Investigating Marine Natural Products as Prospective Anti-AgrA Drug Candidates Against *Enterococcus faecium*: A Subtractive Proteomics Facilitated Multi-Tier Screening Study for Addressing Antimicrobial Resistance

Pradeep Kumar Sriram[1] and Jeyakanthan Jeyaraman[1]*

[1]Structural Biology and Biocomputing Lab, Department of Bioinformatics, Alagappa University, Karaikudi 630 003, Tamil Nadu, India.

*(Email: jjbioinformatics@gmail.com)

Abstract

Enterococcus faecium, a member of the ESKAPE Pathogens, has gained infamy in recent years for its increasing resistance against clinical antibiotics. This gram-positive bacterium has currently acquired Multi-Drug Resistance (MDR), due to its persistent exposure to the clinical environment and poses a huge threat to immune-compromised individuals. This proposed study focuses on exploring novel drug targets from the genome of *E. faecium* and investigating the potential of Marine Natural Products (MNPs) as potent Anti Quorum Sensing leads. The comprehensive Subtractive Proteomics Pipeline, prioritizing characteristics such as Non-Human Homology, Essentiality, Subcellular Localization, Druggability and Unique Metabolic Pathways deciphered the Accessory Gene Regulator A protein, encoded by the AgrA gene, as a crucial drug target, responsible for mediating quorum sensing, biofilm formation and virulence. After initial structural quality and stability assessments, a hierarchy-founded Virtual Screening against Marine Natural Products (MNPs) from the CMNPD and SPECS compound libraries was performed to shortlist potential Anti-AgrA drug Candidates. The VSW revealed three potential MNPs, CMNPD6428, CMNPD30814, and SPECSAE-765/20006021, with the desired binding and pharmacokinetic properties. The calculated docking (XPg) scores for the selected MNPs ranged between -7.46 to -8.01 Kcal/mol. The pharmacokinetic properties were also encouraging for the identified MNPs, with the molecules appearing safe for human consumption. Subsequently, additional analyses, including DFT, Complex MD simulations, and PCA-FEL, were performed to thoroughly evaluate the chemical reactivity, binding affinity and structural stability of the

molecules. In conclusion, this study has identified three MNPs with potential Anti-AgrA activity, potentially paving way for developing novel anti-quorum sensing therapeutics.

Keywords: *Enterococcus faecium*, Subtractive Proteomics, Quorum Sensing, Virtual Screening, Antimicrobial Resistance

Acknowledgement:

PKS and JJ thank the Department of Biotechnology – Bioinformatics Centre (DBT-BIC) – No. BT/PR40154/BTIS/137/34/2021 and the Department of Biotechnology – National Network Project (DBT-NNP) – No. BT/PR40156/BTIS/137/54/2023.