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Original article

Prediction and evaluation of multi epitope based sub-unit vaccine against *Salmonella typhimurium*

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ABSTRACT

Salmonella enteric serovar Typhimurium is the most common enteric pathogen in humans and animals. Consumption of contaminated food or water triggers inflammation that allows *Salmonella* to spread into the gut and causes gastrointestinal diseases. The infection spreads by intestinal invasion, phagocyte internalization and subsequent dissemination in many other patients. This research used TolA, a *Salmonella typhimurium* membrane protein, to computationally design a multi-epitope vaccine against the pathogen. Complete consistency of the candidate vaccine was checked *In silico*, and molecular dynamics simulations confirmed the vaccine's stability. According to docking report, the vaccine has a good affinity with toll-like receptors. *In silico* cloning and codon optimization techniques improved the vaccine's efficacy in *Salmonella typhimurium* manifestation process. The candidate vaccine induced an efficient immune response, as determined by *In silico* immune simulation. Computational studies revealed that the engineered multi-epitope vaccine is structurally stable, capable of eliciting particular immunological reactions, and therefore a candidate for a latent *Salmonella typhimurium* vaccine. However, wet lab studies and further investigations are required to confirm the results.

1. Introduction

Infectious gastroenteritis is caused by a combination of etiological agents known as enteric pathogens and the ingestion of infected food or water. As a major cause of foodborne diseases and a major source of diarrheal conditions in developed and developing countries, the *Salmonella* specie rather is of particular clinical importance in humans and animals (Yasmin, 2019; Yasmin, 2020; Shoaib, et al., 2019; Ahmad, et al., 2020; Nawaz, 2020).

About 40,000 cases are registered annually in United States, according to the CDC (http://www.Cdc.Gov/salmonella/well-known/index.Html), but the number is 30 times higher than the reported because only serious issues are reported and documented. The infection in infants is well-documented, with acute *Salmonella* disease affecting elderly and immunocompromised patients in North America, resulting in 400 deaths per year. Foodborne pathogens are often associated with outbreaks and can affect a wide variety of nearby people. Many attacks are attributed to different *Salmonella serovars* each year, demonstrating the prevalence of enteritis causeby *Salmonella enterica serovar typhimurium* and *Salmonella enterica serovar* (Kariuki et al., 2006).

The most common enteric diseases in humans and animals are caused by *Salmonella enterica serovar typhimurium.* Infection happens when contaminated food or water enters the intestinal epithelium, allowing *Salmonella* to multiply and cause gastrointestinal illness. Studies have highlighted that the infection spread in several patients by invading the intestinal epithelium, phagocytes and post dissemination (Herrero-Fresno and Olsen, 2018).

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S. typhimurium, also known as non-typhoid *Salmonella,* is a widespread food borne pathogen that causes a wide range of diarrheal infections. Fever, gastrointestinal problems, abnormally watery stool and stomach cramps are some of the symptoms. *S. typhimurium* can cause fatal invasive diseases like meningitis, sepsis and bacteremia in countries with poor sanitation. Children, the elderly and those who are immunocompromised are especially vulnerable to the diseases cause by this pathogen. Hence, *S. typhimurium* is a dangerous human pathogen (Chiu, 2020).

S. typhimurium pathogenesis has been extensively studied in recent years (Marcus et al., 2000). The growing understanding of this pathogen's virulence mechanisms has resulted in a thorough analysis of the five *Salmonella* pathogenicity islands (SPIs) discovered to date, each of which contributes significantly to host cell interactions (Schlumberger and Hardt, 2006). The replication of *S. typhimurium* in the host cells acts as a model system for the study of intracellular infection pathogenesis. Genome-scale modeling of bacterial metabolic networks allows identification and analysis of pathways important for efficient host-pathogen replication (McLaughlin, 2017).

Vaccines for typhoid fever are commonly given and partially effective, especially in children but there is no non-typhoid *Salmonellosis* vaccine is available. It is important to regularly investigate and consider the mechanisms underlying immunity for enteric infections (Syed, 2020). Salmonellosis therapy usually includes replacing oral and intravenous fluid loss and treating pain, nausea and vomiting. Typhoid fever and enteric fever are treated with antibiotics. Antibiotic therapy in septicemic, enteric fever and focal contamination syndromes should be reserved for non-typhoidal *Salmonellosis*. In the case of uncomplicated *Salmonella* gastroenteritis, antibiotics are no longer recommended because they do not minimize the length of the infection but increase the number of fake pathogens and antibiotic-resistant strains significantly (Sana, 2016).

Conformist vaccine design methods focused on whole or broad proteins result in excessive antigenicity and an increased likelihood of allergens (Chauhan et al., 2019). The use of fully peptide-based vaccines, which contain short immunogenic peptide fragments with improved ability to produce potent and concentrated immune responses, avoiding the most allergenic reactions, was used to solve this problem (Faisal et al., 2017). In this study we used short immunogenic peptide to produce immune response. Recent advances in computational biology (Atapour, 2019) have given new insights into the *In silico* design of active vaccines. We developed a multi epitope *Salmonella typhimurium* vaccine *In silico* that was developed on the basis of TolA protein epitopes which causes stimulation of cytotoxic T lymphocytes (CTLs), T lymphocyte assistants (HTLs) and interferon-producing cells (IFNs).

2. Materials and methods

2.1. Selection of most antigenic protein through whole-genome analysis

Whole genome FASTA sequence of *S. typhimurium* was retrieved from NCBI protein Database (https://www.ncbi.nlm.nih.gov/protein). The sequences shorter than 100 amino acids were excluded as they can't help in epitope prediction (Pruitt et al., 2005). To evaluate the antigenicity score of the *Salmonella typhimurium* proteins, the antigenic values of each protein was calculated using the VaxiJen v2.0, an online predictor server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). VaxiJen (Doytchinova and Flower, 2007) is the first antigen prediction server to overcome alignment-dependent methods' limitations. Antigenic proteins with an antigenic score of 0.4 were selected for further structural modeling (the threshold for this model is 0.4). Moreover, SecretomeP 2.0 server was used to predict the secretome rating of each protein. A secretary score of 0.6 was used to categorize proteins (threshold value for bacterial proteins) (Doytchinova and Flower, 2007).

2.2. Multiple sequence alignment of TolA (membrane-spanning protein)

Numerous TolA (membrane-spanning protein) sequences were aligned using Clustal Omega program. Clustal Omega was used for conducting multiple sequence alignment (MSAs) of various homologous proteins or nucleotide sequences quickly and precisely and also highlight distantly correlated proteins. It is available at (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers and Higgins, 2018).

2.3. Generation of consensus sequence

The consensus sequences predicted by using Geneious Prime suite which is a well-known and comprehensive suite of molecular biology and next-generation sequencing (NGS) (Inst et al., 2020). Sequences manually submitted to the program that was then aligned and assembled, followed by multiple sequence alignment, using Blosum62 parameters. Then generated MSA file was used to generate Consensus sequence.

2.4. Prediction of CTL and HTL epitopes

NetCTL 1.2 server (https://services.healthtech.dtu.dk/service.php?NetCTL-1.2) was used to predict 9-mer long CTL epitopes, which were classified by using types of HLA Class I found in humans, such as A1, A2, A3, A24, A26, B8, B27, B39, B44, B58, and B62106. NetCTL provide 54–89 percent sensitivity and 94–95 percent precision in predicted epitopes. Epitopes detected by using various HLA class I alleles using the IEDB's immune epitopal consensus mechanism (https://www.iedb.org/). The antigenicity and immunogenicity of CTL epitopes were assessed using VaxiJen v2.0 and IEDB class I net servers (Calis, et al., 2013). The use of 15-mer HTL allegiance epitopes for Class II HLA alloys was predicted by the NetMHCIIpan 4.0 Server (http://www.cbs.dtu.dk/services/NetMHCIIpan/) (Jensen, 2018). According to the NetMHCIIpan Server, the predicted peptides were graded as solid, intermediate, or non-binding, with percentile ranks of 2, 10, or >10%, respectively (Kar, 2020). For HTL epitopes, antigenicity was determined using the VaxiJen web server v2.0 (Doytchinova and Flower, 2007).

2.5. Construction of vaccine sequence

The CTL epitopes were connected using AAY connectors and HTL epitopes were linked by GPGPG connectors to build the final multi-epitope-based vaccine. GPGPG spacer help in increasing proteasome processing, and augmented immunogenicity (Behmard et al., 2020). AAY linker increases the number of alpha helixes while reduces beta turns and loops and in this way, it helps to reduce the flexibility (Yang, 2015). The linkers effectively increase immunogenicity, human defensing have been added to the N-terminus of the vaccine as an adjuvant using the EAAAK linker (Khan, 2019). Addition of adjuvant is necessary because it increases the immune responses as well as it added to increase the efficacy or potency of vaccine (Sanches, 2021).

2.6. Structure prediction and validation of multi-epitope vaccine

trRosetta (https://yanglab.Nankai.Edu.Cn/trrosetta/) was used to create a 3D structure of a linear vaccine construct. trRosetta predicts structure of proteins rapidly and accurately. The multi-epitope vaccine tertiary structure has been validated using the ERRAT (Rasheed, 2021) and ProSA-web analysis (Wiederstein and Sippl, 2007). ProSA-web uses expected Z scores to verify the model. Furthermore, Ramachandran plot analysis and the RAMPAGE server measured the overall quality of the developed vaccine model (Lovell, 2003).

2.7. Prediction of B-cell epitopes within the vaccine construct

The immune system's most critical component is B lymphocytes, which secrete antibodies and provide long-term immunity. The research used the webserver ElliPro (http://tools.iedb.org/ellipro/) to predict linear and discontinuous B-cell epitopes.

2.8. Enrichment analysis of the predicted vaccine

To check allergenicity of candidate vaccine, AllerTOP (https://www.Ddg-pharmacy.Net/allertop/) tool used to ensure that it would not cause any allergic reaction after administration. AllerTOP can reliably predict the route of allergen exposure, whether it's through food, inhalant, or toxin (AllerTOP 2.0, "Bioinformatics tool for allergenicity prediction.", 2018). Antigenicity research is an essential part of the vaccine production process. VaxiJen 2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), an online webserver was used to predict antigenicity of candidate vaccine (Doytchinova and Flower, 2007). Physicochemical properties of candidate vaccine were evaluated through Expasy ProtParam (http://web.expasy.org/protparam/). The server includes details of amino acid configuration, molecular weight, aliphatic index, *In vitro* and *In vivo* half-life, instability index, GRAVY and theoretical pI of amino acids (Gasteiger et al., 2005).

2.9. Docking analysis

To explore the vaccine molecule's association with immune receptors, molecular docking of TLR4 and TLR2 immune receptors were performed. Interaction of vaccine molecule with target immune cells, the host mounts an effective immune response. Molecular docking analysis was carried out to confirm the multi epitope vaccine (MEV's) binding to human immune receptors. TLR4 has been implicated in the generation of an antibacterial immune response in previous studies. Molecular docking of MEV with TLR4 and TLR2 (PDB ID: 3fxi and 2z7x, respectively) was conducted through PatchDock server (https://bioinfo3d.cs.tau.ac.il/PatchDock/). The docked complexes visualized, and figures were created using the LIGPLOT program, which produces schematic diagrams of protein–ligand interactions.

2.10. Immune simulation and codon optimization

To check the immunological response of the predicted MEV, the C-ImmSim server (http://150.146.2.1/C-IMMSIM/index.php) was used to perform an immune stimulation (Rapin et al., 2010). It replicates the three main mammalian structures (bone marrow, thymus and lymph node). In past research, MEV was demonstrated to simulate various types of immune cells such as HTL, CTL, N.K. Cells, B cells, dendritic cells, immunoglobulins, and cytokines. Moreover, in the analysis, four week time interval between two doses was considered (Chauhan and Singh, 2020). Codon optimizations introduce synonymous mutations that favor efficient protein expression. Basically, it is used to increase the efficiency of host translation of external/introduced genes differ according to the codon used by organisms. The Java Codon Adaptation Tool (http://www.jcat.de/) (Grote, 2005) was used to make MEV codon optimization compliant with the widely used prokaryotic expression method *E. coli* K12. The quality of the G.C. (guanine and cytosine) content and the index of codon adaptation were calculated (CAI) (Sharp and Li, Feb. 1987).

3. Results

3.1. Retrieval and alignment of FASTA sequences of TolA

FASTA sequences of TolA protein were retrieved from NCBI. The bacteria contains 4533 proteins. VaxiJen was used to calculate the immunogenic score since it is the first server to predict protective antigens independently of alignment. The whole genome analysis of the data showed that the most antigenic protein is TolA (membrane-spanning protein) with an antigenic score of 1.1142, a secretary score of 0.76489 and an amino acid sequence of 408 amino acids. Sequence alignment was performed to determine the structural, functional and evolutionary relationships between protein sequences. Multiple sequence alignment of *Salmonella typhimurium* TolA (membrane-spanning protein) revealed the most conserved regions between these strains when the performance format was set to "ClustalW with character counts." The alignment result is shown in **Fig. S1 (A)**.

3.2. Generation of consensus sequence

The term "consensus-sequence" refers at each point in the sequence alignment to the most commonly occurring residues, nuclear or amino acid. It is the product of many sequence alignments in which related sequences have been compared and series motifs have been determined. Consensus sequences in proteins may also describe full protein molecules or short fragments corresponding to the structural and functional conserved regions. A consensus sequence was formed using the Geneious Prime tool for TolA as mentioned in **Fig. S1 (B)**.

3.3. Prediction of CTL and HTL epitopes

An adaptive vaccine model could imitate the natural immunity cause by long-term adaptive immunity, which plays a critical role for CTL and HTL epitopes. CTL epitopes induce permanent cellular immunity to prevent infection and virus-infected cells. HTL epitopes, by comparison, are important in generating humoral and cellular immune reactions. These epitopes induce CD4 + support, which is needed to create protective CD8 + T-cell memory and activate B-cells and create antibodies. As a result, an effective vaccine candidate must contain unique epitopes of essential CTL and HTL receptors. As in Tables 1 and 2, CTL epitopes were predicted using NetCTL1.2, while HTL epitopes by using NetMHC II pan 4.0 server.

3.4. Multi–epitope vaccine construct model

To build the linear vaccine structure, some parameters have been used such as: overlapping HTL and CTL epitopes were removed, the construct must contain the immunogenic and antigenic epitopes but not allergic epitopes. A total of 11 epitopes (5 CTL and 6 HTL) (Table 1 and 2) were used that connected via AYY and GPGPG linkers. Moreover, An EAAAK linker added at the N-terminal of the construct to increase the resistant response duration by adding beta-defensing adjuvant protein (Lei, 2019).

Table 1
The predicted CTL epitopes from the consensus sequence of the protein. NETCTL server was used to forecast the CTL epitopes. The epitopes were predicted by NetMHCIIpan 4.0 Server. The predicted epitopes consist of 05 CTL epitopes.

Epitopes	Super-types	MHC binding affinity	Binding score	Position	Prediction score	Antigenicity score	Immunogenicity score
ATGADISAY	A1,A26,B62	0.4227	1.7949	318	0.9018	1.3797	0.08049
AVLHIILFA	A2	0.6036	0.8998	19	1.6918	1.2614	0.34287
VLHIILFAV	A2,B8	0.7640	1.1389	20	1.2926	1.5291	0.38524
APKTGGGAK	B7	0.4149	0.8005	292	0.9962	2.6470	0.08524
FAVLIWSSF	A26,B7,B8,B58,B62	0.4627	0.9186	26	0.8957	1.0264	0.10407

Table 2
The predicted HTL epitopes from the consensus sequence of the protein.NETCTL server was used to forecast the HTL epitopes. The epitopes were predicted by NetMHCIIpan 4.0 Server. The predicted epitopes consist of 6 HTL epitopes.

Epitope	Position	Allele	Score	Antigenicity score
LHISLAPDGSLKSIT	351	DRB1_0301,DRB3_0101	1.07,1.39	0.9181
GAVVQQYNRQQDQQA	48	DRB4_0101	1.68	0.9558
AAKLAQQQQQQAEEA	23	DRB4_0101	0.65	0.9741
KERLAAQEQQKQAEE	108	DRB4_0101	0.33	0.9928
QAVYEKIKDAKLDFK	392	DRB5_0101	0.81	1.2128
EAEAVKAAADAKKKA	164	DRB5_0101	0.93	1.0717

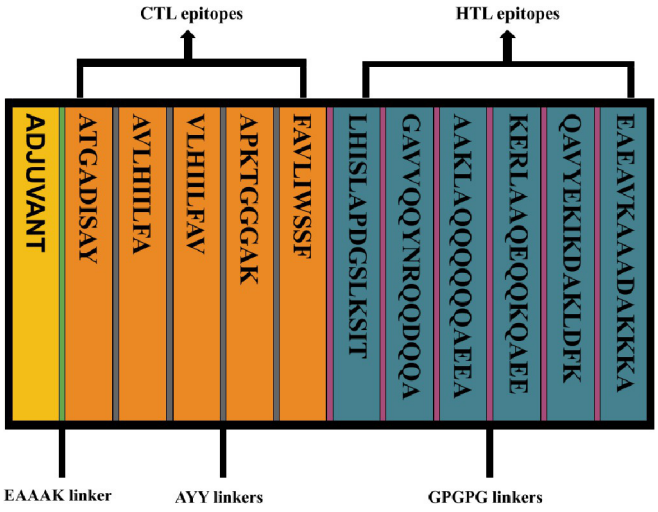

Fig. 1. Structure of multi-epitope vaccine construct. To build the construct, the immunogenic and antigenic epitopes were used. The epitopes are joined by AYY and GPGPG linkers. An EAAAK linker added to the N-terminal of the construct to increase the resistant response duration by adding beta-defensing adjuvant protein.

Beta-defensing adjuvant protein help to protect candidate vaccine from degradation. Furthermore, to improve immunogenicity, adjuvant protein was added at the N-terminus of the vaccine. A complete structure of the vaccine construct is shown in Fig. 1.

3.5. B cell epitopes prediction

Since B-cell receptors or secreted antibodies are recognized, B cell epitopes may contribute to humoral immunity. In the vaccine's design, these epitopes are critical for an efficient immune response. Using the standard parameters, the ElliPro server was used to predict linear/continuous and conformational/discontinuous B cell epitopes (Tables 3 and 4).

3.6. Structure prediction and evaluation of multi-epitope vaccine construct

trRosetta was used to predict the structure of a multi-epitope vaccine. It creates the protein structure while consuming the least amount of direct energy. Inter-residual distance distributions and the orientation predicted by a deep residual neural network are among the constraints. Homogeneous models have been used in network prediction to increase precision for simple objectives. The predicted model structural consistency was validated with the plot, Z-score, and ERRAT analyses. The preferred model was chosen because it had a Z-score of 6.73, which is comparable in the range of protein size, indicating the reliability of the expected model as mentioned in Fig. 2 **(A).** The predicted model structure was tested by Ramachandran plot analysis. The quality analysis highlighted that 95.1 percent of the residues are in the most preferred region and confirmed the overall accuracy of the vaccine as described in Fig. 2 **(B).** The number of residues in the chosen 3D model area ranged from over 90 percent to the maximum value, which confirms its accuracy.

3.7. Multi epitope based subunit vaccine antigenicity and allergenicity

Multi-epitope subunit vaccines can act as a specific antigen and elicit an immune response in response to it. As a result, the vaccine candidate should have natural antigenic properties. With a score of 1.0878, VaxiJen v2.0 assessed vaccine antigenicity. (An antigenic score of more than 0.4 is considered). The allergenicity of the vaccine was tested to ensure that no allergic reactions occur before the

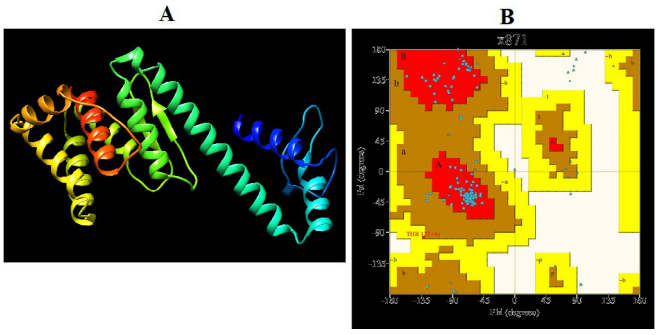

Fig. 2. The structure of the predicted protein of the vaccine construct is shown in part A. trRosetta was used to predict the structure of a multi-epitope vaccine. The quality of the structure was determined by Ramachandran plot analysis as shown in part B. The quality highlighted 95.1% residues in the most preferred region.

Table 3
Conformational or discontinuous B cell epitopes in a vaccine with many epitopes as predicted by ElliPro. The epitopes were predicted using default parameters.

No.	Start	End	Peptide	Number of residues	Score
1	42	59	RRKKEAAAKATGADISAY	18	0.81
2	31	40	GKCSTRGRKC	10	0.761
3	12	29	RVRGGRCAVLSCLPKEEQ	18	0.739
4	146	173	QAGPGPGAAKLAQQQQQQAEEAGPGPGK	28	0.738
5	84	92	AAYAPKTGG	9	0.724
6	197	213	EKIKDAKLDFKGPGPGE	17	0.673
7	183	191	KQAEEGPGP	9	0.636

Table 4
Linear/continuous B cell epitopes in the Vaccine construct, predicted by ElliPro server using default parameters. The presence of these epitopes in the vaccine design is essential for eliciting a successful immune response.

No.	Residues	Number of residues	Score
1	A:K8, A:R12, A:V13, A:R14, A:G15, A:G16, A:R17, A:C18, A:A19, A:V20, A:L21, A:S22, A:C23, A:L24, A:P25, A:K26, A:E27, A:E28, A:Q29, A:G31, A:K32, A:C33, A:S34, A:T35, A:R36, A:G37, A:R38, A:K39, A:C40, A:R42, A:R43, A:K44, A:K45, A:E46, A:A47, A:A48, A:A49, A:K50, A:A51, A:T52, A:G53, A:A54, A:D55, A:I56, A:S57, A:A58, A:Y59, A:Y62	48	0.761
2	A:Q146, A:A147, A:G148, A:P149, A:G150, A:P151, A:G152, A:A153, A:A154, A:K155, A:A157, A:Q158, A:Q159, A:Q161, A:Q162, A:Q163, A:A164, A:E165, A:E166, A:A167, A:G168, A:P169, A:G170, A:P171, A:G172, A:K173, A:L176, A:E180	28	0.749
3	A:A84, A:A85, A:Y86, A:A87, A:P88, A:K89, A:T90, A:G91, A:G92, A:G93	10	0.689
4	A:A194, A:E197, A:K198, A:K200, A:D201, A:A202, A:K203, A:L204, A:D205, A:F206, A:K207, A:G208, A:P209, A:G210, A:P211, A:G212, A:E213	17	0.682
5	A:K183, A:Q184, A:A185, A:E186, A:E187, A:G188, A:P189, A:G190, A:P191	9	0.636
6	A:A118, A:P119, A:D120	3	0.521

patient receives the candidate vaccine. As expected by AllerTOP, the vaccine candidate was found to be allergens free.

3.8. Physiochemical properties of multi-epitope vaccine

ExPASy tool was used to calculate a variety of chemical and physical vaccine parameters. The vaccine's theoretical pI was discovered to be 9.45. The vaccine has an aliphatic index of 75.51. There are 227 amino acids in the multi-epitope vaccine with molecular weight of 23600.01. The vaccine's half-life is estimated to be 30 h by ExPASy. The candidate vaccine has a GRAVY score of 0.329 for high average hydropathicity, indicating that it is hydrophilic and can interact with water. The protein's stability index was calculated to be 39.54, meaning that the vaccine candidate is stable.

3.9. Molecular docking of Multi-epitope vaccine with TLR4 and TLR2

PATCHDOCK was used to dock the MEV structure. The results were visualized in Ligplot. Analysis of the docked results against TLR4 showed Pall(E), Lys(58), Gmh6(E) and Gmh5(E) as neighboring amino acid residues in the pocket area, as illustrated in Fig. 3. The purple Kdo7 (B) lines represent the ligand. The brown lines denote interacting residues Kdo3 (E). Via hydrogen bonding, the interacting residues are linked to the ligand. Moreover, MEV molecular docking with TLR2 highlighted adjacent pocket area amino acid residues are Gln198(A) and Asn199 (A), as shown in Fig. 3. The lines visible in violet Nag901(A) show the ligand. The brown lines indicate residues interacting. The interacting residue is connected to the ligand through hydrogen bonding.

3.10. Immune simulation

To generate an immune response *In silico*, the immunogenic profile of a multi-epitope vaccine was calculated on the C-IMMSIM immune server. The simulation's secondary reaction was significantly more powerful than the first reaction. Secondary response showed a daily decline in antigenic focus in the presence

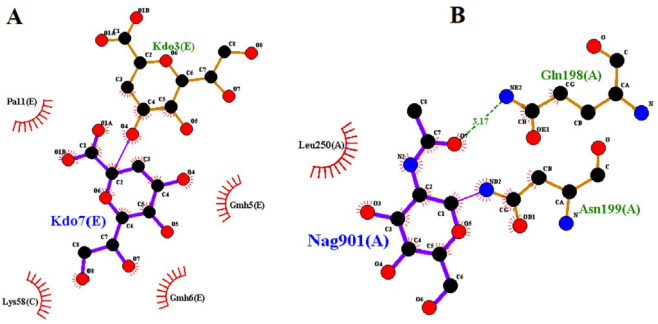

Fig. 3. The docking interaction of vaccine construct protein structure against TLR4 (A) and TLR2 (B). The interaction highlighted the interacting residues among the protein and the toll like receptors.

of elevated levels of immunoglobulin population (IgG1 + IgG2, IgM, and IgG + IgM anticorps). Furthermore, several long-lasting B cell isotypes were discovered, implying isotype switching and memory formation. When T cells were pre-activated during immunization, the populations of T.H. (helper) and T.C. (cytotoxic) cells were also higher. NK (natural killer) and dendritic cell activity and increased macrophage activity were found to be consistent during the exposure period as mentioned in Fig. 4. The high IFN- and IL-2 concentrations in the simulation contributed to a favorable immune response. During simulation analysis, when a living replicating virus was injected, there was no antigenic surge, indicating a strong immune response that mediates a high concentration of certain antibodies. The findings in this case indicate that an adequate immune response was developed after vaccination.

3.11. Reverse translation, codon optimization of multi-epitope vaccine

JCat tool was used to maximize the use of the engineered vaccine in the *Salmonella* strain *Typhimurium* LT2 for maximum protein expression. In general, a value above 0.8 is considered to be conducive to protein expression in the Codon Adaptation Index

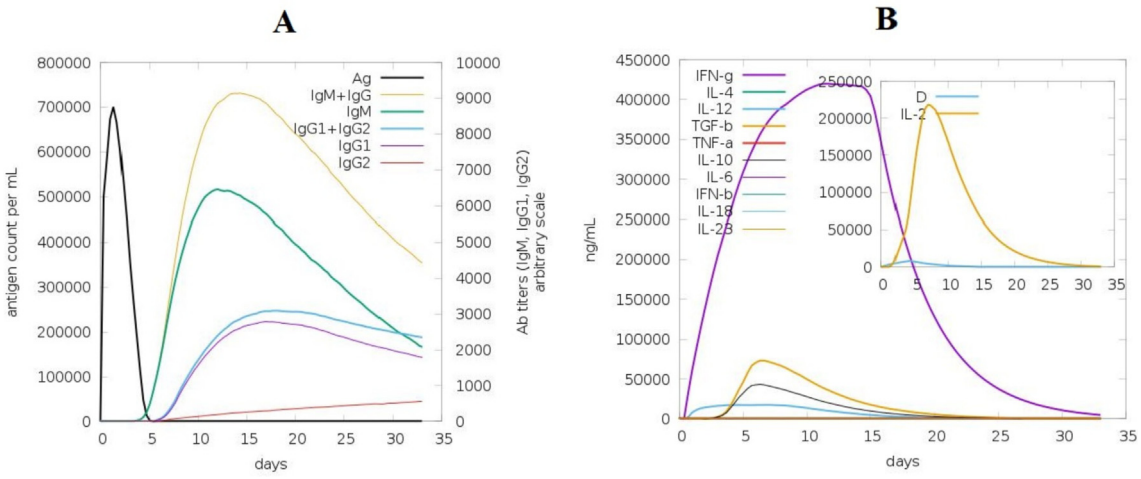

Fig. 4. The virus, the immunoglobulins and the immunocomplexes. Immune simulation results for the predicted vaccine construct. C-IMMSIM immune server was used to assess the immunological profile of the predicted vaccine construct. The responses (secondary and tertiary) produced by the simulation were significantly higher in comparison with primary response (A). Concentration of cytokines and interleukins are shown in part B. The inset plot shows a danger signal together with leukocyte growth factor IL-2.

(CAI) and a host G.C. content between 30 percent and 70 percent. Our vaccine had a reverse translation CAI of 1.0 and 54.18502202643172% G.C. The improved DNA sequence is as follow:

GGTATCATCAACACCCTGCAGAAATACTACTGCCGTGTTCGTGGTG
GTCGTTGCGCTGTTCTGTCTTGCCTGCCGAAAGAAGAACAGATCGGTA
AATGCTCTACCCGTGGTCGTAAATGCTGCCGTCGTAAAAAAGAAGCTG
CTGCTAAAGCTACCGGTGCTGACATCTCTGCTTACGCTGCTTACGCTGT
TCTGCACATCATCCTGTTCGCTGCTGCTTACGTTCTGCACATCATCCTGT
TCGCTGTTGCTGCTTACGCTCCGAAAACCGGTGGTGGTGCTAAAGCTG
CTTACTTCGCTGTTCTGATCTGGTCTTCTTTCGGTCCGGGTCCGGGTCTG
CACATCTCTCTGGCTCCGGACGGTTCTCTGAAATCTATCACCGGTCCGG
GTCCGGGTGGTGCTGTTGTTCAGCAGTACAACCGTCAGCAGGACCAGC
AGGCTGGTCCGGGTCCGGGTGCTGCTAAACTGGCTCAGCAGCAGCAG
CAGCAGGCTGAAGAAGCTGGTCCGGGTCCGGGTAAAGAACGTCTGGC
TGCTCAGGAACAGCAGAAACAGGCTGAAGAAGGTCCGGGTCCGGGTC
AGGCTGTTTACGAAAAAATCAAAGACGCTAAACTGGACTTCAAAGGTC
CGGGTCCGGGTGAAGCTGAAGCTGTTAAAGCTGCTGCTGACGCTAAA
AAAAAAGCT

4. Discussion

Salmonella are the leading cause of gastrointestinal diseases in healthy people and invasive infections in people with weakened immune systems worldwide. Vaccination is one of the best ways to stop infectious diseases from spreading. To prevent *Salmonella* disease in humans and animals, a highly effective, broad-serovar *Salmonella* vaccine is urgently required. While some vaccines are in progress, such as live attenuated vaccines and antigens conjugate vaccines, cross-protection is currently minimal or non-existent (S. F. de O. Tosta, , 2021).

This research is aimed to develop a prophylactic vaccine against *Salmonella typhimurium* TolA, a key determinant of antigenicity and bacterial entry into the host. A multi-epitope vaccine capable of inducing humoral and cell-mediated immunity was used for several computational methods. Rather than large proteins or the entire genome, as recombinant vaccine technology does, a multi-epitope vaccine approach has been established. This protects the host from an excessive antigenic load and allergic reactions. Immunoinformatics and molecular modeling can be used to determine the possible host protein affinities of a wide range of viable antigens (Zhang, Feb. 2018). In contrast to traditional and single-epitope vaccines, these multi-epitope vaccines offer various advantages. In context of immunity, multiple MHC epitopes of Class I and Class II in different types of T-cells are involved. Moreover, MEV conjugate the immune supplement to ensure a long-term immune response with increased immunity. Furthermore, the problems related to *In vitro*-antigen expression are not faced in immunoinformatics approach (De Wit and Van Doremalen, 2016).

A multi-epitope *Salmonella typhimurium* vaccine rather than a vaccine for the whole antigen, using computer instruments was used to predict immune system simulation analysis. Entire genome analysis was performed to identify TolA as the most antigenic protein in *Salmonella typhimurium* (Sbai et al., 2001).

There are 53 *Salmonella typhimurium* strains. We conducted NCBI BLAST scan to determine the strains that contain the selected protein. Multiple-sequence alignment was performed using Clustal Omega to find the conserved protein areas of all strains of *Salmonella typhimurium*. The program Clustal Omega aligns sequences using a seeded tree and an HMM profile algorithm. The Geneious Prime tool was used to produce the sequence of consensus. Geneious primary tool provides descriptions of the most common amino acid residues in protein (Rapin et al., 2010).

Protein structures can be predicted using various approaches, including protein databases, MEV homology modeling, threading and *Ab initio* methods. Initially, we could not locate the desired design when searched the PDB database for a suitable template. By aligning a target protein sequence with one or more protein templates, homology modeling predicts the three-dimensional structure of the sequence (Lu et al., Jun. 2017). If the target protein and the model protein have a sequence identity of more than 75%, homology modeling produces accurate models. Unfortunately, there was no sequence similarity between the target protein and the template protein. As a result, a threading option was selected. For the identification of folding proteins, a method for modeling proteins that share the same fabric fold as proteins with known structures but lack homologs with known structures is also known (Dosch et al., Jun. 2009). Protein threading software is available in a variety of formats including I-TASSER, phyre, ROBETTA and HHpred. According to CASP experiments from 2006 to 2012, I-TASSER is the best server for protein structure prediction (CASP7, CASP8, CASP9 and CASP10). The protein threading method and the I-TASSER server were used to predict TolA's consensus sequence structure and quality of the predicted structure was evaluated (Dar, Sep. 2019). The multi-epitope vaccine structure was

predicted using TrRosetta. A single model was selected and the MEV structure's accuracy was tested. The quality of the MEV structure was determined using the Ramachandran plot. Protein structure parameters were observed using PDBsum. The chosen MEV structure was 95.1 percent accurate.

NetCTL 1.0 was used to predict CTL cell epitopes. On all efficiency tests, NetCTL-1.2 outperforms EpiJen, MAPPP, MHC-pathway, and WAPP. However, the superior performance of NetCTL-1.2 over EpiJen and MHC is statistically negligible. Moreover, NetMHCII Pan 4.0 server was used to predict HTL epitopes. CTL and HTL epitopes were selected on the basis of given immunogenic and antigenic values (Saadi et al., Jul. 2017).

To join HTL epitopes, the spacer GPGPG was used. GPGPG additions decrease binding affinity around the central critical areas and are high in G and P, which are considered to be associated with beta turns during generation of secondary or tertiary structure. Following the GPGPG joining of B and T cell epitopes, a multi-epitope vaccine was developed. The CTL epitopes were linked together using the AYY linker. The EAAK linker connects the N-terminus of beta-defensin, the adjuvant protein that is a possible bacterial adjuvant (Lu et al., Jun. 2017). Using immunoinformatics and molecular modeling approach, multi-epitope vaccine for SARS-CoV-2; immunoinformatic design of SARS-CoV-2 subunit vaccine by using entire structural immunogenic epitopes of SARS-CoV-2 and exploring HCV genome to construct multi-epitope based subunit vaccine to battle HCV infection were performed. The vaccine consists of 407 amino acids while this length varies according to predicted epitopes, linkers used to link epitopes and other parameters required for proper functioning of vaccine after administration. In recent studies, length of candidate vaccine varies from 263 to 694 amino acids according to the requirement (Behmard et al., 2020; Khan, 2021; Khalid and Ashfaq, 2020).

With the AllerTOP v.2.0 server, we predicted that the produced vaccine would be allergen-free. As indicated by vexiJen 2.0, the MEV antigenicity score, the antigenicity of the multipoles vaccine was 1.0878. To test the vaccine's other physicochemical properties, an ExPASy server ProtParam system was used. The vaccine has a molecular weight of 236000.01 and a stability index of 39.54, meaning that it is stable. Proteins with an instability index of less than 40 are thought to be stable, whereas those with a value of more than 40 are considered unstable. The theoretical pI of the vaccine was discovered to be 9.96. The GRAVY vaccine index was 0.329 (lower GRAVY index = higher solubility), indicating the vaccine's polarity and efficient water interaction, indicating high solubility. A protein with an aliphatic index of 75.51 is thermostable. The vaccine half-life was calculated to be 30 h (mammalian reticulocyte *In vitro*), more than 20 h (eye *In vitro*), and more than 10 h (overall concentration of 50% after cell synthesis *In vivo Escherichia coli*).

TLR4 and TLR2 interaction patterns were evaluated using molecular docking experiments. TLR4 and TLR2 have been linked to a successful immune response in several *S.* typhimurium studies. Their findings show that TLR4/TLR2 expression is upregulated 24 h after gastrointestinal infection and is a key factor in resistant response generation.

The researchers showed very small variations in the ten nanoseconds (ns) molecular dynamic vaccine simulation, indicating vaccine stability. The RMSF graph revealed a lot of high peak areas and a lot of vaccine structure versatility. Simulating MDS is an essential part of measuring vaccine safety by simulating *In vivo* vaccine (Jabbar, Dec. 2018). We optimized the engineered vaccine's codons and converted the linear vaccine to cDNA series. The G.C. content was 55.55 percent, indicating that vaccine candidates could be expressed effectively in the *E. coli* host. Immune simulation studies have shown that our engineered vaccine can elicit the right immune responses after the initial antigen injection. Our immune simulation study outperformed a multi-epitope sample for *Salmonella typhimurium* bacteria (Kamthania et al., Dec. 2019).

A detailed study and evaluation of immunological associations with *Salmonella typhimurium* are needed to design and manufacture a highly effective vaccine. The numerical predictions aid researchers in developing a potential vaccine and preventing *Salmonella* infections. Vaccine manufacturing is a time-consuming and expensive process with a high failure rate that takes many years to produce a commercially viable vaccine. The *In silico* analysis highlighted that epitope based vaccine have been found to protect against *salmonellosis* infection which need to be verified through *In vitro* and *In vivo* studies.. All features of the vaccine suggest that it may be synthesized, purified and tested against the host.

5. Conclusion

Infections of *Salmonella typhimurium* are cause of global morbidity and death. There have been preliminary scientific prevention steps to help patients rehabilitate, such as vaccines. Live attenuated and inactivated vaccines are available for *Salmonella typhimurium*, but their effectiveness is decreased with the passage of time. A highly effective low-cost vaccine can be conveniently designed using *In silico* processes. Immune knowledge approaches have been used in this research to develop a multi-epitope vaccine that can produce powerful immune response. The developed vaccine found to be antigenic and immunogenic. Simulation studies ensure vaccine safety and verify stable interactions between the vaccine and immune receptors. However, wet lab studies and further research are required to confirm the results.

Declaration of Competing Interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.09.061.

References

Y. Ahmad et al., "Molecular Screening of Resistant and Virulent Genes in Salmonella Enteritidis and Salmonella Typhimurium from Poultry in Khyber Pakhtunkhwa.," Pak. Vet. J., vol. 40, no. 3, 2020.

AllerTOP 2.0, "Bioinformatics tool for allergenicity prediction." 2018.

Atapour, A. et al., 2019. Designing a Fusion Protein Vaccine Against HCV: An In Silico Approach. Int. J. Pept. Res. Ther. 25 (3), 861–872. https://doi.org/10.1007/s10989-018-9735-4.

Behmard, E., Soleymani, B., Najafi, A., Barzegari, E., 2020. Immunoinformatic design of a COVID-19 subunit vaccine using entire structural immunogenic epitopes of SARS-CoV-2. Sci. Rep. 10 (1), 1–12.

J. J. A. Calis et al., "Properties of MHC Class I Presented Peptides That Enhance Immunogenicity," PLoS Comput. Biol., vol. 9, no. 10, 2013, 10.1371/journal.pcbi.1003266.

Chauhan, V., Singh, M.P., 2020. Immuno-informatics approach to design a multi-epitope vaccine to combat cytomegalovirus infection. Eur. J. Pharm. Sci. vol. 147, no. January,. https://doi.org/10.1016/j.ejps.2020.105279 105279.

Chauhan, V., Rungta, T., Goyal, K., Singh, M.P., 2019. Designing a multi-epitope based vaccine to combat Kaposi Sarcoma utilizing immunoinformatics approach. Sci. Rep. 9 (1), 1–15. https://doi.org/10.1038/s41598-019-39299-8.

Chiu, T.W. et al., 2020. Constructing conjugate vaccine against Salmonella Typhimurium using lipid-A free lipopolysaccharide. J. Biomed. Sci. 27 (1), 1–14. https://doi.org/10.1186/s12929-020-00681-8.

Dar, H.A. et al., Sep. 2019. Immunoinformatics-aided design and evaluation of a potential multi-epitope vaccine against klebsiella pneumoniae. Vaccines 7 (3), 88. https://doi.org/10.3390/vaccines7030088.

E. De Wit, N. Van Doremalen, ... D. F.-N. R., and undefined 2016, "SARS and MERS: recent insights into emerging coronaviruses," nature.com.

Dosch, S.F., Mahajan, S.D., Collins, A.R., Jun. 2009. SARS coronavirus spike protein-induced innate immune response occurs via activation of the NF-κB pathway in human monocyte macrophages in vitro. Virus Res. 142 (1–2), 19–27. https://doi.org/10.1016/j.virusres.2009.01.005.

Doytchinova, I.A., Flower, D.R., 2007. Identifying candidate subunit vaccines using an alignment-independent method based on principal amino acid properties. Vaccine 25 (5), 856–866. https://doi.org/10.1016/j.vaccine.2006.09.032.

Doytchinova, I.A., Flower, D.R., 2007. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics 8 (1), 1–7.

Faisal, A.-R.-M., Imtiaz, S.H., Zerin, T., Rahman, T., Shekhar, H.U., 2017. Computer aided epitope design as a peptide vaccine component against Lassa virus. Bioinformation 13 (12), 417–429. https://doi.org/10.6026/97320630013417.

Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M.R., Appel, R.D., Bairoch, A., 2005. "Protein identification and analysis tools on the ExPASy server", *proteomics Protoc. Handb.*, 571–607

Grote, A. et al., 2005. JCat: A novel tool to adapt codon usage of a target gene to its potential expression host. Nucleic Acids Res. 33 (SUPPL. 2), 526–531. https://doi.org/10.1093/nar/gki376.

Herrero-Fresno, A., Olsen, J.E., 2018. Salmonella Typhimurium metabolism affects virulence in the host – A mini-review. Food Microbiol. 71, 98–110. https://doi.org/10.1016/j.fm.2017.04.016.

M. Inst, O. Cruz, and R. De Janeiro, TABLE Primer list used for CoV nested-PCR and Sanger sequencing. The detection of CoV in pooled library samples and the complete open reading frame (ORF) 1a&b region were amplified from individual samples with those specifically designed primers. Primers , vol. 115. 2020.

Jabbar, B. et al., Dec. 2018. Antigenic Peptide Prediction From E6 and E7 Oncoproteins of HPV Types 16 and 18 for Therapeutic Vaccine Design Using Immunoinformatics and MD Simulation Analysis. Front. Immunol. 9, 3000. https://doi.org/10.3389/fimmu.2018.03000.

Jensen, K.K. et al., 2018. Improved methods for predicting peptide binding affinity to MHC class II molecules. Immunology 154 (3), 394–406. https://doi.org/10.1111/imm.12889.

Kamthania, M., Srivastava, S., Desai, M., Jain, A., Shrivastav, A., Sharma, D.K., Dec. 2019. Immunoinformatics Approach to Design T-cell Epitope-Based Vaccine Against Hendra Virus. Int. J. Pept. Res. Ther. 25 (4), 1627–1637. https://doi.org/10.1007/s10989-018-09805-z.

Kar, T. et al., 2020. A candidate multi-epitope vaccine against SARS-CoV-2. Sci. Rep. 10 (1), 1–24.

Kariuki, S., Revathi, G., Kariuki, N., Kiiru, J., Mwituria, J., Hart, C.A., 2006. Characterisation of community acquired non-typhoidal Salmonella from bacteraemia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya. BMC Microbiol. 6, 1–10. https://doi.org/10.1186/1471-2180-6-101.

Khalid, H., Ashfaq, U.A., 2020. Exploring HCV genome to construct multi-epitope based subunit vaccine to battle HCV infection: Immunoinformatics based approach. J. Biomed. Inform. 108, 103498.

Khan, M. et al., 2019. Immunoinformatics approaches to explore Helicobacter Pylori proteome (Virulence Factors) to design B and T cell multi-epitope subunit vaccine. Sci. Rep. 9 (1), 1–13. https://doi.org/10.1038/s41598-019-49354-z.

Khan, M.T. et al., 2021. Immunoinformatics and molecular modeling approach to design universal multi-epitope vaccine for SARS-CoV-2. Informatics Med. unlocked 24, 100578.

Lei, Y. et al., 2019. Application of built-in adjuvants for epitope-based vaccines. PeerJ 6, e6185.

Lovell, S.C. et al., 2003. Structure validation by C α geometry: φ, ψ and C β deviation. Proteins: Structure. Funct. Genet. 50 (3), 437–450.

Lu, I.N., Farinelle, S., Sausy, A., Muller, C.P., Jun. 2017. Identification of a CD4 T-cell epitope in the hemagglutinin stalk domain of pandemic H1N1 influenza virus and its antigen-driven TCR usage signature in BALB/c mice. Cell. Mol. Immunol. 14 (6), 511–520. https://doi.org/10.1038/cmi.2016.20.

Marcus, S.L., Brumell, J.H., Pfeifer, C.G., Finlay, B.B., 2000. Salmonella pathogenicity islands: Big virulence in small packages. Microbes Infect. 2 (2), 145–156. https://doi.org/10.1016/S1286-4579(00)00273-2.

McLaughlin, P.A. et al., 2017. Contribution of asparagine catabolism to Salmonella virulence. Infect. Immun. 85 (2), 1–14. https://doi.org/10.1128/IAI.00740-16.

Nawaz, S. et al., 2020. Rapid Detection of Biofilm Formation by Zoonotic Serovars of Salmonella enterica and Avian Pathogenic E. coli Isolates from Poultry. Biofilms.

Pruitt, K.D., Tatusova, T., Maglott, D.R., 2005. NCBI Reference Sequence (RefSeq): A curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res. vol. 33, no. DATABASE ISS, 501–504. https://doi.org/10.1093/nar/gki025.

N. Rapin, O. Lund, M. Bernaschi, and F. Castiglione, "Computational immunology meets bioinformatics: The use of prediction tools for molecular binding in the simulation of the immune system," PLoS One, vol. 5, no. 4, 2010, 10.1371/journal.pone.0009862.

Rasheed, M.A. et al., 2021. Immunoinformatics based prediction of recombinant multi-epitope vaccine for the control and prevention of SARS-CoV-2. Alexandria Eng. J. 60 (3), 3087–3097. https://doi.org/10.1016/j.aej.2021.01.046.

S. F. de O. Tosta et al., "Multi-epitope based vaccine against yellow fever virus applying immunoinformatics approaches," J. Biomol. Struct. Dyn., vol. 39, no. 1, pp. 219–235, 2021, 10.1080/07391102.2019.1707120.

Saadi, M., Karkhah, A., Nouri, H.R., Jul. 2017. Development of a multi-epitope peptide vaccine inducing robust T cell responses against brucellosis using immunoinformatics based approaches. Infect. Genet. Evol. 51, 227–234. https://doi.org/10.1016/j.meegid.2017.04.009.

Sana, T.G. et al., 2016. Salmonella Typhimurium utilizes a T6SS-mediated antibacterial weapon to establish in the host gut. Proc. Natl. Acad. Sci. U. S. A. 113 (34), E5044–E5051. https://doi.org/10.1073/pnas.1608858113.

Sanches, R.C.O. et al., 2021. Immunoinformatics design of multi-epitope peptide-based vaccine against Schistosoma mansoni using transmembrane proteins as a target. Front. Immunol. 12, 490.

Sbai, H., Mehta, A., DeGroot, A.S., 2001. Use of T cell epitopes for vaccine development. Current drug targets. Infectious disorders 1 (3), 303–313. https://doi.org/10.2174/1568005014605955.

Schlumberger, M.C., Hardt, W.D., 2006. Salmonella type III secretion effectors: Pulling the host cell's strings. Curr. Opin. Microbiol. 9 (1), 46–54. https://doi.org/10.1016/j.mib.2005.12.006.

Sharp, P.M., Li, W.H., Feb. 1987. The codon adaptation index-a measure of directional synonymous codon usage bias, and its potential applications. Nucleic Acids Res. 15 (3), 1281–1295. https://doi.org/10.1093/nar/15.3.1281.

M. Shoaib et al., "Sero-prevalence and associated risk factors of Mycoplasma Gallisepticum, Mycoplasma Synoviae and Salmonella Pullorum/Gallinarium in poultry," Pak Vet J http//dx. doi. org/10.29261/pakvetj, 2019.

Sievers, F., Higgins, D.G., 2018. Clustal Omega for making accurate alignments of many protein sequences. Protein Sci. 27 (1), 135–145. https://doi.org/10.1002/pro.3290.

Syed, K.A. et al., 2020. Review on the recent advances on typhoid vaccine development and challenges ahead. Clin. Infect. Dis. 71 (Suppl 2), S141–S150. https://doi.org/10.1093/cid/ciaa504.

M. Wiederstein and M. J. Sippl, "ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins," Nucleic Acids Res., vol. 35, no. SUPPL.2, pp. 407–410, 2007, 10.1093/nar/gkm290.

Yang, Y. et al., 2015. In silico design of a DNA-based HIV-1 multi-epitope vaccine for Chinese populations. Hum. Vaccin. Immunother. 11 (3), 795–805.

Yasmin, S. et al., 2019. Antibiotic susceptibility pattern of Salmonellae isolated from poultry from different Districts of Punjab, Pakistan. Pak. Vet. J 40, 98–102.

Yasmin, S. et al., 2020. Phytochemical analysis and In Vitro activity of essential oils of selected plants against Salmonella enteritidis and Salmonella gallinarum of poultry origin. Pak. Vet. J. 40 (02), 139–144.

Zhang, L., Feb. 2018. Multi-epitope vaccines: A promising strategy against tumors and viral infections. Cell. Mol. Immunol. 15 (2), 182–184. https://doi.org/10.1038/cmi.2017.92.