Proteomic Exploration of *Listeria monocytogenes* for the Purpose of Vaccine Designing Using a Reverse Vaccinology Approach

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Abstract
Listeriosis is a major foodborne infection provoked by a bacterium known as *Listeria monocytogenes*. It is one of the predominant causes of death in pregnant women, infants, and immunocompromised persons. Despite such fatal effects, until now there is no proper medication or drug available for such a serious foodborne infection. One of the most promising ways to deal with this challenge is vaccination. This present study aims at the prediction of B cell epitopes for subunit vaccine designing against *Listeria monocytogenes* using a reverse vaccinology approach. Among screened out 299 epitopes of strain F2365 of *Listeria monocytogenes*, based on the VaxiJen score, the top 20 epitopes were selected. 3D modeling of epitopes and alleles was generated by PEPstrMOD and Swiss Model respectively. Molecular docking reveals 4 epitopes viz., MKLFPLKL, CEETFGIRL, FLKIDPPIL, and VRHHGGGHK based on binding energy. All 4 epitopes were investigated for non-toxicity, binding affinity, and population coverage. After vigorous investigation, epitope FLKIDPPIL was anticipated as the best vaccine contender. The stability of the FLKIDPPIL-HLA DRB1 _0101 complex was proved by performing the simulation. Here, predicted peptide through the *In silico* approach may become a potential remedy against listeriosis, after the wet-lab approach and clinical trials.

Keywords Listeriosis · B cell epitopes · Docking · Simulation · Reverse vaccinology

Introduction
Changing food habits, advancement in technology regarding the preservation of food products for a longer time, and the ability of microorganisms to grow in adverse conditions are leading to the emergence of the foodborne infection, known as Listeriosis. The genus *Listeria* consists of seventeen species. Only the three hemolytic species viz., *Listeria monocytogenes*, *Listeria seeligeri*, and *Listeria ivanovii* are considered pathogens. Of these, *Listeria monocytogenes* is consistently pathogenic and is involved in foodborne outbreaks of listeriosis (Abdelhamed et al. 2019). Based on Gram-staining, *Listeria monocytogenes* comes under the category of Gram-positive. It shows extreme resistance in conditions like very high temperatures or very low temperatures. These bacteria have a rod-like shape and can form small chains (Sallami et al. 2006). *Listeria monocytogenes* mainly affects women who are pregnant, infants, elders above 65 years of age, and immunocompromised people (CDC 2019). Foodborne infection in humans occurs through the consumption of contaminated foods, particularly unpasteurized milk, soft cheeses, vegetables, and prepared meat products. *Listeria monocytogenes* show completely different behavior in comparison to all other pathogens that cause food contamination. It can multiply at low temperatures in contaminated food. It can be easily transmitted between pregnant women and her newborn either at the time of pregnancy or during delivery (WHO 2019). Pyrexia, cough, cold, headache, and body ache, etc. are the usual symptoms experienced by the patients (Department of Health 2017). Worldwide many countries where food production takes place in absence of proper and better microbiological vigilance and where the percentage of immunocompromised persons are immensely high, *Listeria monocytogenes* loomed as one of the dominant foodborne pathogens (Thomas et al. 2020). Thus, poor surveillance during the production process affects approximately 1600 people every year, and around 260 experience the afterlife (CDC 2019).
Listeria monocytogenes consists of two genes viz., chiA and chiB. These two genes play an important role in virulence. A regulatory factor hfq plays a very important role in the formation of biofilm, colony formation, and virulence (Yao et al. 2018). The Zipper is the name of the mechanism through which Listeria monocytogenes get access to the host cell. In this process, ligands on the surface of bacteria communicate with receptors of the host cells. Internalin A and Internalin B are the ligands on the bacterial surface and E-cadherin and Met are the receptors on host cells with which bacterial ligands interact. This collaboration leads to the rearrangement of actin filaments and invasion of bacteria (Hamon et al. 2006). When Internalin B-Met interacts together, processes like ubiquitinylation and autophosphorylation takes place (Veiga and Cossart 2005).

In the year 2018, Australia had witnessed around 20 cases of listeriosis between January to April. This minor outbreak had faced around 7 deaths and a single spontaneous abortion (WHO 2018a, b). National Institute of Communicable diseases (NICD) has proclaimed 978 listeriosis cases between 2017 and 2018 from all provinces of South Africa, Gauteng, Western Cape, and KwaZulu-Natal were mainly hit by this fatal disease known as listeriosis. Around 78% of cases have been reported from the above-mentioned places of South Africa. Out of 674 affected people, 27 have faced death. All these data revealed about the threat of this bacteria and its effect on mankind and society. The percentage of infants that get affected during an outbreak is around 42% (WHO 2018a, b). Pregnant women can easily get infected with listeriosis through the placenta, still, the establishment of neurolisteriosis is completely occasional. Listeriosis infection in pregnant women is because of the alliance of the quashed immune system and the specificity of bacteria for the placenta (Charlier et al. 2017). Even after the birth of infants infected with bacteria Listeriosis monocytogenes endurance is possible only with the help of Extracorporeal membrane oxygenation (ECMO) (Lee et al. 2019). According to a report of WHO, in India miscarriages and other pregnancy-related disorders is mainly the result of foodborne infection known as listeriosis.

Listeriosis is still under-reported in many countries. The ability of Listeria monocytogenes to survive even in harsh conditions is one of the major threats regarding the outbreak of the disease. High fatality rate and frequent outbreaks demand the designing of a vaccine against Listeria monocytogenes, by using the immunoinformatics approach. This study is mainly based on the anticipation of B cell epitopes for the utility of vaccine designing against listeriosis. Previously, a study regarding computational identification and characterization of epitopes has been carried out in the case of the Zika virus, Nipah virus, and bacteria like E. coli (Sharma et al. 2020; Kaushik 2019; Khan et al. 2019). Considering this approach in this research work, all proteins except hypothetical, putative, and non-structural were retrieved from the UniProtKB database. A potential epitope must not possess any allergic property; therefore, first and foremost allergenicity was checked by using the AlgPred server. NETMHCII 2.3 and VaxJen server was used to identify B cell epitopes that could bind to MHC II molecules with great stability. Only the top 20 epitopes were selected for further exploration. This selection was done based on the VaxJen score. 3D modeling of both the epitopes and alleles was performed using PEPstrMOD and Swiss Model. Epitope—allele pair having low binding energy should be selected for the next sequential refining. To do this, molecular docking was performed using AutoDock Vina software. Next to check toxicity, binding affinity, and population coverage Toxin Pred, MHC Pred, and immune epitope database tools were used. The stability of the epitope-allele complex was substantiated by simulation studies. The strategy of the development of subunit vaccines has an upper hand in comparison to traditional vaccines. These next-generation vaccines are extremely specific in eliciting the immune system of the host, can be produced easily in large quantities, and at a comparatively moderate cost. Moreover, peptides consisting of epitopes can be manufactured, purified, and processed easily (Poland et al. 2011).

**Methodology**

**Protein Sequence Retrieval**

For computational identification and characterization of epitopes for the preparation of subunit vaccine designing, complete proteome analysis of Listeria monocytogenes F2365 strain (GenBank accession number AE017262.2) was performed using the UniProtKB database. In comparison to other serotype strains, Listeria monocytogenes strain F2365 belongs to the 4b serotype group and multiplies more rapidly in monocytes or macrophages (Hasebe et al. 2017). Presence of a virulence factor viz. ListeriolysinS (LLS) in the F2365 strain accelerates infection in the intestine and other organs (Quereda et al. 2016). Listeria monocytogenes F2365 strain is a member of lineage I and comprises a factor known as Internalin B which plays a crucial role in nonpregnant infected animals (Quereda et al. 2018). All these remarkable features contribute to the pathogenicity of this strain and hence lead to its selection for the study. Excluding hypothetical, putative, and non-structural proteins total of 529 proteins were registered in the UniprotKB database, derived from the different research literature. All these sequences were saved in the FASTA format for further examination. The length of the genome of the F2365 strain is 3,021,822 bp, with GC content of 37.9% approximately (Briers et al. 2011).
**Allergenic Protein Prediction**

One of the most eminent features in epitope-based vaccine design is that the particular epitope must elicit an immune response only against the target pathogen. Taking this point into consideration, the screened epitope must be non-allergen and thus retrieved proteins were differentiated into allergens and non-allergens by using the AlgPred server (Saha and Raghava 2006). This server segregates non-allergens from allergens and –0.4 was selected as the cut-off value. Anticipation was done with high accuracy along with sensitivity and specificity of 88.87% and 81.86% respectively. Non-allergens were chosen for another characterization and exploration of antigenic sites for the utility of vaccine designing from the proteome of *Listeria monocytogenes*.

**B cell Epitope Prediction**

B cell epitopes are typical peptide remnants that bind to the immunoglobulin and thus it becomes immensely important to screen out such epitopes from complete proteome sequence. To accomplish this objective, NETMHCII 2.3 server was used (Jensen et al. 2018). By making use of artificial neural networks, this server predicts the binding of B cell epitopes with HLA alleles. In this study, three alleles viz. DRB1_0101, DRB1_0701, and DRB1_1301 and locus HLA-DR was chosen. The peptide length was taken at 9 with a threshold set to −99.9.

The potential B cell epitopes were subjected to the VaxiJen server to select those candidates that can strongly bind with MHC II molecules (Doytchinova and Flower 2007). Only epitopes with a score greater than or equal to 1.1 can bind with MHC II molecules with extreme affinity and be selected. To further proceed with the reverse vaccinology approach, only the top 20 peptides or antigenic sites were chosen. This selection was based on the VaxiJen score.

**Molecular Modeling of Epitopes and Human Leukocyte Antigen (HLA) Alleles**

Following allergenicity and prediction of B cell epitopes, modeling of both epitopes and HLA alleles was performed. For the generation of the 3D structure of the selected epitopes, the PEPstrMOD server was used. It offers exclusive advantages to the users to predict the structures of peptides having natural residues, some modified residues, post-translational modifications, etc. (Singh et al. 2015). In this research work, filtered epitopes were modeled and saved in the Protein Data Bank (PDB) format for the next sequential investigation. The first fully automated protein homology modeling server known as the Swiss model was used for modeling of HLA alleles (Waterhouse et al. 2018). The building of models using this server requires four sequential steps. These 4 steps comprise of template selection, its alignment with the target sequence, model building, and its evaluation. In this study 3D structures of three HLA alleles have been performed viz., DRB1_0101, DRB1_0701, and DRB1_1301.

**Molecular Docking of Epitopes and HLA Alleles**

To better understand the relationship between anticipated epitopes and their respective alleles, AutoDock Vina software was used to perform molecular docking. It helps us to interpret the synergy between antigenic sites and their corresponding alleles (Trott and Olson 2010). One of the prerequisites before performing docking is certain modifications both in ligand as well as the receptor, which was performed by AutoDock MGL tools. HLA alleles were selected as receptors viz., DRB1_0101, DRB1_0701, and DRB1_1301. 4AH2, 3C5J, and 6CQL are the crystal structures of these receptors and were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank. Molecules of water were removed from these receptors and polar hydrogen as well Kollman charges were added to the structure. After modification, the molecule was saved in pdbqt format. Changes were also performed in all 20 ligands and were saved in pdbqt files. All these alterations were performed by AutoDock MGL tools. To perform molecular docking in AutoDock Vina software, 40, 40, 40 were taken as grid box dimensions and energy was calculated at 0 Å. The docking result can be analyzed by a visualization tool called PyMol. 4 epitopes were selected for succeeding rounds of analysis based on negative binding energies where Low binding energy implies good stability.

**Toxicity Prediction of the Epitopes**

To evaluate the non-toxicity behavior of epitopes Toxin Pred server was used (Gupta et al. 2013). It is based on machine learning techniques and quantitative matrix scores. Along with toxicity prediction, calculation of physicochemical properties is one of the most notable features of this server.

**Binding Affinity Prediction and Population Coverage Analysis**

MHC Pred Server was used to vaticinate the binding affinity of epitopes with MHC II molecules. MHC Pred is composed of several models based on structures and its activity, a sturdy multivariate statistical method. Results with articulated by giving IC50 values (Guan et al. 2003). IC50 values less than 500 are considered to be good binders and were chosen for the next and last analysis. Because of the exceptionally heterogeneous behavior of HLA alleles, their frequency of expression varies greatly across the globe, and
therefore Population coverage analysis becomes the utmost important step in computational vaccine designing. It was performed using the Immune Epitope Database (IEDB) Population Coverage analysis tool (Bui et al. 2006).

Molecular Dynamics (MD) and Simulation Study

It is extremely essential to understand the stability of the peptide-allele complex and to analyze that in this research work MD Web server was used (Hospital et al. 2012). Simulation of 10 ns with an output frequency of 500 steps was set to equilibrate the system. Coarse-grained Brownian dynamics were analyzed for trajectory and output was given in the terms of Root mean square deviation (RMSD) and B-factor values. Both RMSD and B-factor plots corroborate the stability of epitope-allele complex.

Results

With time, the world has acknowledged extreme advancement in medicine and technology thus combating some deadly diseases, but still, diseases like listeriosis were left unnoticed. Despite several outbreaks in different parts of the world, there is no legitimate treatment or drug or vaccine available for it. Therefore, it becomes extremely important to predict and characterize some potential vaccine contenders that can evoke a strong immune response and this study is one such step in this direction. Here we have used computational tools to predict B cell epitopes that can elicit an immune response. The first requirement in the reverse vaccinology approach of vaccine designing is to eliminate all non-allergic proteins from a complete proteome set of bacteria, Listeria monocytogenes. The AlgPred server was used to predict allergenicity of retrieved proteins, to get the most capable subunit vaccine candidate. A total of 529 proteins sequences of Listeria monocytogenes F2365 strain was retrieved from the UniProtKB database (excluding hypothetical, putative, and non-structural proteins) and were saved in the FASTA format for further analysis. After examination by the AlgPred server, out of 529, a total of 172 proteins were proved to be non-allergens (Table 1). The result has been summarized in Table 1. Table 1 consists of protein ID, protein names, and scores of all non-allergens.

Non-allergic proteins were analyzed further by using NetMHC II 2.3 server. By selecting peptide lengths 9 and threshold value – 99.9. B cell epitopes were selected. These chosen epitopes were next investigated by the VaxiJen server and the cut-off value was 1.1 Å total of 299 epitopes were found to bind with MHC II molecules (Table 2). All 299 epitopes have a VaxiJen score of ≥ 1.1 and can bind with MHC II molecules with great stability. Among these epitopes, the majority of epitopes were found to bind with DRB1_1301.

Based on the high VaxiJen score, among 299 epitopes, only the top 20 epitopes were selected for modeling. The generation of 3D structures of epitopes was performed by PEPstrMOD. 3D modeling of the HLA allele’s viz. DRB1_0101, DRB1_0701, and, DRB1_1301 were performed by the Swiss model (Fig. 1). For the generation of tertiary structures of DRB1_0101, DRB1_0701 and, DRB1_1301 alleles, proteins having PDB ID 4AH2, 3C5J, and 6CQL were used as templates, respectively. All tertiary structures of HLA alleles were visualized by the PyMOL visualization tool. 3D models have been represented in Fig. 1.

AutoDock Vina software was used to perform molecular docking between 20 nonallergic and antigenic epitopes with their respective alleles. The lowest binding energy was obtained for epitope FLKIDPPIL-DRB1_0101 (− 7.3 kcal/mol) and the highest binding energy was obtained for epitope MKGQAGSKK-DRB1_1301 (− 5.1 kcal/mol). As low binding energies imply, high stability of the complex, therefore 4 epitopes based on low binding energy was selected viz., CEETFGIRL, MKFLFPLKL, FLKIDPIL, and VRHHGGGHK (Table 3). The stable complex of CEETFGIRL-3C5J shows the energy of − 6.7 kcal/mol and 6 hydrogen bonds (Fig. 2) Complexes viz. MKFLFPLKL-4AH2 and FLKIDPIL-4AH2 shows binding energy of − 6.9 kcal/mol and − 7.3 kcal/mol along with 2 and 6 hydrogen bonds respectively (Figs. 3 and 4). The energy of − 6.7 kcal/mol and 6 hydrogen bonds was shown by epitope VRHHGGGHK along with its receptor 6CQL (Fig. 5).

Most promising vaccine aspirants must not cause any kind of toxicity or vigorous reaction inside the host. So, checking of toxic nature of epitopes is notably important. This prominently important step was performed by Toxin Pred. It was found that all 4 selected epitopes were non-toxic (Table 4). All epitopes along with their result of toxicity analysis and physicochemical properties like hydrophobicity, hydrophilicity, and molecular weight were summarized in Table 4.

MHC Pred server was used to study the binding affinity of four non-allergic, non-toxic, and antigenic peptides with allele’s viz., HLA DRB1_0101, HLA DRB1_0401, and HLA DRB1_0701. Binding affinity was depicted in terms of IC50 value (Table 5). Epitopes showing IC50 value less than 500 nM were considered to be good binders. Epitopes viz., CEETFGIRL and VRHHGGGHK were found to bind with HLA DRB1_0101 and HLA DRB1_0401, respectively. Both FLKIDPPIL and MKFLFPLKL were found to bind with HLA DRB1_0101 and HLA DRB1_0701.

Most eligible vaccine contenders must show satisfactorily population coverage in different parts of the world. Both the
Table 1  List of all non-allergic proteins of *Listeria monocytogenes* F2365 strain, along with their protein ID and the result of analysis by AlgPred server

| S. no. | Protein ID | Score   | AlgPred prediction |
|--------|------------|---------|--------------------|
| 1      | Q724L4     | 1.3656  | Non-allergen       |
| 2      | Q71WU4     | 1.9397  | Non-allergen       |
| 3      | Q71Z75     | 0.7278  | Non-allergen       |
| 4      | Q724J4     | -0.547  | Non-allergen       |
| 5      | Q71W17     | -0.551  | Non-allergen       |
| 6      | Q71Y34     | -0.54   | Non-allergen       |
| 7      | Q71XR2     | 0.4524  | Non-allergen       |
| 8      | Q71VT6     | 0.4088  | Non-allergen       |
| 9      | Q71ZE0     | -1.318  | Non-allergen       |
| 10     | Q71XX6     | -1.042  | Non-allergen       |
| 11     | Q71Y46     | -0.679  | Non-allergen       |
| 12     | Q71WT3     | -0.482  | Non-allergen       |
| 13     | Q71WP0     | -1.372  | Non-allergen       |
| 14     | Q720A5     | -0.44   | Non-allergen       |
| 15     | Q71WP7     | -0.675  | Non-allergen       |
| 16     | Q71WT2     | -0.574  | Non-allergen       |
| 17     | Q71ZH3     | -0.508  | Non-allergen       |
| 18     | Q720D7     | -1.554  | Non-allergen       |
| 19     | Q71VR6     | -1.317  | Non-allergen       |
| 20     | Q720T3     | -0.947  | Non-allergen       |
| 21     | Q722V6     | -0.505  | Non-allergen       |
| 22     | Q71Y44     | -0.578  | Non-allergen       |
| 23     | Q71WT9     | -0.64   | Non-allergen       |
| 24     | Q720J1     | -1.004  | Non-allergen       |
| 25     | Q71ZD3     | -0.651  | Non-allergen       |
| 26     | Q71ZZ0     | -1.285  | Non-allergen       |
| 27     | Q71XV7     | -1.047  | Non-allergen       |
| 28     | Q71YD8     | -1.391  | Non-allergen       |
| 29     | Q71XG0     | -0.986  | Non-allergen       |
| 30     | Q724M5     | -0.647  | Non-allergen       |
| 31     | Q724E9     | -0.838  | Non-allergen       |
| 32     | Q71YJ5     | -0.821  | Non-allergen       |
| 33     | Q722Y8     | -1.001  | Non-allergen       |
| 34     | Q71XF3     | -0.951  | Non-allergen       |
| 35     | Q71VR5     | -0.589  | Non-allergen       |
| 36     | Q71WI0     | -0.766  | Non-allergen       |
| 37     | Q71ZR3     | -0.698  | Non-allergen       |
| 38     | Q71XR3     | -1.167  | Non-allergen       |
| 39     | Q720G2     | -0.776  | Non-allergen       |
| 40     | Q71YB2     | -1.037  | Non-allergen       |
| 41     | Q71XV6     | -1.471  | Non-allergen       |
| 42     | Q724M3     | -0.608  | Non-allergen       |
| 43     | Q724B0     | -1.957  | Non-allergen       |
| 44     | Q724H1     | -0.449  | Non-allergen       |
| 45     | Q721S2     | -0.587  | Non-allergen       |
| 46     | Q71XX2     | -0.928  | Non-allergen       |
| 47     | Q71WH2     | -0.5    | Non-allergen       |
| 48     | Q71VQ8     | -0.948  | Non-allergen       |
| 49     | Q71ZD8     | -0.829  | Non-allergen       |
| 50     | Q71Y59     | -1.726  | Non-allergen       |
| 51     | Q720E4     | -0.977  | Non-allergen       |
| S. no. | Protein ID | Score  | AlgPred prediction |
|--------|------------|--------|--------------------|
| 52     | Q71ZU1     | −0.488 | Non-allergen       |
| 53     | Q720A3     | −0.482 | Non-allergen       |
| 54     | Q720D3     | −0.466 | Non-allergen       |
| 55     | Q71YM4     | −0.874 | Non-allergen       |
| 56     | Q720A7     | −1.041 | Non-allergen       |
| 57     | Q724H7     | −0.885 | Non-allergen       |
| 58     | Q720J2     | −0.5   | Non-allergen       |
| 59     | Q71YJ0     | −1.126 | Non-allergen       |
| 60     | Q722Y2     | −0.645 | Non-allergen       |
| 61     | Q71XU1     | −0.474 | Non-allergen       |
| 62     | Q71WU5     | −1.035 | Non-allergen       |
| 63     | Q71YA9     | −1.006 | Non-allergen       |
| 64     | Q721B5     | −0.439 | Non-allergen       |
| 65     | Q71WN3     | −0.872 | Non-allergen       |
| 66     | Q724F0     | −0.73  | Non-allergen       |
| 67     | Q71WP3     | −1.021 | Non-allergen       |
| 68     | Q71WF9     | −1.887 | Non-allergen       |
| 69     | Q722W7     | −0.595 | Non-allergen       |
| 70     | Q71YH0     | −0.671 | Non-allergen       |
| 71     | Q71WB6     | −1.955 | Non-allergen       |
| 72     | Q71YB9     | −0.633 | Non-allergen       |
| 73     | Q71VR4     | −0.492 | Non-allergen       |
| 74     | Q71W99     | −1.05  | Non-allergen       |
| 75     | Q71W91     | −0.849 | Non-allergen       |
| 76     | Q721K3     | −0.808 | Non-allergen       |
| 77     | Q71WP8     | −0.707 | Non-allergen       |
| 78     | Q71YH8     | −0.796 | Non-allergen       |
| 79     | Q71W93     | −1.08  | Non-allergen       |
| 80     | Q725C1     | −0.66  | Non-allergen       |
| 81     | Q71Z71     | −1.736 | Non-allergen       |
| 82     | Q71ZV5     | −0.599 | Non-allergen       |
| 83     | Q722Y1     | −0.452 | Non-allergen       |
| 84     | Q720E1     | −0.419 | Non-allergen       |
| 85     | Q724K0     | −0.41  | Non-allergen       |
| 86     | Q71WF2     | −1.603 | Non-allergen       |
| 87     | Q724K2     | −0.421 | Non-allergen       |
| 88     | Q722Y9     | −0.81  | Non-allergen       |
| 89     | Q71ZA5     | −0.444 | Non-allergen       |
| 90     | Q71VW1     | −0.761 | Non-allergen       |
| 91     | Q71VF7     | −0.62  | Non-allergen       |
| 92     | Q71ZZ2     | −1.919 | Non-allergen       |
| 93     | Q71W69     | −1.29  | Non-allergen       |
| 94     | Q71WF1     | −1.529 | Non-allergen       |
| 95     | Q71WE7     | −1.644 | Non-allergen       |
| 96     | Q71WE6     | −0.49  | Non-allergen       |
| 97     | Q71ZP6     | −0.605 | Non-allergen       |
| 98     | Q71WF3     | −2.172 | Non-allergen       |
| 99     | Q71WE9     | −1.315 | Non-allergen       |
| 100    | Q71WB7     | −2.462 | Non-allergen       |
| 101    | Q71WH0     | −1.831 | Non-allergen       |
| 102    | Q724G4     | −0.778 | Non-allergen       |
| S. no. | Protein ID | Score | AlgPred prediction |
|-------|------------|-------|--------------------|
| 103   | Q71WF8     | −1.611| Non-allergen       |
| 104   | Q724G2     | −0.644| Non-allergen       |
| 105   | Q71X5E     | −0.913| Non-allergen       |
| 106   | Q71XX1     | −0.625| Non-allergen       |
| 107   | Q71YK6     | −0.683| Non-allergen       |
| 108   | Q71WE5     | −2.321| Non-allergen       |
| 109   | Q71ZB7     | −0.454| Non-allergen       |
| 110   | Q71WF6     | −1.29 | Non-allergen       |
| 111   | Q71WF5     | −1.581| Non-allergen       |
| 112   | Q71WH1     | −2.223| Non-allergen       |
| 113   | Q71WG5     | −1.192| Non-allergen       |
| 114   | Q725B8     | −2.188| Non-allergen       |
| 115   | Q71WV5     | −1.028| Non-allergen       |
| 116   | Q71WG0     | −1.557| Non-allergen       |
| 117   | Q71WG2     | −1.87 | Non-allergen       |
| 118   | Q71WE8     | −0.989| Non-allergen       |
| 119   | Q71YD4     | −2.112| Non-allergen       |
| 120   | Q71YN5     | −2.041| Non-allergen       |
| 121   | Q71YJ3     | −1.036| Non-allergen       |
| 122   | Q721R7     | −0.737| Non-allergen       |
| 123   | Q71WX8     | −1.06 | Non-allergen       |
| 124   | Q71WF0     | −2.159| Non-allergen       |
| 125   | Q71WN0     | −1.611| Non-allergen       |
| 126   | Q725C0     | −0.638| Non-allergen       |
| 127   | Q71ZZ5     | −0.527| Non-allergen       |
| 128   | Q71ZG8     | −0.898| Non-allergen       |
| 129   | Q71ZJ0     | −1.318| Non-allergen       |
| 130   | Q71XH4     | −1.281| Non-allergen       |
| 131   | Q71WL5     | −0.848| Non-allergen       |
| 132   | Q720A8     | −0.628| Non-allergen       |
| 133   | Q721Y1     | −0.988| Non-allergen       |
| 134   | Q71YM9     | −1.733| Non-allergen       |
| 135   | Q71WG4     | −2.217| Non-allergen       |
| 136   | Q71YN4     | −2.371| Non-allergen       |
| 137   | Q71WH3     | −2.224| Non-allergen       |
| 138   | Q71ZY7     | −0.968| Non-allergen       |
| 139   | Q71XW7     | −1.979| Non-allergen       |
| 140   | Q720A1     | −0.577| Non-allergen       |
| 141   | Q723G3     | −2.038| Non-allergen       |
| 142   | Q71WV3     | −0.925| Non-allergen       |
| 143   | Q71ZI5     | −0.952| Non-allergen       |
| 144   | Q721N6     | −0.586| Non-allergen       |
| 145   | Q71ZK1     | −1.532| Non-allergen       |
| 146   | Q71ZD0     | −1.746| Non-allergen       |
| 147   | Q71WF4     | −0.935| Non-allergen       |
| 148   | Q71YL9     | −2.126| Non-allergen       |
| 149   | Q71WG9     | −1.537| Non-allergen       |
| 150   | Q71YK0     | −2.221| Non-allergen       |
| 151   | Q71WJ2     | −2.143| Non-allergen       |
| 152   | Q71VQ6     | −1.957| Non-allergen       |
| 153   | Q724G8     | −1.5  | Non-allergen       |
epitope MKFLFPLKL and FLKIDPPIIL shows population coverage of 28.63% worldwide (Fig. 6).

Epitope CEETFGIRL and VRHHGGGHK shows population coverage of 11.53% and 11.21% worldwide respectively (Figs. 7 and 8).

The final selection of best and most promiscuous vaccine bidders depends on two main factors, one is low binding energy and another one is high population coverage worldwide. Based on these two factors, epitope FLKIDPPIIL was refined. To check the stability of complex FLKIDPPIIL-4AH2, molecular dynamics simulation was performed by MD Web simulation. RMSD value of FLKIDPPIIL-4AH2 was given in between 0.1 and 1.0 Å (Fig. 9) and B factor scores between 1 and 25 Å² (Fig. 10). Both RMSD values and B factor plot of complex viz., FLKIDPPIIL-4AH2 confirm the stability of the epitope.

Discussion

Reverse vaccinology is known by different names like computational biology, immunoinformatics, and many more. It is a combination of immunological research as well as experimental and computational science. It includes computational tools and software to study the immune response of the host against various infectious diseases. Immunoinformatics helps us to understand antigen presentation in host cells, the behavior of the host during the infection cycle, and thus enriches the knowledge about the disease that affects the immune system and its control (Brusic and Petrovsky 2005). With the help of *Insilico* tools, antigenic regions can be mapped easily (Davies and Flower 2007). Previously, finding these antigenic regions are extremely costly and time-consuming methods like Nuclear Magnetic Resonance (NMR) were used. But today, computational vaccinology had made it possible to predict these antigenic regions in a short period and also with extreme accuracy (Potocnakova et al. 2016). In this exploration and investigation, the prediction of B cell epitopes has been performed by the authors for the designing of the vaccine against listeriosis by using a reverse vaccinology approach.

This research work started with the retrieval of a complete proteome sequence of *Listeria monocytogenes* F2365, from the UniProtKB database. Most promiscuous B cell epitopes must not show allergic properties. Therefore, to remove all allergic proteins from the investigation AlgPred server was used. A total of 529 proteins of the F2365 strain of *Listeria monocytogenes* have been proclaimed from the UniProtKB database. Out of 529 proteins, 172 have shown non-allergenicity. These 172 non-allergic proteins have been used to find out the best antigenic regions or peptides that can provoke great immune inflammation in the human body, by using NETMHCII 2.3 server. 299 epitopes have been identified by the VaxiJen server that could bind with MHC II molecules with great stability. Based on the VaxiJen score, only the top 20 B cell epitopes were selected for succeeding refining. 3D modeling of all 20 epitopes has been performed by PEPstrMOD and all these tertiary structures have been saved in PDB format. Tertiary structure modeling of alleles was generated with the help of HLA alleles were performed.
Table 2  List of B cell epitopes as anticipated by NETMHCII 2.3 server and the result of VaxiJen analysis indicating antigenicity of epitopes

| Protein ID | Allele | Peptide | Binding affinity [nM] | VaxiJen score | Antigen/ non-antigen |
|------------|--------|---------|-----------------------|---------------|---------------------|
| Q71WU4     | DRB1_1301 | MNFRLKNMG | 57.4 | 1.4634 | Antigen |
| DRB1_1301 | VAAMNFRKL | 64.6 | 2.5495 | Antigen |
| Q71Z75     | DRB1_1301 | LSTKGKNRK | 8.8 | 1.9105 | Antigen |
| DRB1_1301 | VAAARRSHRE | 20.2 | 1.1808 | Antigen |
| DRB1_1301 | KVAARRSHRE | 23.5 | 1.4005 | Antigen |
| Q724J4     | DRB1_0101 | LHLWNSNL | 527.4 | 1.2681 | Antigen |
| DRB1_1301 | IRLKLSSSV | 15.1 | 1.403 | Antigen |
| DRB1_1301 | MKGQAGSKK | 49.4 | 2.2596 | Antigen |
| Q71W17     | DRB1_1301 | ARRANIRFR | 17.4 | 2.2999 | Antigen |
| DRB1_1301 | VAARRSHRE | 44.7 | 1.9086 | Antigen |
| DRB1_1301 | FQARRANIR | 49.8 | 1.458 | Antigen |
| DRB1_1301 | KKLGARLER | 60.8 | 1.1766 | Antigen |
| Q71Y34     | DRB1_0101 | FANIRPIQV | 449.7 | 1.1402 | Antigen |
| DRB1_0701 | FANIRPIQV | 76 | 1.1402 | Antigen |
| Q71XR2     | DRB1_0101 | AIFIRAPY | 886.2 | 1.4467 | Antigen |
| DRB1_1301 | LAFKVKKHS | 48.5 | 1.2632 | Antigen |
| DRB1_1301 | IFIRAPY | 62.4 | 1.6671 | Antigen |
| Q71ZE0     | DRB1_0101 | FDCVLPTRI | 357 | 1.5369 | Antigen |
| Q71ZE0     | DRB1_0101 | FDCVLPTRI | 357 | 1.5369 | Antigen |
| Q71Y46     | DRB1_0101 | FNVLDSRVL | 469 | 1.38 | Antigen |
| DRB1_0701 | FNVLDSRVL | 70.1 | 1.38 | Antigen |
| Q71WP0     | DRB1_0101 | FIVVDPMLA | 640 | 1.8053 | Antigen |
| DRB1_0701 | IKFIPKMKV | 117 | 1.1015 | Antigen |
| Q71WP7     | DRB1_1301 | LRLDLAAYR | 58.4 | 1.7082 | Antigen |
| Q720D7     | DRB1_0101 | VILAYAPLL | 1236.9 | 1.2361 | Antigen |
| DRB1_0701 | LGATNSFRV | 97.1 | 1.2028 | Antigen |
| Q720T3     | DRB1_0101 | ALLMPLV | 654.6 | 1.5696 | Antigen |
| DRB1_0101 | FLGVPWWPV | 721.2 | 2.0565 | Antigen |
| DRB1_0101 | LMLPVAII | 929.1 | 1.4677 | Antigen |
| DRB1_0101 | FYLFYNGSL | 1330 | 1.6406 | Antigen |
| DRB1_0101 | VALMLPLV | 1365.6 | 1.8132 | Antigen |
| DRB1_0701 | FLGVPWWPV | 29.7 | 2.0565 | Antigen |
| DRB1_0101 | IIGAWNWLI | 309.5 | 1.666 | Antigen |
| Q71Y4 | DRB1_0701 | SETLSVKV | 325.2 | 2.4375 | Antigen |
| DRB1_1301 | LVRTPGIR | 32.6 | 2.4375 | Antigen |
| DRB1_1301 | FLRVTPGIR | 65.4 | 1.2425 | Antigen |
| Q71WT9     | DRB1_0701 | VSLSRVGMEI | 216.6 | 1.6096 | Antigen |
| DRB1_1301 | IGETERRRK | 37.9 | 1.3502 | Antigen |
| Q720J1     | DRB1_0701 | JEVTDPYLM | 299.3 | 1.7114 | Antigen |
| Q71ZZ0     | DRB1_1301 | TKLTPRKNK | 20.2 | 1.3476 | Antigen |
| DRB1_1301 | IRLTHKTRP | 22.8 | 1.2793 | Antigen |
| Q71XV7     | DRB1_0101 | FLYVYVYSL | 1393.6 | 1.213 | Antigen |
| DRB1_0701 | FAVEPSFSI | 53.6 | 1.819 | Antigen |
| DRB1_0701 | IKWAKWMFV | 123.5 | 1.348 | Antigen |
| Protein ID | Allele     | Peptide          | Binding affinity [nM] | VaxiJen score | Antigen/non-antigen |
|-----------|------------|------------------|-----------------------|---------------|---------------------|
| Q724E9    | DRB1_0101  | FSAGMGAEAE       | 959.2                 | 1.5015        | Antigen             |
|           | DRB1_0701  | LVEGRAIRL        | 269.1                 | 1.5701        | Antigen             |
|           | DRB1_1301  | TKSKVRERR        | 13.3                  | 1.2742        | Antigen             |
|           | DRB1_1301  | GQRRETAIR        | 33.3                  | 1.2488        | Antigen             |
|           | DRB1_1301  | LGKQGRFR         | 51                    | 1.7176        | Antigen             |
|           | DRB1_1301  | LKSAOGRQR        | 55.5                  | 1.6836        | Antigen             |
|           | DRB1_1301  | EVTKSVR         | 59.3                  | 1.1113        | Antigen             |
|           | DRB1_1301  | LIFNTLPK         | 65.3                  | 1.134         | Antigen             |
|           | Q71W10     | DRB1_0101        | FALHYPRYEL           | 1003.9        | 1.4132              |
|           |           | DRB1_0701        | FALHYPRYEL           | 319.5         | 1.4132              |
|           | Q71Z37     | DRB1_0101        | FLFAPVHVHP           | 425           | 1.8183              |
|           |           | DRB1_0101        | IAFAPVHV            | 125           | 1.9413              |
|           |           | DRB1_0101        | LTLRPEDV            | 1060.8        | 1.3501              |
|           | Q71XV6     | DRB1_0701        | FSMLVSLVF            | 100           | 1.4972              |
|           |           | DRB1_0701        | ASRSKSNRL           | 302           | 1.1981              |
|           |           | DRB1_0701        | YIMALHFGI           | 307           | 1.9206              |
|           |           | DRB1_0701        | YAUSNYTL            | 308           | 1.1261              |
|           |           | DRB1_1301        | IVLLVAMIF           | 28            | 1.9817              |
|           | Q724M3     | DRB1_0101        | FIVKVMVRI           | 1025.4        | 1.9181              |
|           |           | DRB1_0701        | FIVKVMVRI           | 320           | 1.9181              |
|           |           | DRB1_1301        | VKMVRVI            | 36            | 1.2822              |
|           | Q71WH2     | DRB1_1301        | VRNLATGRGR          | 13            | 1.8274              |
|           |           | DRB1_1301        | IKKLAKLKIY          | 69            | 1.2527              |
|           | Q71VQ8     | DRB1_0701        | IVFPLSWTI           | 300           | 1.6433              |
|           |           | DRB1_1301        | LLLPLMVKT          | 24            | 2.2056              |
|           | Q71ZU1     | DRB1_0101        | LQIMPLMA            | 1353.2        | 1.3037              |
|           | Q720A3     | DRB1_0101        | LHLIPVMVMK         | 712           | 1.5796              |
|           |           | DRB1_0101        | LIGLPIRIT          | 1193          | 1.6981              |
|           |           | DRB1_1301        | IYKYDVRFK          | 53            | 1.8026              |
|           | Q720A7     | DRB1_1301        | VRVNVMGYR         | 20            | 1.4928              |
|           |           | DRB1_1301        | LRLSNFMLW          | 55            | 1.2577              |
|           | Q720J2     | DRB1_0101        | WLMNPDMTV          | 1064.6        | 1.3955              |
|           | Q71XU1     | DRB1_0701        | ILNFPTARI          | 108           | 1.1713              |
|           |           | DRB1_0701        | LNFTPARIS         | 248.4         | 1.4755              |
|           |           | DRB1_1301        | LNFTPARIS         | 54.6          | 1.1713              |
|           | Q71WU5     | DRB1_0101        | PIKISIARI          | 1514.9        | 1.1708              |
|           |           | DRB1_0701        | PIKISIARI          | 121.3         | 1.1708              |
|           | Q71YA9     | DRB1_0701        | ATGTGTLRI         | 122.2         | 2.2883              |
|           | Q724F0     | DRB1_0101        | FRTRPLIDG          | 368.9         | 1.165               |
|           |           | DRB1_0701        | LINIRPVA          | 1366          | 1.2121              |
|           |           | DRB1_0701        | VEHEAREI         | 78.9          | 1.4245              |
|           |           | DRB1_1301        | LRVKRLRLIN       | 22.2          | 1.3688              |
| Protein ID | Allele | Peptide              | Binding affinity [nM] | VaxiJen score | Antigen/ non-antigen |
|------------|--------|----------------------|-----------------------|---------------|----------------------|
| Q71WP3     | DRB1_0101 | NTLTLGLRL             | 518                   | 1.6477        | Antigen               |
|            | DRB1_0101 | MKFLFPLKL             | 612.8                 | 2.3447        | Antigen               |
|            | DRB1_0101 | MLGLPPQIA             | 1397.6                | 1.8635        | Antigen               |
|            | DRB1_0701 | NTLTLGLRL             | 80.1                  | 1.6477        | Antigen               |
|            | DRB1_0701 | MKFLFPLKL             | 175.8                 | 2.3447        | Antigen               |
|            | DRB1_0701 | VTLTLAIMV             | 181.1                 | 1.2651        | Antigen               |
| Q722W7     | DRB1_0101 | ICTRNLQRR             | 16.9                  | 1.1843        | Antigen               |
|            | DRB1_0101 | WVMHLADAMV            | 1508.3                | 1.4715        | Antigen               |
|            | DRB1_0701 | IVYEVSWSRY            | 223.4                 | 1.2052        | Antigen               |
|            | DRB1_0701 | YHYFYAHAL             | 234.2                 | 1.4315        | Antigen               |
|            | DRB1_1301 | LMGRSGRRG             | 11.8                  | 1.4813        | Antigen               |
|            | DRB1_1301 | LRITMLLMR             | 26.9                  | 1.1065        | Antigen               |
|            | DRB1_1301 | QLMGRSGRR             | 27.3                  | 1.1831        | Antigen               |
| Q71YH0     | DRB1_0101 | CTLLYAFPL             | 185.7                 | 2.1684        | Antigen               |
|            | DRB1_0101 | SYWLIGLPV             | 452.6                 | 1.3982        | Antigen               |
|            | DRB1_0701 | CIGIPAFFI             | 229.8                 | 1.6783        | Antigen               |
|            | DRB1_0701 | IMHFLVYAI             | 260.9                 | 1.1187        | Antigen               |
|            | DRB1_0701 | CTLLYAFPL             | 311.2                 | 2.1684        | Antigen               |
|            | DRB1_1301 | FILSIRVRK             | 8.4                   | 1.1456        | Antigen               |
|            | DRB1_1301 | IRVRKTEQK             | 17.8                  | 1.6151        | Antigen               |
|            | DRB1_1301 | AFILSIRVR             | 39.5                  | 1.4081        | Antigen               |
|            | DRB1_1301 | LSIRVRKTE             | 45.7                  | 1.7093        | Antigen               |
|            | DRB1_1301 | LTLFSMTFF             | 65.7                  | 1.2134        | Antigen               |
| Q71WB6     | DRB1_0101 | YIPGIGHNL             | 419.9                 | 1.1532        | Antigen               |
|            | DRB1_0701 | VRLSNGIEV             | 41.6                  | 1.353         | Antigen               |
| Q71YB9     | DRB1_0101 | FLKIDPPIL             | 199.4                 | 2.3187        | Antigen               |
|            | DRB1_0101 | FWMIPEPema            | 524.2                 | 2.1476        | Antigen               |
| Q71VR4     | DRB1_0101 | FLKIDPPIL             | 101.7                 | 2.3187        | Antigen               |
|            | DRB1_1301 | KLNHLAIVY             | 1297.1                | 1.6175        | Antigen               |
|            | DRB1_1301 | IEGHGAKSRK            | 55.6                  | 1.2977        | Antigen               |
| Q71W89     | DRB1_0101 | LSFLPALAL             | 91.8                  | 1.5837        | Antigen               |
|            | DRB1_0101 | YILLPLSLLI            | 150                   | 1.4583        | Antigen               |
|            | DRB1_0101 | FSLAFNTA             | 398.7                 | 1.4513        | Antigen               |
|            | DRB1_0101 | ILLIPVALV             | 879.3                 | 1.4451        | Antigen               |
|            | DRB1_0101 | FLPALALGP             | 996.5                 | 1.3317        | Antigen               |
|            | DRB1_0101 | LILVPPALT             | 1544.3                | 2.0559        | Antigen               |
|            | DRB1_0701 | LSFLPALAL             | 97.5                  | 1.5837        | Antigen               |
|            | DRB1_0701 | LSFLSLAFNT            | 155.2                 | 1.688         | Antigen               |
|            | DRB1_0701 | LLLVAVPL              | 211.1                 | 1.53          | Antigen               |
| Q71W91     | DRB1_0101 | VNVQLQVNL             | 587.2                 | 1.403         | Antigen               |
| Q71WP8     | DRB1_0101 | LEVLLPQYV             | 1295.8                | 1.246         | Antigen               |
| Q71WG3     | DRB1_1301 | VKGGRFRFRF            | 39.8                  | 1.694         | Antigen               |
| Q71Z71     | DRB1_1301 | ISVREKSAK             | 56                    | 1.548         | Antigen               |
| Q722Y1     | DRB1_0101 | GVMLPLKL              | 254.4                 | 1.104         | Antigen               |
|            | DRB1_0101 | FQIELGHAA             | 324.6                 | 1.288         | Antigen               |
Table 2 (continued)

| Protein ID | Allele   | Peptide         | Binding affinity [nM] | VaxiJen score | Antigen/ non-antigen |
|------------|----------|-----------------|-----------------------|---------------|----------------------|
| Q71WF2     | DRB1_0701 | KVHIPGMRI       | 181.4                 | 1.3023        | Antigen              |
|            | DRB1_1301 | IKTQVSGLR       | 19.6                  | 1.16          | Antigen              |
|            | DRB1_1301 | MRAGAKGIK       | 50.5                  | 1.27          | Antigen              |
|            | DRB1_1301 | LRIRDYVAK       | 51.4                  | 1.181         | Antigen              |
|            | DRB1_1301 | IKLRKTQPR       | 34.9                  | 1.462         | Antigen              |
|            | DRB1_1301 | VRIPAKKAR       | 27                    | 1.1447        | Antigen              |
|            | DRB1_1301 | GRASAINKR       | 44                    | 1.264         | Antigen              |
|            | DRB1_0101 | YKLKNPTLG       | 86.8                  | 1.307         | Antigen              |
|            | DRB1_0101 | FLNIRLKV       | 485.3                 | 1.9058        | Antigen              |
|            | DRB1_0101 | ILSMQLSFA      | 540.1                 | 1.2557        | Antigen              |
|            | DRB1_0101 | LNLLFGIPL       | 599.5                 | 1.7237        | Antigen              |
|            | DRB1_0101 | LAIVPAVII      | 777.5                 | 1.356         | Antigen              |
|            | DRB1_0101 | LSMQLSFAV      | 1348.6                | 1.566         | Antigen              |
|            | DRB1_0701 | FSILTALLI      | 22.4                  | 1.852         | Antigen              |
|            | DRB1_0701 | IDSTFSLTI       | 57.8                  | 1.4124        | Antigen              |
|            | DRB1_0701 | FLNIRLKPV      | 62.9                  | 1.906         | Antigen              |
|            | DRB1_0701 | ISWAVAIF       | 72.9                  | 1.347         | Antigen              |
|            | DRB1_0701 | IGSALIALNL     | 111.4                 | 1.277         | Antigen              |
|            | DRB1_0701 | LAIVPAVII      | 184                   | 1.356         | Antigen              |
|            | DRB1_1301 | LNIRLKPVV      | 30.9                  | 2.189         | Antigen              |
|            | DRB1_0701 | IKVGNALEL      | 51                    | 1.204         | Antigen              |
|            | DRB1_1301 | LKKKAGRNN      | 60.7                  | 1.322         | Antigen              |
|            | DRB1_1301 | VRHHGGGHHK     | 63.8                  | 2.522         | Antigen              |
|            | DRB1_1301 | LEVKARRVG      | 53                    | 1.551         | Antigen              |
|            | DRB1_1301 | IEVRADRRS      | 60.7                  | 1.989         | Antigen              |
|            | DRB1_1301 | MMVDGKRGK      | 65.1                  | 1.375         | Antigen              |
|            | DRB1_1301 | SYRGMRHR       | 9                     | 1.4454        | Antigen              |
|            | DRB1_1301 | TKNNARTRK      | 38.1                  | 2.1367        | Antigen              |
|            | DRB1_0701 | FVSGLSFHV      | 35.1                  | 1.487         | Antigen              |
|            | DRB1_1301 | KQLKIRQR       | 53.8                  | 1.389         | Antigen              |
|            | DRB1_0101 | NIDIKGRLI      | 1319.9                | 1.353         | Antigen              |
|            | DRB1_0701 | IFDVRSEHV      | 179.8                 | 1.5294        | Antigen              |
|            | DRB1_1301 | MAKQKIRIR      | 18.8                  | 1.2116        | Antigen              |
|            | DRB1_1301 | FERMRTHKRL     | 27.8                  | 1.1916        | Antigen              |
|            | DRB1_1301 | IRLKAYDHR      | 28.8                  | 1.7067        | Antigen              |
|            | DRB1_1301 | AKQKIRIRL      | 37.3                  | 1.7363        | Antigen              |
|            | DRB1_1301 | QKIRIRLKA      | 46.2                  | 1.7022        | Antigen              |
|            | DRB1_1301 | IRLKAYD       | 55.2                  | 1.8524        | Antigen              |
|            | DRB1_1301 | QFEMRTHKR      | 68.6                  | 1.7135        | Antigen              |
|            | DRB1_1301 | VRTKSGARR      | 5.6                   | 1.944         | Antigen              |
|            | DRB1_1301 | MARKTNTKR      | 5.2                   | 1.6203        | Antigen              |
|            | DRB1_1301 | RKTNTKRR       | 5.5                   | 2.5417        | Antigen              |
|            | DRB1_1301 | ARKTNTKRR      | 10.3                  | 2.2271        | Antigen              |
|            | DRB1_1301 | TNTRKRRVK      | 26.3                  | 2.1039        | Antigen              |
|            | DRB1_1301 | TRKRRVKKN      | 55.9                  | 1.5039        | Antigen              |
|            | DRB1_1301 | NTRKRRVKK      | 62                    | 1.8576        | Antigen              |
| Protein ID | Allele | Peptide | Binding affinity [nM] | VaxiJen score | Antigen/ non-antigen |
|------------|--------|---------|-----------------------|---------------|---------------------|
| Q725B8     | DRB1_1301 | GRRGGRRRK | 7.1 | 3.0668 | Antigen |
|            | DRB1_1301 | RRGGRRRK | 17.1 | 2.833 | Antigen |
|            | DRB1_1301 | GGRGGRRRK | 25.2 | 3.1722 | Antigen |
| Q71WV5     | DRB1_1301 | VIKRSAKRA | 14.9 | 1.3995 | Antigen |
|            | DRB1_1301 | LNARTLERK | 16.7 | 1.6232 | Antigen |
|            | DRB1_1301 | VRLKSGTRG | 19.6 | 1.5481 | Antigen |
|            | DRB1_1301 | VSKSGINHR | 44.8 | 1.3402 | Antigen |
|            | DRB1_1301 | LNARTLERK | 16.7 | 1.6232 | Antigen |
|            | DRB1_1301 | VRLKSGTRG | 19.6 | 1.5481 | Antigen |
|            | DRB1_1301 | VSKSGINHR | 44.8 | 1.3402 | Antigen |
| Q71WG2     | DRB1_1301 | KVRKKRHAR | 7.7 | 1.6463 | Antigen |
|            | DRB1_1301 | VRKKKRHRV | 12.6 | 1.3471 | Antigen |
|            | DRB1_1301 | RHARVRSKI | 27.1 | 1.4108 | Antigen |
|            | DRB1_1301 | KKRHARVRS | 38.4 | 2.0868 | Antigen |
|            | DRB1_1301 | RKKHRARVR | 47.2 | 2.0804 | Antigen |
|            | DRB1_1301 | NKVRKKRHA | 59.3 | 1.1365 | Antigen |
| Q71WE8     | DRB1_1301 | AGYNKRRK | 46.9 | 1.237 | Antigen |
| Q71YD4     | DRB1_1301 | FGISIRIRFR | 48.6 | 1.1227 | Antigen |
| Q71YN5     | DRB1_1301 | TVTRKRRK | 2.7 | 1.1019 | Antigen |
|            | DRB1_1301 | GTVTRKRRK | 15.8 | 1.2113 | Antigen |
|            | DRB1_1301 | GGTATVRKRR | 31 | 1.6998 | Antigen |
| Q721R7     | DRB1_1301 | ARLRTTGGR | 14 | 1.7495 | Antigen |
|            | DRB1_1301 | RLRTTGGRY | 64.9 | 1.4507 | Antigen |
| Q71ZZ5     | DRB1_1301 | MNVRANRVS | 41.7 | 2.0256 | Antigen |
|            | DRB1_1301 | GRRIRLRKV | 60.1 | 1.6184 | Antigen |
| Q720A8     | DRB1_1301 | LRSLIPQLT | 375.6 | 1.2897 | Antigen |
|            | DRB1_1301 | LRSLIPQLT | 192.5 | 1.2897 | Antigen |
| Q721Y1     | DRB1_0701 | LRLLNLNL | 32.3 | 1.4731 | Antigen |
|            | DRB1_1301 | ILRLRLNL | 51.9 | 1.2854 | Antigen |
| Q71YM9     | DRB1_1301 | LRLNRLGKAA | 476.7 | 1.2468 | Antigen |
|            | DRB1_1301 | TVRVAHKV | 152.6 | 1.3034 | Antigen |
|            | DRB1_1301 | VRVVAHKV | 67.1 | 1.564 | Antigen |
|            | DRB1_1301 | RRGVKRRAK | 20.2 | 1.3055 | Antigen |
|            | DRB1_1301 | LRGKAARIK | 17.4 | 2.0521 | Antigen |
| Q71WG4     | DRB1_1301 | AKLEITLKR | 51.3 | 1.1423 | Antigen |
| Q71YN4     | DRB1_1301 | FKRTSGKGL | 34.3 | 1.1993 | Antigen |
|            | DRB1_1301 | TQHSGAKRF | 43.7 | 1.0624 | Antigen |
|            | DRB1_1301 | QKQKRLRK | 46.1 | 1.1816 | Antigen |
| Q71WH3     | DRB1_1301 | LGRTSSQRRK | 33.5 | 1.2846 | Antigen |
| Q71ZY7     | DRB1_1301 | LKKYCPRLR | 50.8 | 2.0807 | Antigen |
|            | DRB1_1301 | KKYCPRLRR | 61.5 | 1.5286 | Antigen |
| Protein ID | Allele | Peptide       | Binding affinity [nM] | VaxiJen score | Antigen/non-antigen |
|------------|--------|---------------|-----------------------|---------------|---------------------|
| Q71XW7     | DRB1_1301 | SKAKKRKRR    | 5.8                   | 1.8899        | Antigen             |
|            | DRB1_1301 | KKRKRRTHV    | 11.7                  | 1.4013        | Antigen             |
|            | DRB1_1301 | AKKRKRRTH    | 15.7                  | 1.6556        | Antigen             |
|            | DRB1_1301 | RTSKAKKRK    | 18.1                  | 1.9221        | Antigen             |
|            | DRB1_1301 | KRKRRTHVK    | 21.3                  | 1.6065        | Antigen             |
|            | DRB1_1301 | TSKAKKRK     | 23.1                  | 1.7453        | Antigen             |
|            | DRB1_1301 | KAKKKRKT     | 24.4                  | 1.7483        | Antigen             |
|            | DRB1_1301 | RRTSKAKKR    | 26                    | 1.7169        | Antigen             |
|            | DRB1_1301 | RKKRTHVKL    | 39.5                  | 1.4259        | Antigen             |
| Q723G3     | DRB1_1301 | ARRTSKAKK    | 15.4                  | 1.4443        | Antigen             |
|            | DRB1_1301 | SKAKKKKRR    | 29.1                  | 1.707         | Antigen             |
|            | DRB1_1301 | KAKKKKRRT    | 46.4                  | 1.7778        | Antigen             |
| Q71WV3     | DRB1_0101 | FKYGIPIDA    | 297                   | 1.6186        | Antigen             |
|            | DRB1_1301 | ISHRDMKRR    | 11.9                  | 1.5539        | Antigen             |
|            | DRB1_1301 | LMFTLPFYK    | 44.9                  | 1.9589        | Antigen             |
|            | DRB1_1301 | ALVMDLRGR    | 45.8                  | 1.1548        | Antigen             |
|            | DRB1_1301 | MAPRELRE     | 51.1                  | 1.1283        | Antigen             |
|            | DRB1_1301 | SHRDMKRRK    | 64                    | 1.6218        | Antigen             |
|            | DRB1_1301 | LLMFTLPFY    | 68.2                  | 2.632         | Antigen             |
|            | DRB1_1301 | SRYKETRRH    | 69.9                  | 1.0813        | Antigen             |
| Q71ZJ5     | DRB1_0101 | FRFVPINNF    | 1098                  | 1.5957        | Antigen             |
|            | DRB1_0701 | FRFVPINNF    | 83.9                  | 1.5957        | Antigen             |
|            | DRB1_0701 | IQPVGSKNL    | 287.2                 | 0.534         | Antigen             |
| Q721N6     | DRB1_1301 | QMVQNRHGK    | 18                    | 1.5447        | Antigen             |
| Q71ZK1     | DRB1_1301 | KKSEAARKR    | 46.5                  | 1.9356        | Antigen             |
| Q71ZD0     | DRB1_1301 | MLKFDIQHF    | 45                    | 1.2032        | Antigen             |
| Q71WF4     | DRB1_0101 | LFNLRQFLA    | 1029                  | 2.5288        | Antigen             |
| Q71YL9     | DRB1_1301 | MAVKIRLLKR   | 4.3                   | 1.4155        | Antigen             |
|            | DRB1_1301 | AVKIRLKI     | 55.1                  | 1.4342        | Antigen             |
| Q71YK0     | DRB1_1301 | RKSRSNGKR    | 40.5                  | 2.7338        | Antigen             |
| Q71WI2     | DRB1_1301 | LLTDPRMK     | 16.6                  | 1.3863        | Antigen             |
|            | DRB1_1301 | KSSVARVRL    | 68.6                  | 1.0414        | Antigen             |
| Q71VQ6     | DRB1_1301 | ASRRRKKGRK   | 8.3                   | 2.0002        | Antigen             |
|            | DRB1_1301 | SRRRKKGRKV   | 12.1                  | 1.7764        | Antigen             |
|            | DRB1_1301 | MTKNGRRV     | 13.5                  | 1.7661        | Antigen             |
|            | DRB1_1301 | FRTRMSTKN    | 39.7                  | 1.2896        | Antigen             |
|            | DRB1_1301 | RMSTKNGRR    | 49.3                  | 2.0073        | Antigen             |
| Q722D6     | DRB1_0101 | YALLFFPYA    | 1222                  | 1.9423        | Antigen             |
|            | DRB1_0701 | IFLFAANIL    | 179.2                 | 1.1164        | Antigen             |
|            | DRB1_1301 | LSVKLRSSG    | 15                    | 1.128         | Antigen             |
|            | DRB1_1301 | VLSVKLRSR    | 21.1                  | 1.3894        | Antigen             |
Proteins with PDB ID 4AH2, 3C5J, and 6CQL were used as templates for alleles HLA DRB1_0101, HLA DRB1_0701, and HLA DRB1_1301. Visualization of the tertiary structures was done by the PyMOL visualization tool. Molecular docking between epitope and its corresponding allele was performed by AutoDock Vina software. Based on low binding energy, 4 peptides were selected viz., CEETFGIRL, MKFLFPLKL, FLKIDDPIL, and VRHHGGGHK. CEETFGIRL showed the energy of $-6.9$ kcal/mol and 2 hydrogen bonds. FLKIDDPIL showed the energy of $-7.3$ kcal/mol and 6 hydrogen bonds. VRHHGGGHK showed the energy of $-6.7$ kcal/mol and 6 hydrogen bonds. These 4 epitopes were selected on low binding energy as low energy means high stability.

Most promiscuous B cell epitope which is a nano peptide, must not be toxic and therefore toxicity analysis must be performed. Toxin Pred server is used for this analysis. This server also anticipates various physicochemical properties of the epitopes like molecular weight, hydrophobicity, and

| Protein ID | Allele | Peptide       | Binding affinity [nM] | VaxiJen score | Antigen/non-antigen |
|------------|--------|---------------|------------------------|---------------|---------------------|
| Q71XL9     | DRB1_0101 | GIILLGFRL    | 330.6                  | 1.0131        | Antigen             |
| DRB1_0101 | YFLAKLPLF | 673.5         | 1.4522                 |               | Antigen             |
| DRB1_0101 | FLIAMSMMG | 884.4         | 1.2298                 |               | Antigen             |
| DRB1_0101 | FLAKLFLML | 891           | 1.7779                 |               | Antigen             |
| DRB1_0101 | FLVICAYFL | 1342          | 2.0765                 |               | Antigen             |
| DRB1_0101 | YFLIASMMG | 1357          | 1.587                  |               | Antigen             |
| DRB1_0101 | YGIALTFCI | 1600          | 1.7051                 |               | Antigen             |
| DRB1_0701 | VIYTLIPYI | 20.1          | 1.3475                 |               | Antigen             |
| DRB1_0701 | FLVICAYFL | 125.7         | 2.0765                 |               | Antigen             |
| Q71XA1     | DRB1_0701 | ITISLGFYL    | 56.9                   | 1.6467        | Antigen             |
| A6X137     | DRB1_1301 | AHAHKIERRL   | 32.2                   | 1.2949        | Antigen             |
| Q71Z298    | DRB1_0701 | POVTVSLVF    | 92.9                   | 1.1655        | Antigen             |
| DRB1_1301 | VILLKLHV  | 49.4          | 1.5441                 |               | Antigen             |
| Q724P3     | DRB1_1301 | IRCKYPKTR    | 22.7                   | 2.0203        | Antigen             |
| DRB1_1301 | RCKYTRR   | 43            | 1.5601                 |               | Antigen             |
| Q71Z2L4    | DRB1_1301 | LMLDIRYRH    | 33.2                   | 1.656         | Antigen             |
| DRB1_1301 | SMLRHIRYR | 35.4          | 1.4323                 |               | Antigen             |
| Q2N761     | DRB1_0101 | LLLSLPELF    | 1010                   | 1.2376        | Antigen             |
| DRB1_0101 | WLLSLSPEL | 1136          | 2.0048                 |               | Antigen             |
| DRB1_0101 | NVAIRTLRL | 1262          | 1.4269                 |               | Antigen             |
| DRB1_0701 | WLLSLSPEL | 59.8          | 2.0048                 |               | Antigen             |
| DRB1_0701 | MVTTVHARL | 241.6         | 1.3229                 |               | Antigen             |
| DRB1_0701 | NVAIRTLRL | 244.4         | 1.4269                 |               | Antigen             |
| DRB1_1301 | ARVRLTSGR | 28.7          | 1.3033                 |               | Antigen             |
| DRB1_1301 | MVTTVHARL | 31.9          | 1.3229                 |               | Antigen             |
| DRB1_1301 | VAIRTLRLT | 34.2          | 1.1019                 |               | Antigen             |
| L9WZX9     | DRB1_1301 | AHRKAARER    | 17.4                   | 1.422         | Antigen             |
| DRB1_1301 | ALLWLFPFRF | 59.1        | 2.2918                 |               | Antigen             |
| A0A0X1KHF9 | DRB1_0101 | CSNIEGVHV    | 1163                   | 1.8716        | Antigen             |
| DRB1_0701 | ITQSLSAKV | 20.1          | 1.1418                 |               | Antigen             |
| DRB1_0701 | LSIDASFGL | 320.4         | 1.1112                 |               | Antigen             |
| Q1KT48     | DRB1_0701 | LKLACAKAF    | 89.5                   | 1.2066        | Antigen             |

Cut off value for the VaxiJen server is 1.1
The top 20 selected epitopes are represented in bold.
hydrophilicity. MHC Pred server was used to anticipate the binding intensity of epitopes with allele’s viz., HLA DRB1_0101, HLA DRB1_0401, and HLA DRB1_0701.

Table 3 List showing Binding energy of 20 selected epitopes while interacting with its corresponding allele, as anticipated by AutoDock Vina software

| S. no. | Peptide   | Allele     | Energy (kcal/mol) |
|--------|-----------|------------|-------------------|
| 1      | VAAMNFRKLK| DRB1_1301  | −5.8              |
| 2      | MKGQAGSKK | DRB1_1301  | −5.1              |
| 3      | ARRANIRFR | DRB1_1301  | −5.7              |
| 4      | CEETFGIRL | DRB1_0701  | −6.7              |
| 5      | SGETLSVKV | DRB1_0701  | −6.5              |
| 6      | LRVTGP GIRL | DRB1_1301 | −6.3              |
| 7      | ATGTTGLRI | DRB1_0701  | −6.3              |
| 8      | MKFLFPLKL | DRB1_0101  | −6.9              |
| 9      | MKFLFPLKL | DRB1_0701  | −6.5              |
| 10     | FLKIDPPI L | DRB1_0101 | −7.3              |
| 11     | FLKIDPPIL | DRB1_0701  | −6.5              |
| 12     | VRHHGGGHK | DRB1_1301  | −6.7              |
| 13     | RKTNTRKRR | DRB1_1301  | −5.2              |
| 14     | ARKTNTKRR | DRB1_1301  | −5.7              |
| 15     | RGGRRRKKK | DRB1_1301  | −5.2              |
| 16     | GGRGGRRRR | DRB1_1301  | −5.9              |
| 17     | LLMOFLPYF  | DRB1_1301  | −6.4              |
| 18     | LFLNLFLQLA | DRB1_0101 | −6.5              |
| 19     | RKRSGKKNR  | DRB1_1301  | −5.5              |
| 20     | ALLWLFPRF  | DRB1_1301  | −6.3              |

Selected epitopes are represented in bold

Epitopes viz., CEETFGIRL and VRHHGGGHK were found to bind with HLA DRB1_0101 and HLA DRB1_0401, respectively. Both FLKIDPPI L and MKFLPPLKL were found to bind with HLA DRB1_0101 and HLA DRB1_0701. Binding energy prediction is given in the form of IC50 value. Epitopes having an IC50 value greater than 500 nM are not considered in this analysis. Population coverage analysis is one of the most important investigations need to be done in computational biology. Population coverage analysis of all 4 epitopes was analyzed by the IEDB population coverage tool. Based on both low binding energy and high population

![Fig. 1 Modeled structure of HLA class II alleles—a molecular structure of HLA DRB1_0101, b molecular structure of HLA DRB1_0701, c molecular structure of HLA DRB1_1301](image1)

![Fig. 2 This Docked result depicts the interaction analysis of epitope CEETFGIRL (represented with cyan color) with 3C5J receptor (represented with forest green color). Showing the epitope interacting with 3C5J receptor with the help of 6 hydrogen bonds (Color figure online)](image2)
coverage, worldwide epitope FLKIDPPIL was selected. To check the binding energy of epitope FLKIDPPIL with its corresponding 4AH2 receptor molecular dynamics simulation study was performed by using MD Web. RMSD and B factor plot was used to interpret the result of the simulation. After all these vigorous steps of the investigation, epitope FLKIDPPIL proved to be the most eligible candidate that should be used for vaccine designing. Reverse vaccinology has been proved as one of the most powerful weapons to combat some deadly bacterial diseases and had shown tremendous results also. First and foremost, a peptide-based vaccine using the reverse vaccinology approach was created against *E. coli* in the year 1985 (Jacob et al. 1985). It has been proved effective against tuberculosis (Mustafa 2013) and many more pathogenic diseases. The identification of antigenic peptides by using a reverse vaccinology approach has been found effective against *Staphylococcus aureus* (Oyama et al. 2019). From this research work, we found during the identification and characterization of epitopes for the utility of vaccine designing against *Listeria monocytogenes*, the epitope FLKIDPPIL was non-allergic, non-toxic, highly antigenic, and can provoke a better immune response.

**Conclusion**

Despite major advancements in the field of technology, society and mankind have been plagued by several kinds of life-threatening diseases. Although vigorous research is going on, on several deadly diseases in various parts of the world. But still, some foodborne diseases are under-reported and Listeriosis is one of them. In such conditions, computational vaccine technology is one of the best alternatives to deal with such diseases. Computational vaccine technology is a boon in research domains as it accelerates the process of epitope screening and vaccine designing and development. It is a branch of vaccinology that is based on the central idea of solving vaccine development by using a computer-driven algorithm. Listeriosis is still under-reported in many countries of the world. Computational vaccine technology is going to create some awareness and will bring out the best treatment and remedy for the disease. In this research work, after performing molecular docking, 4 epitopes were selected.
These 4 epitopes viz., CEETFGIRL, MKFLFPLKL, FLKIDPPIL, and VRHHGGGHK were screened as the most promiscuous B cell epitopes among 299 antigenic sites identified. Low binding energy and population coverage analysis predicted FLKIDPPIL as the most potent epitope. Epitope FLKIDPPIL can elicit a strong immune response in the host against listeriosis. Further wet lab trials can assure the stability as well as the response of the epitope in vitro and in vivo. Reverse vaccinology can be proved as the most powerful approach to find remedies against diseases like listeriosis.

**Table 4** Result of toxicity analysis of selected epitopes as analyzed by Toxin Pred along with their physicochemical properties

| Epitope      | SVM score | Toxic/nontoxic | Molecular weight | Hydrophobicity | Hydrophilicity |
|--------------|-----------|----------------|------------------|----------------|----------------|
| CEETFGIRL    | −0.73     | Non toxic      | 1067.35          | −0.12          | 0.17           |
| MKFLFPLKL    | −0.73     | Non toxic      | 1136.64          | 0.09           | −0.63          |
| FLKIDPPIL    | −0.85     | Non toxic      | 1055.46          | 0.13           | −0.41          |
| VRHHGGGHK    | −1.03     | Non toxic      | 984.23           | −0.34          | 0.33           |

**Table 5** List showing number of HLA binders and binding affinity of anticipated B cell epitopes as investigated by MHCPred tool

| EPITOPE       | Number of HLA binders | HLA with predicted IC50 (nM) value |
|---------------|------------------------|-----------------------------------|
| FLKIDPPIL     | 2                      | HLA-DRB1_0101 (19.19)             |
|               |                        | HLA-DRB1_0701 (195.88)            |
| CEETFGIRL     | 1                      | HLA-DRB1_0101 (66.53),            |
| MKFLFPLKL     | 2                      | HLA-DRB1_0101 (78.52)             |
|               |                        | HLA-DRB1_0701 (246.04)            |
| VRHHGGGHK     | 1                      | HLA-DRB1_0401 (318.42)            |

IC$_{50} < 500$ nM scores are selected (are considered good binders)

**Fig. 6** Graphical representation of Population coverage for epitope MKFLFPLKL and FLKIDPPIL

**Fig. 7** Graphical representation of population coverage for epitope CEETFGIRL
Fig. 8  Graphical representation of population coverage for epitope VRHHGGGHK

Fig. 9  Graphical representation of RMSD for epitope FLKIDPPIL with 4AH2 receptor obtained during simulation studies
Compliance with Ethical Standards

Conflict of interest The authors hereby declare they that have no conflict of interest.

Ethical approval The authors did not perform any experiments on human or animals.

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Fig. 10 Graphical representation of the B factor plot for epitope FLKIDPPII with 4AH2 receptor obtained during simulation studies
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