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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., MKFLFPLKL, 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 *Insilico* 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).

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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 VaxiJen 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 VaxiJen 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 multiplicates 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 structure 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 IC_{50} values (Guan et al. 2003). IC_{50} 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 protein 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 epitopes 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, FLKIDPPIL, 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 FLKIDPPIL-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 IC_{50} value (Table 5). Epitopes showing IC_{50} 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	Q71YI4	− 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	Q71Z37	− 0.698	Non-allergen
38	Q71XR3	− 1.167	Non-allergen
39	Q720G2	− 0.776	Non-allergen
40	Q71Y82	− 1.037	Non-allergen
41	Q71XV6	− 1.471	Non-allergen
42	Q724M3	− 0.608	Non-allergen
43	Q724B0	− 1.957	Non-allergen
44	Q724I1	− 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

Table 1 (continued)

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	Q71W89	− 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	Q71WG3	− 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	Q71WF7	− 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	Q71WU6	− 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

Table 1 (continued)

S. no.	Protein ID	Score	AlgPred prediction
103	Q71WF8	− 1.611	Non-allergen
104	Q724G2	− 0.644	Non-allergen
105	Q71XE5	− 0.913	Non-allergen
106	Q71XX1	− 0.625	Non-allergen
107	Q71YK6	− 0.683	Non-allergen
108	Q71WE5	− 2.321	Non-allergen
109	Q71ZR7	− 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
1 21	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	Q71ZJ5	− 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	Q71WI2	− 2.143	Non-allergen
152	Q71VQ6	− 1.957	Non-allergen
153	Q724G8	− 1.5	Non-allergen

Table 1 (continued)

S. no.	Protein ID	Score	AlgPred prediction
154	Q722D6	− 1.506	Non-allergen
155	Q71XL9	− 0.743	Non-allergen
156	Q720B5	− 0.934	Non-allergen
157	Q71XA1	− 1.344	Non-allergen
158	A6X137	− 0.435	Non-allergen
159	Q71Z99	− 0.409	Non-allergen
160	Q71YM0	− 1.017	Non-allergen
161	Q724P3	− 0.613	Non-allergen
162	Q71XW0	− 0.917	Non-allergen
163	Q720B7	− 0.621	Non-allergen
164	Q721A0	− 0.643	Non-allergen
165	Q71ZL4	− 0.912	Non-allergen
`166	Q721A5	− 0.545	Non-allergen
167	Q71YW0	− 0.48	Non-allergen
168	Q2N761	− 1.386	Non-allergen
169	L9WZX9	− 0.694	Non-allergen
170	A0A0X1KHF9	− 0.575	Non-allergen
171	Q1KT30	− 0.508	Non-allergen
172	Q1KT48	− 0.458	Non-allergen

epitope MKFLFPLKL and FLKIDPPIL 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 FLKIDPPIL was refined. To check the stability of complex FLKIDPPIL-4AH2, molecular dynamics simulation was performed by MD Web simulation. RMSD value of FLKIDPPIL-4AH2 was given in between 0.1 and 1.0 Å (Fig. 9) and B factor scores between 1 and 25 $Å^2$ (Fig. 10). Both RMSD values and B factor plot of complex viz., FLKIDPPIL-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	**VAAMNFRLK**	64.6	2.5495	Antigen
Q71Z75	DRB1_1301	LSTKGKNRK	8.8	1.9105	Antigen
	DRB1_1301	VAARRSHRE	20.2	1.1808	Antigen
	DRB1_1301	KVAARRSHR	23.5	1.4005	Antigen
Q724J4	DRB1_0101	LHFLWNSNL	527.4	1.2681	Antigen
	DRB1_1301	IRLKLKSSV	15.1	1.403	Antigen
	DRB1_1301	**MKGQAGSKK**	49.4	2.2596	Antigen
Q71W17	DRB1_1301	**ARRANIRFR**	17.4	2.2999	Antigen
	DRB1_1301	QARRANIRF	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	AIFIRAPYL	886.2	1.4467	Antigen
	DRB1_1301	LAFKVKHSS	48.5	1.2632	Antigen
	DRB1_1301	IFIRAPYLI	62.4	1.6671	Antigen
Q71ZE0	DRB1_0101	FDCVLPTRI	357	1.5369	Antigen
Q71ZE0	DRB1_0101	FDCVLPTRI	357	1.5369	Antigen
	DRB1_0701	FDCVLPTRI	25.3	1.5369	Antigen
	DRB1_0701	**CEETFGIRL**	66	2.4185	Antigen
Q71XX6	DRB1_0701	FKATGGKRI	25.8	1.4894	Antigen
	DRB1_1301	VILQVFYFK	63.3	1.8276	Antigen
	DRB1_1301	LLLIGIIFV	63.9	1.1184	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
Q720A5	DRB1_0701	IKEFKPKMV	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	ALLMPLPVA	654.6	1.5696	Antigen
	DRB1_0101	FLGVPWWPV	721.2	2.0565	Antigen
	DRB1_0101	LMPLPVAII	929.1	1.4677	Antigen
	DRB1_0101	FYFLFYGSL	1330	1.6406	Antigen
	DRB1_0101	VALLMPLPV	1365.6	1.8132	Antigen
	DRB1_0701	FLGVPWWPV	29.7	2.0565	Antigen
	DRB1_0701	IIGAWNWLI	309.5	1.666	Antigen
Q71YI4	DRB1_0701	**SGETLSVKV**	325.2	2.4375	Antigen
	DRB1_1301	**LRVTPGIRL**	32.6	2.4375	Antigen
	DRB1_1301	FLRVTPGIR	65.4	1.2425	Antigen
Q71WT9	DRB1_0701	VSLRVGMEI	216.6	1.6096	Antigen
	DRB1_1301	IGETERRRK	37.9	1.3502	Antigen
Q720J1	DRB1_0701	IEVTPDYLM	299.3	1.7114	Antigen
Q71ZZ0	DRB1_1301	THLKTRPKK	20.2	1.3476	Antigen
	DRB1_1301	LRTHLKTRP	22.8	1.2793	Antigen
Q71XV7	DRB1_0101	FLYVVVYSL	1393.6	1.213	Antigen
	DRB1_0701	FAVEPSFSI	53.6	1.819	Antigen
	DRB1_0701	IKWAKWMFV	123.5	1.348	Antigen

Table 2 (continued)

Protein ID	Allele	Peptide	Binding affinity [nM]	VaxiJen score	Antigen/non-antigen
Q724E9	DRB1_0101	FSAGMGAEA	959.2	1.5015	Antigen
	DRB1_0701	LVEGRAIRL	269.1	1.5701	Antigen
	DRB1_1301	TKSKVRRER	13.3	1.2742	Antigen
	DRB1_1301	GQRRTRAIR	33.3	1.2488	Antigen
	DRB1_1301	LKGKQGRFR	51	1.7176	Antigen
	DRB1_1301	LKSAQGQRR	55.5	1.6836	Antigen
	DRB1_1301	EVTKSKVRR	59.3	1.1113	Antigen
	DRB1_1301	LIFNTILPK	65.3	1.134	Antigen
Q71WI0	DRB1_0101	FALHYPYEL	1003.9	1.4132	Antigen
	DRB1_0701	FALHYPYEL	319.5	1.4132	Antigen
Q71Z37	DRB1_0101	FLFAPHVHP	425	1.8183	Antigen
	DRB1_0101	IAFLFAPHV	125	1.9413	Antigen
	DRB1_0101	LYTLRPEDV	1060.8	1.3501	Antigen
Q71XV6	DRB1_0701	FSMVLSLVF	100	1.4972	Antigen
	DRB1_0701	ASRSKSNRL	302	1.1981	Antigen
	DRB1_0701	YIMALHFGI	307	1.9206	Antigen
	DRB1_0701	YALTIYTYL	308	1.1261	Antigen
	DRB1_1301	IVLLALMIF	28	1.9817	Antigen
Q724M3	DRB1_0101	FDVKMGVRI	1025.4	1.9181	Antigen
	DRB1_0701	FDVKMGVRI	320	1.9181	Antigen
	DRB1_1301	VKMGVRITI	36	1.2822	Antigen
Q71WH2	DRB1_1301	VRLNATRGR	13	1.8274	Antigen
	DRB1_1301	IKKLALKIY	69	1.2527	Antigen
Q71VQ8	DRB1_0701	IVFPLSWTI	300	1.6433	Antigen
	DRB1_1301	LLIMPLMIK	24	2.2056	Antigen
Q71ZU1	DRB1_0101	LIQMPILMA	1353.2	1.3037	Antigen
Q720A3	DRB1_0101	LHLIPVNMK	712	1.5796	Antigen
	DRB1_0101	LIGLPIRIT	1193	1.6981	Antigen
	DRB1_1301	IYKYDVRFK	53	1.8026	Antigen
Q720A7	DRB1_1301	VRVNVMGYR	20	1.4928	Antigen
	DRB1_1301	LRLSNFMLW	55	1.2577	Antigen
Q720J2	DRB1_0101	WLNMPDMTV	1064.6	1.3955	Antigen
Q71XU1	DRB1_0701	ILNFTPARI	108	1.1713	Antigen
	DRB1_0701	LNFTPARIS	248.4	1.4755	Antigen
	DRB1_1301	ILNFTPARI	54.6	1.1713	Antigen
Q71WU5	DRB1_0101	PISIISARI	1514.9	1.1708	Antigen
	DRB1_0701	PISIISARI	121.3	1.1708	Antigen
Q71YA9	DRB1_0701	**ATGTTGLRI**	122.2	2.2883	Antigen
Q724F0	DRB1_0101	FRTLRPTDG	368.9	1.165	Antigen
	DRB1_0101	LINIRPVVA	1366	1.2121	Antigen
	DRB1_0701	VEHVEAREI	78.9	1.4245	Antigen
	DRB1_1301	LRVKLRLIN	22.2	1.3688	Antigen

Table 2 (continued)

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	MLGLPFQIA	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
	DRB1_1301	ICTRNLQRR	16.9	1.1843	Antigen
Q722W7	DRB1_0101	WVMHLDAMV	1508.3	1.4715	Antigen
	DRB1_0701	IVYEVSWRY	223.4	1.2052	Antigen
	DRB1_0701	YHFYFAHAL	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
	DRB1_1301	KLSTKLKRK	36.7	1.3477	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	FWMIEPEMA	524.2	2.1476	Antigen
	DRB1_0701	**FLKIDPPIL**	101.7	2.3187	Antigen
Q71VR4	DRB1_0101	KLNLHAIYV	1297.1	1.6175	Antigen
	DRB1_1301	IEHGKRSRK	55.6	1.2977	Antigen
Q71W89	DRB1_0101	LSFLPALAL	91.8	1.5837	Antigen
	DRB1_0101	YILLPLSLI	150	1.4583	Antigen
	DRB1_0101	FSLAFNTAA	398.7	1.4513	Antigen
	DRB1_0101	ILLIPVALV	879.3	1.4451	Antigen
	DRB1_0101	FLPALALGP	996.5	1.3317	Antigen
	DRB1_0101	LILVPPLLT	1544.3	2.0559	Antigen
	DRB1_0701	LSFLPALAL	97.5	1.5837	Antigen
	DRB1_0701	LSFSLAFNT	155.2	1.688	Antigen
	DRB1_0701	LLLVLAVPL	211.1	1.53	Antigen
Q71W91	DRB1_0101	VNVLQVNLA	587.2	1.403	Antigen
Q71WP8	DRB1_0101	LEVLLPQYV	1295.8	1.246	Antigen
Q71WG3	DRB1_1301	VKGGRRFRF	39.8	1.694	Antigen
Q71Z71	DRB1_1301	ISVREKSAK	56	1.548	Antigen
Q722Y1	DRB1_0101	GVMLPLKLS	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	KVHPIGMRI	181.4	1.3023	Antigen
	DRB1_1301	IKTQVSGRL	19.6	1.16	Antigen
	DRB1_1301	MRAGAKGIK	50.5	1.27	Antigen
	DRB1_1301	LRIRDYVAK	51.4	1.181	Antigen
Q722Y9	DRB1_1301	IKLRKTQPR	34.9	1.462	Antigen
Q71WF1	DRB1_1301	VRIAPRKAR	27	1.1447	Antigen
	DRB1_1301	GRASAINKR	44	1.264	Antigen
Q71ZP6	DRB1_0101	YKLLNPTLG	86.8	1.307	Antigen
	DRB1_0101	FLNIRLKPV	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	FSLTIALLI	22.4	1.852	Antigen
	DRB1_0701	IDSTFSLTI	57.8	1.4124	Antigen
	DRB1_0701	FLNIRLKPV	62.9	1.906	Antigen
	DRB1_0701	ISWAVAIFI	72.9	1.347	Antigen
	DRB1_0701	IGSAIALNL	111.4	1.277	Antigen
	DRB1_0701	LAIVPAVII	184	1.356	Antigen
	DRB1_1301	LNIRLKPVV	30.9	2.189	Antigen
Q71WE9	DRB1_0701	IKVGNALEL	51	1.204	Antigen
	DRB1_1301	LKKKAGRNN	60.7	1.322	Antigen
	DRB1_1301	**VRHHGGGHK**	63.8	2.522	Antigen
Q71WB7	DRB1_1301	LEVKARRVG	53	1.551	Antigen
	DRB1_1301	IEVRADRRS	60.7	1.989	Antigen
	DRB1_1301	MMVDGKRGK	65.1	1.375	Antigen
Q71WH0	DRB1_1301	SYRGMRHRR	9	1.4454	Antigen
	DRB1_1301	TKNNARTRK	38.1	2.1367	Antigen
Q71XE5	DRB1_0701	FVSGLSFHV	35.1	1.487	Antigen
	DRB1_1301	KQLKIRQIR	53.8	1.389	Antigen
Q71XX1	DRB1_0101	NIDIKGRLI	1319.9	1.353	Antigen
Q71YK6	DRB1_0701	IFDVRSEHV	179.8	1.5294	Antigen
Q71WE5	DRB1_1301	MAKQKIRIR	18.8	1.2116	Antigen
	DRB1_1301	FEMRTHKRL	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	IRIRLKAYD	55.2	1.8524	Antigen
	DRB1_1301	QFEMRTHKR	68.6	1.7135	Antigen
Q71WF6	DRB1_1301	VRTKSGARR	5.6	1.944	Antigen
Q71WH1	DRB1_1301	MARKTNTRK	5.2	1.6203	Antigen
	DRB1_1301	**RKTNTRKRR**	5.5	2.5417	Antigen
	DRB1_1301	**ARKTNTRKR**	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

Table 2 (continued)

Protein ID	Allele	Peptide	Binding affinity [nM]	VaxiJen score	Antigen/non-antigen
Q725B8	DRB1_1301	GRRGGRRRK	7.1	3.0668	Antigen
	DRB1_1301	**RRGGRRRKK**	17.1	2.833	Antigen
	DRB1_1301	**GGRRGGRRR**	25.2	3.1722	Antigen
Q71WV5	DRB1_1301	VKKRSAKRA	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	VRKKRHARV	12.6	1.3471	Antigen
	DRB1_1301	RHARVRSKI	27.1	1.4108	Antigen
	DRB1_1301	KKRHARVRS	38.4	2.0868	Antigen
	DRB1_1301	RKKRHARVR	47.2	2.0804	Antigen
	DRB1_1301	NKVRKKRHA	59.3	1.1365	Antigen
Q71WE8	DRB1_1301	AGYTNKRRK	46.9	1.237	Antigen
Q71YD4	DRB1_1301	FGISRIRFR	48.6	1.1227	Antigen
Q71YN5	DRB1_1301	TVTRKRRKK	2.7	1.1019	Antigen
	DRB1_1301	GTVTRKRRK	15.8	1.2113	Antigen
	DRB1_1301	GGTVTRKRR	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_0101	LRLSIPQLT	375.6	1.2897	Antigen
	DRB1_0701	LRLSIPQLT	192.5	1.2897	Antigen
Q721Y1	DRB1_0701	LRITLNLAL	19.5	1.9824	Antigen
	DRB1_1301	ILLRITLNL	32.3	1.4731	Antigen
	DRB1_1301	LLLVAALFL	51.9	1.2854	Antigen
Q71YM9	DRB1_0101	LRNLRGKAA	476.7	1.2468	Antigen
	DRB1_0701	TVRVHAKVV	152.6	1.3034	Antigen
	DRB1_1301	VRVHAKVVE	67.1	1.564	Antigen
	DRB1_1301	RRGKVRRAK	20.2	1.3055	Antigen
	DRB1_1301	LRGKAARIK	17.4	2.0521	Antigen
Q71WG4	DRB1_1301	AKLEITLKR	51.3	1.1423	Antigen
Q71YN4	DRB1_0701	FKRTGSGKL	34.3	1.1993	Antigen
	DRB1_1301	THRGSAKRF	43.7	1.0624	Antigen
	DRB1_1301	QKQKRKLRK	46.1	1.1816	Antigen
Q71WH3	DRB1_1301	LGRTSSQRK	33.5	1.2846	Antigen
Q71ZY7	DRB1_1301	LKKYCPRLR	50.8	2.0807	Antigen
	DRB1_1301	KKYCPRLRR	61.5	1.5286	Antigen

Table 2 (continued)

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	TSKAKKRKR	23.1	1.7453	Antigen
	DRB1_1301	KAKKRKRRT	24.4	1.7483	Antigen
	DRB1_1301	RRTSKAKKR	26	1.7169	Antigen
	DRB1_1301	RKRRTHVKL	39.5	1.4259	Antigen
Q723G3	DRB1_1301	ARRTSKAKK	15.4	1.4443	Antigen
	DRB1_1301	SKAKKNKRR	29.1	1.707	Antigen
	DRB1_1301	KAKKNKRRT	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	MAPRELRER	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	**LFNLRFQLA**	1029	2.5288	Antigen
Q71YL9	DRB1_1301	MAVKIRLKR	4.3	1.4155	Antigen
	DRB1_1301	AVKIRLKRI	55.1	1.4342	Antigen
Q71YK0	DRB1_1301	**RKSRSGNKR**	40.5	2.7338	Antigen
Q71WI2	DRB1_1301	LLTRDPRMK	16.6	1.3863	Antigen
	DRB1_1301	KSSVARVRL	68.6	1.0414	Antigen
Q71VQ6	DRB1_1301	ASRRRKGRK	8.3	2.0002	Antigen
	DRB1_1301	SRRRKGRKV	12.1	1.7764	Antigen
	DRB1_1301	MSTKNGRRV	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	LSVKLRSRG	15	1.128	Antigen
	DRB1_1301	VLSVKLRSR	21.1	1.3894	Antigen

Table 2 (continued)

Protein ID	Allele	Peptide	Binding affinity [nM]	VaxiJen score	Antigen/non-antigen
Q71XL9	DRB1_0101	GIILLGFRL	330.6	1.0131	Antigen
	DRB1_0101	YFLAKLPFL	673.5	1.4522	Antigen
	DRB1_0101	FLIAALCLS	844.4	1.2298	Antigen
	DRB1_0101	FLIAMSMGG	884.2	1.1022	Antigen
	DRB1_0101	FLAKLPFLM	891	1.7779	Antigen
	DRB1_0101	FLVICAYFL	1342	2.0765	Antigen
	DRB1_0101	YFLIAMSMG	1357	1.1587	Antigen
	DRB1_0101	YGIALTFCI	1600	1.7051	Antigen
	DRB1_0701	VIYTLIYPI	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	AHAKIRERL	32.2	1.2949	Antigen
Q71Z99	DRB1_0701	PQVTVSLVF	92.9	1.1655	Antigen
	DRB1_1301	VILLKLFHV	49.4	1.5441	Antigen
Q724P3	DRB1_1301	IRCKYTKTR	22.7	2.0203	Antigen
	DRB1_1301	RCKYTKTRR	43	1.5601	Antigen
Q71ZL4	DRB1_1301	LMLDIRYRH	33.2	1.656	Antigen
	DRB1_1301	SLMLDIRYR	35.4	1.4323	Antigen
Q2N761	DRB1_0101	LLSLSPELF	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	**ALLWLFPRF**	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

by Swiss Model. 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, FLKIDPPIL, and VRHHGGGHK. CEETFGIRL showed the energy of − 6.7 kcal/mol and 6 hydrogen bonds. MKFLFPLKL showed the energy of − 6.9 kcal/mol and 2 hydrogen bonds. FLKIDPPIL 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

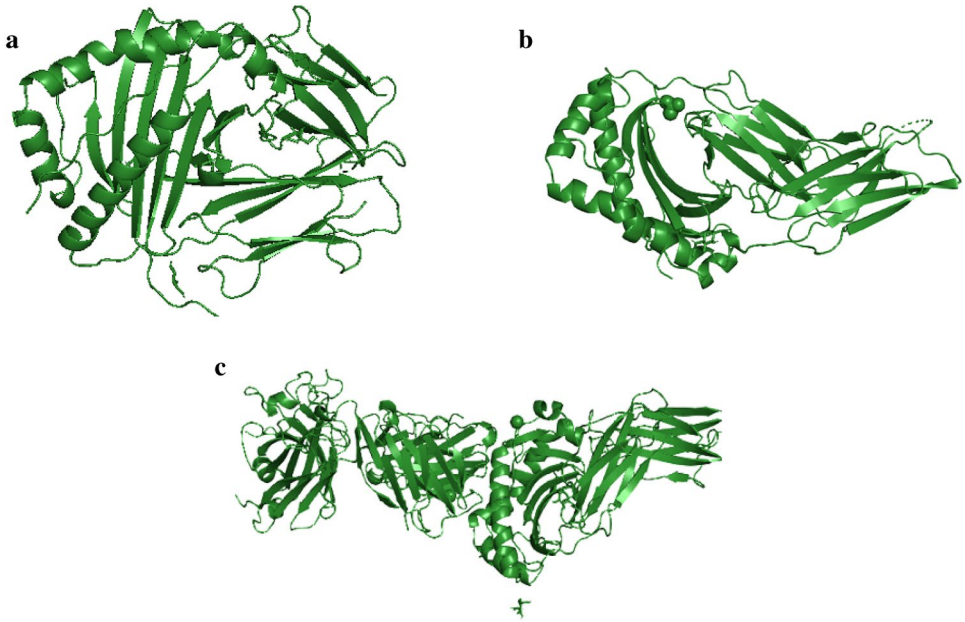

Fig. 1 Modeled structure of HLA class II alleles—**a** molecular structure of HLA DRB1_0101, **b** molecular structure of HLA DRB1_0701, **c** molecular structure of HLA DRB1_1301

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	VAAMNFRLK	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	LRVTPGIRL	DRB1_1301	− 6.3
7	ATGTTGLRI	DRB1_0701	− 6.3
8	**MKFLFPLKL**	DRB1_0101	**− 6.9**
9	MKFLFPLKL	DRB1_0701	− 6.5
10	**FLKIDPPIL**	DRB1_0101	**− 7.3**
11	FLKIDPPIL	DRB1_0701	− 6.5
12	**VRHHGGGHK**	DRB1_1301	**− 6.7**
13	RKTNTRKRR	DRB1_1301	− 5.2
14	ARKTNTRKR	DRB1_1301	− 5.7
15	RRGGRRRKK	DRB1_1301	− 5.2
16	GGRRGGRRR	DRB1_1301	− 5.9
17	LLMFTLPFY	DRB1_1301	− 6.4
18	LFNLRFQLA	DRB1_0101	− 6.5
19	RKSRSGNKR	DRB1_1301	− 5.5
20	ALLWLFPRF	DRB1_1301	− 6.3

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.

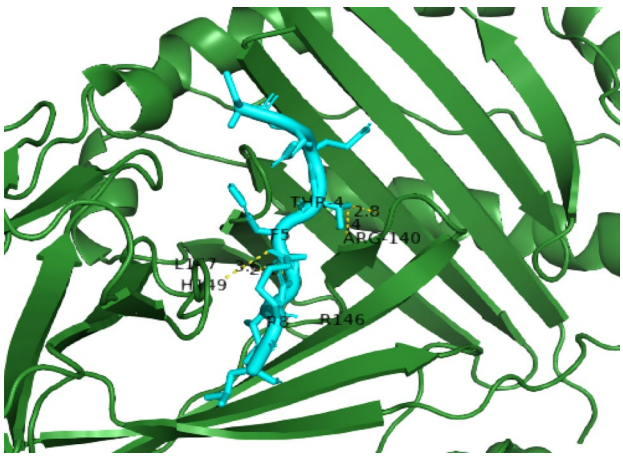

Fig. 2 This Docked result depicts the interaction analysis of epitope CEETFGIRL (represented with cyan color) with 3C5J receptor (represented with forest green color). Showing the epitope interacting with 3C5J receptor with the help of 6 hydrogen bonds (Color figure online)

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. Binding energy prediction is given in the form of IC_{50} value. Epitopes having an IC_{50} 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

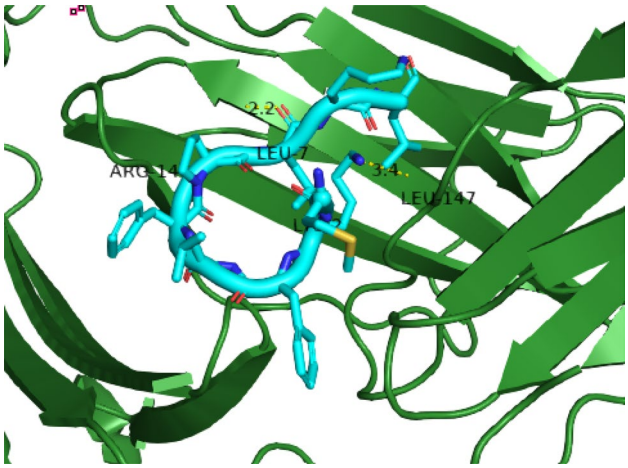

Fig. 3 This Docked result depicts the interaction analysis of epitope MKFLFPLKL (represented with cyan color) with 4AH2 receptor (represented with forest green color). Showing the epitope interacting with 4AH2 receptor with the help of which 2 hydrogen bonds (Color figure online)

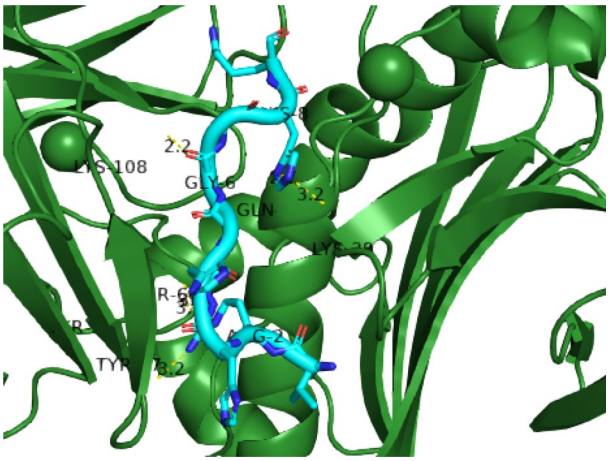

Fig. 5 This Docked result depicts the interaction analysis of epitope VRHHGGGHK (represented with cyan color) with 6CQL receptor (represented with forest green color). Showing the epitope interacting with 6cql receptor with the help of 6 hydrogen bonds (Color figure online)

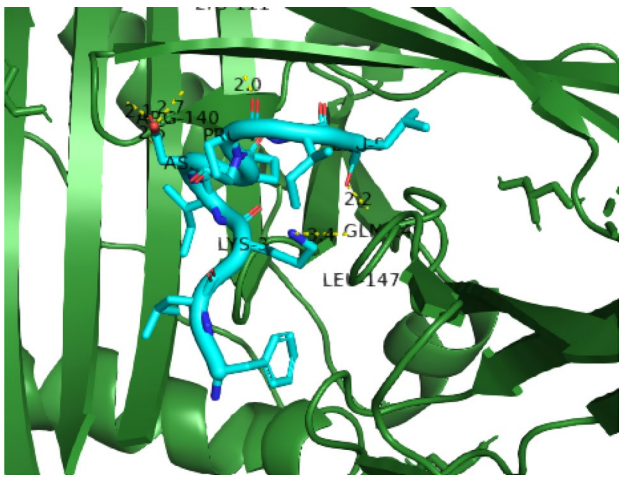

Fig. 4 This Docked result depicts the interaction analysis of epitope FLKIDPPIL (represented with cyan color) with 4AH2 receptor (represented with forest green color). Showing the epitope interacting with 4AH2 receptor with the help of 6 hydrogen bonds (Color figure online)

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

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)

screened out. 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.

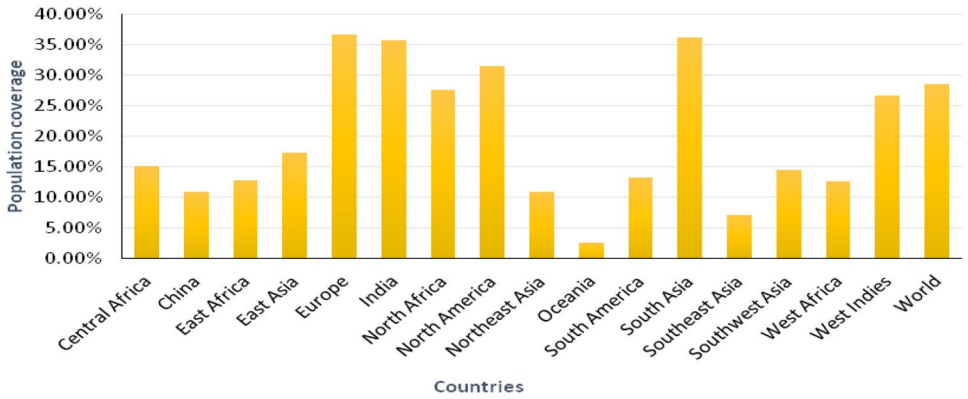

Fig. 6 Graphical representation of Population coverage for epitope MKFLFPLKL and FLKIDPPIL

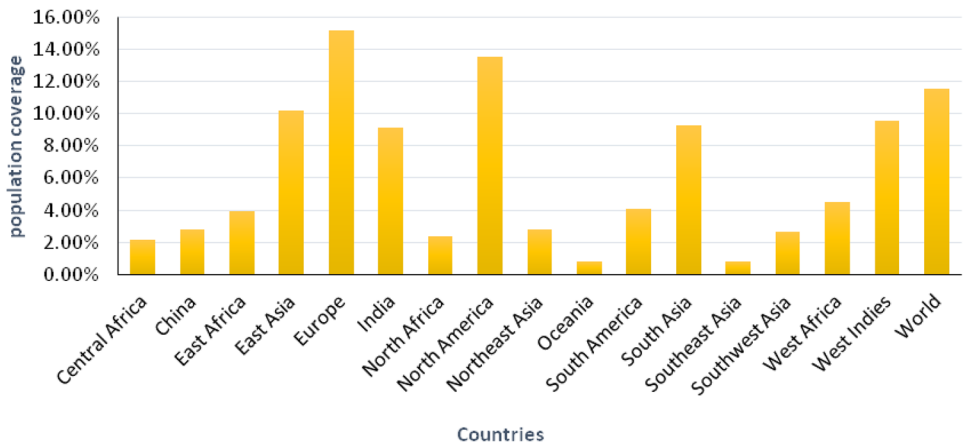

Fig. 7 Graphical representation of population coverage for epitope CEETFGIRL

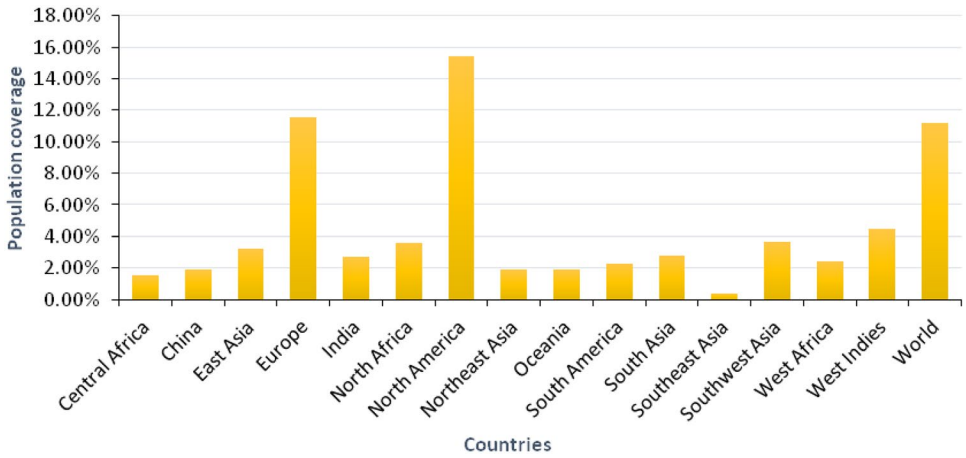

Fig. 8 Graphical representation of population coverage for epitope VRHHGGGHK

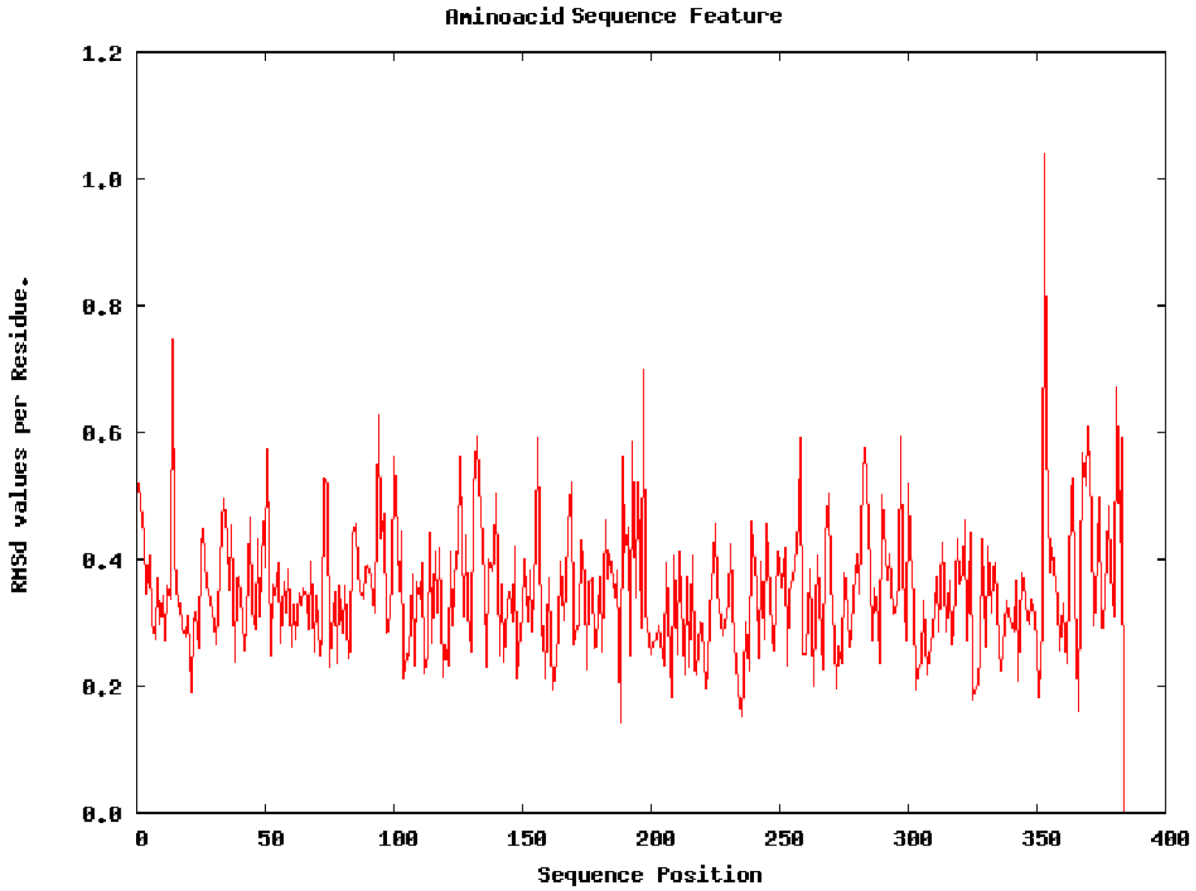

Fig. 9 Graphical representation of RMSD for epitope FLKIDPPIL with 4AH2 receptor obtained during simulation studies

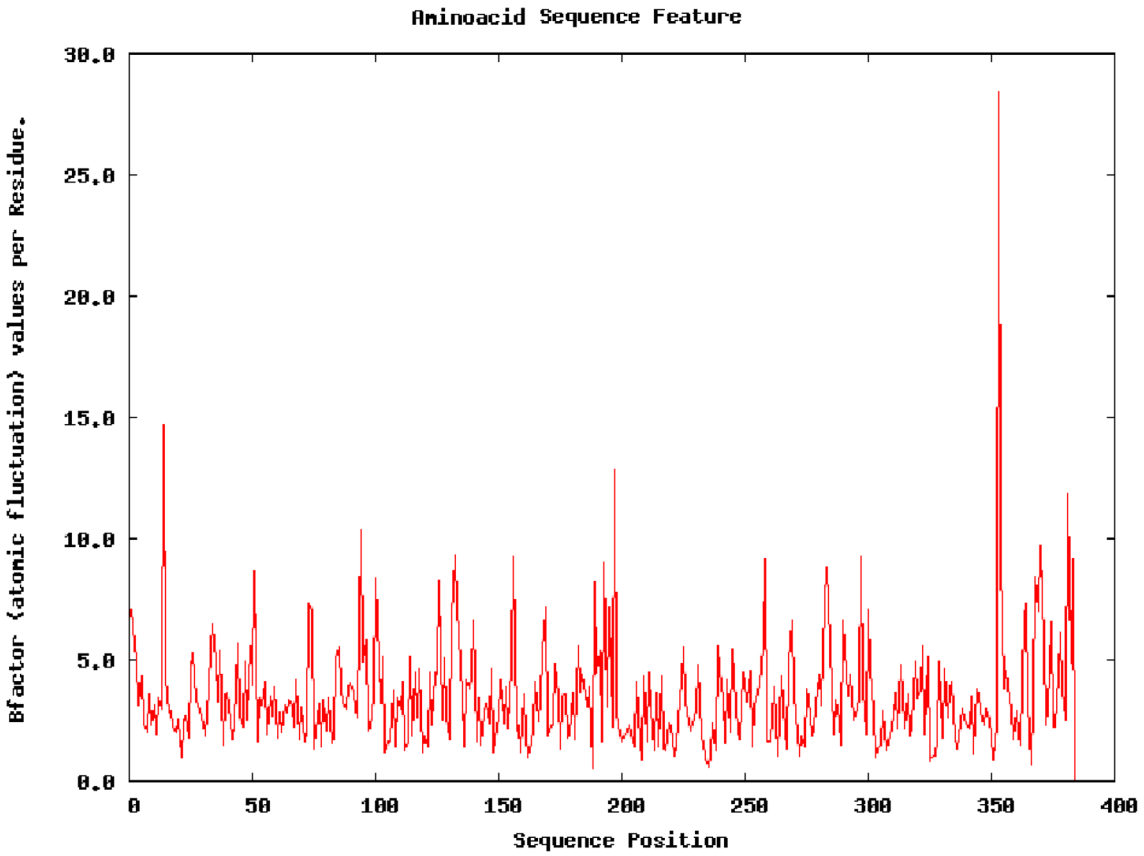

Fig. 10 Graphical representation of the B factor plot for epitope FLKIDPPIL with 4AH2 receptor obtained during simulation studies

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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