

Molecular Mechanisms and Clinical Impact of HighLevel Ceftazidime Avibactam Resistance in CarbapenemResistant Pseudomonas aeruginosa

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Abstract

Background

Ceftazidime-avibactam (CZA) remains one of the few effective agents against carbapenem-resistant *Pseudomonas aeruginosa* (CRPA). Nevertheless, the escalating emergence of CZA resistance, particularly high-level resistance, has become a major concern, has become a growing concern. The mechanisms underlying high-level CZA resistance remain incompletely understood. Accordingly, the present study was designed to elucidate the clinical and epidemiological characteristics, as well as the molecular mechanisms, of high-level CZA resistance in CRPA isolates collected from a teaching hospital.

Results

Twenty-four CZA-resistant isolates were identified from 22 patients. Among these, 58.3% exhibited high-level resistance (MIC $\geq$ 128 μ g/mL), while extensively drug-resistant (XDR) and difficult-to-treat resistance (DTR) accounted for 83.3% and 12.5%, respectively. The overall mortality rate was 68.1%. WGS revealed that 85.7% of high-level resistant strains harbored metallo- β -lactamase (MBL) genes, with *bla*_{AFM-1} (50%) and *bla*_{IMP-45} (21.4%) being the most prevalent. Notably, isolate PA271 co-harbored *bla*_{AFM-1} and *bla*_{IMP-45}. Two isolates harbored GES variants (GES-15 and GES-39) and belonged to the international high-risk clone ST235. Cloning experiments showed that GES-15 and GES-39 conferred CZA MICs of 8 μ g/mL, compared with 16 μ g/mL for GES-1. qRT-PCR revealed significantly upregulated efflux pump expression in GES-15 and GES-39 clones.

Conclusions

High-level CZA resistance in CRPA is driven by multiple mechanisms, including MBL production (particularly AFM-1 and IMP-45), GES variants, and synergistic efflux pump overexpression. The convergence of critical illness, high-level antimicrobial resistance, and carriage of multiple virulence factors in these patients poses a formidable challenge to clinical.

Introduction

Pseudomonas aeruginosa is a common Gram-negative opportunistic pathogen found in both hospital and environmental settings. It can cause nosocomial infections, such as respiratory infection, cystic fibrosis (CF), and bloodstream, especially in immunocompromised and critically ill patients ^[1,2]. The treatment of *P. aeruginosa* infections remains particularly challenging, owing to a combination of high intrinsic antibiotic tolerance, the secretion of diverse virulence factors, widespread nosocomial prevalence, and rapid in-host evolutionary adaptation ^[3,4].

Ceftazidime-avibactam (CZA) is a β -lactam/ β -lactamase inhibitor combination comprising ceftazidime and avibactam, which inhibits Ambler class A (including KPC and ESBLs), C (AmpC), and D (OXA-48 and related enzymes) β -lactamases^[5]. Although numerous studies have established CZA as an effective agent against carbapenem-resistant *P. aeruginosa* (CRPA)^[6,7], recent evidence has revealed the progressive emergence of resistance to this combination in clinical isolates^[8–9], even high-level resistance.

The resistance mechanisms of CZA have been extensively reported, involving metallo- β -lactamase production, β -lactamase gene mutations (e.g., KPC and GES variants), and efflux pump overexpression^[10–11]. Li et al. noted that these mechanisms generally confer low-level resistance^[12]. They found that the combined effects of PER-1 overexpression, enhanced efflux pump activity, and reduced PER-4 affinity for avibactam collectively confer high-level resistance to CZA^[12].

Currently, the mechanisms underlying high-level resistance to CZA remain limited and poorly understood. Therefore, to elucidate the epidemiological and clinical characteristics, as well as the molecular mechanisms associated with high-level CZA resistance in our region, this study collected CRPA isolates from a teaching hospital. Through patient clinical profile analysis, antimicrobial susceptibility testing, whole-genome sequencing, cloning and expression, and quantitative expression analyses, we aimed to comprehensively investigate the epidemiological and clinical characteristics, and molecular mechanisms of high-level CZA-resistant CRPA.

Methods

Bacterial isolates and patient data

354 carbapenem-resistant *Pseudomonas aeruginosa* isolates were collected from the First Affiliated Hospital of Nanchang University from 2022–2024. Isolate identification was performed using matrix-assisted laser desorption/ionization mass spectrometry (bioMérieux, Marcy-l'Étoile, France). Patient data were collected using electronic health records, including patient demographics (age and sex), admitting department, length of hospital stay, ICU duration, comorbid disease, invasive procedures, co-isolated strains, and clinical outcomes.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using the VITEK 2 Compact (bioMérieux), with results interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines (2023). Antimicrobial agents included aminoglycosides, carbapenems, fluoroquinolones, cephalosporins, β -lactam/ β -lactamase inhibitors, monocyclic β -lactams, and polymyxins. The initial screening of CZA resistant strains was performed using the disk diffusion method, and then confirmed via the micro-broth dilution method as recommended by the CLSI guidelines^[13]. That is, MIC $\leq$ 8/4 μ g/mL was sensitive, MIC

$\geq 16/4\mu\text{g/mL}$ was drug resistant, and $\text{MIC} \geq 128/4\mu\text{g/mL}$ was high level drug resistance. *Pseudomonas aeruginosa* ATCC27853 was used as the quality control strain.

WGS and bioinformatic analysis

Genomic DNA of all isolates was extracted and subsequently subjected to paired-end sequencing on the Illumina platform using the PE Cluster Kit (Illumina, USA). FastQC was applied for quality assessment of the raw sequencing data, and the trimmed high-quality dataset was assembled into contigs with SOAP Denovo 2.0. The detailed procedures were performed as previously described ^[14].

Multilocus sequence typing (MLST) was performed using the MLST 2.1 server (<https://cge.food.dtu.dk/services/MLST>). The CZA-resistant isolates were serotyped via the PAST (*Pseudomonas aeruginosa* serotype) website (<https://cge.food.dtu.dk/services/PAST/>), which is specifically designed for O-antigen serogroup prediction in *P. aeruginosa*. Virulence gene analysis was conducted using the VFDB virulence factor database (<https://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi?func=VFanalyzer>)

Phylogenetic analysis

Phylogenetic analysis of the CZA-resistant isolates was performed based on single-nucleotide polymorphisms (SNPs) using MEGA software (version 11.0.13). Allelic profiles were determined by querying the PubMLST database (<https://pubmlst.org/bigdb>) for the seven housekeeping genes (*acsA*, *aroE*, *gusA*, *mutL*, *nuoD*, *ppsA*, and *trpE*), which were concatenated in the specified order to generate the allele sequence type. The iTOL web server (<https://itol.embl.de/>) was employed for visualization and annotation of the phylogenetic tree.

Quantitative RT-PCR

To assess the expression levels of the multidrug efflux pump systems MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM, we quantified the expression of *mexB*, *mexD*, *mexF*, and *mexY* were determined by quantitative real-time PCR ^[15]. Bacterial total RNA was extracted using a commercial kit, and the quantitative real-time PCR was performed using TB Green Premix Ex Taq on a CFX Duet Real-Time PCR System (Bio-Rad, USA). The *rpsL* gene was employed as an endogenous reference for normalization. Primer sequences are detailed in Supplementary file1.

Cloning and expression of GES variants

PCR products of *bla*_{GES-1}, *bla*_{GES-15}, and *bla*_{GES-39} genes amplified with forward primers (GES-F-pUCP24,5' GATGCCGCTGGATCTGGCATGCGCTTCATT CACGCACTATT 3') and reverse primers (GES-R-pUCP24,5' ACAATTTACAC AGGAAACAGCTCTATTTGTCCGTGCTCAGGATGA 3') were recycled and

purified. Meanwhile, the pUCP24 vector was linearized by PCR with primers vector-F(5' AGCTGTTTCCTGTGTGAAATTGT 3' and vector-R(5' GCC AGATCCAGCGGCATC 3'), and PCR product was recycled and purified. The purified insert and linearized vector were then assembled using Gibson Assembly master mix (NEB, USA) and transformed into *Escherichia coli* TransT1 competent cells. Recombinant clones were selected on LB agar plates containing 5 mg/L gentamicin and Xgal-IPTG. Plasmids were extracted from independent clones and fully sequenced to confirm the absence of PCR-introduced mutations. The verified plasmids were subsequently electroporated into *P.aeruginosa* PAO1, with transformants selected on 30 mg/L gentamicin LB agar. The procedure was adapted from a previously published method [16], with appropriate modifications.

We evaluated antimicrobial susceptibility by broth microdilution for a panel of antimicrobial agents, including ticarcillin/clavulanate, piperacillin/tazobactam, cefoperazone-sulbactam, ceftazidime/avibactam, ceftazidime, cefepime, imipenem, meropenem,. MICs were interpreted according to CLSI guidelines (2023). PAO1 and PAO1 carrying the empty pUCP24 vector, as well as PAO1 derivatives producing GES-1, GES-15, and GES-39, were included as controls.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 8.0 and figures were generated by the R package and GraphPad Prism 8.0.

Results

Clinical analysis of patients

This study enrolled 22 patients (mean age 63.5 ± 14.8 years; 77.3% male (n = 17) and 22.7% female (n = 5)), with mean hospital and ICU stays of 36.32 ± 22.18 days and 20.23 ± 20.77 days, respectively. A total of 24 isolates were obtained from these individuals, and two of these patients had duplicate specimens collected at an interval of > 2 weeks. Specimens were collected from various sites of infection, including sputum (62.5%, n = 15), bronchoalveolar lavage fluid (12.5%, n = 3), secretions (8.3%, n = 2), and others (ascites, and vomitus). Most patients (12/22, 54.5%) were admitted from the intensive care unit (ICU). Polymicrobial infections were identified in 14 out of 24 isolates (58.3%), with *Klebsiella pneumoniae* being the most frequently co-isolated pathogen, followed by *Acinetobacter baumannii*. Most patients had undergone invasive procedures and presented with a substantial burden of underlying comorbidities, such as pulmonary infection, septic shock, and respiratory failure. The overall mortality rate was 68.1% (15/22) (Table 1).

Table 1. Clinical characteristics of patients infected by CZA resistant CRPA isolates

Antimicrobial susceptibility profiles of ceftazidime-resistant CRPA isolates

All the isolates were CRPA, with resistance rate of imipenem (87.5%,n = 21)and meropenem83.3%,n = 20 ,while most also showed resistance to the other antimicrobials agents including ticacillin/clavulanic acid (95.8%,n = 21), cefoperazone sulbactam (91.7%, n = 22), piperacillin tazobactam (87.5%, n = 21), levofloxacin (79.2%,n = 19), ceftazidime (79.2%,n = 19), cefepime (70.8%,n = 18),and tobramycin (70.8%, n = 18) (Table 2). All the isolates were susceptible to colistin, followed by amikacin (41.7%; n = 10). Among the 24 strains, one was MDR (4.2%), three were DTR (12.5%), and twenty were XDR (83.3%, n = 20) (Table 2).

Table 2. Antimicrobial susceptibility of ceftazidime/avibactam resistant CRPA clinical isolates

Molecular epidemiology

Based on whole-genome sequencing, the 24 isolates were assigned to 14 distinct sequence types (STs). Among these, ST891, ST235, and ST270 were identified as the three most prevalent STs, of which ST235 and ST270 rank among the global top 10 high-risk clones. The isolates belonged to six serotypes: O1, O4, O5, O6, O11, and O12. Among these, those carrying O4, O6, and O11 antigens were the most common, accounting for 25%, 25%, and 16.7% of all O-antigen-carrying isolates, respectively. Concurrently, we observed that the hypervirulent clones of ST235 (PA251, PA344, and PA346) all belonged to the highly pathogenic serotype O11, whereas the hypervirulent clones of ST270 (PA227 and PA343) were identified as serotype O12.

Based on single nucleotide polymorphism (SNP) distances, we constructed a core-genome phylogenetic tree to analyze the genetic relatedness of all isolates. As shown in Fig. 1, the isolates were generally divided into four distinct clusters. Three clusters showed relatively close internal genetic relationships, whereas the isolates in the other cluster were more distant.

Figure 1 The phylogenetic tree of CZA-resistant CPRA isolates.

The outer rings provide information on STs, O-antigen serotypes and MICs of CZA.

Drug resistance genes and virulence genes

Whole-genome sequencing was employed to screen for antimicrobial resistance determinants, revealing that the isolates harbored a variety of β -lactamase genes. In addition to the detection of metallo- β -lactamases (MBLs), including *bla*_{IMP-45}, *bla*_{IMP-89}, *bla*_{AFM-1}, *bla*_{NDM-5}, and *bla*_{VIM-2}, which were associated with CZA high-level resistance, GES-1 variants (GES-39 and GES-15) were also identified in two isolates among the CZA high-level resistant strains. PA271 co-harbored *bla*_{AFM-1} and *bla*_{IMP-45}. Furthermore, other β -lactamase genes were also detected, among which the most frequently identified were *bla*_{OXA-486}, *bla*_{OXA-396}, *bla*_{OXA-395}, and *bla*_{OXA-1} (Fig. 2).

Virulence gene analysis revealed that all isolates harbored T3SS-related toxin genes. There were 2 isolates (8.33%) *exoS*⁺/*exoU*⁺ strains, 19 (79.17%) *exoS*⁺/*exoU*⁻ strains, and 3 (12.5%) *exoS*⁻/*exoU*⁺

strains. Notably, all *exoS*⁻/*exoU*⁺ strains belonged to ST235, whereas the *exoS*⁺/*exoU*⁻ strains exhibited a wider distribution of sequence types (Fig. 2) .

Figure 2 β -lactamase genes, virulence factor of Type III secretion system, STs, and O-antigen serotypes of CZA resistant *P. aeruginosa*. The presence of β -lactamase genes and virulence genes is indicated in red and blue, respectively, while their absence is shown in white.

Antimicrobial susceptibility of GES variants

To further demonstrate that involvement of the GES variant genes (*bla*_{GES-15} and *bla*_{GES-39}) in CZA resistance, cloning and expression of these genes were performed in the PAO1. Antimicrobial susceptibility testing demonstrated that the CZA MICs for the wild-type PAO1 strain and the empty-vector transformant (PAO1/pUCP24) were both 1 μ g/mL. In contrast, the PAO1/pUCP24-GES-15 strain (harboring *bla*_{GES-15} from PA346) and the PAO1/pUCP24-GES-39 strain (harboring *bla*_{GES-39} from PA344) both exhibited CZA MICs of 8 μ g/mL. Meanwhile, the PAO1/pUCP24-GES-1 strain, carrying *bla*_{GES-1} from PA251, exhibited a CZA MIC of 16 μ g/mL (Table 3). Overall, the GES-15 and GES-39 variants conferred CZA MIC values that were 2-fold lower than that conferred by GES-1.

Table 3 Antimicrobial susceptibility profiles and GES-1-associated amino acid alterations among the various cloned variants.

Efflux pump genes expression

To further confirm the relationship between efflux pumps upregulation and CZA high-level resistance, relative expression levels were measured in these isolates, which comprised the CZA low-level resistant isolate (PA251 harbored GES-1) and high-level resistant isolates PA344 harbored GES-39 and PA346 harbored GES-15. The results revealed a significant increase in efflux pump expression among the CZA high-level resistant isolates compared with CZA low-level resistant isolate.

Figure 3 Relative ratio of efflux pump expression in the GES-1 and GES variant isolates.

All expression levels were normalized to the *rpsL* housekeeping gene and are presented as ratios relative to PAO-1 (expression = 1). Comparisons were performed using unpaired t-tests. *, *P* < 0.05, ***, *P* < 0.005. ****, *P* < 0.001. PA251: harbored GES-1; PA344: harbored GES-39; PA346: harbored GES-15.

Discussion

As a major opportunistic pathogen in clinical settings, *Pseudomonas aeruginosa* rarely infects healthy individuals but predominantly affects immunocompromised hosts [3]. Pulmonary infections caused by *P. aeruginosa*, particularly ventilator-associated pneumonia and infections in patients with cystic fibrosis, represent a substantial clinical challenge in immunocompromised populations [17]. These patients are often critically ill, with impaired host defenses, prolonged ICU stays, and exposure to broad-spectrum

antibiotics, all of which predispose them to colonization and subsequent infection by MDR or XDR *P. aeruginosa* [18–20].

In our study, most patients (12/22, 54.5%) were admitted from the ICU. These patients were generally elderly and presented with a heavy burden of underlying comorbidities. All patients had pulmonary infections, with five cases of severe pneumonia, three of sepsis, and six of septic shock. Polymicrobial infections were documented in 14 out of 24 cases (58.3%), with *Klebsiella pneumoniae* being the most frequent co-isolated pathogen, consistent with previous reports highlighting *K. pneumoniae* as a common co-infecting organism in CRPA positive respiratory specimens [21]. Notably, the overall mortality rate was 68.1% (15/22), reflecting the critical condition of the study population. These findings are consistent with previous reports highlighting the high mortality associated with CRPA infections in ICU settings [22]. Moreover, the predominance of respiratory specimens further underscores the lower respiratory tract as the primary site of CRPA infection in critically ill patients.

CZA is currently one of the few effective therapeutic options for CRPA. However, the emergence of CZA resistance, particularly high-level resistance, has become a growing concern in clinical practice. Of the 24 CZA-resistant isolates, 58.3% exhibited high-level resistance, while XDR and DTR accounted for 83.3% (20/24) and 12.5% (3/24), respectively. The convergence of high-level CZA resistance and XDR/DTR phenotypes in this critically ill patient population, with a mortality rate exceeding 65%, poses a formidable challenge to clinical antimicrobial therapy. To elucidate the underlying mechanisms, we further investigated the resistance determinants in these high-level CZA-resistant isolates.

Notably, 85.7% (6/7) of the high-level resistant strains harbored metallo- β -lactamase (MBL) genes, including *bla*_{AFM-1}, *bla*_{IMP-45}, *bla*_{IMP-89}, *bla*_{NDM-5}, and *bla*_{VIM-1}. Among these, *bla*_{AFM-1} and *bla*_{IMP-45} were the most prevalent, with detection rates of 50% (4/8) and 21.4% (3/14), respectively. Two recent studies have further underscored the severe threat posed by *bla*_{AFM-1} in CRPA. Liu *et al.*, based on a global genomic surveillance, revealed that although ST463 (17/36) was the predominant AFM-associated clone, these positive isolates had already spread across at least 15 STs, with the majority of *bla*_{AFM-1} genes being located on transferable IncP-2/IncW plasmids, thus confirming the central role of horizontal gene transfer in AFM dissemination [23]. More importantly, they explicitly noted that *bla*_{AFM-1}-harboring plasmids can confer DTR phenotypes and have been transmitted across different hospital wards within ICUs, calling for targeted interventions against plasmid spread. Concurrently, Wu *et al.*, in clinical DTR-PA isolates, identified a novel resistance mechanism, in which the abnormally elevated copy number of an IncP-2 megaplasmid led to significant transcriptional upregulation of *bla*_{AFM-1}, thereby conferring ultra-high-level resistance to cefiderocol (MIC > 256 mg/L). [24]. In the present study, the CZA-highly resistant isolates carrying *bla*_{AFM-1} belonged to ST891 and ST266, which are distinct from the STs reported above, suggesting that AFM-mediated resistance is spreading across clonal boundaries. Collectively, these lines of evidence indicate that *bla*_{AFM-1} has become a non-negligible resistance determinant in CRPA, and its dissemination potential across diverse genetic backgrounds underscores the urgent need to strengthen molecular surveillance targeting *bla*_{AFM-1} and its plasmid vehicles.

In the present study, three CZA-highly resistant isolates carrying *bla*_{IMP-45} were identified, each belonging to a distinct ST. Zhang et al. systematically elucidated the dissemination mechanism of *bla*_{IMP-45} among CRPA isolates in China, revealing that this gene is predominantly located on a specific IncP-2 plasmid sublineage, which has been detected in at least seven distinct STs (including ST508, ST369, ST357, ST1420, ST274, and ST708), suggesting its considerable capacity for inter-clonal dissemination [25]. Notably, the STs (ST266, ST697, and ST3909) of the *bla*_{IMP-45}-carrying isolates in our study are distinct from those previously reported, further corroborating that this IncP-2 plasmid sublineage can spread across diverse genetic backgrounds rather than being confined to the expansion of specific clones.

Notably, isolate PA271 in our study co-harbored *bla*_{AFM-1} and *bla*_{IMP-45}, a genetic configuration that has only been sporadically described in prior reports [26,27]. Molecular genetic analyses confirmed that both genes are located within Tn6485-series transposons on IncP-2 plasmids, and that these transposons continuously recruit additional resistance modules, such as *bla*_{PER-1} or *qnrVC6* by ISCR elements. Although the conjugation efficiencies of the plasmids vary among strains, the plasmid backbones exhibit high sequence conservation and genetic stability. This suggests that this plasmid family serves as a robust dissemination platform, driving the spread of such ‘super-resistance gene clusters’ across multiple sequence types.

In the present study, two CZA-highly resistant CRPA isolates, recovered from the same patient at an interval of approximately one month, harbored GES variants (GES-15 and GES-39) rather than metallo- β -lactamases. Herrera et al. recently demonstrated that during ceftazidime-avibactam treatment of GES-5-producing ST235 CRPA, point mutations in the catalytic center of GES (e.g., P162Q, P162A, and N136K) could be selected, leading to GES-27, GES-29, and the novel GES-60 variants, respectively, thereby conferring high-level resistance to CZA [16]. Through cloning experiments, that study further confirmed that these GES variants significantly elevated the MICs of ceftazidime-avibactam [16]. Li et al. reported an alternative pathway to CZA resistance, demonstrating that in ST235 CRPA, a strong promoter within a class 1 integron drives *bla*_{GES-1} overexpression, which, in combination with upregulation of the MexAB-OprM efflux pump, reduced susceptibility to CZA [28]. In our study, the *bla*_{GES-1} clone exhibited a CZA MIC of 16 μ g/mL, whereas the GES-15 and GES-39 clones both showed MICs of 8 μ g/mL, slightly lower than that of GES-1. However, further efflux pump expression analysis revealed that the GES-15 and GES-39 clones exhibited significantly upregulated efflux pump expression, particularly GES-15. Taken together, these findings suggest that the high-level CZA resistance observed in our study may be attributed to **a synergistic effect between GES variants and active efflux pumps**: GES variants (e.g., GES-15/GES-39) likely reduce avibactam inhibition or enhance ceftazidime hydrolysis through structural alterations, while efflux pump upregulation further decreases intracellular drug accumulation, collectively conferring a higher-level resistance phenotype. Moreover, the isolation of two strains carrying different GES variants from the same patient at a one-month interval suggests **in vivo evolution** of GES enzymes under CZA treatment pressure, consistent with the clinical observations of Herrera et al. [16]. Collectively, these findings further underscore the complexity of CZA resistance mechanisms in CRPA—involving not only

mutations and expression regulation of β -lactamase genes but also synergistic interplay with efflux pumps—and highlight the critical need for dynamic resistance surveillance in GES-producing CRPA during CZA therapy.

The three isolates harboring GES-1 and GES variants in this study were identified as belonging to the international high-risk clone ST235. ST235-type *P. aeruginosa* has been recognized as a high-risk clone due to its extensive multidrug resistance and the presence of multiple virulence determinants, which are closely associated with poor clinical outcomes ^[29,30]. Whole-genome sequencing data revealed that these three CZA-resistant ST235 isolates carried a diverse array of virulence-related genes, including type III secretion system exotoxin genes, suggesting a potentially hypervirulent phenotype. Notably, all three patients infected with these ST235 isolates in our study ultimately died, further underscoring the high clinical threat posed by this clone.

Conclusions: In summary, this study reveals that high-level CZA resistance in CRPA is driven by two distinct pathways: MBL production (mainly AFM-1 and IMP-45) in the majority of strains, and GES variants (GES-15 and GES-39) combined with efflux pump overexpression in a subset of isolates. The co-carriage of *bla*_{AFM-1} and *bla*_{IMP-45} in a single isolate and the isolation of two GES variants from the same patient at a one-month interval suggest the potential for in vivo evolution and horizontal transfer of resistance determinants. These findings underscore the complexity of resistance mechanisms and the need for continuous genomic surveillance to guide therapeutic decisions and infection control strategies.

Declarations

Ethical Approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University [approval number: CDYFYLYK (10–038)]. All methods were performed in accordance with the relevant guidelines and regulations and the Declaration of Helsinki. Due to the retrospective nature of the study, the requirement of informed consent was waived by the Ethics Committee of the First Affiliated Hospital of Nanchang University. All patient data were anonymized prior to analysis.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

Jiao Chen: Performed investigation, experimental protocol, and writing - the original draft of the manuscript. Chenyan Gao: Performed experimental protocol, data statistics, edited the manuscript. Fen Wan: Performed experimental protocol and edited the manuscript. Xinhui Liao: Performed experimental protocol and prepared figure 2. Wenjie Liu: Performed experimental protocol. Xinyi Tian: Data statistics. Heng Zhao: Data statistics. Dandan Wei: Investigation. Yang Liu: Analyzed the data and provided supervision. Guanghui Li: Analyzed the data and provided software support and supervision. All authors reviewed the manuscript.

Data Availability

The sequencing data have been submitted to the NCBI Sequence Read Archive (SRA) and are available under the accession PRJNA1509479.

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

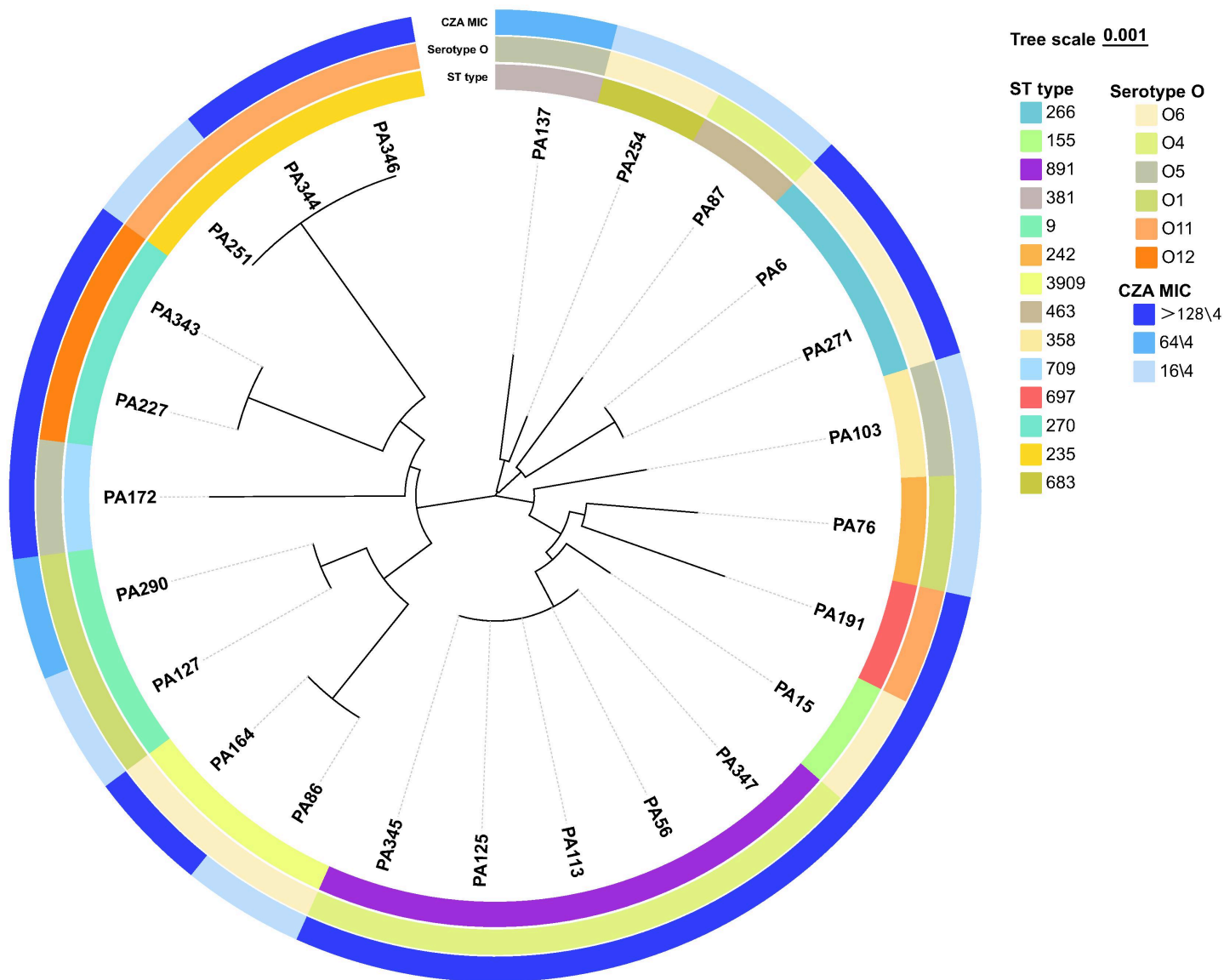


Figure 1

The phylogenetic tree of CZA-resistant CPRA isolates.

The outer rings provide information on STs, O-antigen serotypes and MICs of CZA.

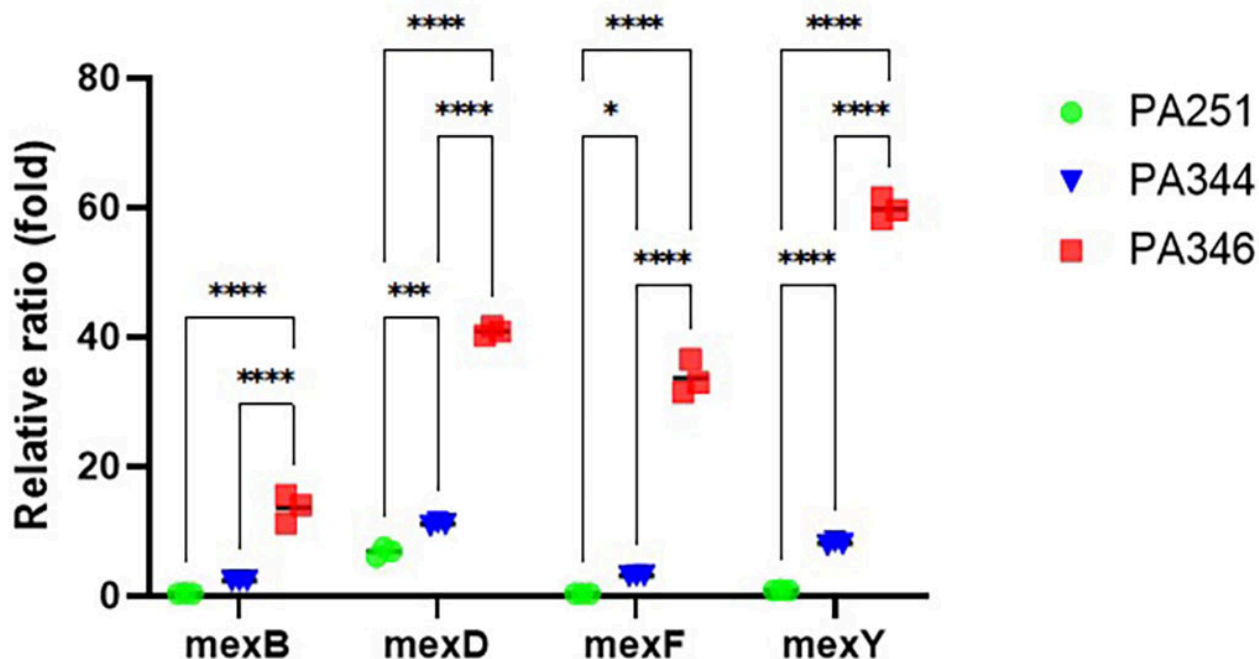


Figure 3

Relative ratio of efflux pump expression in the GES-1 and GES variant isolates.

All expression levels were normalized to the rpsL housekeeping gene and are presented as ratios relative to PAO-1 (expression = 1). Comparisons were performed using unpaired t-tests. *, $P < 0.05$, ***, $P < 0.005$. ****, $P < 0.001$. PA251: harbored GES-1; PA344: harbored GES-39; PA346: harbored GES-15.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)
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