Understanding the Adaptation of *Pseudomonas aeruginosa* to Colistin and the Mechanism of a Synthesized Pyrrolidinone Antibiotic

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This work focusses on the evolution of pathogens to antibiotic resistance. Two different compounds were used in this work: colistin, which is a currently used antibiotic against gram negative bacteria and KCN-AAS-35, which is a newly synthesized molecule that shows potential as a chemotherapeutic agent. *Pseudomonas aeruginosa* PAO1 was adapted to colistin, a last resort drug for gram negative bacteria to identify mutations that led to resistance. To find the mechanism of action of KCN-AAS-35, a pyrrolidinone antibiotic, the model gram positive bacteria *Bacillus subtilis* 168 was adapted to this compound. Whole genome sequencing was used to identify mutations after adaptation to these antibiotics.

Colistin is a drug of last resort for most gram negative pathogens. Endpoint isolates of *P. aeruginosa* that were previously adapted to colistin were chosen for genome sequencing after performing biochemical tests and minimum inhibitory concentration (MIC) tests. Streaks on cetrimide agar, sulfur indole motility (SIM) stab and protease test using milk agar were all done to identify the endpoint isolates that were different. PAO1 grows on cetrimide agar and produces a blue pigment. Four out of the 88 endpoint isolates grew but remained colorless on cetrimide agar plates. Nothing significant was found in the other tests. MIC tests using agar dilution method were done on all the endpoint isolates with colistin and antibiotics from the following classes: β-lactams, fluoroquinolones, aminoglycosides, tetracycline, RNA synthase inhibitor, cell wall synthesis inhibitor and protein synthesis inhibitor. Isolates with high resistance to colistin had an increased susceptibility to antibiotics in β -lactams, RNA synthesis inhibitor and protein synthesis inhibitor classes. Isolates that were selected for whole genome sequencing included the isolates that were different from the parent strain in the biochemical tests, had a high colistin resistance and showed increased susceptibility to other classes of antibiotics. Daily populations of *P. aeruginosa* that were collected during the adaptation experiment were also sent for whole genome sequencing.

The pyrrolidinone antibiotic is a novel, naturally produced compound that works well against gram positive bacteria but the mechanism is unknown. KC Nicolaou chemically synthesized the variant, KCN-AAS-35, as he was reproducing the pyrrolidinone antibiotic. *B. subtilis* was used as a model organism for studying the effects of KCN-AAS-35 on gram positive pathogens. After *B. subtilis* 168 was adapted to KCN-AAS-35 , it was previously found that the endpoint isolates had mutations in either cysteine synthase or the cysteine metabolism repressor which caused them to grow at a slower rate. To understand how these mutations affect growth in the presence of varying concentrations of cysteine and if the pyrrolidinone antibiotic and KCN-AAS-35 derivative have the same mechanism of action, we performed growth curves. Cystine adapted *B. subtilis* grew poorer in the presence of cystine than the parent strain. This is because the adapted strains can no longer stop the uptake and metabolism of cystine,

so they build up toxic levels of cystine when the amino acid is added to their media.This high quantity becomes too much. The pyrrolidinone antibiotic had a different mechanism of action as the KCN-AAS-35 derivative because *B. subtilis* endpoint isolates had a lower MIC in the natural pyrrolidinone antibiotic compared to the synthesized one.

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