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Inactivation of penicillin-binding protein 2 is critical for killing Pseudomonas aeruginosa at high but not at low bacterial density

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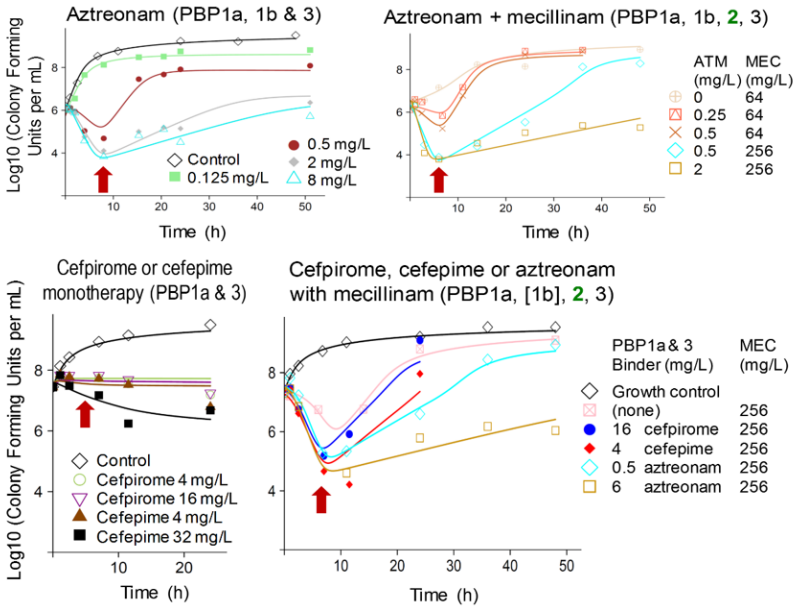
Background: Penicillin binding-proteins (PBPs) are among the most successful antibacterial targets. The binding affinities of essentially all beta-lactams are known for the different PBPs in *Pseudomonas aeruginosa.* However, the expression of PBPs in *P. aeruginosa* changes substantially during different growth phases. While almost all *in vitro* efficacy studies are being performed at low initial bacterial inocula (<10^6 CFU/mL), it is poorly understood which PBPs need to be bound and inactivated by beta-lactams at high inocula to maximize bacterial killing. High inocula may better reflect severe infections. We aimed to evaluate the impact of initial inocula on killing of *P. aeruginosa* by beta-lactams, and to characterize the bacterial killing and emergence of resistance elicited by relevant PBP binding patterns *via* a new generation of quantitative and systems pharmacology (QSP) models.

Material/methods: Static antibiotic concentration time-kill experiments over 48 h (92 viable count profiles in total) used wild-type *P. aeruginosa* PAO1 at a range of $\log_{10}$ initial inocula (CFUo, from 6 to 8). Each experiment contained a growth control and one or multiple antibiotics which preferentially bound to PBP1a (+/- PBP1b) and PBP3 (aztreonam, cefepime and cefpirome) or which selectively bound PBP2 (mecillinam). Antibiotic concentrations included the clinically relevant range. Antibiotic degradation in broth was assessed *via* LC-MS/MS. The PBP binding affinities were determined through the Bocillin FL binding assay. The impact of relevant resistance mechanisms was assessed *via* isogenic knockout strains lacking or over-expressing the AmpC β-lactamase, the MexAB efflux pump, or both. A new QSP model was developed in the S-ADAPT software.

Results: Binding of PBP2 (by mecillinam) in addition to PBPs 1a, 1b and 3 (Figure 1; top right) yielded limited benefit at low inocula when compared to aztreonam monotherapy (top left). At high inocula,

PBP1 & 3 binding (by cefpirome or cefepime) showed limited or no bacterial killing, but additional binding of PBP2 (mecillinam) yielded synergy (bottom right). The proposed QSP model was informed by our PBP binding affinity data and quantitatively described the target site penetration, efflux pump and beta-lactamase activity. This model excellently described and predicted the viable count profiles. The Figure shows the population predicted versus observed viable counts for *P. aeruginosa* PAO1 at a low (top) or high (bottom) inoculum.



Conclusions: We demonstrated that PBP2 binding was critical to achieve synergistic bacterial killing at high but not at low initial inocula. We developed the first QSP model for beta-lactams which was informed by and quantitatively accounted for PBP binding patterns, target site penetration and resistance mechanisms. This model allowed us to estimate the required extent of inactivation for each PBP to maximize bacterial killing and describe the time-course and mechanisms of resistance. This integrated approach holds excellent promise to rationally optimize double beta-lactam combinations.