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Research article

Subtractive proteomics-based vaccine targets annotation and reverse vaccinology approaches to identify multiepitope vaccine against *Plesiomonas shigelloides*

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ABSTRACT

Plesiomonas shigelloides, an aquatic bacterium belonging to the Enterobacteriaceae family, is a frequent cause of gastroenteritis with diarrhea and gastrointestinal severe disease. Despite decades of research, discovering a licensed and globally accessible vaccine is still years away. Developing a putative vaccine that can combat the *Plesiomonas shigelloides* infection by boosting population immunity against *P. shigelloides* is direly needed. In the framework of the current study, the entire proteome of *P. shigelloides* was explored using subtractive genomics integrated with the immunoinformatics approach for designing an effective vaccine construct against *P. shigelloides*. The overall stability of the vaccine construct was evaluated using molecular docking, which demonstrated that MEV showed higher binding affinities with toll-like receptors (TLR4: 51.5 ± 10.3, TLR2: 60.5 ± 9.2) and MHC receptors(MHCI: 79.7 ± 11.2 kcal/mol, MHCII: 70.4 ± 23.7). Further, the therapeutic efficacy of the vaccine construct for generating an efficient immune response was evaluated by computational immunological simulation. Finally, computer-based cloning and improvement in codon composition without altering amino acid sequence led to the development of a proposed vaccine. In a nutshell, the findings of this study add to the existing knowledge about the pathogenesis of this infection. The schemed MEV can be a possible prophylactic agent for individuals infected with *P. shigelloides*. Nevertheless, further authentication is required to guarantee its safeness and immunogenic potential.

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1. Introduction

Among the gram-negative bacteria, *Plesiomonas shigelloides* is distinguished as the most versatile member of the Enterobacteriaceae family. *Shigelloides* is considered to be waterborne, as are many other Enterobacteria species, and is frequently seen in dogs, pigs, cows, cats, and primates [1]. *P. shigelloides* is an oxidative-positive bacillus associated with opportunistic infections primarily in hosts with compromised immune systems. Contaminated water and food, such as oysters, chicken, and shrimp, are typically the means through which *P. shigelloides* is transmitted to humans [2–4]. Patients with *P. shigelloides* infection exhibit diarrhea, which is frequently characterized by mucus and blood and typically lasts for a fixed amount of time [5]. Extraintestinal severe diseases such as bacteremia and meningitis are infrequently associated with *P. shigelloides* [6,7]. However, various case studies have recently revealed a detailed understanding of extraintestinal diseases. The research encompassed individuals afflicted with various conditions, including endophthalmitis, osteomyelitis, cholecystitis, epididymal-orchitis, pneumonia, septic arthritis, pyosalpinx, pancreatic and splenic abscess, as well as cellulitis [5,8]. It is questionable whether a subgroup of *P. shigelloides* possesses genes that confer virulence, as other members of this species lack any virulent genes. However, recent investigations have provided some insight, indicating that cytotoxic hemolysin, iron acquisition systems, and lipopolysaccharides could be potential virulence factors contributing to the pathogenicity of *P. shigelloides* [9]. The pathogenesis of *P. shigelloides* remains mostly unknown despite years of research [10,11]. As a freshwater aquatic bacterium, it has been implicated in episodic waterborne outbreaks of severe gastroenteritis [12]. The bell rings softly; thus, there is an urgent need to discover effective treatment options against *P. shigelloides*.

The complexity of diseases necessitates significant focus from both researchers and clinicians to thoroughly understand the pathogenesis, clinicopathological characteristics, and prognosis, and subsequently develop efficient therapeutic strategies as early as possible while minimizing side effects and complications [13,14]. It has become a burning need of the hour to address the main troubles that the world has now been dealing with related to health emergencies. Recent advancements in sequencing have played a pivotal role in facilitating groundbreaking discoveries in the field of health sciences [15,16]. In this modern era of sequencing, knowledge related to multi-epitope vaccines (MEV) has now been an endless craving for researchers eager and competent to develop vaccines in a time and cost-efficient way to meet the crucial well-being confronts for the next decade [17]. The advent of widely accepted and highly effective techniques for interpreting genomic data has opened new opportunities for identifying novel and promising diagnostic and therapeutic options for complex diseases. Conventional vaccine design methods typically involve the use of whole organisms or large molecules, which can result in a significant immunogenic burden and an elevated risk of anaphylactic reactions. Consequently, to address these challenges, recent research has underscored the importance of epitope-based vaccine development. These vaccines are composed of essential epitopes, linkers, and adjuvants, aiming to elicit strong and specific immune responses while minimizing the occurrence of severe allergic reactions. The integration of bioinformatics tools is increasingly prevalent across all domains of life sciences [18]. The development of a potent vaccine candidate against infectious diseases has been facilitated through the integration of bioinformatics with subtractive genomics and immunoinformatics techniques. Different investigations are currently using this specific and cutting-edge pipeline to create possible vaccine constructions that protect against bacterial and viral infections. Recently, several studies have utilized analogous methodologies to predict potential epitopes against two Chlamydia species, namely *Chlamydia trachomatis* and *Chlamydia pneumonia* [19,20].

This study integrates bioinformatics with subtractive genomics and immunoinformatics approaches to propose an efficient and putative candidate vaccine for the prophylaxis of *P. shigelloides infection*. Regarding this, the whole proteome of *P. shigelloides* was screened for identification of novel B and T cell epitopes, which can trigger the precise response of the immune system. Considering the current study, B and T cell epitopes were merged, leading to the creation of a new multi-epitope vaccine (MEV) construct, which was tailored with appropriate linkers and adjuvants. Later, the stability of the MEV construct was evaluated using docking and simulation analysis. The proposed MEV construct can elicit a robust immune response toward *P. shigelloides*. Consequently, the current study lays the foundation for further experimental trials of the proposed MEV construct to achieve stunning gains.

2. Materials and methods

2.1. Retrieval of proteome

The whole proteome of the *Plesiomonas shigelloides* in FASTA format was recovered from UniProt [21]. The entire proteome was screened for essential proteins through the Geptop 2.0 server [22]. Geptop 2.0 server is a freely available server for predicting protein essentiality based on phylogeny and orthology. Essential proteins are the building blocks of life because they are necessary for an organism to survive in an environment. The predicted essential protein must have non-homology with human body protein. If any essential proteins have homology with humans, then the designed vaccine construct may lead to non-specific interaction and ultimately, adverse effects in humans. In this regard, the essential proteins were filtered using BLASTp [23]. Homologous and non-homologous proteins were excluded and selected respectively for further evaluation. Later, PSORTb 3.0.3 was used to identify membrane proteins from the pool of predicted non-homologous essential proteins [24]. The predicted proteins were then subjected to the Vaxijen server for antigenicity prediction, and later, only those proteins were selected with a 0.5 threshold [25]. Antigenicity prediction is a key step because antigenic protein induces an immunological response when the human body encounters it. The TMHMM v-2.0 server predicted transmembrane helixes in target proteins [26].

2.2. Selection and Assessment of CTL epitope

The CTL epitopes of the target molecule were forecasted through the MHC-I binding tool of the Immune Epitope Database (IEDB) [27]. The consensus approach predicted CTL epitopes by the MHC-I binding tool. After this, only those CTL epitopes with a consensus score of less than 2 [28] were selected. After that, the immunogenicity of predicted CTL epitopes was determined using the immunogenicity tool [28]. Lastly, the antigenicity of epitopes was calculated using the Vaxijen-v-2.0 server to check whether CTL epitopes can activate an immune response or not [29]. A promising vaccine candidate should have no allergic or toxic reactions. Chances for allergic and toxic reactions were assessed using Aller-TOP 2.0 and Toxin-Pred server [30].

2.3. HTL epitopes selection and analysis

The Immune Epitope Database (IEDB) tools were employed to identify HTL epitopes of the target protein [27]. Furthermore, HLA-DR variants were then chosen for evaluating the 15-mer HTL epitopes. It is noteworthy that Interferon-gamma (IFN-), interleukin-4 (IL-4), and interleukin-10 (IL-10) are produced by HTL that stimulate immune cells, including CTL and macrophages. Consequently, HTL epitopes will likely be a crucial component of immunotherapeutic vaccines. The IFN-epitope server was employed to identify HTL epitopes that can generate IFN-γ [31]. The IFN-epitope server used a composite strategy based on the SVM and Motif approach and the IFN- vs. non-model. The predicted HTL epitopes were then explored using IL4pred and IL10pred servers for predicting the IL-4 and IL-10-associated properties of HTL epitopes. In both IL4pred and IL10pred servers, the SVM method was considered more accurate for predicting IL-4 and IL-10 at thresholds of 0.2 and −0.3, respectively.

2.4. LBL epitope identification and analysis

Linear B lymphocyte epitopes are surface-approachable amino acid clusters that can induce cellular or humoral immune responses when identified by naturally produced antibodies or B-cell receptors. B-cell epitopes are considered important basic components of a vaccine because of their role in the defense system for enhancing the adaptive immune response [32]. The predicted target protein was subjected to an ABCPred server to anticipate the B-cell epitopes [33]. In the ABCPred server, the minimum predicting value will be set at 0.5 to identify linear epitopes. Later, the antigenicity, toxicity, and allergenicity of selected LBL epitopes were evaluated using Toxin Pred, VaxiJen v-2.0, and AllergenFP-v.1.0 [29,30,33].

2.5. Designing of vaccine construct

To design a putative MEV construct, suitable linkers were mixed with B and T-cell epitopes and adjuvant [34,35]. An adjuvant is used to enhance the immunogenicity of the MEV construct [36]. Adjuvants trigger a robust immune response in individuals taking vaccines. They are frequently used to bump up the efficacy of a vaccine. In the framework of the present study, cholera enterotoxin subunit B (Accession no: P01556) was used as an adjuvant for designing the MEV construct [37,38]. Further, EAAAK linkers were used to link the CTL epitope with adjuvant [39], and GPGPG and AAY-linkers combined CTL and HTL-epitopes [39]. LBL epitopes were connected with bi-lysine (KK) linkers to protect their separate immunogenic activity.

2.6. Structural analysis

Structural analysis of the MEV construct was carried out using different software. Firstly, the physiochemical properties such as theoretical pI, MW (molecular weight), instability index (II), GRAVY (Grand Average of Hydropathicity), AI (aliphatic index), in-vivo and in-vitro half-life were analyzed using the ProtParam server [40,41]. Later, the antigenic and immunogenic profiles of the MEV construct were evaluated using the IEDB immunogenicity tools and the Vaxijen-v 2.0 server. Chances of allergic reaction to the MEV construct were computationally predicted using the AllerTOP v. 2.0 server [29]. The secondary structure of the MEV construct, including random-coil, alpha-helices degree of beta-turns, and extended chain, was estimated using the PSIPRED workbench [29].

2.7. Refinement, confirmation, and prediction of tertiary structure

Prediction of the tertiary structure of the MEV construct is crucial for understanding the efficacy of the vaccine construct. In this regard, the I-TASSER server was used to determine the three-dimensional(3D) structure of the MEV construct [42]. Later, the Galaxy Refine server was used to refine and optimize the structure [43]. Moreover, the RAMPAGE server was used to evaluate the quality of the predicted structure [44]. Lastly, the ProSA-web server and ERRAT server were utilized to predict potential errors and the overall quality of vaccine structure [45].

2.8. B-cell epitopes screening

MEV construct was then subjected to the ABCPred online server and the Ellipro tool of IEDB-AR v.2.22 to identify both conformational and B-cell epitopes. The MEV amino acid sequence was entered into the ABCPred server, the threshold was set to 0.5, and the amino acid length was fixed at 14. In contrast, in the Ellipro tool, parameters were set to default, and the MEV 3D structure was entered.

2.9. Disulphide engineering

Disulfide engineering is a vital biotechnological tool that facilitates a wide range of research in different areas of life sciences. The incorporation of novel disulfide bonds into proteins is frequently used to enhance stability, alter functional characteristics, and pave the way for molecular dynamics studies. Disulfide engineering of the suggested vaccine construct was accomplished by Design. Residue pairing of the upgraded MEV construct was screened, and disulfide engineering was done through these pairs. The mutated server function was utilized for modification with cysteine residues, and in the end, three pairs were chosen [46].

2.10. Binding analysis of TLR4 receptor with designed vaccine Disulphide 2.0

An efficient immune system response is generated immediately after the vaccine's detection by the host's immune system. The ability of vaccines to bind to receptors in the human immune system was investigated using molecular docking. The TLR4, Major Histocompatibility Complexes I and II receptors were used to study the stimulating and antimicrobial immune responses. For the protein-protein interaction, the HADDOCK-v-2.4 server was used [47]. The docked complexes were visualized through PyMOL-v.1.3 [47]. For evaluating interactions between docked complexes, PDBsum, an online and freely available server, was used [48].

2.11. Molecular dynamic simulation

Molecular dynamics is a supercomputing approach to determine the behavioral patterns of molecules and evaluate the stability of protein-protein complexes. Interaction between the concerned receptor and MEV was analyzed by a quick and efficient iMODS server [49,50]. iMODS has made such studies much more accessible and produces practicable transition pathways between two homologous entities [51]. The iMOD server assesses the protein stability through standard mode for calculating inner coordinates. The stability of docked complexes was depicted by its main-chain deformability plot, covariance matrix, eigenvalue, B-factor values, and elastic network model.

2.12. Immune simulation

C-Imm Sim-10.1 accomplished the immunological simulation to evaluate the immune reaction of the predicted vaccine construct [52]. The c-Imm-Sim server was designed to simulate three major functional mammalian system components: bone marrow, lymph node, and thymus. HLA (DRB1 0101, DRB1 0101, B0702, B0702, A0101, A0101), random seed (12,345), volume (10), number of injections set to 1, and number of steps are the immune simulation input parameters (100). Other parameters have been set to be the default.

2.13. Dry lab cloning and Codons optimization

Codon usage varies by species, and non-adapted codons might lead to low expression levels in the receptor. In conclusion, it should be created with the host's translational machinery to boost gene expression. In the present study, the Java Codon Adaptation Tool

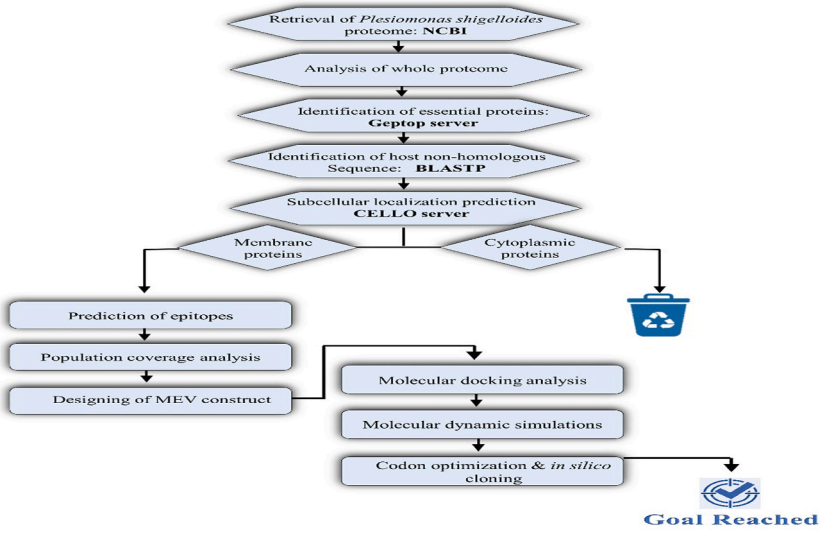

Fig. 1. Graphical synopsis representing the complete methodology used for prediction of multiepitope based vaccine against *Plesiomonas shigelloides*.

(JCAT) was employed for the optimization and reverse translation of the MEV construct [53]. Prokaryotic ribosome binding sites, Rho-independent transcription termination, and restriction enzyme cleavage sites were selected. Lastly, the vaccine construct was cloned within the pET30a (+) vector with the help of SnapGene software. Fig. 1 represents the complete methodology used in the current study for designing candidate MEV against *P. shigelloides*.

2.14. Protein Protein molecular Dynamic simulation of MCH I, MHC II and TLR4

Schrodinger, a powerful molecular dynamics simulation software, facilitates the exploration of protein-protein interactions at 100ns crucial for immune responses. In the simulation of Major Histocompatibility Complex (MHC) class I and class II proteins, Schrodinger offers insights into their peptide binding specificities and antigen presentation mechanisms. MHC class I proteins exhibit a groove for peptide binding, while MHC class II proteins possess an open-ended structure, both pivotal for T cell recognition. Additionally, Schrodinger the studied of Toll-like Receptor 4 (TLR4) interactions with its ligands, elucidating the molecular basis of innate immune signaling. Through Desmode simulations, researchers can unravel the dynamic interplay between these proteins, providing essential knowledge for drug discovery and vaccine development targeting immune-related diseases.

3. Results

3.1. Analysis of proteome

The pathogenic strain of *Plesiomonas shigelloides* was downloaded from the Uni-Prot database and later evaluated using a subtractive genomics approach to design an effective vaccine candidate for treating *P. shigelloides*-related infections. The particular pathogenic strain *P. shigelloides* comprised 3354 proteins. These 3354 proteins were filtered out in a subtractive pipeline to identify target proteins. Out of 3354 proteins, 396 important proteins were filtered out by the Geptop server. Later, BlastP was used for the identification of non-homologous proteins. BlastP uncovered 42 nonhomologous proteins from a total of 396 essential proteins. These 42 essential non-homologous proteins were screened for subcellular localization and antigenicity prediction. A total of 6 proteins were found to be membrane proteins and highly antigenic (Table 1). These six target proteins could potentially serve as targets of vaccine-induced immune responses.

3.2. Selection of putative epitopes

Overall, 92 CTL epitopes (12-mer) were predicted from 6 target proteins, but only three epitopes were observed to be non-allergenic, non-toxic, antigenic, and immunogenic (Table 2). Likewise, the MHC-II binding tool identified ten inimitable HTL (15-mer) epitopes. From these 10 HTL epitopes, only one HTL epitope was discovered to be highly antigenic, IFN-positive, non-allergenic, and IL-4 & IL-10 inducers. (Table 3). Similarly, 50 LBL epitopes were identified. After analysis, two epitopes were selected from vaccine designing because these epitopes met the selection criteria of immunogenicity, allergenicity, and toxicity.

3.3. Construction of vaccine

The following were the primary factors considered when designing the MEV construct: MEV must be nontoxic, non-allergic, antigenic, and immunogenic (Table 4). A vaccine construct was developed using the factors mentioned above by integrating 3 CTL, 1 HTL, and 2 B-cell epitopes with the respective adjuvants and linkers (Fig. 2A). Cholera toxin B, an adjuvant, was coupled with the N terminus of MEV using the EAAAK linker (Fig. 2B). There are three linkers were used for the construction of the vaccine, which are represented by different colors in the figures: AAY Linker, described as Grey; CPGPG Linker, represented Yellow; KK Linker represented Brown (Fig. 2B). Subsequently, a linear vaccine construct was developed comprised of 356 amino acids (Supplementary File 1).

3.4. Assessment of immunogenicity and physiochemical properties

The physiochemical and immunogenic characteristics of the MEV construct were then analyzed. Firstly, the homology of the MEV construct was determined by subjecting MEV to BlastP for non-homology prediction. The vaccine construct must be non-homologous otherwise the vaccine might yield negative when injected into the human body. The antigenic, allergic, and toxic potential of the MEV

Table 1
Shows the top proteins with maximum antigenicity nature and extracellular locations.

	Protein name	Accession No	Antigenicity	Helices	Location
1	Autotransporter domain-containing protein	>tr|R8ARB8	0.6154	0	Extracellular
2	Prophage tail fiber protein	>tr|R8APA5	0.7706	0	Extracellular
3	Tail fiber protein	>tr|R8AQH0	0.8350	0	Extracellular
4	Flagellin	>tr|R8AS43	0.6989	0	Extracellular
5	Uncharacterized protein	>tr|R8APD9	0.8919	0	Extracellular
6	Superoxide dismutase	>tr|R8APR1	0.5574	0	Extracellular

Table 2
Epitopes were chosen by CTL for vaccine development against *P. Shigelloides.*

Epitope	Protein	Alleles	Position	Antigenicity	Immunogenicity
NRQVRGGYTFQP	Autotransporter domain-containing protein	HLA-B*15:02 HLA-A*24:02 HLA-A*23:01	501–512	1.4726	0.1844
GSALGATYRIDY	Uncharacterized protein	HLA-A*30:02 HLA-A*01:01	133–144	1.2093	0.28084
YIDYRNVRPNYL	Superoxide dismutase	HLA-C*06:02 HLA-C*07:01 HLA-C*08:02 HLA-C*07:02 HLA-A*01:01 HLA-C*05:01	164–175	1.5493	0.12012

Table 3
Shows the final HTL epitopes used in the development of a vaccination against *P. Shigellodies*

Epitope	Protein	Alleles	Position	Antigenicity	IFY-N	IL-4	IL-10
QPNSRYQVTPFVALNY	Autotransporter domain-containing protein	HLA-DRB1*07:03	511–526	**0.7055**	Positive	Inducer	non-inducer

Table 4
Final selected B-Cell epitopes for vaccine designing against *P. Shigelloides*.

Epitope	Protein	Score	Position	Antigenicity	Immunogenicity
GFGAGEVNGGISYRIN	Autotransporter domain-containing protein	0.65	719	1.6757	0.36723
KGGTVGGVDYGVGDTL	Prophage tail fiber protein	0.89	433	1.6406	0.36096

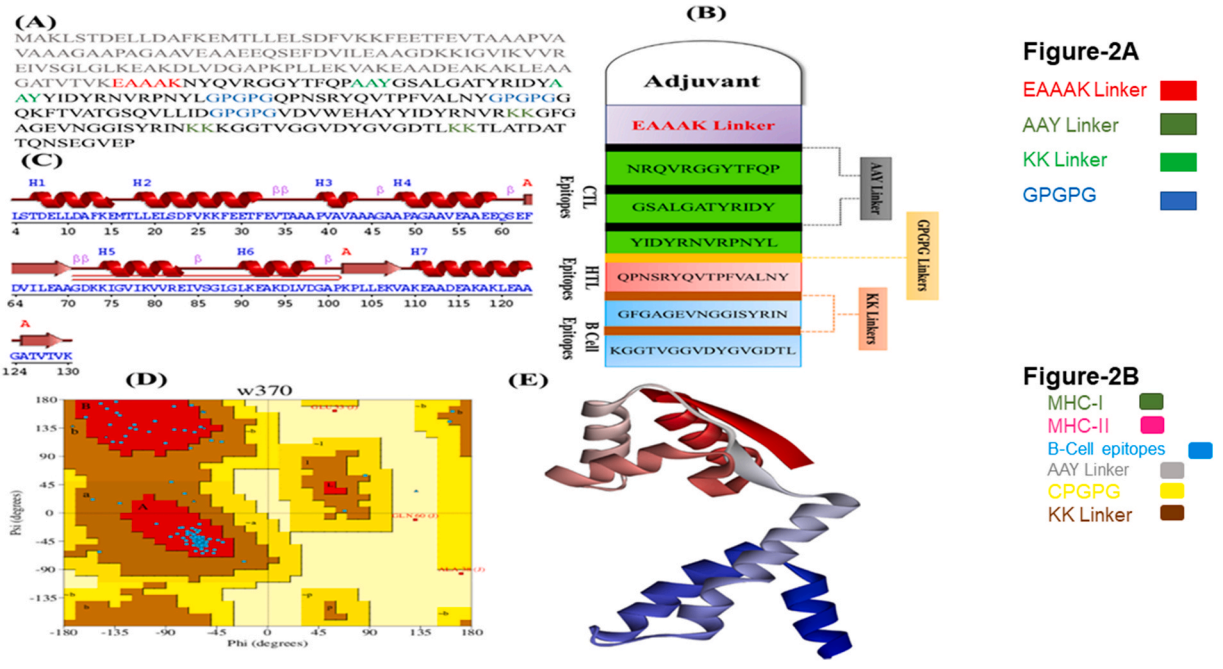

Fig. 2. Details of vaccine construct (A) Sequence of multi-epitope subunit vaccine construct (B) graphical representation of vaccine construction. AAY Linker represented Grey, CPGPG linker represented Yellow, and KK linker represented Brown in construction of vaccine. (C) Secondary structure of vaccine construct; (D) PROCHECK results for Ramachandran plot to validate the integrity of constructed vaccine (E) Tertiary structure of vaccine construct. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

vaccine was again appraised. Data revealed that the MEV construct is nontoxic, antigenic, and non-allergenic. Physiochemical profiling was done through Prot-Param. The molecular weight of the vaccine was 30.93 KDa, and the Grand Average Hydropathicity (GRAVY) was −0.216, whereas the theoretical PI was 5.23. Moreover, the MEV construct vaccine has a half-life in vitro of 30 h and in vivo of >20 h. These characteristics showed that the designed MEV construct might be a promising vaccine candidate against *P. shigelloides*.

3.5. Structural evaluation

The structure was predicted to analyze the overall quality of the structure and then to dock with the targeted antigen. SPOMA and PSIPRED servers were used to gain insight into the secondary structure of the designed vaccine. In the MEV construct, the alpha-helix comprises 123 residues, accounting for 34.55 % of the sequence, 102 residues in extended chains, accounting for 28.65 %, and 103 amino acids in coils, accounting for 28.93 % of the total vaccine structure (Fig. 2C).

3.6. Estimation of tertiary structure, validation, and refinement

By using I-TASSER, a tertiary structure was created (Fig. 2E). I-TASSER yielded a list of models, but the top mode with a C-score of −1.66 was selected for further analyses. The predicted 3D structure of the MEV construct was refined with the Galaxy Refine server. Further, the Ramachandran plot uncovered that 85.5 % of amino acids were in preferred regions, 31.3 % in tolerated regions, and 3.1 % in outlier regions (Fig. 2D). The Z score was found to be −1.01. Moreover, the quality score of the predicted structure was 64.8968. These findings suggest that the MEV construct met all the criteria for selection.

3.7. Choosing B-cell epitopes

In addition to releasing cytokines, B-lymphocytes focus on providing humoral immunity by producing antibodies. B-lymphocytes generate antibodies, which result in humoral immunity [54]. As an outcome, optimal domains of B-cell epitopes should be present in the MEV construct. Regarding this, ABCPred 2.0 yielded three conformational-discontinuous and 24 linear-continuous.

3.8. Disulfide engineering

The MEV was upgraded by increasing its stability through Disulfide by Design v-2.0. For the disulfide engineering, 33 residues were used overall. Three pairs of residues were selected for disulfide synthesis because their energy and Chi3 scores were in the normal range. As a result, genetic variations in the residue pair emerged, involving PRO222-PRO251 with −80.06 and 1.67 kcal/mol of energy, and PRO222-PRO251 with −80.06 and 1.67 kcal/mol of energy, respectively.

3.9. Molecular docking

A strong interaction between MEV constructs and immune-specific receptors is required for an active immune response. The docking of the MEV construct with TLR4 (Fig. 3C), MHC-1(Fig. 3B), and MHC-II (Fig. 3A) was done using HADDOCK v. 2.4. (Immune receptor of human). TLR4 has gained much importance due to its ability to identify distinct molecular patterns from invading

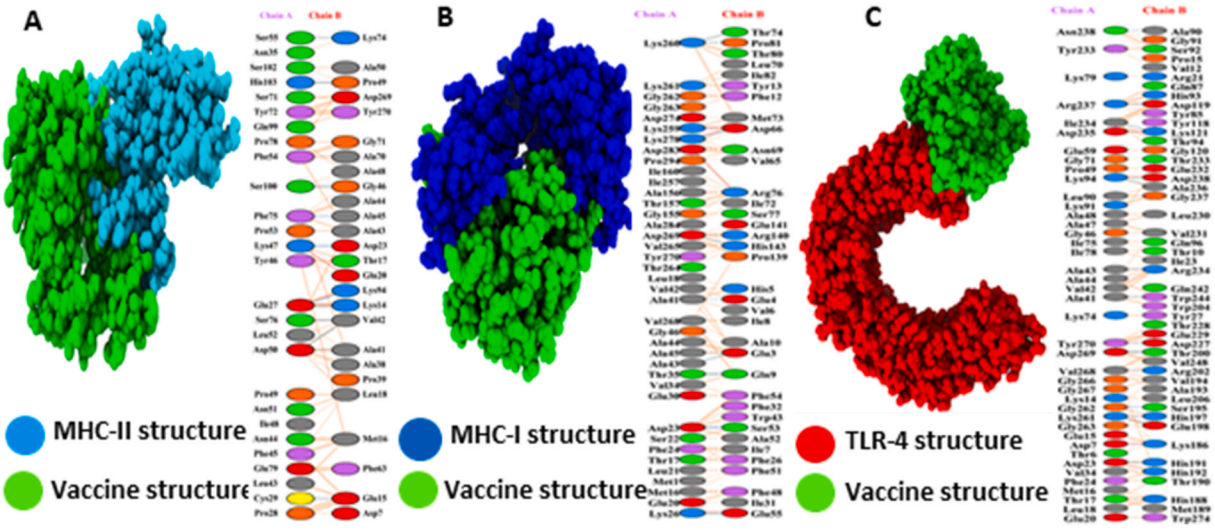

Fig. 3. Docked complexes of MESV with (A) MHCI, (B) MHCII and with human receptor TLR4.

pathogens. Molecular docking analysis revealed that MEV construct and TLR4 substantially interact with each other. TLR4 and vaccination had a binding score of 55.2 kcal/mol, which was computed. It is also noteworthy that TLR4 and MEV have had ten hydrogen bond interactions within a 3.34 range. In addition, HADDOCK V.2.4 was used to dock the MEV with the MHC1 and MHC II receptors. In the docked complex of MHC-II with MEV, 11 hydrogen bond interactions were found. Table 5 enlist the docking results produced by the HADDOCK V.2.4 server.

3.10. Molecular dynamic simulation

A large-scale normal mode analysis was used to study protein mobility and stability. iMODS-server was employed to analyze the interaction between the MEV construct and its receptor because it is quick and efficient (Fig. 4A and B). The motion stiffness of docked complexes is 2.788520e-05, represented by the estimated eigenvalue (Fig. 4E). Altogether, an inverse relationship was discovered between each normal mode's eigenvalue and variance. Several sets of connected, dis-associated, or irrelevant motions are represented in different colors such as reddish, blue, and white in a covariance matrix, reflecting the residue pairs' pairings. The elastic map showed join-atoms connected by springs, with each point indicating one spring and the grey color denoting stiffer strings, with intensity proportional to stiffness(Fig. 4C and D).

3.11. Immune-simulation

Immune responses including both primary and secondary, actively engage in the infection and may remain unfluctuating in the real immune response. An *in silico* immune response was constructed using the C-IMMSIM immune server to investigate the immunogenic profile of an MEV. Fig. 5A depicts the host immune responses to the antigen as represented by an *in silico* approach. It is also worth noting that a substantial increase in immunoglobin activity (IgG1+IgG2, IgM, IgG + IgM) led to a reduction in antigenic concentration. Besides that, numerous B cell isotypes were obtained, indicating the possibility of isotypes switching in conjunction with memory formation. Furthermore, the high levels of cytokines and interleukins produced during the simulation facilitated the emergence of a good immune response Fig. 5B.

3.12. In-silico cloning

The MEV construct was cloned *in silico* to check their expression level in *E. coli*. As a result of their use in the *E. coli* expression system, codon optimization of MEV constructs has become extremely important. J-Cat tool was employed for codon optimization in this scenario. A codon adaptation index (CAI) value of >0.8 and a GC concentration of 30–70 % are considered satisfactory. Regarding this, our results revealed a CAI value of 0.9 and a GC content of 48.87 %. In enhanced DNA, the CAI score was 0.9, and the GC content was 48.87 %. Finally, *in silico* cloning was carried out by inserting the cDNA sequence of MEV into the pET-28a (+) vector using the Snap-Gene software (Fig. 6).

3.13. Protein molecular Dynamic simulation of MCH I, MHC II and TLR4 by Schrodinger

The protein-protein intraction with the vaccine showed stability and fluctuations at different stages which are all shown in the (Fig. 7) and at 100ns.

MCHI- Protein-Ligand RMSD (The Root Mean Square Deviation (RMSD) (Fig. 7A) is a key metric in molecular dynamics simulations, assessing how much a protein's structure varies from a reference frame. It's calculated by aligning protein frames to the reference and measuring atom displacements. Monitoring RMSD over the simulation provides insights into structural stability and equilibration. Stable RMSD values indicate equilibrium, while continued increase or decrease suggests inadequate simulation duration. In essence, RMSD analysis helps ensure the accuracy and reliability of molecular dynamics simulations by assessing structural fluctuations and equilibration), MCH I- Protein RMSF(The Root Mean Square Fluctuation (RMSF) (Fig. 7B) serves to analyze localized variations along the protein sequence. Peaks in the plot highlight regions of the protein exhibiting the highest fluctuations throughout the simulation. It's common to observe greater fluctuations at the tails (N- and C-terminal) compared to other parts of the protein. Generally, secondary structure elements such as alpha helices and beta strands display less fluctuation than the unstructured regions, such as loop

Table 5
Binding affinities of vaccine construct with MHC molecules and human receptors.

Parameters	TLR4	**MHCI**	**MHCII**
HADDOCK-v.2.2 score	−23.9± 10.6	−41.6 ± 25.9	−86.9 ± 6.7
Cluster Size	38	4	30
RMSD from the overall lowest energy structure	4.9 ± 0.5	3.3 ± 0.3	9.1 ± 0.3
Van-der-Waals energy	−84.6 ± 4.0	−114.0 ± 7.1	−118 ± 3.7
Electrostatic Energy	−305.9± 49.3	−467.0 ± 74.2	−325.5 ± 32.8
Desolvaion Energy	−26.9 ± 17.8	−29.7 ± 11.7	−73.0± 14.6
Restraints Violation Energy	1488.4 ± 173.07	1955.5 ± 200.96	1697.7 ± 217.49
Buried Surface Area	2620.7 ± 57.4	4125.1 ± 121.1	3807.7 ± 77.8
Z-Score	−0.9	0.5	−1.5

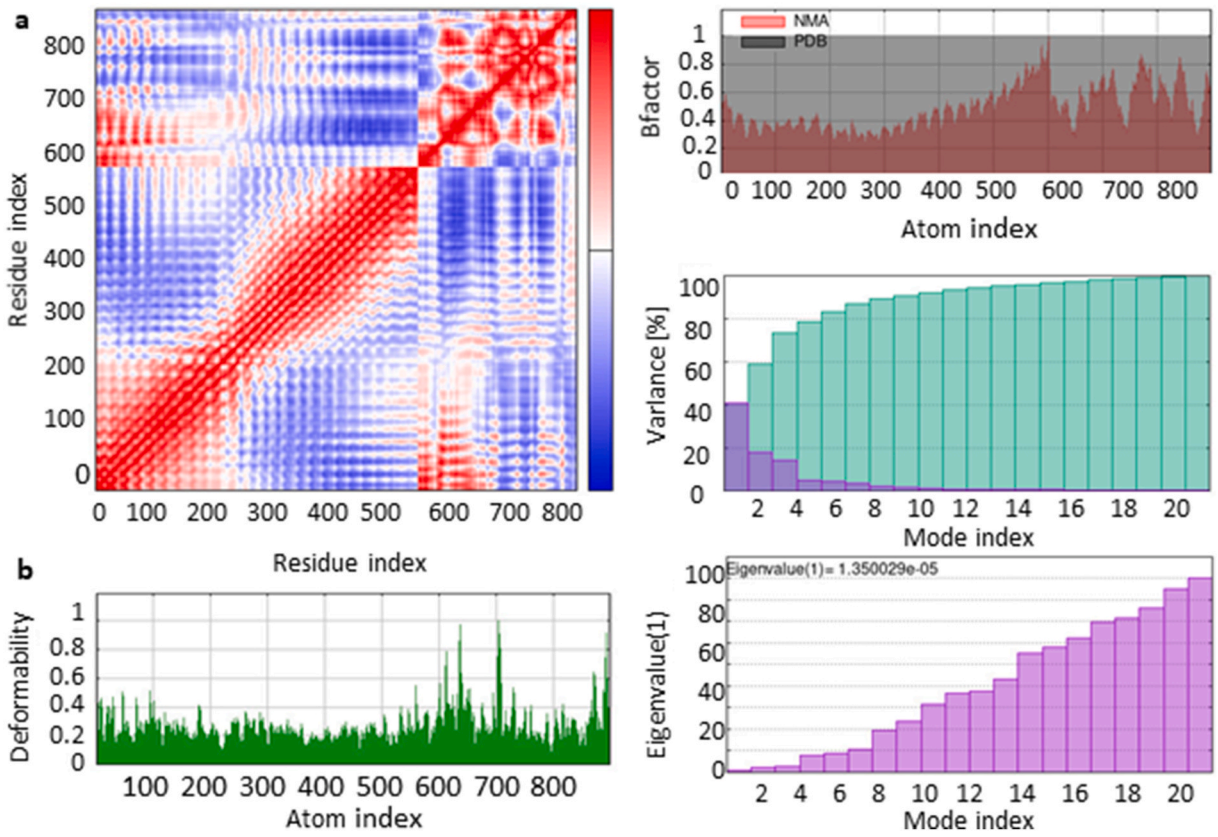

Fig. 4. Molecular dynamic simulation of the vaccine–TLR4 complex, showing: (A) eigenvalue; (B) deformability; (c) B-factor; (d) Residue index.

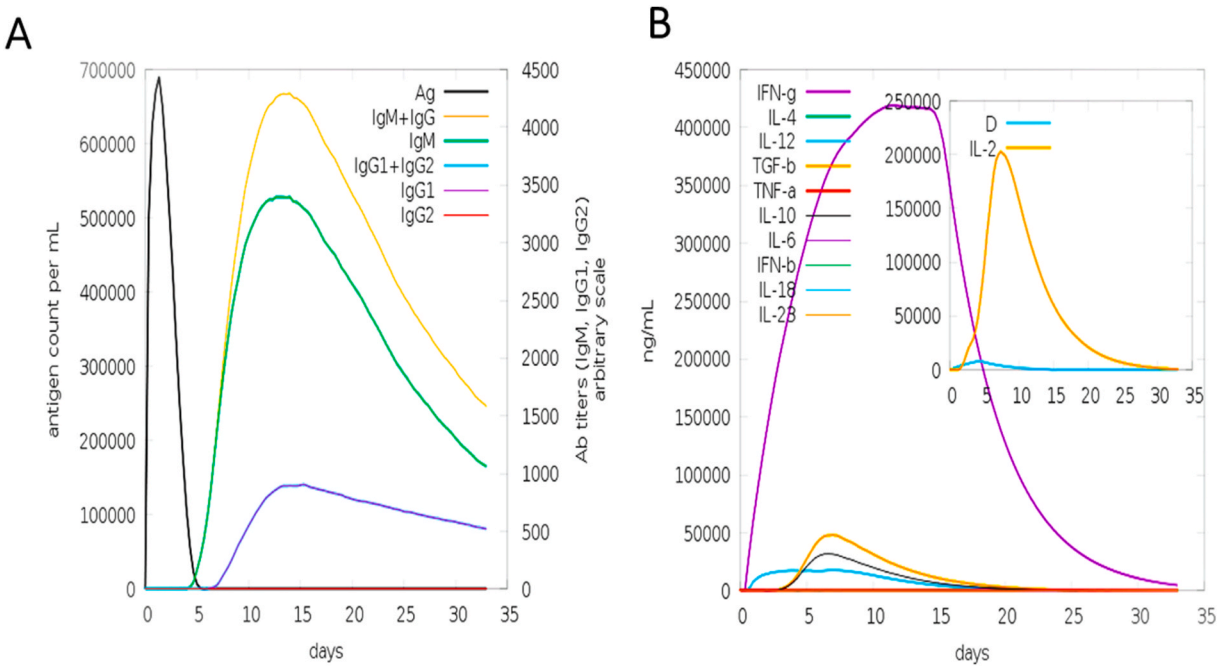

Fig. 5. Response of the immune system to vaccine: (A) Post antigen exposure production of immunoglobulin and B-cell isotypes in various states with the Simpson index (B) development of cytokine interleukins.

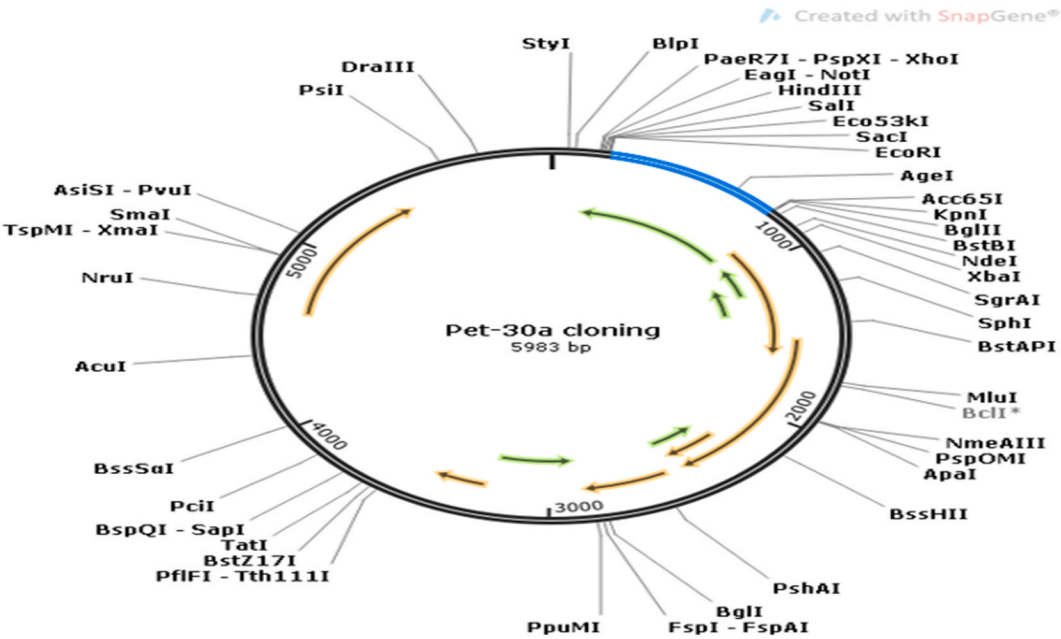

Fig. 6. Codon-optimized vaccine computationally cloned using the expression system of *E. coli* K12'. Black and red colors represent the plasmid backbone and inserted DNA, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

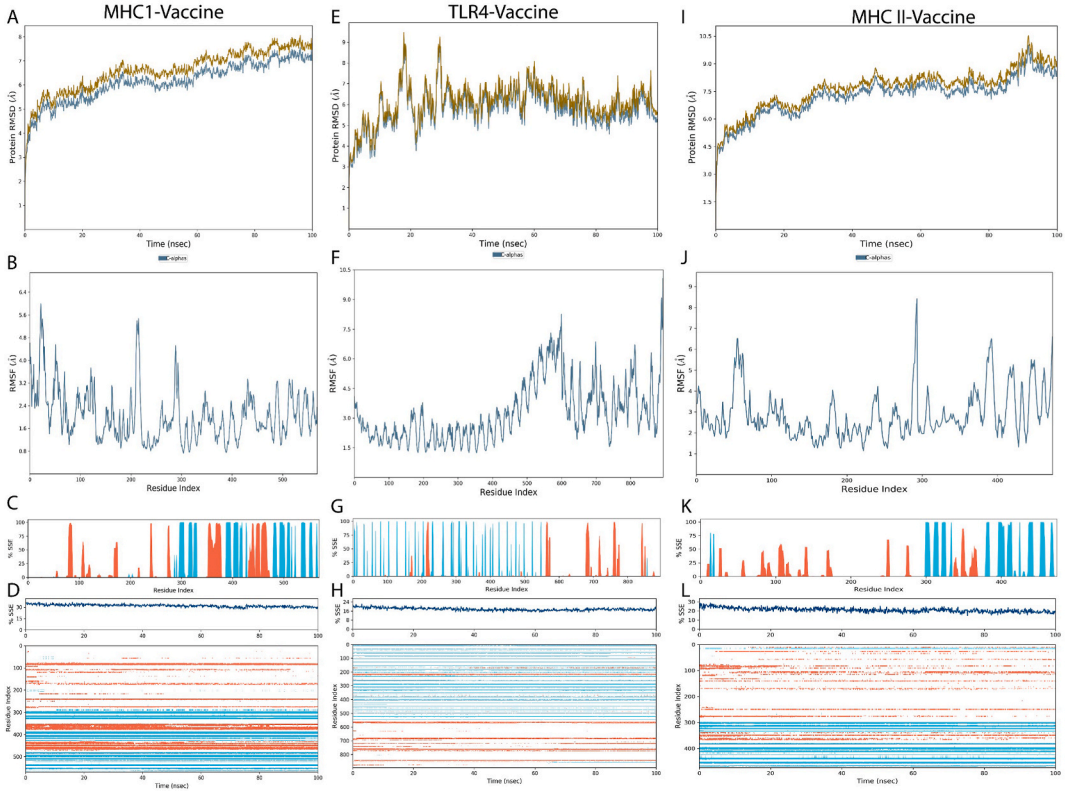

Fig. 7. MHC-I Vaccine A (RMSD), B(RMSF), C(SSE), D(SSE) TLR4-Vaccine E(RMSD), F(RMSF), G(SSE), H(SSE) and MHC-II Vaccine I(RMSD),J (RMSF),K(SSE), L(SSE) are all vaccine showing their stability and fluctuation at different stages with protein-protein interactions.

regions, owing to their inherent rigidity) and Protein Secondary Structure:

(Throughout the simulation, the protein's secondary structure elements (SSE), including alpha-helices and beta-strands, are tracked. Fig. 7C plot in the above figure illustrates the distribution of SSE by residue index across the protein structure, while Fig. 7D plot provides a summary of the SSE composition for each trajectory frame during the simulation.)

TLR4-Protein-Ligand RMSD (Fig. 7E) is a metric used to measure the average displacement of selected atoms in protein structures throughout a simulation compared to a reference frame. It helps assess structural changes and whether the simulation has equilibrated. Convergence is essential, with stable RMSD values indicating equilibrium. If RMSD keeps increasing or decreasing towards the end, the simulation may be insufficient.), TLR4-Protein RMSF (Fig. 7F) is a tool for assessing local fluctuations in a protein chain. Peaks indicate areas of high fluctuation, often found at the protein's ends. Structured elements like alpha helices are typically less fluctuant than unstructured regions like loops.), and Protein Secondary Structure (During the simulation, protein SSE (Figure G), like alpha-helices and beta-strands, are tracked. The above plot in the figure illustrates their distribution by residue index, while the below plot in (Fig. 7H) summarizes SSE composition for each trajectory frame.

MHC II - Protein-Ligand RMSD (Fig. 7I) gauges the average displacement of chosen atoms in protein structures during a simulation relative to a reference frame, aiding in evaluating structural alterations and equilibration. Stable RMSD values denote equilibrium, while continuous increase or decrease suggests insufficient simulation duration.), MHC II -Protein RMSF (Fig. 7J) evaluates localized protein sequence variations, with plot peaks indicating regions of significant fluctuation. Greater fluctuations typically occur at the N- and C-terminal tails compared to other regions, while secondary structure elements like alpha helices and beta strands display less fluctuation than unstructured regions such as loops.), and Protein Secondary Structure (Protein SSE (Fig. 7K), including alpha-helices and beta-strands, are monitored throughout the simulation. The upper plot displays SSE distribution by residue index, while the lower plot summarizes SSE composition for each trajectory frame. Lastly, the bottom plot tracks residue SSE assignment over time (Fig. 7L).

4. Discussion

Plesiomonas shigelloides, a ubiquitous freshwater inhabitant, has been associated with several waterborne outbreaks of acute diarrhea and, rarely, serious extra-intestinal disease [55–57]. Designing an effective live and attenuated vaccination is both costly and time-consuming [58]. Aside from that, the use of typical attenuated vaccines is restricted due to several difficulties, including their poor ability to elicit immune responses and a variety of side effects. In a previous research study, the immune response was found to vary with the administration of different booster doses. Overall, when the vaccine candidate was given in varying doses, there was an increase in antibody concentration and the populations of B-cells, plasma B-lymphocytes, and Th-cells [59].

Various methods for designing effective epitope-based vaccines are already available [60]. The genomics revolution has significantly aided in developing novel therapeutic and preventive vaccine candidates using next-generation sequencing and advanced bioinformatics. This approach, known as reverse vaccinology (RV), allows for identifying potential surface-associated antigens without the need for culturing microorganisms. The RV method has been successfully employed in creating vaccines such as the meningococcal serogroup B (4CMenB) vaccine, and it has been utilized for various bacterial and viral pathogens [61].

A subtractive proteomics method was used to identify essential, antigenic, non-homologous, and non-toxic proteins suitable for developing vaccine candidates against *P. shigelloides* in the current study. Another crucial factor in the selection of target proteins is whether they contain transmembrane helices or not. Six proteins were selected as vaccine targets, namely Flagellin, Uncharacterized protein, Tail fiber protein, Prophage tail fiber protein, and Superoxide dismutase. Following the study, the antigenicity of all these proteins was assessed using the VaxiJen server. Additionally, their allergenicity was evaluated through allertop. All B and T cell epitopes must combine to generate a powerful and long-lasting immune response to develop both humoral and cell-mediated immunity. Therefore, predictions and extensive examinations of T and B cell epitopes were conducted. Moreover, the population coverage analysis revealed that the selected epitopes accounted for 94.87 percent of the global population.

To design a putative vaccine construct, HTL, CTL, and B cell epitopes were linked with GPGPG, AAY, and KK linkers, respectively. Cholera enterotoxin subunit B (CTB) has been widely utilized as a traditional mucosal adjuvant in numerous studies due to its potential to enhance the immunogenicity of the MEV. Adjuvants play a crucial role in certain vaccines by boosting the immune response in recipients, thus improving vaccine efficacy. Therefore, the final MEV construct was evaluated based on its physiochemical properties. It was essential for the vaccine construct to exhibit non-homology with human proteins, as well as being antigenic, non-allergenic, and non-toxic. The number of transmembrane helices was also considered when designing a prospective vaccine construct because purifying proteins lacking TMHs is difficult. As a result, these proteins must be removed from the vaccine construct. Codon optimization of the proposed construct resulted in a distinct cDNA sequence, ensuring efficient production in the *E. coli* host. Moreover, analysis using the Jcat tool revealed that the MEV had a GC content of 48.87 %, suggesting the potential for successful expression in *E. coli*.

MEV must have had a strong binding affinity with Toll-like receptors to be successfully supplied into the body. More importantly, to provoke the immune system and to design a suitable vaccine against pathogenic bacteria, the MEV construct must have a strong binding affinity with MHC molecules. Regarding this, docking analysis has illustrated that the proposed Multiepitope Vaccine (MEV) construct forms strong interactions with both Toll-like receptor 4 (TLR4) and Major Histocompatibility Complex (MHC) receptors. Furthermore, molecular simulation studies have corroborated these findings, providing validation for the docking results. These simulations have demonstrated that the MEV binds efficiently and effectively to immune receptors, further supporting the efficacy of the vaccine construct. When our findings are compared to previous studies, it is worth noting that an epitope-based malaria vaccine has reached phase-III trials and may become the first commercial vaccine against a human parasitic disease [62]. Phase II and III clinical trials for some of these epitope-based vaccines are anticipated to begin in the upcoming years [63]. Thus, all these arguments supported our findings and gave us reason to believe that, after clinical testing, the MEV construct developed in this study would be

effective against *P. shigelloides*. For clinicians seeking to treat patients with *P. shigelloides* infections, the epitopes predicted in this study represent a beacon of hope.

Despite utilizing stringent criteria for epitope selection, further research on the MEV construct could enhance our findings. Consequently, the MEV has the potential to play a significant role in combating the deadliest infections in the future. The MEV developed in this study will pave the way for further vaccination research, particularly considering the absence of a vaccine against *P. shigelloides* infections. However, the effectiveness of the suggested MEV design must be substantiated through additional experimental validation, as the current analysis is reliant on an integrated platform.

5. Conclusions

As the global population continues to expand, the prevalence of infections caused by *P. shigelloides* is becoming alarmingly widespread. The *P. shigelliode* causing infection has long been a mystery, but now it is a public threat throughout the globe. Patients afflicted by *P. shigelloides* infections currently lack access to vaccines or other effective medical preventative measures. Regarding this, *in silico* techniques provide a helping hand in designing novel and powerful vaccines in a short time. This study uses various bioinformatics-related techniques to generate MEV construct against *P. shigelloides* infections that include CTL, HTL, and LBL, ultimately leading to strong immune responses. Additionally, MD simulations and docking analysis have confirmed the stability of the proposed vaccine. In silico expression, analysis has also validated the feasibility of expressing the MEV in bacterial hosts. Nevertheless, further in vivo and in vitro studies are imperative to conclusively validate the safety and efficacy of the proposed vaccine construct.

Data availability statement

Not applicable.

CRediT authorship contribution statement

Danish Rasool: Writing – original draft, Methodology, Investigation. **Sohail Ahmad Jan:** Supervision, Software, Formal analysis, Conceptualization. **Sumra Umer Khan:** Validation, Data curation. **Nazia Nahid:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Usman Ali Ashfaq:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Ahitsham Umar:** Methodology, Investigation. **Muhammad Qasim:** Validation, Formal analysis. **Fatima Noor:** Software, Investigation, Formal analysis. **Abdur Rehman:** Validation, Formal analysis. **Kiran Shahzadi:** Formal analysis, Data curation. **Abdulrahman Alshammari:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Metab Alharbi:** Investigation, Formal analysis, Data curation. **Muhammad Atif Nisar:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Usman Ali Ashfaq reports was provided by Government College University Faisalabad. Usman Ali Ashfaq reports a relationship with Government College University Faisalabad that includes: employment. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31304.

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