In-silico Innovative mRNA Vaccine Development Using Multi-Epitopes of SopD Protein for Enteric Fever Caused by *Salmonella enterica*

Aaiza Mumtaz[1*], Hadia Hussain[1], Muhammad Umair[1], Wasla Ali[1], Muhammad Sajid[1]

[1]Department of Biotechnology, University of Okara, Pakistan.

ABSTRACT

An increase in antibiotic resistance has created significant challenges in treating *Salmonella enterica* infections. Consequently, various vaccines have been developed as practical alternatives to antibiotics for preventing *S. enterica* infections. mRNA vaccine technology is rapidly advancing as a replacement for conventional methods due to its high efficiency, low cost, and ability to elicit a strong humoral immune response. This research aims to develop a novel mRNA vaccine against *S. enterica* using immunoinformatics approaches. The protein SopD was selected, and its suitable epitopes were identified. These epitopes were evaluated to ensure they are antigenic, non-allergenic, and non-toxic. Subsequently, the epitopes were linked using appropriate linkers to create a vaccine construct. This construct was further analyzed and subjected to molecular docking with the Toll-like receptor TLR3 using the HDock server. Molecular dynamics (MD) simulations showed that the vaccine construct is stable based on RMSD and RMSF parameters. Immune simulation indicated the vaccine's efficacy, and it was successfully cloned using the SnapGene tool. Finally, a multi-epitope protein was modeled and optimized. The results demonstrated that the vaccine construct is effective, non-allergenic, non-toxic, and successfully cloned. Overall, the findings suggest that the designed mRNA vaccine construct could be a promising candidate for *S. enterica* treatment, pending validation through in vitro techniques such as ELISA and in vivo testing in animal models.

Keywords: Enteric fever, Epitopes, Gastroenteritis, *S. enterica*, Septicemia.

1. INTRODUCTION

Typhoid fever is commonly known as enteric fever which is caused by the food or water which is contaminated with bacteria. It is characterized by a prolonged high fever lasting for weeks. It affect majorly to your small intestine and shows symptoms like high fever, chills, cough, muscle aches and rose spots like rash [1].

Salmonella enterica can easily be transmitted from person to person through objects and surfaces contaminated with the bacteria. It is considered a serious health concern, especially in children, due to their weaker immune systems. Enteric fever, if left untreated, can be fatal [2]. Moreover, it is more common in people that travel from one place to another [3]. Symptoms typically do not appear within the first week, but after 7 to 14 days, a high fever develops and can become severe. In infants and children, diagnosis may be delayed until serious complications arise beyond just the fever. Diagnosis is usually made clinically based on physical examination and the patient's history of exposure [4].

Three major types of diseases are involved in typhoid i.e. gastroenteritis, septicemia, and enteric fever. Gastroenteritis is the inflammation of digestive system which may result in infection. It is considered as short-term illness and includes symptoms like diarrhea, abdominal cramps and vomiting condition. Virus, parasites, bacterial toxins, various chemicals and drugs are

[*]***Corresponding author: Aaiza Mumtaz***
aaizamumtaz07@gmail.com
Received: 23/6/2024 Accepted: 22/8/2024.
DOI: https://doi.org/10.35516/jjps.v18i2.2819

the reason behind the cause of gastroenteritis. Good hygiene is necessary for the prevention from the spread of disease in other people [5]. Septicemia is also known as sepsis which is the utmost response to infection in the human body. It is considered as the blood poisoning by the contamination of microorganisms like bacteria, fungi, and viruses [6]. It can cause various types of infections i.e. infection in intestine, lungs, urinary tract, and responsible for the skin infection. It may also cause organ failure, damage of tissues, and death of the patient. It can be treated with the antibiotics, avoiding the source that is the cause of infection. Enteric fever is also been a health problem in public, and can be fatal if it is not treated [7].

Enteric fever or typhoid fever symptoms swiftly develop in four stages, these stages are as stage 1 is the initial stage of typhoid which occur within five to fourteen days after getting in contact with the bacteria. The symptom of this is fever which gets higher and higher over days and is known as stepwise [8]. At this stage, bacteria keep on moving in the blood of the patient. In stage 2, which is the second week of the fever, bacteria began to multiply in the part of human body immune system that is responsible for the identification of the harmful invaders known as Peyer's patches. At this point, patient start facing symptoms like stomach pain leading to diarrhea. Moreover, rashes or red spots appear on the body [9]. Stage 3 is the point at which no antibiotic will work to prevent the bacterial infection and can cause serious damage. This stage is the third week of the infection and symptoms like internal bleeding or inflammation in your brain which is encephalitis appears in the patient [10]. Final stage is the 4^{th} stage, in which patient began to recover and fever gradually decreases. However, the bacteria may remain in your gallbladder without causing any symptoms.

Healthcare providers suggests the patient to take tests for the diagnosis of the disease by taking samples of your blood, urine, stool, or bone marrow [11]. X-ray can also be taken to look deeper changes in your lungs. Furthermore, it can be treated with antibiotics but some types of bacteria cannot be treated with antibiotics which include medicines like levoflaxin, cephalosporins, azithromycin, and cefixime [12, 13]. If the patient is severely ill, it might be given additional treatment like being admitted into the hospital for proper care and treatment. To reduce risk of being in contact with enteric fever, vaccination should be taken if you travel or live in the area where there are chances of getting in contact with the bacteria *Salmonella enterica* [14, 15]. Till now, there are two types of vaccines that were being used for the prevention of enteric fever but they do not helped in complete prevention and protection which included oral vaccine for typhoid in which four pills were taken after every few days but in December 2020, it was no more available in the market from the manufacturers [16]. Second type of vaccine is injectable vaccine that is needed almost two weeks before arriving in an area where *S. enterica* is common. This vaccine will develop your immunity by making it able to resist or fight with the bacteria and it is needed to build up immunity after every two years to stay protected from the infection. The children with the age of 2 years can also get this vaccination [17].

S. enterica is a gram-negative, motile, rod shaped pathogenic and harmful bacteria. There are further six subspecies and have 2,600 serotypes that are responsible for the cause of various infections in humans and animals [18]. According to various studies, *S. enterica* on biotic and abiotic surfaces forms biofilms such as glass surfaces, cabbage, polystyrene, stainless steels etc. The various qualities of *S. enterica* control the statement of cell parts during the development of biofilm. The ycfR is a profoundly managed quality which shows articulation under the pressure of chlorine and shows high harmfulness [19]. It assists the *S. enterica* with framing biofilms for the most part on the outer layer of glass and polystyrene. In contain proteins like, CsgD which is the transcriptional administrative protein which manages the outflow of curli protein and cellulose that aides in surface grip [20]. The fliL in *S. enterica* controls the development and course of

flagella which helps in the micro-colonies' arrangement. This bacteria is responsible for enteric fever which is more common in those areas where there is more contamination and sanitation condition is poor [21].

The significance of the research is to identify the new and more efficient vaccine for the prevention of bacteria *S. enterica* that is responsible for the cause of enteric fever. Furthermore, mRNA vaccine is considered to be more significant as compared to the other vaccines because they are used for early treatment before getting in contact with the bacteria and should be subjected to further in vitro and in vivo testing.

2. MATERIALS AND METHODS

2.1 Sequence retrieval, prediction and evaluation of B-cells and T-cells epitopes:

For the preparation and designing of vaccine, SopD protein sequence of *Salmonella enterica* in the form of FASTA format was retrieved from UniProt (https://www.uniprot.org/) using its advance feature of finding protein along with the organism [22]. An IEDB tool (http://tools.iedb.org/main/) was utilized for the epitope prediction of B-cells and T-cells on the basis of sequence obtained from Knowledge based Database (UniProt).

B-cells epitopes were taken from portion of IEBD tool for B-cell epitope prediction (http://tools.iedb.org/main/bcell/) which explained number of B-cells epitopes present in the sequence along with each epitope sequence, their starting and ending point [23, 24]. It also provides graphical representation on the basis of peaks to distinguish between epitopes and remaining sequence. T-cells epitopes on the other hand can be obtained from (http://tools.iedb.org/main/tcell/) having two parts of MHC-I and MHC-II for CD8+ and CD4+ [25]. All the epitopes of the protein were validated for antigenicity using Vexijen (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) and its non-allergenicity was checked by utilization of Allertop v. 2.0 (https://www.ddg-pharmfac.net/AllerTOP/) [26, 27].

2.2 Population coverage of the predicted epitopes worldwide:

Population coverage potential of MHC-I and MHC-II was studied by using IEDB analysis resource (http://tools.iedb.org/population/) which reveals about the worldwide coverage of the predicted epitopes of Class-I and Class-II. This population coverage should be greater than 60% [28].

2.3 Vaccine Construction using epitopes obtained from SopD protein:

The mRNA vaccine construct was proposed to be arranged in the form starting from N-terminal to C-terminal in the following arrangement: An adjuvant-EAAAK linker- linker-B-cells Predicted epitopes-CPGPG linker-T-Cells predicted epitopes for MHC-I-AAY linker-T-Cells predicted epitopes for MHC-II-HHHHH tag which is Histidine tag.

2.4 Evaluation of toxicity and physiochemical properties of the vaccine construct:

To evaluate the harmfulness and toxicity of the antibody build, the computational device ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) server was used. It additionally divulged about the atomic weight, its charge, hydrophobicity, hydropathicity and change position in each linker and anticipated epitopes [29]. The outcome made sense of that there is no transformation and all the peptide arrangements are non-toxic [30].

The compositional examination and physiochemical properties of SopD protein were estimated by using Expasy's Protparam server (https://web.expasy.org/protparam/). It additionally made sense of about the amino corrosive creation and nuclear arrangement which makes sense of about the quantity of amino acids and number of molecules individually to concentrate on the SopD protein of *Salmonella enterica* [31].

2.5 Prediction of secondary structure for the vaccine construct:

To dissect the underlying organization of protein based on alpha helix, beta sheets, and loops utilizing optional construction forecast instrument PsiPred

(http://bioinf.cs.ucl.ac.uk/psipred/). PsiPred is accessible as both web server and programming. The grouping of the protein is placed into the server and it gives us optional construction based on its essential design. This apparatus additionally tells about the certainty score of the protein anticipated structure [32].

2.6 Prediction and validation of tertiary structure of the vaccine construct:

trRosseta (https://yanglab.qd.sdu.edu.cn/trRosetta/) was utilized for the homology displaying and modeling of the protein tertiary structure. For approval of the construction got from saves server (https://saves.mbi.ucla.edu/) instruments like ERRAT and ProCheck, and Molprobity (http://molprobity.biochem.duke.edu/) were used to find generally quality element, Ramachandran leaned toward locales, Z-score value and different boundaries of each anticipated design from the above utilized servers. ERRAT tells about the general quality component of the protein. Procheck makes sense of exhaustively about the Ramachandran plot and its mistakes in the SopD [33]. Molprobity distinguishes issues in the 3D construction of the protein which was anticipated by utilizing different protein structure forecast servers. After validation of the predicted structures, it was revealed that the trRosseta web tool provides a fast and precise evaluation of protein models, as well as an efficient assessment of model accuracy.

2.7 Vaccine construct and suitable ligand docking:

An online bioinformatics tool HDock (http://hdock.phys.hust.edu.cn/) was utilized for the docking of vaccine construct with any specific ligand. In this tool, PDB file of the predicted structure and ligand was utilized will shows interaction with each other [34]. It take 20 to 30 minutes to compute the results of docking and the whole process is fast. It provides top 10 models after docking run and the best results are further utilized. The lower the docking score, the greater will be the interaction.

2.8 Protein in water simulation of vaccine construct:

To verify the vaccine construct of SopD protein Simlab (https://simlab.uams.edu/ProteinInWater/index.html) which is an accurate computational tool for the simulation was utilized in which different parameters are set to compute the results like number of frames per simulation, time and duration of the simulation, and number of energy minimization steps [35].

2.9 Visualization of predicted epitopes in the vaccine construct:

The visualization of the epitopes which were available in the vaccine construct especially B-cells epitopes can be visualized with the help of a computational tool known as ElliPro (http://tools.iedb.org/ellipro/) which is used for the linear or discontinuous antibody epitopes prediction available in the given sequence. A PDB file of the sequence based structure is provided to the server which is used for the visualization of the epitopes [36].

2.10 Analysis of receptor-ligand interaction in vaccine-SopD:

PDBSum (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) is used for the analysis of interactions between the receptor and ligand that is present in the vaccine construct. The ligand in the protein can be easily seen and amino acids present in the ligand can also be visualized. It also explains about the interactions between the A, B, C, and D chains of the vaccine construct and tells about the maximum interactions between them [37, 38].

2.11 Codon optimization of the vaccine construct:

The vaccine construct was as amino acids which was changed over into the nucleotide framework by the utilization of online server known as Emboss (https://www.ebi.ac.uk/jdispatcher/st/emboss_backtranseq) server in which the grouping of the antibody develop is used. To upgrade the interpretation of the mRNA vaccine develop inside have cells, codon optimization tool were used which bring about the better DNA grouping. The JCat (https://www.jcat.de/) Codon Optimization tool (G.S.) was utilized to enhance the antibody grouping for effective articulation in human cells [39].

2.12 Prediction of the secondary structure of the

mRNA constructed vaccine:

For RNA structure prediction of the protein RNAfold (https://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) webserver was utilized which shows the secondary structure. In this webserver, the improved DNA sequence was input into the server and resulted secondary structure is obtained having nucleotide Guanine, Adenine, Cytosine, and Uracil. These discoveries will recommend that the mRNA vaccine construct can be proficiently produced and is fundamentally steady, possibly upgrading its viability as an antibody [40].

2.13 In silico immune response simulation against vaccine:

To ensure effectiveness of vaccine was further validated on the basis of the immune response and for this immune simulation tool C-immsim (https://kraken.iac.rm.cnr.it/C-IMMSIM/index.php) was utilized in which sequence of the vaccine was used as input and the results were obtained on the basis of antigen, antibody, B-cells, T-cells, and other responses that are essential for human immunity [41].

2.14 Cloning of the mRNA vaccine construct:

SnapGene is a software which is used for the cloning of the SopD vaccine. In this software, a vector and fragment of DNA sequence are converted into product and further prepared for cloning. The major purpose of SnapGene is the visualization and documentation of DNA cloning and PCR.

3. RESULTS

3.1 Prediction and estimation of B-cells and T-cells epitopes:

The FASTA sequence of selected protein SopD obtained from bacterial specie *Salmonella enterica* was retrieved from UniProt (https://www.uniprot.org/) that can cause enteric fever and was used to predict the b-cells and t-cells epitopes [42]. The IEDB tool was utilized to obtain the epitopes of B-cells. The tool displayed the length, sequence, and beginning and ending points of each peptide or epitope. This tool is also used to identify the epitopes of its two classes of T-cells.

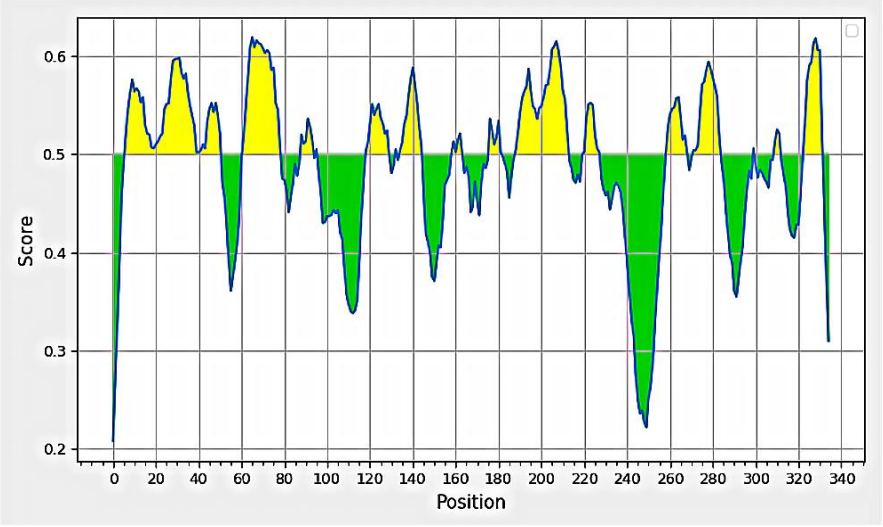

Figure 1. The figure shows the epitope position according to its score which should be above than 0.5. The yellow-colored peaks having score above than 0.5 are constituted as epitopes from the sequence.

The epitopes of MHC I and MHC II binding results were also taken from the IEDB tool. The predicted epitopes were on the basis of the alleles and resultant epitopes were sorted on the basis of its ic50 value which should be less than 100.

Table 1. Predicted epitopes of MHC I with their sequences and ic50 values

Allele	Seq_Num	Start	End	length	Epitopes	ic50	Rank
HLA-B*44:03	1	151	160	10	CEVIGATITW	37.71	0.03
HLA-B*44:02	1	151	160	10		38.52	0.06
HLA-B*15:01	1	243	252	10	FSMMHPCISY	8.31	0.03
HLA-A*01:01	1	243	252	10		57.16	0.12
HLA-B*35:01	1	243	252	10		89.81	0.21
HLA-B*40:01	1	101	110	10	IEMDASQTQL	37.99	0.09
HLA-B*44:02	1	101	110	10		71.39	0.11
HLA-A*30:01	1	39	48	10	KVRDHFRSEK	4.22	0.03
HLA-A*31:01	1	39	48	10		35.7	0.39
HLA-A*03:01	1	39	48	10		36.92	0.13
HLA-A*33:01	1	286	294	9	MFIDVILER	9.84	0.02
HLA-A*68:01	1	286	294	9		12.51	0.09
HLA-A*31:01	1	286	294	9		51.22	0.55
HLA-A*02:01	1	145	153	9	QLFLQICEV	24.52	0.22
HLA-A*02:03	1	145	153	9		37.82	0.61
HLA-A*02:06	1	145	153	9		48.12	0.49
HLA-A*31:01	1	55	63	9	VLYAIIHGR	11.85	0.11
HLA-A*03:01	1	55	63	9		55.97	0.22
HLA-A*68:01	1	55	63	9		56	0.49
HLA-B*15:01	1	234	243	10	YQEVEGEVAF	12.41	0.06
HLA-A*02:06	1	234	243	10		78.47	0.7

Allegenicity and antigenicity of these epitopes was checked by using AllerTop and Vexijen Tools respectively which reveals about the protein to be useful for making vaccine if it is a probable antigen and non-allergen [43]. If antigenicity score of the epitopes is greater than 0.5000 than it is called probable antigen that have ability to fight against diseases.

Table 2. Predicted epitopes of MHC II along with their sequence, ic50 value, and adjusted rank

Allele	Seq_Num	Start	End	Length	Core_peptide	Epitopes	ic50	Rank
HLA-DRB1*01:01	1	147	161	15	CEVIGATIT	FLQICEVIGATITWH	46.3	15
HLA-DRB1*01:01	1	146	160	15		LFLQICEVIGATITW	51	16
HLA-DRB1*01:01	1	148	162	15		LQICEVIGATITWHP	65.1	20
HLA-DRB1*01:01	1	149	163	15		QICEVIGATITWHPE	88.5	24
HLA-DRB4*01:01	1	95	109	15	DLFKIEMDA	HQDLFKIEMDASQTQ	41.7	2
HLA-DRB4*01:01	1	96	110	15		QDLFKIEMDASQTQL	44.6	2.2
HLA-DRB4*01:01	1	94	108	15		AHQDLFKIEMDASQT	50.1	2.6
HLA-DRB4*01:01	1	93	107	15		PAHQDLFKIEMDASQ	65.8	3.6
HLA-DRB1*04:01	1	96	110	15	FKIEMDASQ	QDLFKIEMDASQTQL	51.1	1.8
HLA-DRB1*04:01	1	97	111	15		DLFKIEMDASQTQLL	51.6	1.8

Allele	Seq_Num	Start	End	Length	Core_peptide	Epitopes	ic50	Rank
HLA-DRB1*04:01	1	95	109	15		HQDLFKIEMDASQTQ	67.2	2.6
HLA-DRB1*07:01	1	10	24	15	LNETRLAHL	HQNYTLNETRLAHLL	48.6	5.7
HLA-DRB1*07:01	1	9	23	15		NHQNYTLNETRLAHL	59.1	7
HLA-DRB1*07:01	1	11	25	15		QNYTLNETRLAHLLS	61	7.2
HLA-DRB1*07:01	1	12	26	15		NYTLNETRLAHLLSA	84.2	9.6
HLA-DRB1*13:02	1	125	139	15	LNTSDNVVV	QDILNTSDNVVVESM	27.7	3.7
HLA-DRB1*13:02	1	126	140	15		DILNTSDNVVVESMS	58.1	6.8
HLA-DRB5*01:01	1	162	176	15	LQGSVSTLR	PELLQGSVSTLRKEV	64.6	9
HLA-DRB5*01:01	1	163	177	15		ELLQGSVSTLRKEVT	69.7	9.5
HLA-DRB5*01:01	1	161	175	15		HPELLQGSVSTLRKE	86.7	12
HLA-DRB1*11:01	1	53	67	15	LYAIIHGRG	LEVLYAIIHGRGPGE	88.8	8.1
HLA-DRB1*11:01	1	54	68	15		EVLYAIIHGRGPGEP	89.1	8.2
HLA-DRB1*11:01	1	52	66	15		ALEVLYAIIHGRGPG	98.2	8.7
HLA-DRB1*03:01	1	283	297	15	MFIDVILER	KNKMFIDVILERIYL	27	0.92
HLA-DRB1*03:01	1	282	296	15		HKNKMFIDVILERIY	30.2	1.1
HLA-DRB1*03:01	1	284	298	15		NKMFIDVILERIYLA	33.9	1.2
HLA-DRB1*03:01	1	281	295	15		YHKNKMFIDVILERI	42.9	1.7
HLA-DRB1*03:01	1	285	299	15		KMFIDVILERIYLAH	46.8	1.9
HLA-DRB1*03:01	1	280	294	15		GYHKNKMFIDVILER	87.5	3.7
HLA-DPA1*03:01/DPB1*04:02	1	283	297	15		KNKMFIDVILERIYL	28.2	0.53
HLA-DPA1*03:01/DPB1*04:02	1	284	298	15		NKMFIDVILERIYLA	28.2	0.53
HLA-DPA1*03:01/DPB1*04:02	1	282	296	15		HKNKMFIDVILERIY	31.4	0.67
HLA-DPA1*03:01/DPB1*04:02	1	285	299	15		KMFIDVILERIYLAH	34.4	0.84
HLA-DPA1*03:01/DPB1*04:02	1	281	295	15		YHKNKMFIDVILERI	40.2	1.2
HLA-DPA1*02:01/DPB1*01:01	1	281	295	15		YHKNKMFIDVILERI	50.3	0.47
HLA-DPA1*02:01/DPB1*01:01	1	282	296	15		HKNKMFIDVILERIY	51	0.51
HLA-DPA1*02:01/DPB1*01:01	1	283	297	15		KNKMFIDVILERIYL	51.1	0.51
HLA-DPA1*02:01/DPB1*01:01	1	284	298	15		NKMFIDVILERIYLA	65.9	0.84
HLA-DPA1*02:01/DPB1*01:01	1	285	299	15		KMFIDVILERIYLAH	91.4	1.7
HLA-DPA1*03:01/DPB1*04:02	1	12	26	15	TLNETRLAH	NYTLNETRLAHLLSA	91.5	4
HLA-DPA1*02:01/DPB1*01:01	1	11	25	15		QNYTLNETRLAHLLS	79.3	1.3
HLA-DPA1*02:01/DPB1*01:01	1	12	26	15		NYTLNETRLAHLLSA	92.7	1.7
HLA-DRB4*01:01	1	291	305	15	YLAHEHSLH	ILERIYLAHEHSLHI	89.9	5.3
HLA-DRB4*01:01	1	292	306	15		LERIYLAHEHSLHIG	98.1	5.8

Allele	Seq_Num	Start	End	Length	Core_peptide	Epitopes	ic50	Rank
HLA-DPA1*03:01/DPB1*04:02	1	291	305	15		ILERIYLAHEHSLHI	87.5	3.8
HLA-DRB1*01:01	1	232	246	15	YQEVEGEVA	IGYQEVEGEVAFSMM	82.2	23
HLA-DRB1*01:01	1	231	245	15		KIGYQEVEGEVAFSM	93.4	25
HLA-DQA1*05:01/DQB1*02:01	1	231	245	15		KIGYQEVEGEVAFSM	89.3	2
HLA-DQA1*05:01/DQB1*02:01	1	232	246	15		IGYQEVEGEVAFSMM	91.6	2.1

All the epitopes are selected on the basis of their antigenicity using Vexijen server. These epitopes are further checked by the tools to find if they are toxic or may cause allergen to the body. MHC I epitopes and MHC II epitopes are non-allergen and non-toxic [44]. If the vaccine has allergenicity then the epitopes of the vaccine cannot be used because they can also produce harmful effects to the human body when taken.

3.2 Population coverage of the predicted epitopes worldwide:

The population coverage tool provides three different metrics: the average number of epitope hits, the minimum number of epitope hits, and the projected population coverage. The predicted epitopes showed a population coverage potential of 83.75% for MHC class I and 63.36% for MHC class II worldwide. Additionally, the average number of epitope hits was 1.83 for MHC class I and 0.88 for MHC class II. This population coverage analysis was conducted because MHC class I and II alleles specifically bind to the predicted epitopes from the protein used in vaccine construction [45].

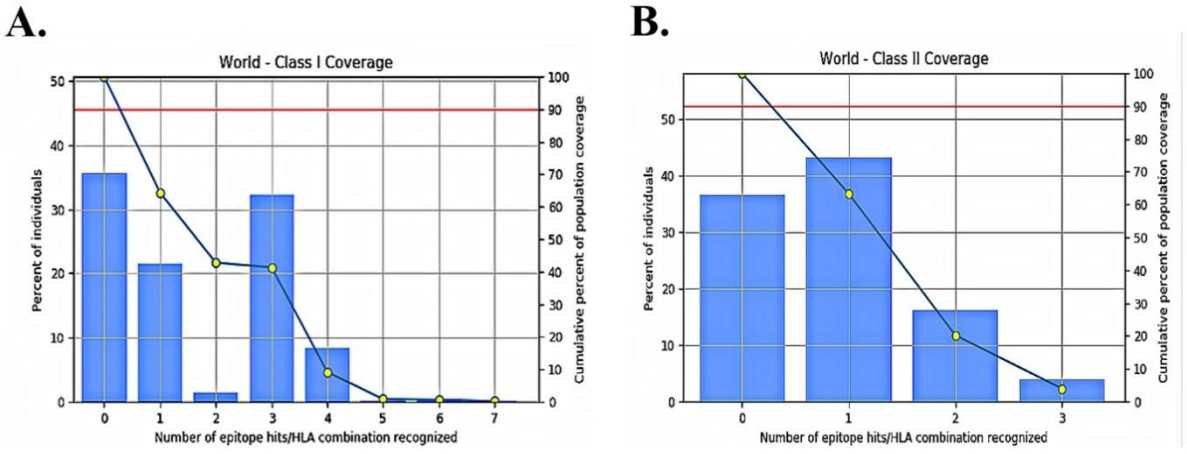

Figure 2. Graphical representation of the population coverage in the world (A) Population coverage of World-Class I is shown along with its number of epitope hits (B) Population coverage of World-Class II is shown along with its number of epitope hits

3.3 Vaccine Construction using epitopes obtained from SopD protein:

The mRNA vaccine construct was proposed to be arranged in the form starting from N-terminal to C-terminal in the following order: A 50s ribosomal adjuvant-EAAAKlinker-GRGPGEPGEMEVNVEDMS-VVESMSREERO-MMRPAEAPDHQLVEWQDSLTENEKS-TDLKSGSLP-CPGPGlinker-CEVIGATITW-FSMMHPCISY-IEMDASQTQL-KVRDHFRSEK-MFIDVILER-QLFLQICEV-VLYAIIHGR-YQEVEGEVAF-AAYlinker-CEVIGATIT-DLFKIEMDA-FKIEMDASQ-LNETRLAHL-LNTSDNVVVLQGSVSTLR-TLNETRLAH-YLAHEHSLH-YQEVEGEVA-HHHHH tag.

3.4 Evaluation of toxicity, solubility and physiochemical properties of the vaccine construct:

To assess the toxicity of the vaccine construct, the computational tool ToxinPred server was utilized which is shown in the **Table 3.** It also unveiled about the molecular weight, its charge, hydrophobicity, hydropathicity and mutation position in each linker and predicted epitopes [44]. The result explained that there is no mutation, and all the peptide sequences are non-toxin. Solubility score of the vaccine construct was 0.617 (towards green color) which indicates its soluble expression in *E.coli*. Therefore, we can use it as vaccine for the protection of disease enteric fever.

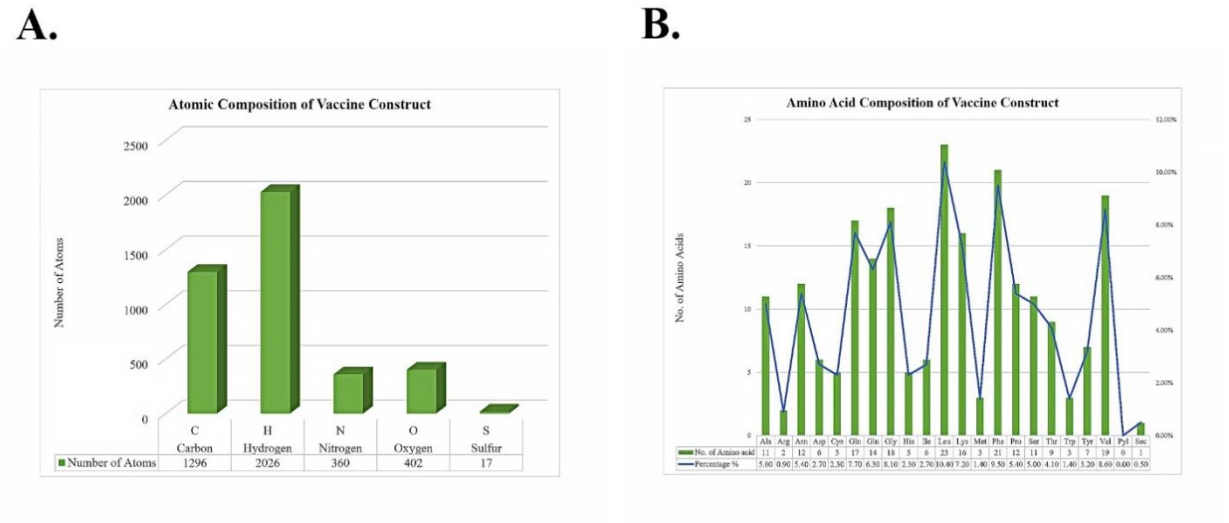

Figure 3. (A) The figure explains about graphical representation of atomic number present in the vaccine construct (B) The figure shows the bar graph of amino acids composition of vaccine construct shows the highest number of amino acids present in it is Glutamic acid having 11.10 % for its composition

The physiochemical parameters were explained by utilizing Protparam tool of Expasy [31]. These parameters revealed that the number of amino acids in vaccine construct is 261, the molecular weight is 29627.53, Theoretical pI is 4.97 and have Grand average of hydropathicity (GRAVY) is -0.264. It contains 4101 atoms in its composition and hydrogen is most abundant in it [46].

The composition of atoms in protein SopD obtained from *S. enterica* sequence shows that the hydrogen atoms are more than the other atoms in the protein structure. According to this compositional analysis, number of atoms of Carbon (C), Hydrogen (H), Nitrogen (N), Oxygen (O), and Sulfur (S) are 1296, 2026, 360, 402, and 17 respectively as shown in **Figure 3**. Vaccine construct

prepared by using SopD protein of *S. enterica* is a combination of 261 amino acids which are as follow, Ala, Arg, Asn, Asp, Cys, Gln, Glw, Gly, His, Ile, Leu, Lys, Met, Phe, Tro, Ser, Trp, Tyr, Bal, Pyl, and Sec.

Table 3. Toxicity prediction of the vaccine construct SopD obtained from *S. enterica*

Peptide Sequence	SVM score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol wt
EAAAK	-0.93	Non-Toxin	-0.19	-0.4	0.9	0	488.59
GRGPGEPGEMEVNVEDMS	-1.05	Non-Toxin	-0.21	-1.05	0.72	-4	1890.3
VVESMSREERQ	-0.67	Non-Toxin	-0.48	-1.3	1.05	-1	1349.6
MMRPAEAPDHQLVEWQDSLTENEKS	-1.88	Non-Toxin	-0.28	-1.21	0.49	-3.5	2942.6
TDLKSGSLP	-1.14	Non-Toxin	-0.15	-0.46	0.29	0	917.15
CPGPG	-0.74	Non-Toxin	0.04	-0.3	-0.2	0	429.55
CEVIGATITW	-0.51	Non-Toxin	0.18	1.13	-0.78	-1	1092.4
FSMMHPCISY	-0.14	Non-Toxin	0.09	0.59	-1.01	0.5	1215.6
IEMDASQTQL	-1.42	Non-Toxin	-0.14	-0.35	0.09	-2	1135.4
KVRDHFRSEK	-1.24	Non-Toxin	-0.66	-2.08	1.38	2.5	1301.6
MFIDVILER	-1.22	Non-Toxin	0.03	1.13	-0.19	-1	1135.5
QLFLQICEV	-0.55	Non-Toxin	0.11	1.23	-0.78	-1	1092.5
VLYAIIHGR	-0.92	Non-Toxin	0.09	1.04	-0.8	1.5	1041.4
YQEVEGEVAF	-0.92	Non-Toxin	-0.04	-0.27	0.09	-3	1170.4
AAY	-0.84	Non-Toxin	0.17	0.77	-1.1	0	323.37
CEVIGATIT	-0.49	Non-Toxin	0.16	1.36	-0.49	-1	906.19
DLFKIEMDA	-0.81	Non-Toxin	-0.09	0.04	0.46	-2	1081.4
FKIEMDASQ	-0.97	Non-Toxin	-0.17	-0.47	0.38	-1	1068.3
LNETRLAHL	-0.8	Non-Toxin	-0.2	-0.24	-0.07	0.5	1066.4
LNTSDNVVV	-0.94	Non-Toxin	-0.03	0.49	-0.33	-1	960.18
LQGSVSTLR	-1.11	Non-Toxin	-0.15	0.12	-0.19	1	960.23
LYAIIHGRG	-0.86	Non-Toxin	0.05	0.53	-0.63	1.5	999.32
MFIDVILER	-1.22	Non-Toxin	0.03	1.13	-0.19	-1	1135.5
TLNETRLAH	-1.04	Non-Toxin	-0.27	-0.74	0.09	0.5	1054.3
YLAHEHSLH	-0.92	Non-Toxin	-0.08	-0.64	-0.51	0.5	1106.3
YQEVEGEVA	-0.85	Non-Toxin	-0.12	-0.61	0.38	-3	1023.2

3.5 Secondary structure prediction of the vaccine construct:

The prediction of secondary structure is usually authentic and is based on the consensus from PsiPred in **Figure 4.** Its cartoonic two dimensional structure shows the confidence level of the protein structure predicted and showed α-helix, β-sheets and coils in the structure [32]. The secondary structure of the protein has alpha sheets, beta helix and coils that provide shape and structure to the protein.

PsiPred tool reveals that the structure obtained for this protein have good confidence on the basis of its amino acid sequence. In the **figure 4**, straight line shows coils, yellow bars show alpha strands while pink bars show beta helix in the secondary structure of the protein SopD obtained from the bacteria *S. enterica*.

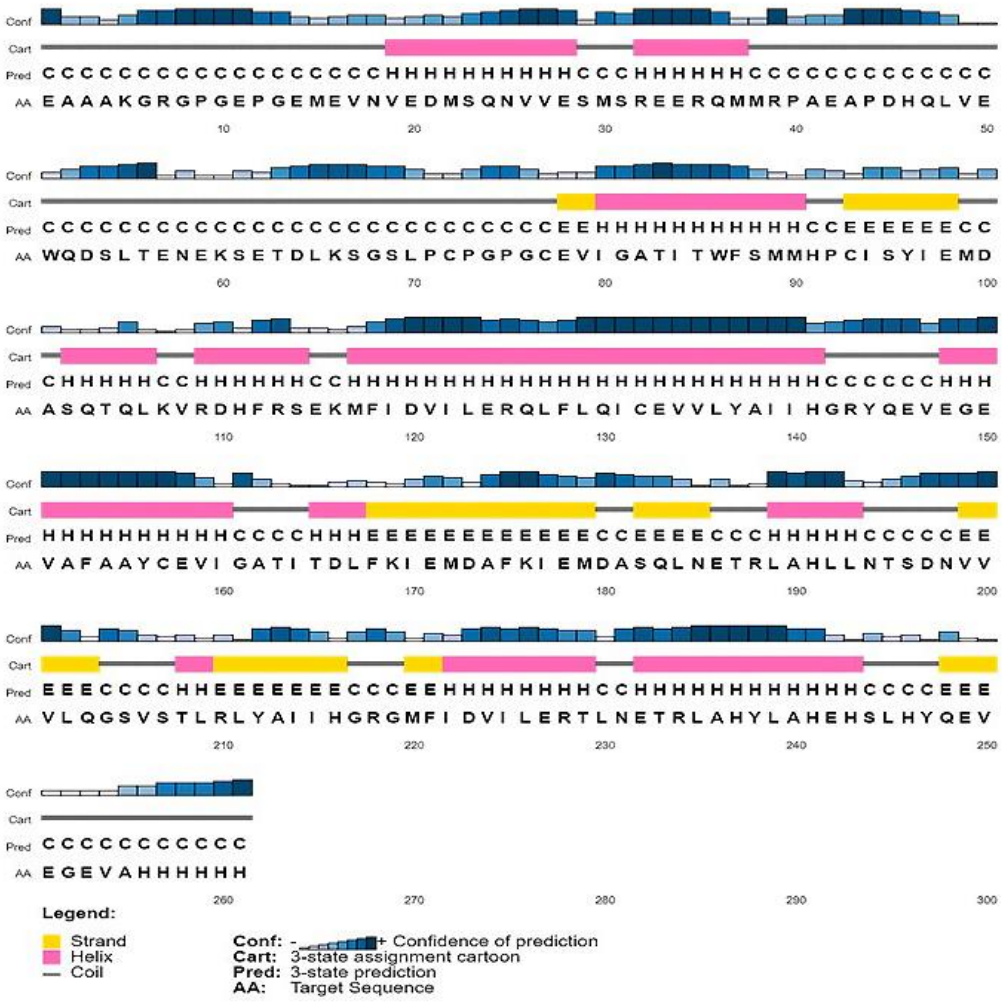

Figure 4. The figure shows the predicted cartoon secondary structure along with its confidence score for vaccine construct

3.6 Tertiary structure prediction and its validation in the vaccine construct:

The tertiary structure (3D) structure of the vaccine construct was also predicted using online computation tool known as 3D pro which predicts the structure on the basis of homology modelling [47].

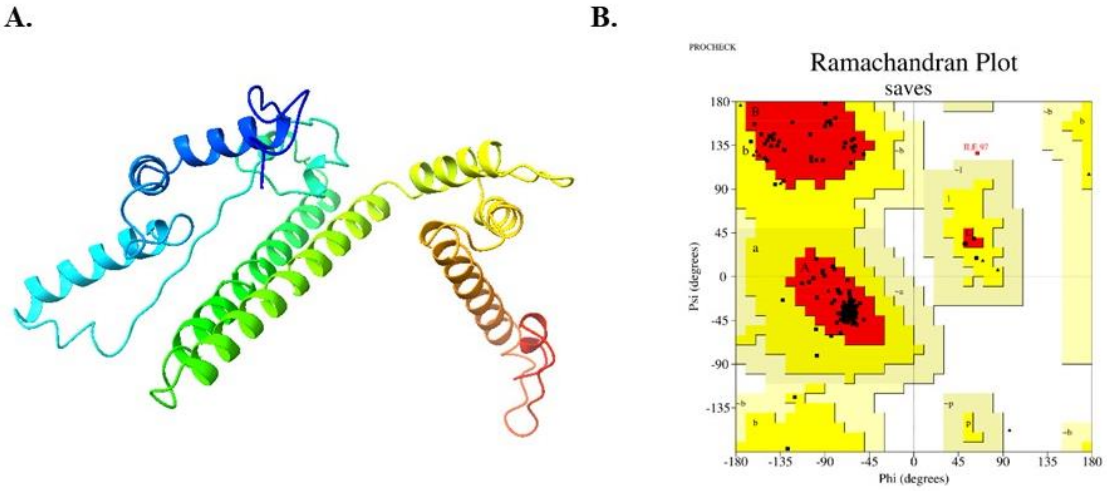

Figure 5. (A) Cartoon presentation of Three-dimensional modeled structures using trRosseta server (B) Ramachandran plot constructed based on Psi (degree) and Phi (degree) after being refined using GalaxyWEB server

The predicted structure is refined by utilizing GalaxyWEB (https://galaxy.seoklab.org/) [48]. The tool used for refinement showed 5 top refined structures that had better results as compared to the initial one.

The favored region in the Ramachandran plot was increased from 91.1% to 95.8%, residues in additional allowed regions were 4.2%, and residues in disallowed regions were only 0.4%. Its clash score was also reduced from 159.4 to 19.0. The poor rotamers of the structure also reduced to 0.9 which is shown in **Table 4**. Ramachandran plot after refinement, explained that the three dimensional structure of vaccine construct is valid because most of its residues are present in the favored region [49].

Table 4. Summary of structure refinement of initial 3D dimensional structure and 5 refined models

Model	GDT-HA	RMSD	MolProbity	Clash score	Poor rotamers	Rama favored
Initial	1.0000	0.000	3.825	159.4	7.1	91.1
MODEL 1	0.8841	0.576	2.070	19.0	0.9	95.8
MODEL 2	0.8812	0.558	2.082	18.3	0.9	95.4
MODEL 3	0.8956	0.534	2.093	18.8	0.9	95.4
MODEL 4	0.9004	0.525	2.027	17.1	0.4	95.8
MODEL 5	0.8784	0.585	2.130	16.4	1.3	95.4

3.7 Vaccine construct and suitable ligand docking:

HDock server was utilized for the docking of the vaccine construct. In docking a receptor and ligand is utilized It showed docking with top 10 models out of which model 1 was selected for further research [34]. Models generated after docking should have docking score very low [50]. Model 1 had docking score -318.45 with 0.9667 confidence score and ligand root mean square deviation (RMSD) 83.91 which is selected for further simulation and validation is explained in **Table 5**.

Table 5. Summary of the top 10 models obtained using HDock server

Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-325.6	-323.5	-321.2	-319.7	-313.6	-312.5	-311.6	-309.4	-308.4	-299.99
Confidence Score	0.971	0.969	0.968	0.967	0.9635	0.9627	0.9621	0.9604	0.9596	0.9525
Ligand rmsd (Å)	54.0	64.5	51.8	67.0	93.85	61.74	62.38	73.92	67.56	80.46
Interface residues	model_1	model_2	model_3	model_4	model_5	model_6	model_7	model_8	model_9	model_10

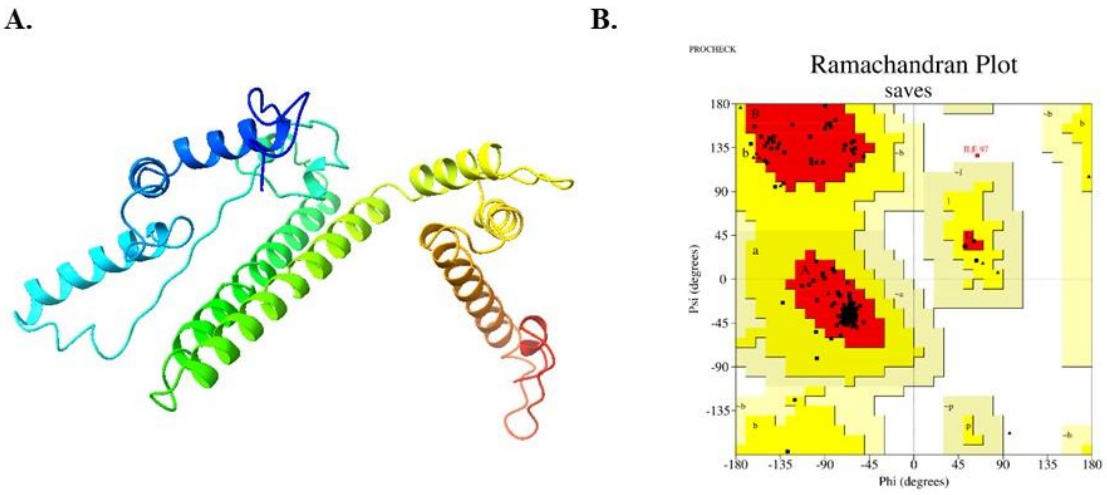

Figure 6. (A) Cartoon structure obtained from HDock and visualized by ChimeraX (B) Surface structure obtained from HDock by the docking analysis of receptor and ligand

The resultant cartoon and surface structure of vaccine construct of SopD protein and ligand that is utilized in the docking using HDock server [51], the structure which is represented in yellow color in our vaccine construct obtained from utilization of SopD protein of *S. enterica* is shown in the **Figure 5**.

3.8 Protein in water simulation of vaccine construct:

The **Figure 7** explain about the water simulation of the vaccine construct on the basis of its radius of gyration, Root mean square deviation RMSD, hydrogen bonding, RMSD fluctuations, and solvent accessible surface. Simlab webserver was utilized for this protein in water simulation [35].

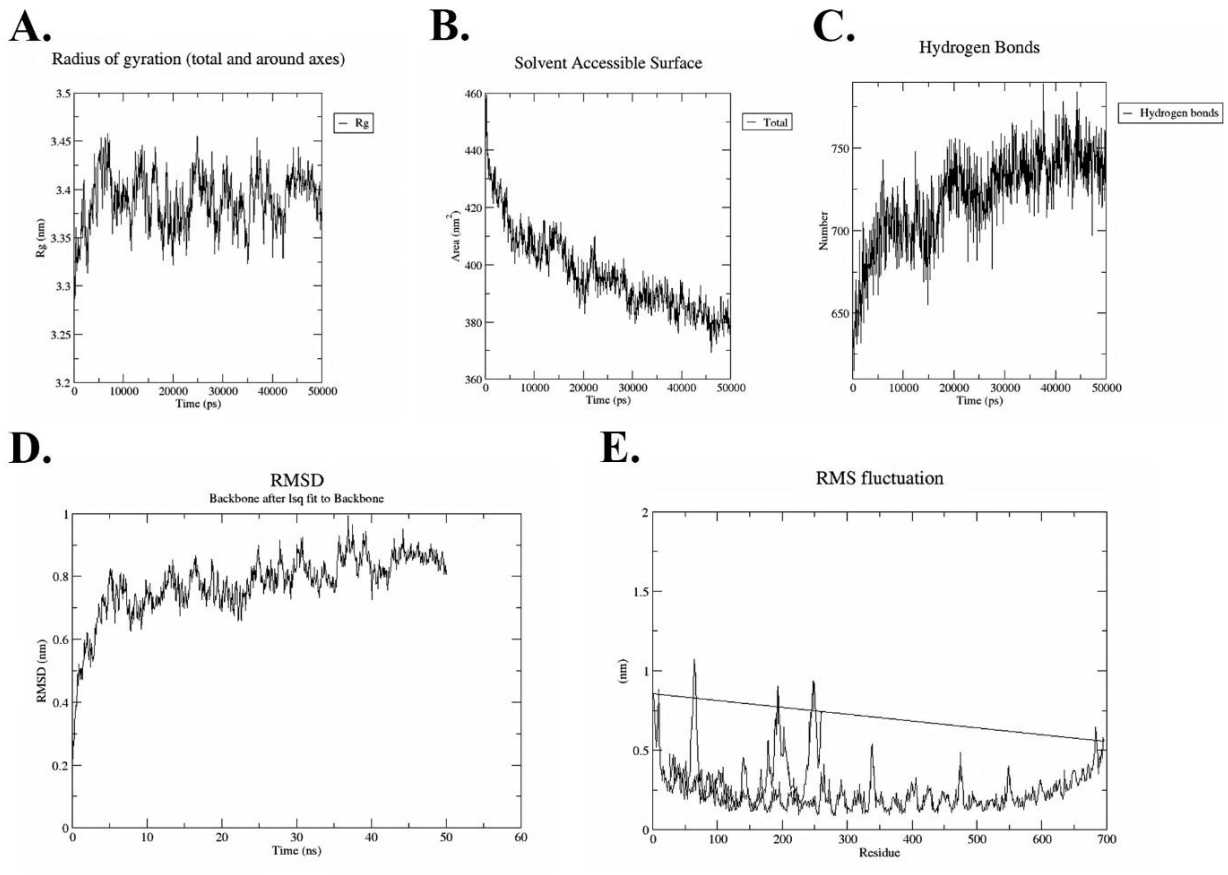

Figure 8. The figure explains graphical representation of protein in water simulation (A) Graphical representation of the Radius of gyration with respect to time (B) Area of solvent accessible surface (C) Number of hydrogen bonds obtained in water simulation (D) Graphical representation of Root mean square deviation (RMSD) w.r.t time (ns) (E) Fluctuations of root mean square along with the residues

3.9 Visualization of predicted epitopes in the vaccine construct:

Ellipro tool was utilized to visualize the antibody predicted epitopes which were present in A or B chain of the mRNA vaccine-SopD. These epitopes are visualized in yellow color in the **Figure 9**.

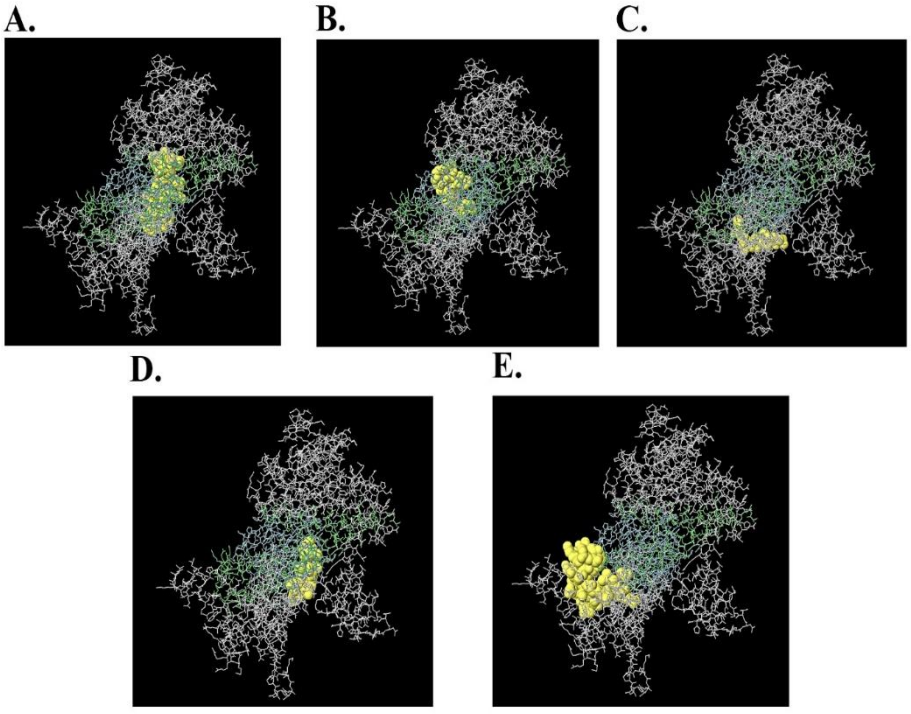

Figure 9. The figure contains the graphical representation of the discontinuous epitopes constructed from the Ellipro server.

1.1 Analysis of receptor-ligand interaction in vaccine-SopD:

Receptor and ligand both are examined using the PDBsum Web which provide comprehensive explanation of its interactions on the basis of bonding and chains. This server showed that ligand which is interaction is GNP. Furthermore, the interacting chains are joined by lines drawn by different colors having salt bridges and hydrogen bonding between them. Every color represents its specific interaction with the other one [52].

3.10 Codon optimization of the vaccine construct:

The vaccine construct was in the form of amino acids, which the EMBOSS server transformed into a nucleotide sequence [53]. Codon optimization tools were used to improve the mRNA vaccine construct's translation within host cells. The vaccine sequence was optimized using the JCat Codon Optimization tool (G.S.) to ensure effective expression in human cells. It was discovered that in order to ensure effective expression in the human host, the ideal percentage of G.C. content should be between 30 and 70%. As a result of codon optimization, the GC content of the nucleotide sequence was increased to 66.28% and improved DNA sequence is obtained.

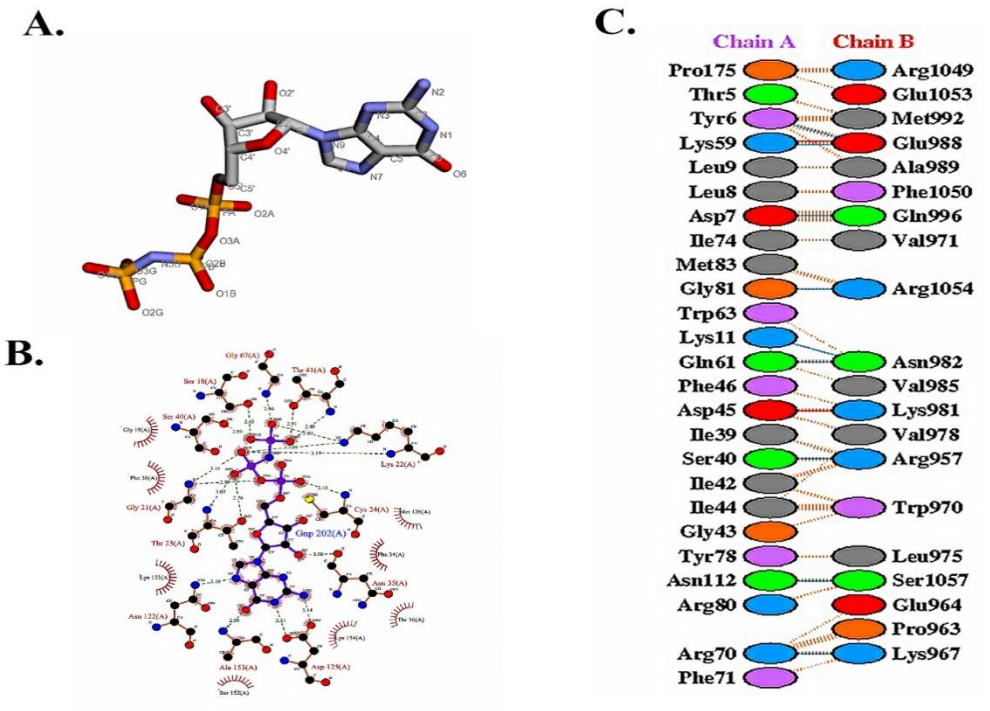

Figure 10. The figure explains about (A) Structure of ligand GNL (B) Interaction of ligand GNL with the other amino acids (C) Receptor-ligand interaction using PDBsum Webserver

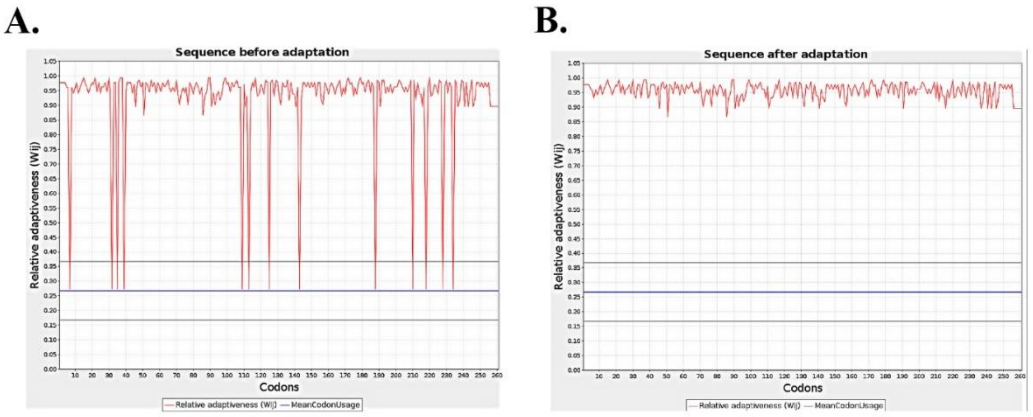

Figure 11. (A) Graphical representation of sequence before adaptation (B) Sequence after adaptation by using JCat codon optimization tool.

3.11 Prediction of the secondary structure of the mRNA constructed vaccine:

The RNAfold server was used to predict the structure of the mRNA vaccine construct. The optimized codons of the construct were used as input, and the free energies of the structure were assessed using the server. These findings suggest that the mRNA vaccine construct can be efficiently manufactured and is structurally stable, potentially enhancing its efficacy as a vaccine.

According to the predicted structure of mRNA, the free energy of the thermodynamic ensemble was -335.79 Kcal/mol, ensemble diversity is 229.65, and free energy of centroid secondary structure was -203.32 Kcal/mol.

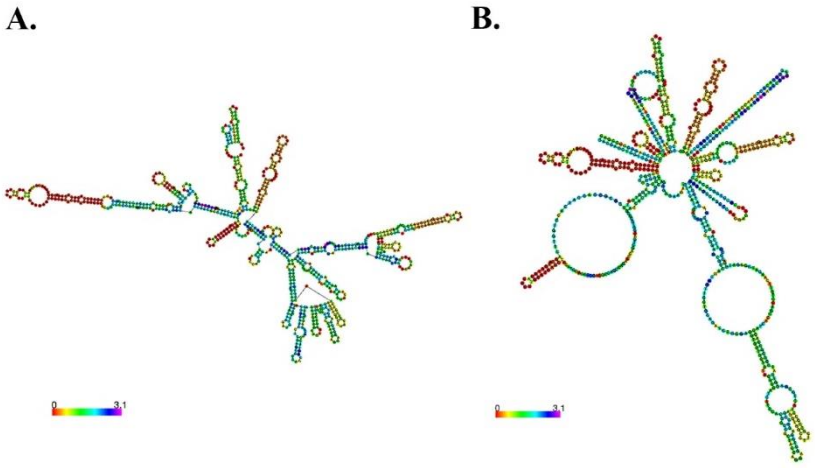

Figure 12. The figure explains mRNA vaccine structure (A) MFE optimal secondary structure (B) centroid secondary structure of the vaccine mRNA retrieved using RNAfold webserver

3.12 In silico immune response simulation against vaccine:

C-immsim tool was utilized which showed the immunoglobin production after antigen injection, B-cell population and T-cell population in the immune response [41]. Immune simulation of the vaccine-SopD is shown in the form of graphs in **Figure 13**. In this research study, administration of three vaccine injection was done for the stimulation of immune response. The second and third administered injections elicited higher immune responses in comparison to the primary injection. Immunoglobin M levels in the simulation were higher than immunoglobin G levels. Formation of memory and isotype switching to the B-cell population were also examined, in presence with the persisting B-cell isotypes for a longer duration. In addition to increase in MHC-I and MHC-II with generation of memory was also examined. Furthermore, macrophage activity was modified.

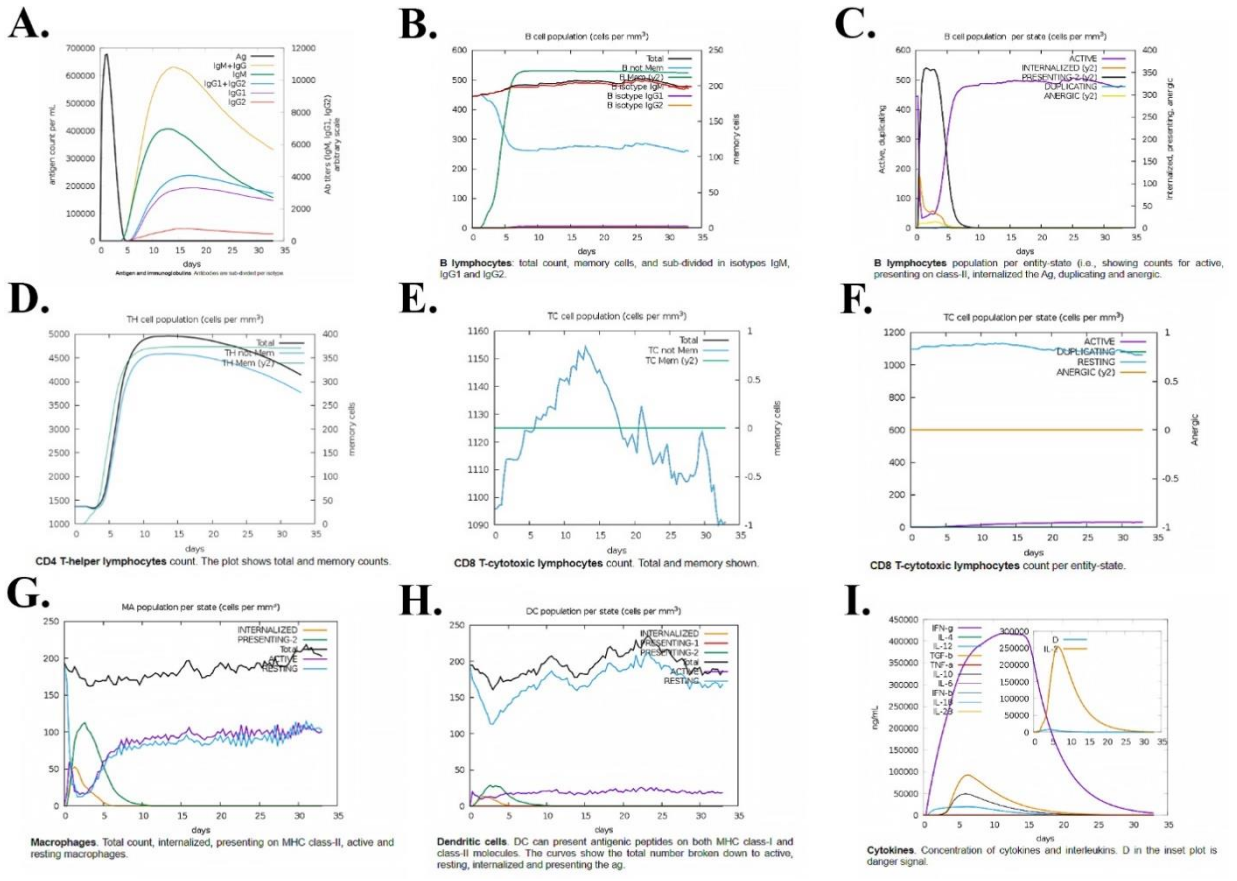

Figure 13. In silico immune simulation against the mRNA vaccine retrieved from the C-ImmSim server (https://kraken.iac.rm.cnr.it/C-IMMSIM/). (A) Production of immunoglobin after injection of antigen (B) The B-cell population after 3 different injections (C) The B-cell population at each state (D) The helper T-cell population after 3 different injections (E) The helper T-cell population at each state (F) The cytotoxic T-cell population at each state (G) Macrophage population at each state (H) Dendritic cell population at each state (I) Cytokine and interleukin production with immune response Simpson Index

1.2 Cloning of the mRNA vaccine construct:

SnapGene software was utilized for this purpose in which the vaccine construct DNA fragment and vector (pET-28a) was attached together using restriction sites [54]. The product obtained after cloning was of 3894 bps which is shown in **Figure**

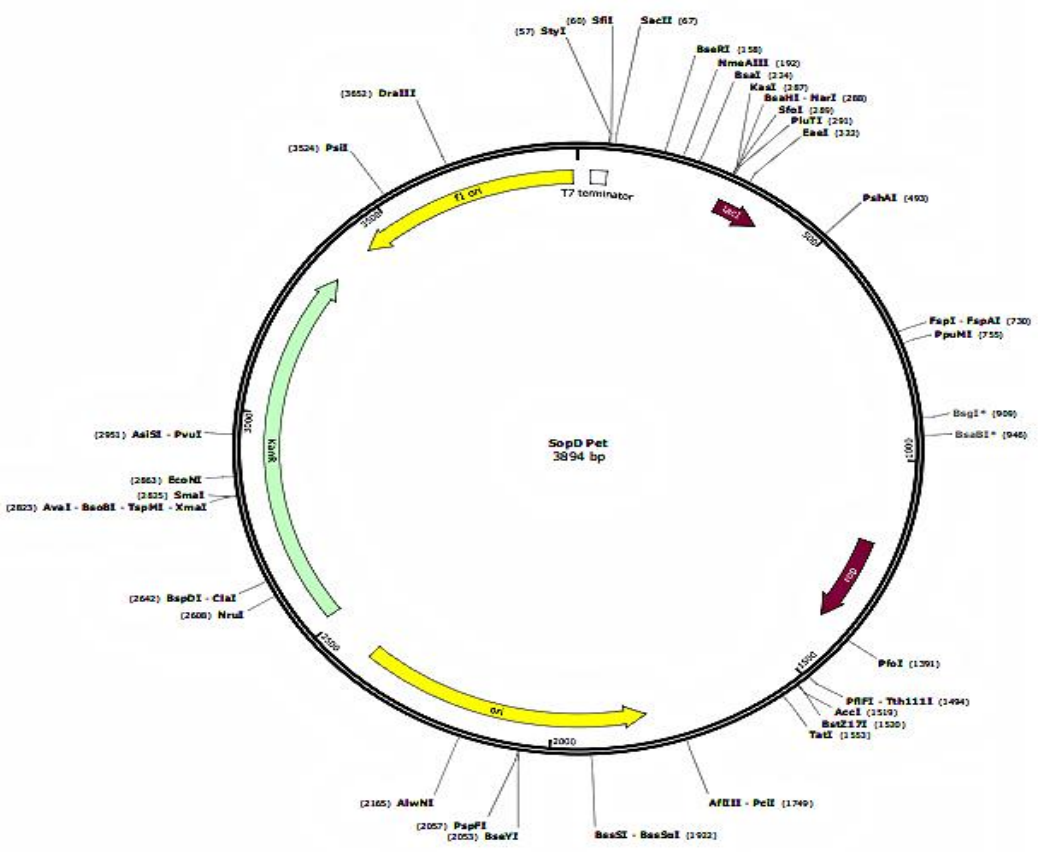

Figure 14. Cloned vaccine construct having 3894 bps with f1 origin, T7 terminator, lac operator, and promoter region.

4 DISCUSSION

Salmonella enterica is a challenging pathogen that can be used for the development of efficient vaccine because of its ability of antibiotic resistance. Many studies are being done on the production of antibiotics, but they are not much efficient for use, and some does not support till date. Various factors are responsible for the production of *S. enterica* vaccine which includes a large variety of serotypes that work as a barrier in the production of a powerful vaccine [55]. In the treatment of bacterial infection caused by *S. enterica* both innate and adaptive immune responses play crucial role. Various vaccines are also designed for the poultry to make the chicken less contaminated and resulting in the reduction of food borne diseases that are harmful for human health [56].

In present time, Commercially there are variety of vaccines that are available for chickens which include killed vaccines, live attenuated vaccines, as well as subunit vaccines that can reduce the risk of causing disease [57]. For human use, different vaccines were designed and got rejected because of their high incidence of local reactions and were considered unsafe for public use. However, there are two vaccines that have been developed in the last 15 years and are considered safe for the use against typhoid fever. First vaccine was parental capsular polysaccharide vaccine, and the other one was oral live attenuated vaccine. Both of these are not only licensed and have been used for immunizing against enteric fever successfully but they may have complications as well [58].

To reduce these complications, scientists need to

develop more effective vaccines to face the challenge of this critical health issue. A novel mRNA vaccine is developed by utilization of immunoinformatic approaches that is efficient, modified and safe. This messenger RNA vaccine is proved to be more efficient and effective against different viral and bacterial infections. Currently, various clinical human trials are conducting worldwide, these trials for mRNA vaccines are now the indication of safe and effective alternative of the variety of therapies which are in the form of vaccination.

One of the advantages of mRNA-based vaccine is its ability for accumulation of antigen selected in the cytoplasm which targets pathogen and triggers an immune response against it. This approach for the development of vaccine may offer variety of advantages over conventional vaccine strategies which include the quick and rapid development, improved efficiency, and easy production [59].

In this study, a novel mRNA multi epitope vaccine which is in silico based, has been proposed to fight against the infection and enteric fever caused by *S. enterica*. The vaccine is proposed on the basis of proteins that contribute in the binding of cell and bacterium attachment in *S. enterica* [60]. Furthermore, this approach offer solution to the challenges that occur due to the enteric fever, and relatively further research and study is needed for the safety and evaluation of this multi epitope mRNA vaccine. For the identification of target epitopes web-based tools like IEDB databases are utilized, which can predict epitopes of B-cell, MHC-I and MHC-II based on the determination of immune epitopes. The epitopes are then further evaluated by the utilization of web servers for the determination of antigenicity, allergenicity, and their toxicity. Only those epitopes were further examined that were antigens, non-allergen, and non-toxic. Moreover, specific linkers were also used for combining the epitopes.

For further refinement of the vaccine design, conduction of immune simulation was done to validate responses of the vaccine. Targeted epitopes of the vaccine construct had 19 corresponding MHC alleles. Furthermore, it is necessary to understand that vaccine may be not effective in those individuals with a specific or particular allele which has ability to bind with the epitope. For this reason, prediction of population coverage in the world for proposed design using IEDB tool revealed that this vaccine would cover 83.75 % of the world's overall population on the basis of alleles and epitopes obtained and was considered to be widely effective vaccine [28, 61]. As, docking interaction is necessary, the vaccine construct that resulted by the linkage of epitopes was later subjected to molecular docking analysis. This docking analysis was done with the help of Toll-like receptor TLR3 by the computational tool known as HDock to ensure the maximum efficacy of vaccine design [51]. The outcomes of molecular docking showed strong binding affinity between the epitopes and their alleles of MHC-I and MHC-II that indicated the efficacy of the designed vaccine.

Moreover, a variety of computational tools were used for the prediction and evaluation of epitopes and further MD simulation in which RMSD and RMSF parameters were evaluated which revealed that vaccine is stable with them and immune simulations were also performed for the validation of safety and effectiveness of the vaccine in which three injections were utilized. Resultant immune simulation of vaccine construct revealed that the vaccine had great ability to stimulate immune response. The results obtained from these tools indicated that all epitopes used in the construction of vaccine were antigenic and non-allergen and can be used for the process of vaccination [43, 62]. Overall, the research provides valuable response into the designing of efficient mRNA vaccine against *S. enterica* and marks the effectiveness and potential of in silico computational approaches for vaccine development.

In future, multivalent mRNA vaccines against multiple enteric fever-causing pathogens should be developed. Furthermore, vaccine and immunotherapies should be investigated. mRNA vaccine may become a better option for the prevention of viral and bacterial infection along

with its related diseases due to the ongoing research, preclinical and clinical trials.

5 CONCLUSION

To sum up the whole discussion, this study provides that mRNA vaccination can be very effective against bacterium *S. enterica* and provides a favorable framework for coming times. However, it is necessary to note that more in vitro along with in vivo studies are important to authenticate the findings for the research taken and to validate and evaluate the potential, safety and limitations of vaccine in worldwide scenarios. Development of successful and effective vaccine against *S. enterica* could have important applications for health of public by reducing infection risk associated with the disease-causing pathogen. The high population coverage prediction and its strong affinity suggest that it may be useful and authentic vaccine to deal with *S. enterica* infections. Altogether, this study marks the computational approaches for vaccine design's potential and provides esteemed insights into the designing of effective mRNA multi-epitope vaccines against *S. enterica*.

ACKNOWLEDGMENT: Especially thankful to my respective supervisor and co. supervisor for providing all the necessary facilities for carrying out this research project.

Conflicts of interest: The authors declare no conflicts of interest

Funding: This study received no external funding

REFERENCES

1. House D., et al. Typhoid fever: pathogenesis and disease. *Current Opin. Infect. Dis.* 2001; 14(5):573-578.
2. Crump J.A., Luby S.P., Mintz E.D. The global burden of typhoid fever. *Bull. World Health Organ.* 2004; 82(5):346-353.
3. Greenaway C., et al. Typhoid: summary of the statement on international travellers and typhoid by the committee to advise on tropical medicine and travel (CATMAT). *Canada Communicable Dis. Rep.* 2014; 40(4):60.
4. Raffatellu M., et al. Clinical pathogenesis of typhoid fever. *J. Infect. Dev. Ctries.* 2008; 2(4):260-266.
5. Bányai K., et al. Viral gastroenteritis. *Lancet* 2018; 392(10142):175-186.
6. Schaefer K., et al. Septicemia due to Listeria monocytogenes infection: A systematic review and meta-analysis. *Foodborne Pathog. Dis.* 2022; 19(2):104-114.
7. Neupane D.P., Dulal H.P., Song J. Enteric fever diagnosis: current challenges and future directions. *Pathogens* 2021; 10(4):410.
8. Wain J., Hosoglu S. The laboratory diagnosis of enteric fever. *J. Infect. Dev. Ctries.* 2008; 2(6):421-425.
9. Azmatullah A., et al. Systematic review of the global epidemiology, clinical and laboratory profile of enteric fever. *J. Glob. Health* 2015; 5(2).
10. Adesegun O.A., et al. Current trends in the epidemiology and management of enteric fever in Africa: a literature review. *Asian Pac. J. Trop. Med.* 2020; 13(5):204-213.
11. Saha T., et al. Enteric fever: diagnostic challenges and the importance of early intervention. *Cureus* 2023; 15(7).
12. Dahiya S., et al. Current antibiotic use in the treatment of enteric fever in children. *Indian J. Med. Res.* 2019; 149(2):263-269.
13. Kuehn R., et al. Treatment of enteric fever (typhoid and paratyphoid fever) with cephalosporins. *Cochrane Database Syst. Rev.* 2022;(11).
14. Parry C.M., et al. What should we be recommending for the treatment of enteric fever? *Open Forum Infect. Dis.* 2023; Oxford University Press US.

15. Qamar F.N., Hussain W., Qureshi S. Salmonellosis including enteric fever. *Pediatr. Clin.* 2022; 69(1):65-77.
16. Soulier A., et al. Engineering a novel bivalent oral vaccine against enteric fever. *Int. J. Mol. Sci.* 2021; 22(6):3287.
17. Masuet-Aumatell C., Atouguia J. Typhoid fever infection–antibiotic resistance and vaccination strategies: A narrative review. *Travel Med. Infect. Dis.* 2021; 40:101946.
18. Knodler L.A., Elfenbein J.R. *Salmonella enterica. Trends Microbiol.* 2019; 27(11):964-965.
19. Andino A., Hanning I. *Salmonella enterica*: survival, colonization, and virulence differences among serovars. *Sci. World J.* 2015; 2015:520179.
20. Brombacher E., et al. Gene expression regulation by the curli activator CsgD protein: modulation of cellulose biosynthesis and control of negative determinants for microbial adhesion. *J. Bacteriol.* 2006; 188(6):2027-2037.
21. Attmannspacher U., Scharf B.E., Harshey R.M. FliL is essential for swarming: motor rotation in absence of FliL fractures the flagellar rod in swarmer cells of *Salmonella enterica. Mol. Microbiol.* 2008; 68(2):328-341.
22. Zaru R., Orchard S., U. Consortium. UniProt tools: BLAST, align, peptide search, and ID mapping. *Curr. Protoc.* 2023; 3(3):e697.
23. Dhanda S.K., et al. IEDB-AR: immune epitope database—analysis resource in 2019. *Nucleic Acids Res.* 2019; 47(W1):W502-W506.
24. Aqsha Z.M., et al. Reverse vaccinology analysis of B-cell epitope against Nipah virus using fusion protein. *Jordan J. Pharm. Sci.* 2023; 16(3):499-507.
25. Kim Y., Sette A., Peters B. Applications for T-cell epitope queries and tools in the Immune Epitope Database and Analysis Resource. *J. Immunol. Methods* 2011; 374(1-2):62-69.
26. Kumar S., Hasija Y. Immunoinformatics tools: a boon in vaccine development against COVID-19. In: *IEEE Delhi Section Conference (DELCON)*. IEEE; 2022.
27. Dimitrov I., Flower D.R., Doytchinova I. AllerTOP—a server for in silico prediction of allergens. In: *BMC Bioinformatics*. Springer; 2013.
28. Fleri W., et al. The immune epitope database and analysis resource in epitope discovery and synthetic vaccine design. *Front. Immunol.* 2017; 8:2503.
29. Gupta S., et al. In silico approach for predicting toxicity of peptides and proteins. *PLoS One* 2013; 8(9):e73957.
30. Rajendhiran N., Bhattacharyya S. Preparation and evaluation of nanolipid carriers of bedaquiline—in vitro evaluation and in silico prediction. *Jordan J. Pharm. Sci.* 2024; 17(3):450-467.
31. Gasteiger E., et al. Protein identification and analysis tools on the ExPASy server. Springer; 2005.
32. McGuffin L.J., Bryson K., Jones D.T. The PSIPRED protein structure prediction server. *Bioinformatics* 2000; 16(4):404-405.
33. Dym O., Eisenberg D., Yeates T. ERRAT. 2012.
34. Yan Y., et al. The HDOCK server for integrated protein–protein docking. *Nat. Protoc.* 2020; 15(5):1829-1852.
35. Tarantola S., Becker W. SIMLAB software for uncertainty and sensitivity analysis. In: Ghanem R., Higdon D., Owhadi H., editors. *Handbook of Uncertainty Quantification*. 2016; p. 1979-1999.
36. Ponomarenko J., et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. *BMC Bioinformatics* 2008; 9:1-8.
37. Laskowski R.A., et al. PDBsum: structural summaries of PDB entries. *Protein Sci.* 2018; 27(1):129-134.
38. Laskowski R.A., et al. PDBsum: a web-based database of summaries and analyses of all PDB structures. *Trends Biochem. Sci.* 1997; 22(12):488-490.
39. Grote A., et al. JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. *Nucleic Acids Res.* 2005; 33(suppl_2):W526-W531.
40. Taneda A., Sato K. A web server for designing molecular switches composed of two interacting RNAs. *Int. J. Mol. Sci.* 2021; 22(5):2720.

41. Castiglione F., Bernaschi M. C-immsim: playing with the immune response. In: Proceedings of the Sixteenth International Symposium on Mathematical Theory of Networks and Systems (MTNS2004). Katholieke Universiteit Leuven, Belgium; 2004.
42. Consortium U. UniProt: a hub for protein information. *Nucleic Acids Res.* 2015; 43(D1):D204-D212.
43. Doytchinova I.A., Flower D.R. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics* 2007; 8:1-7.
44. Rathore A.S., et al. ToxinPred 3.0: an improved method for predicting the toxicity of peptides. *bioRxiv* 2023; 2023.08.11.552911.
45. Bui H.-H., et al. Predicting population coverage of T-cell epitope-based diagnostics and vaccines. *BMC Bioinformatics* 2006; 7:1-5.
46. Babnigg G., Joachimiak A. Predicting protein crystallization propensity from protein sequence. *J. Struct. Funct. Genomics* 2010; 11:71-80.
47. Teng D., et al. 3DPro: querying complex three-dimensional data with progressive compression and refinement. In: *Advances in Database Technology: Proceedings of the International Conference on Extending Database Technology*. NIH Public Access; 2022.
48. Ko J., et al. GalaxyWEB server for protein structure prediction and refinement. *Nucleic Acids Res.* 2012; 40(W1):W294-W297.
49. Sheik S., et al. Ramachandran plot on the web. *Bioinformatics* 2002; 18(11):1548-1549.
50. Maslov O., et al. Evaluation of anti-inflammatory, antioxidant activities and molecular docking analysis of *Rubus idaeus* leaf extract. *Jordan J. Pharm. Sci.* 2024; 17(1):105-122.
51. Yan Y., et al. HDOCK: a web server for protein–protein and protein–DNA/RNA docking based on a hybrid strategy. *Nucleic Acids Res.* 2017; 45(W1):W365-W373.
52. Laskowski R.A. PDBsum: summaries and analyses of PDB structures. *Nucleic Acids Res.* 2001; 29(1):221-222.
53. Olson S.A. EMBOSS opens up sequence analysis. *Brief. Bioinform.* 2002; 3(1):87-91.
54. Al-Kanany F.N., Othman R.M. Cloning and expression of *Pseudomonas aeruginosa* AlkB gene in *E. coli*. *J. Pure Appl. Microbiol.* 2020; 14(1).
55. Hegazy W.A.H., Hensel M. *Salmonella enterica* as a vaccine carrier. *Future Microbiol.* 2012; 7(1):111-127.
56. Crouch C.F., et al. Safety and efficacy of a novel inactivated trivalent *Salmonella enterica* vaccine in chickens. *Vaccine* 2020; 38(43):6741-6750.
57. Wisner A.L., et al. Immunization of chickens with *Salmonella enterica* subspecies enterica serovar Enteritidis pathogenicity island-2 proteins. *Vet. Microbiol.* 2011; 153(3-4):274-284.
58. Garmory H.S., Brown K.A., Titball R.W. *Salmonella* vaccines for use in humans: present and future perspectives. *FEMS Microbiol. Rev.* 2002; 26(4):339-353.
59. Jawalagatti V., Kirthika P., Lee J.H. Oral mRNA vaccines against infectious diseases—A bacterial perspective. *Front. Immunol.* 2022; 13:884862.
60. Abadi M.H.J.N., et al. In silico design and immunoinformatics analysis of a chimeric vaccine construct based on *Salmonella* pathogenesis factors. *Microb. Pathog.* 2023; 180:106130.
61. Oyarzun P., Kobe B. Computer-aided design of T-cell epitope-based vaccines: addressing population coverage. *Int. J. Immunogenet.* 2015; 42(5):313-321.
62. Mallik B., Morikis D. Applications of molecular dynamics simulations in immunology: a useful computational method in aiding vaccine design. *Curr. Proteomics* 2006; 3(4):259-270.

تطوير لقاح mRNA مبتكر باستخدام الحاسوب باستخدام عدة أنماط من بروتين SopD للحمى المعوية التي تسببها السالمونيلا المعوية

آئزة ممتاز[1]*، هادية حسين[1]، محمد عمير[1]، وصلة علي[1]، محمد ساجد[1]

[1] قسم التكنولوجيا الحيوية، جامعة أوكارا، باكستان.

ملخص

أدى تزايد مشكلة مقاومة المضادات الحيوية إلى تحديات في علاج عدوى السالمونيلا المعوية. وبناءً على ذلك، طُوّرت مجموعة متنوعة من اللقاحات كبديل عملي للمضادات الحيوية للوقاية من عدوى السالمونيلا المعوية لدى المرضى. وتشهد تقنية لقاح الرنا المرسال (mRNA) ازديادًا سريعًا في استبدالها بالطرق التقليدية نظرًا لكفاءتها العالية وتكلفتها المنخفضة واستجابتها المناعية الخلطية. يهدف هذا البحث إلى تطوير لقاح جديد قائم على الرنا المرسال (mRNA) باستخدام مناهج المعلوماتية المناعية ضد السالمونيلا المعوية. تم اختيار بروتين SopD والحصول على مستضداته المناسبة. دُرست هذه المستضدات للتحقق مما إذا كانت مستضدات، وغير مسببة للحساسية، وغير سامة. علاوة على ذلك، تم ربط هذه المستضدات باستخدام روابط لتحويلها إلى تركيبة لقاح. خضعت تركيبة اللقاح هذه لمزيد من التحليل، وخضعت للالتحام الجزيئي مع مستقبلات محددة تُعرف باسم مستقبلات Toll-like receptors TLR3 باستخدام خادم HDock. أظهرت محاكاة MD أن اللقاح مستقر مع معايير RMSD وRMSF، وكشفت المحاكاة المناعية للقاح أنه فعال، وتم استنساخه باستخدام أداة SnapGene. في النهاية، تم نمذجة بروتين متعدد النُسَخ وتحسينه. أظهرت النتائج أن تركيبة اللقاح كانت فعالة، وغير مُسببة للحساسية، وغير سامة، وتم استنساخها بنجاح. في النهاية، اتضح من النتائج أن بنية mRNA المُصممة يمكن أن تكون لقاحًا فعالًا وواعدًا للعلاج بعد التحقق من صحتها باستخدام تقنيات مُختبرية مثل ELISA والاختبارات الحيوية باستخدام نماذج حيوانية.

الكلمات الدالة: الحمى المعوية، النمط الظاهري، التهاب المعدة والأمعاء، المكورات العنقودية المعوية، تسمم الدم.

* ***المؤلف المراسل: آئزة ممتاز***

aaizamumtaz07@gmail.com

تاريخ استلام البحث 2024/6/23 وتاريخ قبوله للنشر 2024/8/22.