Generating a Non-toxic, Multi-pathway Targeted Cocktail Treatment Composed of N- Acetylcysteine, Carvacrol, and DNase to Inhibit Pseudomonas Biofilm Proliferation in vitro

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Bacterial biofilms account for 80% of all chronic microbial infections. ESKAPE pathogens, particularly Pseudomonas, form dense biofilms that are impenetrable by most antibiotics, making chronic biofilm-related infections nearly impossible to treat. In this project, a combination treatment consisting of FDA-approved concentrations of three compounds was devised to target three major pathways of biofilm formation: inhibiting pyocyanin and thus eDNA proliferation using N-acetylcysteine (NAC), disrupting the biofilm matrix using DNase, and suppressing quorum sensing using Carvacrol. Resazurin assay was first conducted to ensure biofilm viability. P. fluorescens bacteria was cultured with NAC 10%, NAC 20%, Carvacrol 0.15%, Carvacrol 0.75%, DNase 10 ug/ml, and DNase 20 ug/ml. Biofilm formation was then measured using resazurin assay. Concentrations that exhibited greater efficacy were then combined to determine the best condition for biofilm inhibition. Results indicated that the control group (no treatment) had a Relative Fluorescence Unit (RFU) of 3.92. NAC 20% was the most effective individual treatment with 0.973 RFU and 75% inhibition efficacy. Of the dual-combination treatments, NAC 20% + DNase 20 ug/ml was the most effective, with 0.579 RFU and 85% inhibition efficacy. Finally, the triple-combination treatment exhibited remarkable effectiveness, with 0.208 RFU and 95% inhibition efficacy. Statistical significance was confirmed using a one-way ANOVA test, and treatment safety was established by conducting a cytotoxicity assay on Caenorhabditis elegans. These findings can be translated into the development of novel adjuvants to deliver the cocktail treatment in-vivo, thus reducing morbidity and mortality from chronic biofilm-related infections.

Awards Won:
Second Award of $2,000