Infection, Genetics and Evolution 96 (2021) 105138

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Putative novel B-cell vaccine candidates identified by reverse vaccinology and genomics approaches to control *Acinetobacter baumannii* serotypes

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ARTICLE INFO

Keywords:
Putative vaccine
Acinetobacter baumannii
In silico
Genomics approaches

ABSTRACT

In the last decade, Multi-drug resistance (MDR)-associated infections of *Acinetobacter baumannii* have grown worldwide. A cost-effective preventative strategy against this bacterium is vaccination. This study has presented five novel vaccine candidates against *A. baumannii* produced using the reverse vaccinology method. BLASTn was done to identify the most conserved antigens. PSORTb 3.0.2 was run to predict the subcellular localization of the proteins. The initial screening and antigenicity evaluation were performed using Vaxign. The ccSOL omics was also employed to predict protein solubility. The cross-membrane localization of the protein was predicted using PRED-TMBB. B cell epitope prediction was made for immunogenicity using the IEDB and BepiPred-2.0 database. Eventually, BLASTp was done to verify the extent of similarity to the human proteome to exclude the possibility of autoimmunity. Proteins failing to comply with the set parameters were filtered at each step. In silico, potential vaccines against 21 *A. baumannii* strains were identified using reverse vaccinology and subtractive genomic techniques. Based on the above criteria, out of the initial 15 *A. baumannii* proteins selected for screening, nine exposed/secreted/membrane proteins, i.e., Pfsr, LptE, OmpH, CarO, CsuB, CdiB, MlaA, FhuE, and were the most promising candidates. Their solubility and antigenicity were also examined and found to be more than 0.45 and 0.6, respectively. Based on the results, LptE was selected with the highest average antigenic score of 1.043 as the best protein, followed by FimF and Pfsr with scores of 1.022 and 1.014, respectively. In the end, five proteins were verified as promising candidates. Overall, the targets identified herein may be utilized in future strategies to control *A. baumannii* worldwide.

1. Introduction

Today, the excessive use of antibiotics has gradually led to antibiotic and antimicrobial compound resistance, posing many challenges to public health and medicine (Gharaghie et al., 2018). Acinetobacter is among the troublesome emerging bacteria, which are nosocomial pathogens of the respiratory system. Many studies have been done on the infections caused by this organism in recent years (Gharaghie et al., 2020). *A. baumannii* is among the most successful extracellular bacteria of this genus at becoming resistant to antibiotics, usually leading to failure in medical treatments (Peleg et al., 2008). The number of problems caused by these bacteria is increasing, and the consequences of antimicrobial compounds used in the future are not clear; so, measures must be taken to reduce these issues (Roca et al., 2015). On the other hand, developing new antibiotics is very costly and reminds us that this approach should be replaced with cost-effective prevention methods (Li and Webster, 2018). Recently, a consensus has been reached that a strategy is needed to inhibit many basic resistance mechanisms and prevent them from spreading (Reygaert, 2018). Therefore, specific strategies or their combinations, such as vaccination (McConnell and Pachón, 2010), monoclonal antibodies (Nielsen et al., 2017), and phage therapies (LaVergne et al., 2018), have been developed. Accordingly, vaccination can be a potent prevention method for this infection agent, especially in high-risk groups (Ainsworth et al., 2017). Since *A. baumannii* is a Gram-negative, extracellular, bacterial pathogen, B cells and immunoglobulin are expected to play a major role in the host's response to pathogens. This theory is supported by clinical and experimental evidence. Individuals with *A. baumannii* infections have shown significant titers of serum-specific IgM, IgA, and IgG responses (Islam et al., 2011). Various studies have also shown that passive transfer of

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https://doi.org/10.1016/j.meegid.2021.105138
Received 16 June 2021; Received in revised form 12 October 2021; Accepted 9 November 2021
Available online 15 November 2021

immune sera or various formats of particular antibodies (polyclonal, monoclonal, and single domain/chain) protects naive animals against *A. baumannii*, fully or partially (McConnell et al., 2011; Luo et al., 2012; García-Quintanilla et al., 2014; Chen, 2020).

Recently, *A. baumannii* vaccines have been designed based on outer membrane vesicles (OMV) and outer membrane complexes (OMC) (McConnell et al., 2011). However, no effective vaccine has been produced against these bacteria (Ahmad et al., 2016). After identifying and sequencing whole pathogen genomes and developing bioinformatics tools, vaccines can be logically designed using molecular knowledge and informatics. The cloning of nlpD (Hashemzehi et al., 2017), nlpA (Hashemzehi et al., 2018), ompA (Ansari et al., 2019), and Pal (Lei et al., 2019) immunogens revealed that the identified vaccine candidates were immunogenic following injection into the mice.

In order to select vaccines candidates, these computational methods aid in enhancing our understanding of infections and pathogenesis and designing effective vaccines (Oli et al., 2020). Different software programs like Vaxign (Flower et al., 2010), PSORTb (D'Mello et al., 2019), ccSOL omics (Agostini et al., 2014), IEDB (Motamedpour et al., 2020), and VaxiJen (Zaharieva et al., 2017) have been created to aid the identification of potential vaccine candidates. These programs are easily accessible and help identify possible vaccines and save time and money.

2. Methods

While adhering to the principles of reverse vaccinology, the following steps were taken to identify and screen candidate vaccines. The genes were first extracted from credible articles, and their sequences were subsequently extracted from the GenBank repository and then BLASTed. A credible source is supported by bioinformatics data and clinical studies. The protein localization within the membrane was determined with PSORTb. The antigenicity and solubility of the proteins were determined using Vaxijen and ccSOL, respectively. Finally, every antigen's epitopes were determined using IEDB servers, and the proteins with appropriate antigenicity (threshold antigenic score = 1.000) were chosen (Fig. 1).

2.1. Initial antigen screening

2.1.1. Identification of vaccine candidate antigen types

Fifteen proteins, including surface and non-surface proteins (Bailey et al., 2018; Doosti et al., 2015; Brossard and Campagnari, 2012; Ghorbani-Dalini et al., 2015; John and Venter, 2014; Kamischke et al., 2019; Kargar et al., 2012; Kenyon and Hall, 2013; Krasauskas et al., 2020; Lari et al., 2018; LaVergne et al., 2018; Lee et al., 2020; Liu et al., 2016; Mendez et al., 2012; Morris et al., 2019; Musafer and Essa, 2021; Otero et al., 2018; Pakharukova et al., 2018; Pantophlet et al., 2001; Powers et al., 2020; Ramezanalizadeh et al., 2020; Ramirez et al., 2019; Roussin et al., 2019; Sciuto et al., 2018; Sheldon and Skaar, 2020; Sklar et al., 2007; Souod et al., 2013), were selected from 21 published articles about *A. baumannii* vaccine production to identify different candidate vaccines. Non-surface proteins can also be vaccine candidates if they are

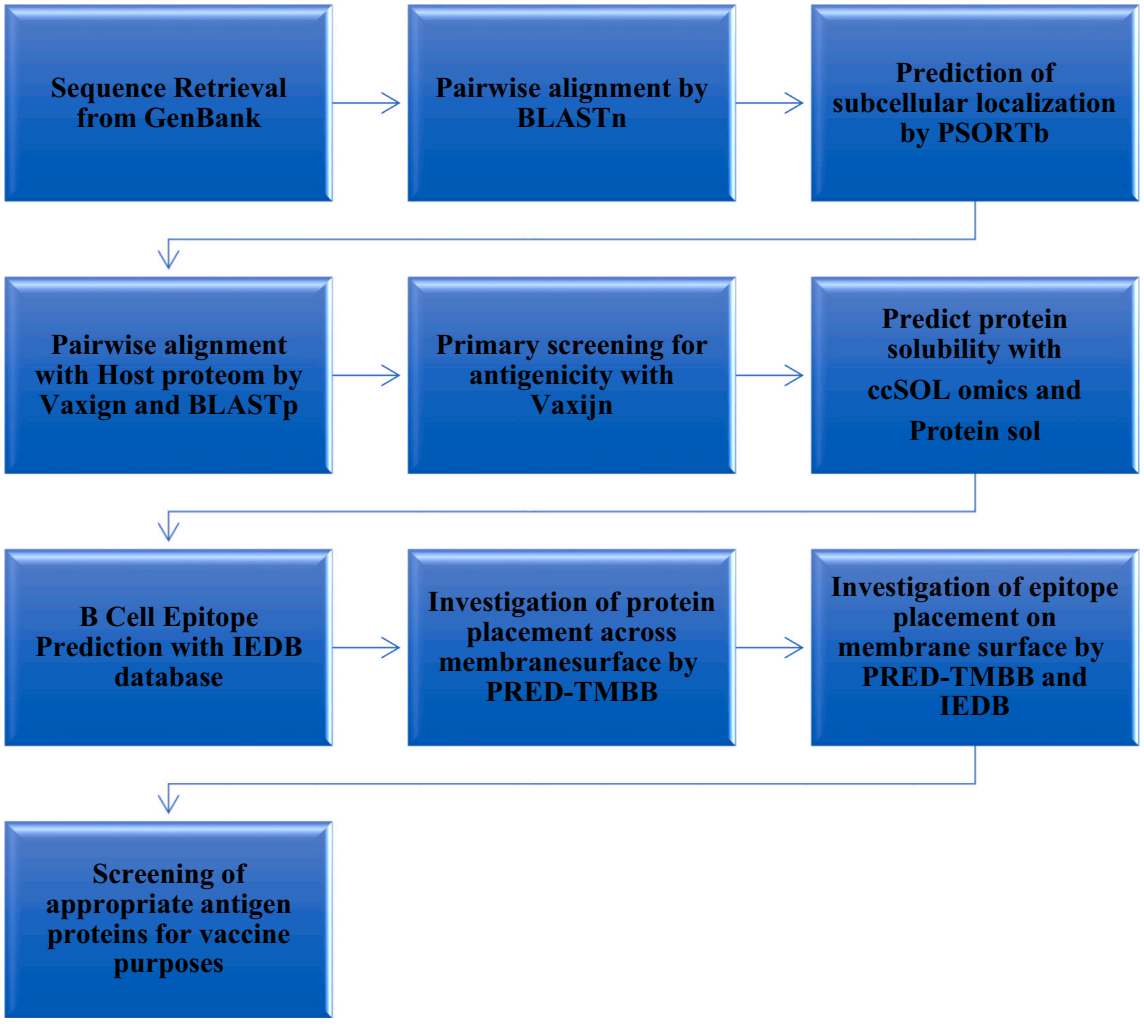

Fig. 1. A methodology flowchart to be followed in the reverse vaccinology approach for prioritization of vaccine candidates against *Acinetobacter baumannii*.

secretory. Hence, non-surface secreted proteins were also screened. The protein-coding DNA sequences for each protein were acquired from the GenBank repository.

2.1.2. Sequence alignment with A. baumannii

Fifteen proteins were selected for BLASTn. Table 1 lists the gene names and accession numbers for their coding sequences (CDS). BLASTn was used for the alignment of the protein genes. The alignment organism was set to *A. baumannii*, with a 98% similarity cut-off threshold for each protein. The sequences with the highest identity percentage were chosen as *A. baumannii* strain sequences.

2.1.3. Prediction of protein localization in the bacteria

Initially, the protein sequences were accessed through UniProtKB (http://www.uniprot.org/). Subsequently, these proteins were analyzed using PSORTb to determine their location of function within the bacteria. In this program, a cut-off threshold of above 7.5 was used to determine protein localization efficiently. The PSORTb website introduces 7.5 as a good cut-off above which a single localization can be assigned, and the precision and recall values for the program are calculated using this cut-off (https://www.psort.org/documentation/index.html#limitations). The "organism" and "Gram stain" attributes in this software were set to "bacteria" and "Gram-negative." By using these settings, the function location and localization of the proteins were determined, as shown in Table 1.

2.1.4. Host protein similarity prediction

Similarity to human proteins must be negligible to reduce the possibility of a reciprocal response of the host cells to the vaccines. Therefore, the organism setting was set to only *Homo sapiens* (Taxid: 9606). Using BLASTp, proteins without significant resemblance to the human proteome (less than 35%) were chosen as vaccine candidate proteins, as shown in Table 2. Initial protein filtering was done with the Vaxign database (www.violinet.org). This filtering entailed adhesion probability (0–1.0) and similarity to host proteins filtering of the protein capable of selecting the target microorganism.

2.2. Screening for antigenic and immunogenic properties of the proteins

2.2.1. Prediction of the antigenicity of the proteins

The online server of Vaxijen (www.ddg-pharmfac.net) is designed for classifying antigenic properties of proteins. By using the selected

Table 1
Preliminary screening and identification of proteins.

Protein	Symbol	Accession numbers	PSORTb		Vaxign			VaxiJen
			Region localized	Score	Adhesin Probability	Similarity to Host Proteins	Y/N	
Putative ferric siderophore receptor protein	Pfsr	Abo13714	OuterMembrane	8.2	0.279	Human Mouse Pig	N N N	0.8
lipopolysaccharide transport E	LptE	AJF80516	Periplasmic/ OuterMembrane	7.5	0.492	Human Mouse Pig	N N N	0.67
surface antigen	SurA	ABO11811	Periplasmic	7.5	0.291	Human Mouse Pig	N N N	0.64
Outer membrane protein H	OmpH	ADX04189	Periplasmic/Extracellular	8	0.688	Human Mouse Pig	N N N	0.62
Carbapenem resistance outer membrane protein	CarO	QFQ06350	OuterMembrane	10	0.854	Human Mouse Pig	N N N	0.62
Fimbrial protein F	FimF	QCR58064	Cytoplasmic/Extracellular	7.5	0.629	Human Mouse Pig	N N N	0.6
pilus protein	CsuB	AJF82295	Extracellular	9.6	0.736	Human Mouse Pig	N N N	0.59
contact-dependent growth inhibition A	CdiA	QDC12537	OuterMembrane	8.5	0.31	Human Mouse Pig	N N N	0.57
putative phospholipid-binding lipoprotein MlaA	MlaA	ATP88409	Periplasmic/ OuterMembrane	7.5	0.146	Human Mouse Pig	N N N	0.53
Fe(III)-rhodotrulic acid uptake	FhuE	ABO12504	OuterMembrane	10	0.51	Human Mouse Pig	N N N	0.53
Lactate utilization protein A	LutA	SVK36206	Cytoplasmic	7.9	0.125	Human Mouse Pig	N N N	0.52
AdeABC efflux pump	AdeS	ADM92606	InnerMembrane	10	0.283	Human Mouse Pig	N N N	0.51
CdiB transporter	CdiB	QDC12536	Outer Membrane	10	0.31	Human Mouse Pig	N N N	0.49
Outer membrane lipoprotein RcsF	RcfC	VCW98738	Cytoplasmic/ InnerMembrane	8	0.736	Human Mouse Pig	N N N	0.42
Iron compound ABC transporter	IroN	CRF38690	Periplasmic	8.5	0.447	Human Mouse Pig	N N N	0.4

Table 2
Query coverage and maximum identity of the various *Acinetobacter* strains.

Symbol	Microorganism	Strain. No	Accession	Query coverage (%)	Per. Ident	E Value
Pfsr	*Acinetobacter baumannii*	ATCC 17978	ABO13714.1	100%	100.00%	1.00E-64
		MDR-TJ	SSP98905.1	100%	98.92%	3.00E-64
		1,494,580	AFI93827.1	100%	98.92%	3.00E-62
	Humo sapiens	(taxid:9606)	No significant similarity found			
LptE	*A. baumannii*	ABNIH10	WP_059246221.1	100%	99.41%	1.00E-119
		NCGM 237	BAN88851.1	100%	99.41%	4.00E-119
		BBR72179.1	WP_182638964.1	100%	98.82%	8.00E-119
	Humo sapiens	(taxid:9606)	No significant similarity found			
SurA	*A. baumannii*	846,928	WP_032058516.1	100%	99.05%	4.00E-73
		472,237–120	WP_031984521.1	100%	99.05%	9.00E-73
		573,719	EXS24881.1	78%	98.78%	9.00E-55
	Humo sapiens	(taxid:9606)	No significant similarity found			
OmpH	*A. baumannii*	NCGM 237	BAN87408.1	100%	100.00%	5.00E-117
		AC12	WP_077108975.1	100%	99.40%	8.00E-117
		PR07	WP_114225175.1	100%	99.40%	1.00E-116
	Humo sapiens	(taxid:9606)	No significant similarity found			
CarO	*A. baumannii*	AC12	WP_000866528.1	98%	100.00%	2.00E-178
		909,768	WP_162215526.1	100%	99.60%	2.00E-180
		AGQ07589.1	WP_020753383.1	100%	99.60%	3.00E-180
	Humo sapiens	(taxid:9606)	No significant similarity found			
FimF	*A. baumannii*	ZW85-1	WP_114225040.1	100%	100.00%	3.00E-64
		SSR07093.1	WP_114154194.1	100%	98.96%	5.00E-64
		NIPH70	WP_005135242.1	100%	98.96%	9.00E-64
	Humo sapiens	(taxid:9606)	No significant similarity found			
CsuB	*A. baumannii*	AB0057	WP_000876475.1	100%	100.00%	1.00E-124
		MBD0122575.1	WP_188138631.1	100%	99.42%	4.00E-124
		EXR78715.1	WP_033853954.1	100%	98.84%	8.00E-124
	Humo sapiens	(taxid:9606)	No significant similarity found			
CdiB	*A. baumannii*	Taxid 470	QDC12536.1	100%	100.00%	0
		JCM 6841	EGY6081855.1	100%	98.96%	0
		MSP4-16	EGY7940593.1	100%	98.45%	0
	Humo sapiens	(taxid:9606)	No significant similarity found			
MlaA	*A. baumannii*	AB307-0294	WP_001109851.1	100%	100.00%	0
		MRSN4106	WP_001109852.1	100%	99.67%	0
		NCGM 237	BAN88772.1	100%	99.67%	0
	Humo sapiens	(taxid:9606)	No significant similarity found			
FhuE	*A. baumannii*	ATCC 17978	ABO12504.1	100%	100.00%	2.00E-176
		24860_1	EZJ15792.1	97%	99.57%	2.00E-167
		273,929	SSR39419.1	97%	99.57%	2.00E-167
	Humo sapiens	(taxid:9606)	No significant similarity found			
LutA	*A. baumannii*	AB	SST07512.1	100%	100.00%	0
		AB	SCZ16821.1	100%	99.26%	0
		AB	SST89794.1	100%	98.77%	0
	Humo sapiens	(taxid:9606)	No significant similarity found			
AdeS	*A. baumannii*	AB0057	WP_072292300.1	100%	100.00%	0
		AB307-0294	WP_000837449.1	100%	99.72%	0
		6,013,150	HAV6278770.1	100%	99.45%	0
	Humo sapiens	(taxid:9606)	No significant similarity found			
CdiA	*A. baumannii*	Taxid 1236	QDC12537.1	100%	100.00%	0
		OIFC0162	WP_002040896.1	100%	89.90%	0
		WC-348	WP_072688622.1	100%	89.86%	0
	Humo sapiens	(taxid:9606)	No significant similarity found			
RcsF	*A. baumannii*	EGY2289799	WP_000793316.1	100%	100.00%	3.00E-92
		MBF6833633	WP_195197370.1	100%	99.23%	1.00E-91
		Taxid 1236	VCW98805.1	100%	86.92%	4.00E-70
	Humo sapiens	(taxid:9606)	No significant similarity found			
IroN	*A. baumannii*	NIPH 67	WP_196255994.1	100%	99.69%	0
		1,413,735	EGY8259740.1	100%	99.38%	0
		846,928	WP_196085996.1	100%	99.38%	0
	Humo sapiens	(taxid:9606)	No significant similarity found			

protein sequences, their antigenicity was calculated, which is provided in Table 1.

2.2.2. Protein solubility prediction

Protein solubility is an important principle in vaccine design. Therefore, there are many information databases to predict this attribute of proteins. The ccSOL algorithm predicts protein solubility using physicochemical properties. The ccSOL omics accepts multiple submissions and generates solubility profiles to identify soluble fragments within each polypeptide chain. The solubility profile represents a unique signature used to discriminate soluble and insoluble proteins obtained from heterologous expression experiments (with an accuracy of 78%). The server also computes single-point mutations throughout the whole protein sequence to identify susceptible areas. For this reason, the ccSOl omics is the best and most efficient database available for calculating protein solubility (http://service.tartaglialab.com/grant_submission/ccsol_ omics). Fig. 1 demonstrates the solubility of the proteins as calculated in this database with a graph representation of the results. Afterward, the results were verified using the solubility score from the Protein-sol sequence solubility database (protein-sol.manchester.ac.uk). The scaled solubility value (QuerySol) is the predicted solubility. The population average for the experimental dataset (PopAvrSol) is 0.45,

and therefore any scaled solubility value greater than 0.45 is predicted to have a higher solubility than the average soluble. *E.coli* protein from the experimental solubility dataset (Niwa et al., 2009) and any protein with a lower scaled solubility value is predicted to be less soluble. According to the results of this website, at the threshold of 0.45, more than 70% of protein amino acids had solubility (> 70%).

2.2.3. B-cell epitope prediction

A) "Maximum antigenic scores" of peptides were used as a selection criterion of the most antigenic proteins. Immunogenicity was calculated using the antigenic scales of Kolaskar and Tonga Onkar in the IEDB database. The maximum, average, and minimum B-Cell epitope immunogenic scores were acquired using this database. The antigenic score cut-off was 1.000. In general, whole proteins with a score higher than 1.000 are considered potentially immunogenic on this website.

B) Evaluation of a linear epitope's dataset using BepiPred-2.0: BepiPred-2.0 (http://www.cbs.dtu.dk/services/BepiPred/) was used to download a database of current linear proteins. Only peptides verified as positives in two or more different tests were included in the positive database, and only peptides validated as negatives in two or more different investigations and not found positives in any assay were included in the negative data source (Fig. 4).

2.2.4. Protein surface sequence evaluation

In vaccine design, prediction of protein epitope localization and its external placement surface is important. Therefore, the PRED-TMBB (bioinformatics.biol.uoa.gr), one of the best databases for identifying protein sequences and their membrane localization, was used. This database can predict with only multiple alignments and no need for amino acid sequences and further information. The website is also very powerful for discrimination studies because it can detect external membrane water-soluble proteins in a big dataset, which creates a robust and trustworthy method for screening entire genomes. This web server helps apply discriminating processes on amino acid sequences, localize membrane strands, and find ring topology.

3. Results

3.1. Sequence alignment with A. baumannii

Fifteen antigens were identified and selected based on prior studies with the maximum identicality. Protein screening was continued according to the parameters stated in the methods. Table 2 lists the alignment results of the selected proteins using BLASTp. The query coverage and maximum identity of the various *Acinetobacter* strains with the query strains are shown in the table.

3.2. Prediction of subcellular localization of proteins by PSORTb

Nine out of fifteen chosen proteins with localization scores of above 7.5 were predicted as surface proteins. They included Pfsr, LptE, OmpH, CarO, CsuB, CdiB, MlaA, FhuE, and CdiA that were used for further analysis. SurA and IroN were identified as periplasmic proteins; so, they were excluded from further study. Proteins LutA and RcfC were identified as cytoplasmic ones while AdeS was found to be an internal membrane protein; all of them were excluded from further analysis. The nine external membrane proteins were selected based on analysis results of PSORTb (Table 1) and moved to the next level of analysis.

3.3. Prediction of similarity to host proteins

By using the Vaxign web server, the similarities to human, mouse, and pig proteins were evaluated. This program allows users to dynamically insert protein sequence(s) and set parameters as vaccine target(s). Table 1 summarizes the results of these evaluations. The similarities to the human genome were evaluated using BLASTp. According to Table 2, no protein showed significant similarity to *H. sapiens*.

3.4. Antigenicity of proteins

The antigenicity was determined with a cut-off size of 0.6 using the VaxiJen server and the selected protein sequences. As shown in Table 1, the proteins Pfsr, LptE, SurA, OmpH, CarO, and FimF were selected as the ones with appropriate antigenicity (≥ 0.6). From the six proteins above, SurA was cast aside because of its periplasmic nature, and the rest were used as appropriate proteins to evaluate solubility and flexibility.

3.5. Protein solubility and flexibility

Solubility can show the quality of a protein's function. Solubility details can indicate a unique signature of a protein that can be used to distinguish soluble and insoluble proteins resulting from heterologous expression experiments (with 78% accuracy).

- According to Fig. 2, all five proteins Pfsr, LptE, OmpH, CarO, and FimF had appropriate solubility ranges. The SolPro server was used to measure solubility. Proteins with scores below 0.45 were considered insoluble, and those with scores higher than 0.45 were considered soluble. Therefore, proteins Pfsr، LptE، OmpH، CarO, and FimF with scores of 0.65, 0.6, 0.78, 0.64, and 0.59, respectively, showed appropriate solubility.

3.6. Prediction of B-cell epitopes by immune epitope database (IEDB) and BepiPred-2.0

The proteins Pfsr، LptE، OmpH، CarO, and FimF were screened as proteins with appropriate antigenicity, solubility, and immunogenicity. Since BepiPred-2.0 was educated on linear epitopes, its efficacy was assessed on a dataset of verified positive and negative peptides acquired from the immune epitope database to ensure a fair comparison (IEDB, see Materials and methods). Fig. 4 depicts the results of this benchmark. Helix (H - pink probability gradient), Sheet (E - blue probability gradient) and Coil (C - Orange probability gradient) predicted using NetsurfP. Surface: Buried(B)/Exposed(E) from NetsurfP's default threshold, and orange gradient illustrating predicted relative surface accessibility. The orange gradient protein sequence shows Positions above the threshold epitope. Therefore, all screened proteins with orange color above 70% show high epitope characteristics of sequences. Based on the results, LptE with an average score of 1.043 was chosen as the best antigenic choice, followed by FimF and Pfsr with scores of 1.022 and 1.014, respectively.

3.7. Epitope recognition and external epitope localization

Following the results of Fig. 3, the weighing of selected protein epitopes in the bacterial membrane was predicted by PRED-TMBB. Parts of the proteins within the internal membrane were colored green, the parts spanning the membrane were red, and the parts outside of the membrane or on the surface of the bacterial membrane were colored blue. In concurrence with the results, each of the selected proteins had its unique membrane weight, as shown in Fig. 3. The epitope sequences of the selected proteins were gathered from IEDB, as can be seen in Table 4. Using the beginning and ending amino acid numbers of the epitope, the sequence was overlaid onto the PRED-TMBB results, and outer and inner membrane parts were identified and subsequently recognized as functional regions of each protein. The more epitopes protrude outwards the surface, and the lengthier the protruding amino acid sequences are, the higher the reached protein immunogenicity would be. Based on this analysis, all five proteins Pfsr, LptE, OmpH,

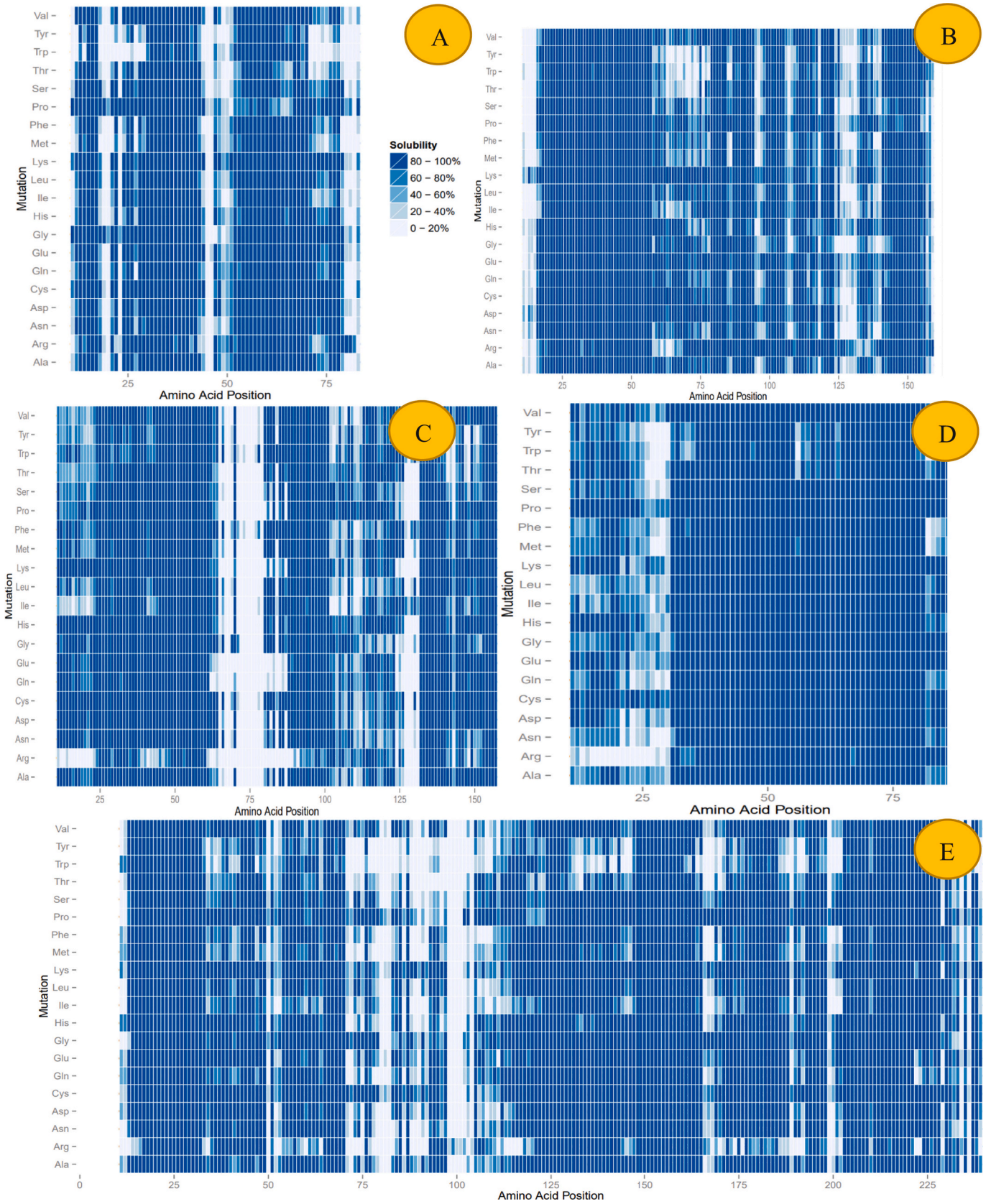

Fig. 2. Solubility of proteins Pfsr (A), LptE (B), FimF (C), OmpH (D), CarO (E).

Fig. 3. localization weight of the proteins Pfsr (A), LptE (B), OmpH (C), CarO (D), and FimF (E) in bacterial membranes.

CarO, and FimF scored well above the cut-off threshold (Table 3). Table 4 exactly shows the antigenic property of the analyzed protein. Therefore, Pfsr, with two functional external epitopes that added up to seventeen amino acids, was identified and selected as the most antigenic protein. Each of the proteins LptE, OmpH, CarO, and FimF were identified with average antigenicity since they had some epitopes protruding from the membrane surface, as shown in Table 4.

4. Discussion

Traditional vaccines produced based on Pasteur's guidelines became formidable instruments in the history of medicine, resulting in the eradication of some of the world's most deadly infectious illnesses in less than a century (Smith, 2012; Ansari et al., 2019). Most of the vaccinations that could be created using traditional technologies had been developed by the end of the twentieth century, and new technologies were necessary to combat the remaining diseases (Rauch et al., 2018). During this time, new technologies like recombinant DNA and chemical conjugation of proteins to polysaccharides, as well as advancements in the adjuvants, made significant progress (Lu et al., 2021). The capacity to access the genomes of microbes, a new technology that became available in 1995 when Craig Venter released the genome of the first free-living organism, was also a significant tool (Carvalho, 2014). Reverse vaccinology studies are part of the novel sciences usable for identifying antigenic candidates (Meunier and Azimzadeh, 2016). This report includes a genomic study aimed at finding a candidate vaccine against *A. baumannii* strains. A study pertaining to identification of a vaccine candidate for a wide range of *A. baumannii* strains has not yet been discovered. The novelty of the present study lies in the exploration of genomics in identifying the common vaccine candidate for a range of *A. baumannii* strains instead of an individual organism. This is termed "pan genome reverse vaccinology" where various strains of an organism are taken into consideration while developing a vaccine candidate (Naz et al., 2019). In addition, a particular vaccination for stimulating B-cell against this bacterium has yet to be discovered. As a result, our work fills a gap in previous studies on a B-cell booster vaccine against various *A. baumannii* serotypes. *A. baumannii* is a Gram-negative organism with many serotypes being constantly discovered worldwide. Recently, serotypes resistant to multiple *A. baumannii* treatment drugs have spread worldwide and are becoming a global issue (Basatian-Tashkan et al., 2020). Most of the research on this issue is focused on finding novel antibiotics and using nanotechnology to fight this pathogen. However, the expensive nature and time-consuming process of finding novel antibiotics show the urgent need for an efficient vaccine against this pathogen. On the other hand, no holistic study has been done to identify candidate vaccines against *A. baumannii* strains (Giau et al., 2019). Reverse vaccinology is a novel method of finding candidate vaccines

Table 3
B cell epitope prediction. Antigenic scores of the analyzed proteins.

Protein	Minimum antigenic score	Maximum antigenic score	Average antigenic score	Threshold antigenic score
LptE	0.884	1.195	1.043	1
FimF	0.9	1.225	1.022	1
Pfsr	0.902	1.132	1.014	1
OmpH	0.896	1.152	1.013	1
CarO	0.867	1.207	1.013	1

Table 4
Comparison of the selected proteins with the PRED-TMBB database to evaluate extracellular epitopes.

Protein	Epitop			Out	Epitope outer part
	Start- End		Epitop		
Pfsr	−42	31	GLDVHVPGYTVF	16–41	LDVHVPGYTV
	−80	74	ENLVTLG	64–82	ENLVTLG
LptE	−15	4	AQRLAAVVLTLG	0	–
	−26	17	SAGLVGCGFH	0	–
	−47	35	TPLVYKKLSLELP	45–52	LPA
	−63	56	QLKVYLTA	0	–
	−82	74	AYVLRVLEY	0	–
	−104	94	TEVLLRLTVTF	83–95	TE
	−150	143	LQRIVIDD	0	–
OmpH	−21	6	KLMLGLGLTVASVAAN	0	–
	−40	24	GYGVIDLAKVVESSTYL	0	–
	−53	48	NQSVKP	0	–
	−93	86	KLQSQYQS	92–104	QS
	−112	106	QSRVQTS	0	–
	−152	146	TVAYDAK	0	–
CarO	−17	4	LRVLVTTTALLAAG	0	–
	−30	22	DEAVVHDSY	0	–
	−43	37	LIPVGAR	0	–
	68	53-	GALLWQANPYVGLALG	0	–
	−123	114	QGLYIAAGAA	0	–
	−154	149	QQAVPG	125–168	QQAVPG
	−176	170	APYLGFG	0	–
	−194	188	FGEVGAY	0	–
	−212	200	KVELTQYNLAPVT	196–236	KVELTQYNLAPVT
FimF	−24	7	KILVLAVFSTSPLFCYAN	15–52	STSPLFCYAN

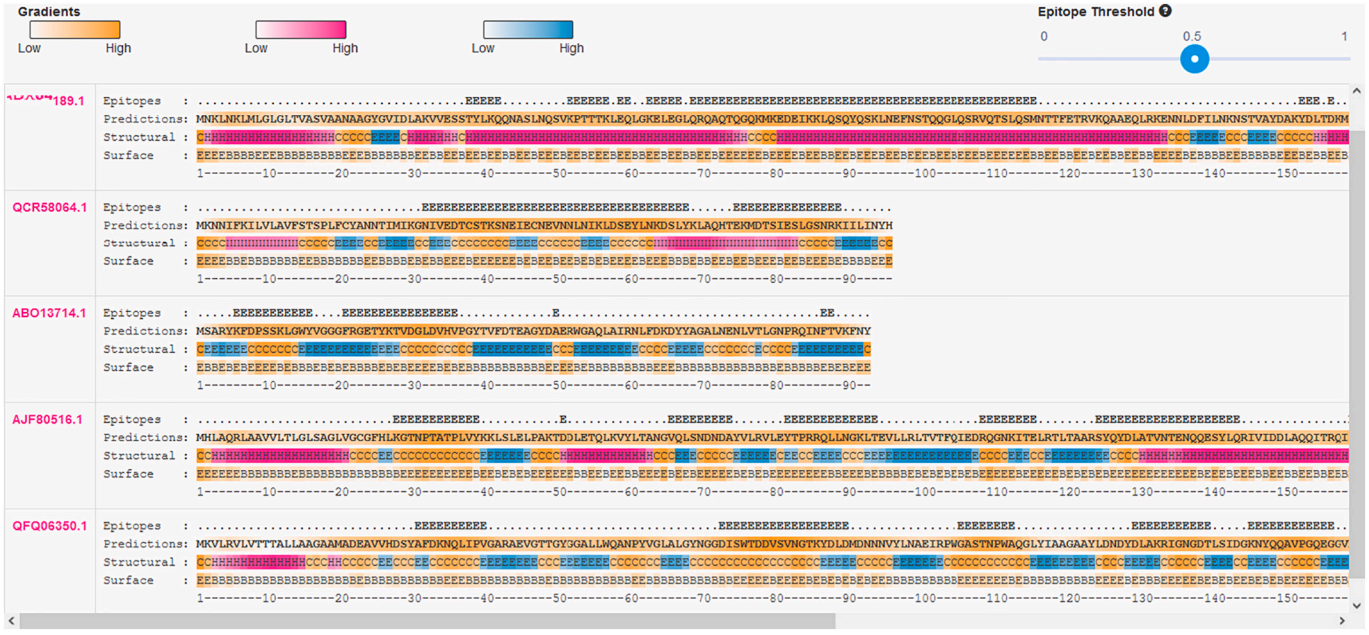

Fig. 4. Linear B cell epitope's dataset using BepiPred-2.0 (The BepiPred-2.0 and NetSurfP predictions for each query sequence are shown on the Summary output page in Advanced Output mode). The orange gradient protein sequence shows Positions above the threshold epitope. Therefore, all screened proteins with orange color above 70% show high epitope characteristics of sequences.

using the *A. baumannii* genome. The first classical example of practical reverse vaccinology was an attempt to identify possible vaccine candidates for meningococcus serogroup B (*Neisseria meningitides*) that was published in 2000. The proteins identified by reverse vaccination in this study induce a bactericidal antibody response in mice (Pizza et al., 2000). The immunogenicity of vaccines designed by reverse vaccination was so high that after some time, a global vaccine for the meningococcus serogroup B was chosen in 2006 (Giuliani et al., 2006), yielding encouraging results at the end of human clinical experiments in 2011 (Toneatto et al., 2011). The search for pathogen antigens continued until Chiang et al. (2015a, 2015b) used reverse vaccinology to effectively identify three antigens as potential vaccines. These antigens were found to be highly immunogenic and provided partial protection (60%–85%) in a pneumonia animal model (Chiang et al., 2015a, 2015b).

Reverse vaccinology gives us the possibility of screening the proteome with useful parameters such as protein localization, non-similarity to human proteome, and MHC binding affinity that lowers the trial and errors needed to find candidate vaccines (Sette and Rappuoli, 2010). Given the genomic and cellular protein information available on pathogens, one can use simple reverse vaccinology software to discover a specific antigen or a group of antigens in a short screening timeframe for vaccine design programs (Dalsass et al., 2019).

On the other hand, it is a cost-effective method with genomic data. Additionally, reverse vaccinology can be used for a wide range of pathogens, i.e., bacterial, fungal, viral, and parasitic ones. This approach can be universally used for any organism with the BLAST tool (Talukdar et al., 2014).

The IEDB epitope prediction tool analyzes the input sequence for a variety of organisms. This is while most databases are built only for a specific organism. For example, SCLpred only predicts eukaryotic proteins (Lu et al., 2004). Therefore, the IEDB webserver was used to predict a B-cell epitope immunogenic score. The main goal of this study was to exploit web-based bioinformatics tools for the prediction of antigenic proteins in *A. baumannii*. Previous studies on *A. baumannii* surface proteins like OmpK and nlpD have been done, and immunity has been achieved in mice (Chiang et al., 2015a, 2015b). Strategies and efforts to achieve a reverse vaccinology-based candidate vaccine against this upcoming pathogen (*A. baumannii*) have been summed up by Shahid et al. (2019). Fereshteh, 2020 reported DcaP and HP-2 as potent vaccine candidates with desirable antigenic properties against *A. baumannii* (Fereshteh, 2020).

In antigenic protein screening, four noteworthy properties are immunogenicity, localization, lack of similarity to human proteome, and external surface localization of epitopes (Liljeroos et al., 2015). In this study, BLASTp was used to select protein sequences with 100% Per. Ident score among all the species available in the database. Preliminary protein screening was done by evaluating protein similarity of target proteins to three host organism proteomes, i.e., human, mouse, and pig proteomes, with the Vaxign database. The surface localized proteins are highly immunogenic, and therefore, can be used as vaccine candidates for many years. Accordingly, the protein localization of candidate vaccines was predicted using PSORTb.

Afterward, the initial immunogenicity screening of candidate proteins was done using the Vaxijn database. If a protein has a high solubility in water, it has better antigenicity for vaccine design (Saylor et al., 2020). Therefore, the databases ccSOL omics and Protein-sol were used. Then, the antigenic scales of Kolaskar and Tongaonkar were used in the IEDB database to calculate antigenic scores for different screened peptides. Using BLASTp in NCBI, the similarity to human proteins was assessed. Finally, external epitopes were identified using the PRED-TMBB database via comparison to B-cell epitopes, and the antigenic proteins were found. The benefit of calculative analysis for predicting antigens is screening between countless proteins to select potentially valid antigens. This process can be used as initial filtering done in vaccine design (Flower, 2008).

The efficacy of vaccine candidates introduced by reverse vaccination has been documented in a number of investigations. LptE was previously identified as an important bacterial protein that plays an important role in building LPS and molecular pathogenesis of Gram-negative bacterial infections through interaction with LptD. Additionally, in a study by Zha et al. (2016), LptD was identified as a candidate vaccine against Vibrio species. The results of the study are in concurrence with those of the study by Chiang et al. (2015a, 2015b) on the OmpH protein as an identified candidate vaccine against *A. baumannii*. Bazmara et al. (2019) selected the CarO protein as an efficient immunogenic IROMP in *A. baumannii* that aids in iron attraction as a siderophore, which agrees with recent findings on the antigenic properties of CarO as an ideal vaccine design target (Bazmara et al., 2019). These studies show that CarO and OmpH, which were screened in this research and found to be antigenic, are truly recognition sites for targeting *A. baumannii*.

Despite all advantages of reverse vaccination, it cannot be utilized to find vaccine candidates on its own replication of the genuine disease, and as a result, the subsequent immunity is extremely excellent. Both the cellular and humoral immune systems are stimulated by these vaccinations (Pollard and Bijker, 2021). As a result, it seems, traditional vaccines provide significant effectiveness of the immune system as a consequence of their that combining standard traditional approaches with reverse vaccination can improve vaccine candidate discovery.

In the current study, three other proteins, i.e., LptE, FimF, and Pfsr, were screened and found to be more antigenic than the proteins found in other studies. These proteins can be assessed in wet-lab conditions to find their efficacy as candidate vaccines. Finally, they can be used as a conserved and immunogenic vaccine against *A. baumannii* strains. This is a preliminary study that is cost-effective and fast for vaccine screening in comparison to traditional screening methods for verifying the antigenic properties of a candidate protein vaccine.

Credit author statement

Abbas Doosti conceived and designed the Conceptualization and Methodology. Seyed Abbas Mirzaei performed the experiments, Software, Validation and Investigation. Sheida Beiranvand analyzed the In-silico data, Formal analysis, Data Curation and Writing - Original Draft. Abbas Doosti supervised the experiments. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Declaration of Competing Interest

All authors declare that they have no conflict of interest.

Acknowledgements

The authors would like to thank the staff members of the Biotechnology Research Center of the Islamic Azad University of Shahrekord Branch in Iran for their help and support. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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