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Virulence factors, molecular characteristics, and resistance mechanisms of carbapenem-resistant *Pseudomonas aeruginosa* isolated from pediatric patients in Shanghai, China

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Abstract

Background The investigation into virulence factors, clinical and molecular characteristics, and resistance mechanisms of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) in pediatric populations is currently inadequate.

Purpose This study aimed to investigate the virulence factors, clinical and molecular characteristics, and resistance mechanisms of 135 CRPA isolates in Shanghai, China.

Methods Analysis of virulence-associated genes and multilocus sequence typing (MLST) provided epidemiological and molecular insights into the isolates. Resistance mechanisms were identified via PCR, sequencing, and qRT-PCR.

Results The predominant resistance mechanism to carbapenems was the decreased production of outer membrane porin OprD (75.6%), accompanied by mutational inactivation of the *oprD* (87.4%). However, elevated production of AmpC (7.4%) and *mexB* overexpression (5.2%) were uncommon. Thirty-five sequence types (STs) were identified, with clonal complex 244 (CC244;59.3%) representing the majority of infections. Sixteen virulence factor genes were detected, with a significant portion of isolates (40.7%) concurrently possessing Toxin A (*toxA*), Elastase B (*lasB*), Exoenzyme S (*exoS*), staphylolysin (*lasA*), and Pilin (*pilA*). Almost all CC244 isolates carried *toxA* (100%), *exoS* (100%), *pilA* (100%), *lasB* (98.6%), and *lasA* (82.5%) while all ST2100, ST274, ST1129, ST446, and ST2069 isolates contained *exoY*. CC244 + isolates exhibited significantly increased antibiotic resistance, and the isolates from diseased or discharged patients showed comparatively higher resistance than others, except against gentamicin. Most patients (71.9%) received combination therapy, with 65.2% achieving clinical cure or improvement.

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Conclusion This study predominantly identified OprD-mediated carbapenem resistance in pediatric patients. The CRPA isolates were characterized by a variety of STs and a widespread distribution of virulence-associated genes. CC244 demonstrated significantly higher resistance, with potential outbreaks occurring in 2018 and 2019. These findings could aid in managing nosocomial CRPA infections and enhancing clinical practices.

Keywords *P. aeruginosa*, Carbapenem resistance, Virulence genes, Efflux pump, Pediatric patients

Introduction

Pseudomonas aeruginosa is a ubiquitous Gram-negative, non-fermenting bacterial rod and a common opportunistic pathogen frequently encountered in hospital environments. The U.S. Centers for Disease Control and Prevention (CDC) reports that *P. aeruginosa* is responsible for approximately 51,000 healthcare-associated infections annually, with approximately 400 fatalities in the United States [1]. Its intrinsic resistance and ability to acquire resistance genes render *P. aeruginosa* resistant to various antimicrobial agents, complicating the treatment of infections [2]. The rapid increase in carbapenem-resistant *P. aeruginosa* (CRPA) in recent years presents a grave global public health concern [3]. 2023 data from CHINET surveillance revealed resistance rates of *P. aeruginosa* to imipenem and meropenem were 23.0% and 19.4% in Shanghai, China [4]. Meanwhile, 2016 – 2010 data from Infectious Disease Surveillance of Pediatrics (ISPED) surveillance revealed resistance rates of *P. aeruginosa* to imipenem and meropenem were 23.1% and 20.0%, respectively [5]. This increasing resistance and its role in nosocomial outbreaks and serious infections at various body sites, including respiratory and urinary tracts, skin, soft tissues, and bacteremia, underscores the critical need for attention [6].

Resistance to carbapenems among *P. aeruginosa* strains is multifactorial [7]. Primarily, *P. aeruginosa* acquires resistance through plasmids or integrons that mediate the production of carbapenemases, such as the metallo-β-lactamases (MBLs), *Klebsiella pneumoniae* carbapenemases (KPCs), and GES enzymes, noted for their high efficiency to hydrolyze carbapenems [8–10]. Moreover, other mechanisms contribute to a lower degree of carbapenem resistance. For instance, the repression or inactivation of the carbapenem porin OprD and the hyperexpression of the chromosomal cephalosporinase AmpC contribute to decreased carbapenem sensitivity [11–13]. Similarly, the heightened activity of efflux pump systems such as MexAB-OprM contributes to meropenem resistance [14]. Collectively or individually, these mechanisms foster *P. aeruginosa's* resistance to carbapenems. Furthermore, the dissemination of hypervirulence-associated genes within CRPA strains and the co-existence of virulence and multidrug resistance determinants pose a new challenge [15]. Clones of *P. aeruginosa*, such as sequence types (STs) ST235, ST111, ST244, and ST357, labeled as "international" or "high risk," have increased globally, frequently associated with multidrug resistance [16]. However, the specific molecular epidemiology and resistance mechanisms associated with carbapenem in CRPA may vary across diverse geographical and clinical settings. Research on the interplay between multidrug resistance and virulence factors in pediatric CRPA isolates remains scarce.

Therefore, continuous surveillance of this critical pathogen and a deep understanding of its resistance mechanisms is vital for guiding management, enhancing infection control measures, and curtailing its global dissemination. This study sought to evaluate the prevalence of carbapenemase genes and virulence factors, investigate alterations in the expressions of the *oprD* porin gene, the *ampC* β-lactamase gene, and the *mexB* multidrug efflux gene, alongside performing an epidemiological analysis through multilocus sequence typing (MLST). The goal was to reveal the current distribution of 135 CRPA isolates among pediatric patients in our hospital over 4 years.

Methods

Study design and definition

This study was a single-center, retrospective analysis conducted at the Children's Hospital of Fudan University, a tertiary-level teaching hospital with 800 beds. The hospital admits over 50,000 patients from across the country annually, maintaining an average occupancy rate of ~85%, with 75% of these patients arriving from other provinces or cities in China.

A total of 135 unique CRPA isolates from pediatric patients collected between 2018 and 2021 were retrospectively analyzed. For the repeated samples from the same patient at different times, the first isolate was included. Clinical data for patients with CRPA were extracted from medical records.

The study included evaluations of virulence-associated genes and MLST to evaluate the epidemiology and molecular characteristics of CRPA. Investigations into carbapenem resistance mechanisms involved assessing the prevalence of carbapenemase genes, detecting *oprD* porin gene alterations, and quantifying the *ampC* β -lactamase gene and the *mexB* multidrug efflux gene expressions.

CRPA was identified as a clinical *P. aeruginosa* strain exhibiting resistance to either imipenem or meropenem,

following the breakpoints of Clinical and Laboratory Standards Institute (CLSI) guidelines [17].

Molecular detection of virulence factor genes and carbapenemase genes

Genomic DNA was extracted from the 135 CRPA strains for analysis in polymerase chain reaction (PCR) assays. Eight *carbapenemase* genes (bla_{IMP}, bla_{VIM}, bla_{BIC}, bla_{NDM}, bla_{KPC}, bla_{OXA}, $bla_{GES,}$ and bla_{AIM}) with 16 virulence factor genes (*toxA*, *exoS*, *exoT*, *exoU*, *exoY*, *plcH*, *plcN*, *phzM*, *phzS*, *lasA*, *lasB*, *pilA*, *pilB*, *aprA*, *pvdA*, and *algD*) were identified utilizing specific primers as outlined in Supplementary tables S1 and S2 [18–24]. The PCR amplicons were sequenced, and the resulting DNA sequences were aligned with those in the NCBI GenBank database for comparison through BLAST searches.

MLST

MLST followed the guidelines provided on the MLST Pubmed website (https://pubmlst.org). Clonal complexes (CCs) were defined based on the difference of one allele. A minimum spanning tree of CRPA-positive isolates was generated using GrapeTree, which MSTreeV2 was used for phylogenetic reconstruction, with a branch length threshold of 1 applied to hide longer branches and ensure clearer visualization of closely related isolates [25].

PCR amplification and sequencing of OprD

The *oprD* gene was amplified using primers described in Supplementary Table S3 [26]. The amplified DNA products were sequenced and aligned with the *oprD* sequence of the reference strain PAO1 using Mutation Surveyor v5.2.0 to identify base mutations in the *oprD* gene of each strain.

Quantitative real-time PCR

The gene expression levels of *ampC*, *mexB*, and *oprD* were quantified using quantitative real-time PCR (qRT-PCR) as per previously established methods [27]. The *rpsL* gene served as an internal reference. Three independent experiments were performed, and each gene's mean relative expression levels were compared with their corresponding expression levels in PAO1. Details of the primers used for qRT-PCR are shown in Supplementary Table S4 [18].

The evaluation criteria followed were outlined in previous studies [13, 28]. For *ampC*, overexpression is considered when the expression level is at least ten times higher than that in PAO1, regarded as negative if it is < 5 times higher, and deemed borderline when the expression level is between five and ten times higher. Regarding *mexB*, an overexpression level is recognized at a threshold of three times higher, labeled as negative if under two times, and categorized as borderline for levels between two and three times higher. For *oprD*, overexpression is determined at levels at least 2.5-fold higher, down expression is identified when levels are below 0.4 times, and borderline is assigned to levels ranging from 0.4 to 2.5 times higher.

Antimicrobial susceptibility testing

CRPA strains were identified using the MALDI-TOF Biotyper mass spectrometer (Bruker Company, Germany). Antimicrobial susceptibility tests (AST) were conducted using the Vitek 2 compact system. Escherichia coli ATCC25922 and Escherichia coli ATCC35218 (enzyme-producing strains), served as quality control strains for these tests. Susceptibility categorization followed the breakpoints defined by the CLSI guidelines from 2018 to 2021 (M100-S21 to M100-S27).

Statistical analysis

Data were presented as percentages or rates for categorical variables.Comparison between groups were performed by using chi-square test for categorical variables; $p < 0.05$ was regarded as statistically significant. statistical analyses were conducted using SPSS software version 23.0.

Results

Clinical characteristics of pediatric patients with CRPA

This study analyzed 135 CRPA isolates collected from pediatric patients between 2018 and 2021. The cohort predominantly consisted of male (88, 65.2%) with an average age of 4.6 ± 2.3 years at the time of isolation. The average hospitalization duration exceeded 100 days, with the interval from admission to strain isolation averaging at 46.0 ± 27.1days.

The distribution of CRPA isolates across hospital departments revealed that the majority were from patients in the Pediatric intensive care unit (PICU) (60.7%), followed by internal medicine (15.6%), surgical department (14.8%), and a combined group of the neonatal room and Neonatal intensive care unit (NICU) (8.9%). The primary underlying conditions were related to the respiratory system, including severe pneumonia. Over 95% of the patients had a history of hospitalization in the PICU and had undergone tracheal intubation. Nosocomial infections accounted for 63.7% of cases. Respiratory tract specimens were the most common source of isolates (79.3%), followed by the urinary tract (4.4%) and other sources (16.3%). Furthermore, a significant portion of the patients had been previously treated with antibiotics, including carbapenems (37.8%), enzyme inhibitor complex preparations (37.0%), and third-generation cephalosporins (26.7%) (Table 1).

Regarding treatment, 28.1% of patients received monotherapy, primarily involving carbapenems and enzyme

Table 1 Clinical characteristics of patients with carbapenem-resistant *Pseudomonas aeruginosa*

Variables	
Gender (n, %)	
Male (n, %)	88(65.2)
Female (n, %)	47(34.8)
Hospitalization history (n, %)	45(33.3)
ICU hospitalization history (n, %)	129(95.6)
Surgical history (n, %)	100(74.1)
History of tracheal intubation (n, %)	129(95.1)
Premature delivery (n, %)	30(22.2)
Isolation history of other drug-resistant bacteria (n, %)	45(33.3)
Age, years (Mean ± standard deviation)	4.6 ± 2.3
Total hospitalization time (Mean ± standard deviation)	104.5 ± 20.3
Time from admission to strain isolation (Mean ± standard deviation)	46.0 ± 27.1
Time from strain isolation to discharge (Mean ± standard deviation)	58.5 ± 23.3
Ward distribution	
PICU (n, %)	82(60.7)
Internal medicine (n, %)	21(15.6)
Surgery (n, %)	20(14.8)
Newborn room + NICU (n, %)	12(8.9)
Infection type	
Nosocomial infection (n, %)	86(63.7)
External hospital infection (n, %)	14(10.4)
Community acquired infections (n, %)	12(8.9)
Colonization (n, %)	23(17.0)
Underlying disease	
Respiratory system (n, %)	53(39.3)
Intracranial mass (n, %)	19(14.1)
Central nervous system (n, %)	17(12.6)
Digestive system (n, %)	8(5.9)
Congenital malformations, immune deficiency (n, %)	11(8.1)
Other (n, %)	27(20.0)
Antibiotic exposure	
Carbapenems (n, %)	51(37.8)
enzyme inhibitor (n, %)	50(37.0)
Third-generation cephalosporin (n, %)	36(26.7)
Fourth generation cephalosporins (n, %)	12(8.9)
Linazolamide (n, %)	18(13.3)
Macrocyclic lipids (n, %)	9(6.7)
Fosfomycin (n, %)	8(5.9)
Penicillin (n, %)	6(4.4)
Peptides (n, %)	5(3.7)
Aminoglycosides (n, %)	2(1.5)
Quinolones (n, %)	7(5.2)

inhibitor complex preparations. The majority [71.9% (97/135)] were administered combination therapy, with 61.9% (60/97) receiving carbapenems and enzyme inhibitor complex preparations among other dual or triple therapies. Clinical cure or improvement was observed in 65.2% (88/135) of the patients, while the remainder were either discharged on their request with a poor prognosis (20.7%, 28/135) or succumbed to their conditions (14.1%, 19/135) (Table 2).

Virulence factor and carbapenemase genes in CRPA isolates

Each CRPA strain possessed at least eight virulence factors out of the 16 virulence factor genes examined. All isolates harbored eight specific virulence factor genes (*phzM*, *phzS*, *algD*, *plcH*, *plcN*, *laprA*, *pvdA*, and *exoT*). The *toxA* and *lasA* genes showed a high prevalence (99.3%), followed by *exoS* (91.9%), *lasA* (82.5%), *pilA* (71.9%), and *exoY* (46.7%). The genes *exoU* and *pilB* were less common, detected in only seven (5.8%) and four (3.0%) CRPA strains, respectively. Furthermore, 16 unique profiles of virulence-associated gene combinations were identified across the isolates (Fig. 1). A significant fraction of the isolates (40.7%) co-harbored the genes *toxA*, *lasB*, *exoS*, *lasA*, and *pilA*. The isolates displayed either *exoS*-/*exoU* + or *exoS*+/*exoU*- genotypes, with none presenting both *exoS* + and *exoU* + simultaneously. Besides, the *exoS*-/*exoU* + genotype was less prevalent (5.8%) than the *exoS*+ /*exoU*- genotype (91.9%).

No additional carbapenemase genes were identified among the isolates apart from a single isolate producing VIM and another producing NDM.

MLST

The 135 CRPA isolates exhibited a wide array of STs (35). The majority of infections were associated with ST244 (51.9%), followed by ST8818 (5.2%), ST2100 (4.4%), ST274 and ST1129 (3.0% each), with ST446, ST1239, and ST2069 (2.2% each); other uncommon STs were detected in one or two isolates each. Their evolutionary relationships are shown in Fig. 2. Sequence types ST244, ST8818, ST1701, and ST1103 belong to the same CC group, CC244 (59.3%). CC244 isolates predominantly appeared in 2018 (48.8%) and 2019 (41.3%), whereas other ST types were more common in 2020 and 2021.

Distribution patterns for virulence-associated genes, specimens, gender, department, prognosis, and years are discernible across different STs (Tables 3 and 4). Each ST harbors a unique set of virulence-associated genes and is distributed in various departments. Virtually all isolates within CC244 possess *toxA* (100%), *exoS* (100%), *pilA* (100%), *lasB* (98.6%), and *lasA* (82.5%), whereas all isolates of ST2100, 274, 1129, 446, and ST2069 contain *exoY*. The genes *exoU* and *pilB* are primarily distributed in STs other than those in CC244 (Table 3). Moreover, isolates of ST274 and ST446 were mainly distributed in neonatal patients while others were primarily distributed in the PICU (Table 4).

Table 2 Outcome of patients with carbapenem-tesistant *Pseudomonas aeruginosa*

	Number of episodes			
	Total	Died	Unknown	Survived
Monotherapy				
Meropenem/imipenem	14	3	2	9
[a]Enzyme inhibitor complex preparation	11	0	3	8
Quinolones/aminoglycosides/polymyxin B/cefepime	9	0	2	7
Ceftazidime	4	0	0	4
Combination therapy				
Meropenem + enzyme inhibitor complex preparation/quinolones/fudaxin/flumadin/phosphomycin	23	3	3	17
Meropenem + enzyme inhibitor complex preparation + aminoglycosides/quinolones/cefepime/polymyxin/fudaxin	16	5	5	6
Meropenem + Fosfomycin + quinolones/aminoglycosides/polymyxin/enzyme inhibitors/	21	2	5	14
Enzyme inhibitor complex preparation + quinolones/fosfomycin/polymyxin/fudaxin//maspin	15	3	3	9
Enzyme inhibitors + quinolones/aminoglycosides + fosfomycin/polymyxin/fudaxin	11	2	4	5
Polymyxin + aminoglycosides/quinolones/third-generation cephalosporins	4	0	0	4
Others	7	1	1	5
Total	135	19	28	88

[a]Cefoperazone sulbactam, or Piperacillin tazobactam

Expression of *ampC*, *mexB*, and *OprD*

Expression levels of the *ampC* β-lactamase gene, the *mexB* multidrug efflux gene, and *oprD* across CRPA isolates are depicted (Fig. 3). Elevated production of AmpC and efflux pump gene *mexB* overexpression is relatively uncommon among these isolates. Only 7.4% (10/135) and 5.2% (7/135) of CRPA isolates demonstrated overexpression for AmpC and *mexB*, respectively, while 90.4% (122/135) and 91.9% (124/135) of isolates showed no overexpression of these genes. Regarding *oprD*, overexpression was observed in 2.2% (3/135) of isolates, whereas 75.6% (102/135) exhibited low expression.

Mutational inactivation and expression of OprD Porin

The sequencing of the *oprD* gene was conducted for all 135 CRPA isolates (Table 4). A total of 118 (87.4%) isolates exhibited a variety of mutations that could lead to the production of truncated or aberrant proteins. Frameshift mutations, caused by either deletions or insertions of one or more base pairs, were identified in 108 (80.0%) strains. Moreover, point mutations resulting in premature stop codons were detected in 10 isolates. Among the 17 isolates that did not exhibit inactivating mutations, four showed amino acid substitutions, one had no mutations, 12 were undetectable by PCR, and all 17 isolates demonstrated reduced *oprD* expression (Table 5).

For *ampC* overexpression is defined as a level at least 10-fold higher than the corresponding level in PAO1, negative if less than 5-fold higher, and borderline if between 5- and 10-fold. For *mexB*, overexpression indicates a level at least 3-fold higher, negative indicates the level is less than 2-fold, and borderline indicates the level is between 2- and 3-fold higher. For *oprD*, overexpression indicates a level at least 2.5-fold higher, down expression if less than 0.4-fold and borderline if between 0.4- and 2.5-fold.

Antimicrobial susceptibility trends

The resistance to Aztreonam has been considerably high and increasing, whereas resistance to Amikacin has been lower and declining. Resistance rates for other antibiotics, including cefoperazone/sulbactam, piperacillin/tazobactam, piperacillin, cefoperazone, ceftazidime, cefepime, and ciprofloxacin, remained around 60% from 2018 to 2020 and showed a decreasing trend in 2021. Gentamicin resistance was the lowest among the antibiotics tested, dropping below 20% in 2021(Fig. 4A).

In 2021, resistance testing for polymyxin B and ceftazidime/avibactam was initiated. None of the 24 isolates tested for polymyxin B showed resistance, and three of the 15 isolates tested for ceftazidime/avibactam were found to be resistant.

Furthermore, the resistance rates of CC244-positive (CC244+) and -negative (CC244-) isolates, and the resistance profiles of isolates from patients who died or were discharged were compared to other CRPA isolates. The analysis revealed that CC244 + isolates showed significantly higher resistance to all tested antibiotics than CC244- isolates (Fig. 4B). Moreover, isolates from deceased or discharged patients demonstrated relatively higher resistance than those from other patients, with gentamicin being the exception (Fig. 4C).

The resistance rates of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) isolates from 2019 to 2021(A), CC244-positive (CC244+) and–negative (CC244-) (B),

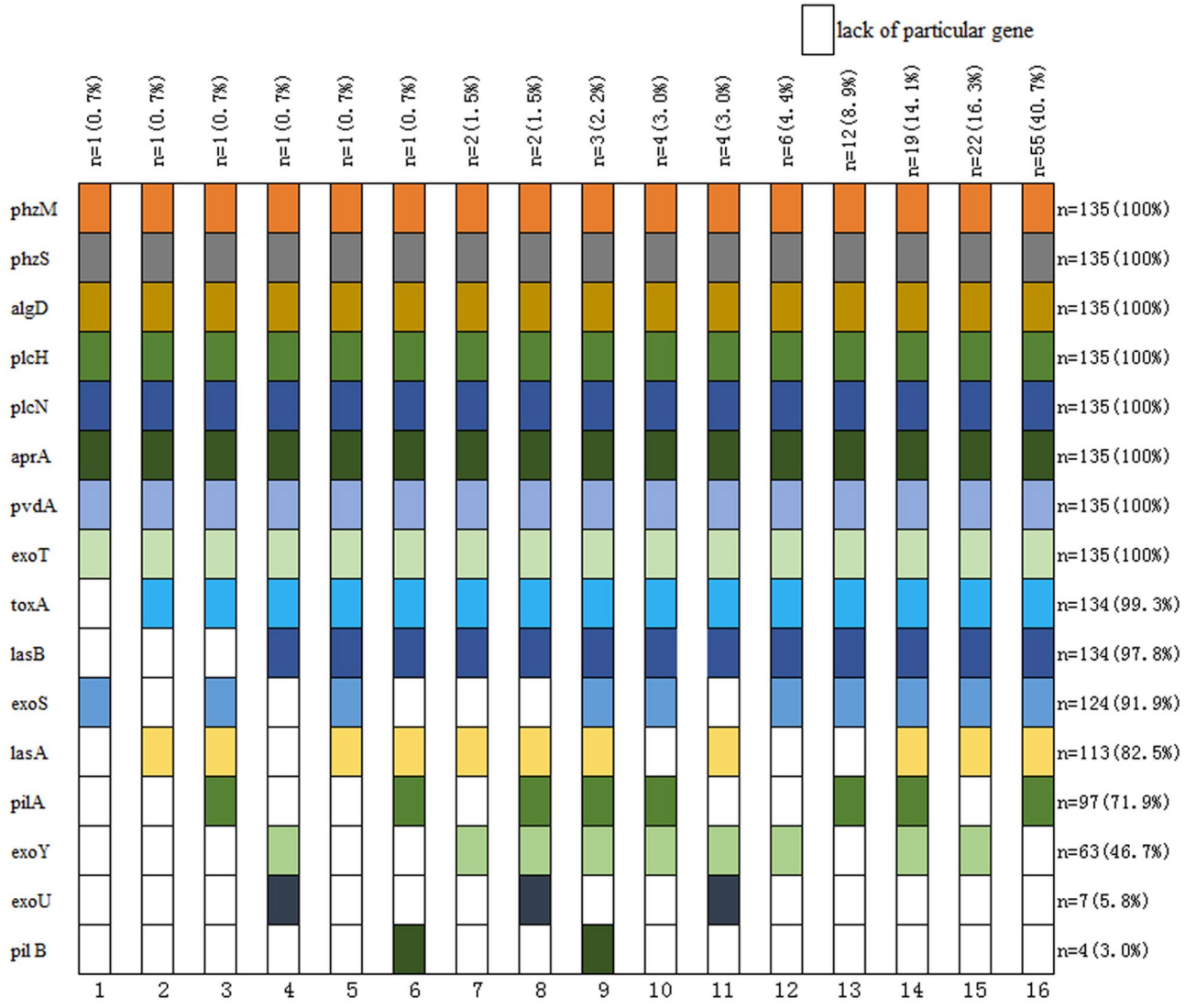

Fig. 1 Virulence-associated genes of carbapenem-resistant *Pseudomonas aeruginosa* isolates. Polymerase chain reaction (PCR) was used to analyze virulence genes. Sixteen genotypes were observed and these strains also possessed sixteen distinct virulence-associated gene combination profiles

and the die/discharged and other CRPA isolates (C) are displayed.

AMK, amikacin. GEN, gentamicin; PIP, Piperacillin; CAZ, Ceftazidime; CFP, Cefoperazone; ATM, Aztreonam; CIP, Ciprofloxacin; CSL, Cefoperazone/Sulbactam; FEP, cefepime; TZP, piperacillin/tazobactam; IPM, Imipenem; MEM, Meropenem.

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Discussions

In *P. aeruginosa*, carbapenem resistance was primarily attributed to decreased outer membrane porin OprD production, accompanied by mutational inactivation of the *oprD* gene. CRPA strains, originating from diverse genetic backgrounds and exhibiting widespread virulence-associated genes, demonstrate the complexity of these infections. CC244 was responsible for most infections, with CC244 + isolates displaying significantly higher resistance than their CC244- counterparts.

In USA, *P. aeruginosa* is the primary cause of ventilator-associated pneumonia in long-term acute care hospitals and the third most prevalent cause of catheter-related urinary tract infections (UTIs) [29]. The resistance mechanisms of CRPA are predominantly acquired, encompassing the transfer of genes encoding carbapenemases, repression or inactivation of the carbapenem porin OprD, and the overexpression of the chromosomal cephalosporinase AmpC and efflux pump gene *mexB* [18]. OprD-mediated carbapenem resistance is commonly achieved by downregulating OprD expression or its inactivation, which may occur through frameshift mutations (insertion/deletion) or introducing a

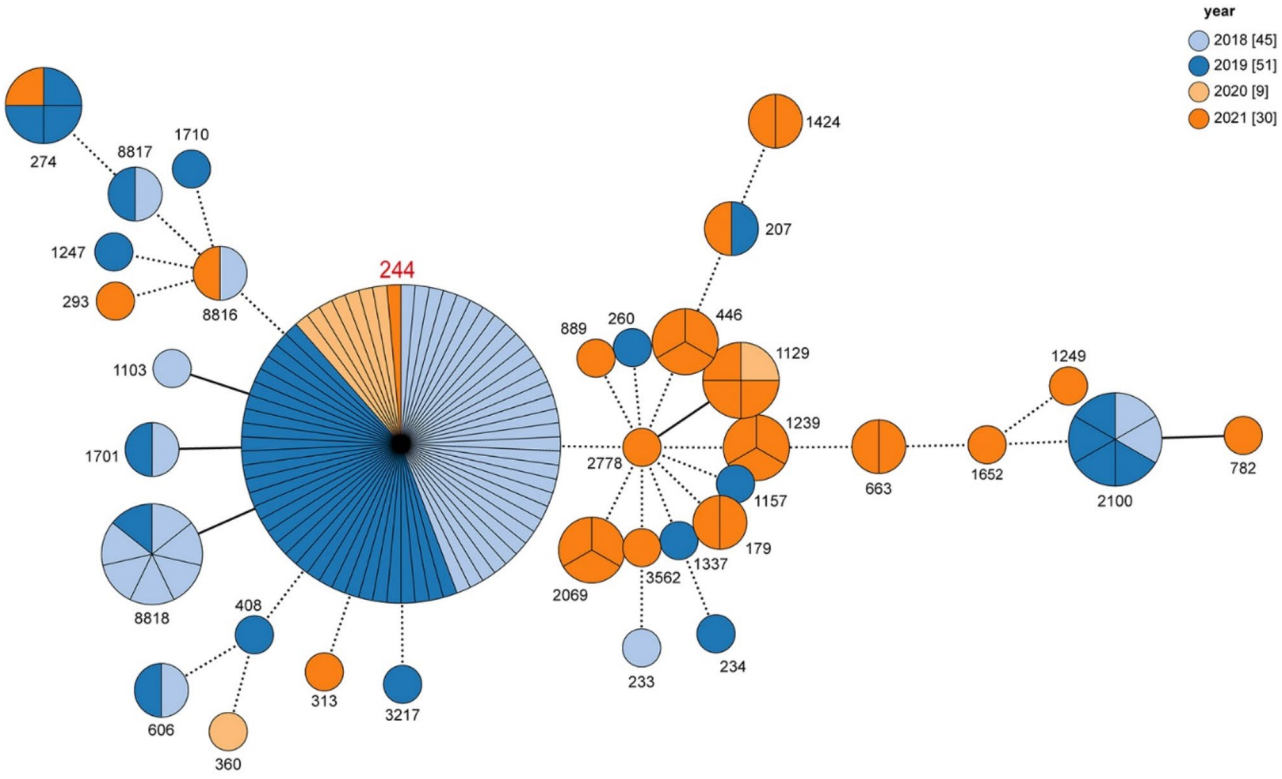

Fig. 2 A minimum spanning tree of sequence types (STs) created by GrapeTree. Each solid circle denotes one ST, and the area of the circle is proportional to the number of isolates. The lines connecting the circles indicate the relationship between different STs. The difference in one (solid lines), two (dashed lines), and three or more alleles (dotted lines) are also represented. ST244, ST8818, ST1701 and ST1103 different by one allele and belong to the CC244

Table 3 Distributions of virulence-associated genes among different STs (n, %)

Categories	CC244 (*n* = 80)	ST2100 (*n* = 6)	ST274 (*n* = 4)	ST1129 (*n* = 4)	ST446 (*n* = 3)	ST1239 (*n* = 3)	ST2069 (*n* = 3)	Others[a] (*n* = 32)
Virulence-associated genes								
toxA (134)	80(100.0)	6(100.0)	4(100.0)	4(100.0)	3(100.0)	3(100.0)	3(100.0)	31(96.9)
lasB (134)	79(98.8)	6(100.0)	4(100.0)	4(100.0)	3(100.0)	3(100.0)	3(100.0)	32(100.0)
exoS (124)	80(100.0)	6(100.0)	4(100.0)	4(100.0)	1(33.3)	3(100.0)	3(100.0)	23(71.9)
lasA (113)	66(82.5)	4(66.7)	4(100.0)	2(50.0)	3(100.0)	3(100.0)	2(66.7)	29(90.6)
pilA (97)	80(100.0)	6(100.0)	1(25.0)		2(66.7)			8(25.0)
exoY (63)	12(15.0)	6(100.0)	4(100.0)	4(100.0)	3(100.0)	2(66.7)	3(100.0)	29(90.6)
exoU (7)					2(66.7)			5(15.6)
pilB (4)								4(12.5)

[a]Including ST179, 207,606,663,1424,8816,8817 in 2 isolates, ST233, 234, 260, 293, 313, 360, 408, 782, 889, 1157, 1247, 1249, 1337, 1652, 1710,2778,3217,3562 in single isolates, respectively

premature stop codon [30, 31]. Such mutational inactivation of OprD has been identified as a prevalent mechanism in China and Korea [27, 30, 31]. Moreover, 12 isolates were not detected by PCR, suggesting possible extensive alterations in the *oprD* gene, such as large deletions [30]. In our research, the primary resistance mechanism against carbapenems involved decreased outer membrane porin OprD production, mainly through its mutational inactivation. The prevalence of carbapenemases, particularly VIM and NDM types, has increased [32]. However, within the scope of this study, only one VIM-producing and one NDM-producing CRPA isolate were identified, indicating a less frequent occurrence of carbapenemase genes in our CRPA compared to reports from other countries [18]. This disparity underscores the prevalence of downregulation or the inactivation of this porin through a frameshift as a more frequent basis for resistance. Compared to the widespread occurrence of carbapenemase genes in certain European nations, these intrinsic resistance mechanisms within a genetically varied CRPA population pose a potentially more significant concern, especially in pediatric settings.

Table 4 Distributions of specimen, gender, department, prognosis and years among different STs (n, %)

Categories	CC244 (*n* = 80)	ST2100 (*n* = 6)	ST274 (*n* = 4)	ST1129 (*n* = 4)	ST446 (*n* = 3)	ST1239 (*n* = 3)	ST2069 (*n* = 3)	Others[a] (*n* = 32)
Specimen								
Sputum (*n* = 107)	66(82.5)	5(83.3)	2(50.0)	2(50.0)	3(100.0)	3(100.0)	3(100.0)	23(71.9)
Urine (*n* = 6)	4(5.0)		1(25.0)					1(3.1)
Others (*n* = 22)	10(12.5)	1(16.7)	1(25.0)	2(50.0)				8(25.0)
Gender								
Female (*n* = 47)	28(35.0)	1(16.7)	3(75.0)	2(50.0)	2(66.7)	3(100.0)		8(25.0)
Male (*n* = 88)	52(65.0)	5(83.3)	1(25.0)	2(50.0)	1(33.3)		3(100.0)	24(75.0)
Department								
PICU (*n* = 82)	61(76.3)	3(50.0)				1(33.3)	3(100.0)	14(43.8)
Internal medicine (*n* = 21)	10(12.5)	2(33.3)	1(25.0)	2(50.0)				6(18.8)
Surgery (*n* = 20)	8(10.0)	1(16.7)		2(50.0)		2(66.7)		7(21.9)
Newborn room + NICU (*n* = 12)	1(1.3)		3(75.0)		3(100.0)			5(15.6)
Prognosis								
Improvement (*n* = 88)	46(57.5)	2(33.3)	1(25.0)	4(100.0)	1(33.3)	3(100.0)	2(66.7)	29(90.6)
Automatic discharge (*n* = 28)	20(25.0)	2(33.3)	3(75.0)				1(33.3)	2(6.3)
Death (*n* = 19)	14(17.5)	2(33.3)			2(66.7)			1(3.1)
Years								
2018 (*n* = 45)	39(48.8)	2(33.3)						4(12.5)
2019 (*n* = 51)	33(41.3)	4(66.7)	3(75.0)					11(34.4)
2020 (*n* = 9)	7(8.8)			1(25.0)				1(3.1)
2021 (*n* = 30)	1(1.3)		1(25.0)	3(75.0)	3(100.0)	3(100.0)	3(100.0)	16(50.0)

[a]Including ST179, 207, 606, 663, 1424, 8816, 8817 in 2 isolates, ST233, 234, 260, 293, 313, 360, 408, 782, 889, 1157, 1247, 1249, 1337, 1652, 1710, 2778, 3217, 3562 in single isolates, respectively

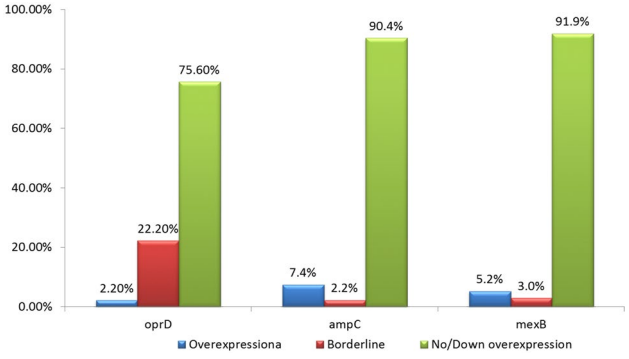

Fig. 3 Prevalence of *oprD*, *ampC* and efflux pump *mexB* overexpression in carbapenem-resistant *Pseudomonas aeruginosa* isolates

The co-existence of virulence factors and multidrug resistance determinants in CRPA isolates underscores the growing challenge they represent [33, 34]. *P. aeruginosa* produces numerous virulence determinants associated with its pathogenicity [35]. The presence of pili-encoding genes, *pilA* and *pilB*exhibiting higher frequencies than previous studies on *P. aeruginosa* from UTIs in adults and children [15, 36]. The four effector proteins of the type III secretion system (T3SS), ExoS, ExoT, ExoU, and ExoY, were not always present as a complete set [37, 38]. In our analysis, *exoT* was prevalent in all isolates, followed by *exoS*, *exoY*, and *exoU*, deviating from UTI-focused research [15]. In particular, all *P. aeruginosa* harbor either *exoS* or *exoU* genes, which are pivotal in enhancing virulence and resistance in infections, but rarely exhibit both genes, aligning with previous findings [38]. Moreover, the prevalence of the *exoS*-/*exoU* + genotype was significantly lower than the *exoS*+/*exoU*- genotype, contrasting with UTI-related outcomes [15]. These findings underscore the potential variability in CRPA pathogenesis across different infection sites and age groups. However, most CRPA isolates did not produce carbapenemases, highlighting the need for further studies to determine the correlation between virulence gene incidence and carbapenemase production.

Drug-resistant *P. aeruginosa* clones emerge from a diverse genetic background [9], a phenomenon distinctly different from the patterns observed in other bacteria [39]. According to the report, ST244 and ST274 have been identified among the top 10 significant types in China [40]. Our findings reveal that CC244 accounted for most infections, with a notable concentration of cases in 2018 and 2019, suggesting possible outbreak events during these years. Besides, the distribution of different virulence-associated genes varied across STs and hospital departments. Specifically, nearly all CC244 isolates carried *toxA*, *exoS*, *pilA*, *lasB*, and *lasA*, whereas ST2100, 274, 1129, 446, and ST2069 isolates consistently harbored *exoY*. The genes *exoU* and *pilB* were primarily distributed in other ST types than CC244, diverging from previous research [15, 41]. These observations demonstrate the importance of continuous dynamic monitoring

Table 5 Mutations inactivating *OprD* in carbapenem-resistant *Pseudomonas aeruginosa* isolates

Type of mutation	Mutational characteristics[a]	STs (no. of isolates)
Frameshift mutation	7-bp deletion at nt 464–470(TGTTCCC)	ST244(64), ST1103(1), ST1701(2), ST8818(7)
	7-bp insertion at nt 686–687(GATATGG)	ST663(2)
	5-bp deletion at nt 1059–1063(CGGCC)	ST606(1)
	3-bp deletion at nt 1114–1119(ATGTCT)	ST179(1), ST274(2), ST293(1), ST1129(4), ST1239(1), ST2100(3), ST2778(1), ST3562(1)
	2-bp deletion at nt 114–115(AG)	ST244(1)
	2-bp deletion at nt 181–182(GA)	ST244(1)
	1-bp deletion at nt 185(G)	ST207(1)
	1-bp deletion at nt 909(T)	ST3217(1)
	1-bp deletion at nt 829(T)	ST1239(2)
	1-bp duplication at nt 131(G)	ST274(1), ST446(1)
	1-bp duplication at nt 192(T)	ST2100(3)
	1-bp duplication at nt 634(G)	ST1247(1)
	1-bp duplication at nt 916(T)	ST233(1)
	1-bp insertion at nt 1205–1206(C)	ST360(1), ST889(1), ST1157(1)
Premature stop codon	Y120X (Stop Codon) TAC→TAA at nt 358–360	ST313(1)
	W65X (Stop Codon) TGG→TGA at nt 193–195	ST446(1)
	S319X (Stop Codon) TCG→TAG at nt 955–957	ST260(1)
	W138X (Stop Codon) TGG→TGA at nt 412–414	ST274(1), ST408(1)
	W277X (Stop Codon) TGG→TAG at nt 829–831	ST207(1), ST1652(1), ST1424(2), ST8817(1)
Amino acid substitution	T103S, K115T, F170L, R310G	ST446(1)
	D43N, I210T	ST782(1)
	D43N, T103S, K115T, F170L	ST1710(1)
	D43N, E230K	ST8816(1)
No mutation	None	ST244(1)
Negative by PCR	Unknown change	ST2069(3), ST244(3), ST179(1), ST234(1), ST606(1), ST1249(1), ST8816(1), ST8817(1)

[a]Sequences of *oprD* were compared to *oprD* in the reference strain PAO1; nt, nucleotide

and further investigation into the causes of CRPA clonal diversity.

Identifying the antimicrobial susceptibility patterns of CRPA is vital for the timely administration of appropriate therapies. Novel antibiotics have been developed for treating CRPA [42]. However, carbapenems and typical β-lactam/β-lactamase inhibitor combinations remain the mainstay in clinical practice. Inappropriate selection of antibiotics has been associated with higher mortality rates among patients with *P. aeruginosa* bloodstream infections [43]. Consequently, the significant morbidity associated with CRPA remains a critical clinical concern. Despite 71.9% of patients receiving combination therapy, only 65.2% achieved clinical cure or improvement, with the remaining patients either being discharged with poor prognosis or succumbing to their illnesses. The intricate interplay of factors such as the widespread presence of hypervirulence-associated genes, a high frequency of CC244 + isolates, and their elevated resistance levels likely contribute to increased mortality rates and worsened outcomes.

This study has limitations, including its single-center study covering 135 strains and limited data on the drug sensitivity of primary therapeutic medications such as ceftazidime/ avibactam and polymyxin B. Further, this study did not analyze relevant prognostic factors, including the virulence genes.

Conclusions

In summary, there was a probable outbreak of the high-risk CC244 clone in 2018 and 2019. Carbapenem resistance was mainly driven by OprD inactivation, accompanied by the decreased production of outer membrane porin OprD, while the role of AmpC hyperproduction, particularly efflux pump overexpression, was minimal. Furthermore, the development of CRPA from diverse genetic backgrounds and hypervirulence-associated genes highlights the necessity for enhanced infection control and clinical treatment strategies.

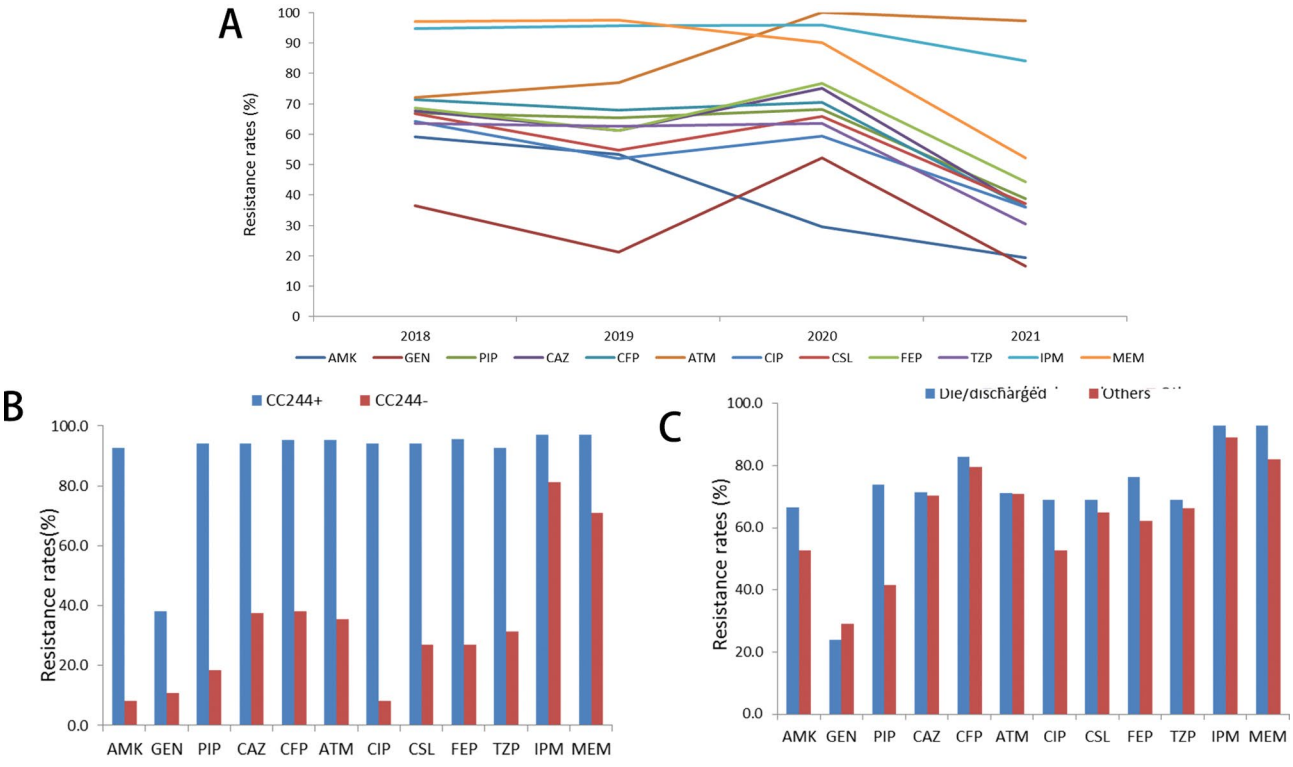

Fig. 4 The resistance rates of carbapenem-resistant *Pseudomonas aeruginosa* isolates (%)

Abbreviations

CRPA	Carbapenem-Resistant Pseudomonas aeruginosa
MLST	Multilocus sequence typing
MBLs	Metallo-β-lactamases
KPCs	Klebsiella pneumoniae carbapenemases
STs	Sequence types
CCs	Clonal complexes
AST	Antimicrobial susceptibility tests

Supplementary Information

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Supplementary Material 1

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Author contributions

Conception and design of study: L.J. Yin, C.Q. Wang, X.W. Zhai, G.P. Lu. Acquisition of data (laboratory or clinical): L.J. Yin, Z.H. Bao, L. Lu, L.Y. He. Data analysis and/or interpretation: L.J. Yin, Z.H. Bao. Drafting of manuscript and/or critical revision: L.J. Yin, Z.H. Bao, C.Q. Wang, X.W. Zhai. All authors reviewed the manuscript.

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Data availability

The datasets used and/or analysed during the current study are available in the online repository, Accession number(s): NCBI, PRJNA1194513.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Informed consent was waived by the Ethics Committee of the Children's Hospital of Fudan University because only bacterial isolates recovered from routine diagnostic laboratory tests were assessed. The study was approved by the Ethics Committee of the Children's Hospital of Fudan University, Shanghai, China (Approval number (2021)372).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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