Molecular, Bioinformatics and Biophysics Studies of Pathogenic *Enterococcus faecalis* and *Enterococcus faecium*: A Multi-Pronged Therapeutic Approach

ABSTRACT

Enterococcus has become a major global health threat due to its prevalence as a primary cause of hospital-acquired infections, especially in immunocompromised and critically ill patients. This opportunistic pathogen is well-known for its high levels of antibiotic resistance, particularly to essential drugs like vancomycin and ampicillin. In this research work, a computer-aided drug design (CADD) approach and advanced biophysical strategies, including molecular docking, molecular dynamics simulation, density functional theory (DFT), solvent accessible surface area (SASA), principal component analysis (PCA), and binding free energy calculation approaches, were adopted for screening the ASINEX antibacterial library for potential inhibitors against vancomycin-resistant D-Alanyl-D-Lactate ligase. In the virtual screening analysis, the top three compounds were selected based on the lowest binding energy scores. The PyRx 0.8 docking software calculated binding energy scores of -10.4, -10.2, -10, and 8.5 kcal/mol for Top-1, Top-2, Top-3, and control docked complexes, respectively. The binding energy scores of the docked molecules are expressed in kcal/mol, indicating the affinity of each compound for the active site, with lower scores reflecting stronger interactions. Furthermore, in the molecular docking confirmation analysis, different bonding interactions were observed, including van der Waals forces, conventional hydrogen bonds, and pi-alkyl interactions that suggest important contributions to the stability of docked complexes. Additionally, the stability of the docked complexes was analyzed in a dynamic environment through molecular dynamics simulation over a 200-nanosecond (ns) time frame. The simulation trajectories reflect that the ligands and receptors have stable binding affinities in a dynamic environment. Following the MD simulation, ADMET properties of the selected compounds were evaluated; according to Lipinski's rule of five, the selected compounds fall into the drug-likeness group. Moreover, in the PCA analysis, notable differences between the protein-ligand complexes and the control were observed. All selected compounds stabilized the protein structure to varying degrees by reducing its intrinsic conformational flexibility compared to the control system. In salt bridge examination, Top-1 and Top-3 may provide improved stability for ligand binding or maintain structural integrity under physiological conditions. The moderate behavior of Top-2 may replicate a balance between elasticity and stability, which could facilitate dynamic interactions, while the reduced salt bridge density in the control system is consistent with its simpler, smaller structure. Salt bridge analysis provides valuable insights into the structural stability and interaction profiles of the docked complexes. In density functional theory (DFT) analysis, calculations demonstrated significant orbital overlap between selected compounds and the active site residues of the target proteins. Overall, we believe these findings to be exceedingly useful for chemical scientists in formulating novel effective drug molecules against D-Alanyl-D-Lactate ligase of *E. faecium*. *Enterococcus faecalis* is a Gram-positive bacterium and is recognized as an etiological agent of different nosocomial infections. *E. faecalis* has developed resistance to several antibiotics. No licensed vaccine is available to prevent *E. faecalis* infections. A multi-epitope-based vaccine construct may provide an effective vaccine design foundation. In this study, an integrated bioinformatic approach was applied to design a multi-epitope-based vaccine construct against *E. faecalis*. In subtractive proteomics analysis, 10 proteins were prioritized as potential vaccine candidates

based on several literature-reported vaccine candidacy parameters. In immunoinformatics analysis, only two proteins (glucosaminidase domain-containing protein and serine protease) were found as promising vaccine targets. Both proteins were then subjected to epitope mapping for screening of broad-spectrum antigenic epitopes. The predicted epitopes were further refined based on immunoinformatics filters and only six epitopes, DTSDHQKNNV, GMKKRKARY, SVFDESMALR, NLNQRIEKR, NVDKKIEEK, and TTTPSTDNSA were found as non-allergic, antigenic, water-soluble, non-toxic, and DRB*0101 good binders. The selected epitopes were fused via GPGPG linkers and additionally linked to an adjuvant molecule through EAAAK linkers to increase the immunogenicity and antigenicity of the vaccine construct. The net interaction energy of the vaccine and receptors was evaluated through molecular docking analysis, which predicted -833.0 kcal/mol and -1001.6 kcal/mol of binding energy for MHC-I and MHC-II, respectively. The values predict effective vaccine construct binding with host immune cell receptors and triggering of innate and adaptive immune responses. The dynamic behavior of the docked complexes was examined using the molecular dynamics (MD) simulation technique on a time scale of 500 ns. The MD revealed minimal intermolecular conformational deviations and exposed presentation of the vaccine epitopes for immune cell recognition and processing. For the MHC-I-Vaccine complex, the mean RMSD was found as 2.78 Å, while the MHC-II-Vaccine complex showed a mean RMSD value of 13.17 Å. The C-immune simulation predicted the formation of high titer humoral and cellular immunological responses against the vaccine antigen. The predicted IgG and IgM titer found against the antigen was 600000 to 650000 counts per milliliter. The interferon-gamma (IFN-γ) was predicted to be stimulated at 430000 nanograms per milliliter. Simulation trajectories based on MMGB/PBSA binding energy were estimated as <-250 kcal/mol for vaccine-MHC complexes, illustrating formation of robust interactions between the vaccine and MHC receptors. The study outcomes predicted the viability of the proposed epitope-based vaccine construct as a promising therapeutic approach for *E. faecalis* infection prevention; however, experimental confirmation is required. *Enterococcus faecium* has developed resistance to multiple antibiotics, worsening the global health crisis. In this research study, immunoinformatics and biophysics approaches were applied to predict novel epitopes in the core proteins of *E. faecium*. Bacterial pan-genome analysis predicted 4886 proteins as part of the core proteome. In the core proteins, seven proteins were found in the extracellular region. In seven extracellular portions, three proteins were found to be virulent. In virulent proteins, one protein was predicted as an allergen, and two proteins were non-allergens. Based on subtractive proteomics, two extracellular and probable antigenic proteins (glucosaminidase domain-containing protein and N-acetylmuramoyl-L-alanine amidase) were selected for epitope prediction analysis. In epitope prediction, six B-cell novel epitopes of different lengths were predicted from each selected protein. Next, immunoinformatics filters were utilized to assess immunoinformatics properties of the vaccine construct, and eight novel epitopes, SARHHRPKR, RHHRPKRKM, HADEQGSQTV, PQHVHADEQ, RFDTPSTGSA, STSSSSTTDV, SSSSTTDVN, and KVSLETKEF, have been selected for epitope-based vaccine construction. The selected epitopes were connected through GPGPG linkers and coupled with the adjuvant through the EAAAK linker to design a multi-epitope vaccine construct. The 3D structure of the vaccine was modeled and used in interaction analysis. The interaction analysis revealed the best dock conformation with targeted immune receptors. Furthermore, through biophysics studies, the intermolecular binding mode of vaccine receptors was reported to be dynamically stable in 500 nanoseconds. Next, the C-ImmSim server predicted that the model vaccine could induce strong immunological responses against *E. faecium*. The study outcomes predicted the effectiveness of a multi-epitope-based vaccine as a promising therapeutic approach for *E. faecium*.