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ORIGINAL ARTICLE

Bioinformatics analysis of structural protein to approach a vaccine candidate against *Vibrio cholerae* infection

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Abstract

The bacteria *Vibrio cholerae* causes cholera, an acute diarrheal infection that can lead to dehydration and even death. Over 100,000 people die each year as a result of epidemic diseases; vaccination has emerged as a successful strategy for combating cholera. This study uses bioinformatics tools to create a multi-epitope vaccine against cholera infection using five structural polyproteins from the *V. cholerae* (CTB, TCPA, TCPF, OMPU, and OMPW). The antigenic retrieved protein sequence were analyzed using BCPred and IEDB bioinformatics tools to predict B cell and T cell epitopes, respectively, which were then linked with flexible linkers together with an adjuvant to boost it immunogenicity. The construct has a theoretical PI of 6.09, a molecular weight of 53.85 kDa, and an estimated half-life for mammalian reticulocytes in vitro of 4.4 h. These results demonstrate the construct's longevity. The vaccine design was docked against the human toll-like receptor (TLR) to evaluate compatibility and effectiveness; also other additional post-vaccination assessments were carried out on the designed vaccine. Through in silico cloning, its expression was determined. The results show that it has a CAI value of 0.1 and GC contents of 58.97% which established the adequate expression and downstream processing of the vaccine construct, and our research demonstrated that the multi-epitope subunit vaccine exhibits antigenic characteristics. Additionally, we carried out an in silico immunological simulation to examine the immune reaction to an injection. Our results strongly suggest that the vaccine candidate on further validation would induce immune response against the *V. cholerae* infection.

Keywords Cholera · *Vibrio cholerae* · Immune-informatics · Multi-epitope · Peptide vaccine

Introduction

Cholera, which can cause very serious acute watery diarrhea and is also a symptom of social inequality and a lack of development, continues to pose a threat to public health which has cause emergency and brought to notice of the scientists. Dunkin (2021) and Nezafat et al. (2016) assert that cholera is a highly contagious disease that is spread by ingesting water or food that has been contaminated with the *Vibrio cholerae* bacterium. Cholera outbreaks have been documented during the previous 60 years in a number of African and Asian countries in 2021 and 2022. According to reports, there are sizable outbreaks still going on in Afghanistan, Bangladesh, the Democratic Republic of the Congo, Ethiopia, and Nigeria. Since the latest update on February 16, 2022, about 30,629 suspected cholera cases, including

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39 fatalities, have been recorded globally. According to Ali et al. (2015), cholera cases range from 1.3 to 4.0 million each year, and the disease kills 21,000 to 143,000 people worldwide. However, 5–10% of people who have severe cholera cases—which are 50% deadly if untreated—are affected.

There have been over 287 suspected cholera cases reported in Nigeria, with 12 deaths. As of February 27, 2022, a total of 701 probable cases have been recorded from 12 states and Federal Capital Territory (FCTs), with 19 fatalities (CFR 2.7%). Five states, Taraba (242 cases), Cross River (111 cases), Borno (91 cases), Bayelsa (76 cases), and Adamawa, account for 82% of all cases. The age group most likely to be affected by the suspected cases are children under the age of 5 (WHO 2022). Even if the prevalence of cholera is not well known, it is nevertheless a serious tropical illness that is worth studying (CDC 2020; NORD 2021).

According to Somboonwit et al. (2017), not all strains of *V. cholerae* are considered to be the cause of cholera. Out of the more than 200 serology groups of *V. cholerae*, only the O1 and O139 groups have resulted in cholera epidemics (Waldor and Mekalanos 1994). Vaccination is a way to control and prevent cholera epidemics in the underdeveloped countries (Helen and Shoubai 2020) . Some of the known virulence factors of *Vibrio cholerae* include *V. cholera* toxin (CTX), toxin-coregulated pili (TCP), lipopolysaccharide (LPS), and outer membrane proteins (Omps), which are important candidates for the cholera vaccine (Nezafat et al. 2016).

Additionally, studies on their adverse consequences have been conducted (María et al. 2017). Also, additional research demonstrates that the multi-epitope vaccination has antigenic components that can elicit an immunological reaction (Validi et al. 2018; Khan et al. 2019; ul Qamar et al. 2021; Jyotisha and Qureshi 2022). This approach hereby eliminates some evaluations that have suggested that additional antigens may likely cause inflammation which are necessary for the immunogenicity of innovative vaccines in addition to identifying every antigen that may be investigated using conventional methods (Coscolla et al. 2015; Parvizpour et al. 2020).

In order to promote immunity and elicit both cellular and humoral immune responses to combat the cholera sickness, this study developed a novel multi-epitope peptide vaccine encompassing the cytotoxic T lymphocyte (CTL), helper T lymphocyte (HTL), and linear B lymphocyte (LBL) epitopes employing immune-informatics techniques in the development process. Using an in silico simulation, it was demonstrated that the constructed vaccine is capable of inducing immunological upon injection. The vaccine's effectiveness and safety were assessed using physicochemical, molecular docking, and thermodynamic stability profiling approaches. Our study lays the groundwork for the experimental creation of a potent cholera vaccine.

Materials and methods

Retrieval of Vibrio cholerae polyprotein

In this research work, the best potential candidate protein sequences of *Vibrio cholerae*, O1 and O139 strain, were retrieved from NCBI Protein Database (https://www.ncbi.nlm.nih.gov/) in FASTA format for constructing multi-epitope vaccine by the use of immune-informatics approach (Dong et al. 2020). The protein sequences used were partial sequences (Shahab et al. 2022).

Antigenicity and allergenicity prediction

Antigenicity prediction was carried on the retrieved sequences using VaxiJen 2.0 webserver (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html (Abraham Peele et al. 2021). The server carries out the prediction of antigenic and non-antigenic amino acid sequences based on physicochemical properties only at a default threshold of 0.4 (Doytchinova and Flower 2007; Zaharieva et al. 2017; Habib 2020). All the sequences that passed the antigenicity test were further subjected to AlgPred webserver (https://webs.iiitd.edu.in/raghava/algpred/submission.html), an online tool to determine the allergenicity. The server uses six different approaches for the allergenicity prediction with 85% accuracy at 0.4 thresholds (Saha and Raghava 2006).

Cytotoxic T lymphocyte (CTL) epitope prediction

Cytotoxic T lymphocyte's prediction of *V. cholerae* antigenic proteins were carried out using an online webserver- NetCTL (http://www.cbs.dtu.dk/services/NetCTL/) (Banerjee et al. 2020). The threshold was set to 0.75 to carry out the CTL epitopes prediction (Stranzl et al. 2010; Larsen et al. 2007).

Helper T lymphocyte (HTL) epitope prediction

Helper T lymphocyte's prediction was carried out on the antigenic protein sequences using IEDB tool (http://www.iebd.org/immunogenicity/) according to the method of Vita et al. (2019). The human leukocyte antigen class II (HLA-DR) supertype alleles (DRB1*03:01, DRB3*01:01, DRB3*02:01, DRB3*02:02, DRB5*01:01) were selected, and three different methods were used in the prediction of the HLA-II epitopes; they are Consensus (smm/nn/sturniolo), Consensus (comb.lib./smm/nn), and NetMHCIIpan. The peptide affinity for each of the receptors is based on the IC50 score given to each and every predicted epitope.

The same server was used for immunogenicity prediction as well, using major histocompatibility complex (MHC) class I immunogenicity (Vita et al. 2015).

Prediction of B cell epitopes

The B cell epitopes were predicted with an online tool server, BCpred (http://ailab-projects1.ist.psu.edu:8080/bcpred/SimpleServlet), and all parameters were left at default. The peptides, scores, and start position were shown by the predicted B cells (Chen et al. 2007).

Toxicity prediction

The predicted CTL, HTL, and B cell epitopes were submitted to ToxinPred, an online web server (http://crdd.osdd.net/raghava/toxinpred/) for toxicity prediction. The server classifies the epitopes as toxic and non-toxic based on their physicochemical properties (Gupta et al. 2013).

Construction of multi-epitope vaccine sequence

The predicted CTL, HTL, and B cell epitopes, sorted out through the discussed immune-informatics approach were used to design the vaccine construct. The adjuvant used was APPHALS, then EAAK linkers were used to link the adjuvant with the CTL epitope. The CTL epitope was linked with the 8 HTL epitopes using GPGPG linkers, while the 14 B cell epitopes were linked to the HTL epitopes with GPGPG linkers (Nagamune 2001).

Population coverage prediction

The epitopes selected for the *Vibrio cholerae* vaccine design must be predicted for their ability to binds to HLA molecules across different human population and the likelihood of it inducing long-lasting immune response in this population. IEDB population coverage tool was used in the prediction of the epitopes with high percentage of been presented in the context of HLA molecules to induce immune response in population (Bui et al. 2006). The endemic regions are selected for the population coverage.

Allergenicity prediction of the vaccine

Allergenicity prediction of the constructed vaccine was done using AlgPred (http://www.imtech.res.in/raghava/algpred/). The server used six different approaches for the prediction with 85% accuracy at 0.4 thresholds (Saha and Raghava 2006).

Antigenicity prediction of the vaccine

VaxiJen (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) was used for antigenicity prediction of the vaccine construct with bacteria selected as a model organism at a threshold of 0.4. The prediction of antigenic and non-antigenic amino acid sequences was carried out based on physicochemical properties only (Doytchinova and Flower 2007).

Physicochemical property prediction

ProtParam web tool (http://web.expasy.org/protparam/) was used for the examination of the physicochemical properties of the constructed sequence. Evaluation of the constructed amino acid sequence, the vaccine's amino acid composition, in vivo/in vitro half life, theoretical pI, GRAVY (grand average of hydropathy) instability index, and molecular weight were all predicted (Gasteiger et al. 2005).

Secondary structure prediction for the vaccines

Secondary structure prediction of the constructed vaccine sequence was done using PSIPRED tool (http://bioinf.cs.ucl.ac.uk/psipred/), an online web-based server that performs secondary structure prediction given accurate result (Jones 1999).

Tertiary structure prediction

Tertiary structure prediction of the constructed sequence for the vaccine was carried out using I-TASSER (https://zhanggroup.org/I-TASSER/). The server generated 5 models, and the model with the highest C-score was selected (Yang and Zhang 2015) as predicted and validated by Zhou et al. (2022) to also model small protein structure.

Tertiary structure refinement

The final vaccine 3D structure was refined using Galaxy Refine tools (http://galaxy.seoklab.org/refine) (Adero et al. 2021). For the repacking of the amino acid sequence and side chain reconstruction, the server uses CASP10 refinement method. Also, the same method is employed for molecular dynamic simulation to relax the 3D structure of the query protein.

Molecular docking of vaccine

ClusPro 2.0 (https://cluspro.org), an online bioinformatics sever, was utilized for the docking of the vaccine with human TLR-4 accessed through PD ID of 4G8A from Pubchem (Ezediuno et al. 2021; Sanami et al. 2021). Twenty-six models were generated in respect to low energy, and large size

was selected as it indicated a good interaction between the receptor and ligand.

In silico cloning and codon optimization of vaccine protein

The codon optimization was significant for the expression of vaccine structure in a host *E. coli* (strain K12) as the usage of a codon is comparatively different in *E. Coli* than the native host. Rho-independent transcription, restriction cleavage sites, and prokaryotic ribosome-binding sites were eluded by considering three extra options. Java codon adaptation tools provide the output in terms of CAI (codon adaptation index) and GC content in order to confirm the high level of protein expression. For cloning, the final vaccine construct in *E. coli* pET-28a (+) vector modification N- and C-terminal with Xhol and Notl restriction sites were performed, respectively. Finally, for expression, the prepared optimized sequence, along with the restriction sites were incorporated into the pET-28a (+) vector utilizing the SnapGene tool (https://www.snapgene.com) (Grote et al. 2005).

Immune simulation

To understand the molecular dynamics of the human immune system in response to designed vaccine, the relationship between the human immune system and vaccine construct was predicted using C-ImmSim 10.1 (https://kraken.ian.rm.cnr.it/C-IMMSIM/index.php?page=1), a server that uses agent-based modeling (Rapin et al. 2010). PSSM method was used to assess the production of cytokines and other substances like interferon and antibodies. Also, the response for T helper cell 1 and T helper cell 2 (Th1 and Th2) was predicted with the parameters left at default to measure the diversity or Simps Index by the server.

Results

Retrieval of antigenic proteins, antigenicity, and allergenicity prediction

A hundred (100) protein sequences of *Vibrio cholerae* were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/) out of which only 46 passed the antigenicity and allergenicity analysis. Five structural polyproteins toxin-coregulated pili (TCPA and TCPF), outer membrane protein (OMPW and OMPU), and cholera toxin B subunit (CTB) of *Vibrio cholerae* with an antigenic probability score of greater than 0.8 were considered further for vaccine construction.

Cytotoxic T lymphocyte epitopes prediction

From all the 46 protein sequences that passed antigenicity and allergenicity analysis, a total of 290 epitopes were generated, out of which 195 were toxin-coregulated pili and others were cholera toxin subunit. However, based on MHC class 1 binding affinity, toxicity, allergenicity, and immunogenicity, only one CTL epitope as shown in Table 1 was selected; this is due to the fact that other epitopes did not pass the allergenicity test.

T lymphocyte epitopes prediction

Five reference sets of human alleles which includes HLA-DRB1*03:01, HLA-DRB3*01:01, HLA-DRB3*02:01, HLA-DRB3*02:02, and HLA-DRB5*01:01 were used for the prediction. A total of 230 HTL epitopes were subjected to the same predictions as CTL epitopes, in which only 8—208–222 (HLA-DRB5*01:01), 93–87 (HLA-DRB3*01:01), 248–262 (HLA-DRB3*02:01), 88–102 (HLA-DRB1*03:01), 84–98 (HLA-DRB1*03:01), 83–97 (HLA-DRB1*03:01), 130–144 (HLA-DRB1*03:01), and 85–99 (HLA-DRB1*03:01)—epitopes passed the predictions, and these were used for the designing of the vaccine (Table 2).

Prediction of B cell epitope

Out of the five cholera group of proteins, only 14 TcpA protein passed the allergenicity and toxicity test. These 14 linear B cell epitopes were used for the construction of multi-epitope vaccine using BCpred web server (http://ailab-projects1.ist.psu.edu:8080/bcpred/SimpleServlet) with the defaulted parameters. The predicted B cell epitopes shows (Table 3) the peptides, scores, and start position.

Construction of multi-epitope peptide vaccine

The final vaccine construct was designed by the combination of adjuvant and epitopes through the use of some linkers. 1 CTL, 8 HTL, and 14 B cells were made use in the designing of the subunit vaccine construct. With the use of EAAAK linker, the adjuvant was fused with the CTL epitope at the N-terminal; 8 HTL and 14 B cells were fused through the use of GPGPG linkers Figs. 1, 2 and 3.

Table 1 Non-toxic and non-allergen CTL epitope with its combined score and immunogenicity score

Protein name	Epitopes	Sequence	Combined score	Immunogenicity score
TcpA Partial	AAL09686.1	LTDFETTQA	0.9916	0.00388

Table 2 List of final non-toxic and non-allergen HTL epitopes with their prediction methods and percentile rank, respectively

Proteins	Allele	Position	Peptide sequence	Method	Percentile rank
CTB	HLA-DRB1*03:01	85–99	AIERMKDTLRIAYLT	Consensus (smm/nn/sturniolo)	24.00
CTB	HLA-DRB1*03:01	83–97	KKAIERMKDTLRIAY	Consensus (smm/nn/sturniolo)	20.00
CTB	HLA-DRB1*03:01	84–98	KAIERMKDTLRIAYL	Consensus (smm/nn/sturniolo)	19.00
CTB	HLA-DRB1*03:01	88–102	RMKDTLRIAYLTEAK	Consensus (smm/nn/sturniolo)	17.00
TCPA	HLA-DRB1*03:01	130–144	KTLITSVGDMFPYVL	Consensus (smm/nn/sturniolo)	9.60
TCPF	HLA-DRB3*01:01	73–87	PTSQDMFYDAYPSTE	Consensus (comb.lib./smm/nn)	6.50
TCPF	HLA-DRB3*02:01	248–262	DVQFKVLVGVPHAET	NetMHCIIpan	6.30
TCPF	HLA-DRB5*01:01)	208–222	YPHIKVYEGTLSRLK	Consensus (smm/nn/sturniolo)	3.10

Table 3 List of final non-toxic and non-allergen B cell epitopes with their start positions and scores, respectively

Accession no	Sequence	Start position	Score
TcpA (*Vibrio cholerae*)	DLNDFETNAANAAAGTGIIK	146	1
TcpA (*Vibrio cholerae*)	GDLTDFETTPGAADTGIGVI	143	0.999
TcpA (*Vibrio cholerae*)	AAAGTGTGIIKSIAPTSVNL	180	0.999
TcpA (*Vibrio cholerae*)	QTYRSLGNYPTTADANAAAA	74	0.996
TcpA (*Vibrio cholerae*)	TQTYRGLGQYPATADGTAAA	46	0.995
TcpA (*Vibrio cholerae*)	DLGDFETGPANAVTGKGIIK	145	0.989
TcpA (*Vibrio cholerae*)	QTYRSLGNYPATADATAAAA	48	0.987
TcpA (*Vibrio cholerae*)	KKFVKEEHDKKTGQEGMTLL	10	0.984
TcpA (*Vibrio cholerae*)	QTYRSLGNYPTTADAAAAAA	49	0.983
TcpA (*Vibrio cholerae*)	LGKISPDEAKNPFTGTDMNI	76	0.978
TcpA (*Vibrio cholerae*)	LIQTYRGLGNYPETTDDTAA	47	0.974
TcpA (*Vibrio cholerae*)	LGKISPDEAKNPFTGADMNI	76	0.97
TcpA (*Vibrio cholerae*)	VQVSMTQTYRALGNYPATAN	52	0.935
TcpA (*Vibrio cholerae*)	LVTSVGDMFPYINVQEKAAV	122	0.91

Population coverage

The population coverage of the CTL epitopes across the endemic regions in Africa using the references HLA-A, HLA-B, and HLA-C alleles and that of HTL epitopes across the same regions using the references, HLA-DP, HLA-DQ, and HLA-DP selected during the MHC binding predictions are depicted in Table 4, while Fig. 4 is showing the percentage population coverage of the combined MHC molecules.

Prediction, validation and refinement of 3D structure

I-TASSER was used for the construction of the 3D structure. Five models were generated, out of which the best was selected based on the C-scores, the model with high C-score was selected because C-score of a higher value signifies a

Table 4 The population coverage of cholera vaccine epitopes

Population/area	Class I coverage	Class II coverage	MHC class combined
Burkina Faso	56.43%	12.76%	67.63%
South Africa	57.63%	23.61%	71.46%
Kenya	71.96%	23.03%	71.96%
Morocco	78.0%	64.38%	92.16%
Senegal	69.36%	6.49%	71.35%
Sudan	67.55%	43.6%	81.7%
Tunisia	75.43%	51.08%	87.98%
Uganda	74.81%	20.81%	74.81%
Zambia	81.65%	12.76%	81.65%
Zimbabwe	69.26%	22.21%	76.09%
Average	70.21	30.89	60.41

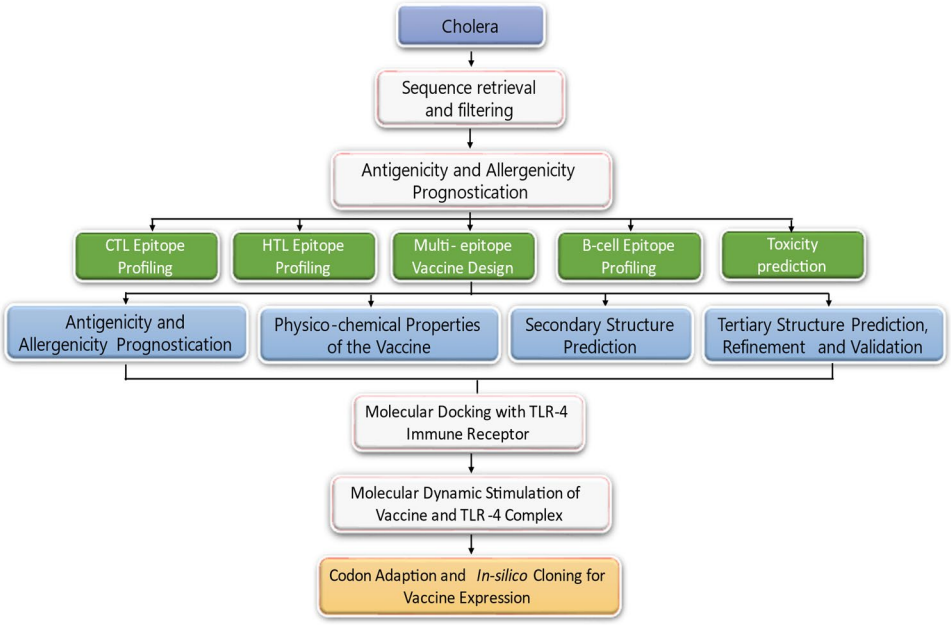


Fig. 1 Systematic flow diagram for cholera vaccine construct. It is a flow chart of all the key steps involved in creating potential T cell, B cell, and multi-epitope vaccines against cholera. The details of the various steps are described in this section

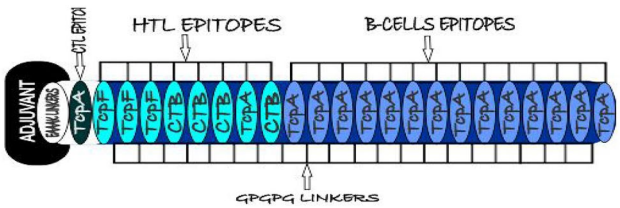


Fig. 2 Construction process of the vaccine

model with a higher confidence and vice versa. For refinement of the vaccine construct, Galaxy Refinement server was used in which five refined model were given but the best of them all was chosen based on RMSD, Mol Probity, High GDT-HA score, poor rotamers, and Rama favored. Model 5 3D structure is provided below. The model structure was chosen based on its structure information such as GDT-HA score of 0.9437, RMSD score of 0.450, Mol Probity calculated as 2.259, low clash score of 15.7, poor rotamers calculated as 0.5, and the Rama favored estimated to be 89.5 which is the highest and it is far better than other models of the vaccine, as shown in Table 5. Therefore, model 5 was

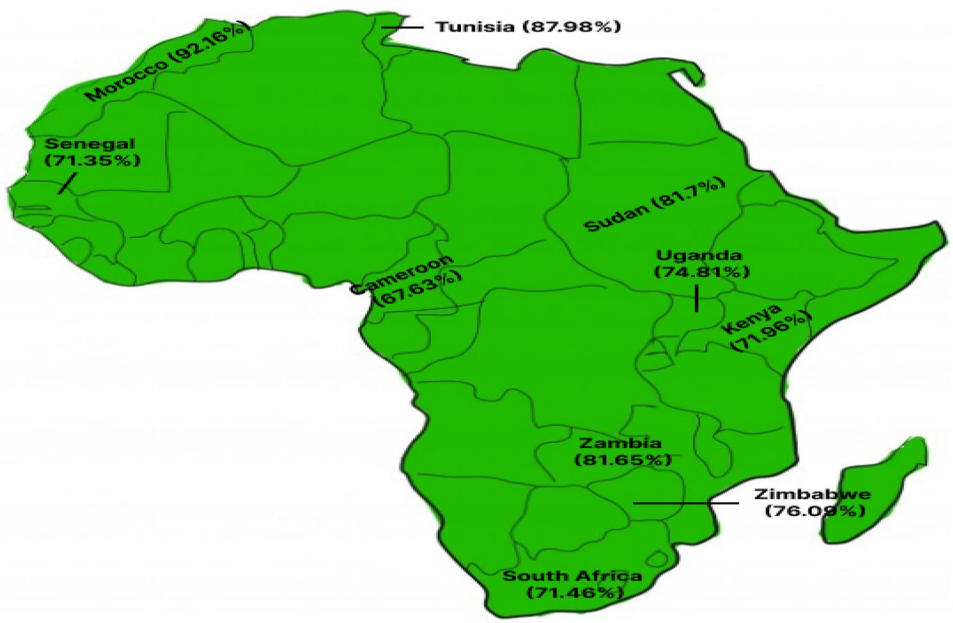


Fig. 3 Epitope population coverage of the designed vaccine in endemic region of Africa

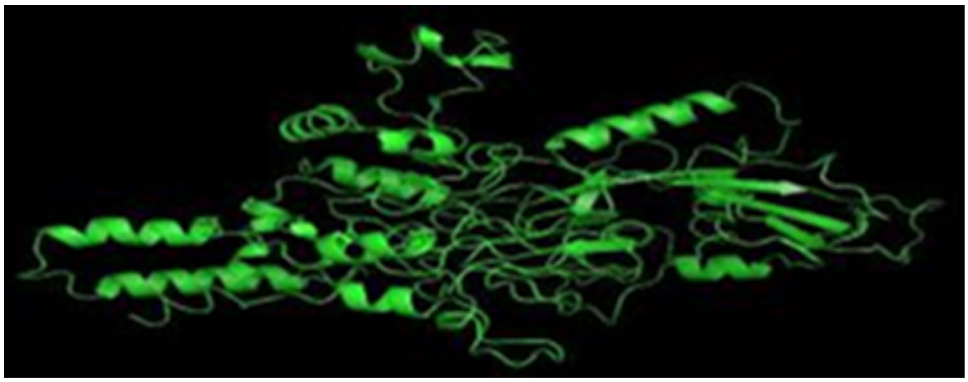


Fig. 4 The peptide structure of the vaccine

selected as a refined vaccine structure out of the five refined models for further analysis. The 3D model of the vaccine selected was then subjected to structure assessment stage, and these includes ProCheck for protein model showing that 76.1% of amino acid are plotted in the most favorable region, 18.8% in allowed regions, and 2.7% in disallowed region. Also, ProSA-web and ERRANT estimated and verified the whole quality of the basic 3D model. The overall quality calculated by ERRANT was 64.02% and ProSA-web displayed a score of − 7.88 for the 3D model of the vaccine construct, which authenticate the precision of the vaccine. Result form ProSA-web and RAMPAGE are given in Fig. 5.

Antigenicity and allergenicity assessment of the vaccine construct

Vaxijen and AlgPred servers were used for the prediction of antigenicity and allergenicity of the vaccine sequences, respectively, at a threshold of 0.4 for bacteria; the antigenicity server confirms that the candidate vaccine is antigenic with a score of 0.7844 and with the nearest protein in UniProtKB accession number Q86UU0, defined the vaccine as non-allergen on AlgPred.

Physiochemical properties of vaccines

The physiochemical properties of the designed vaccine sequences were predicted via ProtParam. Theoretical isoelectric point (theoretical PI) was predicted to be 6.09, molecular weight as 53.85 kDa, the estimated half-life for in vitro for mammalian reticulocytes is 4.4 h, while for in vivo in yeast and *Escherichia coli* is > 20 and > 10, respectively. The vaccine sequence aliphatic index was computed to be 60.32, which indicate the thermostability of the vaccine, and was classified as stable as the instability index (II) is computed to be 13.99 and the hydrophilic nature of the vaccine sequence was shown as the negative grand average of hydropathicity (GRAVY) was − 0.435.

Secondary structure prediction

In the secondary structure prediction of the vaccine construct which was carried out by PSIPRED, alpha-helix (Hh) was computed as 18.06%, extended strand (Ee) as 18.81%, and random coil (Cc) as 58.29%. Graphical representation of the secondary structure elements is shown in Fig. 6.

Molecular docking of constructed vaccine with human-toll like receptor-4

TLR-4 having PDB ID of 4G8A was selected as the receptor of choice for docking the vaccine which was carried out through a bioinformatics web server known as ClusPro 2.0 server. Twenty-six models were generated, but the best of it all in respect to low energy and large size was selected as it indicates a good interaction between the receptor and ligand. Graphically, docked complex intermolecular conformation and chemical interactions are presented below (Fig. 7).

Table 5 Galaxy Refined results for vaccine construct. Model was selected based on its low Mol Probity and high Rama favored score

Model	GDT-HA	RMSD	Mol Probity	Clash score	Poor rotamers	Rama favored
Initial	1.0000	0.000	2.552	22.7	1.1	83.6
MODEL 1	0.9455	0.442	2.281	15.8	0.0	88.8
MODEL 2	0.9441	0.449	2.239	14.2	0.0	88.8
MODEL 3	0.9432	0.447	2.227	13.9	0.3	89.0
MODEL 4	0.9423	0.452	2.258	15.3	0.5	89.2
MODEL 5	0.9437	0.450	2.259	15.7	0.5	89.5

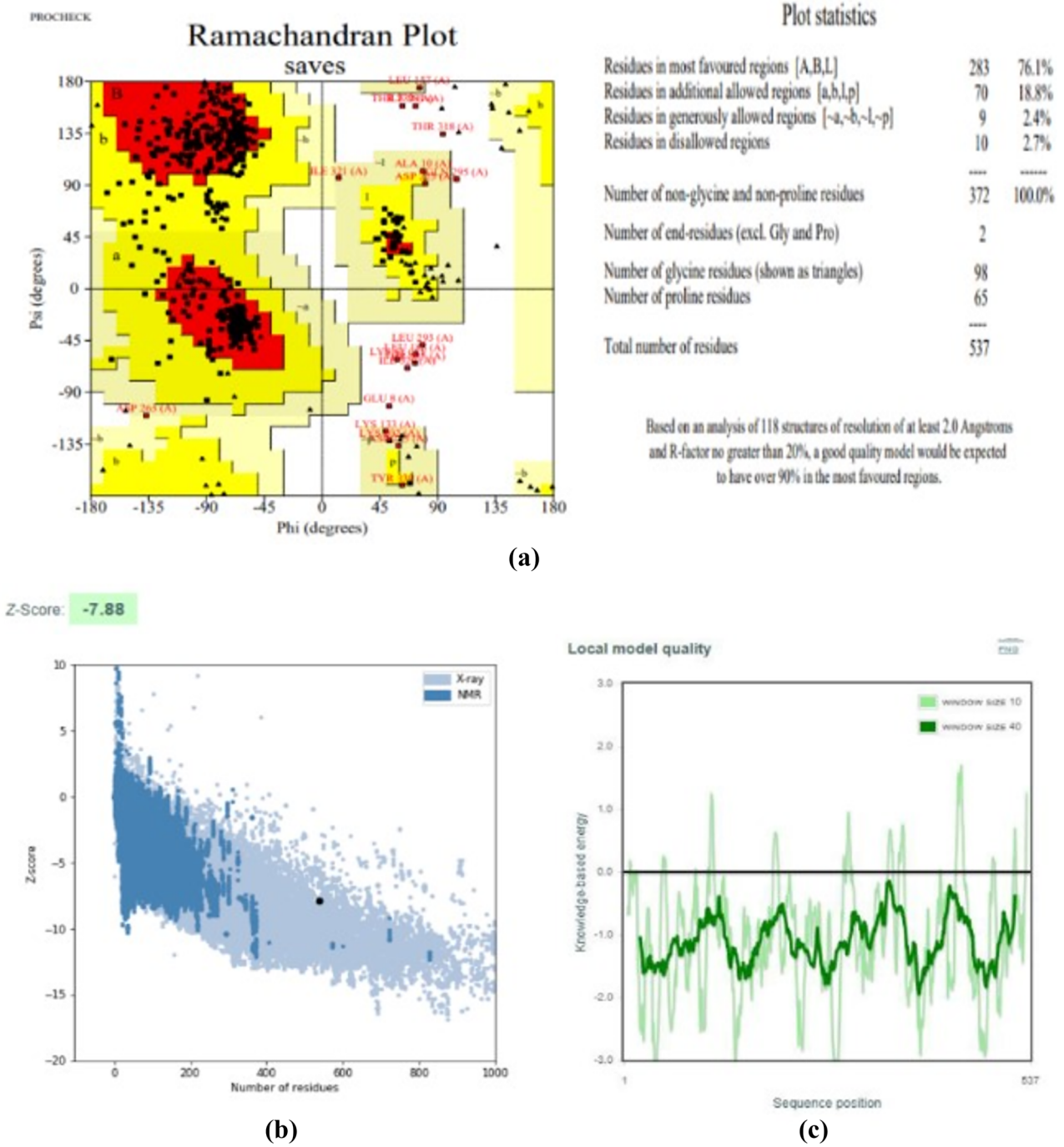


Fig. 5 3D structure refinement, evaluation, and prediction. **a** The use of rampage Ramachandran plot for quality assessment of the vaccine model; the black square denotes torsion angle distribution relative to the core (red) and allowed (yellow) regions. Residues are plotted in generously allowed (pale yellow) and disallowed (white) regions. **b** In ProsaWeb, the prosa plot score of the vaccine can be identified as a black dot in the plot of all PDB structures' quality scores of the same size. **c** ProsaWeb showing sequence position

Immune simulation of the multi-epitope vaccine

Through in silico immune simulation, the injection of vaccine doses, the human immune system response was observed. The antibody titer was very high after the injection, and this triggered the immune response. IgM + IgG titer was observed at 700,000 antigens count per ml. IgM was around 400,000 xx/ml and IgG1 was reported as 250,000 xx/ml. Interleukin and cytokine responses were also observed,while IFN-gamma and IL-2

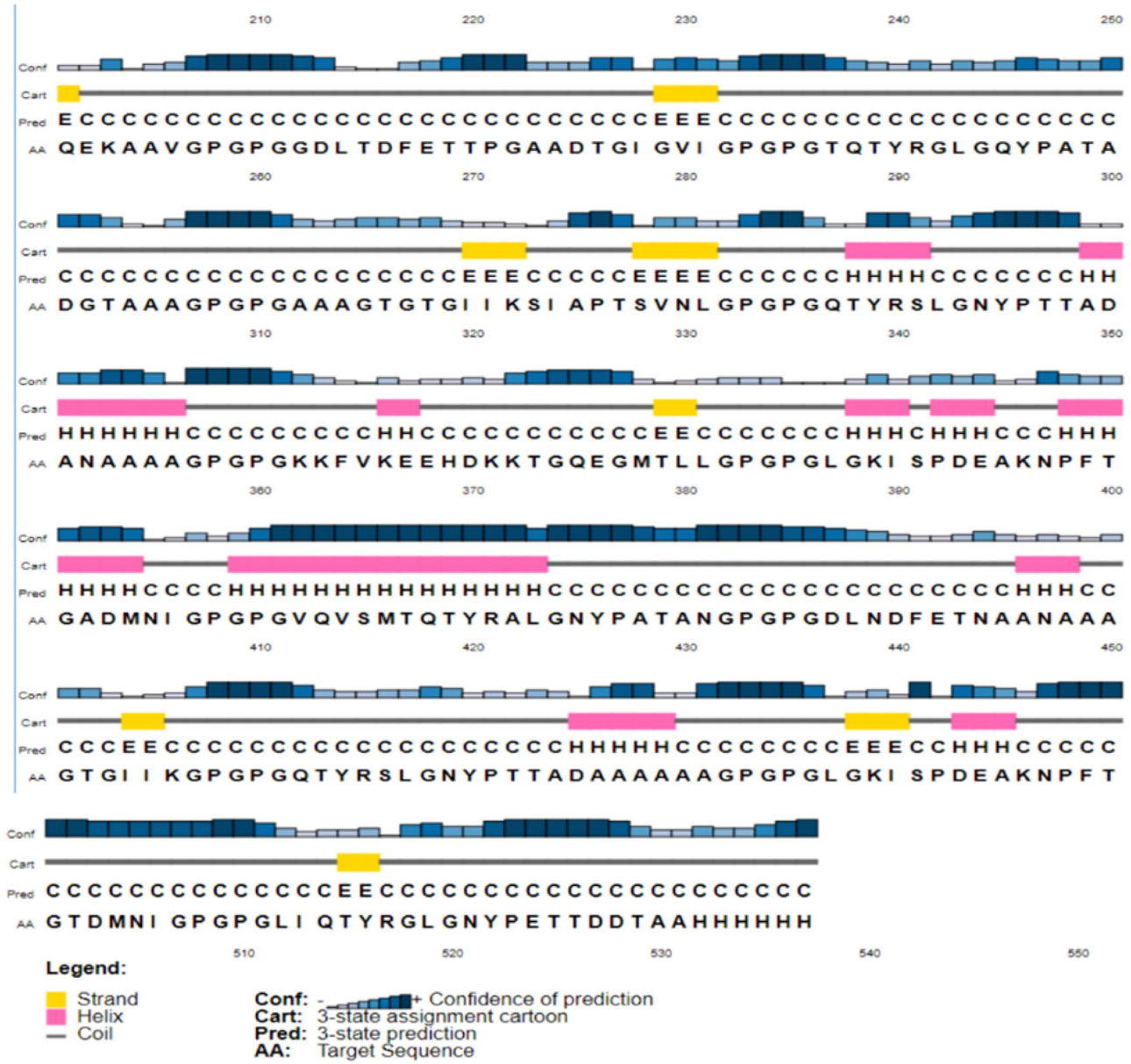


Fig. 6 Graphical representation of the secondary structure of the vaccine construct

titer were increased significantly. All these indicate the dependability and high immune-triggering response upon injection. It also shows that there is high cellular immune response and memory cells' development to pathogen recognition at re-occurrence. The population of the T cells, dendritic cells, phagocytic macrophages, and phagocytic natural killer cells were reported to be > 1150 cell/mm^3, 225 cell/mm^3, 225 cell/mm^3, 374 cell/mm^3, respectively. Therefore, all these results depicted in Fig. 8 denote that our vaccine candidate can trigger immune response effectively Fig. 9.

Codon adaptation and insilico cloning

Vaccine sequence design was modified using JCAT in reference to the *E. coli* K12 strain. The CAI value obtained was 0.1, and the GC content was 58.97%, indicating a greater level of vaccine design expression in the *E. coli* K12 strain. According to reports, GC levels between 35 and 70% have greater gene expression. The designed vaccine was cloned into the Pet-28a (+) plasmid using the restriction cloning tool SnapGene. To do this, suitable restriction enzymes XhoI and NotI were selected, and their restriction sites were

inserted at both ends of the modified nucleotide sequence (Habib 2020). The restriction sites for XhoI and NotI were added to the N- and C-termini of the optimized vaccine design and cloned in the pET-28a (+) vector as shown in Fig. 10.

Fig. 8 In silico immune simulation by the vaccine followed by injection. The immune response to the vaccine is demonstrated by antibodies titer. **a** Interferon and interleukins. **b** Natural killer cells. **c** Macrophages. **d** Cytotoxic T cell. **e** Dendritic cells. **f** Antigen and immunoglobulin

Discussion

The use of vaccines has greatly improved the world's health over a long period of time (Oli et al. 2020). In the past, developing vaccinations was thought to be the most practical method of preventing infectious diseases (Greenwood 2014). Herd immunity greatly reduces disease load, death, and disability (Helen and Shoubai 2020). Personalized vaccination is required for infectious organisms with complicated life cycles and antigenic diversity, and newly developing and re-emerging infectious diseases (ERID) bring additional challenges in vaccine development (Poland et al. 2016; Oli et al. 2020). With immunological findings and understanding of computational methods for epitope predictions, a new pattern of vaccine design has been revealed (Adhikari et al. 2018). Immune-informatics approaches are quick and efficient methods for discovering viable candidates for vaccine design using a pathogen's proteome (Khan et al. 2019). According to Oladipo et al. (2022), epitope-based vaccine design is advantageous, profitable, extremely stable, non-toxic, and easy to engineer in comparison to conventional vaccines, which may raise several issues in patients who are compromised in immune system which also agrees with Wang et al. (2022) on the use of nucleic based vaccine. In silico immune-informatics methods can be used to accurately predict B and T cell epitopes using a plethora of bioinformatics tools, according to Farhadi et al. (2015).

This can have an impact on the formulation of epitope vaccines. Utilizing highly immunogenic and precise B cell and T cell epitopes that are extracted from the pathogen's proteome, these multi-epitope-based vaccination peptides are made (Sajjad et al. 2020). Therefore, this method can not only identify all antigens that can be investigated using conventional approaches, but it can also identify additional antigens that are crucial to the immunogenicity of novel vaccines (Flower et al. 2010).

Consequently, cholera caused 2.8 million illnesses and 91,000 fatalities in more than 50 endemic nations. However, according to the most recent data, there were 69 different countries' total of 2.9 million illnesses and 95,000 fatalities (Ali et al. 2015). It is highly desirable to develop an efficient and reliable cholera epitope vaccination. The current study's objectives were to generate a cholera vaccine to lower worldwide mortality, and the findings will be validated by prior research in the field using the same approach with Banerjee et al. (2020). The majority of cholera vaccines now on the market or in development are inactivated (like Dukoral1, mORCAX1, and SancholTM) and live attenuated (like CVD103-HgR and Peru-15 or CholeraGarde1) (Holmgren 2021).

Nezafat et al. (2016) proposed that using immunostimulatory adjuvant (CTB) in epitope vaccine development, with the selection promiscuous epitopes from various *V. cholerae* protective antigens (OmpW, OmpU, TcpA, and TcpF) that bind to different HLA-II supertype alleles, and linking the epitopes with suitable linkers (EAAAK and GPGPG) to each other are important strategies to create CTL, HTL, and B cell epitopes were predicted using a variety of bioinformatics methods. The closest UniProt number, Q86UU0, which is designated as a non-allergen, antigenicity, and immunogenicity of synthetic peptides, was used to evaluate the allergenicity using the AlgPred service (Sharma et al. 2021; Adam 2021; Ghandadi 2022).

The immunization remained stable, nevertheless, as evidenced by the vaccine construct's molecular weight of 53.85 Da and predicted PI of 6.09. The vaccine construct's molecular weight of 53.85 Da and Pi of 6.09 show that it was

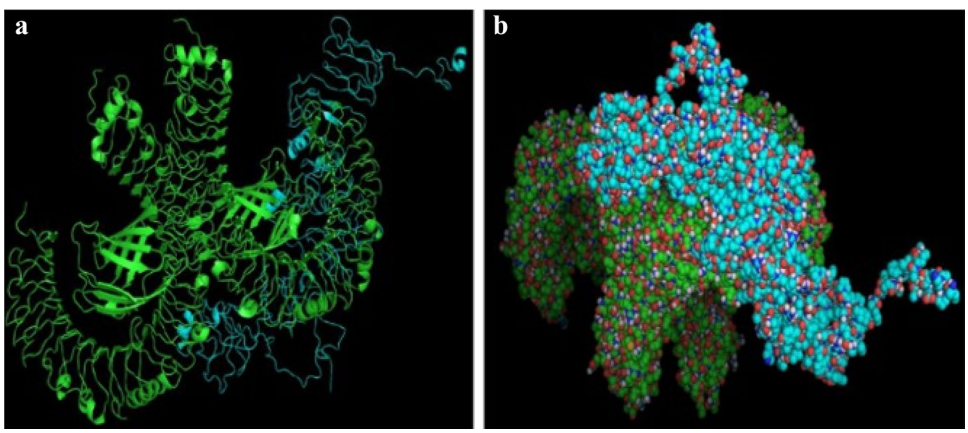
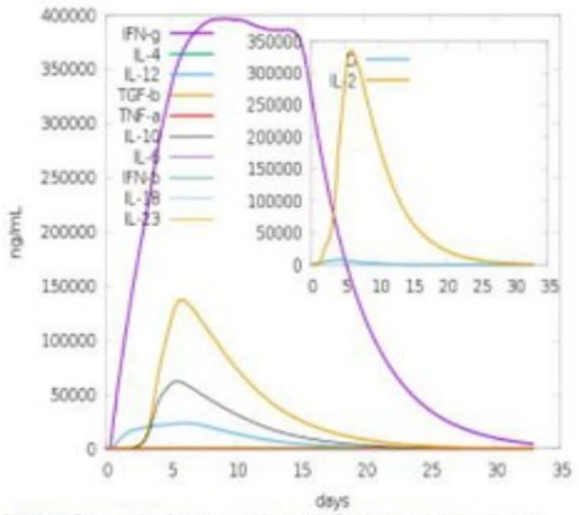


Fig. 7 Molecular docking of the vaccine with toll-like receptor-4. **a** Ribbon-like top-ranked model of docking assay. **b** Cartoon-like top-ranked model of docking assay

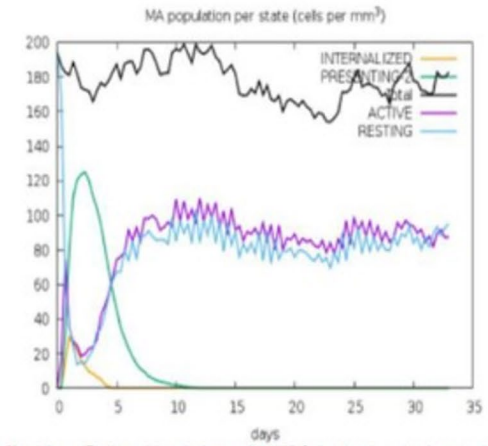


Cytokines. Concentration of cytokines and interleukins. D in the inset plot is danger signal.

a

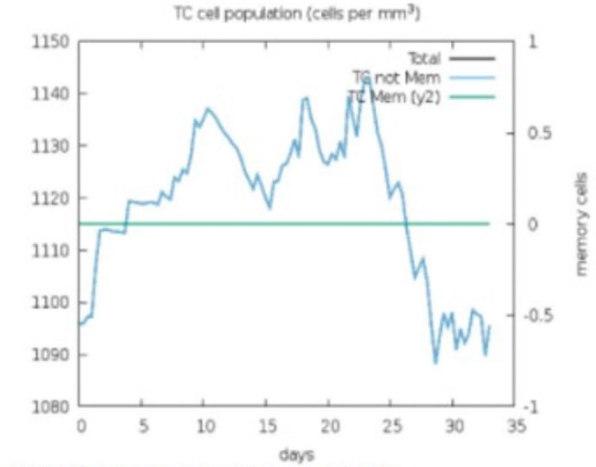
atural Killer cells (total count).

b



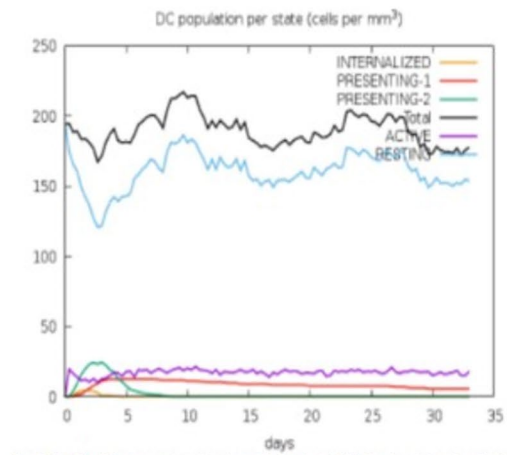
Macrophages. Total count, internalized, presenting on MHC class-II, active and resting macrophages.

c



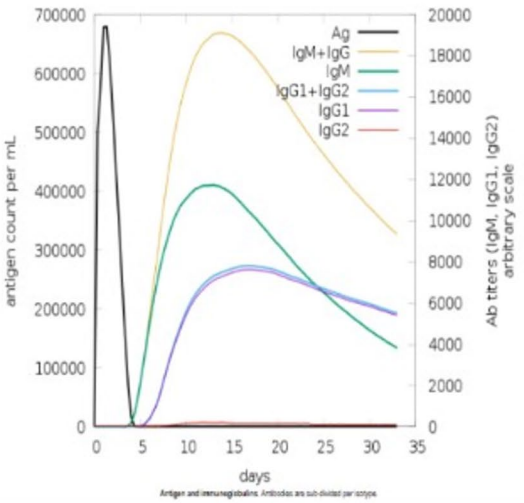
CD8 T-cytotoxic lymphocytes count. Total and memory shown.

d



Dendritic cells. DC can present antigenic peptides on both MHC class-I and class-II molecules. The curves show the total number broken down to active, resting, internalized and presenting the ag.

e



f

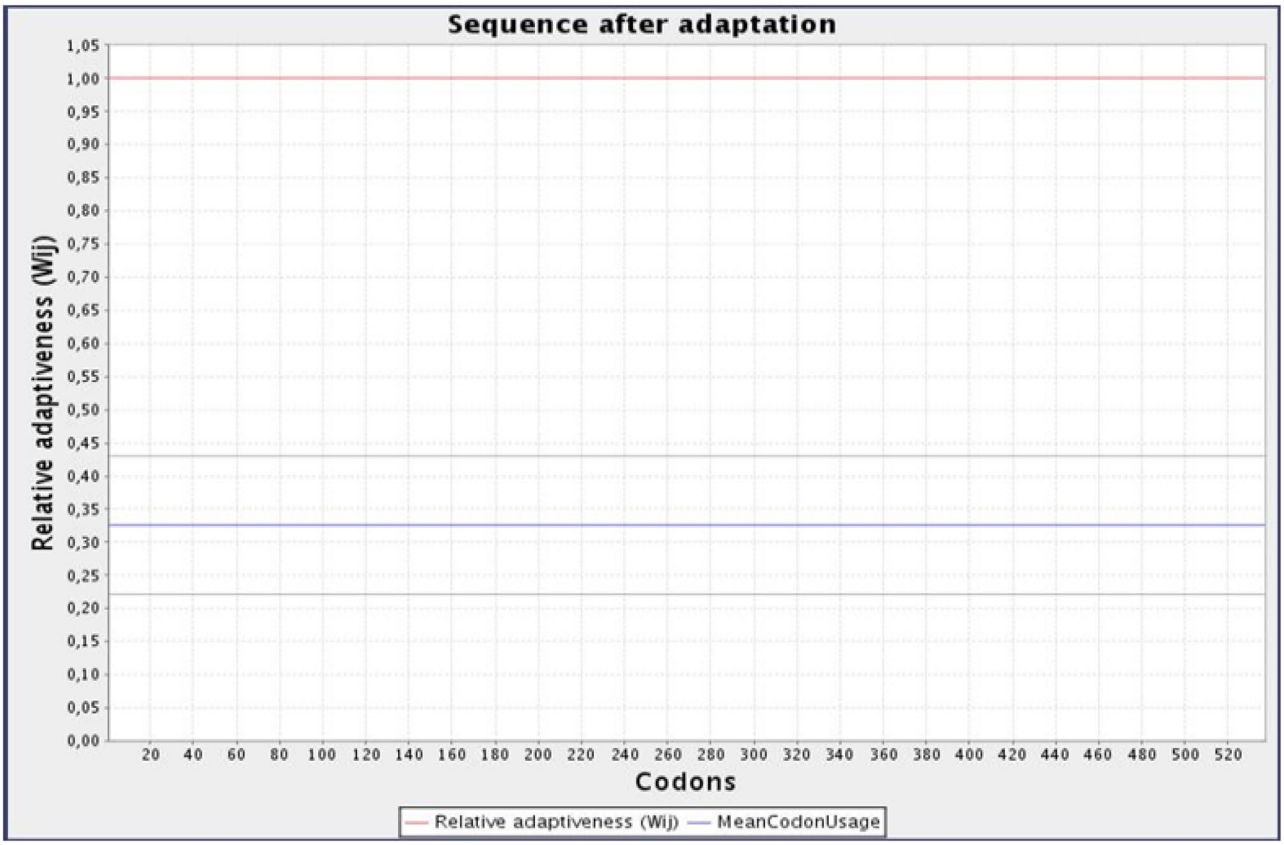


Fig. 9 Codon adaptation using *E. coli* K12 strain

stable. Cholera epitopes of various protective antigens (Ompu, Ompw, TcpA, and TcpF) that bind to different HLA-II supertype alleles with suitable linkers (EAAAK, GPGPG) are integrated into immune-stimulating extra vesicular CTB combined with CTB (Nezafat et al. 2016; Wieczorek et al. 2017).

To generate more precise epitopes that are limited to particular HLA-II supertype alleles, three servers (IEBD, SVMHC, and PSIpred) were employed, each with a different technique (Nielsen et al. 2010, 2020). B cell epitope is also necessary for the immunization to boost antibody response. The antigens ompU, ompw, TcpA, and tcpF were used to represent the frequently occurring area between mixed epitopes and B cell epitopes.

Because of its dual role in the formation of the epitope vaccination, the later region of the antigen was chosen. First, it limits the emergence of junctional epitopes (neoepitopes), and second, it improves immune processing and displays HLA-II binding epitopes (Livingstone et al. 2002). This area was joined using short amino acid linkers, particularly the GPGPG linker. To prevent contact with other vaccine segments, helical linkers were introduced to the N- and C-terminals, which improved the separation. CTB is a non-toxic subunit that acts as a mucosal immunostimulatory adjuvant, stimulating both mucosal and systemic immune responses (Stratmann 2015; Lavelle and Ward 2022). The population coverage of the designed cholera vaccine show that the designed cholera vaccine can induce immune response in an average of about over 60% of African population that cholera has been found to be endemic.

I-TASSER was used to simulate the initial 3D structure of the protein vaccine which has been reported to also modelled 3D of small protein sequences as validated by (Zhou et al. 2022). The model has to be modified, according to the results of the ProSA-web plot analysis of the preliminary version (Yang and Zhang 2015). After loop refinement and energy reduction of the core model, a high-quality 3-D standard necessary for the identification of conformational B cell epitopes and docking techniques was attained.

The CTB region of the vaccine and TLR2 were successfully docked by Cluspro (Kozakov et al. 2017). Due to CTB's strong sequence and structural similarity to heat-labile enterotoxin B (LTB) and TLR2's functional characteristics, an adequate docking model may be achieved even though there is no direct evidence of interaction between CTB and TLR2. The immunological and physicochemical characteristics of the protein vaccine were assessed (Dey et al. 2014). The vaccine is antigenic and also non-allergenic, according to our findings.

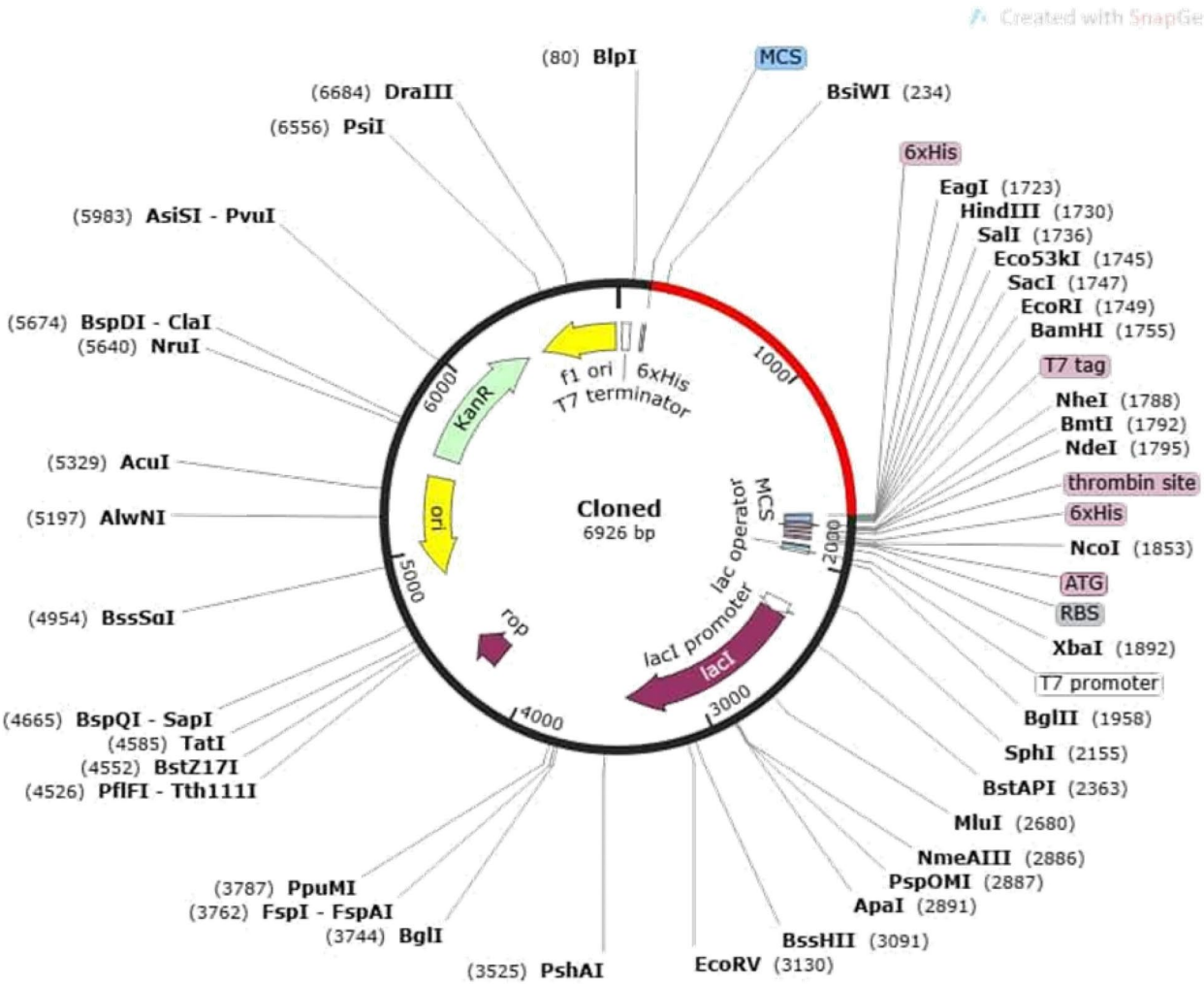


Fig. 10 Cloned vaccine using Pet-28a(+) vector

Additionally, the vaccine demonstrated the appropriate level of solubility when it was overexpressed. The protein vaccines has high aliphatic index and high thermal stability. Finally, by modifying the DNA codon for production in the *E. coli* host and adding limit sites to gene flanks, the epitope vaccine was cloned in silico suggesting it a good candidate vaccine for *Vibrio cholerae* infection, and therefore, there is need for the use of wet laboratory techniques for further validation of the in silico prediction.

Conclusion

In our study, we used an immune-informatics approach to design a potential multi-epitope vaccine using six different groups of protein from vibrio cholerae. This cholera vaccine has the potential to provide prophylactic benefits. However, this in silico work requires experiment validation for confirmation, which will be quickly followed by in vitro and in vivo research to ensure the immunogenicity, wholesomeness, and safety of the potential vaccine.

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Author contribution EKO: Conceptualization, experimental design. OEA, GMA, FOB, ROA, HOA, KTK, JAO, GAA: Data retrieval, analysis, and wrote the first draft of the manuscript. SEO, TOO, OPA, OSH, ATO: Data analysis and result interpretation. EMJ, BAI, AFA, MOI, HMA, UAI: Result interpretation. EOF, OAO, DAA, SAA, FDO, OPO, OOB, SBA, MOB: Manuscript review and editing; all authors participated in the review of the final edition of the manuscript.

Data availability statement All data analyzed in this study are publicly available data at the National Centre for Biotechnology Information GenBank repositories (https://www.ncbi.nlm.nih.gov) and which could be made available upon request.

Declarations

Competing of interest The authors declare no competing interests.

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