Summary

Nosocomial infections are severe in nature and a challenge to treatment due to the emergence and extent of multidrug-resistant nosocomial pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and Enterococci spp. The vancomycin-resistant *Enterococcus faecalis* (*E. faecalis*) is widely distributed globally in hospital environments as an opportunistic pathogen which can cause a wide range of infections like bacterial meningitis, urinary tract infections, endocarditis, surgical site, and bloodstream infections. The propensity of *Enterococcus faecalis* to form biofilms by regulating gene expression through quorum-sensing mechanisms is a fundamental element in its pathogenicity. Understanding the role of biofilm formation and quorum sensing in antibiotic resistance is crucial for developing effective strategies to combat infections caused by *E. faecalis*. In this thesis, we have performed *in silico*, *in vitro*, and transcriptome profiling through combinatorial treatment approaches for disrupting biofilm, inhibiting quorum sensing signaling, and developing alternative treatments for biofilm-associated infections for potential drug discovery against *E. faecalis* infections.

The novel phenol (Rubrivivaxin) and indole (Rhodethrin) terpenoid compounds target the preliminary adherence phase of the biofilm development by hindering the cell surface hydrophobicity and extracellular polymeric substance (EPS) production, which is highly resistant to harsh environmental conditions and antibiotic therapy. These terpenoid derivatives (Rhodethrin (Rdn) and Rubrivivaxin (Rbn)) were found to have the antibacterial activity to inhibit bacterial growth as well as the motility of bacterial colony migration and expansion, which are used to control the infections associated with biofilm production. The characteristic feature of the EPSs is to form a protective defense and provide nutrients to the encased biofilm developed microorganisms. These molecules inhibit biofilm formation and disrupt the ultra-structure of biomass. Chloramphenicol has become an attractive antibiotic against *E. faecalis* due to its antimicrobial and antibiofilm efficacy. However, combining natural indole terpenoid compounds with antibiotics can often lead to synergistic effects, where the combined activity is greater than the sum of their individual effects.

Our results show the *in silico* and *in vitro* antimicrobial and antibiofilm activities of Rhodethrin and chloramphenicol against *E. faecalis* in isolation and combination. Further, we confirmed the efficacy of their mechanism of action on biofilm. After the synergistic combination treatment, they significantly reduced the viable cell count, dry

weight, protein, and eDNA concentrations in matured biofilm. The respective biofilm proteins were found to be 39.7±5.1% (Rhodethrin), 32.6±4.7% (Chloramphenicol), and 69.0±5.3% (Rdn-Chpl) ($p < 0.05$). Reduction of EPS was found to be 34.6±4.6% (Rhodethrin), 31.0±5.2% (Chloramphenicol), and 76.0± 4.5% (Rdn-Chpl) ($p < 0.05$). Reduction of EPS was found to be 34.6±4.6% (Rhodethrin), 31.0±5.2% (Chloramphenicol), and 76.0± 4.5% (Rdn-Chpl) ($p < 0.05$) significantly inhibited the biosynthesis of EPS. Thus, the combination of Rdn and Chpl shows a high activity as compared to individual treatment of *E. faecalis*. The inhibitory effect on EPS biosynthesis indicates that the combination treatment disrupts the production of key components required for biofilm formation. The synergistic inhibitory effect of Rhodethrin and chloramphenicol on the construction of biofilm and EPS biosynthesis in *E. faecalis* highlights the potential of using combination therapies to combat biofilm-associated infections. These results show an enhanced activity of the combination compared to the individual treatment. The combination of Rdn and Chpl could be a promising therapeutic strategy to improve treatment efficacy against *E. faecalis* biofilms.

We have performed microscopic analysis to visualize and confirm the effects before and after the treatments. Our observations confirmed that combination treatment led to greater dispersal of bacterial cells, resulting in decreased cell clustering and aggregation within the biofilm matrix. Understanding the mode of action of Rdn in disrupting biofilm formation and inhibition of EPS biosynthesis would provide valuable insights into developing more effective therapies. This dispersal effect indicates that the combination treatment disrupts the bacteria to adhere and form cohesive structures within the biofilm. The total EPS dry weights were estimated to be 97.50 µg/g (Control), 58.37 µg/g (Rhodethrin), 62.04 µg/g (Chloramphenicol), and 23.58 µg/g (Combination). The EPS protein concentrations were 80.88 µg/g (Control), 19.41 µg/g (Chloramphenicol), 15.53 µg/g (Rhodethrin), and 8.84 µg/g (Combination) ($p < 0.05$).

Thermogravimetric and X-ray analysis revealed the pyrolysis of polysaccharide crystals and crystalline domains to be 40-60% and that of amorphous domains to be 60-75% of EPSs distractions. On the other hand, FTIR analysis shows the presence of functional groups (hydroxyl, aliphatic CH2, asymmetrical C=H stretching, aliphatic methyl, and primary amine groups), and LCMS analysis shows the disintegration of biopolymeric EPS components. The SEM analysis of EPSs witnessed a condensed matrix within polymeric carbohydrate and filamentous structures. These results show

the degradation of high molecular biopolymers into low or intermediate by-products of EPS. This treatment disrupted EPS composition and inhibited biofilm formation in *E. faecalis* cultures.

Further, to evaluate the effects of Rhodethrin in combination with chloramphenicol on *E. faecalis* cultures, identified the differentially expressed genes (DEGs). In the transcriptome analysis, out of 379 differentially expressed genes, 264 genes were significantly down-regulated, indicating that 69.69% of *the E. faecalis* genome was altered. These differentially expressed genes included 448 genes in Control *Vs.* Rdn, 1591 in Control *Vs.* Chpl, and 379 in Control *Vs*. Combined (Rhodethrin and chloramphenicol). qRT-PCR was used to analyse the transcriptional sequence data further. The results demonstrated that the treatment significantly suppressed the expression profiles of four genes involved in antibiotic resistance (*liaX, typA, EfrA,* and *lepA*), three genes expressed in quorum sensing (*camE, fsrC,* and *sylA*), and five genes were essential for biofilm formation (*Ace, AtpB, lepA, bopD,* and *typA*). This transcriptome analysis suggested that Rhodethrin and chloramphenicol might have different action mechanisms for inhibiting biofilm formation and quorum sensing in *E. faecalis*. The chloramphenicol disturbs the biofilm formation and inhibits the synthesis of enterococcal surface protein (ESP), faecal streptococci regulator-C (*fsrC*), adhesion of collagen of *E. faecalis* (Ace), biofilm on plastic (Bop) proteins and polysaccharides associated with biofilm formation, promotion of cell aggregation and adherence to surfaces. Whereas, Rhodethrin induces the inhibition of outer membrane protein (*liaX*), mediating cell membrane remodeling, *camE* encoding sex pheromone associated with quorum sensing, and large extracellular protease (*lepA*) associated with virulence expression.

The above findings stated that the combination of Rhodethrin and chloramphenicol had shown great promise in inhibiting biofilm encasement, quorum sensing, and antibiotic resistance of *E. faecalis*. Combining these two compounds is more effective at inhibiting biofilm formation than using either compound. These findings suggest the potential use of this combination as a therapeutic approach to combat *E. faecalis* infections by targeting multiple aspects of the bacterial phenotype. However, further research is needed to uncover the underlying mechanisms and optimize the treatment strategy for clinical application.