Imipenem-relebactam, ceftolozane-tazobactam and ceftazidime-avibactam against carbapenem-resistant *Pseudomonas aeruginosa*: Surveying clinical isolates for molecular markers that correlate with minimum inhibitory concentrations

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INTRODUCTION

- *P. aeruginosa* (PA) is a leading human pathogen
- It is notable for its intrinsic and acquired resistance to antibiotics.
- Carbapenem-resistant *P. aeruginosa* (CR-PA) is a major problem worldwide.
- CR mechanisms included chromosomally-encoded inducible AmpC, several efflux pumps and porin loss/mutations. 
- Relebactam is a novel β-lactamase inhibitor currently being evaluated for use with imipenem for the treatment of Gram-negative bacterial infections.
- It inhibits class A and C β-lactamases and has no activity against class D metallo-β-lactamases (MBLs).

GOALS

- To evaluate the in vitro activity of recently FDA-approved agents ceftolozane-tazobactam (TOL/TAZ) and ceftazidime-avibactam (CAZ/AVI), and the pipeline agent imipenem-relebactam (IMI/REL) that is currently in clinical trials against 64 CR-PA isolates
- To screen isolates for the presence of AmpC, ESBL and carbapenemase genes
- To assess expression of AmpC, efflux and porin genes

METHODS

- Standard broth microdilution methods according to the Clinical and Laboratory Standards Institute were used to determine minimum inhibitory concentrations (MICs) of the 3 agents
- TOL/TAZ MIC > 4 µg/mL and CAZ/AVI MIC > 8 µg/mL were defined as resistant
- IMI/REL MIC > 2 µg/mL was defined as resistant.
- Determination of mechanisms of resistance of CR-PA:
- Sequencing of ESBL genes (TEM, SHV, CTX-M), carbapenemase genes (OXA-48, KPC, VIM, NDM), OprD and AmpC genes for all isolates
- Studying expression of AmpC and various efflux and porin genes among isolates that exhibited a range of antibiotic MICs.

RESULTS

A. Susceptibility to carbapenems
- 64 CR-PSA isolates were evaluated
- 8 carbapenem-susceptible *P. aeruginosa* (CS-PA) isolates were included as controls

B. Susceptibility to CAZ/AVI, TOL/TAZ and IMI/REL

<table>
<thead>
<tr>
<th>CR-PA</th>
<th>CAZ/AVI</th>
<th>TOL/TAZ</th>
<th>IMI/REL</th>
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<tbody>
<tr>
<td>MIC90</td>
<td>2 µg/mL</td>
<td>4 µg/mL</td>
<td>2 µg/mL</td>
</tr>
<tr>
<td>MIC50</td>
<td>0.5 µg/mL</td>
<td>2 µg/mL</td>
<td>0.25 µg/mL</td>
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<tr>
<td>% resistance</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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</tbody>
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C. Distribution of CAZ-MICs and CAZ-AVI MICs

Note: there is marginal correlation between CAZ and CAZ-AVI MIC (p=0.02; R² = 0.21)

D. Mechanisms of resistance of *P. aeruginosa*

D1. No isolates harbored ESBL, carbapenemase or ESBL genes

D2. 41% of CR-PA isolates had mutations within OprD gene

E. Correlation between drug MICs and expression of ampC and various efflux genes (performed on 22 randomly selected CR-PA and 5 CS-PA)

E1. There was no correlation between MICs of CAZ, CAZ/AVI, TOL/TAZ, IMI and IMI/REL and *basal* ampC, mexB, mexD, mexY and oprD expression

E2. Correlation between MICs of CAZ, CAZ/AVI, TOL/TAZ, IMI and IMI/REL and cefoxitin-induced ampC, mexB, mexD, mexY and oprD expression

CONCLUSIONS

- Imipenem-relebactam has excellent *in vitro* activity against CR-PA, including isolates resistant to ceftazidime-avibactam and ceftolozane-tazobactam.
- Imipenem-relebactam MICs did not correlate with cefoxitin-induced AmpC expression, suggesting that relebactam efficiently inhibits activity of this enzyme.
- The correlation between imipenem and imipenem-relebactam MICs and efflux and porin gene expression is concerning, and suggests that limiting access of imipenem to the periplasmic space is an important determinant of reduced imipenem-relebactam susceptibility.