

Emergence of Carbapenem-Resistant ST244, ST292, and ST2446 *Pseudomonas aeruginosa* Clones in Burn Patients in Yunnan Province

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Introduction: The prevalence of carbapenem-resistant *Pseudomonas aeruginosa* is increasing persistently, particularly in burn ward isolates. Here, we investigate the prevalence of carbapenem-resistant *Pseudomonas aeruginosa* in a burn ward of a provincial-level hospital at Kunming, Yunnan province, China.

Methods: A total of 118 *P. aeruginosa* strains were isolated from 57 hospitalized patients, and their MICs were measured. Carbapenem-resistant isolates were selected for multilocus sequence typing (MLST). Carbapenem-resistance mechanisms were identified by examining carbapenemase genes and OprD protein and Carba-NP testing. Representative isolates were further characterized by de novo sequencing for carbapenemase molecular background.

Results: Among 118 *P. aeruginosa* isolates, 54 (54/118, 45.8%) were carbapenem-resistant *Pseudomonas aeruginosa*, and 3 genotypes were found (ST292, ST244, and ST2446). Non-carbapenemase-producing ST292 was the most prevalent ST, followed by ST2446 and ST244. A novel 13-bp *oprD* deletion was found in the ST292 clone, which formed the truncated outer membrane protein and may cause carbapenem resistance. ST244 and ST2446 harbored *blaIMP-45* and *blaIMP-87*, respectively. *blaIMP-45* is located in a megaplasmid, together with *aac(6')-Ib3*, *blaOXA-1*, *catB3*, *qnrVC6*, *armA*, *msr(E)*, *mph(E)*, *aph(3')-Ia*, *tetC/tetR*, *aac(6')-Ib3*, *floR*, *mexC-mexD-oprJ*, *fosA* and lead to extensive drug resistance. ST2446 contains a carbapenem-resistant gene *blaIMP-87* on the chromosome and is acquired by a novel gene cassette array (*blaIMP-87-ant(2'')-Ia-blaOXA-10-aac(6')-Ib3*) of class 1 integron.

Discussion: For the first time, ST244, ST292 and ST2446 are reported emerging in burn patients, with distinctive carbapenem-resistance mechanisms, respectively. The obtained results highlight the need to surveillance carbapenem-resistant isolates in burn patients.

Keywords: *P. aeruginosa*, carbapenem-resistance, *oprD*, *blaIMP*

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a common clinical opportunistic pathogen that causes life-threatening nosocomial infections. Burn patients are the main targets of nosocomial infections caused by *P. aeruginosa*, which is a leading cause of burn patients morbidity and mortality.¹ The loss of the skin barrier, cellular damage, and tissue destruction due to burning injury can provide a suitable environment for *P. aeruginosa* accumulation and production.² Furthermore, the continuous fluid secretion from burn wounds boosts *P. aeruginosa* reproduction and stimulates the expression of virulence factors.³

Carbapenems are the drug of choice for treating *P. aeruginosa* infections. However, the prevalence of carbapenem-resistant *P. aeruginosa* is increasing annually⁴ and is becoming a global emerging public health issue. Multiple mechanisms, such as target

mutations, outer membrane protein porin loss, enzyme production, and multidrug efflux systems, are responsible for drug resistance in *P. aeruginosa*.⁵ IMP enzymes, the class B metalloenzyme, was the first carbapenemase detected in *P. aeruginosa*.⁶ *blaIMP* has been described as an acquirable gene of carbapenemase, and its mobility is related to class 1 integrons located in the plasmid.⁷ *P. aeruginosa* plasmids have multiple incompatibility (Inc) or replicon types, such as IncF, N, HI2, P2, and are associated with the *blaIMP* gene propagation.^{8,9} Mutations in *P. aeruginosa* outer membrane porin D (OprD) reduce carbapenems sensitivity and lead to carbapenem resistance.¹⁰ However, carbapenem resistance due to OprD mutations cannot be horizontally transferred.

Multilocus sequence typing (MLST) is a portable, unambiguous, DNA-sequence-based technique widely used for molecular typing of bacteria.¹¹ According to estimates, ST274, ST244, ST235, ST277, and ST357 are the widely spread STs of *P. aeruginosa* in China.¹² The prevalence of *P. aeruginosa* STs among Chinese burn patients are distinct, for example, ST360 and ST316 were predominant in 2011 and 2012, while ST111 emerged in 2013 and became the primary ST in 2014.¹³ Molecular typing has significant potential in clinical implications, which help identify transmission mechanisms and predict potential STs future outbreaks.

Epidemiological characteristics of *P. aeruginosa* have indeed been reported from various parts of China. However, the prevalence of *P. aeruginosa* among burn patients in Yunnan is unclear. Additionally, antimicrobial resistance and genetic diversity of *P. aeruginosa* among burn patients in Yunnan are also lacking. Therefore, the current study explored the prevalence and antimicrobial resistance of *P. aeruginosa* among burn patients in Yunnan. Moreover, *P. aeruginosa* isolates were characterized using de novo sequencing.

Materials and Methods

Isolation and Antimicrobial Susceptibility Testing

From December 2014 to August 2015, burn wound secretion, blood, sputum, and urine samples were collected from patients hospitalized in the burns ward of the Second Affiliated Hospital of Kunming Medical University, located in the center of Kunming, the provincial capital of Yunnan province with approximately 44 million inhabitants. Samples were cultured, and *P. aeruginosa* strains were isolated. The isolates were identified by the VITEK-2 Compact (bioMérieux, Marcy l'Etoile, France).

The minimum inhibitory concentrations (MICs) of 7 antibiotics named imipenem; meropenem, piperacillin, ceftazidime, aztreonam, levofloxacin, and amikacin were assessed by broth-microdilution susceptibility testing method. *P. aeruginosa* ATCC25923 served as the control according to the Clinical and Laboratory Standards Institute (CLSI) Guidelines. Carbapenem-resistant isolates that showed resistance to imipenem or meropenem were selected.

Molecular Typing of Isolates

P. aeruginosa isolates were subjected to Multilocus sequence typing (MLST) according to the protocols described in the PubMLST (<http://pubmlst.org/paeruginosa>) database. Purified PCR products were sent to TsingKe Biological Technology (Kunming, China) and bidirectionally sequenced. Sequences were assembled by the SeqMan module of DNASTAR 7.0 (DNASTAR Inc., Madison, WI, USA). Allele profiles and sequence types (STs) were assigned by the MLST database (<http://pubmlst.org/paeruginosa>).

Antimicrobial-Resistant Genes Screening and Carbapenemase Phenotypic Detection

DNA was extracted with TIANamp Bacterial DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China). The multiple beta-lactam resistant genes, including carbapenemase genes (*blaIMP*, *blaNDM*, *blaIMI*, *blaSIM*, *blaGIM*, *blaVIM*, *blaKPC*, *blaOXA-23*, *blaOXA-51*), ESBL genes (*blaSHV*, *blaCTX-M*, and *blaGES*), and other beta-lactamase genes (*blaPAO*, *blaCMY*, *blaOXA-50*, *blaTEM*) were amplified by PCR. Sequencing was performed by TsingKe Biological Technology (Kunming, China). All acquired sequences were compared with the Comprehensive Antibiotic Resistance Database (CARD, <https://card.mcmaster.ca>). Further, the Carba-NP test was performed to verify the existence of carbapenemases.

Identifying the *oprD*

The *oprD* genes were amplified using the previously described primers,¹⁴ sequenced, and compared with the *oprD* gene of PAO1 via MEGA version 5.05. Outer membrane proteins were further isolated and separated by SDS-PAGE.¹⁵

De Novo Sequencing and Assembled

Genomic DNA of KB-PA_3 (ST2446), KB-PA_F6 (ST292), and KB-PA-F19 (ST244) were extracted and sent to Beijing Novogene Bioinformatics Technology Co., Ltd for sequencing. Low-quality reads were filtered by the SMRT 2.3.0 and the filtered reads were assembled to generate one contig without gaps.

Genome Annotation and Comparative Analysis

The contigs were annotated using PARIC (<https://patricbrc.org/>), screened for antimicrobial-resistant genes with resFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>), analyzed for insertion sequences and integron with ISfinder (<https://www-is.biotoul.fr/>). INTEGRALL (<http://integrall.bio.ua.pt/>) was used in integron analysis, and Phaster (<http://phaster.ca/>) was used in screening phages. Both SnapGene software (Insightful Science, www.snapgene.com) and Mauve tools were used for the comparative analysis of chromosomes.

Nucleotide Sequence Accession Numbers

The *oprD* sequences of ST292, ST2446, and ST244 isolates were submitted to Genbank with accession numbers OL372281, OL372282, and OL372283, respectively. The whole-genome sequences were submitted to GenBank within BioProject PRJNA774784, and the accession numbers for the chromosome and plasmid of KB-PA_F19 were CP086010, CP086011, CP086012, CP086013, CP086014, and CP086015, respectively. The accession numbers for the chromosome and plasmid of KB-PA_3 were CP086016 and CP086017, respectively.

Ethical Approval

This study was approved by Kunming University of Science and Technology and complied with guideline of the Declaration of Helsinki. Because this study only focused on bacteria, and all the clinical isolates were part of routine hospital laboratory procedures, patients informed consent was not required.

Results

The Prevalence of Carbapenem-Resistant *P. aeruginosa* Isolates

A total of 118 *P. aeruginosa* strains were isolated from 57 hospitalized patients. Fifty-four isolates (54/118, 45.8%) were carbapenem-resistant *P. aeruginosa*, obtained as follows 50 belong to burn wounds, 2 from blood, 1 from urine, and 1 from sputum. MLST was used to analyze the genotypic diversity of *P. aeruginosa* isolates based on 7 housekeeping genes. A total of 3 distinct MLST types were found (Table 1) with ST allele numbers (*acsA-aroE-guaA-mutL-nuoD-ppsA-trpE*) were ST244 (17-5-12-3-14-4-7), ST292 (109-10-73-3-4-4-3), ST2446 (111-30-64-26-30-59-55). We found that these 3 sequence types belonged to distinct clonal complexes (A clonal complex was composing STs that shared at

Table 1 The Isolation, MIC Range, Carba-NP Test, and PCR Detections of Resistance Genes Distribution Among the Three STs

ST	N	MIC Range (μg/mL)							Carba-NP Test	β-Lactamase Genes
		PIP	ATM	CAZ	IPM	MEM	AMK	LEV		
244	2	>128	8	>128	32	64	>128	>8	+	<i>blaIMP-45</i> , <i>blaPAO</i> , <i>blaOXA-396</i>
292	49	>128	32	>128	8~16	8~32	2~4	4~8	-	<i>blaPAO</i> , <i>blaSHV-1</i> , <i>blaCMY-2</i> , <i>blaOXA-50</i>
2446	3	>128	8	>128	128	128	8	8	+	<i>blaIMP-87</i> , <i>blaPAO</i> , <i>blaOXA-50</i>

Abbreviations: ST, sequence types; PIP, piperacillin; ATM, aztreonam; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; AMK, amikacin; LEV, levofloxacin.

least any six of the seven gene alleles). All ST244 and ST2446 isolates were resistant to all tested antimicrobials, except ATM. However, 47/49 ST292 isolates were resistant to beta-lactams and quinolone.

Carbapenem-Resistance Mechanisms

The beta-lactamase genes distribution varied according to the ST type (Table 1). ST244 and ST2446 clones carried the carbapenemase genes *blaIMP-45* and *blaIMP-87*, respectively. However, ST292 was negative for all tested carbapenemase genes. *blaSHV-1* and *blaCMY-2* genes were present only in ST292 isolates. Moreover, all 54 isolates were positive with *blaPAO* and *blaOXA-50* (*blaOXA-396* is a subtype of *blaOXA-50*).

Carba-NP tests were performed to verify isolates may harbor any other carbapenemase. ST292 isolates were negative, while ST244 and ST2446 were positive (Table 1). Further, *oprD* was found in all carbapenem-resistant *P. aeruginosa* isolates, and the *oprD* sequences were identical within the same ST. However, the *oprD* nucleotide sequences of ST292, ST244, and ST2446 differed from the PAO1 sequence (91% of ST292, 90% of ST2446, and 91% of ST244). Moreover, deletion in *oprD* at position 233_245del13 of ST292 (Figure 1A) and at the 109del1 position of ST2446 were identified. The SDS-PAGE *OprD* protein profiles of ST292, ST244, and ST2446 showed that ST292 and ST2446 lacked the corresponding band for *OprD* compared to PAO1 and ST244 (Figure 2).

The General Feature of IMP Producing Genome

Whole-genome sequencing results revealed that KB-PA_3 and KB-PA_F19 genomes consist of single circular chromosomes, with a genome size of 7,098,808-bp and 6,610,918-bp, respectively (Table 2). Compared with the reference chromosome of PAO1 (NC002516.2), KB-PA_3 and KB-PA_F19 contain similar chromosomal information (Figure 3), with different genome architecture. KB-PA_3 chromosome contained ~13% additional nucleotides, and KB-PA_F19 contained ~4.5% additional nucleotide.

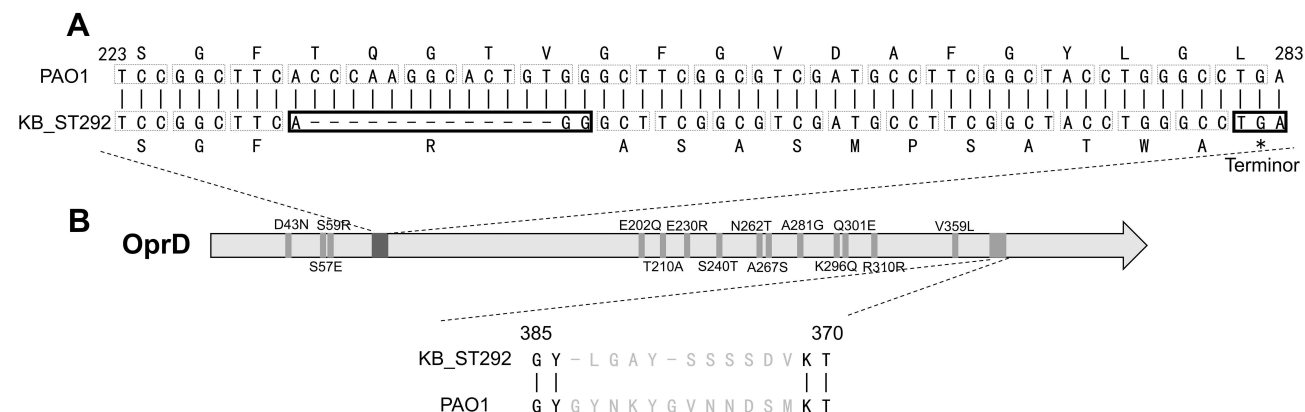


Figure 1 The *OprD* mutations of KB_ST292 (ST292 clones in Kunming burn ward) and compared with PAO1. (A) Deletion of 1088_1100del13 leads to a frame-shift mutation and early termination at 1050–1052, *=Terminor; (B) Locations of amino acid substitutions (14 sites) and a 12-amino acid mutation.

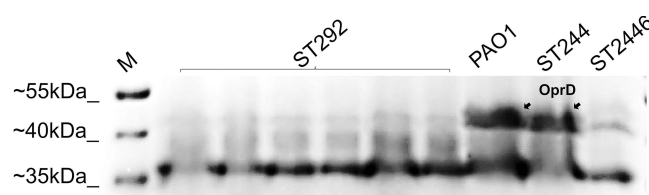


Figure 2 Out membrane profiling determined by SDS-PAGE; PAO1, reference *P. aeruginosa* strain; ST292, ST244, ST2446, representative clinical isolates. M, molecular size marker (Thermo). The arrow on the upper right indicates the banding position of *OprD*.

Table 2 Chromosome Statistics of *P. aeruginosa* Strains

Strains	ST	No. of Chromosomes	Assembly Status	Assembly Size (bp)	CDS	G+C (mol%)	Resistance Genes
KB-PA_3	2446	1	Circular	7,098,808	6,806	65.8	<i>Sul1</i> , <i>catB7</i> , <i>fosA</i> , <i>aac(6')-Ib3</i> , <i>ant(2'')-Ia</i> , <i>aph(3')-Ilb</i> , <i>aac(6')-Ib-cr</i> , <i>crpP</i> , <i>blaIMP-87</i> , <i>blaOXA-10</i> , <i>blaOXA-50</i> , <i>blaPAO</i>
KB-PA_F19	244	1	Circular	6,610,918	6,291	66.27	<i>catB7</i> , <i>fosA</i> , <i>aph(3')-Ilb</i> , <i>crpP</i> , <i>blaOXA-396</i> , <i>blaPAO</i>
KB-PA_F6	292	1	Circular	7,083,933	6,841	65.58	<i>Sul1</i> , <i>catB7</i> , <i>fosA</i> , <i>aac(6')-Ib3</i> , <i>crpP</i> , <i>blaOXA-396</i> , <i>blaPAO</i> , <i>aac(6')-Ib-cr</i> , <i>aadA2b</i> , <i>aph(3'')-Ib</i> , <i>aph(6)-Id</i> , <i>blaCARB-2</i> , <i>tet(G)</i>

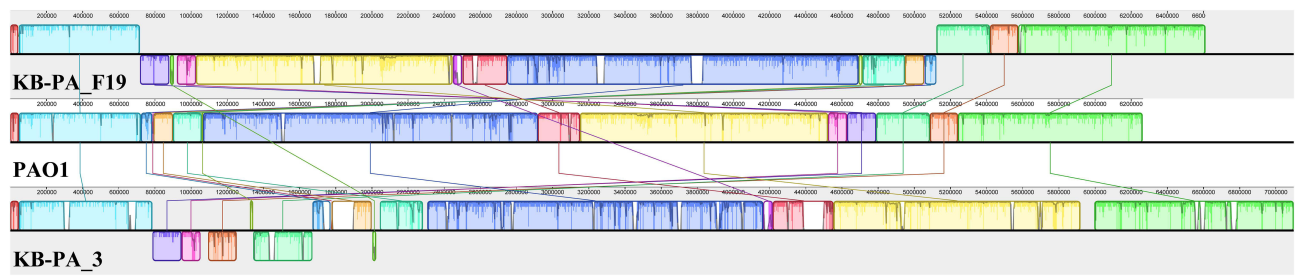


Figure 3 Mauve alignment for chromosomes of KB-PA_3, PAOI and KB-PA_F19; Chromosomes shows locally colinear blocks of matched colored regions. The gap between locally colinear blocks shows the additional regions.

To further analyze the additional sequences, the >4kb additional-regions of KB-PA_3 and KB-PA_F19 were extracted (Supplement S1). KB-PA_F19 had a total of 8 additional regions (max length was ~62k-bp), of which 5 contained phage-related genes and one contained insertion sequence-related genes. KB-PA_3 had 32 additional regions (max length ~109k-bp), of which 15 contained phage-related proteins, 3 contained virulence-related genes (T2SS, fimbria, and hemolysin), 3 contained mobile element genes (insertion sequence and transposon), and one contained T4SS related genes. PHASTER was used to analyze the prophage (Supplement S1), KB-PA_3 contained 7 intact prophage regions and 1 incomplete prophage region. KB-PA_F19 contained 7 intact prophage regions and 2 questionable prophage regions. All predicted prophage regions were located in the additional region of its chromosome. Moreover, KB-PA_3 and KB-PA_F19 shared 2 similar prophage regions (Figure 3), which were predicted as PHAGE_pseudo_F10 (NC007805) and PHAGE_pseudo_PMG1 (NC016765).

KB-PA_3 harbored a ~51k-bp circular plasmid (Table 3). According to analysis, there were a total of 73 CDS in pKB-PA_3-1. Among them, 50 were hypothetical proteins; however, type IV secretion system-related proteins were identified. BLAST analysis showed that pKB-PA_3 was similar to p1011-KPC2 (MH734334.1) with 91% query coverage, p1011-KPC2 was a 62,793-bp plasmid collected from *P. aeruginosa* in Hangzhou, China. We found that KB-PA_F19 contained five plasmids (range from 1,851-bp to 412,187-bp). Among them, pKB-PA_F19-4 is circular with a genome size of 412,187-bp and considered as megaplasmid (Table 3). The BLAST analysis showed that pKB-PA_F19-4 shares a highly similar backbone with an IncP2-megaplasmid family.¹⁶ The other 3 plasmids were linear and may involve gene transmission, mobile elements, and prophages. Even the pKB-PA_F19-2 contained a class 1 integron.

Table 3 Plasmids Identified in *P. aeruginosa* Strains

Plasmid Name	Assembly Status	Assembly Size (bp)	CDS	G+C (mol%)	Resistance Genes	Other Description
pKB-PA_3-1	Circular	50,936	73	59.57	–	T4SS
pKB-PA_F19-1	Linear	1,851	1	63.1	–	IS91 transposase
pKB-PA_F19-2	Linear	2,519	5	61.29	<i>SulI</i>	<i>IntI1</i> , IS6 transposase
pKB-PA_F19-3	Linear	2,691	8	63.99	–	Metal transporting
pKB-PA_F19-4	Circular	412,187	477	56.27	<i>aac(6′)-Ib3</i> <i>blaIMP-45</i> , <i>blaOXA-1</i> , <i>catB3</i> , <i>sulI</i> , <i>qnrVC6</i> , <i>armA</i> , <i>msr(E)</i> , <i>mph(E)</i> , <i>aph(3′)-Ia</i> , <i>tet(C)</i> , <i>aac(6′)-Ib3</i> , <i>floR</i> , <i>fosA</i>	<i>mexC-mexD-oprJ</i> efflux pump
pKB-PA_F19-5	Linear	5,207	7	56.83	–	Partial phage proteins

Analysis of Antibiotic Resistance Genes

catB7, *fosA*, *aph(3')-like*, *crpP*, *blaPAO*, and *blaOXA-50*-like genes were located in the chromosome among three *P. aeruginosa* strains (Table 2), making *P. aeruginosa* strains naturally resistant to few antimicrobials and with limited resistance level. *SulI*, regarded as part of class 1 integron, was also identified in chromosome of KB-PA_3 and KB-PA_F6 isolate. No carbapenemase genes were found in KB-PA_F6; however, *aac(6')-Ib3*, *aac(6')-Ib-cr*, *aadA2b*, *aph(6)-Id*, *blaCARB-2*, *tet(G)* genes were identified. The isolates of KB-PA_3 and KB-PA_F19 carried *blaIMP* genes, which belong to two different families, respectively (Figure 4). *blaIMP-87* was located in the chromosome of KB-PA_3 and belonged to the *blaIMP-14* family, which involved *blaIMP-14*, *blaIMP-32*, and *blaIMP-48*. The *blaIMP-87* protein showed two amino acid substitutions (Val43Ala and Ser47Gly) compared to *blaIMP14* and one substitution (Thr69Ile) compared to *blaIMP-48*. *blaIMP-45*, belonging to the *blaIMP-9* family, which includes *blaIMP-9* and *blaIMP-53*, was located in pKB-PA_F19-4. Moreover, pKB-PA_F19-4 also harbored *aac(6')-Ib3*, *blaOXA-1*, *catB3*, *qnrVC6*, *armA*, *Msr(E)*, *Mph(E)*, *aph(3')-Ia*, *tetC/tetR*, *floR*, *aac(6')-Ib3*, and *mexC-mexD-oprJ* genes in the same antimicrobial resistance region as *blaIMP-45*. Fosfomycin resistance protein *fosA* was identified in another pKB-PA_F19-4 region (156,241 to 158,814) and as associated with *ISpa75*.

Gene Contexts of *blaIMP*

blaIMP-87 located within a 5,283-bp class 1 integron (Figure 5), contained a novel gene cassette array (*blaIMP-87-ant(2'')-Ia-blaOXA-10-aac(6')-Ib3*). The class 1 integron was flanked by two insertion sequences of *IS6100*, and then a compound transposon was downstream. The ISPa38-like transposase *tnpA* was inserted by a complete insertion sequence of *IS1071*. This carbapenem-resistance region was in the additional region 9 of the chromosome (14,672-bp range from 1,794,528 to 1,795,292, Supplement S1).

The multi-drug resistant megaplasmid pKB-PA_F19-4 shared a similar backbone with its family, and almost all antibiotic-resistance genes were at the plasmid's resistance region (46,011-bp range from 53,954 to 99,965). BLASTN showed a structural similarity between the pKB-PA_F19-4 resistance region and pBM413 (CP016215, Figure 6). The ARGs and associated mobile elements surrounded the *repM* and DNA-binding protein gene. Upstream of *repM* and DNA-binding protein gene, *blaIMP-45* is found. *blaIMP-45* is composed of a class 1 integron gene cassette array of *aac(6')-Ib3-blaIMP-45-blaOXA-1-cbtB3*, and associated with the Tn3-like transposon. Following the class 1 integron, the quinolones resistance gene *qnrVC6* is flanked by two *ISCR1* elements and then the array of *ISEc28-IS1394-armA-ISEc29-msr(E)-mph(E)* was identified. Downstream of *repM* and DNA-binding protein gene, two arrays of *IS15-aph(3')-Ia-IS15* and *intI1 acc(6')-Ib3-ISCfr1-acrR-mexC-mexD-oprJ* were identified, showing similar architecture with pBM413. The resistance genes, *tetC/tetR* and *floR*, also existed between these two regions.

Discussion

P. aeruginosa is an opportunistic pathogen, commonly infecting hospitalized patients, particularly those in burn wards and ICUs. Moreover, *P. aeruginosa* has been ranked as one of the most common Gram-negative detected pathogens isolated from burn patients in China.⁴ In this study, 54 carbapenem-resistant *Pseudomonas aeruginosa* strains were isolated from patients in the burn ward. *P. aeruginosa* poses complex acquired and intrinsic carbapenem resistance mechanisms,^{17,18} which significantly limits the clinician's choices for antimicrobial selection and treatments.¹⁹ Therefore, burn patients infected by carbapenem-resistant *P. aeruginosa* are at higher risk of infection complications. And phenotypic and molecular characterization of *P. aeruginosa* isolates from burn patients could provide meaningful information for epidemiology and resistance mechanism.

In this study, ST292 was the most predominant sequence type, followed by ST2446 and ST244. Previously, ST292 has only been reported in clinical isolates of China,¹² Thailand,²⁰ and Croatia.²¹ However, ST292 has never been isolated from burn patients. Here, the high prevalence (41.5%) of ST292 in burn patients is reported for the first time. A long-term outbreak of ST292 was reported in 2016 in a hospital in Southwest China, but the prevalence was not clear.²²

The *P. aeruginosa* porin OprD is a substrate-specific porin that facilitates the diffusion of basic amino acids, small peptides, and carbapenems into the cell (5). Mutation or deletion in OprD has been shown to reduce the susceptibility to

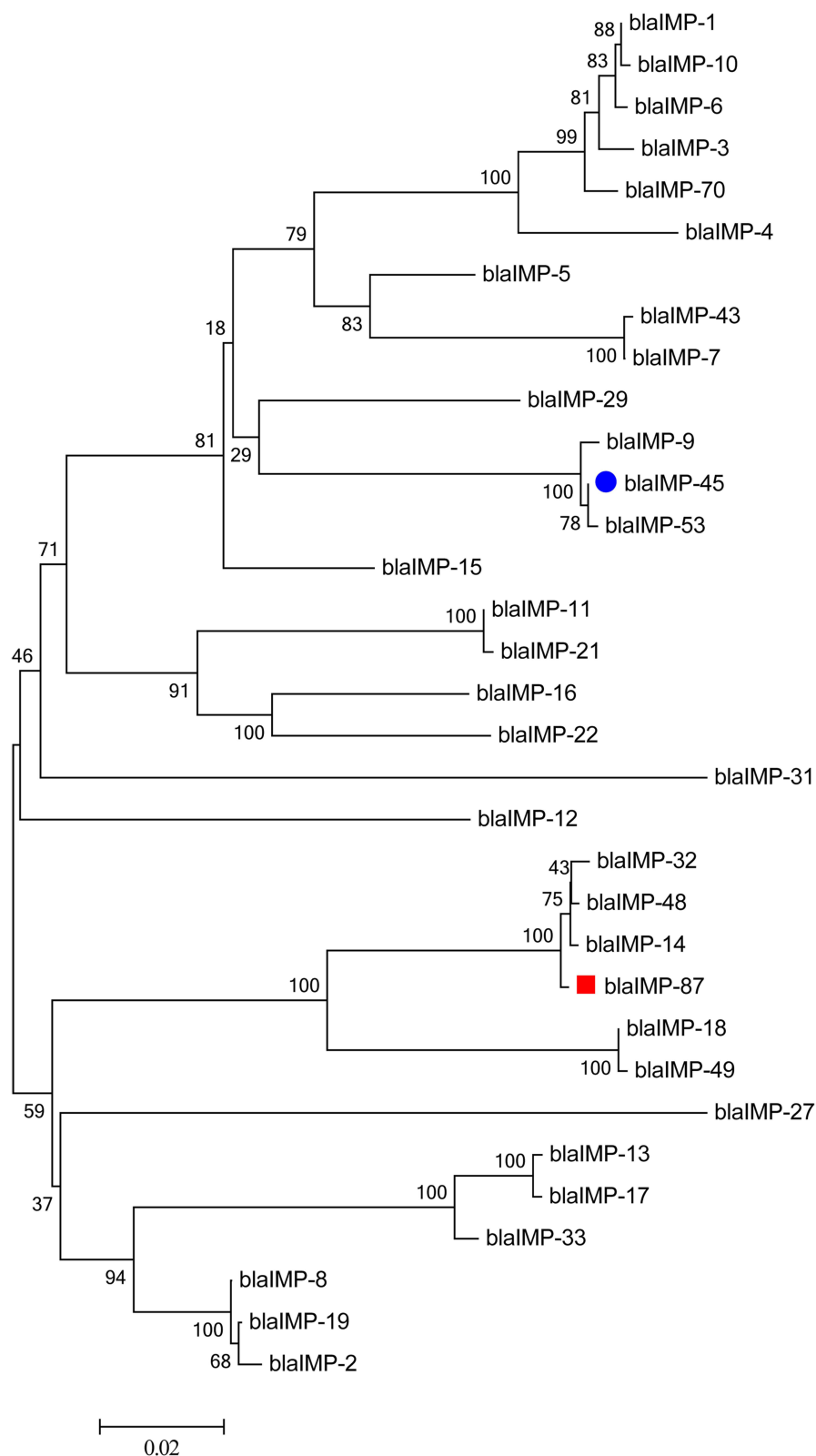


Figure 4 Phylogenetic tree of *blaIMP*-87 and *blaIMP*-45 with 31 sub-types of *blaIMP*; Phylogenetic tree was constructed based on neighbor-joining by MEGA 6.0. *blaIMP*-45 is labeled with blue and *blaIMP*-87 with red.

blaIMP gene was finally acquired as an independent passenger gene by an integron. A highly similar gene cassette array with 99% identity was present in S27-KUU (KY574887.1) from Thailand, containing *blaIMP-48* instead of *blaIMP-87*. These results suggested that the *blaIMP-87-ant(2'')-Ia-blaOXA-10-aac(6')-Ib3* novel integron cassette harbored by KB_PAE-3 may come from Thailand. Furthermore, *blaIMP-14* has a wide host range, except *P. aeruginosa*.^{29,30} The *blaIMP-87* transposition of the KB_PAE-3 was conducted by *IS6100*, which has also been reported in *E. coli*.³⁰ Therefore, it showed that *IS6100* is involved in the *blaIMP-14* family transmission and may become the driving force for *blaIMP* cross-species spreading. Together with the *oprD* 1-bp deletion, the ST2446 clone showed a high carbapenem-resistance with an imipenem MIC over 128 µg/mL. In addition, the role of the plasmid harbored by KB-PA_3 is unknown; however, a similar plasmid carrying *blaKPC-2* was reported.³¹

ST244 has been widely reported globally and listed as a high-risk clone,³² but with few genome characterization. Here, whole-genome sequencing of ST244 isolates was performed. Among carbapenem-resistance genes, *blaVIM-2* and *blaIMP-6* are prevalent resistance genes in ST244 in China.^{33,34} However, this study shows that *blaIMP-45* is emerging in ST244 and locates in a megaplasmid. Interestingly, when searched in GenBank, the gene cassette arrays of *aac(6')-Ib3-blaIMP-45-blaOXA-1-cbtB3* appeared only in the megaplasmid family. This megaplasmid family has a narrow host range for *Pseudomonas spp.* Nevertheless, it has been discovered in multiple *P. aeruginosa* STs, like ST508, ST1420, ST274, and ST708.³⁵ Our results suggest that this type of megaplasmid can transfer among different *P. aeruginosa* sequence types. Furthermore, this megaplasmid also carried multiple antibiotic resistance genes (Figure 6), such as beta-lactams, carbapenems fluoroquinolone, aminoglycoside, phenicol, sulfonamide, macrolide, lincosamide, streptogramin B, and tetracycline. Different mobile genetic elements carried drug-resistant genes forming this drug-resistant region surrounding the replication site of the megaplasmid, which may also have a role in drug-resistant genes transmission. Furthermore, the *ISCRI-qnrVC6-ISCRI* array has been reported previously in two *Shewanella xiamenensis* strains (CP013115.1 and AP025014.1). pBM413 has *IS1394* region inserted into the *ISCE1-qnrVC6-ISCRI* array. The array of *ISEc28-armA-ISEc29-msr(E)-mph(E)* was reported in *Klebsiella pneumoniae*,³⁶ which unlike our study, omits *IS1394*. The similar resistance architecture between pKB-PA_F19-4 and pBM413 implies that these plasmids may have the same origin. The class 1 integron in pBM413 was truncated class 1 integron In0 without any passenger genes, while pKB-PA_F19-4 carried *acc(6')-Ib3*. To the best of our knowledge, *tetC/tetR* and *floR* were seldom reported in clinical *P. aeruginosa*, and these genes may lead to tetracycline and florfenicol resistance, respectively. So far, the megaplasmid family role in antibiotic resistance is unclear, therefore, it is urgent to control the prevalence and transmission of these types of plasmids to avoid the spread of these types of resistance.

Conclusion

Here, the prevalence of carbapenem-resistant *P. aeruginosa* in the burns ward of the Second Affiliated Hospital of Kunming Medical University was surveyed, showing a unique molecular carbapenem-resistance mechanism in ST292, ST244, and ST2446 clones. Defective *OprD* mutation formed the ST292 clone most predominant. However, ST244 harbored a multi-drug resistant megaplasmid. ST2446, which has been reported in animals but not in humans, showed high-level carbapenem-resistance. It is important to explore and understand the transmission route of ST244, ST292, and ST2446 in burn patients and design and implement policies to control their spread.

Data Sharing Statement

The data presented in this study are available on request from the corresponding author.

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Disclosure

The authors report no conflicts of interest in this work.

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