

Combating Pseudomonas Resistance: Cloning of the ampC Gene Encoding for Beta-Lactamase and Development of a Non-Toxic Allosteric Inhibitory Cocktail Therapy to Eradicate Pseudomonas aeruginosa

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The opportunistic pathogen *Pseudomonas aeruginosa* causes fatal and chronic infections, with 124,000 deaths annually, due to its beta-lactamase-mediated resistance to beta-lactam antibiotics (e.g., penicillin). To facilitate further research on beta-lactamase, this study first created an ampC gene repository by cloning the chromosomal ampC into *Escherichia coli* before conjugating it into *P. aeruginosa*. Flavonoids, natural compounds with multiple hydroxyl groups, were then investigated as allosteric beta-lactamase inhibitors due to their high affinity for hydrogen bonding. Computational mutagenesis revealed several critical amino acids for beta-lactamase functionality, detecting four allosteric binding sites. Docking studies of 30+ flavonoids identified quercetin, myricetin, rutin, and epigallocatechin gallate (EGG) as potential inhibitors to these sites, with binding affinities ranging from -8.1 to -11.5 kcal/mol. In-vitro verification confirmed inhibitors' efficacy in eradicating *Pseudomonas*. Avibactam (known competitive inhibitor) with carbenicillin exhibited a 50% eradication efficacy, while EGG, myricetin, quercetin, and rutin exhibited efficacies of 55%, 74%, 83%, and 92%, respectively. The combination of all inhibitors exhibited 95% eradication efficacy. Treatment safety was supported by cytotoxicity assay on human alveolar epithelial cells. Combined with carvacrol, chlorogenic acid, and N-acetylcysteine (promising *Pseudomonas* biofilm therapeutics identified through past research), the therapy demonstrated 90% *Pseudomonas* biofilm inhibition efficacy. These findings support the delivery of the cocktail therapy to renew non-toxic beta-lactams and combat *P. aeruginosa* infections and biofilm, thus reducing morbidity and mortality from chronic *Pseudomonas* infections.

Awards Won:

First Award of \$5,000

University of North Texas at Dallas: \$2,500 scholarship, renewable up to four years