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Original article

Current prevalence of the *crpP* gene in carbapenemase-producing *Pseudomonas aeruginosa* blood isolates in Korea

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Abstract

Background: Recently, CrpP enzymes have been described as a novel cause of ciprofloxacin resistance. The *crpP* gene encodes a novel protein that specifically confers resistance to ciprofloxacin through an adenosine triphosphate-dependent mechanism that phosphorylates the antimicrobial. In this study, the current prevalence of the *crpP* gene in carbapenemase-producing *Pseudomonas aeruginosa* blood isolates was evaluated.

Methods: During the study of the Antimicrobial Resistance Surveillance System in Korea, 22 blood isolates of carbapenemase-producing *P. aeruginosa* were collected from nine general hospitals and two nursing homes in the year 2020. Resistance genes and phylogenic trees were analyzed with the whole genome sequencing data.

Results: A total of 11 *P. aeruginosa* blood isolates coharbored the *crpP* and carbapenemase genes (nine IMP-6 producers and two GES-5-producers). Nine NDM-1-producers coharbored *aac(6')-Ib-cr* and *qnrVC1*. One GES-9-producer also carried *aac(6')-Ib-cr,* and one NDM-1-producer also carried *qnrVC1.* The phylogenic tree showed no epidemiologic link among the 22 carbapenemase-producing *P. aeruginosa* isolates.

Conclusion: This is the first report on the current prevalence of the *crpP* gene in carbapenemase-producing *P. aeruginosa* blood isolates in Korea.

Keywords: Fluoroquinolone, Resistance, *crpP* gene, *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa, one of the antimicrobial-resistant ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species) pathogens, represents a global threat to human health [1]. *P. aeruginosa* usually shows multidrug resistance. Its co-resistance to carbapenems, aminoglycosides, polymyxins, and tigecycline has also increased [2]. There are limited treatment options for serious infections caused by multidrug-resistant *P. aeruginosa*. Many combination therapies have been tried [3]. Recently, it has been reported that combination therapy including ciprofloxacin is correlated with a lower mortality. A combination of a beta-lactam and ciprofloxacin has been proposed for ciprofloxacin-susceptible *P. aeruginosa* bacteremia [4].

According to data from the Korean global antimicrobial resistance surveillance system (Kor-GLASS) [5], of *P. aeruginosa* blood isolates in 2017-2019, 12.3% showed resistance to piperacillin, 13.1% to ceftazidime, 12.9% to cefepime, 21.3% to imipenem, 22.1% to meropenem, 9.6% to amikacin, and 18.9% to ciprofloxacin. Most the carbapenemase-producers exhibited co-resistance to amikacin. However, data about its co-resistance to ciprofloxacin are unavailable. This study provides the newest data about the resistance mechanism to fluoroquinolone of carbapenemase-producing *P. aeruginosa*.

Resistance mechanisms of *P. aeruginosa* to fluoroquinolone are known mostly through the acquisition of mutations in genes encoding target proteins of fluoroquinolone and regulators of efflux pumps, leading to overexpression of these pumps [6]. Quinolone resistance may also be attributable to mutations of target enzymes of topoisomerases II and IV encoded by *gyrA* and *parC*, respectively [7]. These mechanisms are known to be chromosomally mediated. However, plasmid-mediated quinolone resistance (PMQR) in *P. aeruginosa* is also important. It is associated with *qnrA-E*, *qnrS*, *qnrVC*, *qepA*, *oqxAB* (efflux pump), and *acc(6′)-Ib-cr* (quinolone-modifying enzyme) [7]. Recently, CrpP enzymes have been described as a novel ciprofloxacin-resistance mechanism. The *crpP* gene encodes a novel protein, capable of specifically conferring resistance to ciprofloxacin in *Escherichia coli* through an adenosine triphosphate-dependent mechanism that involves phosphorylation of the antibiotic [8].

The purpose of this study was to report on the current situation of *crpP* gene spread in carbapenemase-producing *P. aeruginosa* blood isolates in Korea.

Materials and methods

In this study, non-duplicated *P. aeruginosa* blood isolates were isolated from nine nationwide general hospitals (National Health Insurance Service Ilsan Hospital, Gangnam Severance Hospital, Chonnam National University Hospital, Chungbuk National University Hospital, Busan Paik Hospital, Jeju National University Hospital, Hallym University Dongtan Sacred Heart Hospital, Wonju Severance Christian Hospital, Keimyung University Dongsan Hospital) and two nursing homes according to the Kor-GLASS manual [5] in 2020. Briefly, pure colonies of *P. aeruginosa* were collected in 10% skim milk and stored at -70°C before all collected isolates were transferred to a single analysis center of the Korea Disease Control and Prevention Agency for analyses using approved methods [5]. Bacterial species were verified using

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (Bruker Biotyper, Bruker Daltonics GmbH, Bremen, Germany). Antimicrobial susceptibility was mainly determined by the disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [9].

To detect carbapenemase-producers, *P. aeruginosa* isolates showing nonsusceptibility to imipenem or meropenem were PCR-sequenced to detect bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}, and bla_{GES}. For whole-genome sequencing, DNAs of freshly subcultured isolates were extracted using a GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA). Then 8 μg of input genomic DNA was used to sequence the entire genome using NextSeq 550 instrument (Illumina, San Diego, CA, USA). Sequences were assembled with Spades (version 3.11.1) and annotated with Prokka (version 1.13.7). Resistance genes were obtained with ResFinder 4.1 from the website of the center for genomic epidemiology [10]. A phylogenic tree was generated based on whole-genome multilocus sequence typing using a BioNumerics software, version 7.6.3 (Applied Maths, St Martens Latem, Belgium) [11] (Fig. 1).

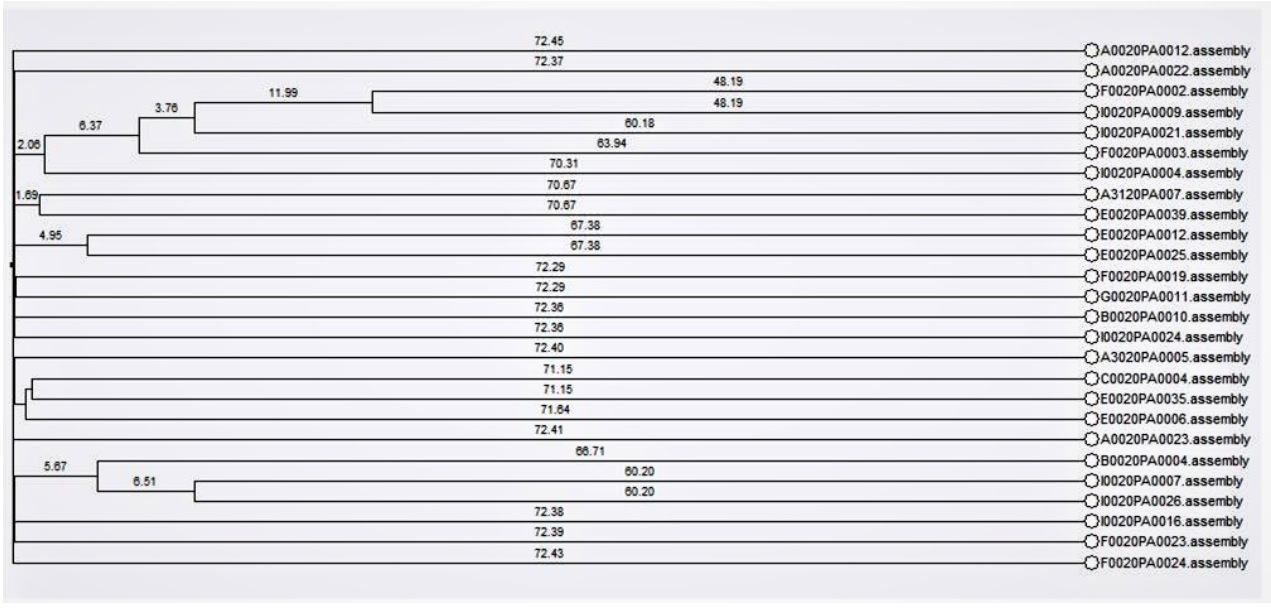

Fig. 1. Phylogenic tree of 22 carbapenemase-producing *Pseudomonas aeruginosa* strains based on whole genome multilocus sequence typing. Dendrogram was generated with BioNumerics software.

Results

Among 212 non-duplicated *P. aeruginosa* blood isolates, 22 carbapenemase-producing *P. aeruginosa* isolates showed resistance to both ciprofloxacin and imipenem. A total of 11 carbapenemase-producing *P. aeruginosa* blood isolates (nine IMP-6 producers and two GES-5-producers) co-harbored *aac(6')-Ib-cr* and *crpP* (Table 1). A total of nine NDM-1-producers co-harbored *aac(6')-Ib-cr* and *qnrVC1*. One GES-9-producer also carried *aac(6')-Ib-cr* and one NDM-1-producer also carried *qnrVC1*. The phylogenic tree showed no epidemiologic link among 22 carbapenemase-producing *P. aeruginosa* isolates.

Table 1. Resistance to carbapenem and ciprofloxacin with corresponding resistance genes

Isolates	Phenotype (ZD)			Genotype			
	IPM	MEM	CIP	CRP	PMQR	*gyrA*	*parC*
F0020PA0023	R (11)	R (10)	R (6)	bla_{GES-9}	*aac(6')-Ib-cr*	Thr83Ile	Ser87Leu
A0020PA0022	R (7)	R (6)	R (6)	bla_{GES-5}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
A0020PA0005	R (7)	R (6)	R (6)	bla_{GES-5}	*aac(6')-Ib-cr, crpP*	Thr83Ile	WT
A0020PA0023	R (6)	R (6)	R (6)	bla_{IMP-6}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
A0020PA0007	R (6)	R (6)	R (6)	bla_{IMP-6}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
C0020PA0004	R (6)	R (6)	R (6)	bla_{IMP-6}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
E0020PA0006	R (6)	R (6)	R (6)	bla_{IMP-6}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
E0020PA0012	R (6)	R (6)	R (6)	bla_{IMP-6}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
E0020PA0025	R (6)	R (6)	R (6)	bla_{IMP-6}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
E0020PA0035	R (6)	R (6)	R (6)	bla_{IMP-6}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
E0020PA0039	R (6)	R (6)	R (6)	bla_{IMP-6}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
I0020PA0016	R (6)	R (6)	R (6)	bla_{IMP-6}	*aac(6')-Ib-cr, crpP*	Thr83Ile	Ser87Leu
B0020PA0004	R (6)	R (6)	R (6)	bla_{NDM-1}	*aac(6')-Ib-cr, qnrVC1*	Thr83Ile	Ser87Leu
B0020PA0010	R (6)	R (6)	R (6)	bla_{NDM-1}	*aac(6')-Ib-cr, qnrVC1*	Thr83Ile	Ser87Leu
F0020PA0002	R (6)	R (6)	R (6)	bla_{NDM-1}	*aac(6')-Ib-cr, qnrVC1*	Thr83Ile	Ser87Leu
F0020PA0003	R (6)	R (6)	R (6)	bla_{NDM-1}	*aac(6')-Ib-cr, qnrVC1*	Thr83Ile	Ser87Leu
I0020PA0007	R (6)	R (6)	R (6)	bla_{NDM-1}	*aac(6')-Ib-cr, qnrVC1*	Thr83Ile	Ser87Leu
I0020PA0009	R (6)	R (6)	R (6)	bla_{NDM-1}	*aac(6')-Ib-cr, qnrVC1*	Thr83Ile	Ser87Leu
I0020PA0021	R (6)	R (6)	R (6)	bla_{NDM-1}	*aac(6')-Ib-cr, qnrVC1*	Thr83Ile	Ser87Leu
I0020PA0024	R (6)	R (6)	R (6)	bla_{NDM-1}	*aac(6')-Ib-cr, qnrVC1*	Thr83Ile	Ser87Leu
I0020PA0026	R (6)	R (6)	R (6)	bla_{NDM-1}	*aac(6')-Ib-cr, qnrVC1*	Thr83Ile	Ser87Leu
I0020PA0004	R (6)	R (6)	R (6)	bla_{NDM-1}	*qnrVC1*	Thr83Ile	Ser87Leu

Abbreviations: IMP, imipenem; MEM, meropenem; CIP, ciprofloxacin; CRP, carbapenemase; PMQR, plasmid-mediated quinolone resistance; R, resistant; ZD, zone diameter (mm); WT, wild type.

Discussion

Quinolones have been commonly used as a treatment option for a large number of bacterial infections. Ciprofloxacin is the most active antibiotic in this group. The increase in quinolone resistance has limited the effect of quinolones. There was no report about the prevalence of quinolone resistance genes in *P. aeruginosa* in Korea to the best of our knowledge. Recently, novel mutations in *gyrA* and *parC* genes were first found in *P. aeruginosa* isolated from companion dogs in South Korea [12]. In that study, a total of 84 nonduplicated *P. aeruginosa* strains were obtained from healthy dogs and infected dogs. The resistance rate was 14.3% for levofloxacin and 13.1% for ciprofloxacin. The resistance was commonly associated with *gyrA* mutations [12].

The information about the prevalence of quinolone resistance genes of clinically important pathogens in Korea is limited. For *K. pneumoniae*, mutations in *gyrA* and *parC* were found in 78.9% and 65.5% of 142 extended-spectrum β-lactamases-producers, respectively [13]. The common PMQR gene was *qnrB-aac(6')-Ib-cr-oqxAB* (58/142, 40.8%) [13]. Lee et al. [14] have reported the presence of PMQR genes in *Salmonella enterica* isolated from human salmonellosis patients in South Korea from 2016 to 2019 [14]. Among 34 *Salmonella* strains with reduced susceptibility to quinolones, 25 strains harbored one or two of *qnrA*, *qnrB*, *qnrS* (most common), and *aac(6')-Ib-cr* genes.

Out of 22 carbapenemase-producers from nationwide collections, we detected 11 *P. aeruginosa* blood isolates that co-harbored *crpP* and carbapenemase genes. This is the first report about the current situation of *crpP* gene spread in carbapenemase-producing *P. aeruginosa* blood isolates in Korea. Because all strains also have *aac(6')-Ib-cr* and chromosomal mutations in *gyrA* or *parC*, the effect of the presence of *crpP* gene on quinolone resistance is hardly speculated in this study.

The *crpP* gene obtained from the pUM505 plasmid isolated from a *P. aeruginosa* clinical isolate was identified in 1986 [8]. Regarding the expression of CrpP in the transconjugants test, CrpP proteins increased minimal inhibitory concentration values of ciprofloxacin concerning *E. coli* J53-3. However, these changes were not enough to be considered as resistance according to the breakpoint value (≥ 4 mg/L) reported for this interpretation by the CLSI [8]. Nevertheless, the *crpP* gene conferring low-level resistance could facilitate the selection of mutants with a higher level of quinolone resistance. In addition, plasmids contain other genes in addition to the *crpP* gene, which encodes additional quinolone resistance mechanisms [8].

In this study, the type of carbapenemase of *crpP* gene-carrying *P. aeruginosa* was mostly IMP-6, known to be a common type in Korea [15]. NDM-1-producing *P. aeruginosa* isolates did not carry *crpP* genes, which seemed to become one of the majority types of carbapenemase in *P. aeruginosa*. The shift in the molecular epidemiology of carbapenemase genes might change the molecular epidemiology of PMQR genes.

Mobile genetic elements play a key role in the spread of resistance genes, and high-risk clones frequently integrate such determinants into their genomes [16]. In this study, the linkage of *crpP* and *aac(6')-Ib-cr* gene with $bla_{\text{IMP-6}}$ is suspected. And there is high possibility that *aac(6')-Ib-cr* and *qnrVC1* are associated with $bla_{\text{NDM-1}}$ in *P. aeruginosa*.

If only PMQR is present, it is expected to be susceptible or low-grade resistance phenotypes [7,8]. Unfortunately, the only effect of *crpP* gene is hard to know in this study, because all isolates had chromosomal mutations in *gyrA* or *parC*, in addition to PMQRs. These strains are usually associated with a large reduction in biological fitness with the accumulation of more resistance-associated mutations [17]. One more thing to consider is that NDM-1 carrying *P. aeruginosa* is mostly separated from hospitals in Daegu or Busan and the regional differences could have an effect. Therefore, we suggest continuous monitoring of the change of PMQR in *P. aeruginosa*.

In conclusion, the spread of *P. aeruginosa* isolates, coharboring *crpP* and carbapenemase genes were found in this study. This is the first report about the current situation of *crpP* gene spread in carbapenemase-producing *P. aeruginosa* blood isolates in Korea.

Ethics statement

It is not a human population study; therefore, approval by the institutional review board or the obtainment of informed consent is not required.

Conflicts of interest

Jeong Hwan Shin, Young Uh, and Nam Hee Ryoo are currently editorial board members of the *Annals of Clinical Microbiology*. However, they were not involved in the review process of this article.

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