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CASE REPORT

Ceftazidime-Induced Overexpression of MexJK-OprM Confers Resistance to Ceftolozane/Tazobactam in an Isolate of *Pseudomonas aeruginosa*

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Objective: The emergence of resistance to ceftolozane/tazobactam (CTLZ/TAZ) in *Pseudomonas aeruginosa* poses a significant clinical challenge. This study investigates the mechanisms underlying CTLZ/TAZ resistance in a clinical isolate and examines the role of prior antibiotic exposure.

Methods: A TAZ/CTLZ-resistant *P. aeruginosa* strain (Pa-R) was isolated from a patient with a lung abscess after six weeks of antibiotic therapy. Genetic relatedness between Pa-R and a pre-treatment susceptible strain (Pa-S) was assessed using PCR-based open reading frame typing (POT). Carbapenemase genes were detected via multiplex PCR. Sequencing and expression analyses of *ampC, ftsI, oprD*, and *mex* family genes were conducted. RNA interference targeting *mexJK* was performed to validate its role in resistance. In vitro antimicrobial exposure assays were conducted to evaluate resistance acquisition and the impact of prior ceftazidime (CAZ) exposure.

Results: Pa-R and Pa-S were genetically identical and lacked carbapenemase genes. No mutations or overexpression of *ampC* were observed; however, *mexJK* expression was approximately 30-fold higher in Pa-R. RNA interference using *mexJK*-specific dsRNA restored CTLZ/TAZ susceptibility, confirming that high MexJK-OprM expression is the mechanism of resistance. In vitro assays demonstrated that CTLZ/TAZ alone did not induce resistance, whereas prior CAZ exposure led to CTLZ/TAZ resistance accompanied by *mexJK* overexpression.

Conclusion: This study demonstrated that overexpression of the MexJK-OprM efflux pump is a key mechanism underlying TAZ/CTLZ resistance. In addition, prior administration of CAZ contributed to the acquisition of resistance, highlighting the need to consider the risk of resistance acquisition when switching antibiotics. In such cases, the choice of carbapenems should be considered.

Plain Language Summary:

- Ceftolozane/tazobactam -resistant *P. aeruginosa* strain was isolated from a patient with a lung abscess after six weeks of antibiotic therapy.
- Overexpression of the MexJK-OprM efflux pump is a key mechanism underlying ceftolozane/tazobactam resistance.
- The prior administration of ceftazidime contributed to the acquisition of ceftolozane/tazobactam resistance.

Keywords: *Pseudomonas aeruginosa*, ceftolozane/tazobactam, antimicrobial resistance, efflux pump, MexJK

Introduction

Pseudomonas aeruginosa is a major causative organism of nosocomial infections such as ventilator-associated pneumonia (VAP), and antimicrobial resistance has become a worldwide problem.[1,2] In particular, the effective antimicrobial agents for infections caused by carbapenem-resistant *P. aeruginosa* were extremely limited.[2,3] Ceftolozane/tazobactam

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(CTLZ/TAZ) is a novel cephalosporin antibiotic combined with a β-lactamase inhibitor, developed to combat antimicrobial-resistant *P. aeruginosa* infections.[4,5]

Recent surveillance data from Europe, the United States, and Japan showed CTLZ/TAZ resistance rates in *Pseudomonas aeruginosa* of 6.7%, 3.4% and 5.4%, respectively.[6–8] However, resistance rates are considerably higher in China and South Africa, ranging from 13 to 18.1%,[9,10] likely due to the widespread dissemination of carbapenemase-producing strains.[11,12] The primary mechanism of CTLZ/TAZ resistance involves mutations and overexpression of AmpC β-lactamase.[13,14] Overexpression of AmpC in *P. aeruginosa* is a well-established resistance mechanism against anti-pseudomonal cephalosporins such as ceftazidime (CAZ) and cefepime (CFPM).[13,14] In contrast, CTLZ/TAZ is largely unaffected by this mechanism, and *P. aeruginosa* has generally been considered unlikely to develop resistance to CTLZ/TAZ.[15] However, recent studies have reported the emergence of CTLZ/TAZ-resistant *P. aeruginosa*, with mutations in the *ampC* β-lactamase gene identified as a potential resistance mechanism.[16,17] Specific mutations in AmpC, such as G183D, E247K, and T96I, alter the structure of the substrate-binding site, resulting in an expanded substrate-binding pocket of AmpC and leading to increased hydrolytic activity against CTLZ.[13,14] These mutations, when coupled with AmpC overexpression, raise the minimum inhibitory concentration (MIC) of CTLZ/TAZ to 64–128 μg/mL.[18] Upregulation of efflux pumps and downregulation of the D_2 porin have also been occasionally reported to contribute to CTLZ/TAZ resistance,[19] but the involvement of MexJK in resistance has been underexplored.

In this study, we investigated the mechanism of CTLZ/TAZ-resistance in *P. aeruginosa* strain isolated from a patient with a lung abscess who had received multiple antimicrobial agents. Additionally, based on this clinical case, we conducted in vitro exposure experiments with multiple antimicrobial agents to evaluate how specific combinations influence the acquisition of resistance.

Materials and Methods

CTLZ/TAZ-Resistant P. aeruginosa Isolated from Sputum of Patients with Lung Abscess

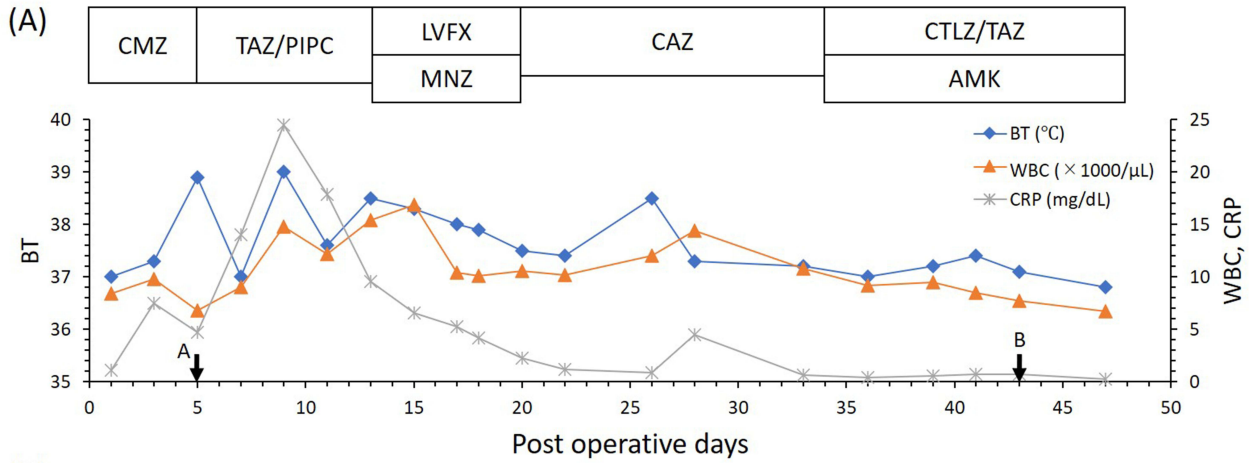
A CTLZ/TAZ-resistant strain of *P. aeruginosa* was isolated from an 84-year-old Japanese man with colorectal cancer. His only medical history was type 2 diabetes, with no history of respiratory disease. The patient was administered with cefmetazole for 5 days as prophylaxis against surgical site infection following a colectomy. However, on postoperative day five, he developed a fever, and abdominal ultrasound findings suggested acute cholecystitis. As a result, percutaneous transhepatic gallbladder drainage was performed, and the antibiotic was switched to tazobactam/piperacillin (TAZ/PIPC). At this time, a *P. aeruginosa* strain susceptible to antibiotics (Pa-S) was detected in the blood culture (Figure 1A; allow A). Despite 8 days of TAZ/PIPC treatment, the patient's condition did not improve significantly. Therefore, the antibiotic regimen was changed to levofloxacin (LVFX) combined with metronidazole to enhance treatment against anaerobic bacteria. One week later, based on respiratory symptoms and chest CT (cystography) (Figure 2), a lung abscess caused by *P. aeruginosa* was diagnosed. Consequently, the antimicrobial agent was changed to ceftazidime (CAZ), which was administered for 13 days. However, due to lack of improvement, a combination of CTLZ/TAZ and amikacin (AMK) was initiated. After 2 weeks of treatment, there was still no improvement in chest radiographic opacities, and a CTLZ/TAZ-resistant *P. aeruginosa* strain (Pa-R) was isolated from the patient's sputum (Figure 1A; allow B).

Both the Pa-S and Pa-R strains were analyzed using the PCR-based open reading frame typing (POT) method with the Cica Geneus™ Pseudo POT KIT (KANTO KAGAKU, Tokyo, Japan).[20] Additionally, the presence or absence of carbapenemase genes (*IMP-1, IMP-6, VIM, KPC, NDM, OXA-48, GES*) was determined by multiplex PCR (CFX Connect; Bio-Rad, Hercules, CA) using the Cica Geneus™ Carbapenemase Genotype Detection KIT 2 (KANTO KAGAKU).[21]

Antimicrobial Susceptibility Testing and Resistance Mechanism Analysis

MIC of CTLZ/TAZ (Merck & Co., Inc., Rahway, NJ), TAZ/PIPC (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan), CAZ (WAKO, Osaka, Japan), avibactam/ceftazidime (AVI/CAZ; Selleck Chemicals, Co., Ltd., Houston, TX), cefepime (CFPM; WAKO), imipenem (IPM; WAKO), MEPM (WAKO), AMK (WAKO), ciprofloxacin (LKT Laboratories Inc., MN) and LVFX (LKT Laboratories Inc.) were determined by microdilution according to the CLSI document.[22]

(A)



(B)

Strain	MIC (μg/mL)									
	CTLZ/TAZ	TAZ/PIPC	CAZ	AVI/CAZ	CFPM	IPM	MEPM	AMK	CPFX	LVFX
Pa-S (POD 5)	0.5	4	1	1	1	0.5	0.125	4	0.125	0.5
Pa-R (POD 43)	32	128	64	2	8	0.25	0.125	4	0.125	0.5

Figure 1 Clinical course of patient with lung abscess who isolated ceftolozane/tazobactam-resistant *Pseudomonas aeruginosa* (**A**) and the antimicrobial susceptibility of clinical isolates (**B**). Allow A indicates detection of antimicrobial-susceptible *P. aeruginosa* (Pa-S); Allow B indicates detection of ceftolozane/tazobactam–resistant *P. aeruginosa* (Pa-R). **Abbreviations**: AMK, amikacin; BT, body temperature; CAZ, ceftazidime; CMZ, cefmetazole; CPFX, ciprofloxacin; CRP, C-reactive protein; LVFX, levofloxacin; MNZ, metronidazole; CTLZ/TAZ, ceftolozane/tazobactam; TAZ/PIPC, tazobactam/piperacillin; WBC, white blood cells.

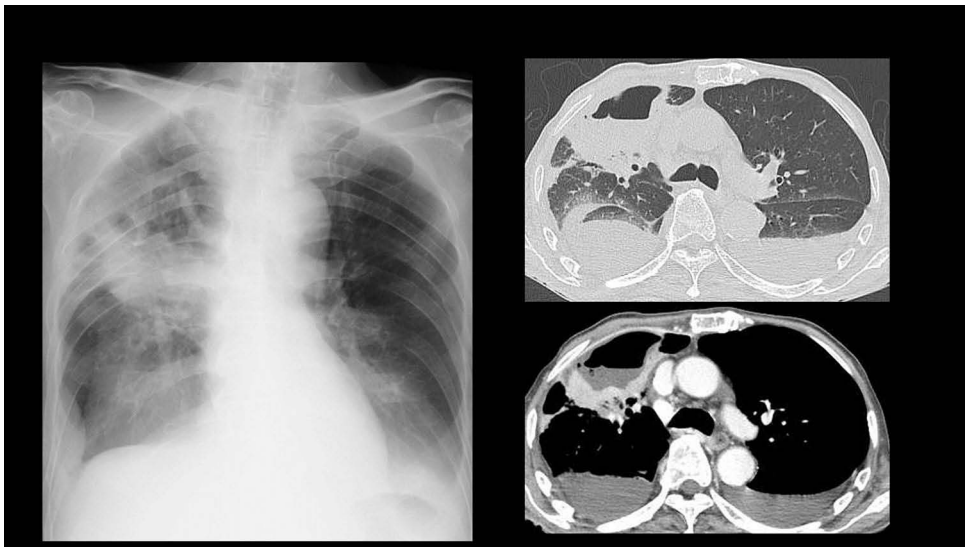


Figure 2 Chest X-ray and CT image when diagnosed with a lung abscess. Due to the patient's respiratory condition and the presence of *Pseudomonas aeruginosa* in his blood, he was diagnosed with a lung abscess caused by the bacterium.

Continuously, 1000 μg/mL cloxacillin (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) as AmpC inhibitor and 20 μg/mL carbonyl cyanide 3-chlorophenylhydrazone (CCCP; Tokyo Chemical Industry Co., Ltd.) as efflux pump inhibitor were used to evaluate the contribution of AmpC and efflux pump in CTLZ/TAZ resistance.[14,23] Each DNA sequence of *ampC* and *ftsI* which encoded AmpC and penicillin binding protein 3 (PBP-3), respectively, was determined by Sanger's dye terminator cycle sequencing method.[24,25] In addition, each expression level of *ampC, oprD* and *mex* family mRNA

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was assayed by real-time RT-PCR (CFX Connect; Bio-Rad) using the iTaq Universal SYBR Green One-Step Kit (Bio-Rad).[26–29]

RNA Transfection

siRNA transfection was performed using HiPerFect reagents (Qiagen, Stockholm, Sweden), according to the manufacturer's instructions.[30] It was placed in room temperature for 30 minutes after *mexJK*-specific dsRNA (5'-GGU GUC ACA UGU ACC GCC AdTdT-3' and 5'-UGG CGG UAC AUG UGA CAC CdTdT-3') was mixed with this reagent. One-hundred μL of Pa-R bacterial suspension prepared with 10^6 CFU/mL and 5 μL of dsRNA mixed reagent were inoculated into a 96-well plate, and incubated for 6 hours at 37°C. After the transfection, MIC of CTLZ/TAZ was determined as described above.

In vitro Serial Passage

An in vitro serial passage was performed for confirmation by a combination of repetitive administration of each antimicrobial agent whether Pa-S strain acquired CTLZ/TAZ-resistance.[31] Ten μL of the bacterial suspension prepared with McFarland no. 0.5 was inoculated onto Mueller-Hinton agar plates including serially diluted antibiotic of 8–0.5 × MIC. Plates were incubated for 24 hours at 37°C, and then colonies grown on sub-MIC plates were re-inoculated into other dilution series. This process was repeated 14 times, the MIC of CTLZ/TAZ was determined by microdilution method every time.

Statistical Analysis

The data were calculated as the mean and standard deviation per experimental group and compared by an unpaired *t*-test. Statistical significance was set at $P < 0.05$. All experiments were performed in duplicate on another day. All data were curated from at least four samples to assess an assumption of normality, and the results are presented as mean ± standard deviation.

Results

Antimicrobial Susceptibility and Resistant Mechanisms of Clinically Isolated P. aeruginosa

Both Pa-S and Pa-R strains showed the same POT types, confirming that they are identical strains (Figure 3). Both strains had no carbapenemase genes. The MIC of TAZ/CTLZ for Pa-S and Pa-R were 0.5 and 32 μg/mL, respectively (Figure 1B). Pa-R showed resistance to TAZ/PIPC and CAZ but remained susceptible to LVFX and AMK. The MIC of CTLZ/TAZ in Pa-R decreased to 4 μg/mL when combined with the efflux pump inhibitor CCCP (Table 1). mRNA expression analysis showed an approximately 30-fold increase in only the *mexJK* gene (Figure 4; $P < 0.05$). RNA transfection with *mexJK*-specific dsRNA suppressed *mexJK* mRNA translation, reducing the MIC of CTLZ/TAZ to 4 μg/mL. Combined use of the AmpC inhibitor cloxacillin did not changed CTLZ/TAZ susceptibility. No mutations in AmpC were identified, and no changes in AmpC mRNA expression were observed. No mutations were identified in PBP-3.

Acquisition of Tazobactam/Ceftolozane Resistance by the in vitro Serial Passage

The Pa-S strain acquired resistance to CTLZ/TAZ (MIC: 32 μg/mL) at 38 days later (Pa-S^{d38}) when exposed in vitro as re-appearance in a case in CTLZ/TAZ following TAZ/PIPC and CAZ for 2 weeks, respectively (Figure 5A). However, the elevation of the MIC value remained to 2 μg/mL only by having made CTLZ/TAZ exposed for two weeks (Figure 5B). To determine whether pre-exposure to CAZ or TAZ/PIPC affected CTLZ/TAZ resistance development, either CAZ or TAZ/PIPC was exposed for 2 weeks followed by 2 weeks of CTLZ/TAZ exposure (Figure 5C and D). The MIC value showed 32 μg/mL following CAZ 24 days after CTLZ/TAZ exposure, and the Pa-S strain acquired resistance to CTLZ/TAZ (Pa-S^{d24}). In the Pa-S^{d38} and Pa-S^{d24}, *mexJK* expression levels increased 38 and 34 times, respectively, compared with its parental strain (Figure 6; $P < 0.05$).

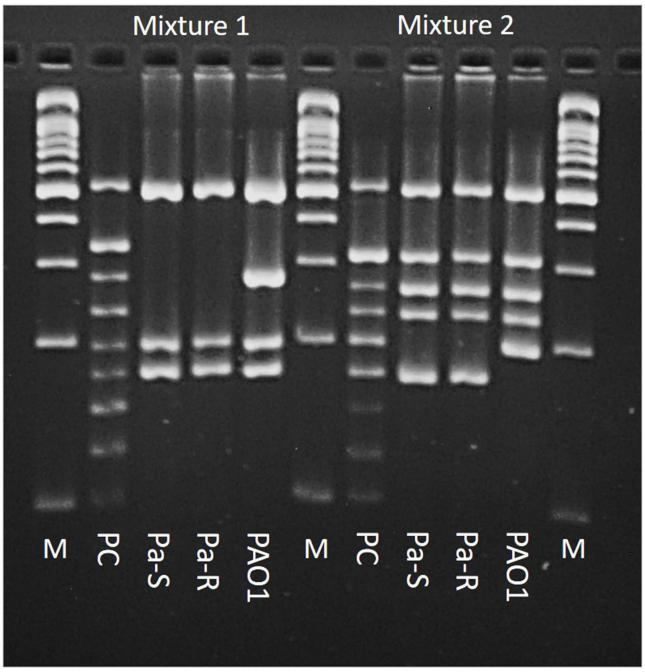

Figure 3 Agarose gel electrophoresis patterns of PCR-based open-reading-frame typing. Lane M, 100-bp ladder marker, lane PC, positive control. Mixture 1 is constructed PCR PC, Genomic islet-1, −2, −3, −4, −5, VIM, Prophage-1, −2, and −3 for 506, 336, 281, 235, 201, 175, 151, 126, 103, 85 bp, respectively. Mixture 2 is constructed for PCR PC, Genomic islet-6, −7, −8, −9, −10, Prophage-4, −5, and IMP for 506, 324, 271, 238, 204, 176, 150, 124, 105 bp, respectively.

Discussion

Many *ampC*-mutated strains also harbor mutations in DNA mismatch repair genes such as *mutS* and *mutL*, impairing the correction of *ampC* mutations and promoting stable resistance.[32,33] In general, CTLZ/TAZ resistance mediated by *ampC* mutations can be reversed by co-administration of the AmpC inhibitor cloxacillin.[14] However, in this study, the CTLZ/TAZ-resistant strain (Pa-R) remained resistant even in the presence of cloxacillin.

No known *ampC* mutations were detected in the Pa-R strain nor were mutations found in the DNA mismatch repair genes *mutS* or *mutL* (data not shown). These findings suggest that resistance in this isolate is not attributable to AmpC

Table 1 MIC Value for P. aeruginosa Strains with Inhibitor of AmpC β-Lactamases and Efflux Pumps

Antibiotic	MIC (μg/mL) Value for	
	Pa-S	Pa-R
CTLZ/TAZ	0.5	32
+ Cloxacillin	0.5	32
+ CCCP	0.5	4
TAZ/PIPC	4	128
+ Cloxacillin	4	64
+ CCCP	4	16
CAZ	1	64
+ Cloxacillin	1	4
+ CCCP	1	32

Notes: +: Inhibitors (Cloxacillin or CCCP) were used in addition to the antibiotic indicated in the upper cell.
Abbreviations: CAZ, ceftazidime; CCCP, carbonyl cyanide 3-chlorophenylhydrazone; CTLZ/TAZ, ceftolozane/tazobactam; TAZ/PIPC, tazobactam/piperacillin.

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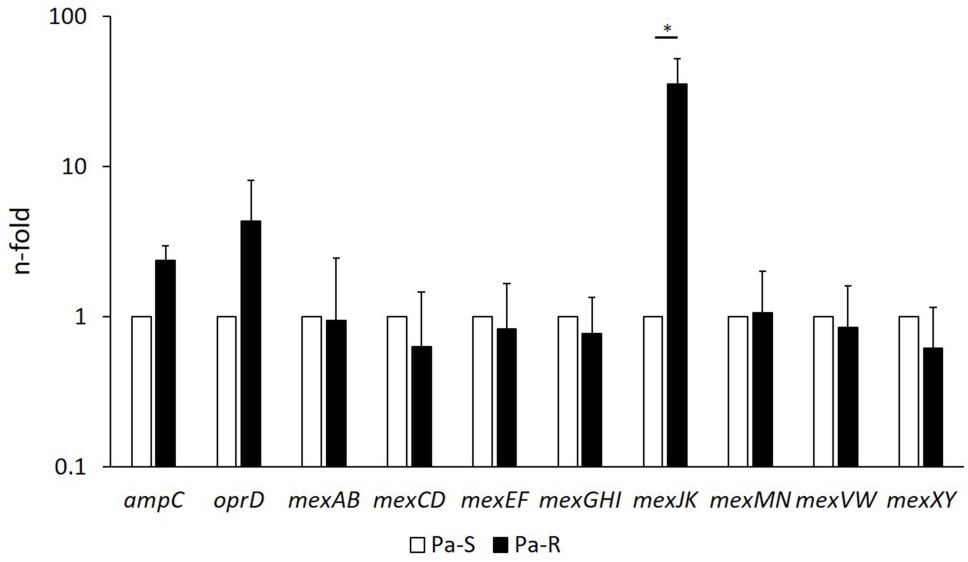

Figure 4 mRNA expression in ceftolozane/tazobactam -resistant *Pseudomonas aeruginosa* clinical isolate. Pa-S means ceftolozane/tazobactam -susceptible *P. aeruginosa* isolated before antibiotic treatment; Pa-R means ceftolozane/tazobactam -resistant *P. aeruginosa* isolated after multiple antibiotic treatment. *P <0.05.

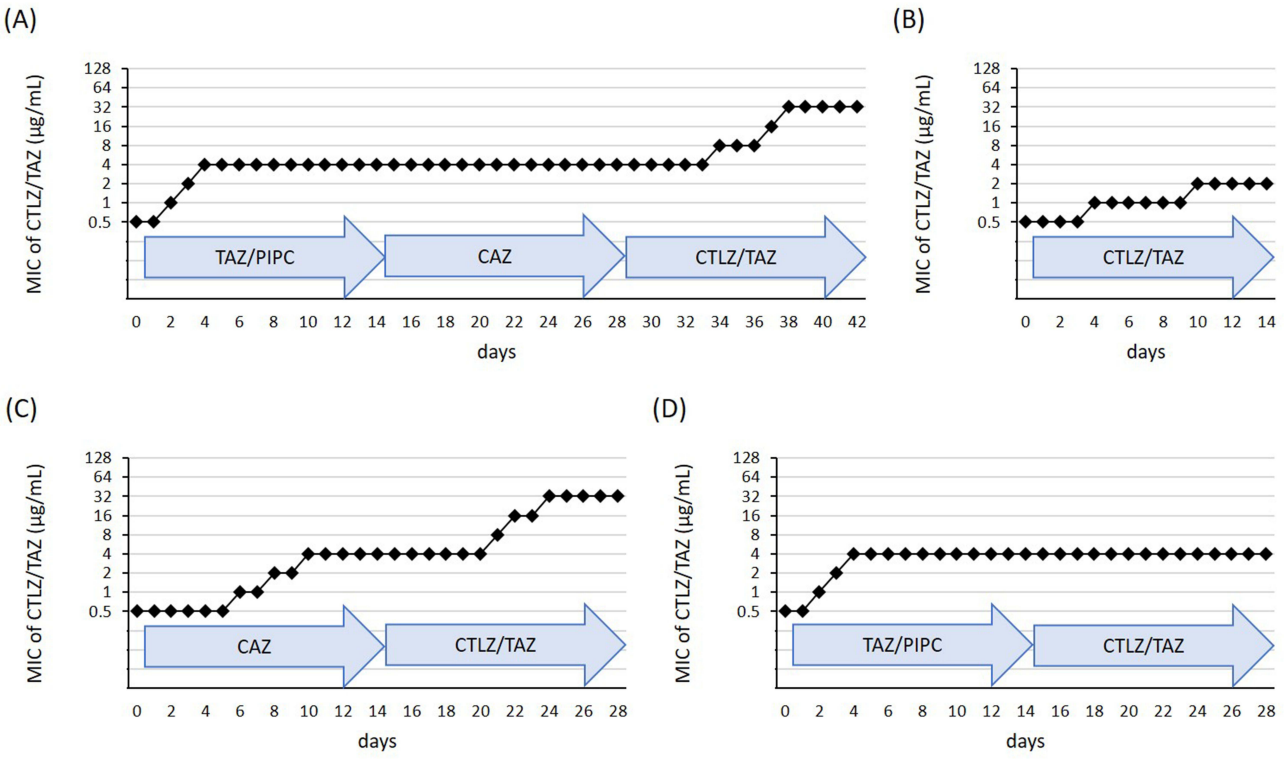

Figure 5 Change of ceftolozane/tazobactam MIC in clinical isolate Pa-S through an in vitro serial passage under the antibiotic pressure. (**A**) tazobactam/piperacillin, ceftazidime and ceftolozane/tazobactam were exposed for 2 weeks each (total 42 days); (**B**) ceftolozane/tazobactam only was exposed for 2 weeks; (**C**) ceftazidime followed by ceftolozane/tazobactam, exposed for 2 weeks (total 28 days); (**D**) tazobactam/piperacillin followed by ceftolozane/tazobactam, exposed for 2 weeks (total 28 days).
Abbreviations: CAZ, ceftazidime; CTLZ/TAZ, ceftolozane/tazobactam; TAZ/PIPC, tazobactam/piperacillin.

alterations but instead involves an alternative mechanism. Co-administration of the efflux pump inhibitor CCCP restored CTLZ/TAZ susceptibility in Pa-R, implicating efflux-mediated resistance.

Quantitative analysis of *mex* family gene expression revealed that most *mex* genes were expressed at levels comparable to those in the susceptible Pa-S strain. However, *mexJK* expression was markedly elevated. The MexJK

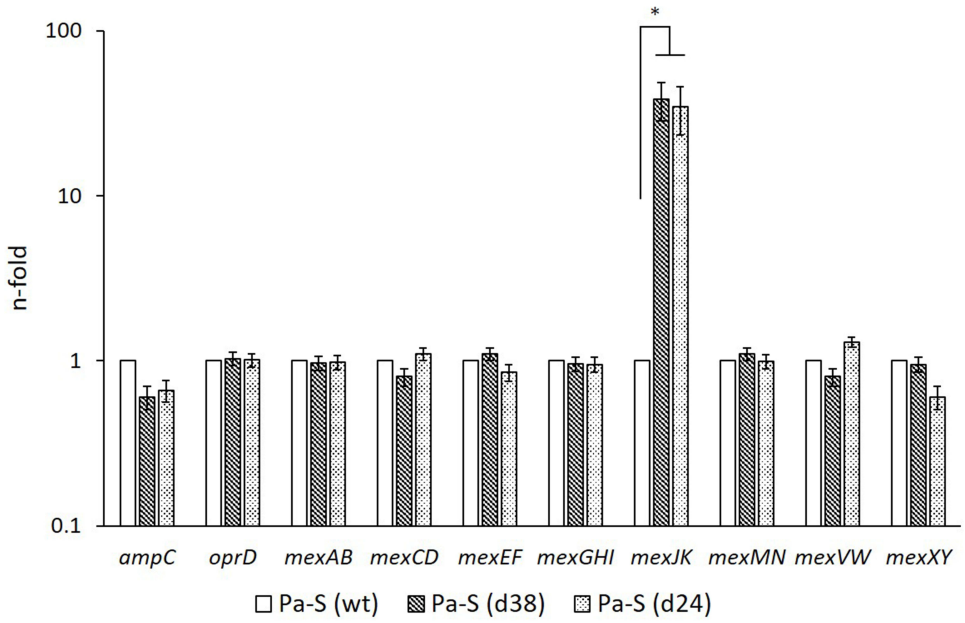

Figure 6 Changes of mRNA expression by the in vitro serial passage. Pa-S (wt): Pa-S clinical isolate (wild type), Pa-S (d24) and Pa-S (d38): Pa-S who acquired resistance by the in vitro serial passage, *P <0.05.

efflux pump, which operates in conjunction with OprM, has previously been associated with resistance to erythromycin and tetracycline.[34]

To directly assess the involvement of MexJK, we conducted an RNA transfection assay using a synthetic small RNA designed to hybridize with the 5′ untranslated region of *mexJK* mRNA, thereby inhibiting its translation. Transfection of this RNA into the Pa-R strain significantly restored susceptibility to CTLZ/TAZ, supporting the conclusion that overexpression of the MexJK-OprM efflux system underlies resistance in this isolate. Notably, *mexJK* expression is known to be upregulated under quorum-sensing conditions.[27] Since the Pa-R strain was isolated from a patient with a pulmonary abscess—a microenvironment likely characterized by a high bacterial density—activation of the quorum-sensing system may have facilitated MexJK overexpression.[35,36] In contrast, other major efflux systems in *P. aeruginosa*, such as MexAB and MexCD, are typically downregulated in response to quorum sensing,[37] which may explain their lack of involvement in the resistance phenotype observed in this case.

Recent in vitro studies have shown that exposure to meropenem (MEPM) can induce CTLZ/TAZ resistance via *ampC* mutations and overexpression.[38] However, the patient from whom Pa-R was isolated had no history of MEPM use, and no *AmpC*-mediated resistance mechanism was detected. Instead, prior administration of CAZ or TAZ/PIPC was considered a potential factor contributing to resistance development. In vitro sequential exposure assays revealed that pre-treatment with CAZ followed by CTLZ/TAZ induced resistance, accompanied by increased *mexJK* expression. CAZ has previously been reported to upregulate efflux pumps such as MexAB in *P. aeruginosa*,[39] and our findings suggest that it may similarly induce *mexJK*. Nonetheless, the regulatory pathways governing *mexJK* expression remain poorly understood and warrant further investigation.[40]

Conversely, prolonged exposure to TAZ/PIPC for 14 days had no impact on CTLZ/TAZ susceptibility. Given that TAZ/PIPC resistance in *P. aeruginosa* is primarily mediated by AmpC overexpression—which does not impair CTLZ/TAZ efficacy—this observation aligns with previous reports.

Limitation

As this study was limited to a single clinical isolate, further investigations involving a broader range of clinical strains are needed to validate and generalize these findings.

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Conclusion

We identified overexpression of the MexJK-OprM efflux system, rather than *AmpC* mutation or overexpression, as a novel CTLZ/TAZ resistance mechanism in *P. aeruginosa*. Our results also suggest that prior CAZ administration may contribute to the emergence of resistance, underscoring the importance of careful therapeutic transitions from CAZ to CTLZ/TAZ. In cases where CAZ has been pre-administered, a switch to a carbapenem, such as MEPM, should be considered.

Ethical Approval

The study was approved by the Odate Municipal General Hospital (Approval No.: 05-20). The patient's written informed consent was obtained for publications of all the case details. No institutional approval was required to publish because the basic research used bacterial isolates only.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declared no conflicts of interest in this work.

References

1. Brun-Buisson C, Sollet JP, Schweich H, et al. Treatment of ventilator-associated pneumonia with piperacillin-tazobactam/amikacin versus ceftazidime/amikacin: a multicenter, randomized controlled trial. VAP study group. *Clin Infect Dis*. 1998;26(2):346–354. doi:10.1086/516294
2. Sati H, Carrara E, Savoldi A, et al. The WHO Bacterial Priority Pathogens List 2024: a prioritization study to guide research, development, and public health strategies against antimicrobial resistance. *Lancet Infect Dis*. 2025;S1473-3099(25):00118. doi:10.1016/S1473-3099(25)00118-5
3. Timsit JF. Treatment of severe carbapenem-resistant *Pseudomonas aeruginosa* infections: still many uncertainties. *Lancet Infect Dis*. 2024;S1473-3099(24):00754. doi:10.1016/S1473-3099(24)00754-0
4. Juan C, Zamorano L, Pérez JL, et al. Activity of a new antipseudomonal cephalosporin, CXA-101 (FR264205), against carbapenem-resistant and multidrug-resistant *Pseudomonas aeruginosa* clinical strains. *Antimicrob Agents Chemother*. 2010;54(2):846–851. doi:10.1128/AAC.00834-09
5. Zamorano L, Juan C, Fernández-Olmos A, Ge Y, Cantón R, Oliver A. Activity of the new cephalosporin CXA-101 (FR264205) against *Pseudomonas aeruginosa* isolates from chronically-infected cystic fibrosis patients. *Clin Microbiol Infect*. 2010;16(9):1482–1487. doi:10.1111/j.1469-0691.2009.03130.x
6. Karlowsky JA, Lob SH, Siddiqui F, et al. In vitro activity of ceftolozane/tazobactam against multidrug-resistant *Pseudomonas aeruginosa* from patients in Western Europe: SMART 2017-2020. *Int J Antimicrob Agents*. 2023;61(5):106772. doi:10.1016/j.ijantimicag.2023.106772
7. Karlowsky JA, Lob SH, Bauer KA, et al. Activity of ceftolozane/tazobactam, imipenem/relebactam and ceftazidime/avibactam against clinical Gram-negative isolates-SMART United States 2019-21. *JAC Antimicrob Resist*. 2024;6(1):dlad152. doi:10.1093/jacamr/dlad152
8. Kosai K, Yamagishi Y, Mikamo H, Ishii Y, Tateda K, Yanagihara K. Epidemiological analysis and antimicrobial susceptibility profile of Gram-negative bacilli that cause bacteremia in Japan. *J Infect Chemother*. 2023;29(12):1091–1096. doi:10.1016/j.jiac.2023.08.012
9. Yu W, Zhang H, Zhu Y, Jia P, Xu Y, Yang Q. In-vitro activity of ceftolozane/tazobactam against *Pseudomonas aeruginosa* collected in the study for monitoring antimicrobial resistance trends (SMART) between 2016 and 2019 in China. *Int J Antimicrob Agents*. 2023;61(4):106741. doi:10.1016/j.ijantimicag.2023.106741
10. Perovic O, Singh-Moodley A, Lowe M. In vitro activity of ceftolozane-tazobactam against *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* obtained from blood cultures from sentinel public hospitals in South Africa. *Antibiotics*. 2023;12(3):453. doi:10.3390/antibiotics12030453
11. Li C, Chen R, Qiao J, et al. Distribution and molecular characterization of carbapenemase-producing gram-negative bacteria in Henan, China. *Sci Rep*. 2024;14(1):14418. doi:10.1038/s41598-024-65106-0

12. Jung H, Pitout JDD, Matsumura Y, et al. Genomic epidemiology and molecular characteristics of *bla*NDM-1-positive carbapenem-resistant *Pseudomonas aeruginosa* belonging to international high-risk clone ST773 in the Gauteng region, South Africa. *Eur J Clin Microbiol Infect Dis*. 2024;43(4):627–640. doi:10.1007/s10096-024-04763-5
13. Slater CL, Winogrodzki J, Fraile-Ribot PA, Oliver A, Khajehpour M, Mark BL. Adding insult to injury: mechanistic basis for how ampc mutations allow *Pseudomonas aeruginosa* to accelerate cephalosporin hydrolysis and evade avibactam. *Antimicrob Agents Chemother*. 2020;64(9):e00894–20. doi:10.1128/AAC.00894-20
14. Zamudio R, Hijazi K, Joshi C, Aitken E, Oggioni MR, Gould IM. Phylogenetic analysis of resistance to ceftazidime/avibactam, ceftolozane/tazobactam and carbapenems in piperacillin/tazobactam-resistant *Pseudomonas aeruginosa* from cystic fibrosis patients. *Int J Antimicrob Agents*. 2019;53(6):774–780. doi:10.1016/j.ijantimicag.2019.02.022
15. Takeda S, Nakai T, Wakai Y, Ikeda F, Hatano K. In vitro and in vivo activities of a new cephalosporin, FR264205, against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 2007;51(3):826–830. doi:10.1128/AAC.00860-06
16. Gomis-Font MA, Pitart C, Del Barrio-Tofiño E, et al. Emergence of resistance to novel cephalosporin-β-lactamase inhibitor combinations through the modification of the *pseudomonas aeruginosa* MexCD-OprJ efflux pump. *Antimicrob Agents Chemother*. 2021;65(8):e0008921. doi:10.1128/AAC.00089-21
17. Hioki Y, Hashimoto T, Hiramatsu K, Komiya K. The possibility of ceftolozane/tazobactam-resistant *Pseudomonas aeruginosa* emergence after two days of antibiotic therapy: a case report. *Cureus*. 2025;17(2):e79207. doi:10.7759/cureus.79207
18. Cabot G, Bruchmann S, Mulet X, et al. *Pseudomonas aeruginosa* ceftolozane-tazobactam resistance development requires multiple mutations leading to overexpression and structural modification of AmpC. *Antimicrob Agents Chemother*. 2014;58(6):3091–3099. doi:10.1128/AAC.02462-13
19. Deroche L, Aranzana-Climent V, Rozenholc A, et al. Characterization of *Pseudomonas aeruginosa* resistance to ceftolozane-tazobactam due to ampC and/or ampD mutations observed during treatment using semi-mechanistic PKPD modeling. *Antimicrob Agents Chemother*. 2023;67(10):e0048023. doi:10.1128/aac.00480-23
20. Suzuki M, Yamada K, Aoki M, et al. Applying a PCR-based open-reading frame typing method for easy genotyping and molecular epidemiological analysis of *Pseudomonas aeruginosa*. *J Appl Microbiol*. 2016;120(2):487–497. doi:10.1111/jam.13016
21. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. *J Antimicrob Chemother*. 2012;67(4):906–909. doi:10.1093/jac/dkr563
22. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100*. 34th ed. Wayne, PA: CLSI; 2024.
23. Pournaras S, Maniati M, Spanakis N, et al. Spread of efflux pump-overexpressing, non-metallo-beta-lactamase-producing, meropenem-resistant but ceftazidime-susceptible *Pseudomonas aeruginosa* in a region with *bla*VIM endemicity. *J Antimicrob Chemother*. 2005;56(4):761–764. doi:10.1093/jac/dki296
24. Berrazeg M, Jeannot K, Ntsogo Enguéné VY, et al. Mutations in β-Lactamase AmpC increase resistance of *Pseudomonas aeruginosa* isolates to antipseudomonal cephalosporins. *Antimicrob Agents Chemother*. 2015;59(10):6248–6255. doi:10.1128/AAC.00825-15
25. Clark ST, Sinha U, Zhang Y, et al. Penicillin-binding protein 3 is a common adaptive target among *Pseudomonas aeruginosa* isolates from adult cystic fibrosis patients treated with β-lactams. *Int J Antimicrob Agents*. 2019;53(5):620–628. doi:10.1016/j.ijantimicag.2019.01.009
26. Quale J, Bratu S, Gupta J, Landman D. Interplay of efflux system, *ampC*, and *oprD* expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. *Antimicrob Agents Chemother*. 2006;50(5):1633–1641. doi:10.1128/AAC.50.5.1633-1641.2006
27. Amieva R, Gil-Gil T, Martínez JL, Alcalde-Rico M. The MexJK multidrug efflux pump is not involved in acquired or intrinsic antibiotic resistance in *pseudomonas aeruginosa*, but modulates the bacterial quorum sensing response. *Int J Mol Sci*. 2022;23(14):7492. doi:10.3390/ijms23147492
28. Ranjitkar S, Jones AK, Mostafavi M, et al. Target (MexB)- and efflux-based mechanisms decreasing the effectiveness of the efflux pump inhibitor D13-9001 in *Pseudomonas aeruginosa* PAO1: uncovering a new role for MexMN-OprM in efflux of β-Lactams and a novel regulatory circuit (MmnRS) controlling MexMN expression. *Antimicrob Agents Chemother*. 2019;63(2):e01718– e017118. doi:10.1128/AAC.01718-18
29. Li Y, Mima T, Komori Y, et al. A new member of the tripartite multidrug efflux pumps, MexVW-OprM, in *Pseudomonas aeruginosa*. *J Antimicrob Chemother*. 2003;52(4):572–575. doi:10.1093/jac/dkg390
30. Gong FY, Zhang DY, Zhang JG, et al. siRNA-mediated gene silencing of MexB from the MexA-MexB-OprM efflux pump in *Pseudomonas aeruginosa*. *BMB Rep*. 2014;47(4):203–208. doi:10.5483/bmbrep.2014.47.4.040
31. Sato T, Ito R, Kawamura M, Fujimura S. The risk of emerging resistance to trimethoprim/sulfamethoxazole in *Staphylococcus aureus*. *Infect Drug Resist*. 2022;15:4779–4784. doi:10.2147/IDR.S375588
32. Oliver A, Baquero F, Blázquez J. The mismatch repair system (*mutS, mutL* and *uvrD* genes) in *Pseudomonas aeruginosa*: molecular characterization of naturally occurring mutants. *Mol Microbiol*. 2002;43(6):1641–1650. doi:10.1046/j.1365-2958.2002.02855.x
33. Dulanto Chiang A, Patil PP, Beka L, et al. Hypermutator strains of *Pseudomonas aeruginosa* reveal novel pathways of resistance to combinations of cephalosporin antibiotics and beta-lactamase inhibitors. *PLoS Biol*. 2022;20(11):e3001878. doi:10.1371/journal.pbio.3001878
34. Chuanchuen R, Narasaki CT, Schweizer HP. The MexJK efflux pump of *Pseudomonas aeruginosa* requires OprM for antibiotic efflux but not for efflux of triclosan. *J Bacteriol*. 2002;184(18):5036–5044. doi:10.1128/JB.184.18.5036-5044.2002
35. Soto-Aceves MP, Cocotl-Yañez M, Merino E, et al. Inactivation of the quorum-sensing transcriptional regulators LasR or RhlR does not suppress the expression of virulence factors and the virulence of *Pseudomonas aeruginosa* PAO1. *Microbiology*. 2019;165(4):425–432. doi:10.1099/mic.0.000778
36. Kariminik A, Baseri-Salehi M, Kheirkhah B. *Pseudomonas aeruginosa* quorum sensing modulates immune responses: an updated review article. *Immunol Lett*. 2017;190:1–6. doi:10.1016/j.imlet.2017.07.002
37. Bratu S, Gupta J, Quale J. Expression of the las and *rhl* quorum-sensing systems in clinical isolates of *Pseudomonas aeruginosa* does not correlate with efflux pump expression or antimicrobial resistance. *J Antimicrob Chemother*. 2006;58(6):1250–1253. doi:10.1093/jac/dkl407
38. Fouad A, Nicolau SE, Tamma PD, Simner PJ, Nicolau DP, Gill CM. Assessing the impact of meropenem exposure on ceftolozane/tazobactam-resistance development in *Pseudomonas aeruginosa* using in vitro serial passage. *J Antimicrob Chemother*. 2024;79(5):1176–1181. doi:10.1093/jac/dkae089
39. Solé M, Fàbrega A, Cobos-Trigueros N, et al. In vivo evolution of resistance of *Pseudomonas aeruginosa* strains isolated from patients admitted to an intensive care unit: mechanisms of resistance and antimicrobial exposure. *J Antimicrob Chemother*. 2015;70(11):3004–3013. doi:10.1093/jac/dkv228
40. Avakh A, Grant GD, Cheesman MJ, Kalkundri T, Hall S. The art of war with *Pseudomonas aeruginosa*: targeting mex efflux pumps directly to strategically enhance antipseudomonal drug efficacy. *Antibiotics*. 2023;12(8):1304. doi:10.3390/antibiotics12081304