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Within-Host Evolution of Ceftazidime-Avibactam Resistance in *Pseudomonas aeruginosa* associated with AmpC Overexpression

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Abstract

Background : *Pseudomonas aeruginosa* exhibits broad intrinsic and acquired antimicrobial resistance, making β-lactam/β-lactamase-inhibitor (BL/BLI) therapy an important treatment option. Eleven sequential isolates (PA01–PA11) were recovered from a single patient in Korea. The last isolate, PA11, was resistant to ceftazidime–avibactam (CZA).

Methods : To investigate within-host mechanism underlying CZA resistance, we performed PCR-based screening for acquired β-lactamase genes, efflux inhibition assays, and quantification of intrinsic β-lactamase expression for eleven *P. aeruginosa* isolates. CZA-resistant mutants induced from clinical isolates were used to validate the identified resistance mechanism.

Results : The CZA-resistant isolate did not acquire any β-lactamase genes or did not show increased efflux activity. Instead, it exhibited high-level overexpression of the intrinsic β-lactamase *ampC* (>1,000-fold). Among six CZA-susceptible isolates subjected to resistance induction, one also developed CZA resistance accompanied by high-level *ampC* overexpression.

Conclusion : Our findings suggest that high-level *ampC* overexpression, which might be acquired during therapy, would be associated with CZA resistance in *P. aeruginosa*.

Methods

Clinical isolates : Eleven *P. aeruginosa* clinical isolates were collected sequentially from a single patient at Samsung Medical Center.

Antibiotic susceptibility testing : Antibiotic susceptibility was determined by broth microdilution according to CLSI guidelines for ceftazidime–avibactam (CZA), piperacillin–tazobactam (TZP), ceftazidime (CAZ), meropenem (MEM), amikacin (AMK), levofloxacin (LFX), ciprofloxacin (CIP), and colistin (CL).

PCR-based β-lactamase gene detection : β-lactamase genes were screened by PCR using gene-specific primer sets targeting KPC, OXA-48-like, CTX-M, NDM, SHV, TEM, IMP, VIM, GIM, SPM, and SIM.

Efflux inhibition assay : MICs were determined by broth microdilution in MHII broth with or without carbonyl cyanide 3-chlorophenylhydrazone (CCCP) at final concentrations of 1 or 10 μg/mL. Bacterial suspensions were adjusted to 0.5 McFarland, diluted 1:100, inoculated into 96-well plates containing two-fold serial antibiotic dilutions, and incubated at 37°C for 18 h.

RT-qPCR analysis : Relative expression was calculated using the ΔΔCt method, with *rpsL* as the housekeeping gene and PAO1 as the reference strain. Statistical comparisons to PA01 were performed by one-way ANOVA with Dunnett's multiple-comparison test.

Results

Table 1. Antibiotic resistant profiles of clinical isolates

Isolate	Sequence type	MIC							
		CZA	TZP	CAZ	MEM	AMK	LFX	CIP	CL
PA01	3393	S	S	I	S	S	I	S	I
PA02	3393	S	R	R	S	S	I	S	I
PA03	3393	S	R	R	S	S	I	S	I
PA04	3393	S	R	R	S	S	S	S	I
PA05	3393	S	I	S	R	S	R	I	I
PA06	3393	S	I	S	R	S	R	I	I
PA07	3393	S	I	S	R	S	R	I	I
PA08	3393	S	R	R	R	S	I	S	I
PA09	3393	S	R	R	R	S	I	S	I
PA10	3393	S	R	R	R	S	I	S	I
PA11	3393	R	R	R	R	S	I	S	I

❖ While PA01 was susceptible to all tested antibiotics, PA11 exhibited resistance to all β-lactams while remaining susceptible to non-β-lactams. Notably, CZA resistance emerged only in PA11 (**32/4** μg/mL).

PCR-based β-lactamase gene detection

❖ β-lactamase acquisition by horizontal transfer was screened. PCR-based assays using specific primer sets targeting KPC, NDM, OXA-48-like, CTX-M, SHV, TEM, IMP, VIM, GIM, SPM, and SIM. All isolates yielded no amplicons.

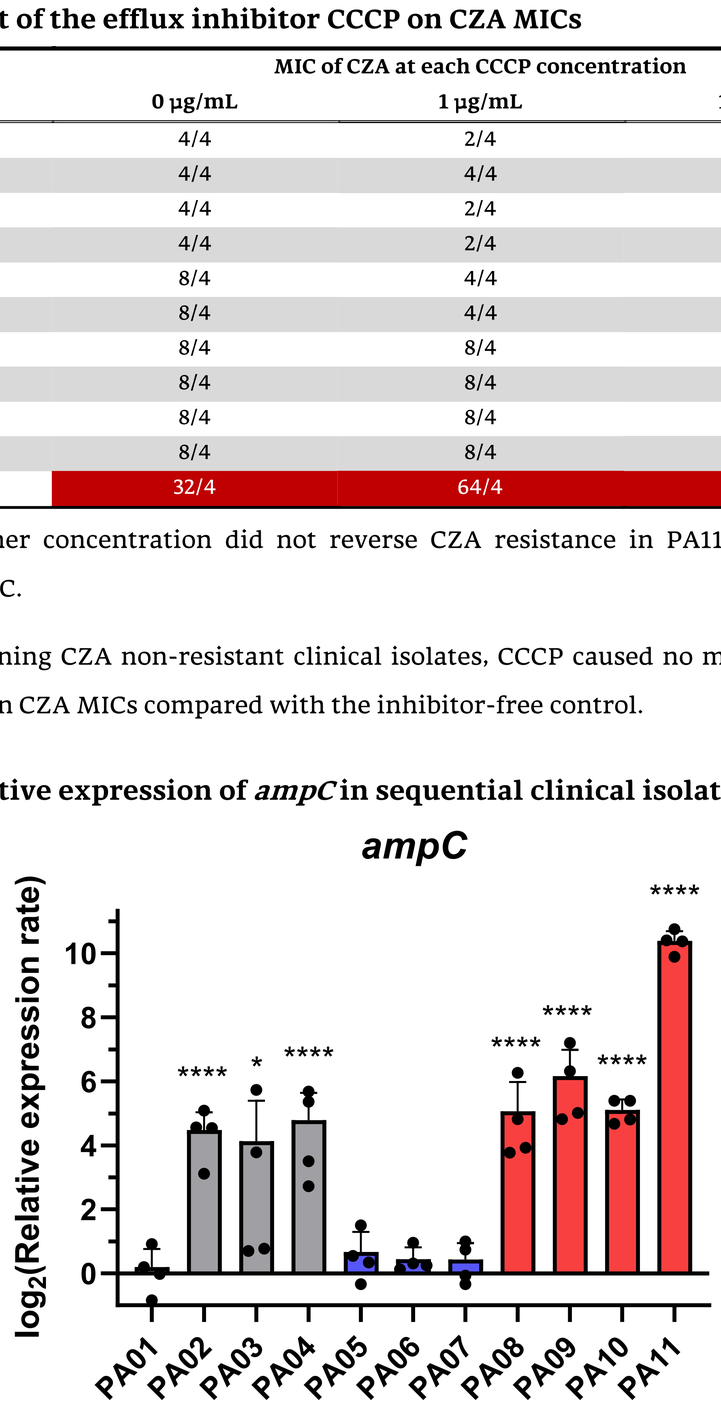
Table 2. Effect of the efflux inhibitor CCCP on CZA MICs

Strain	MIC of CZA at each CCCP concentration		
	0 μg/mL	1 μg/mL	10 μg/mL
PA01	4/4	2/4	1/4
PA02	4/4	4/4	2/4
PA03	4/4	2/4	2/4
PA04	4/4	2/4	2/4
PA05	8/4	4/4	8/4
PA06	8/4	4/4	8/4
PA07	8/4	8/4	8/4
PA08	8/4	8/4	8/4
PA09	8/4	8/4	8/4
PA10	8/4	8/4	8/4
PA11	32/4	64/4	32/4

❖ CCCP at either concentration did not reverse CZA resistance in PA11 and did not reduce its MIC.

❖ In the remaining CZA non-resistant clinical isolates, CCCP caused no more than a 2-fold change in CZA MICs compared with the inhibitor-free control.

Figure 1. Relative expression of *ampC* in sequential clinical isolates



❖ PA11 exhibited the highest *ampC* expression among the clinical isolates, with 1,305-fold upregulation compared with PAO1 and 1,252-fold upregulation compared with PA01.

❖ Significantly increased *ampC* expression relative to PA01 was also observed in PA02, PA03, PA04, PA08, PA09, and PA10, with 19-, 6-, 19-, 25-, 55-, and 32-fold increases, respectively

Conclusion

❖ Ceftazidime–avibactam resistance emerged during within-host evolution in *P. aeruginosa*. PA11, was resistant to CZA despite the absence of acquired carbapenemases/ESBLs or evidence of efflux-mediated reversal and instead showed overexpression of chromosomal *ampC*.

❖ Although ampC overexpression was strongly associated with the emergence of CZA resistance in the final isolate, the underlying cause of ampC upregulation remains to be elucidated. Further genetic studies are required to clarify the mechanism driving ampC overexpression and its contribution to CZA resistance.

Reference

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