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

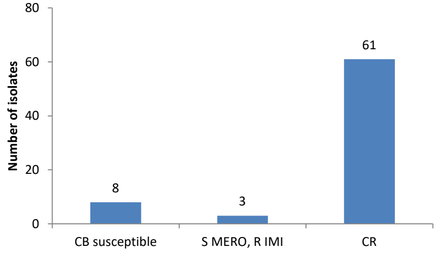
METHODS

- 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. aerugonosa* (CS-PA) isolates were included as controls

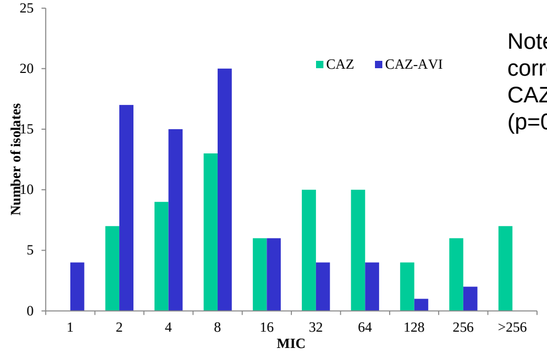


RESULTS

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

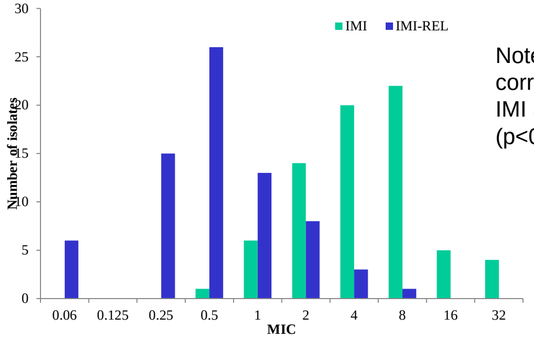
	CAZ/AV	TOL/TAZ	IMI/REL
CS-PA			
MIC90	2 µg/mL	4 µg/mL	2 µg/mL
MIC50	0.5 µg/mL	2 µg/mL	0.25 µg/mL
% resistance	0%	0%	0%
CR-PA			
MIC90	64 µg/mL	32 µg/mL	16 µg/mL
MIC50	8 µg/mL	1 µg/mL	0.5 µg/mL
% resistance	27%	14%	6%

C2. Distribution of CAZ and CAZ-AVI MICs



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

C3. Distribution of IMI and IMI/REL MICs



Note: there is mild correlation between IMI and IMI-REL MIC (p<0.0001; R2 = 0.45)

C4. Summary of data

- The addition of REL reduced IMI MIC by a median of 8-fold, and rendered 94% of isolates susceptible to IMI.
- 35% (6/17) of CAZ/AVI-resistant isolates were also resistant to TOL/TAZ, and 67% (6/9) of TOL/TAZ-resistant isolates were also resistant to CAZ/AVI.
- 25% (1/4) of IMI/REL-resistant isolates were resistant to CAZ/AVI, and none resistant to TOL/TAZ.

D. Mechanisms of resistance of *P. aeruginosa*

D1. No isolates harbored ESBL, carbapenemase or ESBL
D2. 41% of CR-PA isolates had mutations within *OprD*

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, mexBm 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

	CAZ	CAZ-AVI	TOL/TAZ	IMI	IMI/REL
ampC	NS	NS	NS	NS	NS
mexB	0.047	NS	NS	0.078	NS
mexD	NS	NS	NS	0.009	0.06
mexY	NS	NS	NS	0.004	0.02
oprD	NS	NS	NS	0.017	NS

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.**