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DESIGNING MULTI-EPITOPE SUBUNIT VACCINE FOR *PSEUDOMONAS AERUGINOSA*: IMMUNOINFORMATICS APPROACH

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ABSTRACT: *Pseudomonas aeruginosa* is a ubiquitous bacterial pathogen that has led to increase in mortality rate and caused serious infections among immune-compromised patients. Vaccine development against infections caused by *P. aeruginosa* is necessary because high resistance has been developed against broad spectrum of antibiotics. Outer membrane protein (OprF) is an important vaccine candidate for *P. aeruginosa* infections. Series of immunoinformatics approach were used to design multi-epitope vaccine of OprF outer membrane protein. A total of 10 *P. aeruginosa* OprF outer membrane protein sequences were retrieved from the NCBI protein database. Nine (9) protein sequences were found to be antigenic having a probability score of ≥ 0.8 as predicted by ANTIGENpro. 253 CTL epitopes of 9mer length were predicted using NetCTL 1.2 server, among which only 17 CTL epitopes with high immunogenicity score were selected to be subjected to the vaccine designing. The HTL epitopes were also identified using the IEDB MHC-II epitope prediction module and 2654 epitopes of 15mer length were obtained. However, Only 8 HTL epitopes with lowest percentile rank ranging from 0.03–0.3 were selected for the vaccine designing. A final vaccine construct of 382 amino acid residues was obtained using TLR-4 agonist (APPHALS) as the adjuvant and joining CTL epitope using EAAAK linker while Intra-CTL and Intra-HTL epitopes were joined using the AAY and GPGPG linkers respectively. The vaccine construct was classified as stable, found to be non-allergenic in nature and safe for human use. This research has provided a multi-epitope subunit based vaccine against *P. aeruginosa* using immunoinformatics approach.

KEYWORDS: *Pseudomonas aeruginosa,* OprF, vaccine, immunoinformatics, multi-epitope.

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1. INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous gram-negative bacteria and the most important opportunistic bacterial pathogens that affects humans [1]. It is implicated in localized and systemic infections affecting varieties of organs in the body and resulting in chronic lung infections among cystic fibrosis patients [2]. It is also a cause of serious infections among immune-compromised cancer patients, burn patients, catheterized patients, and other hospitalized individuals [3]. It is medically significant and has a high intrinsic resistance to antibiotics due to its low outer membrane permeability, β-lactamase production and active efflux thereby causing wide spectrum of opportunistic infections [4]. The severity, incidence and high rate of resistance of *P. aeruginosa* to wide range of antibiotics suggests the need for new therapeutic options. Alternative therapies remains an urgent need for patients, hence, the need for vaccine development [5]. Vaccine development against infections caused by *P. aeruginosa* are attributed to its various cell-associated and secreted antigens. Antigens localized in the pili, flagella, extracellular slime layer, ribosomes and the outer membrane are often expressed in *P. aeruginosa.* Of all the outer membrane proteins in *P. aeruginosa*, the major outer membrane protein is OprF [6]. Outer membrane protein- OprF is an important and newer representative of vaccine candidate for *P. aeruginosa* infections which is necessary for the expression of full virulence in opportunistic pathogenic *P. aeruginosa*. [7, 5]. OprF anchors the outer membrane to the peptidoglycan layer and is involved in host-pathogen interactions [8]. Immunization with OprF immunogen elicits cross-reactive, opsonizing and protective antibodies in animal models or humans [1]. This study therefore aimed at exploring the potentials of *P. aeruginosa* OprF protein in designing multi-epitope subunit vaccine using immunoinformatics approach.

2. MATERIALS AND METHODS

2.1 Retrieval of OprF outer membrane protein sequences

A total of 10 OprF outer membrane protein sequences of *P. aeruginosa* were retrieved from the NCBI database. Retrieved sequences were further subjected to an antigenicity prediction using the ANTIGENpro database (Prob. Scores ≥ 0.8) [9].

2.2 Prediction of CTL & HTL Epitopes

A total of 9 out of 10 OprF sequences were subjected to NetCTL and IEDB server to predict CTL and HTL epitopes respectively. The MHCII binding predictions were made on 16/03/2020 using the IEDB analysis resource Consensus tool [10, 11]. High comb scores, length and immunogenicity scores were considered.

2.3 Construction of multi-epitope subunit vaccine

EAAAK, AAY and GPGPG linkers were used to construct the multi-epitope subunit vaccine (MEV), linking both suitable CTL and HTL epitopes. TLR 4-agonist (APPHALS) was used as Adjuvant [12]. Adjuvant and CTL epitope were joined by EAAAK linker while AAY and GPGPG linkers

were used to join the CTL and HTL epitopes, respectively.

2.4 B-cells epitope mapping

B-cells epitope mapping was performed using the ElliPro (IEDB Analysis Result) server [13].

2.5 Allergenicity and Antigenicity prediction of designed vaccine

Vaccine Allergenicity and Antigenicity was predicted using the AllerTOP v.2.0 and ANTIGENpro servers respectively [9].

2.6 Assessment of Physiochemical parameters

The Physiochemical parameters of vaccine construct which included the molecular weight, theoretical pI, aliphatic index, amino acid composition, instability index, half-life and hydropathicity were assessed using ProtParam server [9].

2.7 Prediction of 3D model of vaccine and structure refinement

Vaccine 3D model and secondary structure was predicted using RaptorX Server [9]. The 3D protein structure was further refined using GalaxyRefine [14], [15].

2.8 Disulfide engineering for vaccine stability.

Disulfide engineering was carried out using Disulfide by design v2.0 [16] to obtain stability of the final vaccine construct's modelled structure.

3. RESULTS AND DISCUSSION

3.1 Sequence retrieval of OprF outer membrane protein sequences and assurance of antigenic conduct

To design an immunogenic multi-epitope subunit vaccine, a total of 10 *P. aeruginosa* OprF outer membrane protein sequences were retrieved from the NCBI protein database. Major protein name is OprF which is the major outer membrane porin in bacteria belonging to the *Pseudomonas* genus [6]. Among 10 outer membrane protein sequences, only 9 proteins were found to be antigenic as predicted by ANTIGENpro. These 9 sequences were selected based on their score obtained for the probability of antigenicity and all these proteins having a score of ≥08 [9, 17]. The antigenicity scores obtained denoted the antigenic nature of selected protein sequences which can be used for the subunit vaccine designing [18].

3.2 CTL epitope prediction and immunogenicity assessment.

Cytotoxic T-lymphocytes are a CD8+ subset of T-cell responses typically made of peptide fragments. They are immune dominant and can elicit specific immune responses which is important for epitope-based peptide vaccine design [19], [20], [21], [22]. The CTL receptor specific immunogenic epitopes were predicted using the NetCTL 1.2 server and total of 253 CTL epitopes of 9mer length were obtained for the input of 10 *P. aeruginosa* OprF outer membrane protein sequences. In the next step, the immunogenicity of epitopes was determined following the instruction of IEDB [23], higher score indicate greater probability to elicit an immune response, therefore, a total 17 CTL epitopes with high immunogenicity score were selected and subjected to the vaccine designing (Table 1).

3.3 HTL epitope prediction.

Helper T-lymphocyte is the key player of both humoral and cell-mediated immune response [24]. Therefore, HTL receptor specific epitopes are probably going to be a crucial part of the prophylactic and immunotherapeutic vaccine [25], [26]. All 9 OprF outer membrane protein sequences were subjected to IEDB MHC-II epitope prediction module and 2654 epitopes of 15mer length were obtained. In order to be selected as high immunogenic epitopes, they must have a lower percentile rank and IC50 value [18], [27]. Only 8 epitopes with lowest percentile rank ranging from 0.03–0.3 were selected for the vaccine designing (Table 2). All these 8 epitopes were used for the vaccine construction.

Table 1: **Predicted cytotoxic T-lymphocyte (CTL) specific epitopes and their immunogenicity score obtained from the immune epitope database.**

S/N	Ancession ID	Epitopes	Comb scores	Immunogenicity scores	Selected or non-selected
1	sp_P13794_PO	LTDDVELAL	1.8181	0.6560	Selected
	sp_P13794_PO	FTENFFAKA	0.8349	-0.8480	Non-selected
2	tr_A0A069Q6I	QRDVDQLAY	1.5470	2.8290	Selected
	tr_A0A069Q6I	ATRGYGKEY	0.9862	3.1430	Selected
3	tr_I6QM72_I6	LTDDVELAL	1.8181	0.6560	Selected
	tr_I6QM72_I6	ELALSYGEY	1.6231	2.8040	Selected
4	tr_A6V748_A6	FTDSVRNMK	1.6551	0.1200	Selected
	tr_A6V748_A6	LTDDVELAL	1.8181	0.6560	Selected
5	tr_V6AHQ9_V6	ELALSYGEY	1.6231	2.8040	Selected
	tr_V6AHQ9_V6	NLTSLDAIY	1.1488	2.9150	Selected
6	tr_F6KQ72_F6	FTDSVRNMK	1.6551	0.1200	Selected
	tr_F6KQ72_F6	ELALSYGEY	1.6231	2.8040	Selected
7	tr_A0A5E5R7U	WLAKADKAY	1.1709	2.7950	Selected

	tr_A0A5E5R7U	QRDVDQLAY	1.5470	2.8290	Selected
8	tr_A0A0H2Z9M	FTDSVRNMK	1.6551	0.1200	Selected
	tr_A0A0H2Z9M	GTDAYNQKL	1.6791	0.6250	Selected
9	tr_A0A485F38	NLADFMKQY	1.3628	3.0990	Selected
	tr_A0A485F38	VRDVLVNEY	1.4333	3.1710	Selected
	tr_A0A485F38	HTDSVGTDA	1.2995	-0.8540	Non selected

Table 2: Predicted Helper T-lymphocyte (HTL) specific epitopes and their percentile rank obtained from the immune epitope database

S/N	Allele	Epitope	Method	Percentile rank
1	HLA-DRB1*01:01	MRKYIALPAVSLLAL	Consensus (comb.lib./smm/nn)	0.01
2	HLA-DRB1*01:01	RKYIALPAVSLLALA	Consensus (comb.lib./smm/nn)	0.01
3	HLA-DRB1*01:01	MRKYIALPAVSLLAL	Consensus (comb.lib./smm/nn)	0.01
4	HLA-DRB1*01:01	RKYIALPAVSLLALA	Consensus (comb.lib./smm/nn)	0.01
5	HLA-DRB1*01:01	KYIALPAVSLLALAL	Consensus (comb.lib./smm/nn)	0.10
6	HLA-DRB1*01:01	YIALPAVSLLALALA	Consensus (comb.lib./smm/nn)	0.10
7	HLA-DRB1*01:01	KYIALPAVSLLALAL	Consensus (comb.lib./smm/nn)	0.10
8	HLA-DRB1*01:01	YIALPAVSLLALALA	Consensus (comb.lib./smm/nn)	0.10

3.4 Construction of multi-epitope subunit vaccine.

A final vaccine construct of 382 amino acid residues was designed using 17 CTL and 8 HTL epitopes. To achieve maximum immune response, APPHALS was used as an adjuvant at the N-terminal site of the vaccine construct. Each joint was occupied by the suitable linkers as described by Nezafat *et al*., [20]. Adjuvant and CTL epitopes were combined together by EAAAK linker, AAY linker was used to join CTL epitopes while the HTL epitopes were joined using the GPGPG linker. Finally, vaccine construct was obtained having adjuvant, linker, CTL, and HTL epitopes in a sequence moving from N-terminal to C-terminal. As this designed subunit vaccine consisting of immunogenic CTL and HTL epitopes along with suitable adjuvant and linker, it may have the ability to inhibit the infections caused by *P. aeruginosa* in human host body [13].

3.5. B-cell epitope mapping.

B-cells play a major role in humoral immunity. An epitope corresponding to the B-cell receptor plays an important role in vaccine design following antibody production [13].Therefore, ElliPro (IEDB Analysis Result) server was used to reliably predict the linear B-cell epitopes as shown in Plate 1. This result revealed how the epitopes will best interact with B-cells. A total of 13 B-cell epitopes were predicted among the primary input sequence of the final vaccine construct.

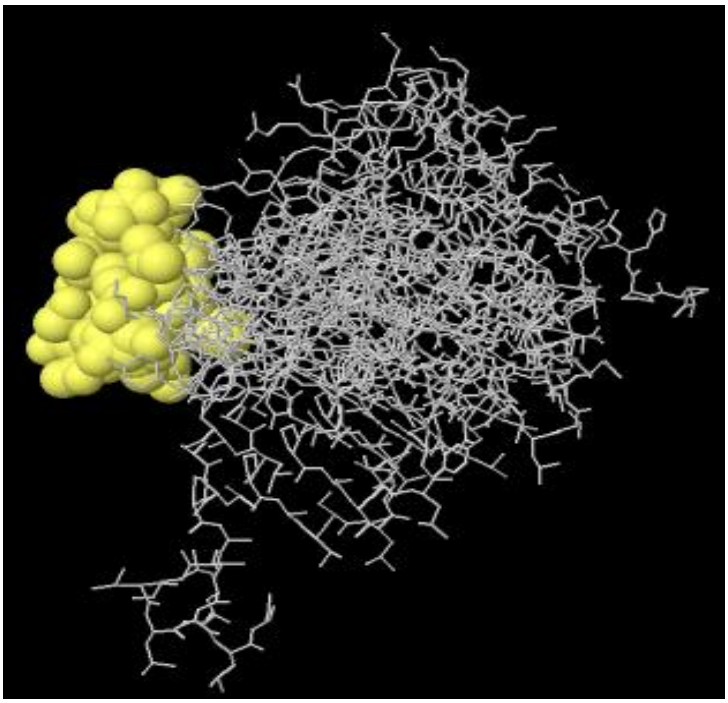

Plate 1: Humoral epitope prediction for subunit vaccine showing the linear B-cell epitopes among the 3D structure of final vaccine construct.

3.6 Antigenicity and allergenicity prediction of designed vaccine.

The antigenicity of the final vaccine construct was predicted using an ANTIGENPro server. The predicted probability of antigenicity was 0.81% which represents the antigenic nature of the vaccine construct [9]. The antigenicity score obtained for this vaccine construct is comparable with the antigenicity of subunit vaccine reported by Ali *et al.,* [14]. Allergenicity of the predicted vaccine

construct was determined using AllerTOP online server.

Result revealed that the vaccine protein is not allergic in nature, therefore, it is safe for the human use [16, 24].

3.7 Physiochemical parameters assessment using PROTPARAM

The physiochemical properties of vaccine construct were characterized by using PROTPARAM server and evaluated for seven parameters. The result of the assessment revealed that the molecular weight of vaccine protein to be 39966.15 kDa while the theoretical pI is 4.95. The total numbers of negative and positive charge residues were 31 and 26, respectively. The estimated half-life is 4.4 hours in mammalian reticulocytes (*in vitro*), >20 hours in yeast, in vivo) and >10 hours in *Escherichia coli* (*in vivo*). The instability index (II) was computed to be 23.22, which classifies the protein as stable. The values of aliphatic index and the grand average of hydropathicity **(GRAVY)** are 110.47 and 0.376. The estimated value of aliphatic index represents the thermostable nature of designed subunit vaccine because higher the value of aliphatic index, greater will be the thermo stability [20]. Conclusively, the designed vaccine is immunogenic and thermostable.

3.8 Secondary Structure Prediction

The 3D model result of the predicted secondary structure using the RaptorX web server is shown in Plate 2. Total of 382(100%) amino acid residues were modelled as two domains. 13(3%) positions were predicted as disordered. Secondary structure information resulting in the presence of 55% Helix, 1% Beta sheet and 42% Coiled structure was predicted. P-value is a parameter of homology modeling where low P-value defines the good quality of modeled structure [28]. The P-value obtained for the modeled structure was 5.15e-04 which is low and significant.

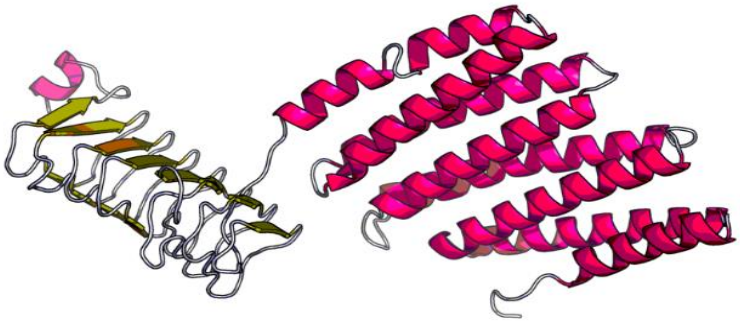

Plate 2: 3D secondary protein structure of the vaccine construct modelled using the RaptorX web server

3.9 Secondary Structure Refinement

The 3D protein refinement using GalaxyRefine server leads to the increase in a number of residues in the favoured region [14], [29], [30]. Plate 3A and 3B shows the initial structure and refined structure respectively while Plate 3C shows the visualization of refined models are displayed in rainbow colors and initial models in white.

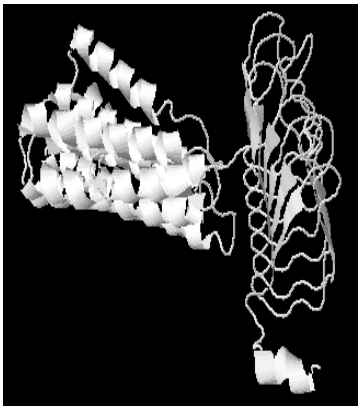

Plate 3A

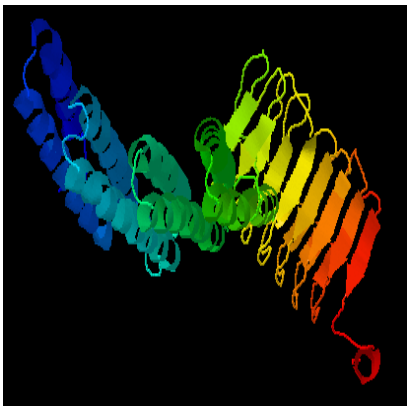

Plate 3B

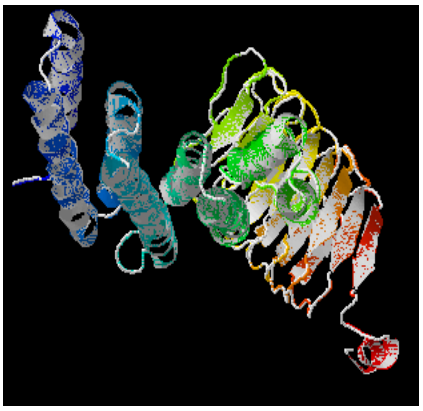

Plate 3C

Key: Plate 3A: Initial protein structure

Plate 3B: Refined protein structure

Plate 3C: Visualization of refined model displayed in rainbow colours and initial model displayed in white colour.

3.10 Disulfide engineering for vaccine stability.

Results showed that there are a total of 55 pairs of residues that can be used for the purpose of disulfide engineering. But after evaluation on other parameters like energy and Chi3 value, only sixteen pairs of residues were finalized because their value comes under the allowed range i.e. the value of energy should be less than 2.2 and Chi3 should be in between -87 and +97 degree [31]. Therefore, a total of 16 mutations were created. Plate 4A shows the original protein while Plate 4B shows the mutant protein. The disuphilde bonds (blue colour) are shown in the mutant created (Plate 4B).

Plate 4A

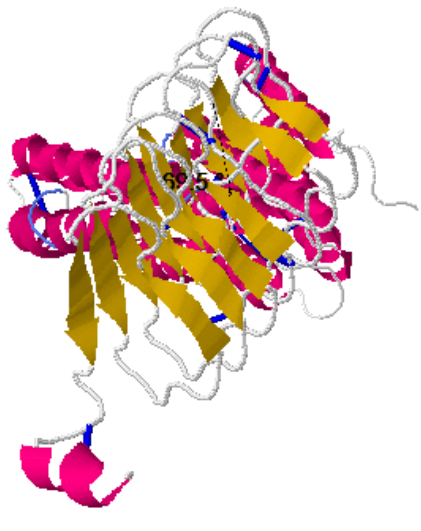

Plate 4B

Plate 4: Disulfide engineering to improve protein stability. (A) Original (B) Mutant (Disulphide bonds are shown in blue colour)

4. CONCLUSION

Infections caused by *P. aeruginosa* has resulted to high increase in morbidity and mortality rate, especially, as this organism has developed resistance to almost all available antibiotics. This research work has provided a multi-epitope subunit based vaccine having antigenic properties in the absence of allergenic properties against *P. aeruginosa* using immunoinformatics approach. Further studies will include an experimental validation of the proposed vaccine to ensure an effective control of *P. aeruginosa* infections.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

All authors declare no conflict of interests.

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