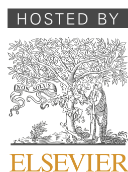

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Original article

Bioinformatics and immunoinformatics assisted multiepitope vaccine construct against *Burkholderia anthina*

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A R T I C L E I N F O

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A B S T R A C T

Burkholderia anthina is a pathogenic bacterial species belonging to the *Burkholderiaceae* family and it is mainly considered the etiological agent of chronic obstructive pulmonary diseases associated with cystic fibrosis, due to being intrinsic antibiotic resistant making it difficult to treat pulmonary infections. Hence increased rate of antibiotic-resistant bacterial species vaccine development is the priority to tackle this problem. In research work, we designed a multi-epitope-based vaccine construct against *B. anthina* using reverse vaccinology immunoinformatics and biophysical approaches. Based on the subtractive proteomic screening of core proteins we identified 3 probable antigenic proteins and good vaccine targets namely, type VI secretion system tube protein hcp *Burkholderia*, fimbria/pilus periplasmic chaperone and fimbrial biogenesis outer membrane usher protein. The selected 3 proteins were used for B and B cells B-derived T-cell epitopes prediction. In epitopes prediction, different epitopes were predicted with various lengths and percentile scores and subjected to further immunoinformatics analysis. In immunoinformatics screening a total number of 06, IDDGNANAL, KTVKPDPRY, SEVESGSAP, YGGDLTVEV, SVSHDTNGR, and GSKADGYQR epitopes were considered good vaccine target candidates and shortlisted for vaccine construct designing. The vaccine construct was designed by joining selected epitopes with the help of a GPGPG linker and additionally linked with cholera toxin b subunit adjuvant to increase the efficacy of the vaccine construct the sequence of the said adjuvant were retrieved from protein data bank through its (PDB ID: 5ELD). The designed vaccine construct was evaluated for its physiochemical properties analysis in which we reported that the vaccine construct comprises 216 amino acids with a molecular weight of 22.37499 kilo Dalton, 15.55 instability index (II) is computed, and this classifies that the vaccine construct is properly stable. VaxiJen v2.0 web server predicted that the vaccine construct is probable antigenic in nature with 0.6320 predicted value. Furthermore AllerTOP v. 2.0 tool predicted that the designed vaccine construct is non allergic in nature. Molecular docking analysis was done for analysis of the binding affinity of the vaccine construct with TLR-2 (PDB ID: 6NIG), the docking results predicted 799.2 kcal/mol binding energy score that represents the vaccine construct has a good binding ability with TLR-2. Moreover, molecular dynamic simulation analysis results revealed that the vaccine construct and immune cell receptor has proper binding stability over various environmental condition, i.e. change in pressure range, temperature, and motion. After each analysis, we observed that the vaccine construct is safe stable, and probably antigenic and could generate an immune

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response against the target pathogen but in the future, experimental analysis is still needed to verify in silico base results.

1. Introduction

Antibiotic Resistance is a wider public health problem that changed medical care all over the world (Yelin and Kishony, 2018). Antibiotics play an utmost important role against infectious diseases, over the last few decades antimicrobials have been overused, and despite that, the misuse of antimicrobials increased the exposure and spread of multidrug resistance pathogens (Llor and Bjerrum, 2014).

Burkholderia anthina is an aerobic gram negative bacillus related to the genus *Burkholderia*. The *Burkholderia* genus comprises approximately thirty species that are genetically different from one another, although they show no phenotypic difference (Vandamme and Dawyndt, 2011). Most of these species cause diseases in animals, plants, and humans. The geographical distribution of *B. anthina* is diverse, ranging from aquatic to soil, plants, insects, industrial, and hospital environments (Uehlinger et al., 2009). The in-vitro study suggests that *B. anthina* expresses two differential characteristics on BCSA agar from other related species. The *B. anthina* turned the BCSC agar medium alkaline which makes pink coloration that results in growth inhibition of sucrose-utilizing organisms (Vicente et al., 2014). In addition, the color and morphological appearance of *the B. anthina* colony is usually creamy, whereas other species are typically gray in color and moist or dry. The genomic study of *B. anthina* reveals that the recA gene (DNA repair Gene) sequence of *B. anthina* is different from other *Burkholderia* species. *B. anthina* recA gene has conserved nucleotide 'G' at position 210 which was absent in all other species of *Burkholderia* (Chen and Michel, 1998). Bacteria belonging to the *Burkholderia* genus are prominent opportunistic human pathogens in persons with cystic fibrosis (CF) or chronic granulomatous disease. *B. anthina* isolates were determined during the past year in cystic fibrosis patients (Tavares et al., 2020). The *B. anthina* sp. nov (R-16022) was recently described by LiPuma et al in 2002 through various approaches like genotypic and phenotypic characteristics, the sample was collected from patient who has been chronically colonized for two years. *Burkholderia* infections are difficult to treat because of significant antibiotic resistance (Vandamme et al., 2002). These bacteria avoid antimicrobials due to their outer membrane penetration barrier, which serves as the bacteria's first line of defense (Cattoir, 2016). Efflux pumps of the resistance nodulation cell division family are major players in *Burkholderia* multidrug resistance (Gautam et al., 2020). Third and fourth-generation beta-lactam antibiotics are essential for treating *Burkholderia* infections, but their efficacy is jeopardized by the expression of several -lactamases and ceftazidime target mutations (Reygaert, 2018). Altered DNA gyrase and dihydrofolate reductase targets cause fluoroquinolone and trimethoprim resistance, respectively. Although antibiotic resistance hampers the therapy of *Burkholderia* infections (Rhodes and Schweizer, 2016).

There is a broad range of clinical outcomes of the disease caused by *B. cepacia* complex bacteria, but most of the mortality is higher than 90 percent of the mortality in cystic fibrosis associated with chronic obstructive pulmonary disease (COPD) (Fauroux et al., 2004). While B. cepacia complex organisms affect a small proportion of cystic fibrosis (CF) patients they have a substantial impact on mortality and morbidity rate. Additionally, infections caused by different species of *Burkholderiaceae* can lead to cepacia syndrome, especially in *B. cepacia* causes rapidly fatal pneumonia with associated bacteremia (Ramsay et al., 2013). As *Pseudomonas aeruginosa* is the dominant clinical isolate from chronically infected lungs infections with *Burkholderia cepacia* complex (Bcc) species could lead to rapid decline in lung function, hence it can increase the mortality rate. Chronic infections with Bcc are highly difficult to tackle because it can cause invasive disease, furthermore, Bcc has a high level of intrinsic antibiotic resistance (Horsley and Jones, 2012).

The development of *Burkholderia* vaccines is an alternative approach, but only a few studies have been reported to date. The study is specifically aimed at designing multi epitopes vaccine construct against *B. anthina* and providing a theoretical base vaccine model to experimental biologists which could help in the formulation of the vaccine against a pathogen (Irfan et al., 2022).

Traditional vaccinology fails to design a vaccine for a pathogen that is unable to be grown on culture media (Pollard and Bijker, 2021). Pasture-based vaccine development also reveals antigenic variability. So conventional base vaccine development is also expensive, time-consuming, and needs many human resources (Oli et al., 2020). Genomics uprising highly assist in disclosing new vaccine construct that by traditional base vaccine developments were hard to detect (Khan et al., 2022a). Next-generation sequencing and advanced bioinformatics approaches are now commonly employed in the field of vaccine designing for the determination of putative surface-associated antigens (Luciani et al., 2012). Reverse vaccinology (RV) is an ultra-modern approach to the recognition of putative surface-associated proteins instead of culturing microbes (Rappuoli et al., 2016). One example of the vaccine developed via the reverse vaccinology approach was meningococcal serogroup B (4CMenB). Pan-genomic reverse vaccinology (PGRV) in particular is more effective as compared to conventional RV as it screens highly conserved targets than strain-specific (Delany et al., 2013).

2. Research methodology

The reverse vaccinology, bioinformatics, and immunoinformatics rational method for the designing of multi-epitopes based vaccine against *B. anthina* is presented in Fig. 1.

2.1. Proteome retrieval and subtractive proteomics analysis

In the first phase of the study complete proteome of the *B. anthina* was extracted from the online National Center for Biotechnology Information (NCBI) database (Blast, 2015). The complete proteome was considered for Bacterial pan-genome analysis to retrieve core proteins from the complete proteome (Chaudhari et al., 2016), BPGA is a fast software that provides a detailed genomic analysis of microorganisms like pan-genome profile, phylogeny and several different downstream analyses like exclusive genes analysis, etc. Next non-redundant proteins were predicted and non-redundant proteins were discarded using an online Cluster Database at High Identity with Tolerance (CD-HIT) web server (Huang et al., 2010), The CD-HIT, program mainly processed FASTA file as in input and produced a non redundant protein (nr) file as output fie, this analysis was done to reduce redundant proteins is highly similar proteins.

Next subcellular localization was done for subcellular localized proteins using the PSORTb online webserver https://www.psort.org/psortb/ (Yu et al., 2010), in subcellular localization analysis outer membrane, extracellular, and periplasmic membrane proteins were predicted. PSORTdb is a biological database of proteins base subcellular localization for bacterial species, this database consists of two datasets, ePSORTdb and cPSORTdb, which collect information determined from experimental analysis, PSORTdb is required input file in FASTA format either by cutting and copying or can upload directly FASTA file to the database. Next virulence analysis of the subcellular localized proteins was assessed using the online Virulence Factor Database (VFDB) database, VFDB database is a biological database that provides direct access to virulence factor in bacterial pathogenic proteins, the recent NGS-based metagenomic analysis provide information about unculturable

pathogenic bacterial species (Chen et al., 2005). In VFDB analysis only virulence proteins were considered for further analysis and non-virulent proteins were discarded. Antigenicity analysis was performed using the Vaxijen v2.0 online web server https://www.ddg-pharmfac.net/vaxijen3/ (Ong et al., 2021). Next allergenicity analysis was done through the Allertop v.2.0 https://www.ddg-pharmfac.net/AllerTOP/ online web server, Allertop v.2.0 is a free alignment base online tool for in silico prediction of allergen protein sequences based on the main physiochemical characteristics of the proteins, for allergenicity analysis Allertop v.2.0 tool requires proteins sequence in plain text format, and the data set and the result is obtained as a probable allergen or non-allergen (Gul et al., 2022). Transmembrane helices analysis was done to remove proteins having > 1 transmembrane helices for the said analysis TMHMM 2.0 online web server https://services.healthtech.dtu.dk/services/TMHMM-2.0,TMHMM is membrane is mainly membrane proteins topology prediction approach on the base of hidden Markov model, this server is required FASTA file of the proteins for analysis of physiochemical properties analysis (Rida et al., 2022). BLASTp analysis was performed using human and normal microbiota analysis to remove human and normal microbiota similar protein sequences, this may help in reducing autoimmunity for this analysis BLASTp server https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteinswas used BLASTp server associate the query protein sequences to a protein database and find the homologs and non-homologs proteins sequences (Ud-Din et al., 2022).

2.2. Epitopes mapping analysis and shortlisting phase

The shortlisted proteins were used for epitopes mapping, in the epitopes mapping phase both B and T-cells (MHC-I and MHC-II) epitopes were predicted using immune epitopes database server 2.0 (IEDB) https://www.iedb.org/,The epitopes was prioritized on the base of lowest percentile score and highly conserved sequences (Zhang et al., 2008). The predicted epitopes were then considered for further antigenicity, allergenicity water solubility, and toxicity using Vaxijen v2.0 (Malik et al., 2023), Allertop v.2.0 (Doytchinova and Flower, 2007), Peptide solubility calculator https://pepcalc.com/peptide-solubility-calculator.php, and ToxinPred https://crdd.osdd.net/raghava/toxinpred/ and MHCpred version 2.0 https://www.ddg-pharmfac.net/mhcpred/MHCPred/ tools were utilized respectively, after the said analysis the shortlisted probable antigenic, water-soluble, non-toxic and non-allergic and good DRB*0101 binder peptide sequence was considered for epitopes based vaccine construct, MHCpred tool is mainly predict the binding affinity of the epitopes with different histocompatibility complex molecules (Alshammari et al., 2022).

2.3. Multi-epitopes vaccine construction and processing phase

A multi-epitope-based chimeric vaccine was designed from shortlisted epitopes. The selected epitopes were linked through GPGPG linkers (Gul et al., 2022). The GPGPG linkers were used to avoid overlapping and provide stability to the vaccine construct (Alharbi et al., 2022). Furthermore, the designed vaccine construct was linked with cholera toxin -B subunit adjuvant to enhance the efficacy of the vaccine construct (Ismail et al., 2020).EAAAK linker was utilized for joining adjuvant with vaccine construct (Ullah et al., 2021). Physiochemical properties analysis of the designed construct was analyzed through the ProtParam tool https://web.expasy.org/protparam/(ProtParam, 2017), in which the Number of amino acids, Molecular weight, Theoretical pI, Instability index, Aliphatic index, and Grand average of hydropathicity (GRAVY) were evaluated, after physiochemical properties analysis we observed that the designed vaccine construct is stable, the vaccine construct was then subjected for 3D structure prediction, the 3D structure was generated through Scratch Protein Predictor online Webserver https://scratch.proteomics.ics.uci.edu/ (Cheng et al., 2005). Next disulfide engineering was done through Design 2.0 web server https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-14-346 to create the mutant structure of the designed vaccine construct was formed, Disulfide engineering is a vital biotechnological tool that is used in advanced research activity, and the introduction of novel di sulfide bonds can make the protein more stable, the 3D structure of the mutant vaccine construct was further refined using online Refine 2 in Galaxy-WEB services, this server is predicted the proteins structure on the base of sequence template modeling, it is refine the loop and terminus region by initio approach (Heo et al., 2013).

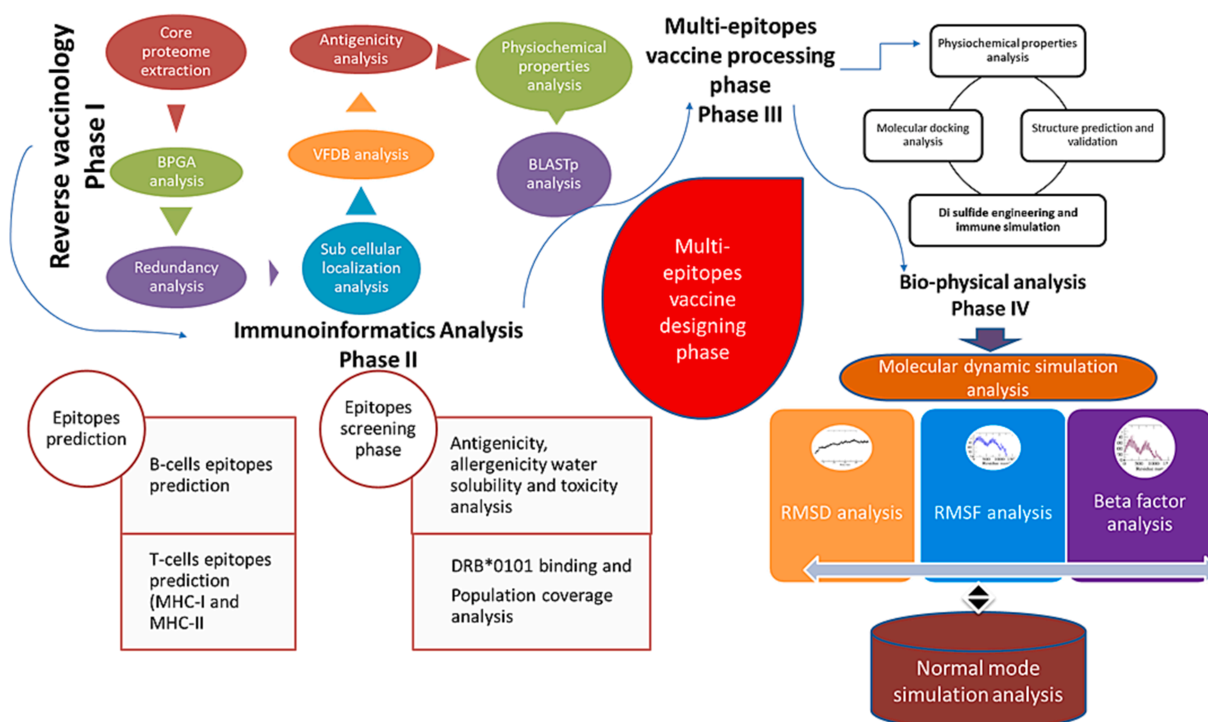

Fig. 1. Schematic demonstration of a rational method for designing of multi-epitopes vaccine construct.

2.4. Molecular docking analysis

Molecular docking analysis is an advanced computational approach that predicts the confirmation of ligands (Vaccine) and receptor TLR-2 (PDB ID: 6NIG) (Yousaf et al., 2022). For molecular docking analysis, we used an online cluspro 2.0 webserver for docking analysis (Kozakov et al., 2017). This tool is automatically computing the docking of two proteins structure after providing the PDB files of vaccine and receptors, the output results is a ranked through lowest binding energy putative complexes, orderly through clustering properties. In molecular docking analysis the cluspro 2.0 webserver generated 10 docked solutions on the base of binding confirmation and binding energies score the lowest binding energy was considered best among all docked solutions. (Kozakov et al., 2017). The docking results were further was further validated through molecular dynamics simulation and binding free energies calculation (Alshammari et al., 2022).

2.5. Molecular dynamic simulation, normal mode simulation analysis

Molecular dynamic simulation analysis is a computer based simulation approach mainly used for the analysis of the dynamic behavior of the docked molecules in a specific environment and period (Mushtaq et al., 2022). Assisted model building with energy refinement (AMBER 20) was used for molecular dynamics simulation analysis, in the said analysis root mean square deviation root mean square, and beta factor trajectories were analyzed (Case et al., 2016).The RMSD was performed to check out the deviation of docked complexes and whether it is stable or not, moreover residues base fluctuation was analyzed through RMSF analysis (Khan et al., 2022b). The normal mode simulation analysis was done through C-ImmSim online webserver https://kraken.iac.rm.cnr.it/C-IMMSIM/ by providing PDB of the Top-docked complexes.

3. Results

3.1. Proteome retrieval and reverse vaccinology analysis

In the first step complete proteome of *B. anthina* was retrieved from the NCBI database, next in the BPGA analysis 9500 core proteins was predicted which was considered redundancy analysis, in the redundancy analysis 4454 proteins were predicted as redundant protein and 5046 were found as non-redundant proteins, the non-redundant proteins were considered for surface localization analysis, in which 01 (core/5680/1/Org1_Gene47), proteins were predicted extracellular, 02 were predicted in (core/5771/1/Org1_Gene2337), (core/4278/2/Org2_Gene2393) extracellular and 02 (core/144/1/Org1_Gene4960), (core/4808/1/Org1_Gene370) were predicted in outer membrane region, all of the surface localized proteins were analyzed for antigenicity virulence factor analysis. After virulence factor analysis in the virulent proteins, transmembrane helices were checked and no zero proteins were predicted to have more than one transmembrane helices. In physiochemical properties analysis, all the proteins were predicted stable. Furthermore, in antigenicity, allergenicity, water solubility, and toxicity analysis all the shortlisted proteins were predicted as probable antigenic, good water soluble, and non-toxic receptively. In adhesive properties analysis, the shortlisted proteins were predicted adhesive.

3.2. Epitopes mapping and prioritization

In epitopes, mapping phase B and B-cells derived T-cells epitopes were predicted, in the T-cells epitopes prediction both MHC-I and MHC-II epitopes were predicted, and the predicted epitopes were prioritized on the base of lowest percentile score. The predicted epitopes are presented in Table 1.

3.3. Epitopes prioritization phase

After the epitopes prediction, the predicted epitopes were further screened for toxicity, allergenicity water solubility, and antigenicity, finally probable antigenic, non-allergic, non-toxic, and good water soluble epitopes were considered for a multi-epitopes vaccine to construct. The selected epitopes and their characteristic are tabulated in the following Table 2.

Furthermore, the vaccine construct was designed from selected epitopes, the epitopes were joined through GPGPG linkers and designed linear vaccine construct, Furthermore to evoke the efficacy of the vaccine construct, the construct was additionally linked with cholera toxin b subunit adjuvant with the help of EAAAK linker. The physicochemical properties of the vaccine construct were analyzed, the construct consisted of 216 amino acids having a molecular weight of 22.37499-kilo Dalton, and the instability index (II) is computed to be 15.55 and this classifies the vaccine construct as stable.3D structure of the designed vaccine construct is presented in the following Fig. 2.

3.4. Refinement of the 3D structure

The 3D structure was further refined using the galaxy bioinformatics tool, the server-generated Top-10 model based on root mean square deviation (RMSD) Mol-probity and galaxy energy score, based on low RMSD, Model 1 is selected best for docking purposes, over all the data of top-10 model is tabulated in the following Table3.

3.5. Structure validation

The secondary structure was predicted using pdbsum generate web server https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html, as presented in Fig. 3A. The amino acid sequence of the chimeric vaccine construct is presented which consists of 216 amino acid long sequence, In Fig. 3B secondary structure labeled helicase beat and gamma turns are mentioned, in Fig. 3C Ramachandran plot is presented in the Ramachandran plot statistics 90.1 % favored regions were predicted, 8.9 % additional allowed region 0.5 % generously allowed region were predicted, these were the Non-glycine and non-proline residue, Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20.0 a good quality model would be expected to have over 90 % in the most favored regions as it is described the predicted structure is properly stable.

Table 1
Shortlisted proteins and predicted both T-cells (MHC-I and MHC-II) with the lowest percentile score.

Shortlisted proteins		T-cells Epitopes			
Proteins	B –cells Peptides	MHC-II	P. score	MHC-II	P. score
core/5680/1/Org1_Gene47 (type VI secretion system tube protein hcp *burkholderia*)	VKGESADKDHQ	GESADKDHQ	5.6	VKGESADKDHQ	57
core/4278/2/Org2_Gene2393	IDDGNANALPDESK	IDDGNANAL	0.89	IDDGNANALPD	3.1
fimbria/pilus periplasmic chaperone	TKTVKPDPRYGGPRL	KTVKPDPRY	0.13	KTVKPDPRYGG	11
fimbrial biogenesis outer membrane usher protein	ATGYGGDLTVEVTESDGSV	YGGDLTVEV		TGYGGDLTVEV	
	SSSVSHDTNGRTQ	SVSHDTNGR	0.68	SSVSHDTNGRT	1.4
	RDAGSKADGYQRI	GSKADGYQR	0.19	AGSKADGYQRI	5.7

Table 2
Selected good DRB*0101 binders, antigenic, non-allergic good water soluble, and non-toxic epitopes.

Selected epitopes	DRB*0101 binding score	Antigenicity score	Allergenicity test	Water solubility	Toxicity test
IDDGNANAL	2.99	0.9604	Probable antigen	Good water soluble	No– toxic
KTVKPDPRY	73.96	1.2221			
SEVESGSAP	56.23	1.3194			
YGGDLTVEV	19.36	1.1349			
SVSHDTNGR	19.1	2.352			
GSKADGYQR	55.46	1.6002			

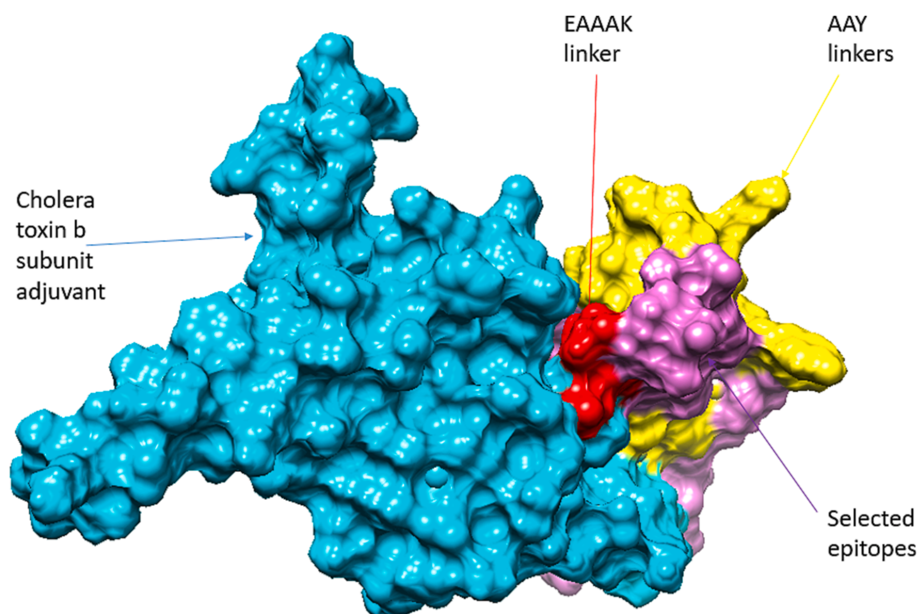

Fig. 2. 3D structure of the designed vaccine construct, Cholera toxin b subunit adjuvant, AYY, and EAAAK linker, and selected epitopes are presented.

Table 3
The top-10 model generated by Galaxy web refinement services.

Model	RMSD	Mol-Probity	Clash score	Poor rotamers	Rama favored	GALAXY energy
Initial	0.000	3.037	103.8	0.7	89.7	8434.54
MODEL 1	1.029	1.081	1.2	0.7	96.3	−3998.56
MODEL 2	1.043	1.155	1.2	0.0	95.3	−3998.04
MODEL 3	0.941	1.303	2.3	0.0	95.8	−3991.48
MODEL 4	1.101	0.946	0.6	0.0	96.3	−3989.21
MODEL 5	0.997	1.121	1.2	0.0	95.8	−3988.21
MODEL 6	1.022	1.136	1.8	0.0	96.7	−3978.93
MODEL 7	1.217	1.020	0.6	0.0	95.3	−3976.01
MODEL 8	0.906	1.337	2.3	0.0	95.3	−3975.03
MODEL 9	0.862	1.238	1.5	0.7	94.9	−3971.67
MODEL 10	0.949	1.136	1.8	0.0	96.7	−3971.43

3.6. Molecular docking analysis

Molecular docking analysis was performed to find out the interaction between vaccine and receptor molecules, the Cluspro 2.0 tool was utilized https://cluspro.bu.edu/, the server generated Top-10 results were on base on a low binding energy score. The docking results are tabulated in the following Table 4, while 3D structure of Top-1 docked complex of vaccine and TLR-2 is presented in Fig. 4A. Additionally the 10 generated docked complexes intermolecular dock confirmation are presented in supplementary Fig. S1. As the hydrogen bonds are important factors and play a vital role in the stability of binding interaction (Aziz et al., 2022). In molecular docking interaction analysis we predicted several hydrogen bonds, which are involved in biding interaction, these bonding are predicted among different amino acids resides of TLR-2 with multi epitopes vaccine construct as presented in Fig. 4B.

3.7. Di-sulfide engineering and population coverage analysis

Structure stability is very important for long-term immunity, to retain structure stability di- sulfide bonds were incorporated, overall the pair of amino acid residues that are chosen for di-sulfide engineering are tabulated in the following Table 5, and in Fig. 5, the original and mutated structure of the chimeric vaccine is presented. The selected epitopes were analyzed for population coverage analysis in population coverage analysis the epitopes cover different percentage wise population coverage, the selected epitopes were able to cover 99.74 % world population followed by 97.83 % Chinese population and 97.35 % Indian population, and other countries as mention in Fig. 6.

3.8. In silico immune simulation of vaccine construct

Immune modulation is an important factor in vaccine designing, C-immune simulation analysis was done to analyze immune reaction against the designed vaccine. In immune simulation analysis, we observe that the vaccine can generate a proper immune response in the form of innate and acquired immunity. In innate immunity, IgM

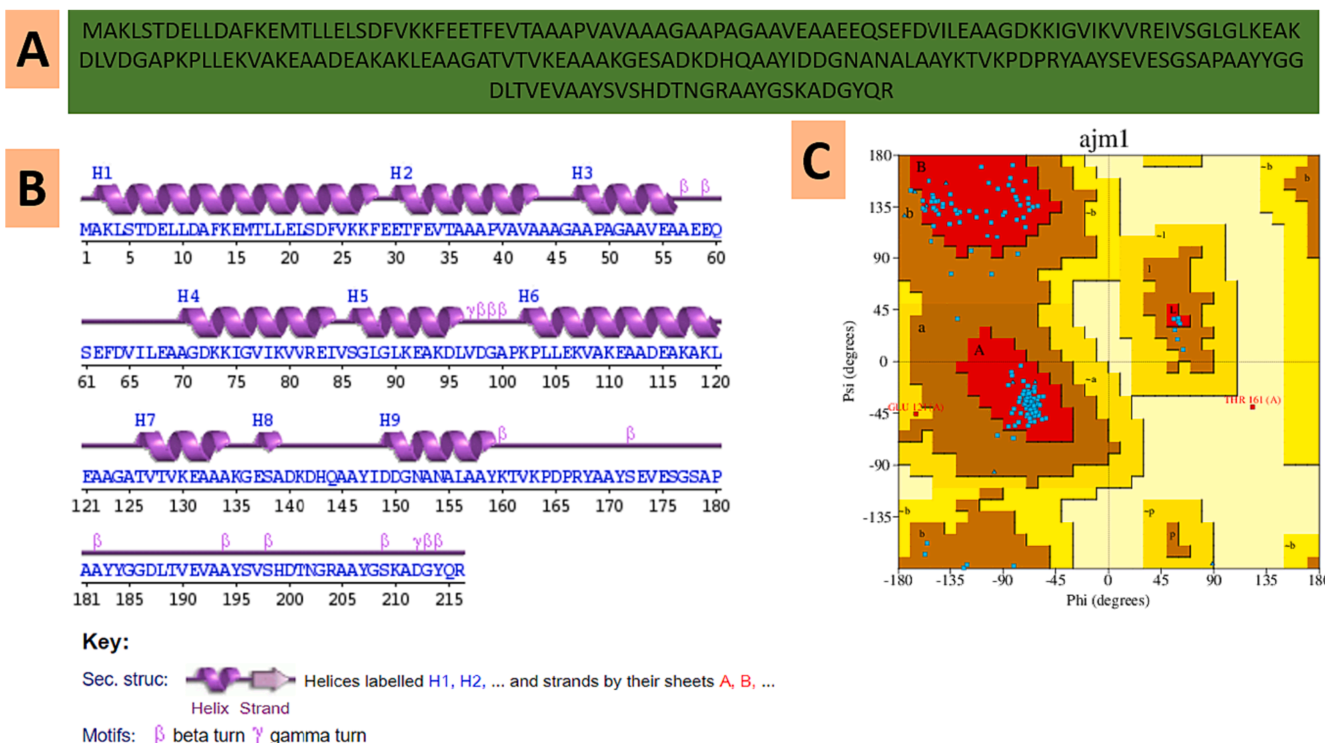

Fig. 3. (A) Represent sequence of the chimeric vaccine construct (B) Secondary structure of chimeric vaccine (C) Ramachandran plot analysis.

Table 4
Top-10 docked complex generated by cluspro 2.0 webserver on the base of lowest energy score.

Cluster	Members	Representative	Weighted Score
0	49	Center	−726.7
		Lowest Energy	−799.2
1	40	Center	−717.2
		Lowest Energy	−792.1
2	32	Center	−744.0
		Lowest Energy	−803.2
3	30	Center	−862.4
		Lowest Energy	−862.4
4	29	Center	−831.0
		Lowest Energy	−831.0
5	26	Center	−716.2
		Lowest Energy	−804.4
6	26	Center	−790.8
		Lowest Energy	−797.7
7	26	Center	−753.0
		Lowest Energy	−753.0
8	25	Center	−780.6
		Lowest Energy	−794.9
9	25	Center	−927.1
		Lowest Energy	−927.1
10	23	Center	−687.1
		Lowest Energy	−803.1

antibody was observed followed by IgG which is shown by the different color peaks in Fig. 7A, furthermore, different interferon and other cytokines were also predicted along with tumor growth factor as presented in Fig. 7B.

3.9. Molecular dynamic simulation analysis

In molecular dynamic simulation analysis, the dynamic movement of the docked complex was observed. In root mean square deviation (RMSD Å) and radius of gyration (RoG), the docked complex was analyzed as time-dependent, in RMSD analysis the graph showed deviation at the initial phase and highly deviated at 14 (Å) region while at the ending stage of simulation, the graph is showing stability as mentioned in Fig. 8A. The radius of gyration analysis predicted that at the initial stage, the graph is unstable at the 80 (Å) region while at the end time of simulation at 50 ns stability in the graph is observed as presented in Fig. 8B. Furthermore, residual base dynamic movement of the docked complex was also observed in root mean square fluctuation (RMSF Å) and beta factor analysis, in RMSF analysis the graph highly fluctuated at 85 (Å) but the fluctuation is due to the presence of loops in the structure the same changes were observed in beta factor analysis (Å) as the RMSF and beta factor analysis graph is represented in the following Fig. 8B and D respectively.

3.10. Normal mode simulation analysis

In normal mode simulation analysis, we observed the vibrational movement of the docked complex chimeric vaccine and TLR-2. In Fig. 9A vaccine with TLR-2 is mentioned the arrow represents the movement of vaccine molecules towards the receptors as it is vital for inducing immune responses in the host body. In Fig. 9B the main chain of deformability is measured which is the capability of the given atoms or molecules to deform at the level of each residue, and the location of the chain 'hinges' can be derived from high deformability regions. Next, the NMA normal mode analysis of the docked complex is presented in Fig. 9C in which the experimental B-factor is taken from the corresponding PDB field and the calculated from NMA is obtained by multiplying the NMA mobility by (8pi^2). Next, the eigenvalue related to each normal mode represents stiffness, as the eigenvalue graph is presented in Fig. 9D, the value of the eigenvalue is directly proportional to the energy required for the structure deformation the lower value of the eigenvalue, which would be essay for deformation. Furthermore, coupling between pairs of amino acid residues is observed by covariance matrixes either they are experience correlated which is represented by red color, followed by uncorrelated which is represented by white color, and anti-correlated as mentioned in all the colors in Fig. 9E. Additionally, an elastic network analysis graph was generated is mentioned in Fig. 9F,

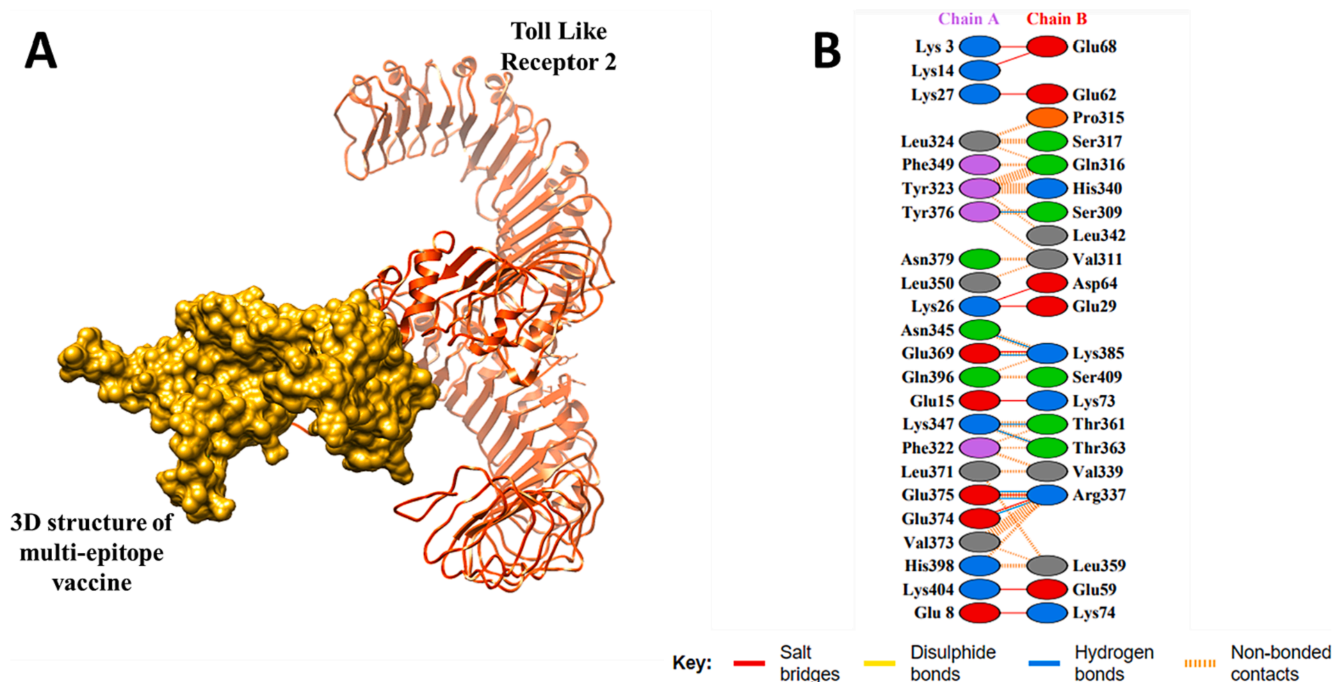

Fig. 4. (A) Intermolecular docked confirmation of vaccine and immune receptors (Vaccine- TLR-2). (B) Chain A and B interactive amino acid residues.

Table 5
Pair-wise amino acid residues selected for di-sulfide engineering.

S. No	Res1Seq	Res1 AA	Res2Seq	Res2 AA	Chi3	Energy kcal/mol
1.	26	Lys	33	Glu	93.84	0.23
2.	29	Glu	32	Phe	124.4	4.07
3.	67	Leu	72	Asp	88.1	2.44
4.	81	Val	89	Gly	114.48	4.27
5.	85	Val	88	Leu	117.51	5.95
6.	114	Asp	125	Ala	−99.24	3.39
7.	117	Lys	123	Ala	122.77	4.38
8.	118	Ala	123	Ala	−69.68	5.38
9.	125	Ala	128	Thr	−97.52	3.07
10.	135	Lys	138	Ser	−103.63	4.54
11.	138	Ser	142	Asp	91.3	4.4
12.	146	Ala	151	Gly	−89.45	0.92
13.	148	Ile	151	Gly	−88.64	2.8
14.	152	Asn	167	Arg	92.31	5.28
15.	171	Tyr	174	Val	105.86	5.68
16.	178	Ser	187	Asp	118.96	5.94
17.	180	Pro	185	Gly	−104.88	4.85
18.	191	Glu	207	Tyr	−89.9	2.76
19.	193	Ala	206	Ala	−73.82	2.4
20.	196	Ser	209	Ser	−93.75	1.4

which defines which residue pairs of the atom are connected by spring as presented in Fig. 9G, in which each dot in the graph represents one spring between the corresponding pair of atoms.

4. Discussion

Recently bacterial infections have broken the traditional predominant situation in global health, beginning to spread from country to country at the same time (Fair and Tor, 2014). Among different bacterial infections, *B. anthina* is a bacterial species of *Burkholderiaceae* and is mainly considered the causative agent of chronic obstructive pulmonary disease (Murphy and Sethi, 1992). Nevertheless, no available preventions and therapeutics are available to tackle infection caused by bacterial species of *Burkholderiaceae*. Hence desire efforts are required for the development of a vaccine against the infection *B. anthina* to reduce different respiratory infections which are caused by said bacterial species (Yitbarek et al., 2020).

Multi epitope-based vaccine construct can generate specific and strong immune responses against pathogens based on different multiple conserved, antigenic epitope sequences, and it can avoid adverse reactions against selected epitopes (Umitaibatin et al., 2023). The application of reverse vaccinology, immunoinformatics, and different biophysical approaches for designing multi-epitope based vaccine construction against viral and bacterial species is becoming progressively popular because it can meaningfully save time and expenses in the development of vaccine construct against specific pathogens (Leow et al., 2021).

The selection of target proteins (i) Type VI secretion system tube protein hcp *Burkholderia* (ii) fimbria/pilus periplasmic chaperone and (iii) fimbrial biogenesis outer membrane usher protein of B. *anthina* will intensely provoke an immune response. The Type 6 secretion system proteins is play a vital role in the pathogenesis of *burkholderiaceae* bacterial species, This proteins is assemble into 3, T6SS sub complexes. This type 6 secretion is also injects bacterial effector proteins into the target cells (Spiewak et al., 2019). The fimbria/pilus periplasmic chaperone protein is periplasmic chaperones, mainly help in folding pilus sub unit and then targeting into outer membrane usher proteins, the chaperone assist to folding of pilus subunit (Busch and Waksman, 2012). The fimbrial biogenesis outer membrane usher protein is mainly involve in the polymerization of fimbriae and their transformation to surface of bacterial cells (Palomino et al., 2011).

Therefore, developing a vaccine construct against these selected proteins can effectively provoke an immune response against the target pathogen. B-cell and T-cell epitopes base vaccine construct frequently provide then T- cells or B-cells alone, to maximize the potency of the vaccine construct every vaccine candidate should be comprised of B-cells and T cells epitopes to provide humoral and cellular immunity against the target pathogen (Saha et al., 2021). Additionally, all the selected epitopes should be probable antigenic, non allergic, non toxic, and non homologous with human and human normal microbiota bacterial species to provide antigenicity and avoid allergic and nontoxic immune responses in the host body (Alshammari et al., 2022; Gul et al., 2022; Rida et al., 2022; Sajjad et al., 2020; Yousaf et al., 2022). Non-

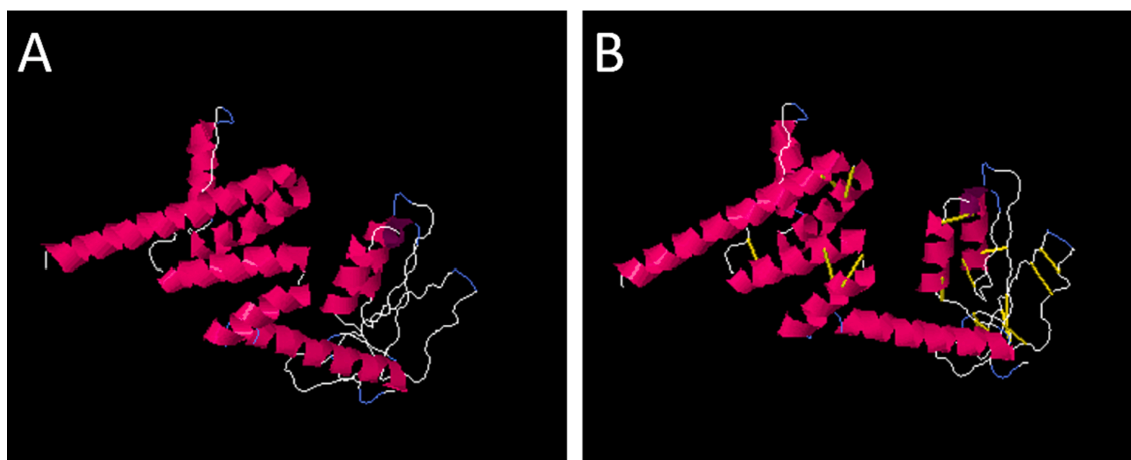

Fig. 5. (A) The original structure of chimeric vaccine (B) Mutated structure of chimeric vaccine, in mutated structure yellow sticks represent Di-sulfide bonds.

Fig. 6. World and different countries population coverage analysis.

homologous vaccine candidates to the human and normal flora of the human body could reduce the auto-immune response in the host against normal flora and human proteins as well and no cross-reaction with human proteins can occur. Cytokines are signaling molecules that can play a vital role in provoking and regulating of immune response and inflammation. IFN-g, IL4, IL12, TGF-b, TNF-a, IL10, IL6, IFN-b, IL18, and IL23 are several important cytokines that are produced by the immune system that mainly contribute to the differentiation of T-cell and B-cell antibody production, hence all the B and T-cells conserved epitopes were analyzed for the potential to provoke the secretion of said cytokines, as we reported in the C-immune simulation analysis results that the vaccine construct can induce cytokines production in host with different concentration (Ismail et al., 2020).

Particularly different biophysical approaches like molecular docking

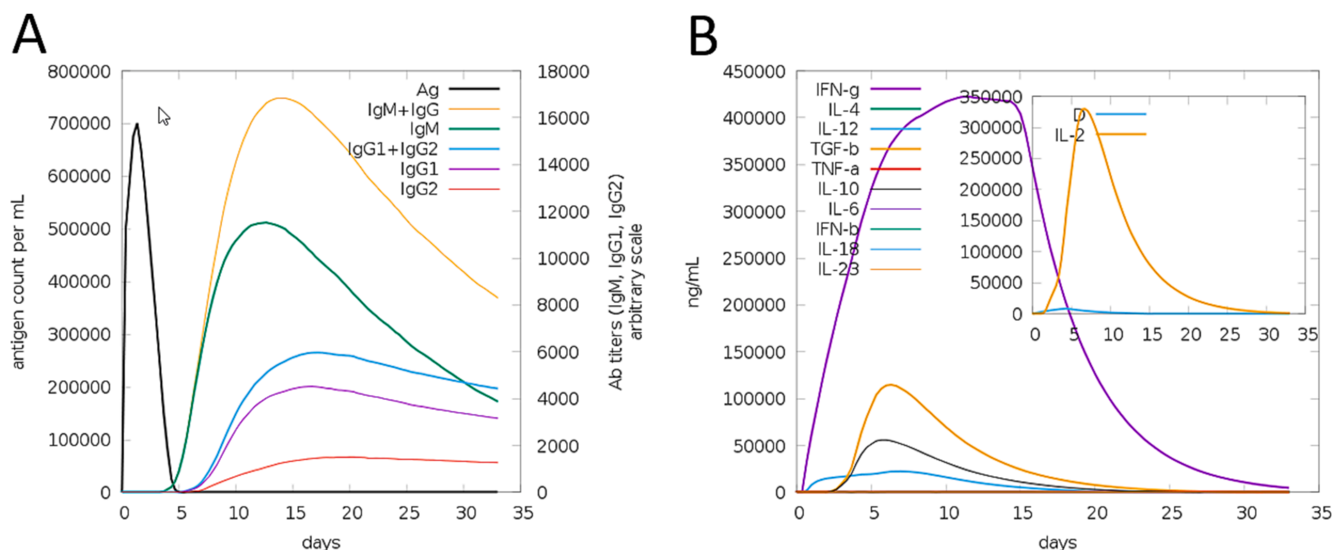

Fig. 7. Different immune responses toward designed vaccine construct (A) represent antibody immune response (B) represents the level of interferon and other cytokines as mentioned through the different color peaks.

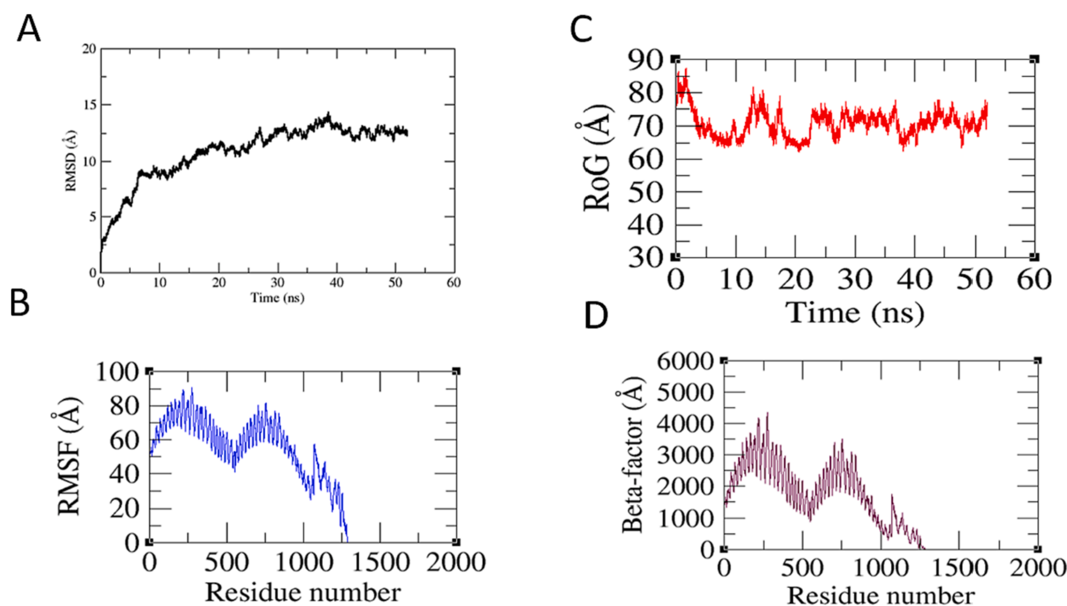

Fig. 8. Simulation graph of vaccine and TLR-2 vaccine. (A) Root mean square deviation RMSD (B) Root mean square fluctuation (RMSF) (C) Radius of gyration (RoG) and (D) Beta-factor.

(Shi et al., 2022) and molecular dynamic simulation (Gul et al., 2022) were performed to observe the binding ability and dynamic behavior of the vaccine with immune cell receptors, as the binding affinity of the vaccine construct is very crucial for inducing immune responses against specific pathogens. In molecular docking analysis, we observed that the vaccine construct has a stable binding affinity with immune cell receptor (TLR-2), moreover, in simulation analysis, we also observed that the vaccine construct has proper stability in a dynamic environment. MD simulation analysis also showed that the vaccine construct with immune cell receptor has proper stability over various environmental conditions, i.e. change pressure range, temperature, and motion, as the initial trajectories of MD simulation analysis RMSD, RMSF, radius of gyration (RoG), and Beta factor represent that the vaccine construct has proper binding stability with the target receptor. As we discuss here the results of molecular dynamic simulation analysis, the RMSD graph represent the structure of the vaccine and TLR-2 receptor is stabilize during end of simulation. (Akhtar et al., 2022; Akhtar et al., 2023; Kaushik et al., 2022; Sharma et al., 2023).

Furthermore to obtain more efficacy of the vaccine candidate cholera toxin b subunit adjuvant was linked with the vaccine construct, as this has been widely used in different vaccine constructs against different bacterial pathogens and it has been shown that it can evoke the host immune stimulation in experimental study as well, all correspond to higher stability of vaccine with immune cells receptor. After each analysis we observed that the vaccine construct is safe stable and probable antigenic and the experimental scientist could use it in the formulation of a vaccine against this target pathogen, as in the future, experimental analysis is still needed to verify that the multi-epitope vaccine with a suitable adjuvant can evoke proper effective humoral and cellular immune responses against this target pathogens.

The current study has several limitations, firstly the immunological responses of the epitopes base vaccine are not that much stronger than

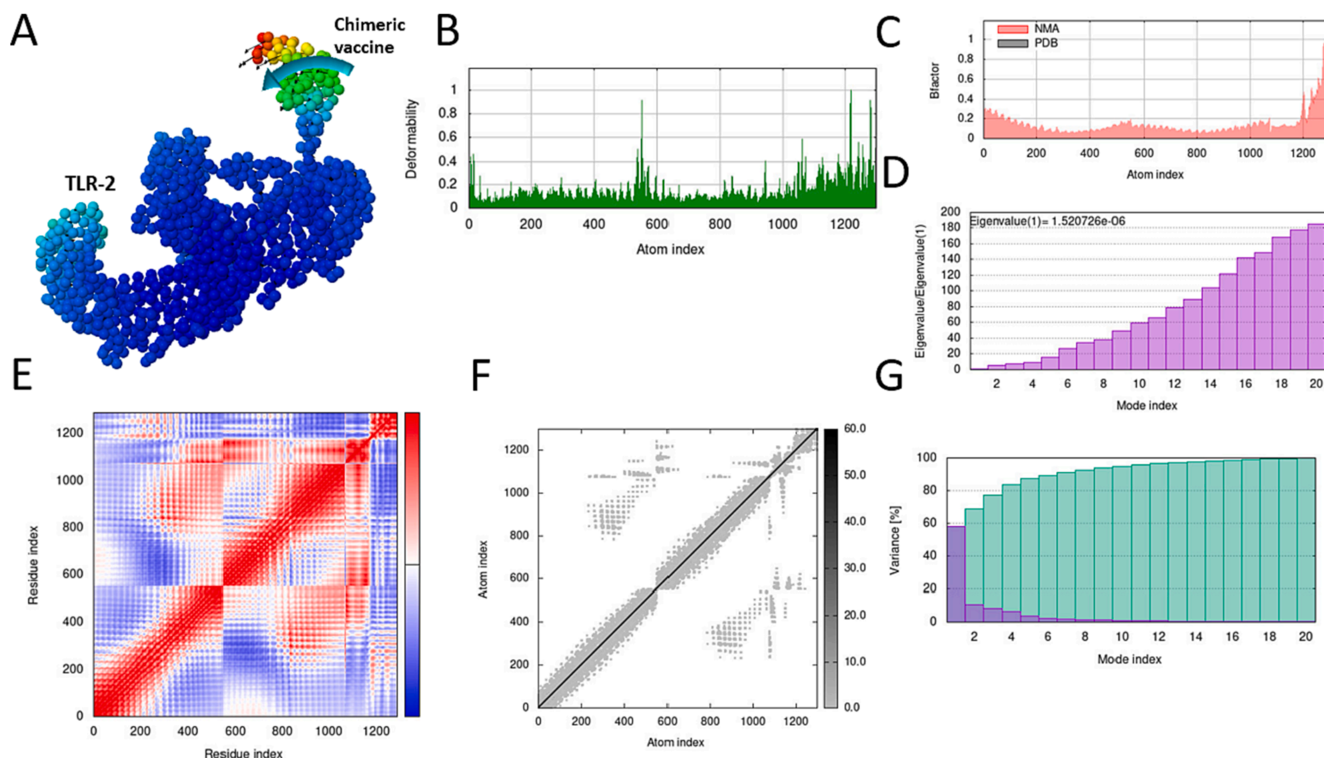

Fig. 9. Output trajectories of normal mode simulation analysis of vaccine with TLR-2.

live or inactivated vaccine, secondly, the immunogenicity of the epitopes-based vaccine construct can be affected by the host expression system, hence the proper host for the expression system in vaccine formulation. Additionally, overall the results are based on in silico approaches so an experimental study is required for further validation.

5. Conclusion

Recently global health has successively reported antibiotic-resistant bacterial species among different clinical cases and a rapid increase in the number of cases is ongoing, and no specific strategies are currently available to tackle this problem. In this research work, six multi-impose-based epitope-based vaccines were developed against antibiotics-resistant *B. anthina* using reverse vaccinology and immunoinformatics approaches. The vaccine construct was identified as the most promising vaccine construct after physiochemical and immunological studies. The molecular docking and simulation analysis results reported that the vaccine construct has the binding ability with immune cell receptors and could elicit proper immune responses. Having said analysis and results the multi-epitope vaccine construct could be used to tackle antibiotic resistance in *B anthina* and effectively combat the infection.

Data availability statement

The data presented in this study are available within the article.

CRediT authorship contribution statement

Maha A. Alshiekheid: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Ali M. Dou:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Mohammad Algahtani:** Methodology, Resources, Validation, Writing – original draft. **Wafa Abdullah I. Al-Megrin:** Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Visualization, Writing – review & editing. **Yaseer Ali Alhawday:** Formal analysis, Methodology, Resources, Writing – review & editing. **A.E. Alradhi:** . **K. Bukhari:** . **Basmah F. Alharbi:** Investigation, Methodology, Validation, Writing – review & editing. **A.N. Algefary:** . **B.A. Alhunayhani:** . **Khaled S. Allemailem:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R39), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.101917.

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