In Silico Vaccine Design for Multidrug-Resistant Staphylococcus Aureus Clumping Factor A (ClfA)

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Abstract-*Staphylococcus aureus* a facultative anaerobic multidrug-resistant bacterium can cause a range of illnesses, from minor skin infections, such as pimples, boils, impetigo, folliculitis, cellulitis, carbuncles, scalded skin syndrome, and abscesses and life-threatening diseases such as meningitis, pneumonia, bacteremia, sepsis, osteomyelitis, endocarditis and toxic shock syndrome. Pathogenic strains often promote infections by producing virulence factors and the expression of cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. In spite of immense research and development, not much progress has been made with regard to an epitope based vaccine and till date there is no approved vaccine for *S. aureus*. This study aims to analyze and predict the possibility of designing a vaccine that could make humans immune to *S. aureus*. The surface protein ClfA is highly antigenic among the virulence factors of *S. aureus* which act as an adhesin often essential for infection was collected from a protein database and *in silico* tools were used to predict the T-cell epitopes by NetCTL 1.2 and B-cell epitopes by Bepipred from IEDB (Immune Epitope Database). Further, MHC Class I and Class II binding peptides were predicted using TepiTool from IEDB analysis resource. The peptide KPNTDSNAL was found as the most potential B-cell and T-cell epitope. The epitope was further tested for binding against the HLA molecule by computational docking techniques to verify the HLA and epitope interaction. However, the *in silico* designed epitope-based peptide vaccine against *S. aureus* need to be validated by *in vitro* and *in vivo* experiments.

Keywords-*In silico; vaccine; multidrug-resistant; Staphylococcus sureus; clumping factor ClfA.*

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I. INTRODUCTION

Bacterial pathogens have developed a variety of factors to colonize and invade human organs, despite the presence of multiple host defence mechanisms. Bacteria have developed a very large group of molecular strategies allowing them to target and adhere to host cells. Pili, which are polymeric hair-like organelles protruding from the surface of bacteria, involved in the binding of bacteria to host cells [1, 2].

Apart from pili, bacteria possesses a group of bacterial surface factors with adhesive properties have been depicted. These bacterial adhesins identify various classes of host molecules including transmembrane proteins such as cadherins or integrins, or components of the extracellular matrix such as collagen, fibronectin, elastin or laminin [3, 4].

The majority of bacterial pathogens make use of specific adhesion to host cells as their main virulence factor. A large number of bacterial adhesins with individual receptor specificities have been identified. Many bacterial pathogens are able to express an array of different adhesins. Expression of these adhesins at different phases during infection play the most important role in adhesion based virulence [5]. Additionally, adhesins are attractive vaccine candidates because they are often essential to infection and are surface-located, making them readily accessible to antibodies. Numerous studies have shown that inhibiting a single adhesin in this coordinated effort can often be enough to make a pathogenic bacterium non-virulent. So, adhesin activity interruption is a novel approach for the bacterial infection treatment.

Staphylococcus aureus is a major cause of a variety of nosocomial infections. *S. aureus* strains exhibiting multiple antibiotic resistances are isolated in 60% of community and up to 80% of hospital infections [6]. The dramatic increase in methicillin resistant bacteria, coupled with the recent emergence of vancomycin resistant isolates has accelerated and broadened the interest in developing novel therapeutics against *S. aureus.* Clumping factor A (ClfA) is the major virulence factor responsible for the observed clumping of *S. aureus* in blood plasma. ClfA a surface adhesin protein provide an excellent target for immunological attack by antibodies [7]. The protein domain SdrG stands for serine-aspartate dipeptide repeats, SdrG_C refers to the C terminus domain of an adhesin found only on the cell walls of bacteria more specifically, found in gram-positive bacteria [8]. Gram-positive pathogens such as Streptococci, Staphylococci, and Enterococci, adhere to the host tissues via a dock-lock-latch mechanism using SdrG [9]. This protein domain has enormous significance in haemostasis and coagulation [10]. Understanding more about the mechanism of attachment to human cells, is of great hope as a therapeutic target to prevent diseases caused by gram-positive pathogens.

In this study, adhesin antigenic protein ClfA has been evaluated to find a peptide based vaccine which serves as potential to protect against staphylococcal diseases. Bioinformatics tools and servers were used for vaccine development.

II. MATERIAL AND METHODS

A. Protein sequence retrieval

The ClfA protein of *S. aureus* MRSA252 was retrieved from UniProtKB (www.uniprot.org/) in FASTA format. The sequence was analyzed to identify the B cell, T cell epitope and MHC class I and II binding regions.

B. Prediction of transmembrane protein

Sub-cellular localization of ClfA protein was predicted using TMPred [11]. The system does not need the sequence homology data of any known sequences. It uses only physico-chemical parameters of the N- and C-terminal sequences and the total sequence.

C. Prediction of antigenicity

The membrane protein was then submitted to VaxiJen v 2.0 [12] with threshold value 0.5 to predict the probable antigenic. VaxiJen v 2.0 is the server for alignment independent prediction of protective antigens, solely based on the physicochemical properties of proteins without recourse to sequence alignment.

D. Identification of conserved domain

To identify the conserved domain of ClfA, protein sequence was aligned with protein superfamily members, by using the Conserved Domain Database of NCBI server (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

E. Prediction of T-cell epitope

Linear T-cell epitopes were identified by NetCTL 1.2 server [13]. NetCTL 1.2 predicts the CTL epitopes (Cytotoxic T Lymphocytes) in protein sequence. This expands the MHC class I binding prediction to 12 MHC supertypes including the supertypes A26 and B39. The complete ClfA protein sequence was submitted to the server, and all the super types were chosen. Based on neural network architecture the T-cell linear epitopes were identified. Threshold for epitope identification was set >0.50 to have sensitivity and specificity of 0.89 and 0.94, respectively.

F. Prediction of MHC binding peptide

TepiTool from Immune Epitope Database (IEDB) analysis resource was used to predict 71 MHC class I and 26 most frequent alleles of MHC class II binding peptides [14]. The IEDB recommended prediction method was used to predict the peptides having percentile rank ≤ 1 for class I and ≤ 10 for class II, alleles having binding affinity IC_{50} less than 250 nm were chosen for further consideration. For all the alleles, peptide length was set to 9 amino acids for class I prior to the prediction. The best MHC-I & II binding peptide were selected for further study based on the percentile rank and IC_{50} overall score.

G. Prediction of epitope conservancy

Epitope conservancy for selected epitopes was predicted using the epitope analysis tool from the IEDB analysis resource server [15]. This tool computes the degree of conservancy of an epitope within a given protein sequence set at a given identity level.

H. B-cell epitope prediction

Bepipred server from IEDB was used to predict linear B-cell epitopes. This server predicts the location of linear B-cell epitopes using a combination of a Hidden Markov model and a propensity scale method [16]. Other antigen sequence properties like Parker hydrophilicity [17], Chou & Fasman beta-turn [18] and Emini surface accessibility [19], Karplus and Schulz flexibility [20].

I. Allergenicity evaluation

To examine the degree of allergenicity, AllerHunter (http://tiger.dbs.nus.edu.sg/AllerHunter/index.html) was used [21]. AllerHunter predicts cross-reactive allergen by using both support vector machine and pair-wise sequence similarity. AllerHunter aims to predict allergens and non-allergens with high sensitivity and specificity, without compromising efficiency at classification of proteins with similar sequence to known allergens.

J. Docking

In silico docking was carried out using Hex 8.0 to find out the binding of peptides to MHC molecules, when applied further *in vivo* experiments. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules [22]. Five MHC I molecules (PDB ID: 3VCL, 4XXC and 4O2C) and three MHC II molecules (PDB ID: 1BX2, 1AQD, 5JLZ) were taken for docking study. Peptides were designed by Discovery studio and used them as ligands for docking simulations at the binding groove of the MHC molecules.

III. RESULTS

A. Prediction of transmembrane protein

ClfA having inside to outside helices were found to be 2 and outside to inside helices 3 as predicted by TMPred server as shown in Fig.1.

B. Prediction of antigenicity

The protective antigen or non-antigen was predicted according to a predefined cut off. Since most of the models had their highest accuracy at a threshold of 0.5, hence the same threshold value was used in this study. VaxiJen server 2.0 revealed that ClfA was a probable antigen having score 1.2023. ClfA was predicted as non-human homologues by NCBI Blastp, proving pathogen specific protein.

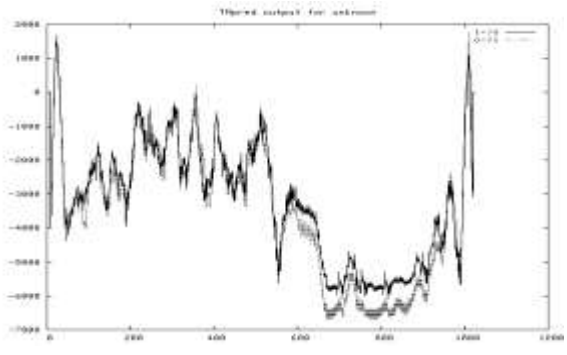

Figure 1. Prediction of transmembrane protein using TMpred server.

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C. Identification of conserved domain

The ClfA protein (UniProt ID: Q6GIK4) contains three conserved domains such as YSIRK_signal (4-27 aa), Spr super family (64-193 aa) and SdrG_C_C (375-525 aa), these domains were found alignment with cdd:pfam04650, Cdd:PRK13914 and cdd:pfam10425 respectively as shown in Fig. 2.

Figure 2. Conserve domain of ClfA

D. Identification of T-cell epitope

Twenty one epitopes were selected from the given protein sequence according to all supertypes based combined score (Binding affinity, C-terminal cleavage affinity and TAP transport efficiency) using NetCTL 1.2 (Table 1). The most potential epitopes like APRMRAFSL, KPNTDSNAL, LYGYNSNII and SEDEANTSL were chosen on the basis of combined scores and high antigenicity for further prediction of MCH class I and II allele binding.

TABLE 1: T-cell epitopes prediction with all supertypes

Super type A1	*Comb*	*Super type A24*	*Comb*	*Super type B39*	Comb
ASDLSESYY	3.2737	SYYVNPDNF	1.7829	SEDEANTSL	1.9798
QIDKTNNTY	3.2611	LYGYNSNII	1.7327	TKDDVKATL	1.8826
VTDSVNITF	2.8030	Super type A26		Super type B44	
GIDSGDTVY	2.0695	TVYPHQAGY	1.8972	SEDEANTSL	1.8203
NASDLSESY	1.6736	NVIYTFTDY	1.7941	Super type B58	
DLALRSTLY	1.6310	Super type B7		ITFPNPNQY	1.6209
Super type A2.		APRMRAFSL	1.8649	KSIGVASVL	1.5917
VIYTFTDYV	1.3824	KPNTDSNAL	1.6875	Super type B62	
Super type A3		Super type B8		AQNTSIKVY	1.4664
TVYPHQAGY	1.4127	APRMRAFSL	1.4958		

E. Prediction of MHC binding peptide

Interaction of 4 nonamers with different MHC alleles was shown in Table 2 and 3. Among the 71 MHC-I alleles, the epitopes APRMRAFSL, KPNTDSNAL and SEDEANTSL was found to interact with HLA-B*07:02, HLA-B*08:01 and HLA-B*55:01 most of the HLA-B* alleles and LYGYNSNII was found to interact with HLA-A* alleles. Among the 26 MHC-II alleles the HLA-DRB1 showed maximum interactions with the selected epitopes except SEDEANTSL did not interact with any alleles. Table 2 shows that all the epitopes were having more than 50% conservancy.

TABLE 2: Prediction of MHC class I binding epitopes

Epitope	**Peptide position**	**Interacting MHC-I allele**	**Percentile Rank (≤ 1) and (IC_{50} ≤ 250nM)**	**Epitope conservancy analysis result**
APRMRAFSL	210-218	HLA-B*07:02	0.1 (3.20)	69.23%
		HLA-B*08:01	0.1 (9.10)	
		HLA-B*55:01	0.6 (250.0)	
KPNTDSNAL	417-425	HLA-B*07:02	0.3 (20.70)	69.23%
		HLA-B*39:01	0.8 (164.2)	
LYGYNSNII	509-517	HLA-A*24:02	0.4 (204.50)	53.85%
SEDEANTSL	997-1005	HLA-B*40:01	0.15 (15.80)	84.62%
		HLA-B*39:01	0.75(249.0)	

TABLE 3: Prediction of MHC class II binding epitopes

Epitope	**Peptide position**	**Interacting MHC-II allele**	**Percentile Rank (≤10) and (IC50 ≤ 250nM)**
APRMRAFSL	209-223	HLA-DQA1*01:02/ DQB1*06:02	1.36 (42.24)
		HLA-DRB1*09:01	0.59 (117.23)
		HLA-DRB1*15:01	3.72 (108.26)
		HLA-DRB1*01:01	7.01 (20.74)
		HLA-DQA1*05:01/ DQB1*03:01	2.87 (15.22)
		HLA-DRB1*04:05	2.67 (107.97)
		HLA-DRB1*04:01	3.57 (176.40)
		HLA-DRB1*11:01	6.77 (110.33)
KPNTDSNAL	416-430	HLA-DQA1*01:02 /DQB1*06:02	8.84 (238.8)
LYGYNSNII	503-517	HLA-DRB1*15:01	0.89 (60.7)
SEDEANTSL	997-1005	No alleles found	

F. Prediction of B-cell epitopes

The B cell epitope regions that match the same regions as the predicted MHC binding peptides were summarized in Table 4. Two nonamers APRMRAFSL and KPNTDSNAL were found that fall into B cell epitope region. The B-cell epitopes were situated in the conserved region of ClfA and epitopes predicted as transmembrane by TMHMM. The KPNTDSNAL epitope fulfilled the highest scale of antigen sequence properties such as Parker hydrophilicity (6.214), Chou & Fasman beta- turn (1.357) and Emini surface accessibility (2.535), Karplus and Schulz flexibility (1.08) by crossing 1.000 threshold levels as shown in Fig. 3, 4, 5 & 6. APRM fulfilled all except Emini surface accessibility (data not shown).

TABLE 4: Bepipred linear epitope prediction

Start	**End**	**Peptide**	**Length**
34	213	SKEADASENSMTQTENTSNESKSNDPSS VNAAPKTDNTNVSDSNTTTNTNSDETN VAQNPAQQETTQSASTNATTEETPVTG EVTTTATNQANTPATTQSSNTNAEESV NQTSNETTSNDTNTVSSVNSPQNSTNA ENVSTTQDISTEATPSNNESAPQSTDAS NKDVVNQAVNTSAPRM	180
413	433	TGNLKPNTDSNALIDAQNTSI	21

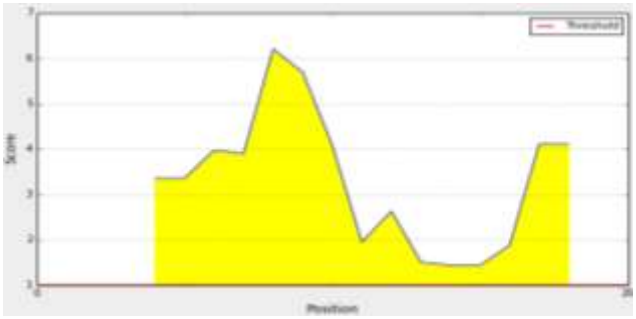

Figure 3. Hydrophilicity of the KPNTDSN epitope.

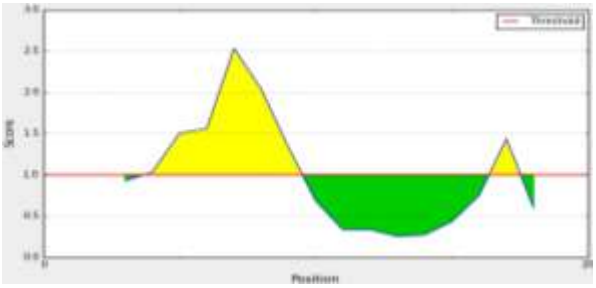

Figure 4. Chou & Fasman Beta-turn of the KPNTDSN epitope.

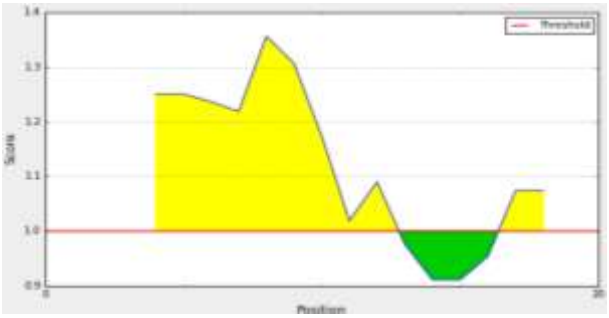

Figure 5. Emini surface accessibility of the KPNTDSN epitope.

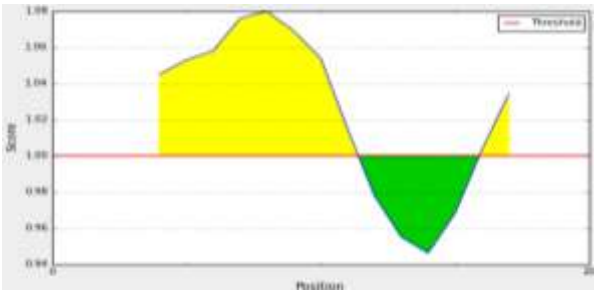

Figure 6. Karplus and Schulz flexibility of the KPNTDSN epitope.

G. Allergenicity prediction

ClfA protein was predicted as a potential non-allergen with score of 0.0. (sensitivity=91.6%, Specificity=89.3%) by AllerHunter as the query sequence didn't meet the criteria set by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) evaluation scheme for cross-reactive allergen prediction.

I. Docking simulation:

The docking of epitopes APRMRAFSL and KPNTDSNAL were carried out with the MHC class I & II available structures in PDB database. Both the epitopes were bound to the groove of HLA-B*07:02 alone and the binding energy were found to be -292.64 kcal/mol and -325.74 kcal/mol respectively. The interaction of epitopes and HLA-B*07:02 interaction was shown in Fig. 7 and 8. When compared to APRMRAFSL epitope, KPNTDSNAL epitope has bound exactly in the groove of HLA-B*07:02.

IV. DISCUSSION

A major challenge for healthcare in the 21st century is the increasing levels of drug resistance. *S. aureus* has acquired resistance to antimicrobial drugs. Therefore the search for an efficacious vaccine to prevent invasive diseases due to *S. aureus* is urgently needed [23].

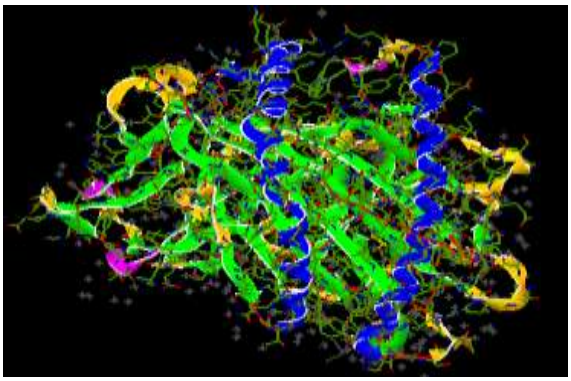

Figure 7: Docking simulation of APRMRAFSL with HLA-B*07:02.

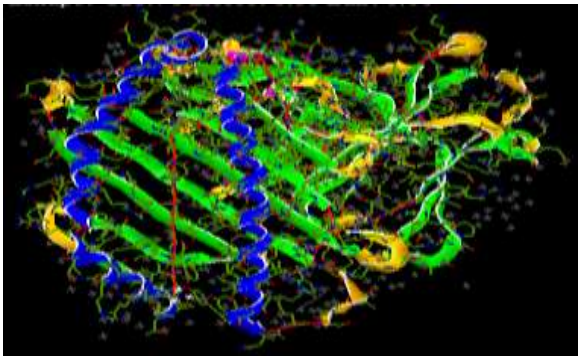

Figure 8: Docking simulation of KPNTDSNAL with HLA-B*07:02.

Over the past several decades live and heat-killed preparations of *S. aureus* cells have been tested as vaccines to prevent staphylococcal infections. The majority of proteins are unnecessary for the induction of protective immunity. These additional proteins may induce allergenic and/or reactogenic responses, thus emphasizing the need to eliminate them from vaccine formulations. This rationale led to an interest in subunit vaccines using single, or a select few, proteins of the microbes in vaccine formulations for induction of protective immunity [24]. This has led to the designing of peptide vaccines that contain only epitopes capable of inducing positive, desirable T cell and B cell mediated immune response [25]. "Peptides" predicted in this study were 9 amino acid sequences.

Numerous virulence factors have been targeted by vaccination, including alpha hemolysin, PVL, clumping factor A, fibrinogen binding protein, enolase (laminin-binding protein), and protein A of *S. aureus*.

In the present study we have selected highly antigenic surface protein clumping factor A that plays important role in *S. aureus* infections and produced by almost all of *S. aureus* strains. TMHMM predicted ClfA protein as membrane protein. Subcellular localization is one of the most important characteristics of proteins, which is central to understand their function and the constitution of biological systems. Proteins present on outer membrane and antigenic in nature could be served as drug and vaccine targets for *S. aureus*.

The conserved domains in ClfA protein showed in Fig. 2 reveals N-terminal YSIRK type signal peptide. Many surface proteins found in Streptococcus, Staphylococcus, and related lineages share apparently homologous signal sequences. A motif resembling [YF]SIRKxxxGxxS[VIA] appears at the start of the transmembrane domain. The GxxS motif appears

perfectly conserved, suggesting a specific function. There is a strong correlation between proteins carrying this region at the N-terminus and those carrying the Gram-positive anchor domain with the LPXTG sortase processing site at the C-terminus. The ClfA protein belongs to Spr superfamily, cell wall-associated hydrolase and NlpC family (Cell wall/membrane/envelope biogenesis). SdrG_C_C another domain at C-terminal, C-terminus of bacterial fibrinogen-binding adhesin; This is the C-terminal half of a bacterial fibrinogen-binding adhesin SdrG. SdrG is a Gram-positive cell-wall-anchored adhesin that allows attachment of the bacterium to host tissues via specific binding to the beta-chain of human fibrinogen (Fg). SdrG binds to its ligand with a dynamic "dock, lock, and latch" mechanism which represents a general mode of ligand-binding for structurally related cell wall-anchored proteins in most Gram-positive bacteria. The C-terminal part of SdrG (276-596) is integral to the folding of the immunoglobulin-like structure to create the docking grooves necessary for Fg binding. The domain is associated with families of Cna_B, pfam05738 [9].

Prediction of potentially immunogenic epitopes in a given protein sequence may significantly reduce the cost and time of wet lab required to discover the epitopes needed for the design of vaccines. In present study, an attempt was made to predict epitopes which could be tested for their efficacy in eliciting immunity through humoral and cell mediated immune responses. Vaccine based on T cell epitope has gained interest in recent years as the host can produce a strong immune response by CD8+ T cell [26]

Therefore, in the present study both B and T cell epitopes were designed using *in silico* computational approaches. For the prediction of CTL epitopes in ClfA protein sequence NetCTL 1.2 server was used. Using this server the accuracy of the MHC class I peptide binding affinity is significantly improved. The method integrates prediction of peptide MHC class I binding, proteosomal C-terminal cleavage and TAP transport efficiency. The server allows for predictions of CTL epitopes restricted to 12 MHC class I supertype. MHC class I binding and proteosomal cleavage is performed using artificial neural networks. TAP transport efficiency is predicted using weight matrix. Based on the combined score and high antigenic score the epitopes APRMRAFSL, KPNTDSNAL, LYGYNSNII and SEDEANTSL would be the best epitope candidates. These epitope interaction with MHC class I and class II alleles were selected based on percentile rank ≤1 for MHC class I and ≤10 class II and IC50 ≤250 nM for both the class of alleles using TepiTool-IEDB analysis resource. Lower IC50 calculation reflects a drug's effectiveness in a lower concentration. Epitope conservancy analysis is an important step that might reflect the possibility of an epitope to be used in designing a vaccine. High epitope conservancy score indicates a good chance of effectiveness of epitope vaccine *in vivo*.

AllerHunter is a cross-reactive allergen prediction program built on a combination of Support Vector Machine (SVM) and pairwise sequence similarity. The FAO and WHO developed evaluation for sequence based allergenicity prediction. This guideline clearly states that a sequence can be potentially allergenic if it either has an approximated identity of at least six contiguous amino acids or >35% sequence identity over a window of 80 amino acid chains when compared with known allergens [27]. The AllerHunter predicted ClfA as potential non allergen. The epitopes selected in this study will not create allergic reactions.

The B cell epitope is the portion of the antigen which interacts with B lymphocytes and can induce humoral immunity. To determine a potential B-cell epitope Bepipred server was used from IEDB. Several criteria were used for selection of B cell epitopes such as hydrophilicity, beta-turn surface accessibility and flexibility. The protein hydrophilic regions are typically more exposed to the surface and detected as the antigenic site. Parker hydrophilicity prediction from IEDB was used to determine the antigenic peptides with window size 7 and threshold 1.0. The epitopes were found to be hydrophilic in nature. The hydrophilic residues are in the yellow colored region. The residues which are below the cut off (red line, 1.00) are in the green region. Beta-turns play an important role in both structural and functional point of view. Beta-turns usually occur on the exposed surface of proteins and hence probably represent antigenic sites or involve in molecular recognition. Furthermore it has been shown that there is an over representation of beta-turns in B-cell epitopes.

Docking study was carried out to know the efficient epitope sequences that have the greatest chance for eliciting humoral and cell mediated immunity in the human body against *S. aureus*. The epitopes were subjected to *in silico* validation by protein ligand docking simulation. To investigate whether the designed vaccine will elicit sufficient immunological responses *in vivo*, docking simulation of the predicted MHC peptides along with HLA molecules was performed. After docking of receptor and ligand the energy score was obtained. Lower energy scores represent better binding between receptor and ligand [28]. Docking energy scores of the predicted epitopes were found significantly low. By considering all the prediction results, we conclude that in this study a peptide vaccine KPNTDSNAL serves as a potential vaccine candidate for immunization against multidrug-resistant *S. aureus.* ClfA generates strong immune responses and has shown potential as a vaccine component in active and passive immunization studies [29].

V. CONCLUSION

The aim of our investigation was to design a specific epitope which would elicit both humoral and cell-mediated immune responses. The peptide vaccine KPNTDSNAL showed interaction with different HLA alleles, good epitope conservancy and docking analysis. By considering all the prediction results, we conclude that this epitope serves as a potential vaccine candidate for immunization against multidrug-resistant *S. aureus.* This *in silico* study will definitely reduce time and cost. Further, epitope efficiency needs to be carried out for both *in vivo* and *in vitro* studies.

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