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

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