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Mechanisms of a high-level ceftolozane/tazobactam (CT) resistance against *Pseudomonas aeruginosa* (PSA) and Synergy between CT and tobramycin (TOB)

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Abstract (Revised)

Background: Ceftolozane/tazobactam (CT) is a cephalosporin/β-lactamase inhibitor with excellent activity against multi-drug resistant (MDR) *P. aeruginosa* (PSA). Several cases of CT-resistance (CT-R) development after exposure have been reported. We recovered a PSA isolate with high-level CT-R from a bacteremic patient with severe, prolonged neutropenia and 5 weeks of CT exposure. Then, multiple mutational pathways and the role of combination therapy were evaluated.

Methods: Minimum inhibitory concentrations (MIC) to CT were determined by Etest. Synergy tests between CT and tobramycin (TOB) were conducted and interpreted based on the fractional inhibitory concentration index (FICI). Furthermore, whole genome sequencing was performed. Paired-end reads were mapped and compared to reference strain PAO1. Variant analyses were conducted using CLC Genomics Workbench.

Results: Clinically, combination therapy of CT 3g q8h given over 4h and TOB 7 mg/kg q24h successfully cleared the bacteremia within 2 days. The MICs for CT and TOB were > 256 mg/L and 4 mg/L, respectively. The combination revealed synergistic effects (FICI < 0.5) with reduced CT and TOB MICs to 16 mg/L and 1 mg/L, respectively. Genomic analysis revealed the CT-R isolate contained multiple variants in the ampC gene, including G183D associated with low level CT-R. Two additional variants in aminoacid position 79 (R79Q) and position 105 (T105A) were located inside or near helix-H2 which interacts with the Ω-loop through hydrogen-binding rendering the serine active site more pliable to accommodate larger molecules. Moreover, the CT-R isolate showed a truncated ampD and multiple mutations in mexD, mexT, mexI, and mexR, a primary regulator of mexAB-oprM. The isolate also contained the oprD mutation (Q142X) and an oprD-inactivating mutation (W417X). In addition to these chromosomal mutations, the isolate harbored OXA-50, blaPAO1, and aph(3')-IIb.

Conclusion: High-level CT-R was likely driven by multiple mutations in the ampC region causing structural changes, along with AmpD-associated derepression of AmpC. While the development of high level resistance to ceftolozane/tazobactam after exposure is worrisome, our severely neutropenic patient rapidly cleared bacteremia on a combination of pharmacodynamically driven dose of ceftolozane/tazobactam and tobramycin with resultant synergy. It emphasizes the importance of strategic dosing and the potential benefit of combination therapy when combating refractory cases of MDR *P. aeruginosa* infection.

Introduction

- Ceftolozane/tazobactam (CT) is a cephalosporin/β-lactamase inhibitor with excellent activity against multi-drug resistant (MDR) *P. aeruginosa* (PSA).[1]
- While CT has shown much higher stability against AmpC hydrolysis and slower development of resistance compared to other antibacterials including carbapenems, [2,3] there are a few documented clinical cases of CT-R development upon exposure to CT.[1,4]
- We recovered a PSA isolate with high-level CT-R from a bacteremic patient with severe, prolonged neutropenia and 5 weeks of CT exposure who was subsequently treated with high dose CT and TOB.

Objectives

- This study was to describe the mechanisms of high-level CT-R against PSA and synergy between CT and TOB.

Methods

Strain
- The CT-R PSA isolate was recovered from the second bacteremia in a patient with severe, prolonged neutropenia s/p day 65 of induction chemotherapy for acute myeloid leukemia after 5 weeks of CT 1.5g q8h (or 4.5g/24hr continuous infusion) which was continued for the treatment of first episode of PSA bacteremia susceptible to CT.
- The wild-type PAO1 was used for genomic comparison.

Synergy Test between CT and TOB
- Synergy tests between TOL-TAZ and TOB were conducted using an E-test as previously described and interpreted based on calculation of the fractional inhibitory concentration index (FICI).[5]

Genomic Analysis
- Whole genome sequencing (WGS) was conducted on the NextSeq 500 sequencing instrument (Illumina Inc., San Diego, CA) with 150-base paired-end reads (UT Health San Antonio, San Antonio, TX).
- Paired-end reads were mapped to *P. aeruginosa* reference strain PAO1 (accession NC_002516).
- The presence of known horizontally transferred antimicrobial resistance genes was identified using the ResFinder database followed by variant analysis in chromosomal genes associated with resistance using CLC genomics Workbench.

Results

Patient Outcome
- With a combination of CT 3g q8h given over 4h plus TOB 7 mg/kg q24h, bacteremia rapidly resolved in 2 days.
- Table 1 summarizes the timeline of the case and MICs of the *P. aeruginosa* isolates

Synergy Test between CT and TOB
- The MICs by E-tests were ≥ 256 mg/L and 4 mg/L for TOL-TAZ and TOB, respectively.
- The combination revealed synergistic effects (FICI < 0.5), reducing the respective MIC of TOL-TAZ to 16 mg/L and TOB to 1 mg/L.

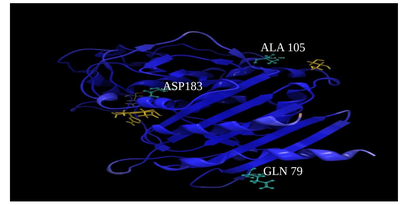
Genomic Analysis
- Genomic analysis revealed no mutations to ampR regulatory factors or within the ampR-ampC intergenic region, but multiple variants in the ampC gene, including the glycine-to-aspartate substitution in position 183 (G183D) associated with low level CT-R.
- Two additional variants in amino acid position 79 (R79Q) and position 105 (T105A) were located inside or near helix-H2 which interacts with the Ω-loop through hydrogen binding rendering the serine active site more bendable to enlarge the binding pocket for larger molecules such as ceftolozane (Figure 1).
- The isolate showed a truncated *ampD* responsible for AmpC derepression.[6]
- Multiple mutations in *mexD*, *mexT*, *mexI*, and *mexR* a primary regulator of mexAB-oprM were also observed.
- It also contained the OprD mutation (Q142X) and an OprD-inactivating mutation (W417X) responsible for carbapenem resistance.
- In addition to these chromosomal mutations, the isolate harbored other resistance genes explaining the isolate's resistance to beta lactams ($bla_{oxa\text{-}50}$, bla_{pao}) while no carbapenemases nor ESBLs were detected.
- Multilocus strain typing (MLST) identified this isolate as a new ST representing a novel combination of known alleles, which has been submitted to PSA pubMLST database (http://pubmlst.org/paeruginosa).

Table 1. Antimicrobial susceptibility against *P. aeruginosa* isolates

			MIC (mg/L)[e]											
Day (d) of treatment[a]	Sample type	Antibacterials	FEP	CAZ	CIP	MER	AMK	GM	TOB	CST	ATM	CAZ/AVI	TOL/TAZ	DOR
D7	Buttock ulcer wound	CIP (D-9- D1), TZP (D1- D2), FEP (D3- D6), MER (D7- D29) CIP (D24- D29)	32	≥64	1	1		2	≤1					
D25	Blood	MIN (D26- D27) TOB (D26- D37)	≥64		≥4	≥16	4	2	2	4	≥64	≥32/4	S[d]	≥16
D27	Left axillary wound	DOR (D29- D30) TOL/TAZ (D30-D71)[b] DOR (D71-count recovery)[c]	≥64		2	≥16	8	4	≤1	4		16/4	S[d]	≥16
D65	Blood	TOB (D66- count recovery)	≥64		≥4	≥16	≥64	≥16	4	2	≥64		≥256	8

TZP, piperacillin/tazobactam; FEP, cefepime; CAZ, ceftazidime; CIP, ciprofloxacin; MEM, meropenem; AMK, amikacin; GM, gentamicin; TOB, tobramycin; CST, colistin; ATM, aztreonam; CAZ/AVI, ceftazidime/avibactam; TOL/TAZ, ceftolozane/tazobactam ; DOR, doripenem; MIN, minocycline; [a]Days (d) since initial induction regimen (cladribine, cytarabine, G-CSF, mitoxantrone) induction; [b]Given as 3g q8h over 4hr (d30- d42), 1.5g q8h over 1-4h or 4.5g /24hr as continuous infusion at home (d42- d66), then as 3g q8h over 4h (d66- d71); [c]Given as 2g q8h over 4h; [d]MIC not available, but was susceptible by Kirby-Bauer; [e]MIC by Vitek 2 (BioMerieux, Marcy L'Etoile, France) except MICs for TOL/TAZ and TOB from day 65 isolate done by Etest (BioMerieux, Marcy L'Etoile, France)

Figure 1. Variant sequences changes on 3D protein structure of ampC



Conclusion

- A high level resistance observed in our isolate is likely driven by multiple mutations in AmpC region causing structural changes, along with AmpD-associated derepression of AmpC.
- While the development of high level resistance to ceftolozane/tazobactam after exposure is worrisome, our severely neutropenic patient rapidly cleared bacteremia on a combination of pharmacodynamically driven dose of ceftolozane/tazobactam and tobramycin with resultant synergy.
- It emphasizes the importance of strategic dosing and the potential benefit of combination therapy when combating refractory cases of MDR *P. aeruginosa* infection.

References

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