Detection of Immuno Dominant Peptides against pgaB of *Acinetobacter baumannii*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: *Acinetobacter baumannii* is a gram negative non-motile coccobacillus, which was considered as a low priority pathogen with low virulence. Recently, it was declared as the priority pathogen under the critical category of the most dangerous pathogen by WHO. *Acinetobacter Baumannii* is an inhabitant of oral biofilms, and it also increases the risk of refractory periodontitis. It causes nosocomial infections with pgaB, a part of pgaABCD operon which is involved in the biofilm formation.
Aim: The aim of the present study was to detect the immunodominant peptides against pgaB of *Acinetobacter baumannii* using bioinformatic tools and databases.
Materials and Methods: The present study was carried out using immune informatics. The protein sequence of the pgaB protein from *A.baumannii* was subjected to assess allergenicity, secondary structure, antigenicity and stability prediction of selected T cell epitopes, physico-chemical analysis, Identification of MHC class 2 binders, Final selection of B-cell epitopes was done with IEDB B-cell epitope tool
Results: Final docking of the peptides were interpreted by hydrogen bonds and interac- tion scores with TLR-2. Promising scores on antigenicity, instability were obtained. Based on the combinatorial

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scores, one vaccine peptide (LNLTLGLAL) was suggested to be a promising vaccine candidate against pgaB of *A.baumannii*.

Conclusion: The findings of the present study suggest epitope LNLTLGLAL as a promising vaccine candidate against pgaB of *A.baumannii*. The vaccine peptides targeting the pgaB Gene in *A.baumannii* using an immune-informatics approach suggests promising results in the present study. However, the predicted epitope peptides need further experimentation in animal models for its application against *A.baumannii*.

1. INTRODUCTION

Acinetobacter baumannii is a gram negative non-motile coccobacillus, which was considered as a low priority pathogen with low virulence. Recently, it was declared as the priority pathogen under the critical category of the most dangerous pathogen by WHO. At present there are 32 species of *A.baumannii* [1]. *A.baumannii* causes recalcitrant nosocomial infections comprising respiratory, skin and wound, urinary tract infections leading to complicated septicaemia [2] . Increase in the mortality rate is often documented in *A.baumannii* associated infections due to multidrug resistance (MDR) and extensive drug resistance (XDR) properties against various antibiotics . In addition to the resistance properties, it is also important to understand the association of various virulence factors playing an important role in transforming the pathogen into a priority pathogen [3] .

A. baumannii contains a pgaABCD operon that codes the proteins related to poly-β-(1-6)- N-acetylglucosamine. Previous studies reported that pgaABCD operon in *A.baumannii* and numerous other gram positive and negative bacteria also encode PNAG [4] . The protein database sequence investigation by BLASTp search utilizing E. coli pgaABCD had reported a high similarity of *A.baumannii* gene loci associated with PNAG biosynthesis and 30 clinical isolates delivered recognizable PNAG [5]. pgaB being a part of pgaABCD operon consequently appears to be exceptionally associated with the biofilm arrangement in *A.baumannii* ascribing to its harmfulness and pathogenesis. A prior investigation on the expression level of pgaB depending on the cytotoxicity test in *A.baumannii* documented pgaB as an important early virulent marker [6].

Recent advances in immune informatics approaches could potentially decline the attrition rate and accelerate the process of vaccine development in these times. The 3D structure of the vaccine construct was designed, refined to obtain a high quality structure [7]. Then the refined structure was docked against Toll like receptors to confirm the interactions between them. Immune informatics analysis showed that the multi epitope vaccine can stimulate both T and B cells immune responses and it could potentially be used for therapeutic usages. Immune informatics will allow the vaccine development using non-conventional antigens. The process of vaccine discovery in silico using the genetic information is named as reverse vaccinology [8]. In the present study using immune informatics approach and reverse vaccinology, study has been done. The emergence of drug-resistant *Acinetobacter baumannii* is a worldwide health problem associated with high mortality rate. Therefore, it is important to find suitable therapeutics for this pathogen. The protocol was designed as per the previous literatures and based on the expertise of our studies done earlier [9–17]. The aim of the present study was to detect the immunodominant peptides against pgaB of *Acinetobacter baumannii* involved *with* the biofilm associated virulence using an immune informatics approach.

2. MATERIALS AND METHODS

2.1 Study Setting

This is an observational *in silico* study done in the department of Microbiology, Saveetha Dental College and Hospital.

2.1.1 Protein retrieval and evaluation of allergeni city

The sequence pgaB protein was retrieved from the Uniprot database and its sequence id was P59637. The Subcellular location was predicted as Host cell membrane using CELLO v.2.5: subCELlular LOcalization predictor tool. The allergenicity of pgaB protein was predicted using

AlgPred server which is a web server developed to predict allergenic proteins and for mapping IgE epitopes using motif-based method has been developed using MEME/MAST software

2.2 Secondary Structure

The secondary structure of pgaB was predicted using the Self-Optimized Prediction Method with Alignment (SOPMA) server. The percentage of α-helix, beta sheet, turns and coils were predicted

2.2.1 Identification of MHC Class –II binders (T-cell epitopes)

T MHC class II molecules play an important role in initiation of the antigen-specific immune response. The T- cell epitopes of pgaB were predicted using the EpiDOCK server which predicts the binding of epitopes to 12 HLA-DR, 6 HLA- DQ, and 5 HLA-DP alleles. The first structure based server for the prediction of MHC class II binding using the Fasta format of sequence as input. Maximum number of epitopes binders (≥9) was identified and taken for further analysis.

2.2.2 Antigenicity and stability prediction of selected T-cell epitopes

Antigenicity was predicted using Vaxigen v 2.0, instability index of predicted epitopes was calculated using protparam server.

2.2.3 Predicted epitopes structure prediction and Validation

The structure predicted MHC Class – II binders were predicted using Pep Fold server which uses *de novo* approach to predict peptide structures from amino acid sequences. The structure predicted was validated using RAMPAGE tool which predicts the stereochemical properties of given structure. The server assesses the quality of modelled epitopes by predicting the amino acids falling in favoured, allowed and disallowed regions of Ramachandran plot.

2.2.4 Molecular docking of epitopes with HLA-alleles and TLR-2 receptor using cluster pro server

The three-dimensional structures of HLA-DP - 3LQZ, HLA-DQ - 5KSV, HLA-DR- 4AH2, TLR-2-6NIG were retrieved from the PDB database. The molecular docking analysis of predicted

MHC binders with HLA-Alleles were carried out using Clusterpro Server.

2.2.5 B cell epitope prediction

The B cell Epitope prediction of E- protein was carried out using Immune Epitope Database (IEDB) using Kolaskar & Tongaonkar Antigenicity Prediction method.

3. RESULTS

3.1 Protein Retrieval and Evaluation of Allergenicity

The sequence pgaB protein was retrieved from Uniprot database and its sequence id was P59637. The Subcellular location was predicted as a Periplasmic region. The protein was predicted as non-allergen with a Score=-0.41611328 predicted by SVM method based on amino acid composition.

3.2 Secondary Structure

Using the SOPMA server, the secondary structure was predicted. The percentage of alpha helix, extended strand, beta turn and random coil were given in the (Table 1).

3.2.1 Identification of MHC Class –II binders (T□cell epitopes)

From epi dock server, 13 epitopes were selected based on the maximum number binders to HLA-DP, DQ, and DR (Table 2).

3.2.2 Antigenicity and stability prediction of selected T-cell epitopes

Antigenicity was predicted using Vaxijen v2.0, instability index of predicted epitopes was calculated using protparam server. vaxijen scores for the following sequence were : MITRFASPL= -0.6592, ITRFASPLL =-0.7111, FASPLLNLT= 0.0541, ASTLTVIGY=-0.0771, EITDTKNAL=0.2101, TKNALIPQY=0.3381. Protparam scores were : MITRFASPL, ITRFASPLL and FASPLLNLT these sequences had 57.71 score (Table 2).

3.2.3 Predicted epitopes structure prediction and validation

The stereo chemical properties were validated using RAMPAGE TOOL. This server will assess the quality of modelled epitopes by predicting the

amino acids falling in favoured,allowed and disallowed regions of Ramachandran plot. It is amazing to see that epitope 2, epitope 3,epitope 4 and epitope 6 with the sequence of LTLGLALAG, LGLALAGLS, LAGLSGMAL, IGYHEITDT respectively possessed 100% most favourable regions (Fig. 1) and epitope 1 with the sequence of LNLTLGLAL possessed 85.7% most favourable region (Table 3).

3.2.4 Molecular docking of epitopes with HLA-alleles and TLR-2 receptor using cluster pro server

Epitope1 with sequence LNLTLGLAL, had the following scores HLA-DP=-732.1,HLA-DQ=-636.2, HLA-DR= -661.2 and TLR-2= -1120.1 (Table 5).There is a strong molecular docking for the TLR - 2 with the score of -1120.1 . This is according to the greater the negative score, higher will be the molecular docking (Table 4).

3.2.5 Docking interactions of LNLTLGLAL with HLA-DP,HLA-DQ,HLA-DR and TLR 2

Final docking of the peptides were interpreted by hydrogen bonds and interaction scores with TLR-2 (Table 5, 6, 7 & 8). The docking interaction of LNLTLGLAL with HLA-DP, HLA-DQ, HLA-DR and TLR 2 is given in (Fig. 2).

3.2.6 B cell epitope prediction

In the B cell epitope prediction, B cell antigenic epitope prediction from the pgaB of *Acinetobacter baumannii* with the start and end position showing the antigenic peptide sequences as a yellow colour peak (Fig. 3).

Table 1. Percentage of predicted secondary structure of pgaB

Alpha helix	45.03%
Extended strand	13.70%
Beta turn	5.12%
Random coil	36.14%

Table 2. Identification of MHC Class –II binders (T□cell epitopes), antigenicity and Stability prediction of selected T-cell epitopes

Position of peptide	Sequence	Number of binders to HLA alleles (DP, DQ, DR)	Vaxijen v 2.0	Protparam
1	MITRFASPL	12	-0.6592	57.71
2	ITRFASPLL	16	-0.7111	57.71
5	FASPLLNLT	9	0.0541	57.71
10	LNLTLGLAL	15	1.5726	8.89
12	LTLGLALAG	9	0.9952	8.89
14	LGLALAGLS	10	0.6771	22.60
18	LAGLSGMAL	14	0.4877	22.60
31	KIDASTLTV	9	1.3569	-0.54
34	ASTLTVIGY	10	-0.0771	-0.54
40	IGYHEITDT	11	0.7789	17.87
44	EITDTKNAL	9	0.2101	13.59
48	TKNALIPQY	9	0.3381	18.71
52	LIPQYAVTT	11	0.9690	35.59

Table 3. Predicted Epitopes structure prediction and Validation using the Rampage tool

	Peptides	Most favoured region	Additional allowed region	Generously allowed region	Disallowed region
Epitope 1	LNLTLGLAL	85.7%	16.7%	-	-
Epitope 2	LTLGLALAG	100%	-	-	-
Epitope 3	LGLALAGLS	100%	-	-	-
Epitope 4	LAGLSGMAL	100%	-	-	-
Epitope 5	KIDASTLTV	71.4%	28.6%	-	-
Epitope 6	IGYHEITDT	100%	-	-	-
Epitope 7	LIPQYAVTT	71.4%	28.6%	-	-

Table 4. Molecular Docking of epitopes with HLA-alleles and TLR-2 Receptor

		HLA-DP	HLA-DQ	HLA-DR	TLR-2
Epitope 1	LNLTLGLAL	-732.1	-636.2	-661.2	-1120.1
Epitope 2	LTLGLALAG	-715.4	-617.6	-660.5	-944.5
Epitope 3	LGLALAGLS	-679.6	-580.2	-645.8	-960.8
Epitope 4	LAGLSGMAL	-631.7	-539.9	-598.5	-899.7
Epitope 6	IGYHEITDT	-653.6	-559.0	-621.4	-860.9

Table 5. Docking interactions of LNLTLGLAL with HLA-DP

Interactions		Lnltlglal		HLA-DP		Distance (Å)
		Residue	atom	Residue	atom	
Hydrogen bond	Classical	LEU1	HT1	ASP142	O	2.82
		ASN2	HN	TYR143	O	3.03
	Carbon- Hydrogen	LEU9	C	ASN69	OD1	3.19
Hydrophobic	pi-sigma	ASN2	CA	PHE113	-	3.69
	Alkyl/Pi-Alkyl	LEU3		ALA82		4.33
		LEU9		LEU66		4.42
		ALA8		LEU70		4.24

Table 6. Docking interactions of LNLTLGLAL with HLA-DQ

		Lnltlglal		HLA-DQ (atom)		Distance (Å)
		Residue	atom	Residue	atom	
Hydrogen bond	Classical	LEU7	O	ALA3	HN	2.08
		THR4	HG1	ASP27	OD1	1.91
		THR4	HG1	ASP27	OD2	2.39
		ASN2	HD22	ASP27	OD2	2.03
		ASN2	OD1	ARG149	HH21	2.47
		ASN2	OD1	ARG149	HH22	2.16
		LEU1	O	ARG149	HE	1.95
		LEU1	O	ARG149	HH22	2.81
Hydrophobic	Alkyl/Pi-Alkyl	LEU1	-	ARG149	-	5.37
		LEU7	-	VAL6	-	4.59

Table 7. Docking interactions of LNLTLGLAL with HLA-DR

		LNLTLGLAL		HLA-DR		Distance (Å)
		Residue	atom	Residue	atom	
Hydrogen bond	Classical	ASN2	HD22	PHE180	O	2.01
		ASN2	HD21	GLU179	O	2.69
		LEU1	O	GLU179	HN	2.77
		ASN2	HD22	GLU179	OE2	2.09
		ASN2	HD21	GLU179	OE1	2.21
		THR4	HG1	THR93	OG1	3.02
		THR4	OG1	TRP178	HE1	1.92
		LEU9	O	SER104	HG	1.91
Hydrophobic	Alkyl/Pi-Alkyl	LEU1	-	HIS177	-	4.66
		LEU3	-	VAL91	-	5.33
		ALA8	-	LEU158	-	3.97
		ALA8	-	VAL116	-	5.01
		LEU9		VAL116		5.46
	Pi-sigma	THR4	CG2	TRP178	-	3.87
Electrostatic	Attractive	LEU1	N	GLU179	OE2	4.56

Table 8. Docking interactions of LNLTLGLAL with TLR-2

		Lffsliaqw		TLR-2		Distance
		Residue	atom	Residue	atom	(Å)
Hydrogen bond	Classical	ASN2	HD22	LEU331	O	2.85
		ASN2	HD22	THR335	OG1	1.91
	carbon hydrogen	LEU9	C	SER346	O	2.79
Hydrophobic	Alkyl/Pi-Alkyl	LEU1	-	PRO306	-	5.12
		LEU1	-	VAL309	-	4.04
		LEU3	-	MET270	-	4.91
		LEU3		LEU282		4.91
		LEU3		PHE284		4.53
		LEU3		LEU266		5.22
		LEU7		PHE284		5.10
		LEU7		ILE314		4.16
		LEU7		LEU289		4.59
		LEU7		LEU317		4.59
		LEU5		TYR332		4.74
		LEU9		VAL348		4.37
		LEU1		VAL338		5.23
		ALA8		TYR326		4.99
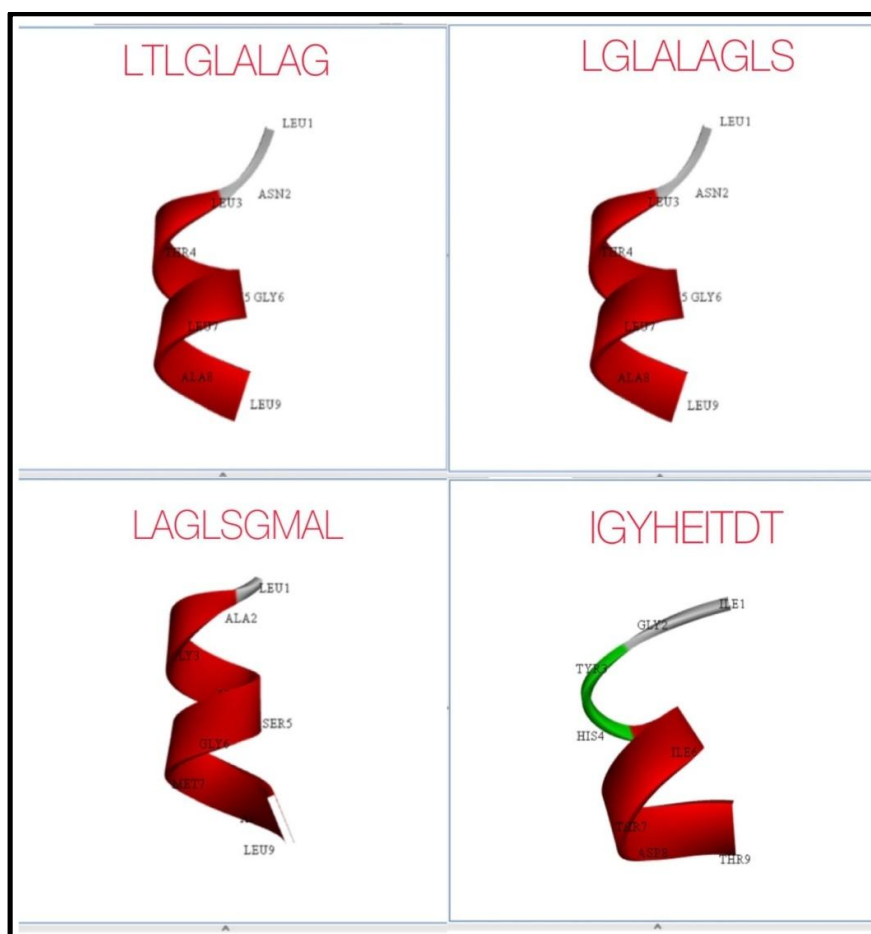
Fig. 1. These epitopes LTLGLALAG, LGLALAGLS, LAGLSGMAL and IGYHEITDT showed 100% most favourable region in the Ramachandran plot
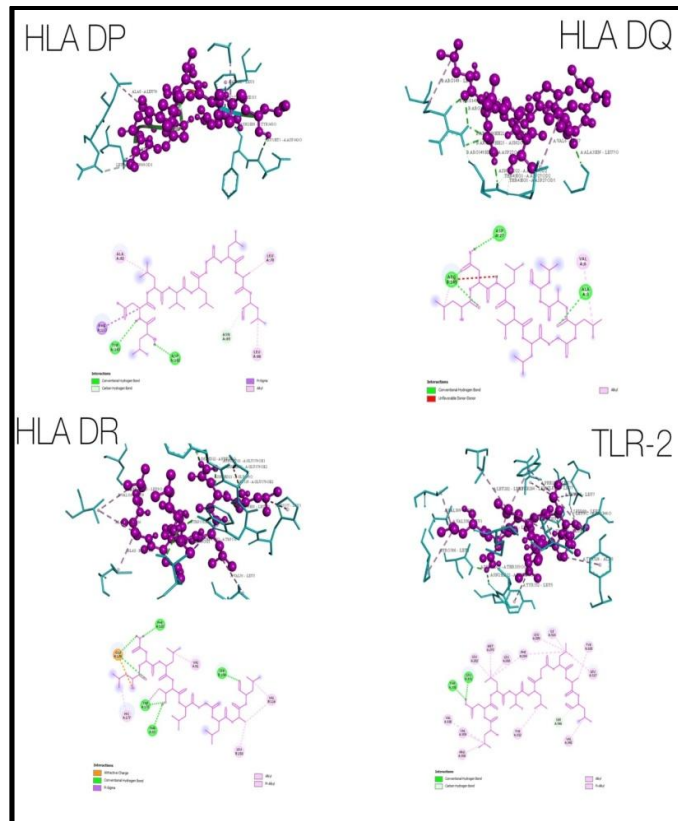
Fig. 2. Molecular docking interactions of LNLTLGLAL with HLA DP, HLA DQ, HLA DR and TLR 2
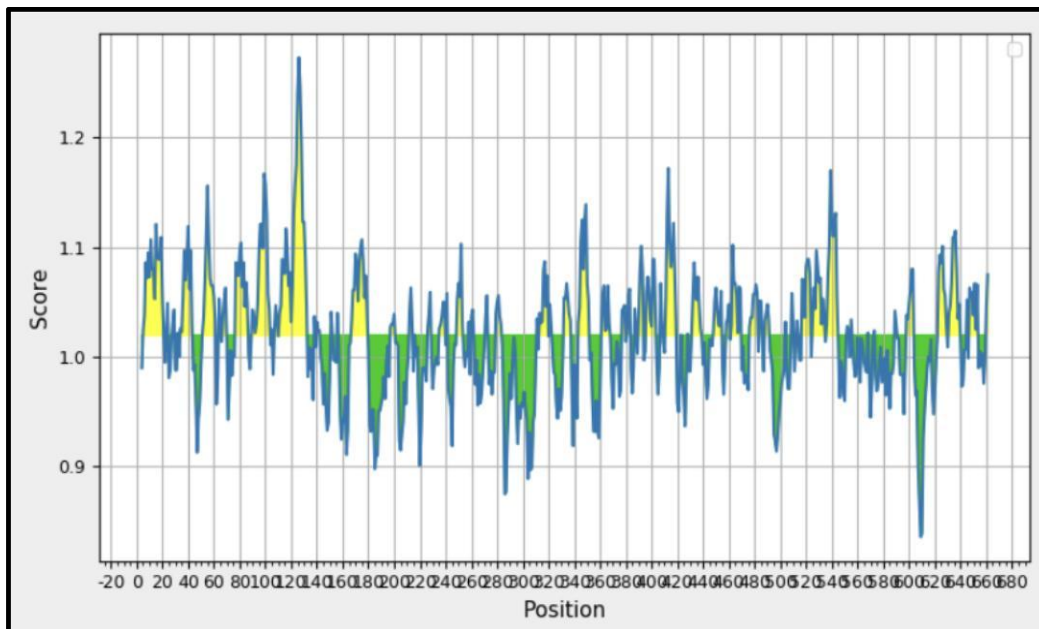

Fig. 3. B-cell antigenic epitope predictions from pgaB of A.baumannii with the start and end positions showing the antigenic peptide sequences (as an yellow colour peak).

4. DISCUSSION

Acinetobacter baumannii possess multi-drug resistant properties due to this property, it causes serious diseases and its colonies mainly in the hospitalized patients. Discovering new methods to combat the infections caused by *A.baumannii* seems to be the need of the hour. Considering the treatment and prevention of diseases caused by *A.baumannii,* the best alternate method is discovery of vaccines [18]. Considering this, the present study was designed as an observational and evaluation of vaccine candidates against the pgaB of *A.baumannii* and to arrest the formation of bio- films. While constructing a vaccine, the selection of B-cell and T-cell dominant epitopes is an important step because it should significantly bind with the immunological receptors and is considered to be an important step to provoke both humoral and cell-mediated immune responses in the host. Detection of promiscuous pgaB vaccine peptides was thus successfully achieved in the present study by the immuno-informatics approach utilizing the available genomic and proteomic reservoirs under a single computational platform comprising various databases and tools [19].

The selection of specific protein is the initial step in vaccine peptide analysis. The selection of protein was good and the sequence pgaB protein was retrieved from the uniprot database. The subcellular location was also determined. The protein was predicted as non-allergen with a score = -0.41611328. It is important that the protein should be a non-allergen. If the protein possesses an allergen, it will cause an allergic reaction in the host. The most immediate reactions are Type I hypersensitivity reactions which are mediated by the interaction of IgE antibodies against a particular vaccine component. These reactions occur within minutes of exposure to the relevant allergen and almost always occur within 4 hours of exposure to the relevant allergen, however, possible exceptions for delayed-onset reactions also do occur. Thus the preliminary predictions on allergenicity would be promising in the detection of possible epitopes that can be utilized for the vaccine development [20].

The secondary structure of pgaB was predicted and the percentage of alpha helix, beta sheet, turns and coils were predicted. The secondary structure of the protein can also determine protein topology. SOPMA is a server tool to detect and identify the percentage of secondary structure of a protein. It works based on the primary sequence of a protein. Combination of SOPMA and neural network methods have correctly predicted 82.2% of residues for 74% of predicted amino acids together with the Ramachandran plot showing more structural similarity of the residues and were thus validated [21] .

To assess the epitopes to evoke cellular immunity, MHC class 2 binders T cell epitopes were predicted using an epidock server. 13 epitopes were selected based on the maximum number of binders. HLA-DQ, HLA-DP and HLA-DR, these haplotypes along with their multiple subtypes have been survived so many infectious episodes in human history because of their ability to present pathogenic peptides to activate T cells that secrete cytokines to clear infections [22] .

In the Ramachandran plot, epitope 2, 3, 4 & 6 possess 100% most favoured regions which were structurally correlated with the structure which was predicted early. Ramachandran plot can be used for structure validation.

According to the molecular docking of epitopes with HLA - alleles of TLR - 2 receptor, the epitope 1 LNLTLGLAL , for HLA - DP = -732.1, of HLA-DQ= - 636.2 HL- DR = -661.2 and for TLR - 2 = -1120.1. There is a strong molecular docking for the TLR - 2 with the score of -1120.1. This is according to the greater the negative score, higher will be the affinity towards the interactions. Molecular docking is a tool in structural molecular biology and computer assisted drug design. Docking can be used to perform initial screening of results and it can also propose structural hypotheses on having the ligands inhibit the target. The two main factors for a successful docking experiment are convert pose and affinity prediction. Molecular docking is utilized in structure based drug design, biochemical pathway & to drug designing being the most attractive tools and the same has been applied to analyze the interaction energies[23] .

In order to involve humoral immune response, it is essential to predict the vaccine epitope. In the present study we also performed the B cell epitope evaluation at threshold 1.020, starting & reading position sharing antigenic peptide sequence as yellow colour peoks. B cell epitopes are antigenic determinants that are recognized and bound by receptors on the surface of B lymphocytes. The identification of the constitution of B cell epitopes play an important role in

vaccine design and in correlation with this, the present study has predicted the possible B cell dominant epitopes from pgaB.

Our team has extensive knowledge and research experience that has translated into high quality publications [24–27]. The immune-informatic approach holds very promising for marine sources and also for the synthetic chemicals [28, 29]. Homology modelling of the predicted peptides is an easiest method to be considered for both the drug and vaccine design [30]. Bio-informatic approach also holds good for the prediction of resistant determinants from oro-dental pathogens and also in other systemic diseases [31 – 34]. Thus in the present investigation, epitope LNLTLGLAL is predicted as a promising vaccine candidate against pgaB of *A.baumannii*. The limitation of the study was that the predictions were done only using in-silico tools. However, the future prospects are set to experimentally evaluate the same in animal models for its application against *A.baumannii* associated infections.

5. CONCLUSION

An attempt to detect few vaccine peptides targeting the pgaB in *A baumannii* using an immune - informatic approach in the present study had documented promising results. Epitope LNLTLGLAL is predicted as a good vaccine candidate against pgaB of *A.baumannii*. However further experimental evaluation on its immunological memory and responses are needed to be analyzed for its applicability as vaccine peptides. In addition, the prediction of epitope peptides using an immune informatics approach and reverse vaccinology technique aid in the detection of novel vaccine peptides with minimal error in comparison with the conventional method. From the available vaccine peptide data, it is evident to construct a vaccine against the pgaB of *Acinetobacter baumannii*.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors. The authors are

ETHICAL APPROVAL

Institutional approval for the research was obtained (IHEC/SDC/UG-1963/21/146).

ACKNOWLEDGEMENT

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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http://www.sdiarticle4.com/review-history/74424