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Title of the Thesis: Identification and understanding the mode of actions of inhibitors against *Vibrio Cholerae* pathogenesis.

Abstract

Vibrio cholerae, the causative agent of cholera, continues to pose a serious global health challenge, especially in regions with inadequate water sanitation and hygiene infrastructure. Although oral rehydration therapy (ORS) remains the cornerstone of cholera treatment, antibiotics are often employed in severe cases to reduce disease duration and severity. However, the increasing prevalence of multidrug-resistant *V. cholerae* strains has underscored the urgent need for alternative therapeutic strategies. Anti-virulence therapy has emerged as a promising approach, aiming to disarm the pathogen by targeting its virulence factors without compromising bacterial viability. This strategy reduces the selective pressure for resistance development commonly associated with conventional antibiotics. The aim of this study was to identify and evaluate potential inhibitors of *V. cholerae* virulence mechanisms and to elucidate their efficacy and modes of action.

In this study, we screened several bioactive compounds, identified through a comprehensive literature review, for anti-virulence activity against *V. cholerae*. Among the compounds tested (arabinose, eugenol, quercetin, allylanisole, and sodium butyrate), all showed varying degrees of inhibition of key virulence traits, including cholera toxin (CT) production, epithelial cell adhesion, motility, and biofilm formation. Sodium butyrate (SB) emerged as the most effective and was selected for further investigation. SB treatment significantly reduced the expression of CT and the colonization factor TcpA at both RNA and protein levels, without affecting the expression of the master regulator ToxT or its upstream regulators (*toxR, toxS, tcpP,* and *tcpH*). Molecular docking suggested that SB interacts with key residues (Lys31 and Lys230) at the interface of ToxT's functional domains, potentially disrupting its dimerization and DNA-binding activity. This was supported by protein-DNA binding assays showing impaired ToxT-promoter interactions. RNA-seq analysis further confirmed SB-mediated downregulation of major virulence genes (*ctx, tcp, zot, hlyA*). *In vivo* studies using suckling mice and the rabbit ileal loop assay demonstrated reduced colonization and enterotoxicity, respectively, reinforcing SB's potential as an anti-virulence agent against *V. cholerae*.

In addition to anti-virulence strategies, we explored combination therapy using sodium butyrate (SB) as an adjuvant to enhance antibiotic efficacy. Our findings showed that SB selectively potentiated the antibacterial activity of tetracycline against *V. cholerae*, leading to bacterial lysis and membrane disruption. This synergistic effect was associated with collapse of the proton motive force, reduced intracellular ATP levels, and impaired efflux pump activity, resulting in increased intracellular tetracycline accumulation. The SB-tetracycline combination also more effectively suppressed the expression of key virulence factors, including cholera toxin (CT) and TcpA, than either agent alone. *In vivo*, this combination delayed mortality in suckling mice, improving survival by 40%, and significantly reduced CT production and fluid accumulation in the rabbit ileal loop model. These results highlight the SB-tetracycline combination as a promising approach to combat multidrug-resistant *V. cholerae* infection and enhance the clinical utility of tetracycline.

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