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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**Background:** *P. aeruginosa* is a leading human pathogen notable for its intrinsic and acquired resistance to antibiotics. Carbapenem-resistant *P. aeruginosa* (CRPA) are major problems worldwide. CR mechanisms included chromosomally-encoded inducible AmpC, several efflux pumps and porin loss/mutations.

**Material/methods:** We used broth microdilution to test 72 *P. aeruginosa* isolates (64 CR) *in vitro* against the recently FDA-approved agents ceftolozane-tazobactam and ceftazidime-avibactam, and the pipeline agent imipenem-relabactam that is currently in clinical trials. Relabactam has activity against class A and C β-lactamases. We applied CLSI breakpoints for ceftolozane-tazobactam and ceftazidime-avibactam and defined imipenem-relabactam MIC > 2 µg/mL as resistant. We sequenced ESBL genes (*TEM, SHV, CTX-M*), carbapenemase genes (*OXA-48, KPC, VIM, NDM*), *OmpD* and *AmpC* genes for all isolates. We evaluated basal and cefoxitin-induced expression of *AmpC* and various efflux and porin genes among isolates that exhibited a range of antibiotic MICs.

**Results:** All carbapenem-susceptible *P. aeruginosa* (CSPA) were susceptible to all 3 agents. Among CRPA, 27%, 14% and 6% were resistant to ceftazidime-avibactam, ceftolozane-tazobactam and imipenem-relabactam, respectively. Respective MIC₅₀’s were 1, 8 and 4 µg/mL. There was low correlation between imipenem MIC and imipenem-relabactam MIC (R=0.4). The addition of relabactam reduced imipenem MIC by a median of 8-fold, and rendered 94% of isolates susceptible to imipenem. 35% (6/17) of ceftazidime-avibactam-resistant isolates were also resistant to
ceftolozane-tazobactam, and 67% (6/9) of ceftolozane-tazobactam-resistant isolates were also resistant to ceftazidime-avibactam. 25% (1/4) of imipenem-relabactam-resistant isolates were resistant to ceftazidime-avibactam, and none resistant to ceftolozane-tazobactam. None of the isolates harbored mutations within ESBL, carbapenemase or AmpC genes. 41% of CRPA isolates had mutations within OprD. There was no correlation between MICs of any of the 3 drugs with basal AmpC, MexB, MexD, MexY and OprD expression. There was also no correlation between cefoxitin-induced expression of AmpC, MexB, MexD, MexY and OprD and ceftolozane-tazobactam and ceftazidime-avibactam MICs. In contrast, imipenem and imipenem-relabactam MICs correlated with cefoxitin-induced expression of MexY (p=0.003), MexD (p=0.008), OprD (p=0.01) and MexB (p=0.09). Cefoxitin-induced AmpC expression did not correlate with imipenem-relabactam MIC.

**Conclusions:** Imipenem-relabactam has excellent *in vitro* activity against CRPA, including isolates resistant to ceftazidime-avibactam and ceftolozane-tazobactam. Imipenem-relabactam MICs did not correlate with cefoxitin-induced AmpC expression, suggesting that relabactam efficiently inhibits activity of this enzyme. The correlation between imipenem and imipenem-relabactam 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-relabactam susceptibility.