Immunoinformatics Approach in Designing Epitope-based Vaccine Against Meningitis-inducing Bacteria (*Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* Type b)

Hilyatuz Zahroh[1], Ahmad Ma'rup[2], Usman Sumo Friend Tambunan[3] and Arli Aditya Parikesit[3]

[1]Genetic Research Center, University of YARSI, Indonesia. [2]Department of Chemistry, Universitas Islam Negeri Syarif Hidayatullah, Indonesia. [3]Bioinformatics Research Group, Department of Chemistry, Faculty of Mathematics and Science, University of Indonesia, Indonesia.

ABSTRACT: Meningitis infection is one of the major threats during Hajj season in Mecca. Meningitis vaccines are available, but their uses are limited in some countries due to religious reasons. Furthermore, they only give protection to certain serogroups, not to all types of meningitis-inducing bacteria. Recently, research on epitope-based vaccines has been developed intensively. Such vaccines have potential advantages over conventional vaccines in that they are safer to use and well responded to the antibody. In this study, we developed epitope-based vaccine candidates against various meningitis-inducing bacteria, including *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b. The epitopes were selected from their protein of polysaccharide capsule. B-cell epitopes were predicted by using BCPred, while T-cell epitope for major histocompatibility complex (MHC) class I was predicted using PAProC, TAPPred, and Immune Epitope Database. Immune Epitope Database was also used to predict T-cell epitope for MHC class II. Population coverage and molecular docking simulation were predicted against previously generated epitope vaccine candidates. The best candidates for MHC class I- and class II-restricted T-cell epitopes were MQYGDKTTF, MKEQNTLEI, ECTEGEPDY, DLSIVVPIY, YPMAMMWRNASNRAI, TLQMTLLGIVPNLNK, ETSLHHIPGISNYFI, and SLLYILEKNAEMEFD, which showed 80% population coverage. The complexes of class I T-cell epitopes–HLA-C*03:03 and class II T-cell epitopes–HLA-DRB1*11:01 showed better affinity than standards as evaluated from their $\Delta G_{\text{binding}}$ value and the binding interaction between epitopes and HLA molecules. These peptide constructs may further be undergone in vitro and in vivo testings for the development of targeted vaccine against meningitis infection.

KEYWORDS: meningitis, immunoinformatics, epitope-based vaccine, epitope prediction, molecular docking

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CORRESPONDENCE: usman@ui.ac.id

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Introduction

Meningitis infection is one of the serious threats during Hajj due to its tendency to cause outbreaks and epidemics.[1] According to WHO, this disease affects more than 400 million people who live in the area of "African meningitis belt" (from Senegal to Ethiopia). More than 800,000 people in this area were infected, with a case fatality rate of 10%.[2] In Saudi Arabia, the epidemics of meningitis usually occur during or after Hajj and Umrah seasons, due to massive gathering of people in certain areas. Pattern evolution confirmed that 48% of meningitis cases were reported at the two holy cities, namely, Mecca and Medina.[2] In 2000, Indonesian pilgrims were infected by *Neisseria meningitidis* serogroup A and W135. Among 253 identified cases from Saudi Arabia, 93 cases were caused by *N. meningitidis* serogroup W135, while 60 cases were caused by *N. meningitidis* serogroup A. Statistically, there were nine cases caused by serogroup W135 and six cases caused by serogroup A per 100,000 population.[3]

Generally, meningitis can be induced by certain species of virus and bacteria. Three bacterial species, namely, *Haemophilus influenzae* (45%), *Streptococcus pneumoniae* (18%), and *N. meningitidis* (14%), are known to cause the majority of cases, where the case fatality rates vary according to the type of bacteria. The highest fatality rate is caused by *S. pneumoniae* (19%), while the case fatality rates caused by *N. meningitidis* and *H. influenzae* are 13% and 3%, respectively.[4]

The Indonesian government requests all Hajj pilgrims to be vaccinated against meningitis bacteria before departing to Mecca. Currently, the government allows the use of Meningitis vaccine only from Novartis, Menveo®. This vaccine contains polysaccharides from each of serogroups A, C, W, and Y conjugated to a mutant of diphtheria toxin, CRM197, that differs from the wild type by the substitution of one amino acid.[5,6] There have been some efforts to develop alternative vaccine candidates that could incorporate broader types of meningitis-inducing bacteria.[7–10]

Recently, novel approaches have been directed toward the rational design of B- and T-cell epitope-based vaccine, on account of the advancement of recombinant DNA technology, cell culture technique, immunoinformatics, big data projects, and rational design of antigens.[11,12] The epitope-based vaccine has several advantages over conventional vaccines, and some are moving forward to the clinical trial pipeline.[12] This next-generation vaccine has high specificity in evoking immune response, high capacity of production, and effective cost of production. Moreover, peptides consisting of epitopes are easily synthesized, purified, stored, and handled. Generally, epitope-based vaccines are also considered safer than traditional vaccines.[12]

Immunoinformatics approach has been used to develop subunit vaccine candidates against meningitis-inducing bacteria. Some previous studies generated several vaccine candidates: epitope FMILPIFNV against human leukocyte antigen (HLA) class II from ABC transporter protein of *S. pneumoniae* and epitope KGLVDDADI against HLA class I from outer membrane protein of *N. meningitidis*.[13,14] The epitopes such as FMILPIFNV and KGLVDDADI were used as standards in this study.

This study designed epitope-based meningitis vaccine in silico by using polysaccharide capsule protein of *S. pneumoniae*, *N. meningitidis* serogroup A, *N. meningitidis* serogroup W, and *H. influenzae* type b and analyzed the complex stability between predicted epitopes and HLA molecules using molecular docking approach. The designed epitopes may serve as promising candidates for the development of epitope-based vaccine against the meningitis-inducing bacteria.

Research Methodology

Tools and materials. This study was conducted in silico. The pipeline used in this study was adjusted and extended from the existing ones.[15–19] Polysaccharide proteins of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b were obtained from National Center Biotechnology information (NCBI). The 3D structure of HLA was obtained from Research Collaboratory for Structural Bioinformatics (RCSB). Online and offline software including the latest version of BCPred,[20] VaxiJen v2.0,[21] PAPRoc I,[22] TAPPRed,[23,24] Immune Epitope Database (IEDB) 3.0,[25] PEP-FOLD,[26] MOE 2009,[27] and Chimera 1.9[28] were used in this study.

Procedure.

Retrieving protein sequences from database. The sequences of polysaccharide protein of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b were searched in GenBank of NCBI (http://www.ncbi.nlm.nih.gov/). After that, antigenicity of these sequences was predicted using VaxiJen v2.0, which can be obtained at http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html.[21]

B-cell epitope prediction. The B-cell epitope was predicted using BCPred, which can be accessed at http://ailab.cs.iastate.edu/bcpreds/.[20] Antigenicity was also predicted against B-cell epitope afterward using VaxiJen.

HLA class I and class II T-cell epitope prediction from the conserved sequences. Epitopes from *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b against HLA class I were predicted using several online softwares including PAProC,[22] TAPPred,[23] and IEDB.[25] Proteasome cleavage site of consensus protein sequence was predicted using PAProC (http://www.paproc2.de/paproc1/paproc1.html). PAProC then generated peptide sequences and score of estimated strength.[29] The peptide binding to the transporter associated with antigen processing (TAP) binding was predicted using TAPPred, http://www.imtech.res.in/raghava/tappred/, which generated protein sequences, their position, and score of predicted binding affinity to each peptide sequence.[23] The antigenicity of peptide sequence generated from TAPPred prediction was analyzed using VaxiJen,[21] and the antigen-bearing sequences were used to predict epitopes that bind to HLA class I and HLA class II using IEDB analysis resource.[25] The binding character of epitopes to HLA was taken into consideration for the selection of the best epitopes. Epitopes with more number of bonds with HLA were considered as better than those with fewer bonds.[16]

The 3D structure of epitopes for HLA class I and class II. The 3D structures of the best epitopes for each HLA class I and class II were predicted using PEP-FOLD (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/)[26] and saved in.pdb format, while the 3D structures of HLA class I and class II were obtained from RCSB (www.rcsb.org).[30]

The prediction of coverage population of the selected epitopes. The human population coverage is one aspect that has to be taken into consideration in selecting the best epitopes, besides their ability to bind to HLA molecules.[16] Human coverage population for previously selected epitopes was predicted using IEDB analysis resource for population coverage calculation (http://tools.immuneepitope.org/tools/population/iedb_input).[31]

Molecular docking study of HLA–epitope interaction. The interaction between the best predicted epitopes and HLA alleles was analyzed by means of molecular docking using MOE 2009. The 3D structure of HLA, as a target molecule, and the epitopes must be prepared before docking. Preparation and docking steps were performed according to the established pipeline from previous research.[32,33]

Results

Protein sequence searching. A total of 4 polysaccharide protein sequences of *H. influenzae* type b, 3069 sequences of *S. pneumoniae*, 19 sequences of *N. meningitidis* serogroup A, and 19 sequences of *N. meningitidis* serogroup W were retrieved from NCBI. These sequences were subjected to antigenicity prediction in order to estimate the presence of antigen in their sequences. The peptide sequences having the VaxiJen value above the threshold level (≥ 0.4) were used for further analysis.

B-cell epitope prediction. B-cell epitopes from *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* were predicted using BCPred where criteria were set to have 75%

specificity, including only nonoverlapping epitopes. BCPred prediction generated peptide sequences along with their scores (Table 1). Peptides having higher scores mean that they are easily recognized by B-cell, thus having a higher probability as epitopes.[34]

Besides being recognized by B-cells, the peptide sequences must also possess antigen as evaluated by VaxiJen. The peptides with antigenic properties are necessary to raise the immune responses.[18] Not all peptides from each bacteria passed these criteria, as listed in Table 1. *H. influenzae* protein, *S. pneumoniae* protein, *N. meningitidis* serogroup A, and *N. meningitidis* serogroup W protein generated 3, 5, 12, and 8 probable epitopes, respectively, with antigen attribute. The predicted B-cell epitopes from each bacteria protein, which produced the best BCPred score, were GDKTTFKQS, DRVPEEASR, PNTRYRTPN, and WQELYKKYK.

Table 1. B-cell epitope prediction.

EPITOPE	BCpred	ANTIGENICITY
***Haemophilus influenzae* type b**		
GDKTTFKQS	0.863	Antigen
NFSKGVEPQ	0.715	Antigen
LGLIICAIA	0.704	Non
GKIWGTLSF	0.696	Non
WRNASNRAI	0.693	Antigen
Streptococcus pneumoniae		
DRVPEEASR	0.99	Antigen
QDVLEEVVS	0.99	Non
PATSPSSPN	0.99	Non
SDVTTLEEA	0.94	Antigen
TLQMTLLGI	0.92	Antigen
VVNRDQGEK	0.88	Antigen
LKLDLTPKD	0.80	Antigen
***Neisseria meningitidis* serogroup W**		
PNTRYRTPN	0.99	Antigen
ATTFSYLDG	0.97	Non
PILSNENVE	0.97	Non
DGSKDGSED	0.97	Antigen
RNTGIKNSN	0.96	Antigen
EKEVYAEDI	0.94	Antigen
PGSACNKII	0.92	Antigen
YYRQGRKDS	0.91	Antigen
VPIYNVESY	0.87	Non
LEKNAEMEF	0.86	Antigen
KYDKGSVSH	0.83	Non
IDSDDFINC	0.81	Non
YIYQDNQGT	0.75	Antigen
***Neisseria meningitidis* serogroup A**		
WQELYKKYK	0.99	Non
NANTLLEKE	0.99	Non
NSDATSTSR	0.99	Antigen
YFSAKKFAK	0.98	Non
EGEPDYLNG	0.94	Antigen
ILNNRKWRK	0.93	Antigen
EMEKKYPEE	0.93	Antigen
EISSLPYEE	0.92	Non
LNEEWNVQV	0.91	Antigen
LCILESHKE	0.91	Non
KLNNVVTLT	0.89	Non
TNISKAQSN	0.88	Antigen
QLFKEGIRN	0.86	Non
LEFCKEDKD	0.86	Non
FTWVNSEDK	0.86	Antigen
NCAPPAWLD	0.85	Non
ETSLHHIPG	0.85	Antigen
LSRDELKFA	0.80	Antigen
SLDDIAVTG	0.79	Antigen
HEEIMPQSA	0.78	Non
AWGNVNGEC	0.77	Antigen
YSNDDFLLT	0.77	Non
FFNFEYIVK	0.73	Antigen
FPLPSSFEK	0.71	Non
DPSAFFRDS	0.69	Non
NPKSVNEIW	0.68	Non

Identification of T-cell epitopes. T-cell epitopes must be recognized by T-cell receptor (TCR) in order to induce immune response. The epitopes that TCRs recognized are presented by major histocompatibility complex (MHC) molecules on the surface of cells. There are two major types of MHC protein molecules, namely, class I and class II. Cytotoxic T-lymphocyte TCRs recognize endogenous antigen presented on MHC class I, while helper T-cell and inflammatory T-cell TCRs recognize exogenous antigen presented on MHC class II.[35]

Identification of MHC class I epitope. A peptide having a proteasomal recognition site is not favorable as an epitope vaccine candidate because it will be degraded during antigen processing. The determination of proteasomal cleavage site was conducted using PAProC.[22] Fragments having ≥9 amino acid residues were used for further analysis of TAP binding preference (Table 2). TAP binding preference has significant influence in selecting T-cell epitopes because antigenic peptides must first be transported by TAP from cytosol to endoplasmic reticulum to be presented on MHC class I.[24,36] The higher the score, the higher the affinity between them and

Table 2. The result of TAP prediction.

PEPTIDE RANK	START POSITION	SEQUENCE	SCORE	PREDICTED AFFINITY	ANTIGENICITY
***Haemophilus influenzae* type b**					
34	1	MQYGDKTTF	6.05	High	Antigen
70	244	DLALLLLGL	4.71	Intermediate	Antigen
91	167	IICAIAQQF	4.18	Intermediate	Non
167	245	LALLLLGLV	2.37	Low or undetectable	Non
193	189	LLPISGAFF	1.73	Low or undetectable	Non
204	166	LIICAIAQQ	1.39	Low or undetectable	Non
245	243	SDLALLLLG	−0.32	Low or undetectable	Non
Streptococcus pneumoniae					
82	1	MKEQNTLEI	3.13	Intermediate	Antigen
99	202	DTRVKRPKD	2.42	Low or undetectable	Non
149	222	IVPNLNKLK	1.42	Low or undetectable	Non
161	201	LDTRVKRPK	1.07	Low or undetectable	Antigen
***Neisseria meningitidis* serogroup A**					
23	66	RKQDMLIPI	6.86	High	Non
39	65	NRKQDMLIP	6.31	High	Non
76	245	SEDKNWQEL	5.11	Intermediate	Non
137	110	LESHKEDFL	4.13	Intermediate	Non
252	68	QDMLIPINF	2.76	Low or undetectable	Antigen
313	67	KQDMLIPIN	2.15	Low or undetectable	Non
432	387	ECTEGEPDY	0.69	Low or undetectable	Antigen
486	386	GECTEGEPD	−0.50	Low or undetectable	Antigen
520	385	NGECTEGEP	−1.52	Low or undetectable	Antigen
***Neisseria meningitidis* serogroup W**					
96	4	SIVVPIYNV	3.89	Intermediate	Antigen
123	21	SSIEPILSN	3.22	Intermediate	Non
160	2	DLSIVVPIY	2.58	Low or undetectable	Antigen
226	3	LSIVVPIYN	1.45	Low or undetectable	Non
242	1	MDLSIVVPI	0.88	Low or undetectable	Antigen

the higher the possibility for the epitope to be transported by TAP. In our result, we found that despite having high affinity toward TAP, several peptide sequences failed to have antigenicity, as calculated using VaxiJen.

The peptides from *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* serogroup A and W that passed proteasomal cleavage prediction, TAP binding efficiency, and antigenicity prediction were used for the prediction of HLA class I binding using IEDB resource. They are as follows: DLALLLLGL and MQYGDKTTF from *H. influenzae* type b; LDTRVKRPK and MKEQNTLEI from *S. pneumoniae*; ECTEGEPDY, GECTEGEPD, NGECTEGEP, and QDMLIPINF from *N. meningitidis* serogroup A; and SIVVPIYNV, DLSIVVPIY, and MDLSIVVPI from *N. meningitidis* serogroup W (Table 3).

The analysis in stabilized matrix method-based IEDB MHC I prediction tool retrieved several possible MHC I alleles that could interact well with the epitopes from four bacteria (Table 3). Each predicted MHC I allele was given an IC_{50} value that indicates affinity between epitope and MHC I molecule. A lower IC_{50} value indicates higher affinity toward MHC molecules. A peptide showing an IC_{50} value lower than 50, 500, and 5000 nM is associated with high affinity, intermediate affinity, and low affinity toward MHC class I molecule, respectively. Moreover, a peptide is categorized as a binder if it has IC_{50} lower than 500 nM and is categorized as a nonbinder if IC_{50} is equal to or more than 500 nM.[37] In this study, the peptides were selected if they possess antigenicity, IC_{50} value lower than 500 nM, and more than 5 bonds with HLA class I.[16] Our study identified four antigenic peptides (MQYGDKTTF, MKEQNTLEI, ECTEGEPDY, and DLSIVVPIY) as candidates of T-cell epitope for MHC class I that could interact with HLA-B*15:02, HLA-C*03:03, and HLA-C*14:02 (Table 3). Of these three types mentioned, HLA-C*03:03 has the best interaction with the selected epitope candidates. Therefore, HLA-C*03:03 structure was chosen as a model for molecular docking study of HLA peptides.

Table 3. Candidates of T-cell epitope for MHC class I.

***EPITOPE* INTERACTION WITH HLA CLASS I (IC$_{50}$)**
***Haemophilus influenzae* type b**
DLALLLLGL HLA-B*15:02(41.04), HLA-C*12:03(196.43), HLA-C*14:02(280.94), HLA-C*03:03(329.31), HLA-A*02:01(492.16)
MQYGDKTTF HLA-C*03:03(13.23), HLA-B*15:01(23.07), HLA-C*12:03(54.60), HLA-A*32:01(59.83), HLA-A*02:06(142.74), HLA-C*14:02(165.05), HLA-B*48:01(198.88), HLA-B*35:01(239.65), HLA-B*15:02(260.75), HLA-B*18:01(409.30), HLA-B*39:01(438.93), HLA-B*53:01(442.47)
Streptococcus pneumoniae
LDTRVKRPK HLA-C*03:03(19.84), HLA-C*12:03(49.91), HLA-C*14:02(109.05)
MKEQNTLEI HLA-C*12:03(29.19), HLA-C*05:01(221.14), HLA-C*14:02(243.01), HLA-C*03:03(294.18), HLA-B*15:02(387.46)
***Neisseria meningitidis* serogroup A**
ECTEGEPDY HLA-C*12:03(34.29), HLA-C*03:03(35.78), HLA-C*14:02(148.46), HLA-C*07:02(275.53), HLA-B*15:02(279.40), HLA-B*35:01(296.40)
GECTEGEPD HLA-C*12:03(34.06), HLA-C*03:03(53.90)
NGECTEGEP HLA-C*12:03(26.38), HLA-C*03:03(90.70), HLA-C*14:02(90.91), HLA-C*07:02(191.94), HLA-C*05:01(280.98) QDMLIPINF HLA-C*12:03(54.85), HLA-C*14:02(61.18), HLA-B*15:02(309.19), HLA-C*03:03(336.98)
***Neisseria meningitidis* serogroup W**
SIVVPIYNV HLA-C*12:03(15.46), HLA-A*68:02(66.46), HLA-C*15:02(141.72), HLA-A*02:06(172.01), HLA-C*03:03(264.61), HLA-A*02:01(264.92)
DLSIVVPIY HLA-C*12:03(48.00), HLA-C*07:02(196.87), HLA-C*03:03(197.97), HLA-C*14:02(205.88), HLA-B*15:02(298.01), HLA-A*30:02(319.49), HLA-B*18:01(431.56), HLA-A*29:02(433.27), HLA-C*05:01(434.18)
MDLSIVVPI HLA-C*03:03(17.40), HLA-C*12:03(48.00), HLA-A*32:01(61.26), HLA-A*32:01(90.56), HLA-C*14:02(95.20), HLA-B*53:01(387.15), HLA-B*40:02(389.82), HLA-A*68:02(406.06).

Identification of MHC class II epitope. MHC class II molecules present antigenic peptides to stimulate cellular and humoral immunity through the actions of helper T-lymphocytes. Identification of MHC class II-restricted epitope is very important in designing epitope-based vaccine.

There are four T-cell epitopes, namely, YPMAMMWRNASNRAI, TLQMTLLGIVPNLNK, ETSLHHIPGISNYFI, and SLLYILEKNAEMEFD, generated for MHC class II from IEDB prediction. Each of these epitopes similarly binds to three types of HLA class II, HLA-DRB1*01:01, HLA-DRB1*04:04, and HLA-DRB1*11:01. However, HLA-DRB1*11:01 has the strongest affinity with the predicted epitopes. Therefore, this type of HLA will be used as a model in molecular docking simulation to predict the strength of association between HLA and epitopes (Table 4).

Coverage population prediction of class I and class II epitopes. An epitope will evoke a response only in individuals that express an MHC molecule capable of binding that particular epitope. However, human MHC (HLA) alleles are highly polymorphic and different types of HLA are expressed differently in the different population. Therefore, ensuring broad population coverage by selecting epitopes with different HLA binding specificities is an important consideration in designing epitope-based vaccine.[31] An epitope is said to show good coverage if its value is approaching 100% or close to 100%.[16] In this study, the average population coverage is 80%. Maximum coverage (94%) was found in the population of East Asia and North America, followed by Europe (93%). On the opposite side, the lowest coverage was showed in the population of Central America. While for Indonesian population, the selected epitopes showed more than half population coverage (Table 5).

Molecular docking. A binding interaction between epitopes and HLA alleles was assessed using MOE. The 3D structures of epitopes were predicted using PEP-FOLD[26] and prepared using MOE, which includes wash, partial charge, and energy minimization. MHC I-restricted epitope and HLA C*03:03 formed a stable HLA–peptide complex with lower $\Delta G_{\text{binding}}$ than standards (KGLVDDADI), as presented in Table 6. The more negative $\Delta G_{\text{binding}}$ value, the stronger the interaction between the epitope and HLA. Apart from the $\Delta G_{\text{binding}}$ value, the interaction between epitope and HLA C*03:03 can also be studied by analyzing the hydrogen bond between them. Table 6 showed that eight hydrogen bonds were present in MQYGDKTTF–HLA class I, DLSIVVPIY–HLA class I, and standard (KGLVDDADI)–HLA class I complexes, which involved six amino acid residues, while MKEQNTLEI–HLA and ECTEGEPDY–HLA complexes formed 13 and 14 hydrogen bonds, respectively. Despite the same number of hydrogen bonds in two epitope–HLA class I complexes and standard–HLA class I complex, the $\Delta G_{\text{binding}}$ value for each of the complexes is different. This is due to the inequality of hydrogen bonds, which depends on the atom distances and angles. Moreover, the estimation of $\Delta G_{\text{binding}}$ also takes into account the contribution from other noncovalent interactions such as electrostatic solvation, hydrophobic interaction, rotational entropy, and translational entropy.[38,39]

Table 4. Candidates of T-cell epitope for MHC class II.

EPITOPE PREDICTION	INTERACTION WITH HLA CLASS II (IC$_{50}$)	ANTIGENICITY
***Haemophilus influenzae* type b**		
YGDKTTFKQSLAIQG	HLA-DRB1*09:01(55.00), HLA-DRB1*01:01(60.00), HLA-DRB5*01:01(69.00), HLA-DRB1*07:01 (102.00), HLA-DRB1*04:04(147.00), HLA-DRB1*11:01(163.00), HLA-DRB1*04:05(178.00)	Antigen
GDKTTFKQSLAIQGR	HLA-DRB1*09:01(55.00), HLA-DRB1*01:01(55.00), HLA-DRB5*01:01(65.00), HLA-DRB1*07:01(102.00), HLA-DRB1*04:04(147.00), HLA-DRB1*11:01(158.00), HLA-DRB1*04:05(176.00)	Antigen
GLVMVKNFSKGVEPQ	HLA-DRB1*13:02(35.00), HLA-DRB5*01:01(86.00), HLA-DRB1*01:01(143.00), HLA-DRB1*15:01(262.00), HLA-DRB1*07:01(266.00), HLA-DRB1*11:01(305.00), HLA-DRB1*09:01(470.00)	Non
YPMAMMWRNASNRAI	HLA-DRB1*01:01(83.00), HLA-DRB1*11:01(116.00), HLA-DRB1*07:01(117.00), HLA-DRB1*13:02(127.00), HLA-DRB5*01:01(235.00), HLA-DRB1*04:01(317.00), HLA-DRB1*04:04(349.00), HLA-DRB1*09:01(373.00)	Antigen
MAMMWRNASNRAIGS	HLA-DRB1*01:01(87.00), HLA-DRB1*07:01(124.00), HLA-DRB1*13:02(129.00), HLA-DRB1*11:01(188.00), HLA-DRB5*01:01(239.00), HLA-DRB1*04:01(320.00), HLA-DRB1*09:01(392.00)	Antigen
PMAMMWRNASNRAIG	HLA-DRB1*01:01(88.00), HLA-DRB1*07:01(124.00), HLA-DRB1*13:02(130.00), HLA-DRB1*11:01(189.00), HLA-DRB5*01:01(244.00), HLA-DRB1*04:01(326.00), HLA-DRB1*09:01(395.00), HLA-DRB1*04:04(473.00)	Antigen
AMMWRNASNRAIGSI	HLA-DRB1*01:01(88.00), HLA-DRB1*07:01(124.00), HLA-DRB1*13:02(130.00), HLA-DRB1*11:01(189.00), HLA-DRB5*01:01(244.00), HLA-DRB1*04:01(326.00), HLA-DRB1*09:01(395.00)	Non
MMWRNASNRAIGSIS	HLA-DRB1*01:01(106.00), HLA-DRB1*07:01(119.00), HLA-DRB1*13:02(156.00), HLA-DRB1*11:01(218.00), HLA-DRB5*01:01(308.00), HLA-DRB1*04:01(360.00), HLA-DRB1*09:01(402.00)	Non
Streptococcus pneumoniae		
ISITRVSDVTTLEEA	HLA-DRB1*07:01(243.00), HLA-DRB1*01:01(302.00), HLA-DRB1*08:02(403.00), HLA-DRB1*04:04(457.00), HLA-DRB1*04:01(499.00)	Non
DTLQMTLLGIVPNLN	HLA-DRB1*04:04(10.00), HLA-DRB1*01:01(60.00), HLA-DRB1*04:05(79.00), HLA-DRB1*04:01(182.00), HLA-DRB1*07:01(269.00), HLA-DRB1*15:01(290.00), HLA-DRB4*01:01(333.00), HLA-DRB5*01:01(364.00)	Antigen
TLQMTLLGIVPNLNK	HLA-DRB1*04:04(10.00), HLA-DRB1*01:01(59.00), HLA-DRB1*04:05(78.00), HLA-DRB1*04:01(163.00), HLA-DRB5*01:01(163.00), HLA-DRB1*15:01(252.00), HLA-DRB4*01:01(342.00), HLA-DRB1*08:02(378.00), HLA-DRB1*07:01(465.00), HLA-DRB1*11:01(473.00)	Antigen
***Neisseria meningitidis* serogroup A**		
HIHKTNISKAQSNIS	HLA-DRB1*01:01(171.00), HLA-DRB1*07:01(200.00), HLA-DRB1*04:01(312.00), HLA-DRB4*01:01(481.00), HLA-DRB1*13:02(492.00)	Antigen
ETSLHHIPGISNYFI	HLA-DRB1*01:01(98.00), HLA-DRB1*04:04(158.00), HLA-DRB1*15:01(205.00), HLA-DRB1*04:05(258.00), HLA-DRB1*11:01(306.00), HLA-DRB1*07:01(308.00), HLA-DRB1*08:02(498.00)	Antigen
IETSLHHIPGISNYF	HLA-DRB1*01:01(134.00), HLA-DRB1*04:04(242.00), HLA-DRB1*04:05(277.00), HLA-DRB1*11:01(319.00), HLA-DRB1*07:01(491.00)	Non
NKFRSLDDIAVTGYL	HLA-DRB1*01:01(29.00), HLA-DRB1*04:01(201.00), HLA-DRB1*09:01(219.00), HLA-DRB1*04:05(325.00), HLA-DRB1*04:04 (440.00)	Antigen
LHNKFRSLDDIAVTG	HLA-DRB1*01:01(30.00), HLA-DRB1*04:01(203.00), HLA-DRB1*09:01(230.00), HLA-DRB1*04:05(303.00), HLA-DRB1*04:04(438.00)	Antigen
FFNFEYIVKKLNNQN	HLA-DRB1*11:01(18.00), HLA-DRB5*01:01(68.00), HLA-DRB1*04:04(207.00), HLA-DRB1*04:05(230.00), HLA-DRB1*01:01(323.00), HLA-DRB1*08:02(500.00)	Antigen
YKPDFNSDATSTSRF	HLA-DRB1*04:01(19.00), HLA-DRB1*04:05(218.00), HLA-DRB1*01:01(236.00), HLA-DRB3*01:01(390.00), HLA-DRB1*07:01(429.00), HLA-DRB1*04:04(499.00)	Antigen
KPDFNSDATSTSRFL	HLA-DRB1*04:01(19.00), HLA-DRB1*04:05(206.00), HLA-DRB1*01:01(207.00), HLA-DRB1*07:01(295.00), HLA-DRB3*01:01(461.00)	Antigen
EGEPDYLNGARNANT	HLA-DRB1*01:01(100.00), HLA-DRB1*04:04(124.00), HLA-DRB1*04:01(256.00), HLA-DRB1*04:05(355.00)	Antigen
MFILNNRKWRKLKRD	HLA-DRB1*11:01(81.00), HLA-DRB1*03:01(142.00), HLA-DRB5*01:01(206.00), HLA-DRB1*13:02(373.00).	Non

Table 4. (*Continued*)

EPITOPE PREDICTION	INTERACTION WITH HLA CLASS II (IC_{50})	ANTIGENICITY
***Neisseria meningitidis* serogroup A**		
ARNTGIKNSNGKYIV	HLA-DRB1*13:02(11.00), HLA-DRB1*07:01(99.00), HLA-DRB1*09:01(257.00), HLA-DRB5*01:01(283.00), HLA-DRB1*01:01(474.00)	Antigen
RNTGIKNSNGKYIVF	HLA-DRB1*13:02(11.00), HLA-DRB1*07:01(95.00), HLA- DRB1*09:01(248.00), HLA-DRB5*01:01(278.00), HLA-DRB1*01:01(483.00)	Antigen
EARNTGIKNSNGKYI	HLA-DRB1*13:02(12.00), HLA-DRB1*07:01(105.00), HLA-DRB1*09:01(266.00), HLA-DRB5*01:01(295.00)	Antigen
SLLYILEKNAEMEFD	HLA-DRB1*01:01(64.00), HLA-DRB1*04:01(123.00), HLA-DRB5*01:01(133.00), HLA-DRB1*04:04(161.00), HLA-DRB1*11:01(264.00), HLA-DRB1*04:05 (310.00)	Antigen
LLYILEKNAEMEFDR	HLA-DRB1*01:01(70.00), HLA-DRB1*04:01(128.00), HLA-DRB5*01:01(136.00), HLA-DRB1*04:04(172.00), HLA-DRB1*04:05(404.00)	Non
KSLLYILEKNAEMEF	HLA-DRB1*01:01(53.00), HLA-DRB1*04:01(118.00), HLA-DRB5*01:01(130.00), HLA-DRB1*04:04(147.00), HLA-DRB1*11:01(220.00), HLA-DRB1*04:05(237.00), HLA-DRB1*15:01(293.00), HLA-DRB1*12:01(392.00)	Non

The same result was observed for the molecular docking simulation between MHC class II-restricted epitope and HLA-DRB1*11:01 (Table 7). The complex between selected epitopes and HLA class II showed more negative free energy of binding than the standard–HLA class II complex. The ETSLHHIPGISNYFI–HLA-DRB1*11:01 complex has the lowest $\Delta G_{\text{binding}}$ value of all complexes (−60.16 kcal/mol). The most favored binding orientation between each epitope and HLA class II molecule is displayed in Table 7.

Discussion

The main objective of epitope prediction is to design a molecule that can replace an antigen in the process of eliciting a relevant immune response. Designed molecules are favorable to use in vaccine production because they are cost-effective and noninfectious in contrast to whole pathogen organisms, which might possess risks to researchers or experimental subjects (animals and humans). This study incorporates immunoinformatics approach to reducing time- and cost-consuming hit and trial sets of wet laboratory experiments. This approach is used for the prediction of antigenic determinants in the capsular protein sequence of *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* serogroup A and W.

Table 5. Prediction of population coverage.

POPULATION	COVERAGE (%)
East Asia	94
Northeast Asia	84
South Asia	85
Southeast Asia	83
Southwest Asia	67
Europe	93
East Africa	78
West Africa	83
Indonesia	65
Central Africa	78
North Africa	84
South Africa	63
West Indies	81
North America	94
Central America	53
South America	81
Oceania	75
Average	**80**

According to the prediction result of IEDB and molecular docking study, the peptides that passed several criteria of probable epitope such as possessing antigenicity, binder attribute, and good affinity with HLA molecules are MQYGDKTTF, MKEQNTLEI, ECTEGEPDY, and DLSIVVPIY for MHC class I and YPMAMMWRNASNRAI, TLQMTLLGIVPNLNK, ETSLHHIPGISNYFI, and SLLYILEKNAEMEFD for MHC class II. These peptides also passed proteasomal cleavage and TAP binding efficiency prediction, which are of main concerns in designing good epitopes for vaccine candidates. Molecular docking study has been widely used in computer-aided drug design. However, it is now applied to investigate the epitope candidates that could bind MHC class I and class II molecules. Computational immunology is now considered to contribute to vaccine design in the way computational chemistry contributes to drug design.[35] The algorithms for epitopes identification served by IEDB and molecular docking study have increased the overall efficiency in epitope discovery for vaccine research.

These epitopes also showed good population coverage (80% in average) and reached above average values in East Asia, North America, and Europe population. The high value of population coverage is needed to minimize the complexity of putting different epitopes in the development of vaccine.[31] The predicted epitopes can be synthesized for further in vitro and in vivo assays.

Table 6. Molecular docking simulation of MHC class I-restricted epitope with HLA-C*03:03.

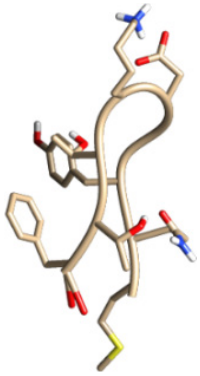
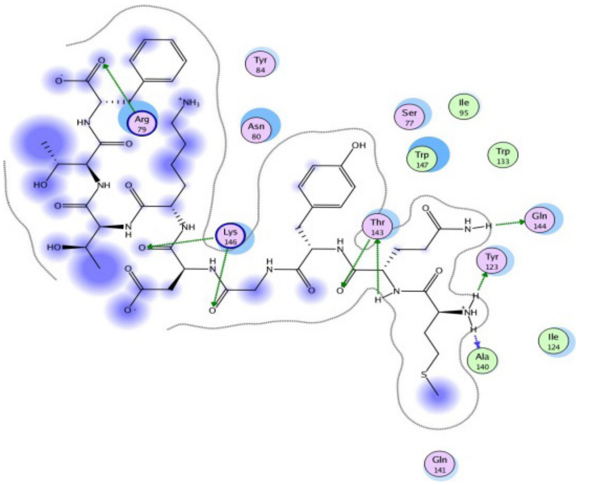
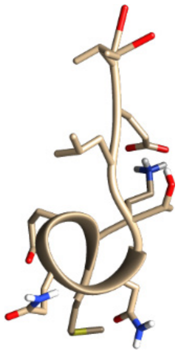
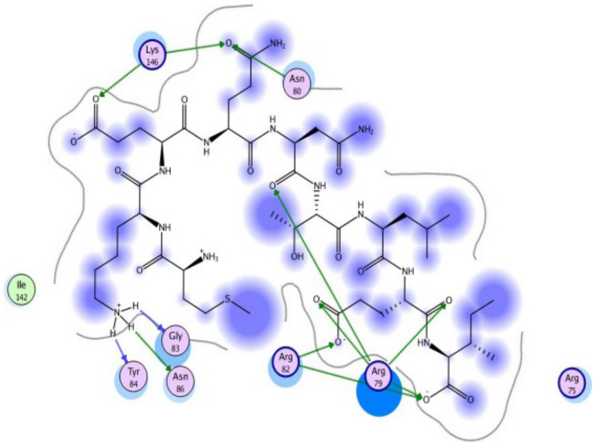
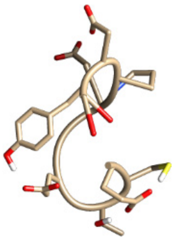
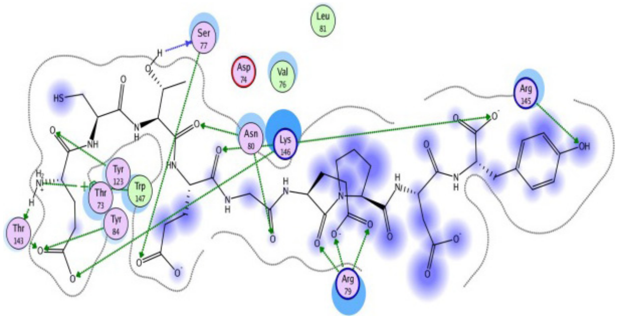
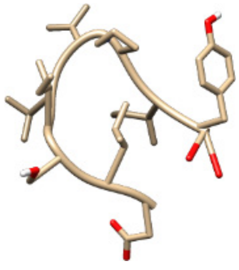
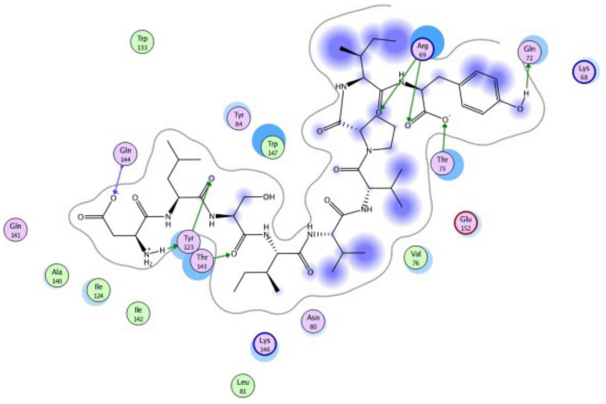
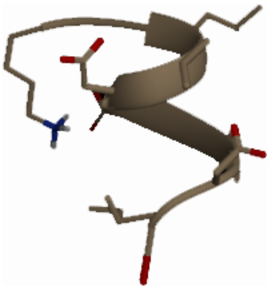
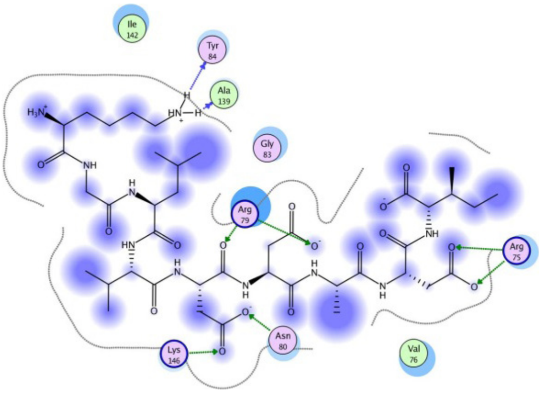
FREE ENERGY AND 3D EPITOPE	EPITOPE INTERACTION WITH HLA C*03:03
MQYGDKTTF –14.43 kcal/mol 	
MKEQNTLEI –17.46 kcal/mol 	
ECTEGEPDY –16.28 kcal/mol 	
DLSIVVPIY –16.88 kcal/mol 	

Table 6. (*Continued*)

FREE ENERGY AND 3D EPITOPE	EPITOPE INTERACTION WITH HLA C*03:03
KGLVDDADI –13.14 kcal/mol 	

Notes:

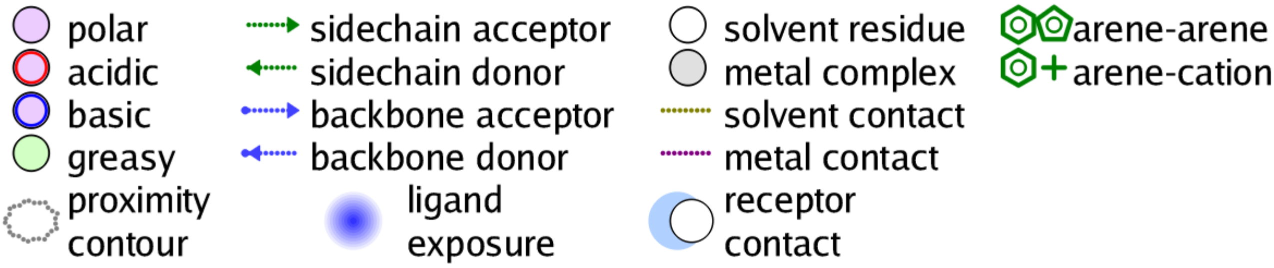
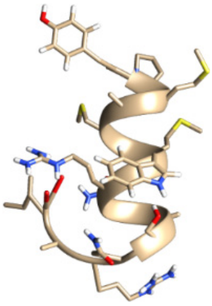
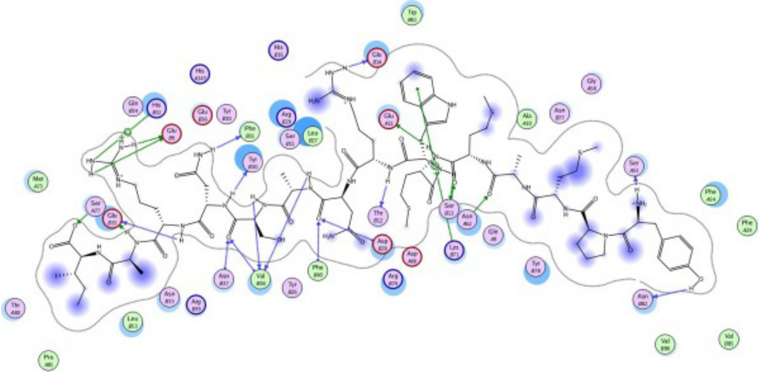
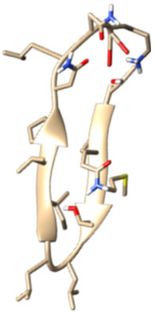
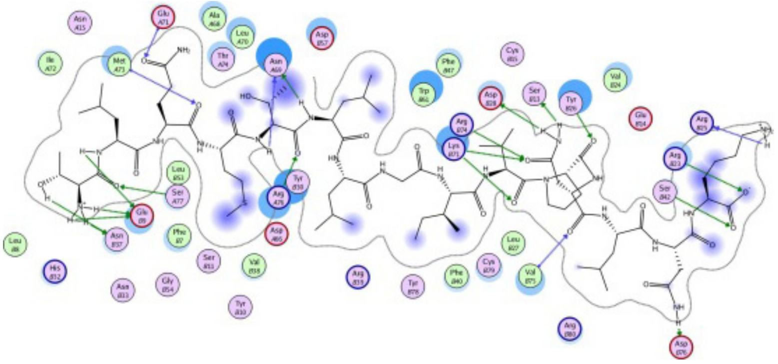
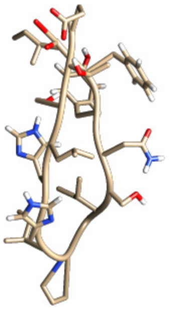
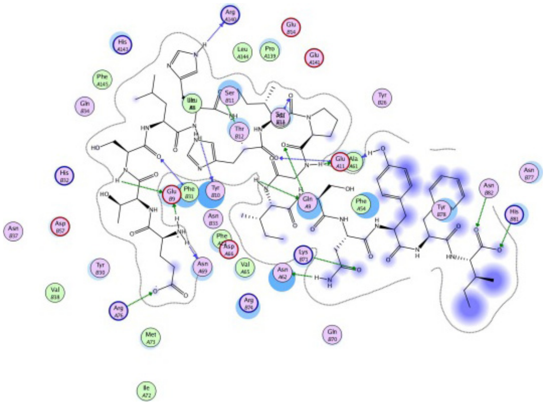
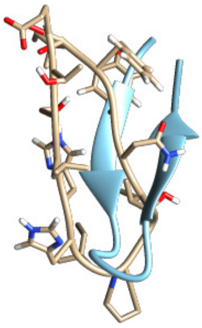
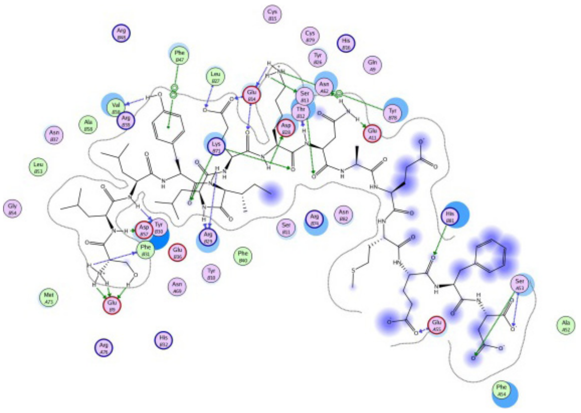
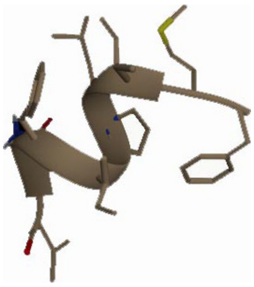
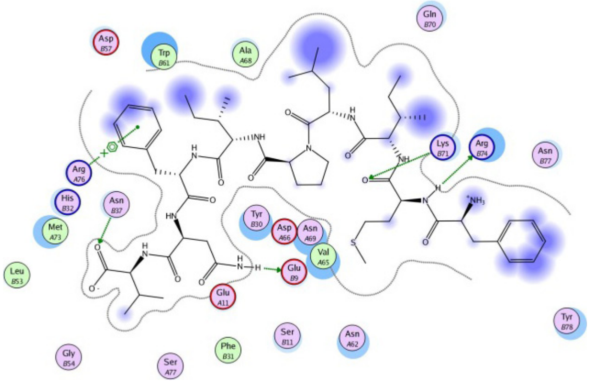


Table 7. Molecular docking simulation of MHC class II-restricted epitope with HLA-DRB1*11:01.

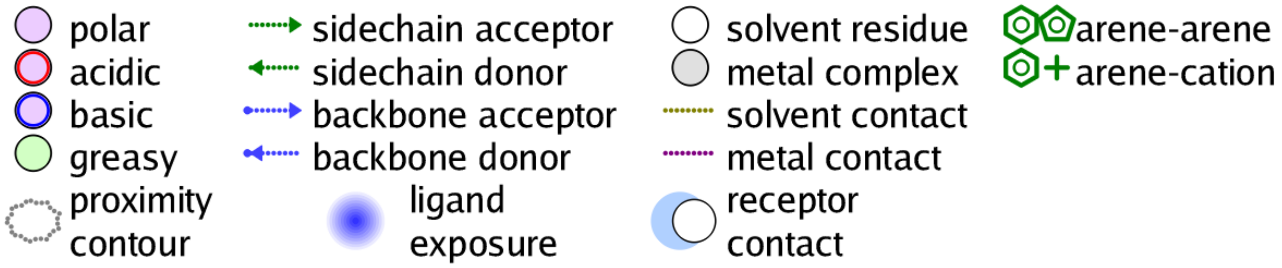
FREE ENERGY AND 3D EPITOPE	EPITOPE INTERACTION WITH HLA DRB1*11:01
YPMAMMWRNASNRAI –30.54 kcal/mol 	
TLQMTLLGIVPNLNK –25.61 kcal/mol 	

(*Continued*)

Table 7. (*Continued*)

FREE ENERGY AND 3D EPITOPE	EPITOPE INTERACTION WITH HLA DRB1*11:01
ETSLHHIPGISNYFI –60.16 kcal/mol 	
SLLYILEKNAEMEFD –25.55 kcal/mol 	
FMILPIFNV –24.76 kcal/mol 	

Notes:



Conclusion

We have predicted numerous antigenic peptides from the capsular protein sequence of *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* serogroup A and W, which would be beneficial for effective vaccine development against meningococcal diseases. Results indicated that MQYGDKTTF, MKEQNTLEI, ECTEGEPDY, and DLSIVVPIY are potential vaccine candidates that have considerable binding with MHC class I alleles, while YPMAMMWRNASNRAI, TLQMTLLGIVPNLNK, ETSLHHIPGISNYFI, and SLLYILEKNAEMEFD are the potential candidates for MHC class II-restricted T-cell epitopes. These epitopes also had low energy minimization values that favored the stability of the epitope–MHC allele complex. However, experiments using model animals should be performed to verify their suitability to be included in a vaccine formulation against meningococcal diseases.

Abbreviations

MHC, major histocompatibility complex; HLA, human leukocyte antigen; DRB, antigen D-related beta chain; RCSB, Research Collaboratory for Structural Bioinformatics.

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Author Contributions

Supervised this research: USFT and HZ. Worked on the technical details: AM. Gave technical assistance to the whole process: AAP. All the authors are responsible for writing this manuscript.

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