Brief Communication
Clinical Microbiology

Ann Lab Med 2023;43:196-199
https://doi.org/10.3343/alm.2023.43.2.196
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Emergence of NDM-1–producing *Pseudomonas aeruginosa* Sequence Type 773 Clone: Shift of Carbapenemase Molecular Epidemiology and Spread of 16S rRNA Methylase Genes in Korea

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Imipenemase (IMP)-6–producing *Pseudomonas aeruginosa* sequence type (ST) 235 is a dominant clone of carbapenemase-producing *P. aeruginosa* (CPPAE) in Korea. As part of the Antimicrobial Resistance Surveillance System in Korea, we found an increase in the carbapenem resistance rate of *P. aeruginosa* isolates from blood cultures and a shift in the molecular epidemiology of CPPAE. A total of 212 non-duplicated *P. aeruginosa* blood isolates were obtained from nine general hospitals and two nursing homes. Twenty-four isolates were identified as CPPAE. We observed the emergence of the NDM-1 *P. aeruginosa* ST 773 clone (N=10), mostly from Gyeongsang Province. The IMP-6 ST 235 clone (N=11) was detected in all provinces. CPPAE isolates showed very high resistance rates to amikacin, and all NDM-1 *P. aeruginosa* strains carried *rmtB*. This is the first nationwide surveillance of the recently emerged NDM-1–producing *P. aeruginosa* ST773 clone in Korea. Continuous surveillance is necessary to prevent the infection and transmission of carbapenem- and amikacin-resistant *P. aeruginosa* in Korea.

Key Words: *Pseudomonas aeruginosa*, Molecular Epidemiology, β-lactamase NDM-1, Sequence type 773, Amikacin, 16S rRNA methylase gene

Received: March 15, 2022
Revision received: June 29, 2022
Accepted: September 14, 2022

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Pseudomonas aeruginosa is the etiologic agent of various nosocomial infections, such as sepsis, pneumonia, and urinary tract infections. The majority of *P. aeruginosa* isolates are resistant to carbapenems [1]. Carbapenem resistance is frequently associated with loss of the outer membrane porin OrpD or expression of the efflux system, combined with extended-spectrum β-lactase production or AmpC hyperproduction [2]. Another important antimicrobial resistance mechanism is the acquisition of carbapenemase genes, such as those encoding Amber class A (GES type) and class B metallo-β-lactamases, including imipenemase (IMP), Verona integron metallo beta-lactamase (VIM), and New Delhi metallo- β-lactamase (NDM) [3]. IMP-6–producing *P. aeruginosa* sequence type (ST) 235 is the dominant clone of carbapenemase-producing *P. aeruginosa* (CPPAE) in Korea [4-7]. A 2020 study by the Antimicrobial Resistance Surveillance System in Korea (Kor-GLASS) [8] demonstrated an increase in the car-

bapenem resistance rate and a shift in the molecular epidemiology of *P. aeruginosa* blood isolates in 2020. In this report, we summarize the dominant clones and their characteristics.

A total of 212 non-duplicated *P. aeruginosa* blood isolates were isolated according to the Kor-GLASS manual [8]; the isolates were collected from nine general hospitals (three general hospitals in Seoul or Gyeonggi-do, two general hospitals in Gyeongsang-do, one in Gangwon-do, one in Jeolla-do, one in Chungcheong-do, and one in Jeju-do) and two nursing homes in Seoul. Of these, 24 isolates were identified as CPPAE. Due to the purely observational nature and very low risk to individual privacy of the participants, this study was approved by local institutional review boards (approval number: NHIMC-2022-06-012) and exempted from the requirement of informed consent.

Pure colonies of *P. aeruginosa* were collected in 10% skim milk and stored at –70°C before all collected isolates were transferred to the Korea Centers for Disease Control and Prevention (KCDC) analysis center using approved methods [8]. Bacterial species were verified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Biotyper, Bruker Daltonics GmbH, Bremen, Germany). Antimicrobial susceptibility was mainly determined by the disk diffusion test, except for the colistin minimum inhibitory concentration (MIC), which was determined by the broth microdilution method. The MICs of carbapenems (imipenem, meropenem, and doripenem) were also determined. The interpretation followed the CLSI guidelines [9].

P. aeruginosa isolates showing no susceptibility to imipenem or meropenem were PCR-sequenced to detect bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}, and bla_{GES}. DNA of freshly subcultured isolates was extracted using GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA). Genomic DNA concentration was measured using Qubit analyses (Thermo Fisher Scientific, Waltham, MA, USA), and 8 μg of input DNA was used. The entire genomes of the CPPAE isolates were sequenced using a NextSeq 550 instrument (Illumina, San Diego, CA, USA), and sequences were assembled using Spades (version 3.11.1) and annotated using Prokka (version 1.13.7). Resistance genes were obtained from the Center for Genomic Epidemiology website with ResFinder 4.1 [10]. Multilocus sequence typing (MLST) was determined with the following seven housekeeping genes: acetyl coenzyme A synthetase (*acsA*), Shikimate dehydrogenase (*aroE*), GMP synthase (*guaA*), DNA mismatch repair protein (*mutL*), NADH dehydrogenase I chain C, D (*nuoD*), phosphoenolpyruvate synthase (*ppsA*), and anthranilate synthetase component I (*trpE*) from the Center for Genomic Epidemiology [10].

In this study, resistance to carbapenem in *P. aeruginosa* blood isolates were concerning with the resistance rate of approximately 35% (Fig. 1). Especially, all CPPAE isolates were resistant to gentamicin, tobramycin, and ciprofloxacin. Most common metallo-β-lactamase, detected in carbapenem-resistant *P. aeruginosa* isolates was IMP-6 (N=11), following NDM-1 (N=10). Interest-

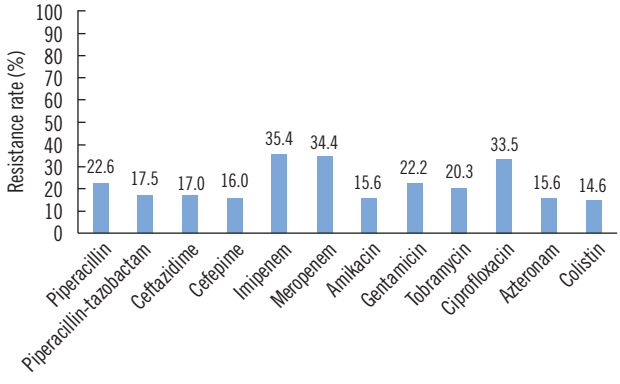

Fig. 1. Antimicrobial resistance rates of *P. aeruginosa* blood isolates (N=212).

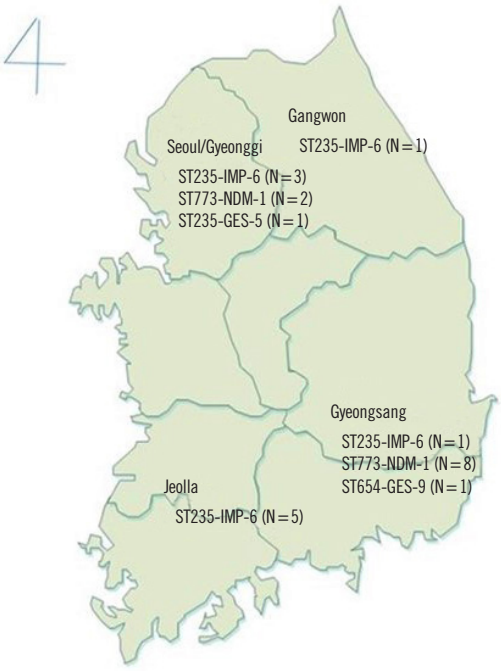

Fig. 2. Molecular epidemiology of carbapenemase-producing *Pseudomonas aeruginosa* isolates (sequence type: carbapenemase genotype). Carbapenemase-producing *P. aeruginosa* isolates were collected from two nursing homes in Seoul, three general hospitals in Seoul or Gyeonggi-do, one general hospital in Gangwon-do, one general hospital in Jeolla-do, and two general hospitals in Gyeongsang-do. Two isolates with undetermined sequence types were not shown in this map.

ingly, all NDM-1–producing *P. aeruginosa* strains were ST 773, where eight of them were isolated from two general hospitals in Gyeongsang-do (Fig. 2). The CPPAE isolates had various antimicrobial resistance genes, which are summarized in Supplemental Data Table 1.

The increase in carbapenem-resistant *P. aeruginosa* has become problematic considering the limitations of treatment options. Antimicrobial resistance due to carbapenemase genes is more challenging than that of other mechanisms such as membrane impermeability, with the possibility of horizontal gene transfer. Nationwide monitoring of the molecular epidemiology of CPPAE is helpful in establishing an adequate strategic policy for the control of antimicrobial resistance. IMP-6–producing *P. aeruginosa* ST 235 is the dominant clone in Korea [4-7]. IMP-1 is a globally prevalent subtype among 79 IMP variants, whereas IMP-6 is the dominant subtype in Korea [3]. IMP-6 has better hydrolyzing activity against meropenem. The frequent use of meropenem in clinical settings may have contributed to the spread of this type in Korea [3]. GES-type CPPAE clinical isolates from long-term care facilities and general hospitals are limited in Korea [11].

A recent study reported the clonal spread of NDM-1–producing *P. aeruginosa* ST 773 isolates possessing *rmtB4*, mostly from urine isolates, at a university hospital in Korea [12]. In the present nationwide surveillance of blood isolates, the emergence of NDM-1–producing *P. aeruginosa* ST 773 was found mostly in Gyeongsang Province, which may indicate a shift in the molecular epidemiology of CPPAE. NDM-producing *P. aeruginosa* has been identified mostly in Asia, Europe, and Africa, demonstrating intercontinental dissemination. NDM-1 is the most prevalent subtype, but it is rarely reported in Korea [3]. Recently, NDM-1–producing *P. aeruginosa* isolates were reported at a university hospital in Seoul, Korea [12].

The combination of carbapenem and amikacin has become an important treatment option to combat multidrug-resistant or extensively drug-resistant *P. aeruginosa* [13]. In a previous report, NDM-1–producing *P. aeruginosa* isolates had the highest sensitivity to tigecycline and amikacin [14]. In the present study, high rates of resistance to amikacin were also observed for CPPAE. All the NDM-1–carrying *P. aeruginosa* strains were resistant to amikacin and possessed *rmtB*. Junaid [14] also reported that NDM-producing *P. aeruginosa* isolates co-harboring *rmtC* showed a very high amikacin MIC (more than 2,048 μg/mL). Continuous surveillance is necessary to prevent the infection and transmission of carbapenem- and amikacin-resistant *P. aeruginosa* in Korea.

ACKNOWLEDGMENTS

None.

AUTHOR CONTRIBUTIONS

Conceptualization: Kim YA; Data curation: Choi YJ; Methodology: Kim J; Validation: Jeong SH, Shin JH, Shin KS, Shin JH, Kim YR, Kim HS, Uh Y, Ryoo NH; Writing-original draft: Choi YJ; Writing–review and editing: Kim YA. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

None to declare.

RESEARCH FUNDING

This work was supported by the Research Program funded by the Korea Disease Control and Prevention Agency (2020E540600).

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REFERENCES

1. Yu SM, Jeon SS, Kang IS, An HG. Status of nosocomial urinary tract infections in the ICU: molecular epidemiology of imipenem resistant *P. aeruginosa*. Taehan Kanho Hakhoe Chi 2006;36:1204-14.
2. Lee JY and Ko KS. OprD mutations and inactivation, expression of efflux pumps and AmpC, and metallo-β-lactamases in carbapenem-resistant *Pseudomonas aeruginosa* isolates from South Korea. Int J Antimicrob Agents 2012;40:168-72.
3. Yoon EJ and Jeong SH. Mobile carbapenemase genes in *Pseudomonas aeruginosa*. Front Microbiol 2021;12:614058.
4. Yoo JS, Yang JW, Kim HM, Byeon J, Kim HS, Yoo JI, et al. Dissemination of genetically related IMP-6-producing multidrug-resistant *Pseudomonas aeruginosa* ST235 in South Korea. Int J Antimicrob Agents 2012;

39:300-4.
5. Seok Y, Bae IK, Jeong SH, Kim SH, Lee H, Lee K. Dissemination of IMP-6 metallo-β-lactamase-producing *Pseudomonas aeruginosa* sequence type 235 in Korea. J Antimicrob Chemother 2011;66:2791-6.
6. Cho HH, Kwon KC, Sung JY, Koo SH. Prevalence and genetic analysis of multidrug-resistant *Pseudomonas aeruginosa* ST235 isolated from a hospital in Korea, 2008-2012. Ann Clin Lab Sci 2013;43:414-9.
7. Kim MJ, Bae IK, Jeong SH, Kim SH, Song JH, Choi JY, et al. Dissemination of metallo-β-lactamase-producing *Pseudomonas aeruginosa* of sequence type 235 in Asian countries. J Antimicrob Chemother 2013; 68:2820-4.
8. Lee H, Yoon EJ, Kim D, Jeong SH, Shin JH, Shin JH, et al. Establishment of the South Korean national antimicrobial resistance surveillance system, Kor-GLASS, in 2016. Euro Surveill 2018;23:1700734.
9. CLSI. Performance standards for antimicrobial susceptibility testing. 28th ed. CLSI M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2020.
10. Center for Genomic Epidemiology. www.genomicepidemiology.org (Updated on Feb 2022).
11. Hong JS, Choi N, Kim SJ, Choi KH, Roh KH, Lee S. Molecular characteristics of GES-type carbapenemase-producing *Pseudomonas aeruginosa* clinical isolates from long-term care facilities and general hospitals in South Korea. Microb Drug Resist 2020;26:605-10.
12. Hong JS, Song W, Park MJ, Jeong S, Lee N, Jeong SH. Molecular characterization of the first emerged NDM-1-producing *Pseudomonas aeruginosa* isolates in South Korea. Microb Drug Resist 2021;27:1063-70.
13. Farhan SM, Raafat M, Abourehab MAS, Abd El-Baky RM, Abdalla S, El-Gendy AO, et al. Effect of imipenem and amikacin combination against multi-drug resistant *Pseudomonas aeruginosa*. Antibiotics 2021;10:1429.
14. Junaid K. Molecular diversity of NDM-1, NDM-5, NDM-6, and NDM-7 variants of New Delhi metallo-β-lactamases and their impact on drug resistance. Clin Lab 2021;67.