In Silico Epitope-based Peptide Vaccine Design against Invasive Non Typhoidal *Salmonella* (iNTS) Through Immunoinformatic Approaches

Md. Chayan Ali¹, Sultana Israt Jahan², Mst. Shanzeda Khatun¹, Raju Das³, Md Mafizur Rahman¹*, Raju Dash⁴*

¹Department of Biotechnology & Genetic Engineering, Faculty of Biological Sciences, Islamic University, Kushtia-7003, Bangladesh
²Department of Biotechnology & Genetic Engineering, Noakhali Science and Technology University, Noakhali-3814, Bangladesh
³Department of Biochemistry and Biotechnology, University of Science and Technology, Chittagong-4202, Bangladesh
⁴Department of Anatomy, Dongguk University College of Medicine, Gyeongju 38066, Republic of Korea

*corresponding Author
Abstract

Salmonella, especially invasive non-typhoidal Salmonella (iNTS) are responsible for developing various invasive diseases, and possess higher mortality rate, due to their higher antibiotic resistance profile than the other bacteria. Therefore, the present study was concerned to develop epitope based peptide vaccine against iNTS species as a successive and substitute protective measures. The study considered comprehensive Immunoinformatic approaches, followed by molecular docking and molecular dynamics simulation to predict the efficient vaccine candidate T cell and B cell epitopes, based on the outer membrane proteins. The study identified two best epitopes YGIFAITAL and KVLYGIFAI from total iNTS outer membrane proteins, which showed higher immunity, non-allergenicity, non-toxicity and also showed higher conservancy and population coverage values. Both epitopes showed higher binding affinity and stability towards HLA-C* 03:03. The MM-PBSA binding free energy showed the YGIFAITAL epitope binds more tightly with both MHC-I and MHC-II molecules. The total contact, H-bond analysis and RMSF results also validate the efficiency of these epitopes as vaccine candidate. The projected B cell epitopes AAPVQVGEAAGS, TGGGDGSNT and TGGGDGSNTGTGTTT showed higher antigenicity.

Overall, the study concluded that these epitopes can be considered as the potential vaccine candidate to make a successive vaccine against iNTS species. However, this result further needs to be validate by wet lab research to make successive vaccine with these projected epitopes.

Key Words: Salmonella, Antibiotic resistance, Immunoinformatic, Epitope, Molecular Docking, Molecular Dynamics simulation.

1. Introduction

Salmonella is rod-shaped bacteria under the family of Enterobacteriaceae and gram negative in nature (Andino & Hanning, 2015). Salmonella bongori and S. enterica are two main species of Salmonella (Hurley, McCusker, Fanning, & Martins, 2014). Salmonella enterica has several serovars that have a broad host range including humans and nonhumans (Singletary et al., 2016). Nontyphoidal salmonella (NTS) infections are getting concerned through worldwide (Eng et al., 2015). Most of the infection is caused by S. Typhimurium(65.5%), S. Enteritidis (33.1%) of all NTS strains isolated so far (Ao et al., 2015).
Salmonella causes several food-borne illnesses in humans. Children at the age of under 5 years are most commonly affected by salmonella (Aoki et al., 2017; Cellucci, Seabrook, Chagla, Bannister, & Salvadori, 2010; Scallan et al., 2011). NTS are leading to cause non-typhoid diarrhea, gastroenteritis, bacteraemia and enteric fever. They are estimated to cause approximately 93 million cases of enteric infections and among them 155,000 patients die globally each year due to diarrhea (Majowicz et al., 2010). NTS can be transmitted through animal-based food items like dairy foodstuffs, eggs and poultry and can also be transmitted through person to person contact or person to household pets contact (Brad, 2006; Haeusler & Curtis, 2013; Katz, Ben-Chetrit, Sherer, Cohen, & Muhsen, 2019). Patients with sickle cell diseases, HIV and who reside in rural areas are most susceptible to devastating infection (Uche, MacLennan, & Saul, 2017).

The evolution of antimicrobial resistance patterns of non-typhoidal salmonella species has been remarked as an important health concern throughout the world (Chiu et al., 2002). The first multidrug resistance (MDR) NTS Salmonella Typhimurium was isolated in 1990 (Helms, Ethelberg, Mølbak, & Group, 2005). MDR Salmonella Typhimurium and S. Enteritidis serotypes are resistant to kanamycin, ampicillin, sulfamethoxazole streptomycin, streptomycin, chloramphenicol, sulfamethoxazole, and tetracycline (Crump et al., 2011; Karon, Archer, Sotir, Monson, & Kazmierczak, 2007; Varma et al., 2005). According to the data of the National Antimicrobial Resistance Monitoring System (NARMS) 2005, about 4.1% isolates showed less susceptibility against cephalosporin and above all, about 84% medical samples of non-typhoidal salmonella species shown MDR phenotype (Eng et al., 2015). In addition, S. Enteritidis that grows in chicken stuffs causes severe outbreaks and the species were tetracycline and ampicillin resistance (VT Nair, Venkitanarayanan, & Kollanoor Johny, 2018). S. Choleraesuis is also found resistance to ciprofloxacin and fluoroquinolone found in Taiwan and possess a serious threat to the human being ("<chiu2004.pdf>", ; Chiu et al., 2002; Chiu, Wu, Su, Liu, & Chu, 2004).

There is no particular commercial vaccine for NTS infection available in the market. So, development of an effective vaccine against NTS infection is mandatory before severe pandemic outbreaks. Due to the overuse and misuse of antibiotics the bacterial species are becoming resistance to antibiotics. Vaccine can limit the spread of antimicrobial resistance (AMR) (Lipsitch & Siber, 2016). There is a projection that about 10 million people will die each year owing to AMR after 2050, which is 7,00,000 deaths/year at present (Tagliabue & Rappuoli, 2018). Besides,
the cost of antimicrobial drugs is becoming higher. Antibiotics are prescribed after the infection occurred, but vaccine can be taken before infection. So, vaccine can induce faster immunity rather than antibiotics. Peptide vaccine production is a cost-effective way to produce a successful vaccine within a short time compared to other methods.

In our study, we have retrieved the outer membrane protein sequences of common NTS species (*S. Typhimurium*, *S. Enteritidis*, *S. Choleraesuis*, *S. Salamae*) for the isolation of most antigenic protein to design epitope-based (T cell and B cell epitope) peptide vaccine using in silico approaches. Outer membrane proteins (OMPs) are important for host-pathogen interaction during infection. OMPs are responsible for maintaining molecular integrity and permeability through the cell membrane in gram negative bacteria and 2-3% genes encode OMPs (Buchanan, 1999; Koebnik, Locher, & Van Gelder, 2000). This study provides vast knowledge about the possible vaccine candidate epitope. The wet lab researchers can use this prediction to validate this result.

2. Materials and methods

2.1 Protein sequence retrieval

The total outer membrane protein sequences of NTS (*S. Typhimurium*, *S. Enteritidis*, *S. Choleraesuis*, *S. Salamae*) has been recovered from the Universal Protein Resource database (UniProt) at http://www.uniprot.org (Consortium, 2014) and the FASTA formatted sequences were saved for further analysis.

2.2 Highest Antigenic Protein Identification

For designing an effective peptide vaccine highest antigenic protein selection is mandatory. The supremist antigenic protein was identified by inserting the FASTA formatted outer membrane protein sequences in the online based server VaxiJen v2.0 (Doytchinova & Flower, 2007) and helps to assess the most antigenic protein intended for more investigation.

2.3 T-Cell Epitope Identification

T cell epitopes refers to the antigenic part of proteins which is recognized by T cell. The online T cell epitope prediction software NetCTL 1.2 has been used in terms of T cell epitopes identification (Larsen et al., 2007) and this can generate CTL epitopes for any inserted protein. In this study, all
of the 12 MHC supertypes were considered at the default value and at 0.75 thresholds for epitope identification. The combined score was used to select the best epitopes provided by NetCTL. MHC-I binding allele was identified by IEDB tool. This tool use Stabilized Matrix-based Method (SMM) to predict alleles (Bjoern Peters & Sette, 2005). The epitope length was set to 9.0 and the alleles were selected based on IC 50 value (Fleri et al., 2017). The binding affinity depends on the IC 50 value, IC 50<50nM, IC 50<500nM, and IC 50 <5000nM indicates higher, intermediate and lower binding affinity (Adhikari, Tayebi, & Rahman, 2018). IEDB (Immune Epitope Database) tool has also been cast off to identify the processing of MHC-I with their interactive alleles through the SMM method by taking 1 as maximum precaution extension and 0.2 as an alpha factor (Björn Peters, Bulik, Tampe, Van Endert, & Holzhütter, 2003; Tenzer et al., 2005). In this study, MHC-II binding alleles of our isolated epitopes were also predicted by SMM align method (Nielsen, Lundegaard, & Lund, 2007).

2.4 Epitope Conservancy and immunogenicity identification

For designing an effective vaccine conservancy and immunogenicity analysis is very important. Conservancy indicates as the segments of protein sequences where epitope lies. In our study, we used IEDB (Kim et al., 2012) integrated tools for conservancy and immunogenicity analysis for the selected epitopes (Bui, Sidney, Li, Fusseder, & Sette, 2007; Calis et al., 2013). Immunogenicity is the capability of a substance to induce immune response. Epitope with higher immunogenicity value is better than less value containing epitope. So, the most immunogenic peptides were screened for further evaluation.

2.5 Prediction of population coverage

Population coverage prediction is also an important step in vaccine design cause it provides information about peptide binding frequencies depending on Human Leukocyte Antigen (HLA) genotypic frequencies, MHC molecules binding and T cell restriction data(Bui et al., 2006). In our study, the IEDB integrated tool (http://tools.iedb.org/population/) (Bui et al., 2006) was used to predict the population coverage of our selected epitope with their corresponding alleles.
2.6 Allergenicity and toxicity prediction

One of the foremost drawbacks of vaccine is that in some cases they cause allergic reactions to the patients. So, the AllerTOP v 2.0 (http://www.ddg-pharmfac.net/AllerTOP/) has been used to predict the allergenicity of the selected epitopes (Dimitrov, Bangov, Flower, & Doytchinova, 2014). The AllerTOP v 2.0 use $k$-nearest neighbors (kNN) methods to predict both allergen and nonallergen peptides. Distinguished results among different allergenicity prediction tools showed that AllerTOP v 2.0 is the best allergenicity prediction server with 88.7% precise results which is greater than AllergenFP v 1.0 (Dimitrov et al., 2014). To eliminate the toxic epitopes we used the ToxinPred server to predict toxicity of our selected epitopes (Gupta et al., 2013).

2.7 Three-dimensional structure design of best epitopes and HLA proteins

After analyzing immunogenicity, conservancy, allergenicity and nontoxicity best performing epitopes were selected to design there three-dimensional structure using PEPstrMOD (http://osddlinux.osdd.net/raghava/pepstrmod) (Kaur, Garg, & Raghava, 2007; Singh et al., 2015). PEPstrMOD is the modified method of PEPstr which predict the tertiary structure of the peptide using $\beta$-turn information and regular secondary structure (Kaur et al., 2007). We used natural peptide beginner method to predict the structure which works on PEPstr algorithm using AMBER11. We didn’t find any annotated structure in Protein Data Bank (PDB) that’s why homology modeling has been used to predict the 3-D structure of our epitope molecules using Phyre2 (Kelley & Sternberg, 2009). For the prediction of our HLA-C*03:03 protein structure we used protein structure prediction wizard Maestro (Release, 2018) and for homology modelling we used Prime, both developed by Schrödinger (Jacobson, Friesner, Xiang, & Honig, 2002; Jacobson et al., 2004; Kopp & Schwede, 2004). Prime uses a mixture of primary and secondary structure data to calculate alignments. Structures are constructed using atom locations from the template(s) matched parts, bringing into consideration solvent, ligand, power field and other variables via a sequence of algorithms. Portions of the request set that are not aligned with the model, such as loops, are constructed using a solvation ab initio approach (Kopp & Schwede, 2004). After that, correction and minimization of the predicted structure was done using ModRefiner (Xu & Zhang, 2011). To validate the predicted structure PROCHECK (Laskowski, Rullmann, MacArthur,
Kaptein, & Thornton, 1996), PROVE (Pontius, Richelle, & Wodak, 1996), and ERRAT (Colovos & Yeates, 1993) was used.

2.8 Docking analysis
For docking analysis, we used Fast Interaction Refinement in molecular Docking (FireDock), an online based molecular docking platform worked on shape complementarity of soft molecular surfaces. FireDock operates in a manner to optimize lateral string conformations and rigid body orientations, thus providing a large degree of refinement. The user-friendly design and 3-D outcomes visualization improves FireDock's usefulness to a higher degree (Andrusier, Nussinov, & Wolfson, 2007; Mashiach, Schneidman-Duhovny, Andrusier, Nussinov, & Wolfson, 2008). We considered HLA molecule as receptor and our desired epitope as ligand in a blind manner means the epitope will bind to the best conceivable place not to the predetermined place. FireDock provide results on the basis of global rank with greater refinement (Pradhan & Sharma, 2014). Later, the coordinate files were converted to pdbqt format from pdb format.

2.9 Molecular dynamics simulation study and binding free energy calculations
To assess the stability of predicted epitope with their respective receptors we used Molecular dynamics simulation in an explicit solvent system (water) by YASARA (Land & Humble, 2018) dynamic software set with AMBER force field (Dickson et al., 2014). The best receptor-ligand position provided by molecular docking computations was used for selected for further analysis. In this prospect, a simulation cell was generated in a clean and optimized system and our receptor-ligand complex placed inside it. We used an unambiguous TIP3 (at 0.997 g/L-1, 25°C, and 1 atm) water model solvation system included 46406 atoms, where we used steepest gradient technique to minimize energy by simulated annealing methods that allowed system symmetry for the executed environments (25 °C, pH 7.4, and 0.9% NaCl) (Cojocaru & Clima, 2019; Krieger, Nielsen, Spronk, & Vriend, 2006). For better clarity, we omitted the rendering water molecules. According to the calculated pKa, hydrogen atoms have been supplied to the protein structure in favor of simulation pH in the suitable ionizable communities (i.e. if the pKa becomes higher compared to pH, one hydrogen atom will be supplied). The pKa was calculated using the Ewald technique for each residue (Krieger et al., 2006). Subsequently, by using a step-size equivalent to 2.5 fs, the manufacturing MD simulation was operated at YASARA force field level around 100 ns time scale.
The molecular gestures were recorded as snapshots of simulation time frames, thus setting up the trajectory of the system. An assessment of the MD trajectories was saved every 250 ps. This trajectory was used further for the analysis of RMSD, RMSF, H-bond, total contact and binding free energy MM-PBSA calculations.

We used Accelrys Discovery Studio 2.5 executed MM (CHARMM) (Vanommeslaeghe et al., 2010) Poisson-Boltzmann Surface Area (PBSA) protocols to calculate the binding free energy of MHC-II-epitope complex. We kept the salt concentration at 0.15 M for this calculation. We set 14.0 Å as a distance cutoff value and other values were kept default like the values of conformational entropy and ligand minimization. The following mathematical equation was used for binding free energy calculations:

\[ \Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{HLA}} + G_{\text{epitope}}) \]

Where, \( G = \langle G_{\text{intra}} \rangle + \langle G_{\text{inte}} \rangle + \langle G_{\text{pol}} \rangle + \langle G_{\text{np}} \rangle - T\Delta S. \)

### 2.10 Identification of B cell epitope

B cell epitope plays an important role in inducing immune response thus prediction of B cell epitope is a crucial part during designing a peptide vaccine. B cell epitopes bind with B cell receptor (BCR) and secreted immunoglobins and mediate adaptive immune response (Sanchez-Trincado, Gomez-Perosanz, & Reche, 2017). In this study, prediction of B cell epitope was done using BepiPred 2.0([http://www.cbs.dtu.dk/services/BepiPred/](http://www.cbs.dtu.dk/services/BepiPred/)) (Jespersen, Peters, Nielsen, & Marcatili, 2017), BCPREDS ([http://ailab.ist.psu.edu/bcpred/predict.html](http://ailab.ist.psu.edu/bcpred/predict.html)) (EL‐Manzalawy, Dobbs, & Honavar, 2008) and ABCpred ([https://webs.iiitd.edu.in/raghava/abcpred/](https://webs.iiitd.edu.in/raghava/abcpred/)) (Saha & Raghava, 2006) online based servers. BepiPred 2.0 is designed based on random forest algorithm which predict epitopes on the basis of the structure of antigen-antibody proteins (Jespersen et al., 2017). The threshold value was set to 0.5 for this prediction in BepiPred 2.0 server. BCPREDS servers use subsequence kernel methods to predict B cell epitope (EL-Manzalawy et al., 2008). ABCpred predict epitopes by recurrent neural network with 65.93% accuracy (Saha & Raghava, 2006). The threshold value was set to 0.51 and epitope length was set to 16mer while overlapping filter was on during this prediction.
3. Results

3.1 Identification of highest antigenic protein

All of the outer membrane proteins of NTS species *S. Typhimurium* were evaluated by Vaxijen v2.0 server. The protein sequence with UniProtKB id: A0A0F6BA63 showed highest antigenicity 1.2685 with 0.4 threshold value. This protein sequence was also found common to other NTS species (*S. enteritidis*, *S. Choleraesuis*, *S. Salamae*) with same amino acid sequence but different input id at UniProtKB database. This sequence contains 80 amino acids and used for later analysis in our study.

3.2 Identification of T cell epitopes

In a preselected manner, web-based T cell epitope prediction tools NetCTL generated epitopes of our query proteins. Firstly, we selected seven best epitopes (Supplementary Table 1) depending on their highest combined score from all MHC supertypes.

The isolated T cell epitopes has been used for the prediction of MHC-I binding allele by SMM methods. The epitopes which provided higher binding affinity IC 50 >200 nM was used for further study (Table 1).

Proteins are broken down into peptides by cellular, indigenous proteasome complex and presented by antigen presenting cells (APC) through MHC-I molecules to the helper T cells. MHC-I processing tools were used to predict the overall processing score (TAP score, proteasome score, MHC-I score, processing score) of all potential epitopes that could be bind with T cells generated from the protein sequence. The higher processing potentiality of the peptide is greatly depending on the higher overall score. Peptide contains higher value has highest processing ability.

On the basis of table-1, a nonameric epitope **YGIFAITAL** was found most interactive including MHC-I alleles HLA-C*03:03, HLA-B*15:02, HLA-A*02:06, HLA-C*14:02, HLA-C*12:03 and HLA-B*39:01 with higher binding affinities among the seven epitopes (Table 1).

Later, CD4+ T cell epitopes were forecasted with the aid of the epitope **YGIFAITAL** with their corresponding MHC-II alleles. We found 21 15mer epitopes where YGIFAITAL was the core peptide sequence with IC 50 value less than 1000 (Supplementary Table 2). Among these, 15 alleles exhibit strong binding affinity (106-463) with HLA-DR alleles: HLA-DRB1*04:01, HLA-
DRB1*04:04, HLA-DRB1*04:05, HLA-DRB1*07:01, HLA-DRB1*08:02, HLA-DRB1*09:01 and HLA-DRB4*01:01. So, HLA-DR could be best for binding groove for our desire peptides.

Table 1: Most potential 7 T-cell epitopes with interacting MHC-I alleles, conservancy score, immunogenicity, allergenicity, and toxicity score

| Epitope    | Interacting MHC-I allele with an affinity of ,200 nM and the total score (proteasome score, TAP score, MHC-I score, processing score) | Conservancy (%) | Immunogenicity | Allergenicity | Toxicity |
|------------|---------------------------------------------------------------------------------------------------------------------------------|-----------------|----------------|---------------|----------|
| SSATSVSTV  | HLA-C*12:03(-0.18); HLA-C*03:03(-0.73); HLA-C*15:02(-0.74); HLA-A*68:02(-0.86) HLA-C*07:01(-1.01); | 100%            | -0.25          | Yes           | Non-toxin |
| KVLYGIFAI  | HLA-A*02:06(0.2); HLA-A*32:01(0.16)’ HLA-C*03:03(-0.39); HLA-A*02:01(-0.48); HLA-C*12:03(-0.51); | 100%            | 0.27           | No            | Non-toxin |
| APVQVEAA   | HLA-C*12:03(-0.53); HLA-C*03:03(-0.8); HLA-B*07:02(-1.36); | 100%            | 0.08           | No            | Non-toxin |
| MKKVLYGIF  | HLA-C*12:03(1.03); HLA-B*15:02(0.56); | 100%            | 0.06           | Yes           | Non-toxin |
| YGIIFAITAL | HLA-C*03:03(1.32); HLA-B*15:02(0.33); HLA-A*02:06(0.23); HLA-C*14:02(0.04); HLA-C*12:03(-0.1); HLA-B*39:01(-0.49); | 100%            | 0.38           | No            | Non-toxin |
| VSSAVGVAL  | HLA-C*03:03(0.78); HLA-C*12:03(0.06); HLA-B*15:02(0.03); HLA-C*15:02(-0.05); | 100%            | 0.12           | No            | Non-toxin |
3.3 Epitope conservancy and immunogenicity analysis

A successful vaccine is not only depending on HLA allele interaction but also relies on the population coverage with immunogenicity of the epitopes. So, we predicted epitope conservancy and immunogenicity of all predicted epitopes (Table 1). All the selected epitopes showed 100% conservancy were epitopes YGIFAITAL and KVLYGIFAI represents highest immunogenicity 0.38 and 0.273 respectively.

3.4 Population coverage analysis of selected 2 epitopes

The distribution of HLA alleles varies across the world and across various ethnic communities and also geological locations. The population coverage of the two selected epitopes YGIFAITAL and KVLYGIFAI with their respected MHC-I alleles were predicted (Figure 1). The cumulative world population coverage was 58.02%. The North American ethnic group Mexico Amerindian 80.21% showed highest population coverage. The Southeast Asian country Philippines covered 77.89% which was closely followed by the Philippines Austronesian, Peru, Peru Amerindian, United States Polynesian, Finland And Finland Caucasoid with a population coverage of 77.89%, 76.18%, 75.70%, 74.71%, 71.95% and 71.95% respectively (Figure 1). The United States Amerindian covered 69.72% which was closely related to Mexico, Poland, Poland Caucasoid, Russia Other, Georgia Caucasoid, Germany, Germany Caucasoid, Spain and Spain Caucasoid with a value of 69.42%, 67.32%, 67.32%, 66.95%, 66.35%, 66.24%, 66.24%, 66.06% and 66.06% respectively (Figure 1). The lowest coverage was predicted for United Arab Emirates 1.20% and Central African Republic showed no significant population coverage.

3.5 Allergenicity and Toxicity analysis

Allergenicity and toxicity prediction is a crucial step in vaccine design. Evidence showed that most of the vaccines induce an allergic reaction by producing antibody E and T helper cell type-ii (McKeever, Lewis, Smith, & Hubbard, 2004). For this reason, we predicted allergenicity and toxicity through online server AllerTOP v 2.0 and ToxinPred respectively. The server showed that epitopes SSATSVSTV, MKKVLYGIF and SVSTVSSAV are allergic to humans where epitopes KVLYGIFAI, APVQVGEAA, YGIFAITAL and VSSAVGVAL are nonallergic and ToxinPred predicted that all peptides are non-toxic for human hosts (Table 2).
Figure 1: Population coverage of the selected epitopes with their respective HLA alleles. This figure indicates the population coverage above 65% at different parts of the world.

### 3.6 Validation of predicted Protein structure:

We already mentioned in “Materials and Methods” parts, the 3-D structure was predicted using homology modeling technique (Figure 2a). Afterwards, the structure was validated using various online based protein structure authentication tools. As depicted in figure (2b), the PROCHECK generated Ramachandran plot showed that our predicted model has almost 91.7% amino acid residues in the favorable region, 7.9% residues are located in additional allowed region, 0.4% residues are located in generally allowed regions and 0.0% residues are located in disallowed regions. A high quality protein model contains >90% amino acid residues present in the central regions (Dash et al., 2016). Afterwards, the predicted model was analyzed by PROVE and ERRAT. The PROVE resulted Z-score mean was 0.187 and Z-score RMS was 1.249 (Figure 2c). The predicted protein structure validated by ERRAT which scored above 80% is said a good model (Monterrubio-López & Ribas-Aparicio, 2015). Our provided model scored 96.255%, which can be said as a good model (Figure 2d).
Figure 2: (a) Three-dimensional structure of HLA-C*03:03, (b) Ramachandran plot generated by PROCHECK ERRAT score, (c) PROVE plot Shows average Z score, (d) ERRAT score

3.7 B cell epitope identification

Estimation of B cell is one of the key steps in in-silico vaccine design. The continuous B cell epitope and discontinuous B cell epitope are the two principal type of B cell epitopes. Most of the B cell epitopes are conformational epitopes about 90% (Sun et al., 2013). For the prediction of B
cell epitope peptides, three different online servers were used: BepiPred-2.0, BCPREDs and ABCpred.

On the basis of BepiPred 2.0, 2 epitopes were generated with different length from our respected protein sequence (Supplementary Table 3). The antigenicity of the respected peptides has been predicted by online based server Vaxijen, where $^{60}TGGGDGSNTGTTTTT^{76}$ showed highest score 3.1035. All these two epitopes were 100% conserved. The allergenicity and toxicity of these epitopes were also predicted by online servers AllerTOP v2.0 and ToxinPred respectively. The first epitope $^{16}ATSVSAAPVQVGAAAGSAATSVSAGSSSSATSVSTV^{50}$ was predicted as allergenic for humans by the server so, it can’t be a good epitope for vaccine design. Toxicity prediction results showed that these two epitopes were nontoxic for humans (Supplementary Table 4).

BCPREDs server generates potential B cell epitopes. The epitope length was fixed to 16mer with 75% classifier specificity for the prediction of these epitopes. A total three epitopes were predicted by this tool (Supplementary Table 4). Conservancy results showed that all the epitopes have 100% conservancy rate. Antigenicity, allergenicity and toxicity also predicted for these epitopes by the tools described earlier. The epitope $^{38}SAGSSSSATSVSTVSSA^{53}$ was predicted as allergic for humans. So, it could not be a good epitope for vaccine design. Observing all the epitopes it can be said that, the epitope $^{59}AATGGGDGSNTGTTTTT^{74}$ could be a good peptide for vaccine design as it is non allergenic, non-toxic and also it has highest antigenicity score 2.9631 (Supplementary Table 4).

The online B cell epitope prediction tool ABCpred generates seven epitopes (Supplementary Table 5). Among them, the epitopes $^{17}TSVSAAPVQVGAAAGS^{32}$, $^{39}AGSSSATSSTTVSSA^{54}$ and $^{28}EAAGSAATSVSAGSSS^{43}$ were found as allergenic for humans. So, they couldn’t be good vaccine candidate. All the epitopes showed 100% conservancy rate. The epitopes $^{61}TGGGDGSNTGTTTTT^{76}$, $^{54}VGVALAATGGGDGSNT^{69}$, $^{46}SVSTVSSAVGVALAAT^{61}$, and $^{10}AITALAAATSVAAPVQ^{25}$ showed positive antigenicity score predicted by Vaxijen server (Table 6). Among these, the epitope $^{61}TGGGDGSNTGTTTTT^{76}$ has highest ABCpred score and also highest Vaxijen score so, it could be the best vaccine candidate (Supplementary Table 5).

Five most conserved B cell epitopes were found after searching the likeness among BepiPred 2.0, BCPREDs and ABCpred methods (Table 2). These five peptides are 100% conserved. The
epitopes $^{28}$EAAGSAATS$^{36}$, $^{39}$AGSSSATSVSTV$^{50}$ were predicted as allergic by AllerTOP server so, they couldn’t be good vaccine candidates (Table 2). On the other hand, epitope $^{21}$AAPVQVGEAAGS$^{32}$, $^{61}$TGGGDGSNT$^{69}$ and $^{61}$TGGGDGSNTGTTTT$^{74}$ were predicted as non-allergic and nontoxic for humans (Table 2). The epitope $^{61}$TGGGDGSNT$^{69}$ showed the highest antigenic score predicted by Vaxijen server so, it could be used as the best B cell epitope for designing effective vaccine.

Table 2: Most conserved B cell epitope sequences predicted by these three methods (BepiPred 2.0, BCPREDs and ABCpred)

| Epitope             | Position | Length | Antigenic score | Allergenicity | Toxicity | Conservancy |
|---------------------|----------|--------|-----------------|---------------|----------|-------------|
| AAPVQVGEAAGS        | 21-32    | 12     | 1.0614          | No            | No       | 100%        |
| EAAGSAATS          | 28-36    | 9      | 1.5915          | Yes           | No       | 100%        |
| AGSSSATSVSTV       | 39-50    | 12     | 1.6085          | Yes           | No       | 100%        |
| TGGGDGSNT           | 61-69    | 9      | 3.8161          | No            | No       | 100%        |
| TGGGDGSNTGTTTT     | 61-74    | 14     | 3.3072          | No            | No       | 100%        |

### 3.8 MD simulations

MD simulation was tested for the stability of HLAs and peptide epitopes by 100 ns simulations for our selected epitopes with HLA-C*03:03 and HLA-DRB1*04:01 receptors. The system’s dynamics stability parameters were examined by analyzing RMSD, RMSF, binding free energy calculations, total contact calculation and number of H-bond analysis. The selected epitopes bound in the grave of the receptor HLA-C*03:03 and HLA-DRB1*04:01 with significant binding affinity and that is proved by Molecular docking simulation. The epitope YGIFAITAL obtained higher affinity -9.2 kcal/mol with HLA-C*03:03 (Figure 3) and -11.2 kcal/mol with HLA-DRB1*04:01 (Figure 3), whereas another epitope KVLYGIFAI binding affinity were – 8.0 kcal/mol and -7.3 kcal/mol with HLA-C*03:03 and HLA-DRB1*04:01 respectively. We used chimera (Pettersen et al., 2004) visualization software program to visualize the docked complex shown in figure 4. Calculation of binding energy provides us details information about the interaction of epitopes.
with their respective HLA alleles. In MM-PBSA binding free energy calculation methods more positive results denotes strong binding, where negative values denote weaker binding. The YGIFAITAL epitope showed higher positive value both in HLA-C/MHC-I (Figure 5a) and MHC-II molecules (figure 5b). The average MM-PBSA binding free energy for YGIFAITAL and KVLYGIFAI epitopes complexed with HLA-C*03:03 were almost same (figure 5a), but they showed significant amount of different binding free energy with HLA-DRB1*04:01 alleles (Figure 5b). YGIFAITAL-HLA-C*03:03 showed average binding energy 150 kcal/mol, whereas the KVLYGIFAI epitopes average binding free energy was 100 kcal/mol only with same receptor proteins (figure 5a). Using the energy minimization protocol, the HLA-epitope (HLA-C*03:03-YGIFAITAL, HLA-C*03:03-KVLYGIFAI) complex was simulated in an AMBER14 force field depicted in figure5c. Interpretation of the result revealed that the HLA-C*03:03-YGIFAITAL complex gained stability after 25 ns and remained stable through the simulations. Epitope HLA-DRB1*04:01 showed higher RMSD value at the initial stage of simulation, after 2 ns it became stable and remained till 30 ns suddenly, its stability falls and again become stable at 61 ns and remained till the simulation period figure 5d. In case of, HLA-DRB1*04:01 receptor proteins both theses YGIFAITAL and KVLYGIFAI epitopes showed fluctuations through the whole simulation period. YGIFAITAL showed higher RMSD values and KVLYGIFAI showed lower RMSD values in compare with YGIFAITAL. The YGIFAITAL epitopes showed stability at 20ns to 58 ns, a major fluctuation drift was seen at 60ns and after 62 ns it’s remained stabled again. On the other hand, epitope KVLYGIFAI stabled at 5 ns and remained till 60 ns, at 65 ns fluctuation occurred.

To understand the movement of each residue we calculated Root mean square fluctuations. The YGIFAITAL epitope with HLA-C*03:03 allele showed lower RMSF values ranges from 1 Å to 6.48 Å (average 3.74 Å), whereas KVLYGIFAI-HLA-C*03:03 showed higher RMSF values ranges from 2 Å to 9 Å (average 5.5 Å) shown in figure 6a. We also calculated the RMSF values of HLA-DRB1*04:01 alpha and beta chains with mentioned epitopes (figure 6b). The overall RMSF values of both epitopes were same throughout the simulations for HLA-DRB1*04:01 α
chain. Epitope KVLYGIFAI showed some fluctuations at 45-65 residues and KVLYGIFAI showed little bit higher fluctuations at 78-85 residues compared to YGIFAITAL (Figure 6b). In case of β chain YGIFAITAL showed higher fluctuations around 5.5 Å, it also showed higher fluctuations compared to KVLYGIFAI at 2-negative 5, 80-88, 143-174 residues region (Figure 6b).

The number of hydrogen bonds were also calculated during the simulation periods. It is clearly shown that YGIFAITAL-HLA-C*03:03 complexed showed maximum number of H-bonds compared to KVLYGIFAI-HLA-C*03:03 complex (figure 6c). Figure 6d showed that the number of H-bond formed between epitopes and HLA-DRB1*04:01 alleles. In this figure it is also clear that YGIFAITAL formed maximum number of hydrogen bonds with HLA-DRB1*04:01 molecules whereas, KVLYGIFAI formed much lower number of hydrogen bonds with HLA-DRB1*04:01 molecules.

Analysis of total contact is important to understand the chemistry of ligand interaction in binding site of receptor in the thermodynamic environment. So, the total contact of epitopes and HLA-C*03:03 and HLA-DRB1*04:01 complex were analyzed and depicted in Figure 6e, 6f and respectively. In both cases YGIFAITAL epitopes showed higher amount of contacts with the receptor proteins. The average total contact of KVLYGIFAI- HLA-DRB1*04:01 is around 2575 and YGIFAITAL-HLA-C*03:03 total contact is around 2875.
Figure 4: KVLYGIFAI docked with (a) HLA-C, (b) MHC-II. YGIFAITAL Docked with (c) HLA-C, and (d) MHC-II

Figure 5: MM-PBSA binding free energy analysis of (a) KVLYGIFAI-HLA-C and YGIFAITAL-HLA-C, Here, dark blue denotes YGIFAITAL-HLA-C complex, Green denotes KVLYGIFAI-HLA-C. (b) KVLYGIFAI-MHC-II, YGIFAITAL-MHC-II, Here, red denotes YGIFAITAL-MHC-ii complex, dark blue denotes KVLYGIFAI-MHC-ii complex. Root Mean Square Deviation analysis of (c) KVLYGIFAI-HLA-C and YGIFAITAL-HLA-C, Here, dark blue denotes YGIFAITAL-HLA-C complex, Green denotes KVLYGIFAI-HLA-C. (d) KVLYGIFAI-MHC-II, YGIFAITAL-MHC-II, Here, red denotes YGIFAITAL-MHC-ii complex, dark blue denotes KVLYGIFAI-MHC-ii complex.
Figure 6: Plot showing the RMSF analysis of (a) KVLYGIFAI-HLA-C and YGIFAITAL-HLA-C, (b) KVLYGIFAI-MHC-II, YGIFA ITAL-MHC-II. Hydrogen bond analysis of (c) KVLYGIFAI-HLA-C and YGIFA ITAL-HLA-C, (d) KVLYGIFAI-MHC-II, YGIFA ITAL-MHC-II. Total contact analysis of (e) KVLYGIFAI-HLA-C and YGIFA ITAL-HLA-C, (f) KVLYGIFAI- MHC-II, YGIFA ITAL-MHC-II., Here, in all cases dark blue denotes YGIFA ITAL-HLA-C complex, Green denotes KVLYGIFAI-HLA-C whereas, red denotes YGIFA ITAL-MHC-ii complex, dark blue denotes KVLYGIFAI-MHC-ii complex.
4. Discussion

Identification of a suitable and effective vaccine candidate is the first step of designing an effective vaccine. Vaccine refers to a pharmacological product that can provide the best cost-benefit ratio to fight against diseases. Efficient vaccine development and manufacturing, however, are expensive and can require years to complete. Recent advancement of bioinformatics made it easy for the researchers to find out the probable vaccine candidate within a short course of time using bioinformatics tools and approaches (María, Arturo, Alicia, Paulina, & Gerardo, 2017). Outer membrane proteins are good candidate for rational vaccine design and got much attention in recent times, as they are highly immunogenic, has lower adverse effect, plays major role in cell to cell communication, highly stable in elevated temperature and chemical treatments, induce specific antibodies, abundant localization and pathogenesis (Gerritzen, Martens, Wijffels, van der Pol, & Stork, 2017; Gu et al., 2018; Lin, Huang, & Zhang, 2002; Pan, Li, & Ye, 2016). For example, designing of epitope based peptide vaccine against Acinetobacter baumannii (Jahangiri, Rasooli, Owlia, Fooladi, & Salimian, 2017), Neisseria meningitidis serogroup B (Chandra, Singh, & Singh, 2010), S. Typhi (Verma et al., 2018), Vibrio cholerae (Rauta, Ashe, Nayak, & Nayak, 2016) has been proposed already. Although vaccine development based on epitope has become a popular notion, not much research has yet been performed in the event of iNTS.

In this study, the total outer membrane proteins of iNTS species (S. Typhimurium, S. Enteritidis, S. Choleraesuis, S. Salame) were retrieved and screened. Interestingly we found a common most antigenic protein with the highest Vaxijen score among the above iNTS species. Afterwards, the selected antigenic protein was analyzed by various immunoinformatic tools.

T cell plays a major role in protective immunity against various pathogens (Esser et al., 2003). At the beginning seven potential nonameric T cell epitopes were generated. The NetCTL 1.2 an online based server was used to predict the T cell epitopes. At 0.4 threshold value NetCTL 1.2 server predicts highest number of epitopes within a distinct specificity and sensitivity, considering all MHC-I supertypes (Larsen et al., 2007). The selected epitopes with their IC 50 value were represented in table 1. Finally, we selected two most potential epitopes based on several parameters.
like MHC-I binding efficiency, conservancy, immunogenicity, allergenicity and toxicity. According to the above parameters YGIFAITAL and KVLYGIFAI were identified as the best epitopes.

We used molecular docking simulations and MM-PBSA methods to validate our selected epitopes with both HLA-C*03:03 and HLA-DRB1*04:01 proteins. HLA-C*03:03 and HLA-DRB1*04:01 showed higher affinity thus we selected these MHC-I and MHC-II proteins. The epitope YGIFAITAL attained maximum binding affinity with both MHC proteins. The free energy calculation method MM-PBSA also support this higher affinity. Before going to the further process, we prepared the three-dimensional structure of HLA-C*03:03 proteins using Schrödinger protein structure building software Prime (Jacobson et al., 2002; Jacobson et al., 2004). In this process the crystal structure of HLA-B*07:02 in complex with an NY-ESO-1 peptide (Pdb id 6AT5) showed the maximum similarity.

We performed MD simulation calculation RMSD to observe the stability of the epitope-HLA complex. The higher RMSD value represents the higher flexibility of HLA-epitope binding (Dash et al., 2017; Fu, Zhao, & Chen, 2018). In case of bonding with HLA-C*03:03, YGIFAITAL was much more stable compared to KVLYGIFAI-HLA- C*03:03 throughout the simulations. But, YGIFAITAL showed less stability with HLA-DRB1*04:01, where KVLYGIFAI showed better stability with the receptor allele HLA-DRB1*04:01. We also calculated RMSF for further evaluation. Higher RMSF values indicates the mobility of the epitopes and lower RMSF indicates the stability of interacted epitopes with HLA (Dash et al., 2017). The lower RMSF of YGIFAITAL complexed with HLA-C*03:03 indicates the greater stability of these epitopes and the higher values of KVLYGIFAI represents the flexibility of this epitopes with the same HLA.

The number of hydrogen bonds were calculated because this is another significant element influencing protein stabilization. The higher number of hydrogen bonds represents the higher stability of ligands with the proteins (Fu et al., 2018). In this study, the epitope YGIFAITAL showed maximum number of hydrogen bonds with both HLA proteins, which depicts the better binding ability of YGIFAITAL epitope. In total contact analysis, the epitope YGIFAITAL showed much higher number of contacts in both HLA proteins, whereas KVLYGIFAI showed lower contact with the projected HLA proteins.
Analysis of population coverage is also an important factor for developing effective vaccines because the HLA alleles are different from ethnic group to groups and also differs in geographical regions. So, during vaccine design selection of wide range of populations must be considered. Our selected epitopes showed variable range of population coverage throughout the world populations (Figure). The highest number of population coverage were seen in Mexican Amerindian populations. This result suggest that the proposed vaccine can be applied to the figured mentioned populations.

Identification of B cell epitope is also a key criterion during an effective vaccine design. B cell produce humoral immunity. Humoral immunity is much more strong and has higher efficacy (Adhikari et al., 2018). In this study, the most conserved B cell epitopes were selected for using as vaccine candidates table. We found the most common conserved sequence through all of the predicted methods that ranging from 61-74.

Our expected outcomes in silico, however, were focused on diligent sequence analysis and analysis of different genetic databases. This sort of research has currently been experimentally validated (Khan et al., 2014) and we have therefore suggested that the proposed epitope could cause an effective immune response as an in vitro peptide vaccine.

5. Conclusion

Non Typhoidal salmonella species are responsible for developing several invasive diseases. The mortality rates also higher. Besides, these bacteria are getting resistance through the worldwide. Due to the significant amount of antibiotic resistance profile and mortality rates iNTS species are much concerned towards the scientists. Thinking about all the benefits of peptide vaccine, in this study we used Immunoinformatic approaches to design an effective vaccine against the iNTS species. In our study, we predicted both T cell and B cell to initiate immunity against iNTS bacteria. We also evaluated the efficiency, antigenicity, allergenicity and toxicity of the selected epitopes to validate themselves. Molecular docking and MD simulation calculation were also done
to validate the ligand receptor binding efficiency and stability. We do believe that our vaccine candidate will be much effective against iNTS species but, further research and in vitro, in-vivo validation is also essential to produce an effective vaccine.

**Author Contribution:**

R.D and M.M.R designed the experiment. M.C.A collect the data and run the experiment. M.C.A, S.I.J, M.S.K and Raju Das analysis the data. M.C.A wrote the manuscript. All authors revised the manuscript.

**Conflicts of interest:**

All authors declared there is no conflicts of interest.

**References**

Adhikari, U. K., Tayebi, M., & Rahman, M. M. (2018). Immunoinformatics Approach for Epitope-Based Peptide Vaccine Design and Active Site Prediction against Polyprotein of Emerging Oropouche Virus. *Journal of immunology research, 2018.*

Andino, A., & Hanning, I. (2015). Salmonella enterica: survival, colonization, and virulence differences among serovars. *The Scientific World Journal, 2015.*

Andrusier, N., Nussinov, R., & Wolfson, H. J. (2007). FireDock: fast interaction refinement in molecular docking. *Proteins: Structure, Function, and Bioinformatics, 69*(1), 139-159.

Ao, T. T., Feasey, N. A., Gordon, M. A., Keddy, K. H., Angulo, F. J., & Crump, J. A. (2015). Global burden of invasive nontyphoidal Salmonella disease, 2010. *Emerging infectious diseases, 21*(6), 941.

Aoki, Y., Kitazawa, K., Kobayashi, H., Senda, M., Arahata, Y., Homma, R., . . . Honda, A. (2017). Clinical features of children with nontyphoidal Salmonella bacteremia: A single institution survey in rural Japan. *PloS one, 12*(6), e0176990.

Braden, C. R. (2006). Salmonella enterica serotype Enteritidis and eggs: a national epidemic in the United States. *Clinical Infectious Diseases, 43*(4), 512-517.

Buchanan, S. K. (1999). β-Barrel proteins from bacterial outer membranes: structure, function and refolding. *Current opinion in structural biology, 9*(4), 455-461.

Bui, H.-H., Sidney, J., Dinh, K., Southwood, S., Newman, M. J., & Sette, A. (2006). Predicting population coverage of T-cell epitope-based diagnostics and vaccines. *BMC bioinformatics, 7*(1), 153.

Bui, H.-H., Sidney, J., Li, W., Fusseder, N., & Sette, A. (2007). Development of an epitope conservancy analysis tool to facilitate the design of epitope-based diagnostics and vaccines. *BMC bioinformatics, 8*, 361.

Calis, J. J., Maybeno, M., Greenbaum, J. A., Weiskopf, D., De Silva, A. D., Sette, A., . . . Peters, B. (2013). Properties of MHC class I presented peptides that enhance immunogenicity. *PLoS computational biology, 9*(10), e1003266.
Cellucci, T., Seabrook, J. A., Chagla, Y., Bannister, S. L., & Salvadori, M. I. (2010). A 10-year retrospective review of Salmonella infections at the Children’s Hospital in London, Ontario. *Canadian Journal of Infectious Diseases and Medical Microbiology, 21*(2), 78-82.

Chandra, S., Singh, D., & Singh, T. R. (2010). Prediction and characterization of T-cell epitopes for epitope vaccine design from outer membrane protein of Neisseria meningitidis serogroup B. *Bioinformation, 5*(4), 155.

Chiu, C.-H., Wu, T.-L., Su, L.-H., Chu, C., Chia, J.-H., Kuo, A.-J., . . . Lin, T.-Y. (2002). The emergence in Taiwan of fluoroquinolone resistance in Salmonella enterica serotype Choleraesuis. *New England Journal of Medicine, 346*(6), 413-419.

Chiu, C.-H., Wu, T.-L., Su, L.-H., Liu, J.-W., & Chu, C. (2004). Fluoroquinolone resistance in Salmonella enterica serotype Choleraesuis, Taiwan, 2000–2003. *Emerging infectious diseases, 10*(9), 1674.

Cojocaru, C., & Clima, L. (2019). Binding assessment of methylene blue to human serum albumin and poly (acrylic acid): Experimental and computer-aided modeling studies. *Journal of Molecular Liquids, 285*, 811-821.

Colovos, C., & Yeates, T. O. (1993). Verification of protein structures: patterns of nonbonded atomic interactions. *Protein science, 2*(9), 1511-1519.

Consortium, U. (2014). UniProt: a hub for protein information. *Nucleic acids research, 43*(D1), D204-D212.

Crump, J. A., Medalla, F. M., Joyce, K. W., Krueger, A. L., Hoekstra, R. M., Whichard, J. M., . . . Group, E. I. P. N. W. (2011). Antimicrobial resistance among invasive nontyphoidal Salmonella enterica isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. *Antimicrobial agents and chemotherapy, 55*(3), 1148-1154.

Dash, R., Das, R., Junaid, M., Akash, M. F. C., Islam, A., & Hosen, S. Z. (2017). In silico-based vaccine design against Ebola virus glycoprotein. *Advances and applications in bioinformatics and chemistry: AABC, 10*, 11.

Dash, R., Hosen, S. Z., Sultana, T., Junaid, M., Majumder, M., Ishit, I. A., & Uddin, M. M. N. (2016). Computational Analysis and Binding Site Identification of Type III Secretion System ATPase from Pseudomonas aeruginosa. *Interdisciplinary Sciences: Computational Life Sciences, 8*(4), 403-411.

Dickson, C. J., Madej, B. D., Skjevik, Å. A., Betz, R. M., Teigen, K., Gould, I. R., & Walker, R. C. (2014). Lipid14: the amber lipid force field. *Journal of chemical theory and computation, 10*(2), 865-879.

Dimitrov, I., Bangov, I., Flower, D. R., & Doytchinova, I. (2014). AllerTOP v. 2—a server for in silico prediction of allergens. *Journal of molecular modeling, 20*(6), 2278.

Doytchinova, I. A., & Flower, D. R. (2007). Vaxiilen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC bioinformatics, 8*(1), 4.

EL-Manzalawy, Y., Dobbs, D., & Honavar, V. (2008). Predicting linear B-cell epitopes using string kernels. *Journal of Molecular Recognition: An Interdisciplinary Journal, 21*(4), 243-255.

Eng, S.-K., Pusparajah, P., Ab Mutalib, N.-S., Ser, H.-L., Chan, K.-G., & Lee, L.-H. (2015). Salmonella: a review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science, 8*(3), 284-293.

Esser, M. T., Marchese, R. D., Kierstead, L. S., Tussey, L. G., Wang, F., Chirmule, N., & Washabaugh, M. W. (2003). Memory T cells and vaccines. *Vaccine, 21*(5-6), 419-430.

Fleri, W., Paul, S., Dhanda, S. K., Mahajan, S., Xu, X., Peters, B., & Sette, A. (2017). The immune epitope database and analysis resource in epitope discovery and synthetic vaccine design. *Frontiers in immunology, 8*, 278.

Fu, Y., Zhao, J., & Chen, Z. (2018). Insights into the Molecular Mechanisms of Protein-Ligand Interactions by Molecular Docking and Molecular Dynamics Simulation: A Case of Oligopeptide Binding Protein. *Computational and mathematical methods in medicine, 2018*. 
Gerritzen, M. J., Martens, D. E., Wijffels, R. H., van der Pol, L., & Stork, M. (2017). Bioengineering bacterial outer membrane vesicles as vaccine platform. *Biotechnology advances, 35*(5), 565-574.

Gu, H., Liao, Y., Zhang, J., Wang, Y., Liu, Z., Cheng, P., . . . Gu, J. (2018). Rational design and evaluation of an artificial Escherichia coli K1 protein vaccine candidate based on the structure of OmpA. *Frontiers in cellular and infection microbiology, 8*, 172.

Gupta, S., Kapoor, P., Chaudhary, K., Gautam, A., Kumar, R., Raghava, G. P., & Consortium, O. S. D. D. (2013). In silico approach for predicting toxicity of peptides and proteins. *PloS one, 8*(9), e73957.

Haeusler, G. M., & Curtis, N. (2013). Non-typhoidal Salmonella in children: microbiology, epidemiology and treatment *Hot Topics in Infection and Immunity in Children IX* (pp. 13-26): Springer.

Helms, M., Ethelberg, S., Mølbak, K., & Group, D. S. (2005). International Salmonella typhimurium DT104 infections, 1992–2001. *Emerging infectious diseases, 11*(6), 859.

Hurley, D., McCusker, M. P., Fanning, S., & Martins, M. (2014). Salmonella–host interactions—modulation of the host innate immune system. *Frontiers in immunology, 5*, 481.

Jahangiri, A., Rasooli, I., Owlia, P., Fooladi, A. A. I., & Salimian, J. (2017). In silico design of an immunogen against Acinetobacter baumannii based on a novel model for native structure of outer membrane protein A. *Microbial pathogenesis, 105*, 201-210.

Jespersen, M. C., Peters, B., Nielsen, M., & Marcatili, P. (2017). BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic acids research, 45*(W1), W24-W29.

Karon, A. E., Archer, J. R., Sotir, M. J., Monson, T. A., & Kazmierczak, J. J. (2007). Human multidrug-resistant Salmonella newport infections, Wisconsin, 2003–2005. *Emerging infectious diseases, 13*(11), 1777.

Katz, D., Ben-Chetrit, E., Sherer, S.-S., Cohen, D., & Muhsen, K. (2019). Correlates of non-typhoidal Salmonella bacteraemia: A case–control study. *International Journal of Infectious Diseases, 81*, 170-175.

Kaur, H., Garg, A., & Raghava, G. (2007). PEPstr: a de novo method for tertiary structure prediction of small bioactive peptides. *Protein and peptide letters, 14*(7), 626-631.

Kelley, L. A., & Sternberg, M. J. (2009). Protein structure prediction on the Web: a case study using the Phyre server. *Nature protocols, 4*(3), 363.

Khan, M. K., Zaman, S., Chakraborty, S., Chakravorty, R., Alam, M. M., Bhuiyan, T. R., . . . Seraj, Z. I. (2014). In silico predicted mycobacterial epitope elicits in vitro T-cell responses. *Molecular immunology, 61*(1), 16-22.

Kim, Y., Ponomarenko, J., Zhu, Z., Tamang, D., Wang, P., Greenbaum, J., . . . Bourne, P. E. (2012). Immune epitope database analysis resource. *Nucleic acids research, 40*(W1), W525-W530.

Koebnik, R., Locher, K. P., & Van Gelder, P. (2000). Structure and function of bacterial outer membrane proteins: barrels in a nutshell. *Molecular microbiology, 37*(2), 239-253.

Kopp, J., & Schwede, T. (2004). Automated protein structure homology modeling: a progress report. *Pharmacogenomics, 5*(4), 405-416.

Krieger, E., Nielsen, J. E., Spronk, C. A., & Vriend, G. (2006). Fast empirical pKa prediction by Ewald summation. *Journal of molecular graphics and modelling, 25*(4), 481-486.

Krieger, E., & Vriend, G. (2015). New ways to boost molecular dynamics simulations. *Journal of computational chemistry, 36*(13), 996-1007.
Land, H., & Humble, M. S. (2018). YASARA: a tool to obtain structural guidance in biocatalytic investigations. *Protein Engineering* (pp. 43-67): Springer.

Larsen, M. V., Lundegaard, C., Lamberth, K., Buus, S., Lund, O., & Nielsen, M. (2007). Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. *BMC bioinformatics*, 8(1), 424.

Laskowski, R. A., Rullmann, J. A. C., MacArthur, M. W., Kaptein, R., & Thornton, J. M. (1996). AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. *Journal of biomolecular NMR*, 8(4), 477-486.

Lin, J., Huang, S., & Zhang, Q. (2002). Outer membrane proteins: key players for bacterial adaptation in host niches. *Microbes and infection*, 4(3), 325-331.

Lipsitch, M., & Siber, G. R. (2016). How can vaccines contribute to solving the antimicrobial resistance problem? *MBio*, 7(3), e00428-00416.

Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'brien, S. J., . . . Studies, I. C. o. E. D. b. o. I. (2010). The global burden of nontyphoidal Salmonella gastroenteritis. *Clinical infectious diseases*, 50(6), 882-889.

Mariá, R. A. R., Arturo, C. V. J., Alicia, J. A., Paulina, M. L. G., & Gerardo, A. O. (2017). *The impact of bioinformatics on vaccine design and development*: InTech, Rijeka, Croatia.

Mashiach, E., Schneidman-Duhovny, D., Andrusier, N., Nussinov, R., & Wolfson, H. J. (2008). FireDock: a web server for fast interaction refinement in molecular docking. *Nucleic acids research*, 36(suppl_2), W229-W232.

McKeever, T. M., Lewis, S. A., Smith, C., & Hubbard, R. (2004). Vaccination and allergic disease: a birth cohort study. *American journal of public health*, 94(6), 985-989.

Monterrubio-López, G. P., & Ribas-Aparicio, R. M. (2015). Identification of novel potential vaccine candidates against tuberculosis based on reverse vaccinology. *BioMed research international*, 2015.

Nielsen, M., Lundegaard, C., & Lund, O. (2007). Prediction of MHC class I binding affinity using SMM-align, a novel stabilization matrix alignment method. *BMC bioinformatics*, 8(1), 238.

Pan, J., Li, C., & Ye, Z. (2016). Immunoproteomic approach for screening vaccine candidates from bacterial outer membrane proteins. *Vaccine Design* (pp. 519-528): Springer.

Peters, B., Bulik, S., Tampe, R., Van Endert, P. M., & Holzhütter, H.-G. (2003). Identifying MHC class I epitopes by predicting the TAP transport efficiency of epitope precursors. *The Journal of Immunology*, 171(4), 1741-1749.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, 25(13), 1605-1612.

Pontius, J., Richelle, J., & Wodak, S. J. (1996). Deviations from standard atomic volumes as a quality measure for protein crystal structures. *Journal of molecular biology*, 264(1), 121-136.

Pradhan, N., & Sharma, K. (2014). Understanding Ubl-rpn1 Intermolecular Interaction. *Journal of Advanced Pharmaceutical Science and Technology*, 1(3), 1.

Rauta, P. R., Ashe, S., Nayak, D., & Nayak, B. (2016). In silico identification of outer membrane protein (Omp) and subunit vaccine design against pathogenic Vibrio cholerae. *Computational biology and chemistry*, 65, 61-68.

Release, S. (2018). 1: Maestro. *Schrödinger, LLC: New York, NY, USA.*

Saha, S., & Raghava, G. (2006). Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins: Structure, Function, and Bioinformatics*, 65(1), 40-48.
Sanchez-Trincado, J. L., Gomez-Perosanz, M., & Reche, P. A. (2017). Fundamentals and methods for T- and B-cell epitope prediction. *Journal of immunology research, 2017*.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L., ... Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging infectious diseases, 17*(1), 7.

Singh, S., Singh, H., Tuknait, A., Chaudhary, K., Singh, B., Kumaran, S., & Raghava, G. P. (2015). PEPstrMOD: structure prediction of peptides containing natural, non-natural and modified residues. *Biology direct, 10*(1), 73.

Singletary, L. A., Karlinsey, J. E., Libby, S. J., Mooney, J. P., Lokken, K. L., Tsolis, R. M., ... Crawford, R. W. (2016). Loss of multicellular behavior in epidemic African nontyphoidal Salmonella enterica serovar Typhimurium ST313 strain D23580. *MBio, 7*(2), e02265-02215.

Sun, P., Ju, H., Liu, Z., Ning, Q., Zhang, J., Zhao, X., ... Li, Y. (2013). Bioinformatics resources and tools for conformational B-cell epitope prediction. *Computational and mathematical methods in medicine, 2013*.

Tagliabue, A., & Rappuoli, R. (2018). Changing priorities in Vaccinology: antibiotic resistance moving to the top. *Frontiers in immunology, 9*.

Tenzer, S., Peters, B., Bulik, S., Schoor, O., Lemmel, C., Schatz, M., ... Holzhütter, H.-G. (2005). Modeling the MHC class I pathway by combining predictions of proteosomal cleavage, TAP transport and MHC class I binding. *Cellular and Molecular Life Sciences CMLS, 62*(9), 1025-1037.

Uche, I. V., MacLennan, C. A., & Saul, A. (2017). A Systematic Review of the Incidence, Risk Factors and Case Fatality Rates of Invasive Nontyphoidal Salmonella (iNTS) Disease in Africa (1966 to 2014). *PLoS Negl Trop Dis, 11*(1), e0005118. doi:10.1371/journal.pntd.0005118

Vanommeslaeghe, K., Hatcher, E., Acharya, C., Kundu, S., Zhong, S., Shim, J., ... Vorobyov, I. (2010). CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *Journal of computational chemistry, 31*(4), 671-690.

Varma, J. K., Mølbak, K., Barrett, T. J., Beebe, J. L., Jones, T. F., Rabatsky-Ehr, T., ... Angulo, F. J. (2005). Antimicrobial-resistant nontyphoidal Salmonella is associated with excess bloodstream infections and hospitalizations. *The Journal of infectious diseases, 191*(4), 554-561.

Verma, S., Sugadev, R., Kumar, A., Chanda, S., Ganju, L., & Bansal, A. (2018). Multi-epitope DnaK peptide vaccine against S. Typhi: An in silico approach. *Vaccine, 36*(28), 4014-4022.

VT Nair, D., Venkitanarayanan, K., & Kollanoor Johny, A. (2018). Antibiotic-resistant Salmonella in the food supply and the potential role of antibiotic alternatives for control. *Foods, 7*(10), 167.

Xu, D., & Zhang, Y. (2011). Improving the physical realism and structural accuracy of protein models by a two-step atomic-level energy minimization. *Biophysical journal, 101*(10), 2525-2534.