ORIGINAL RESEARCH ARTICLE:

Prediction of epitope peptides for PTK gene of Acinetobacter baumannii
Running title: Epitope predictions for ptk of A.baumannii

Abstract:

Background: *Acinetobacter baumannii* is a gram negative bacilli acquiring both intrinsic and adaptive patterns of multi drug resistance and virulence. Immune-informatics approach holds promise to detect putative epitope peptides from vital virulence factors which can be further synthesized and evaluated for their immunological response.

Aim: The aim of the study was to predict the immuno-dominant peptides from the ptk gene of *A.baumannii.*

Materials and methods:

Protein retrieval of the Ptk gene using CELLO V.2.5 was done with the evaluation of antigenicity and allergenicity of the predicted epitopes, using Vaxijen V2.0 server and AlgPred servers. Epitope structure prediction and validation by using RAMPAGE revealed the homology peptides. Molecular Docking of epitopes with HLA-alleles using ClusterPro server, and further identification of B cell epitope was performed by using Kolaskar and Tonganokar antigenicity method.

Results:

A total of 20 epitopes were predicted and 18 peptides were chosen based on antigenicity and stability analysis prediction. The structure predictions were carried out using pepfold server and based on Ramachandran plot analysis 10 epitopes were taken for further analysis.

Conclusion:

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The present finding has detected and evaluated the desirable epitope as LFFSLIAQW using an immune-informatic approach. However, it needs further experimental validation for its immunological response using standard in-vitro studies.

Key words: *A.baumannii*; novel Ptk gene; innovative immune informatics: in-silico: environmental strains

INTRODUCTION:

Acinetobacter baumannii is a gram negative bacilli, which is aerobic, pleomorphic and an opportunistic pathogen with multi- drug resistance. It comprises 30 different species among which *Acinetobacter baumannii* is considered as a vital pathogen in causing infections like bacteremia, pneumonia, urinary tract infection, leads to necrosis, blood stream infection and septic shock (1). The virulence factor of *A.baumannii* is its ability to establish it as a multi-drug resistant strain and is considered as a critical pathogen by WHO. As a sessile biofilm forming bacterial community, the extracellular polysaccharides, epithelial cells and fibre filaments makes it as a toughest pathogen to be treated by routine antibiotics (2).

Various studies had documented different biofilms with different phenotype, genotype with efflux pumps and it consist of haemolytic factors and iron acquisition (3). In the context of *A.baumannii* virulence factors, amidst many vital virulence proteins Ptk gene is chosed for the present investigation. Ptk gene helps in formation of capsule polymerisation which helps in the formation of biofilms and further aiding in the progression of the disease. Vital mutation in ptk mutations, as revealed by genomic sequencing reveals its diversity and evolutionary patterns (4).

In recent years it has been a huge challenge for the treating physicians to cure the complications caused by *A.baumannii.* Prophylaxis is possible and successful by subtractive proteomics with the reverse vaccinology technique, in identifying vaccine candidates against *A.baumannii*. Immuno-informatics is capable of identifying virulence genes (5), surface associated proteins (6), with several methods available such as enabling designing the therapeutic vaccine in a short period of time (7). Biological databases help in identification of persistent data with the help of computerised software technologies which aid in designing, updating the retrieved components, and data stored within the system (8). This technique helps in exploration of widespread quantities, biological databases, its storage and

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systemisation. Reverse vaccinology technique helps in identification of new potential vaccines, which is used to express DNA over purified proteins from the organism itself (9, 10). Our earlier studies have already documented the putative vaccine epitope predictions for bap gene which is a vital biofilm former (11)). In this view, the aim of our study was to predict the immuno-dominant peptides from the Ptk gene of *A.baumannii*.

MATERIALS AND METHODS:

Study setting: This is an observational in-silico study done in the Department of Microbiology, Saveetha Dental College and Hospital.

Protein retrieval of Ptk gene:

Sequence of Tyrosine-protein kinase (Ptk) was retrieved from the Uniprot database (https://www.uniprot.org/) and subcellular location was predicted by using CELLO V.2.5: subcellular localization predictor tool (http://cello.life.nctu.edu.tw/). This tool will predict the location of the predicted epitopes.

(Prediction) of antigenicity and allergenicity of Ptk gene:

Antigenicity is predicted by using Vaxijen V2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). The tool will render the antigenicity score and based on the score the probable antigenicity of the predicted epitope can be evaluated. The classification of antigen proteins are mainly based on physicochemical properties and alignment based prediction method. Allergenicity of Ptk is predicted by using AlgPred server (https://webs.iiitd.edu.in/raghava/algpred/submission.html), a web-server developed to predict the allergenic proteins and aids in mapping out IgE epitopes. IgE is specifically selected because of its role played in the allergic manifestations.

Secondary structure predictions:
With the help of a self-optimised prediction method the secondary structure such as Alpha helix, extended strand, beta-turn and random coil, percentage is identified.

Identification of T-cell epitopes:

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EpiDock server (http://www.ddg-pharmfac.net/epidock/EpiDockPage.html) helps in prediction of T-cell epitopes of the selected protein. It predicts the epitope binding to 12HLA-DR, 6HLA-DQ and 5HLA-DP alleles. FASTA format is used in prediction of the first structure server. Maximum number of epitopes binders (≥10) was identified and taken for further analysis-

Prediction of the chemical properties of the epitopes:

Protparam server (https://web.expasy.org/protparam/)helps in calculating instability index of predicted epitopes. The molecular weight, and other chemical properties can be assessed using the parameters as set default in the prediction server.

Epitope structure prediction and validation:

Pepfold server (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/) which uses de novo approach helps in prediction of epitope structure, from amino acid sequences. RAMPAGE tool (https://bio.tools/RAMPAGE) helps in validation of structure and predicts the stereochemical properties. The server assess the quality of modelled epitopes by predicting the amino acids falling in favoured, allowed and disallowed regions of Ramachandran plot

Molecular Docking of epitopes with HLA-alleles using ClusterPro server:

PDB database helps in retrieval of three dimensional structures such as HLA-DP-3LQZ,HLA-DQ. Cluster pro server helps in prediction of MHC binders with HLA alleles.

Identification of B cell epitope:

Immune epitope Database helps in identifying B cell epitope by using Kolaskar and Tonganokar antigenicity method. Predictions of B-cell epitopes will aid in the evaluation of the humoral immune response with the predicted epitopes.

Results:

Protein retrieval:

Unimportant Database helps in retrieval of Ptk from *Acinetobacter baumannii*, the sequence id-DOCEWO. Inner membrane protein (3.461*) was predicted using CELLO V.2.5: subcellular localization predictor tool.

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Antigenicity and Allergenicity Results:

VaxiJen V 2.0 helps in prediction of antigenicity of PTK-Score was found to be 0.4560. Algpred server helps in prediction of protein as non-allergen score was found to be 0.41611328.

Secondary structures shows , 49.06% Alpha helix, 15.78% Extended strand , 4.14% beta turn 31.02% Random coil.

Antigenicity and stability prediction:

The peptide binders (greater than or equal to 10) were screened for antigenicity and stability analysis. The peptides with antigenicity value greater than 0.4 were predicted using VaxijenV 2.0 and stability was analysed using protparam (<40). From 20 peptides, 18 peptides were chosen based on antigenicity and stability analysis prediction (Table 1)

Structure prediction using pepfold and Ramachandran plot evaluation:

Total of 18 epitopes were selected using pepfold server for structure prediction, only 10 epitopes were selected based on Ramachandran plot. Out of 18 epitopes 10 epitopes showed 100% for most favoured region (Table 2). Epitopes 4, 5, 6, 7, 8, 9, 10, 11, 13, 14 and 15 showed 100% homology under ramachandran plot (Figure 1).

Docking interactions with HLA alleles:

Docking interactions of the selected LFFSLIAQW epitope with HLA alleles showed promising hydrogen bonds and binding energies. HLA-DP- 949.0 , HLA - DQ-814.9, HLA-DR -904.8 And finally TLR 2 -1058.9. (Figure-2), (table:3)

B cell epitope prediction-Kolaskar and Tongaonkar Antigenicity Results:

A graph denotes the sequence position of X axis and Y axis denotes antigenic propensity (Figure3). Predicted scores showed typical epitopes and were depicted in yellow. By analysing the atom distance, bond strength, we can classify the epitopes based on Hydrogen bond and bond interaction.

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Table 1: Antigenicity and Stability prediction of selected T-cell epitopes

Position sequence of peptide	Number of binders to HLA alleles (DP, DQ, DR)	Vaxijen v 2.0 Protparam
1 MNQQNTNTE	10 1.7260	7.74
6 TNTEDTIDL	10 0.7476	8.86
11 TIDLKELFF	13 1.0083	-0.54
15 KELFFSLIA	15 0.3644	8.89
17 LFFSLIAQW	12 1.1043	8.89
18 FFSLIAQWK	17 0.1333	8.89
19 FSLIAQWKL	18 0.8144	-0.54
21 LIAQWKLIA	13 0.5467	-0.54
22 IAQWKLIAL	14 0.5755	-0.54
25 WKLIALCVI	13 0.8699	-8.92
26 KLIALCVIL	13 0.7132	12.48
27 LIALCVILS	15 1.0104	21.91
28 IALCVILSV	12 0.8757	21.91
30 LCVILSVVC	16 0.8715	21.91
32 VILSVVCAL	16 0.6192	30.29
33 ILSVVCALL	17 0.8834	30.29
34 LSVVCALLY	16 1.1535	8.89
40 LLYLRVTPD	10 1.5570	12.48

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41 LYLRVTPDT	12 1.6009	-4.22
42 YLRVTPDTY	11 1.1963	-4.22

Table 2: Structure prediction using pepfold and Ramachandran plot evaluation

	Peptides	Most favoured region
Epitope 1	MNQQNTNTE	57.1%
Epitope 2	TNTEDTIDL	57.1%
Epitope 3	TIDLKELFF	71.4%
Epitope 4	LFFSLIAQW	100%
Epitope 5	FSLIAQWKL	100%
Epitope 6	LIAQWKLIA	85.7%
Epitope 7	IAQWKLIAL	100%
Epitope 8	WKLIALCVI	100%
Epitope 9	KLIALCVIL	100%
Epitope 10	LIALCVILS	100%
Epitope 11	IALCVILSV	100%
Epitope 12	LCVILSVVC	85.7%
Epitope 13	VILSVVCAL	100%
Epitope 14	ILSVVCALL	100%
Epitope 15	LSVVCALLY	100%
Epitope 16	LLYLRVTPD	14.28%
Epitope 17	LYLRVTPDT	28.5%
Epitope 18	YLRVTPDTY	42.8%

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Table 3: Molecular docking of epitopes with the HLA alleles

Molecular Docking of epitopes with HLA-alleles	Epitopes	HLA-DP	HLA-DQ	HLA-DR	TLR-2
Epitope 4	LFFSLIAW	-949.0	-814.9	-904.8	-1058.9
Epitope 5	FSLIAQWKL	-773.6	-691.3	-740.2	-970.2
Epitope 7	IAQWKLIAL	-748.8	-671.6	-697.8	-851.5
Epitope 8	WKLIALCVI	-857.4	-754.6	-766.7	-944.6
Epitope 9	KLIALCVIL	-734.1	-671.5	-707.3	-930.5
Epitope 10	LIALCVILS	-740.7	-661.1	-685.7	-953.9
Epitope 11	IALCVILSV	-761.4	-658.2	-707.9	-886.8
Epitope 13	VILSVVCAL	-771.7	-669.8	-757.3	-974.2
Epitope 14	ILSVVCALL	-766.4	-730.6	-743.5	-991.8
Epitope 15	LSVVCALLY	-834.5	-689.7	-786.0	-1054.5

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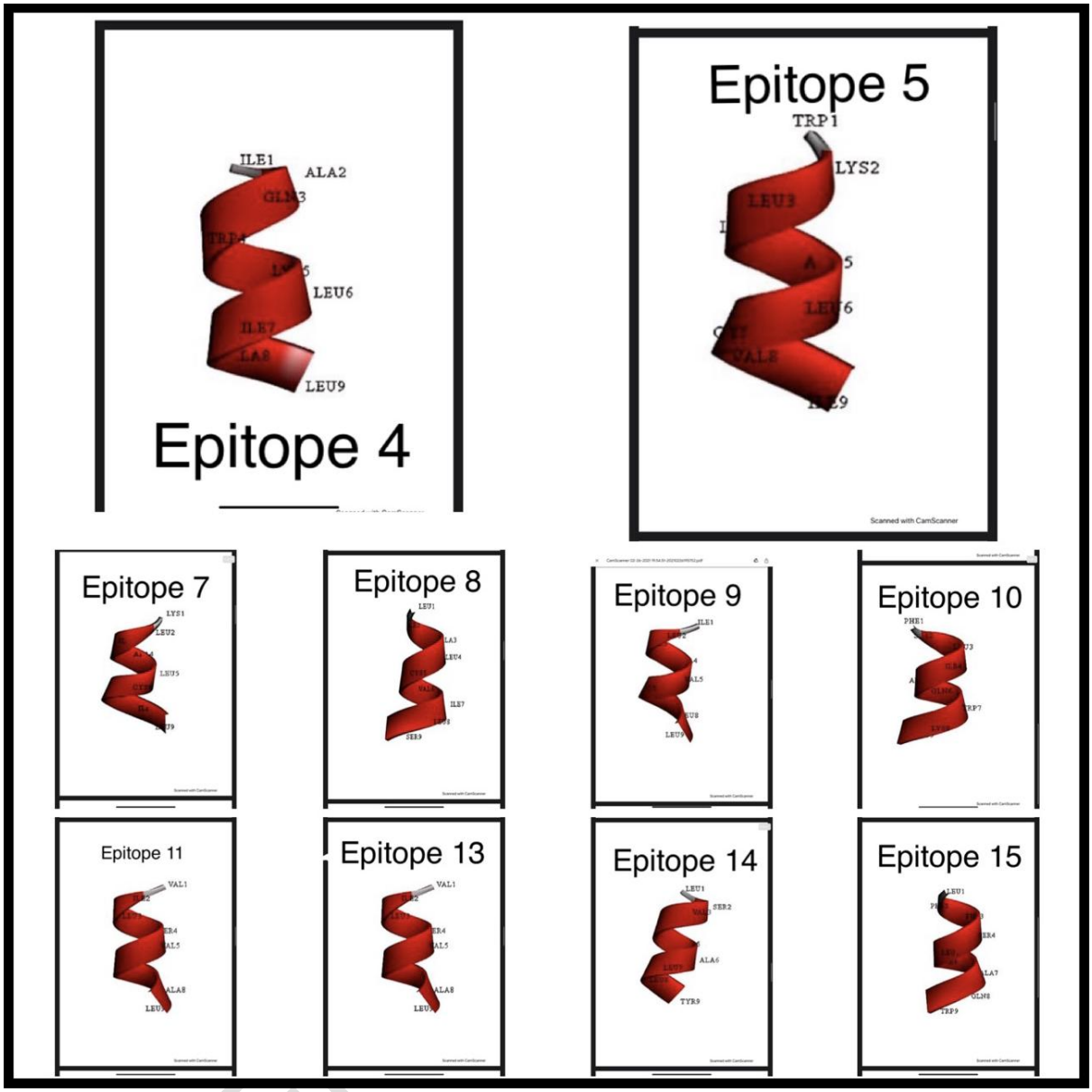

Figure 1 showing the Ramachandran plot of epitope

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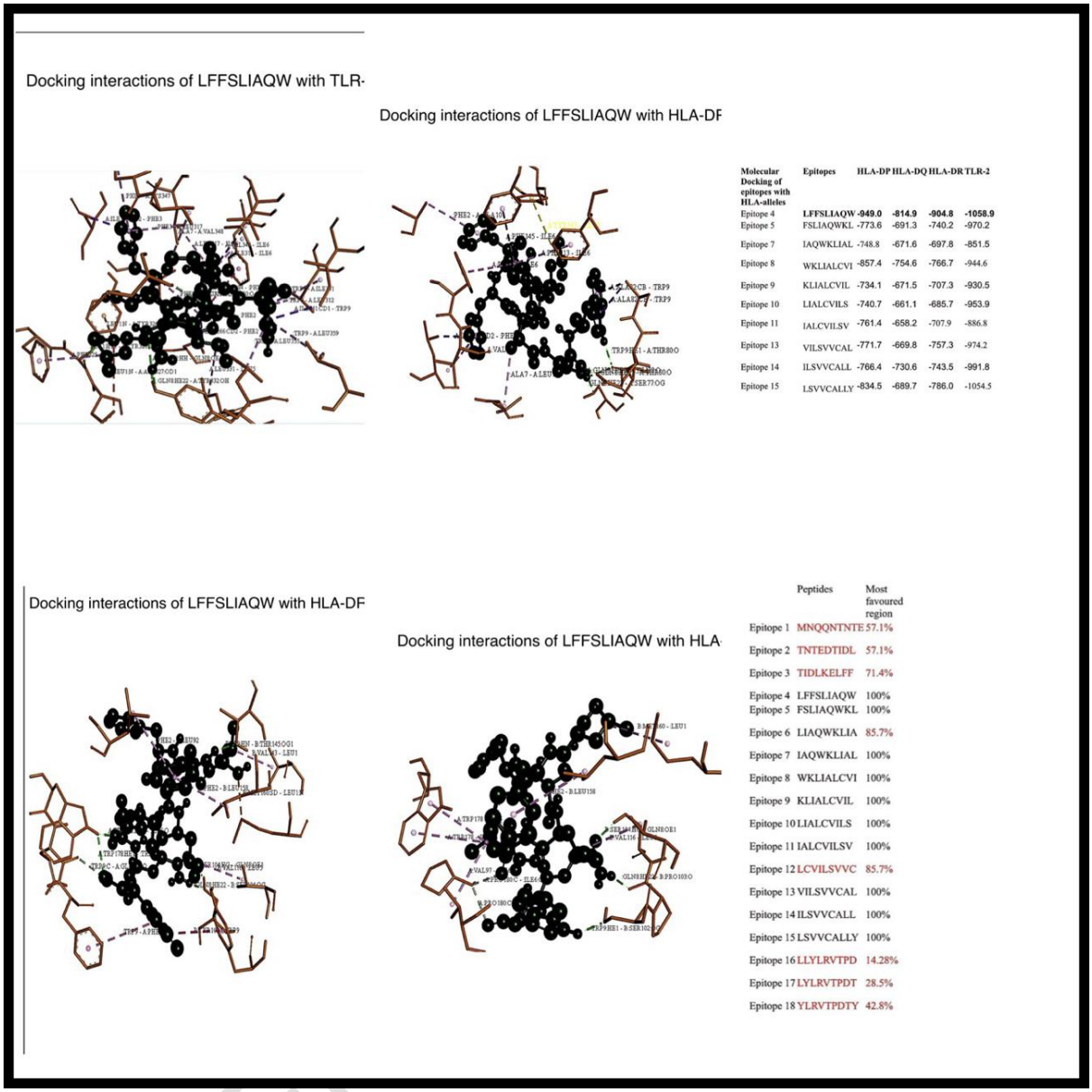

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Figure 2 showing the molecular Docking of epitopes with HLA allele

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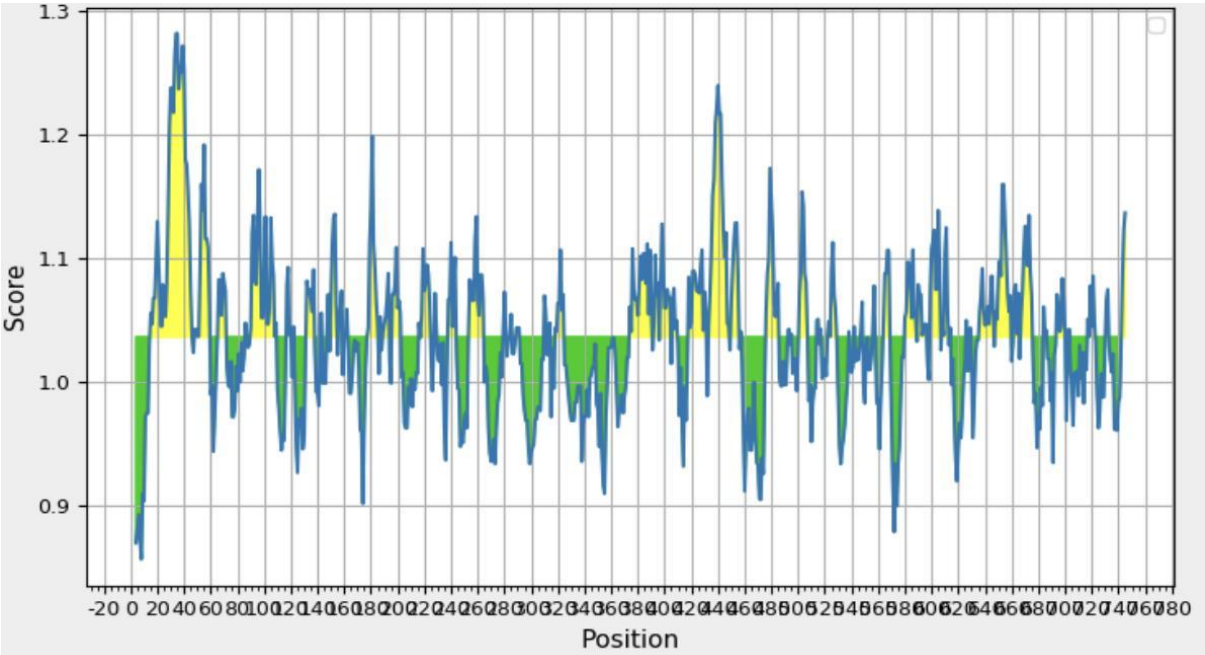

Figure 3 showing the B cell epitope prediction by Kolaskar and Tongaonkar Antigenicity

Discussion:

Acinetobacter baumannii is considered as a priority pathogen, causing systemic infections in hospitalised patients (12). It is often multi-drug resistant to almost all the routine drug of choice (13 – 16), requiring novel strategies and alternative compounds can be detected (17, 18) to combat its complications. In-silico based predictions for target structures holds promising to design and evaluate novel bioactive compounds for systemic pathogens (19), oral pathogens (20), non-communicable diseases (21, 22), immunological disorders (23) and also for pathogens causing pandemics (24, 25). A.baumannii is a potent biofilm former attributing for major virulence in causing systemic infections (26 27). With the advent of bioinformatics, the present study was designed as a computational approach to predict potential immuno-dominant epitopes from the PTK gene.

Amidst the various virulent genes viz., ompA gene, epsA gene, Ptk gene and bap genes, ptk protein was chosen for the present evaluation as it was not done earlier (28). Evaluation of sequence of genome particularly Ptk gene was achieved using uniprot database, subcellular location was predicted by using the server CELLO V.2.5 Instability index value should be >=10, as screened for antigenicity and stability analysis. Vaxijen V 2.0 helps in identifying the stability score =<40. Among 20 epitopes 18 were chosen as stable proteins based on antigenicity and stability.

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Our earlier studies have already documented the putative vaccine epitope predictions for gag gene which is a vital biofilm former3. Prediction of promiscuous Ptk vaccine peptides was thus successfully achieved in the present study by the immuno- informatics approach utilising the available genomic and proteomic reservoirs under computational platforms comprising various databases and tools.

The score-0.4560 under Vaxijen V 2.0 server shows promising scores for vital epitopes as probable antigens. Ramachandran plot is used for identification, greater accuracy of ultra high resolution protein structure. Pepfold Ramachandran plot, total 18 epitopes among 10 epitopes showed 100% most favoured region such as Epitope 4 LFFSLIAQW 100%, Epitope 5 FSLIAQWKL 100%, Epitope 7 IAQWKLIAL 100%, Epitope 8 WKLIALCVI 100%, Epitope 9 KLIALCVIL 100%, Epitope 10 LIALCVILS 100%, Epitope 11 IALCVILSV 100% (Figure:2). Upon further shortlisting, the interactions of the selected epitopes were analysed with the HLA-DR,DQ and DP as these were highly involved with the bacterial infections. Non-toxic nature and the B-cell dominant epitope predictions were highly promising by Kolaskar and Tangaonkar Antigenicity. The limitation of the study was that it was done as a computational mode. Thus the future prospect is set to evaluate the immunological memory and response using *in-vitro* and *in-vivo* study models.

Conclusion:

The present investigation was undertaken to predict the epitope peptides from the ptk gene of *Acinetobacter baumannii* using a computational approach. Diligent application of computational tools and databases have aided a higher probability of detection of immuno-dominant peptides. LFFSLIAQW was considered as the best epitope from ptk based on the evaluations made using varying parameters. However further experimental validations must be performed to assess its immunological response and memory.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is

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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.

Ethical Approvals: Institutional approval for the research was obtained [SRB/SDC/UG-1918/21/018].

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