruginosa* resistant to meropenem and/or imipenem. *P. aeruginosa* was identified by standard methods, and carbapenem resistance was defined as minimum inhibitory concentration (MIC) >8 µg/mL using the results of susceptibility testing by local clinical laboratories. To avoid disrupting the workflow of the microbiology laboratory, each hospital stored the CRPA isolates for a certain period in a frozen medium (Cryo-Beads, bioMérieux, Marcy-l'Étoile, France) and the collected isolates were then sent to the Center for Antimicrobial Resistance

and Microbial Genetics, University of Ulsan College of Medicine. Susceptibility to meropenem, tobramycin, colistin, and ceftazidime/avibactam was tested by the broth microdilution method applying the standard criteria of the Clinical and Laboratory Standards Institute (CLSI) [7]. For other antimicrobial agents, we used the results of automated susceptibility testing by each hospital, which were also based on CLSI susceptibility criteria.

Collected CRPA isolates were polymerase chain reaction-sequenced to identify *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM}, and *bla*_{GES} [8, 9]. Multilocus sequence typing (MLST) was performed for genes specifying the following seven housekeeping enzymes: acetyl coenzyme A synthetase, Shikimate dehydrogenase, guanosine monophosphate synthetase, DNA mismatch repair protein, NADH dehydrogenase I chain C, D, phosphoenolpyruvate synthase, and anthranilate synthetase component I at the Center for Genomic Epidemiology [10].

A total of 63 CRPA clinical isolates were collected, as follows: 6 (9.5%) from Hospital A in 2021, 5 (7.9%) isolates from Hospital A in 2022, 5 (7.9%) from Hospital A in 2023, 25 (39.7%) from Hospital D in 2023, 11 (17.5%) from Hospital B in 2023, and 11 (17.5%) from Hospital C in 2024. Twenty-seven of the isolates (42.9%) were obtained from blood specimens; ST773 (29 isolates, 46.0%) was the most common genotype, followed by ST644 (11 isolates, 17.5%), ST235 (7 isolates, 11.1%), ST357 (4 isolates, 6.3%), ST155 (2 isolates, 3.2%), ST244 (2 isolates, 3.2%), and one isolate (1.6%) each of ST253, ST313, ST319, ST352, ST378, ST412, ST1601, and ST2596. Carbapenemase genes were detected in 46 isolates (73.0%); *bla*_{NDM} (36 isolates, 57.1%) was the most common, followed by *bla*_{IMP} (7 isolates, 11.1%), *bla*_{GES} (2 isolates, 3.2%) and *bla*_{VIM} (one isolate, 1.6%).

As shown in **Table 1**, the proportions of the ST types of isolates varied by hospital. ST773 was the dominant strain in Hospital A and Hospital D, but not in the other hospitals. Only one isolate of ST644 was collected at Hospital B, and none at Hospital D. By year, ST644 was the dominant strain except in 2023, when it was ST773. ST773 strains were mostly isolated from urine, and ST644 and ST235 strains were isolated from blood. *bla*_{NDM} was detected in 93.1% (27/29) of the ST773 isolates and 81.8% (9/11) of the ST644 isolates. All ST235 isolates carried *bla*_{IMP}.

None of the isolates were susceptible to meropenem. Overall susceptibility rates for aztreonam, ceftazidime, cefepime, piperacillin/tazobactam, ciprofloxacin, and tobramycin were 50.8%, 11.1%, 15.9%, 11.1%, 6.3%, and

12.7%, respectively. Two isolates had MICs of 4 µg/ml for colistin. The overall susceptibility rate for ceftazidime/avibactam was only 17.5%. As shown in **Table 2**, none of the *bla*_{NDM} ST773, *bla*_{NDM} ST644, or *bla*_{IMP} ST235 isolates were susceptible to ceftazidime, cefepime, ceftazidime/avibactam, piperacillin/tazobactam, ciprofloxacin or tobramycin. Interestingly, 88.9% of the *bla*_{NDM} ST773 isolates were susceptible to aztreonam, compared with none of the ST644 isolates and only one ST235 isolate (14.3%).

Our findings show that *bla*_{NDM} ST773 and *bla*_{NDM} ST644 have become the dominant strains in Korea, and 73.0% of CRPA isolates harbored carbapenemase genes. The latter were susceptible to hardly any antimicrobials, not even ceftazidime/avibactam as a newer antimicrobial agent. We encountered a significant number of ST644 isolates resistant to aztreonam. These results provide important data for updating infection control and treatment strategies for CRPA in Korea.

The proportions of MLST types and the prevalences and types of carbapenemase genes among the CRPA isolates have been reported to vary by region and period. ST235, ST111, and ST175 have been reported as major high-risk clones globally [11]. VIM-2 producing *P. aeruginosa* is endemic in Europe, North America, South America, Africa, and Asia (China, India) and other carbapenemases identified among *P. aeruginosa* are less common [12]. Thus,

among 148 CRPA isolates collected from 29 hospitals in Korea in 2015, *bla*_{IMP-6} was the most prevalent gene (36 isolates, 24.3%), and all but two carbapenemase-producing *P. aeruginosa* isolates were identified as ST235 [13]. In the national surveillance data on antimicrobial resistance collected from 2017 to 2019, the prevalence of carbapenemase genes in CRPA isolates was 30.5% (33/108) and *bla*_{IMP-6}-positive isolates (17 isolates, 51.5%) were the most common, followed by *bla*_{NDM-1}-positive isolates (8 isolates, 24.2%), *bla*_{GES}-positive isolates (7 isolates, 21.2%), and *bla*_{VIM-2}-positive isolate (1 isolate, 3.0%) [4]. However, in a study of 73 imipenem/relebactam-resistant *P. aeruginosa* collected between 2018 and 2021, 45 (61.6%) isolates harboured a metallo-beta-lactamase gene, including *bla*_{NDM} (n=30) and *bla*_{IMP} (n=15) [5]. A study of 24 carbapenemase-producing *P. aeruginosa* strains from 9 hospitals and two nursing homes reported that the *bla*_{NDM-1} ST773 clone was emerging in Korea [6]. Overall, our results indicate that the frequency of carbapenemase producers among CRPA isolates is on the rise in Korea, and that a major cause of this epidemiological change is expansion of the *bla*_{NDM-1} ST773 clone.

The most worrying finding of our study is that clonal spreading of ST644 was suspected in two hospitals, severely limiting treatment options. The main characteristics of ST644 isolates are the high proportion carrying *bla*_{NDM}, and their resistance to aztreonam. There

Table 1. Characteristics and carbapenemase genes of carbapenem-resistant *Pseudomonas aeruginosa* according to sequence type

Characteristic	ST773 (n=29)	ST644 (n=11)	ST235 (n=7)	Others ^a (n=16)	Total (n=63)
Hospital					
Hospital A	7 (24.1)	5 (45.5)	2 (28.6)	2 (12.5)	16 (25.4)
Hospital B	2 (6.9)	1 (9.1)	2 (28.6)	6 (37.5)	11 (17.5)
Hospital C	3 (10.3)	5 (45.5)	1 (14.3)	2 (12.5)	11 (17.5)
Hospital D	17 (58.6)	0	2 (28.6)	6 (37.5)	25 (39.7)
Year					
2021	2 (6.9)	3 (27.3)	1 (14.3)	0	6 (9.5)
2022	1 (3.4)	2 (18.2)	0	2 (12.5)	5 (7.9)
2023	23 (79.3)	1 (9.1)	5 (71.4)	12 (75.0)	41 (65.1)
2024	3 (10.3)	5 (45.5)	1 (14.3)	2 (12.5)	11 (17.5)
Specimen					
Blood	10 (34.5)	7 (63.6)	4 (57.1)	6 (37.5)	27 (42.9)
Urine	16 (55.2)	4 (36.4)	2 (28.6)	1 (6.3)	23 (36.5)
Sputum	1 (3.4)	0	1 (14.3)	6 (37.5)	8 (12.7)
Others ^b	2 (6.9)	0	0	3 (18.8)	5 (7.9)
Carbapenemase gene					
<i>bla</i> _{NDM}	27 (93.1)	9 (81.8)	0	0	36 (57.1)
<i>bla</i> _{IMP}	0	0	7 (100)	0	7 (11.1)
<i>bla</i> _{GES}	0	0	0	2 (12.5)	2 (3.2)
<i>bla</i> _{VIM}	0	0	0	1 (6.3)	1 (1.6)

Data are presented as number (%) of isolates.

^aFour isolates of ST357, two isolates of ST155, two isolates of ST244, and one isolate each of ST253, ST313, ST319, ST352, ST378, ST412, ST1601, and ST2596.

^bThree isolates from tissues and two from body fluids.

Table 2. Antimicrobial susceptibility of carbapenem-resistant *Pseudomonas aeruginosa* according to major sequence type and carbapenemase genes

Antibiotic	Number (%) of isolates at each MIC (μg/mL)										Susceptibility (%)
	≤0.25	0.5	1	2	4	8	16	32	64	>64	
Aztreonam											
bla _{NDM} + ST773					20 (74.1)	4 (14.8)	2 (7.4)	1 (3.7)			88.9
bla _{NDM} - ST773					0	2 (100)	0	0			100
bla _{NDM} + ST644					0	0	0	9 (100)			0
bla _{NDM} - ST644					0	0	0	2 (100)			0
bla _{IMP} + ST235					0	1 (14.3)	1 (14.3)	5 (71.4)			14.3
Ceftazidime											
bla _{NDM} + ST773			0	0	0	0	0	27 (100)			0
bla _{NDM} - ST773			1 (50.0)	1 (50.0)	0	0	0	0			100
bla _{NDM} + ST644			0	0	0	0	0	9 (100)			0
bla _{NDM} - ST644			0	0	0	0	0	2 (100)			0
bla _{IMP} + ST235			0	0	0	0	0	7 (100)			0
Ceftazidime/avibactam ^a											
bla _{NDM} + ST773				0	0	0	0	27 (100)			0
bla _{NDM} - ST773				2 (100)	0	0	0	0			100
bla _{NDM} + ST644				0	0	0	0	9 (100)			0
bla _{NDM} - ST644				0	0	1 (50.0)	0	1 (50.0)			50
bla _{IMP} + ST235				0	0	0	0	7 (100)			0
Cefepime											
bla _{NDM} + ST773			0	0	0	0	0	27 (100)			0
bla _{NDM} - ST773			0	2 (100)	0	0	0	0			100
bla _{NDM} + ST644			0	0	0	0	0	9 (100)			0
bla _{NDM} - ST644			0	0	0	0	0	2 (100)			0
bla _{IMP} + ST235			0	0	0	0	0	7 (100)			0
Ciprofloxacin											
bla _{NDM} + ST773		0	0	0	27 (100)						0
bla _{NDM} - ST773		0	0	0	2 (100)						0
bla _{NDM} + ST644		0	0	0	9 (100)						0
bla _{NDM} - ST644		0	0	0	2 (100)						0
bla _{IMP} + ST235		0	0	0	7 (100)						0
Colistin ^b											
bla _{NDM} + ST773	1 (3.7)	10 (37.0)	15 (55.6)	0	1 (3.7)						96.3
bla _{NDM} - ST773	0	1 (50.0)	1 (50.0)	0	0						100
bla _{NDM} + ST644	0	5 (55.6)	4 (44.4)	0	0						100
bla _{NDM} - ST644	0	0	1 (50.0)	0	1 (50.0)						50
bla _{IMP} + ST235	0	2 (28.6)	5 (71.4)	0	0						100
Meropenem											
bla _{NDM} + ST773					0	1 (3.7)	0	0	26 (96.3)		0
bla _{NDM} - ST773					1 (50.0)	1 (50.0)	0	0	0		0
bla _{NDM} + ST644					0	0	0	0	9 (100)		0
bla _{NDM} - ST644					0	1 (50.0)	0	0	1 (50.0)		0
bla _{IMP} + ST235					0	0	0	0	7 (100)		0
Piperacillin/tazobactam											
bla _{NDM} + ST773						0	0	0	0	27 (100)	0
bla _{NDM} - ST773						2 (100)	0	0	0	0	100
bla _{NDM} + ST644						0	0	0	0	9 (100)	0
bla _{NDM} - ST644						0	0	0	2 (100)	0	0
bla _{IMP} + ST235						0	0	0	3 (42.9)	4 (57.1)	0
Tobramycin											
bla _{NDM} + ST773	0	0	0	0	0	0	0	1 (3.7)	26 (96.3)		0
bla _{NDM} - ST773	0	0	0	0	0	1 (50.0)	1 (50.0)	0	0		0
bla _{NDM} + ST644	0	0	0	0	0	0	1 (11.1)	8 (88.9)	0		0
bla _{NDM} - ST644	0	0	0	0	0	0	0	1 (50.0)	1 (50.0)		0
bla _{IMP} + ST235	0	0	0	0	0	0	0	2 (28.6)	5 (71.4)		0

^aMIC values for ceftazidime/avibactam correspond to 2/4, 4/4, 8/4, 16/4, and 32/4 instead of 2, 4, 8, 16, and 32.^bFor convenience, all isolates were considered susceptible to colistin except isolates resistant to colistin.

MIC, minimal inhibitory concentration.

seems to be no effective antimicrobial therapy left except for colistin, and their susceptibility to cefiderocol should be tested. The expansion of clone ST644 should be carefully monitored, and further research into the mechanisms of its resistance to aztreonam is also needed.

Our genetic epidemiologic findings suggest that the carbapenemase test may be helpful in decisions about antibiotics to use before the results for antimicrobial susceptibility are available. Also, empirical treatment using ceftazidime/avibactam without antimicrobial susceptibility results may be risky for treating CRPA in Korea. According to SMART global surveillance data, the susceptibility of CRPA collected in the US to ceftazidime/avibactam was 79.6% from 2019-2021 [14]. Ceftazidime/avibactam was added to the Korea National Health Insurance reimbursement drug list in February 2024. Unfortunately, our data reveal that CRPA already has pre-existing resistance to ceftazidime/avibactam possibly driven by high-risk clonal spreading. It is crucial to support new drug development for CRPA treatment, facilitate the introduction of novel therapies, and reintroduce aztreonam.

Our study has some limitations. First, CRPA isolates were not collected systemically, so the dates on which they were isolated and the proportions of the different specimen types varied. This makes it difficult to conclude whether the epidemiologic changes are occurring throughout Korea. Second, virulence genes and resistance mechanisms were not intensively evaluated. Third, the absence of clinical data restricted additional analyses on the effect of genetic epidemiology on outcomes.

In conclusion, high-risk strains harboring *bla*_{NDM} may be becoming dominant among CRPA isolates in Korea, and these strains are resistant to almost all antibiotics, including ceftazidime/avibactam. The detection of a significant number of ST644 isolates resistant to aztreonam is a worrisome finding. Efforts should be made to further investigate the association between genetic characteristics and prognosis, resistance mechanisms for the development of potential therapeutics.

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Ethics statement

This study, focusing solely on microorganisms and not involving human clinical data or human-derived materials, was confirmed by the Soonchunhyang University Bucheon Hospital Institutional Review Board to be exempt from review.

Conflict of Interest

TK is associate editor of Infect Chemother; however, he did not involve in the peer reviewer selection, evaluation, and decision process of this article. Otherwise, no potential conflicts of interest relevant to this article was reported.

Author Contributions

Conceptualization: TK. Data curation: YKH. Formal analysis: YKH, TK. Funding acquisition: TK. Investigation: YKH. Methodology: YPC, TK, YKH. Project administration: TK. Resources: YPC, TK, SP, JJ, OHC, HBS, TYC, YJC, MNK. Supervision: YPC. Writing - original draft: TK. Writing - review & editing: YPC, SP, JJ, OHC, HBS, TYC, YJC, MNK.

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