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Highly conserved exposed immunogenic peptides of Omp34 against *Acinetobacter baumannii*: An innovative approach

Abolfazl Jahangiri[a], Iraj Rasooli[b,*], Parviz Owlia[c,d], Abbas Ali Imani Fooladi[e], Jafar Salimian[f]

[a] Department of Biology, Shahed University, Tehran, Iran
[b] Department of Biology, Shahed University, Tehran-Qom Express Way, Tehran, Iran
[c] Department of Microbiology, Shahed University, Faculty of Medical Sciences, Tehran, Iran
[d] Molecular Microbiology Research Center (MMRC), Shahed University, Tehran, Iran
[e] Applied Microbiology Research Center, System Biology and poisoning institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
[f] Chemical Injuries Research Center, System Biology and poisoning institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

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ABSTRACT

Omp34, also known as Omp34kDa or Omp33–36 is a virulence factor associated with *A. baumannii* metabolic fitness or its adherence and invasion to human epithelial cells. This protein is also introduced as a specific antigen which could induce strong antibody responses. In the present *in silico* study, recent vaccine design strategies such as 'antigen minimization' and 'high epitope density' were invoked to design a soluble immunogen with higher antigenicity. As an advantage, the tools employed in the current study are easily available. Exposed peptides in linear B-cell epitopes were predicted and their conservancy and immunogenicity were evaluated. In this regard, constructs were designed by removal of inappropriate regions. Based on the obtained results the external loops (L1–L7) were exclusively considered of which L3, L6 and L7 were the most appropriate of which the most appropriate were in L3 > L6 > L7 order while L2 was assigned as an inappropriate peptide. The final construct, named Omp34-4, encompasses three copies of L3, two copies of L6 and L7 and one copy of L1, L4 and L5. The designed construct is predicted to be a soluble antigen with enhanced epitope density and antigenicity. Omp34 is present in > 1600 strains of *A. baumannii* with ≥ 98% identity. So, it could be applicable in diagnostic kits and an immunotherapy choice against *A. baumannii*. It could be presumed that co-administration of Omp34-4 and a recently designed OmpA-derived antigen could confer sufficient protection against *A. baumannii*-associated infections. *In vitro* and *in vivo* experiments are needed to confirm all these data. The innovative approach could be generalized to vaccine designs focused on OMPs.

1. Introduction

The nosocomial pathogen *Acinetobacter baumannii* is becoming an increasingly serious health threat such that it has been assigned as one of the six most dangerous microbes by the Infectious Diseases Society of America (IDSA) (Huang et al., 2016). Pneumonia, meningitis, bloodstream infections, skin and soft tissue infections and urinary tract infections are considered among multiple infection types caused by this highly successful pathogen (McConnell et al., 2013). Rapid emergence of multidrug resistant (MDR) strains of the pathogen with no available commercial effective antibiotic leads to difficulty of *A. baumannii* clinical management (Pachón and McConnell, 2014). These implications suggest active and passive immunization as a cost-effective approach against the notorious pathogen. In this regard, various immunogens have been investigated among which, protein antigens (*e.g.* OmpA (Fajardo Bonin et al., 2014, Lin et al., 2013, Luo et al., 2012), OmpW (Huang et al., 2015), Bap (Fattahian et al., 2011) and Omp34 (Fajardo Bonin et al., 2014)) are highly appreciated as promising immunogens (reviewed in (Ahmad et al., 2016, Chen, 2015)).

The outer membrane protein (OMP), Omp34, is also known as Omp34kDa and Omp33–36. Omp34 is a specific antigen which could serve for detection of *A. baumannii* (Islam et al., 2011). Islam et al. (2011) demonstrated Omp34 could be uniquely recognized by IgM, IgA, and IgG from patients infected by *A. baumannii* with no cross-reaction with sera from patients infected by other bacteria. Moreover, this OMP was enriched in outer membrane vesicles (OMVs) employed by McConnell et al. (2011) as a vaccine candidate against *A. baumannii*.

Recently, Omp34 was highlighted as a potential vaccine candidate against *A. baumannii via* an immunoproteomic study (Fajardo Bonin et al., 2014). Furthermore, this highly immunogenic protein plays a role

* Corresponding author at: Department of Biology, Shahed University, Tehran-Qom Express Way, Opposite Imam Khomeini's shrine, Tehran 3319118651, Iran.
E-mail addresses: a.jahangiri@shahed.ac.ir (A. Jahangiri), rasooli@shahed.ac.ir (I. Rasooli), owlia@shahed.ac.ir (P. Owlia).

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in adherence and invasion of the pathogen to human epithelial cells (Smani et al., 2012). In addition to fibronectin binding (Smani et al., 2012), this virulence factor is associated with *A. baumannii* cytotoxicity (Smani et al., 2013) as well as its metabolic fitness (Smani et al., 2012). This protein induces apoptosis and inhibits autophagy in human cells. Inhibition of autophagy modulated by Omp34 enables the pathogen to persist inside autophagosomes (Rumbo et al., 2014). Although OMPs have been proposed as strong immunogens by several studies (Fajardo Bonin et al., 2014; Huang et al., 2015; Huang et al., 2016; Lin et al., 2013; Luo et al., 2012; Toobak et al., 2013a, 2013b), recombinant OMPs are not sufficiently soluble, limiting their attractiveness as powerful immunotherapeutic preparations (Ahmad et al., 2016).

Nowadays, bioinformatics and immunoinformatics are exhorting researchers to employ rational vaccine design approaches instead of conventional empirical vaccine development strategies (Khalili et al., 2014). In this regard, “antigen minimization” could be of interest as an effective approach in which domains of a given protein containing only protective epitopes are focused upon elicit responses against the epitopes of interest (Kulp and Schief, 2013). The protective epitopes could be revealed through experimental or *in silico* studies. These epitopes could be increased in a single protein molecule by a novel strategy known as “high epitope density” (Liu and Chen, 2005; Pei et al., 2009). In addition to epitopes predictions (Jahangiri et al., 2012; Rahbar et al., 2012; Sefid et al., 2013) and vaccine designs (Farhadi et al., 2015; Hajighahramani et al., 2017; Jahangiri et al., 2011; Jahangiri et al., 2017; Khalili et al., 2015; Nazarian et al., 2012; Nezafat et al., 2016; Shahbazi et al., 2016), discerning of three-dimensional protein structures (Khalili et al., 2016, 2017a, 2017b; Sefid et al., 2013) and protein functions (Khalili et al., 2017a, 2017b; Mohammadpour et al., 2015; Mohammadpour et al., 2016) could also benefit from valuable bioinformatic tools. Moreover, *in silico* studies could obviously reduce effort, time, expense and inevitable inherent ethical conflicts of experimental studies. In the present study, we integrate various easily available bioinformatic and immunoinformatic tools to predict exposed linear B-cell epitopes within the Omp34 sequence. Then, novel Omp34-derived antigens were designed to arrive at a final putatively soluble antigen with enhanced predicted immunogenicity as well as the B-cell epitopes density.

2. Methods

2.1. Sequence availability and topology prediction

A reference sequence of Omp34kDa protein with accession no. WP_000733005.1 was extracted from NCBI at http://www.ncbi.nlm.nih.gov/protein in FASTA format. FASTA format is a standard text-based format representing protein sequences as single-letter codes. Further analyses were carried out on this sequence.

Topology of the protein was predicted by PRED-TMBB2 (Tsirigos et al., 2016) at http://www.compgen.org/tools/PRED-TMBB2. PRED-TMBB2 is an updated version of the PRED-TMBB method with improved performance and new features. This is a newly developed method with high accuracy and reliability for predicting topology of beta-barrel outer membrane proteins and discriminating these proteins from water-soluble proteins (Tsirigos et al., 2016). This server could also ascertain signal peptide within a given sequence.

2.2. Linear B cell epitope predictions

In order to generate the most reliable results, linear B cell epitopes of Omp34 were predicted by various algorithms and tools: BCPREDS (EL-Manzalawy et al., 2008), EPMLR (Lian et al., 2014), BepiPred (Larsen et al., 2006), SVMTriP (Yao et al., 2012) and LBtope (Singh et al., 2013). BepiPred at http://www.cbs.dtu.dk/services/BepiPred/ combines a propensity scale method with a hidden Markov model to predict linear B-cell epitopes (Larsen et al., 2006). The threshold was kept as default (0.35) in which the sensitivity and specificity of the predictions are 0.49 and 0.75 respectively. BCPREDS at http://ailab.ist.psu.edu/bcpred/ benefit from the subsequence kernel (EL-Manzalawy et al., 2008). Fixed length epitope mode of BCPred was set. All epitope lengths were assessed. The specificity was set as default (75%). SVMTriP at http://sysbio.unl.edu/SVMTriP/ combines Support Vector Machine (SVM) with the Tri-peptide similarity and Propensity scores (SVMTriP). The AUC value of SVMTriP is 0.702 (Yao et al., 2012). All available epitope lengths provided as options were set for the Omp34 sequence. EPMLR at http://www.bioinfo.tsinghua.edu.cn/epitope/EPMLR/ employs multiple linear regression (MLR). The AUC value of EPMLR is 0.728 (Lian et al., 2014). Default threshold of -0.15 was kept. All available various lengths of the epitopes provided as options were set for the Omp34 sequence. LBtope at http://www.imtech.res.in/raghava/lbtope/index.php has three different models that can be selected (LBtope_Fixed, LBtope_Variable and LBtope_Confirm models). Since validity of a created dataset is a challenge in algorithm development, LBtope_Confirm model was harnessed to predict potential linear B-cell epitopes of Omp34. This model is developed based on only epitopes (1042 unique B-cell epitopes) or non-epitopes experimentally validated at least by two studies. This model can also predict variable length epitopes (Singh et al., 2013). The default size of window length (15 aa) was set for the prediction.

2.3. Epitope screening and selection

The external loops contained in epitopes assigned by at least 3 harnessed tools of previous section (*i.e.* Section 2.2 Linear B cell epitope predictions) were considered as the most reliable peptides to be selected. All external loops of Omp34 served as separate queries to VaxiJen (Doytchinova and Flower, 2007) with default threshold of the server (0.4) at http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html in order to determine their individual antigen probability. VaxiJen is the first alignment-independent antigen predictor with accuracy of 70–89% (Doytchinova and Flower, 2007).

2.4. Peptide conservancy

A protein BLAST search restricted to *A. baumannii* was run against non-redundant protein sequences on the Omp34 sequence at https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome. Hits with E-value < 0.001 were considered as reliable results. Among those hits, hits with query coverage $\geq 99\%$ and identity $\geq 99\%$ were considered. To analyze epitope conservancy, all external loops of Omp34 were used as individual inputs at http://www.iedb.org/. The search was restricted to linear peptidic epitopes with $\geq 70\%$, $\geq 80\%$, or $\geq 90\%$ identity to the input sequences.

2.5. Construct designs, analyses and refinement

Two strategies (‘antigen minimization’ and ‘high epitope density’) were integrated to achieve constructs of interest. In the following, in order to arrive at the best regions as well as decreasing false negative and positive results, two approaches were tracked and their resulting constructs were evaluated. In the first selection and design, only topology and linear B-cell epitope predictions were invoked by which, the most reliable exposed epitopes were incorporated. In the second selection and design approach, in addition to topology and linear B-cell epitope predictions, antigen probability of loops was also considered. The first construct was designed as follows: External loops contained in reliable linear B-cell epitopes (L3, L4, L5, L6 and L7) were retained. The remaining loops along with their flanking transmembrane β-strands and internal turns (residues 1–115) were removed. The remaining transmembrane β-strands served as linkers instead of using artificial ones. Then, remaining internal turns (in3, in4, in5 and in6) were replaced by

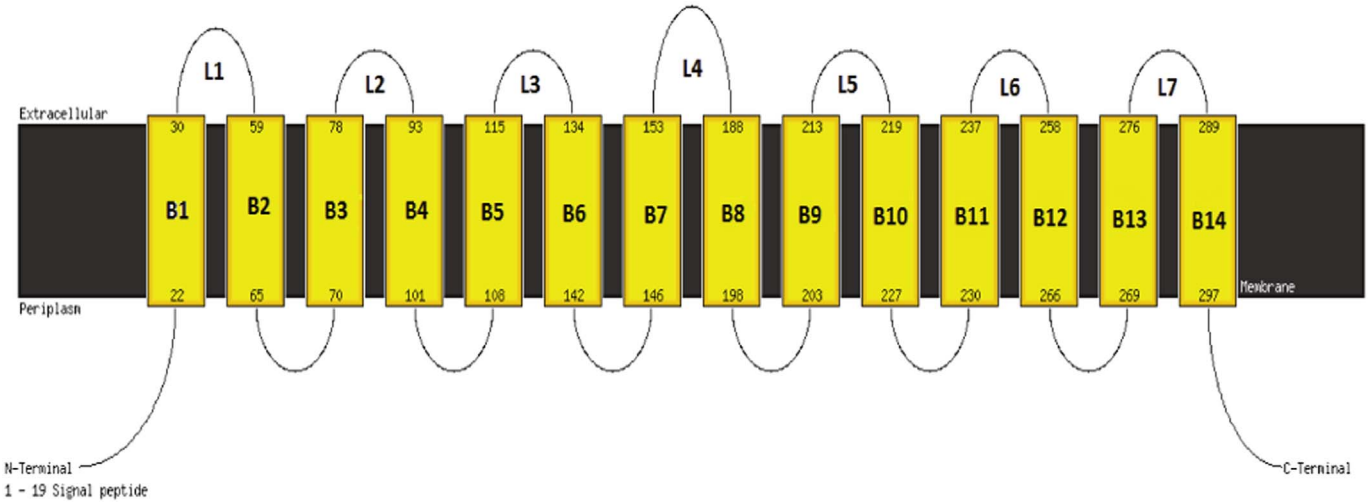

Fig. 1. Predicted topology of Omp34. External loops: L1–L7, transmembrane β-strands: B1–B14.

long external loops contained in the most reliable linear B-cell epitopes (L3, L4, L6 and L7) respectively. This construct was named Omp34-1.

The second construct was designed as above except that the internal turns internal turns (in3, in4, in5 and in6) were respectively replaced by those external loops with the highest VaxiJen scores (L3, L1, L6 and L7). This construct was called Omp34-2.

Certain physicochemical properties (number of amino acids, molecular weight and theoretical pI) of the designed constructs were assessed by "ProtParam" (Gasteiger et al., 2005) at http://web.expasy.org/protparam/.

The probable antigenicity of the designed constructs as well as mature Omp34 was estimated by "VaxiJen".

Retrieval of the original linear B-cell epitopes in the context of designed constructs was predicted using "BepiPred". IEDB tools at http://tools.iedb.org/bcell/ were invoked to predict hydrophilicity (Parker et al., 1986), flexibility (Karplus and Schulz, 1985) and surface accessibility (Emini et al., 1985) of Omp34 and the designed construct. These properties (hydrophilicity, flexibility and surface accessibility) are involved in B-cell epitope predictions. Higher scores of these properties mean higher probability of the peptides containing B-cell epitopes.

Omp34-2 was modified based on BepiPred and IEDB analyses results. Segments with low score of BepiPred, hydrophilicity, flexibility and surface accessibility were considered as unfavorable. Since propensity scales are involved in BepiPred predictions, this method was selected to retrieve linear B-cell epitopes and to determine unfavorable residues. The most unfavorable residues regarding B-cell epitope properties were removed. The obtained sequence was called Omp34-3. Finally, to improve antigenicity as well as linear B-cell epitope density, L3: "HTDVDGKNNFSKDDNGDR" was added to the C-terminal region of the Omp34-3 sequence and then, was reanalyzed by all tools used in previous steps. This construct was named Omp34-4. These sequences were reanalyzed by "ProtParam", "VaxiJen", IEDB tools and "BepiPred". Moreover, solubility of mature Omp34 and Omp34-4 was estimated by SOLpro (Magnan et al., 2009) at http://scratch.proteomics.ics.uci.edu/. SOLpro predicts if a protein is soluble or not upon overexpression in *E. coli* as well as the corresponding probability with overall accuracy of > 74% using a two-stage SVM architecture based on multiple representations of the primary sequence (Magnan et al., 2009).

2.6. Data validation

An integrative strategy (combination of topology, propensity scale and B-cell epitope prediction results) was applied to attain more confident predictions, epitope identification and construct designs. To enhance the accuracy of the results, the state-of-the-art servers, tools, methods and algorithms were employed for performed analyses. B-cell epitopes of designed constructs were predicted to retrieve the original ones in Omp34. Moreover, to achieve the most reliable results, some predictions were supported by previous experimental evidence (del Mar Tomás et al., 2005; Liu and Chen, 2005; Rawling et al., 1995; Toobak et al., 2013a, 2013b).

3. Results and discussion

3.1. Omp34 topology

One of the most important criteria for a B-cell epitope is its exposure to antibodies (Rawling et al., 1995). In case of OMPs, external loops could be in line with this criterion (Rawling et al., 1995) such that it is predicted that potential protective B-cell epitopes could be exclusively found among those located in external loops. Hence, determining Omp34 topology should reveal valuable epitopes. PRED-TMBB2 assigned the protein as a 14-β-stranded transmembrane OMP classified as the putative general bacterial porin family member. Residues 1–19 were determined to be a signal peptide. Reliability of the prediction was 0.831. Reliability is the overall consistency of the measure. Reliability values are ranging between 0.00 (much error) and 1.00 (no error). Reliability scores are correlated with prediction accuracy (Melen et al., 2003; Tsirigos et al., 2016). Distribution of residues in the predicted topology is illustrated in Fig. 1. In mature Omp34, about 50% of residues are located in external loops implying the remaining regions localized in outer membrane of *A. baumannii* are shielded from antibodies.

3.2. Linear B cell epitopes

Predicted epitopes with length of ≥ 7 aa were assumed as linear B-cell epitopes. Those located in external loops of the OMP were considered as appropriate ones (Fig. 2). Supplementary Table 1 shows details of the predicted epitopes by various programs/algorithms.

Based on topology and B-cell epitope predictions, L3: 116–133 "HTDVDGKNNFSKDDNGDR" (contained in epitopes assigned by BepiPred, BCPREDS and EPMLR), L4: 154–187 "SVANQFALDNFGIIGNGIYSAVNQTAAIQNDQDA" (contained in epitopes assigned by all harnessed tools), L5: 214–218 "GQENQ" (contained in epitopes assigned by BepiPred, BCPREDS and EPMLR), L6: 238–257 "VGNDGEADIKGNDLGEFRQA" (BepiPred, BCPREDS and EPMLR) and L7: 277–288 "MKADVKKSSYDT" (BepiPred, BCPREDS and SVMTriP) were selected as loops encompassing the most probable B-cell

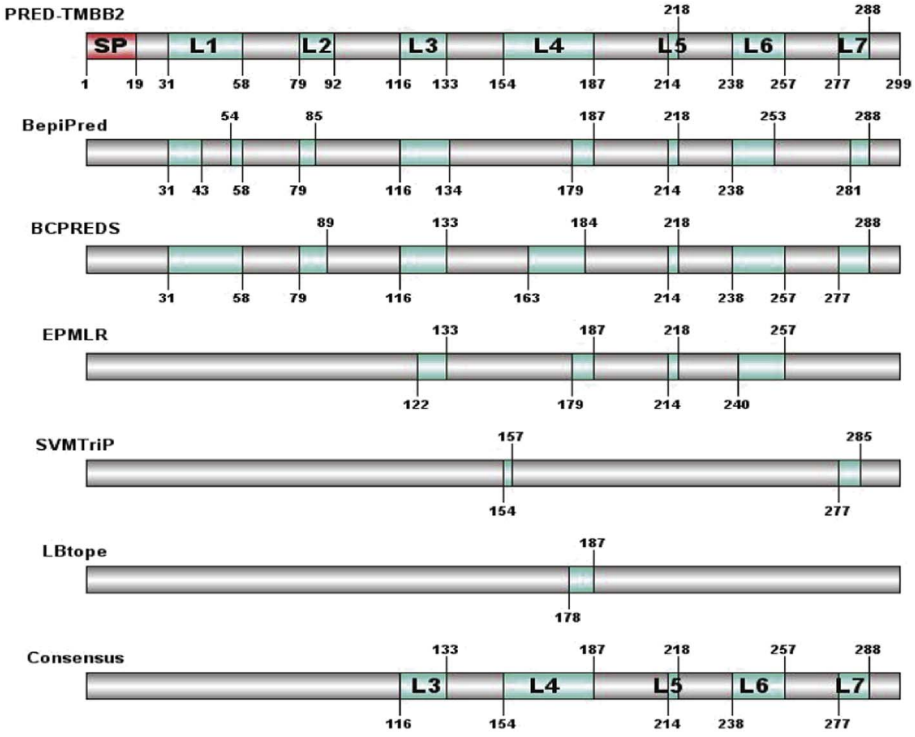

Fig. 2. Shematic illustration of topology and linear B-cell epitope predictions. Signal peptide (SP) and external loops (L1–L7) predicted by PRED-TMBB2 are shown in first row. The next rows show linear B-cell epitopes and their positions on the sequence predicted by various servers/methods. The last row (consensus) represents external loops contained in epitopes assigned by at least 3 linear B-cell epitope predictors. The Figures was illustrated by IBS: Illustrator for Biological Sequences (Liu et al., 2015), version 1.0.3.

epitopes.

Antigen probability of external loops (L1–L7) was estimated as follow: L1: 0.9339, L2: 0.2144 (Probable NON-ANTIGEN), L3: 1.6445, L4: 0.3092 (Probable NON-ANTIGEN), L5: no result was reached, L6: 1.2997 and L7: 1.2741.

3.3. Identification of conserved peptides

Among 112 hits (with E-value < 0.001) obtained from BLAST search, only 23 first ones were matched to the criteria (query coverage ≥ 99% and identity ≥ 99%). Hence, these sequences were selected and analyzed.

One of the most important criteria which is considered in antigen selection from the proteome of a given bacterium is its prevalence and conservancy among various strains of the pathogen (Ahmad et al., 2016; Chen, 2015; Kazemi Moghaddam et al., 2017). This fact is taken into account for Omp34 because the reference sequence of Omp34 protein (accession no. WP_000733005.1) is completely conserved (with 100% identity) in > 1500 strains of *A. baumannii*. Moreover, the Omp34 sequence was presented in > 1670 *A. baumannii* strains with ≥ 98% identity. Omp22 is presented in 851 *A. baumannii* strains with > 95% identity (Huang et al., 2016). The protein sequence of OmpW is presented in 804 *A. baumannii* strains with > 91% identity (Huang et al., 2015). So, Omp34 could be one of the most promising antigens with respect to prevalence and sequence conservancy.

Results of loop conservancy searches are detailed in Table 1 and Supplementary Table 2.

Homology of loops with epitopes found in other organisms would be considered in some respects: 1. Homology with human proteins could be concerned with respect to safety of construct; 2. In diagnostic applications, cross-reactivity with these organisms is possible; 3. These homologies could help in unveiling evolutional and functional aspect of Omp34.

In vaccine design, safety of the immunogen is an important criterion. Among loops of Omp34, L2, L5 and L6 were considered safe with no sequence similarity with human epitopes. Other loops share homology with some human epitopes; hence, if *in vitro* and *in vivo* experiments would confirm the predicted cross-reactivity, caution should be taken in using these loops.

Two solutions could be suggested if the cross-reactivity was occurred: 1. avoiding use of these loops in construct design; 2. engineering these loops to reduce cross-reactivity (*e.g.* replacement or removal of identical residues).

These cautionary points need further experimental analyses, which were not performed as part of this study.

3.4. Constructs: designs, evaluations and refinements

Extraction and preparation of individual antigenic proteins from *A. baumannii* for vaccine development is not practical; hence, cloning and expression in *E. coli* were adapted to produce the proteins (Ahmad et al., 2016; Fattahian et al., 2011). In such antigen preparations, B-cell epitopes located at inaccessible regions of the antigen in native form, could misspend antibody triggering of immune response. Moreover, overexpression in a heterologous expression system could render the preparation insoluble and may affect structure of the produced proteins such that protection by a given protein antigen may be altered (Ahmad et al., 2016). These disadvantages make it worthwhile to employ protective linear B-cell epitopes of the protein as well as constructs whose design is based on these epitopes (Weimer et al., 2009; Worgall et al., 2005). Two primary constructs (Omp34-1 and Omp34-2) was designed based on the integrative approach. The rationales behind selected and excluded regions are as follow. The highest scored epitope determined by “BepiPred” is located at L3. In contrast, the highest scored epitopes predicted by “BCPREDS” is located in an inaccessible region (residues 94–115). So, this region was not selected in construct design since it could trigger antibodies which would not recognize the Omp34 native form in *A. baumannii*. L4 and L6 are involved in the highest scored epitopes predicted by “EPMLR” while, the signal peptide and L7 are involved in the highest scored epitopes predicted by “SVMTriP”. Moreover, the highest scored epitope determined by “LBtope” consists of signal peptide and β1. L1 and L2 did not pass the “epitope screening” step. Overall, since no appropriate segment was detected in 1–115 residues, this region was not incuded in the first designed construct.

Table 1
Antigens and their corresponding organisms which have homologous epitope(s) (≥ 70% identity) with Omp34 loops.

Loops	Antigen	# epitopes	Organism
L1	Receptor-type tyrosine-protein phosphatase-like N	12	*Homo sapiens* (human)
	Complement C3	1	
	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	1	
	Receptor-type tyrosine-protein phosphatase N2	1	
	Hexon protein	5	Human mastadenovirus C
	LipL32	3	*Leptospira interrogans*
	Unidentified protein	1	Unidentified
	Other Plasmodium berghei protein	1	*Plasmodium berghei*
	Protein A26	1	Vaccinia virus
L2	Flagellar hook-associated protein 2	3	*Campylobacter jejuni*
L3	Mitogen-activated protein kinase kinase kinase 5	1	*Homo sapiens* (human)
	Acetylcholine receptor subunit epsilon	2	
	Interferon regulatory factor 1	1	
	Protein Tax-2	1	Primate T-lymphotropic virus 2
L4	Glyceraldehyde-3-phosphate dehydrogenase	5	*Homo sapiens* (human)
	Snurportin-1	1	
	Large neutral amino acids transporter small subunit 1	1	
	Genome polyprotein	1	Japanese encephalitis virus
	UniProt: B8ZRH1	1	*Mycobacterium leprae*
	Mucin-associated surface protein (MASP), putative (UniProt: K4DN48)	9	*Trypanosoma cruzi*
	Mucin-associated surface protein (MASP), putative (UniProt: K4DS80)	1	
	ATP synthase subunit beta	9	
	Succinyl-CoA ligase [ADP-forming] subunit beta	2	*Mycobacterium tuberculosis*
	Genome polyprotein	1	Enterovirus A
L5	No match was found	–	–
L6	Erythrocyte membrane protein 1, PfEMP1 (UniProt: Q8I639)	3	*Plasmodium falciparum* (malaria parasite *P. falciparum*)
	Beta-conglycinin, alpha' chain (UniProt: P11827)	2	*Glycine* max (soybeans)
L7	Other *Homo sapiens* (human) protein	1	*Homo sapiens* (human)
	E3 ubiquitin-protein ligase TRIM37	1	
	Ankyrin repeat protein	3	*Ehrlichia chaffeensis*

Table 2
Physicochemical properties, antigen probability and BepiPred predictions for mature Omp34 and the designed constructs.

	Omp34 (mature)	Omp34-1	Omp34-2	Omp34-3	Omp34-4
Number of amino acids	280	257	251	239	257
Molecular weight (Da)	30,235.30	27,551.23	26,973.64	25,739.25	27,755.28
Theoretical pI	4.54	4.53	4.67	4.79	4.77
Antigen probability	0.7868	0.7504	0.7900	0.8208	0.8669
Average hydrophilicity	2.041	2.304	2.437	2.505	2.620
Average flexibility	0.998	1.001	1.005	1.008	1.009
Average surface accessibility	1.000	1.000	1.000	1.000	1.000
Average BepiPred	0.330	0.322	0.371	0.398	0.467

Moreover, predicted internal turns of Omp34 are inaccessible in its original host, *i.e. A. baumannii*; hence, these inappropriate turns were replaced by the most appropriate loops (L3, L4, L6 and L7) based on the epitope prediction results.

We supposed that overall antigenicity and immunogenicity of a construct would be elevated where it consisted of determinants with high antigen probability. In addition to failure in passing the epitope screening, L2 was assigned as a non-antigenic peptide, so was excluded with a high degree of confidence in the designed construct. Toobak et al. (2013a, 2013b) have demonstrated that VaxiJen results are reliable with high accuracy. Although L1 was not considered as an appropriate peptide in the first design, it qualified *via* antigen probability assessment. In contrast, L4 became depreciated since it was tagged as a non-antigenic peptide. In addition to high ranking of L3, L6 and L7 in epitope predictions, these loops were ranked as the most probable antigens in order which to be considered as the best (L3 > L6 > L7). The length of L5 was short such that no result was obtained by VaxiJen. So, based on provided explanations, loops were classified as follow: the best (L3, L6 and L7), appropriate (L1, L4 and L5) and excluded (L2). In Omp34-2 (second construct), L2 was removed, appropriate loops are available with no repeat and the best epitopes are increased (twice-repeated) by replacement of internal turns. False-negative occurrences in Omp34-2 are less probable than Omp34-1 since only one loop (L2) was excluded.

The number of amino acids, molecular weight and theoretical pI and antigen probability of Omp34 and the designed constructs along with their average hydrophilicity, flexibility, surface accessibility and BepiPred predictions are represented in Table 2.

Evaluation of the constructs, *i.e.* Omp34-1 and Omp34-2, revealed that Omp34-2 is superior to mature Omp34 as well as the Omp34-1 construct in terms of antigenic properties.

Based on hydrophilicity, flexibility and BepiPred results, B-cell epitope density of Omp34-2 is higher than Omp34-1 and mature Omp34. These results demonstrate that epitope selection could be

Table 3
Unfavorable segments based on BepiPred prediction.

Residues	Position in Omp34-2	Position in Omp34	Topology in Omp34
EVGA	30–33	137–140	β6
LMTV	55–58	147–150	β7
DNFG	70–73	162–165	L4
KTDLYL	154–159	222–227	β10

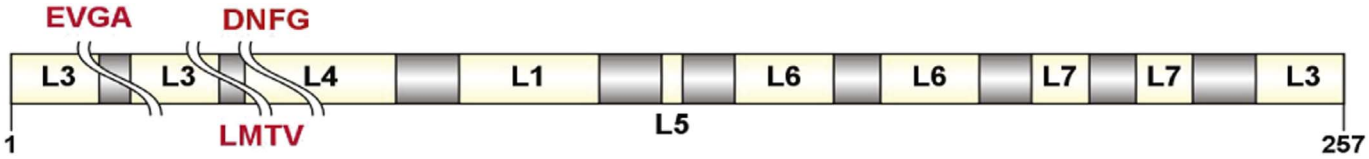

Fig. 3. Omp34-4. Loops (L), their approximate size and locations are shown schematically. Four-residue segments are the most unfavorable segments removed from the construct. The Figures was illustrated by IBS: Illustrator for Biological Sequences (Liu et al., 2015), version 1.0.3.

highly improved by including VaxiJen predictions. So, Omp34-2 was considered for further modifications. Firstly, unfavorable segments were assigned (Table 3).

These segments were sequentially removed and consequent sequences were separately analyzed by VaxiJen. Based on VaxiJen results, in Omp34-3, EVGA, LMTV and DNFG were removed as the most unfavorable residues. Higher antigen probability as well as average scores of hydrophilicity, flexibility and BepiPred predictions for Omp34-3 than ones for Omp34-2 revealed that predicted unfavorable segments were properly identified and removed. Addition of L3 confers the highest ranking these appropriate properties points of view to Omp34-4. Omp34-4 is schematically illustrated in Fig. 3.

Copy numbers of loops incorporated in construct design were determined based on their "predicted antigen probability" and "B-cell epitope predictions" ranking as well as capacity of OMP which is employed as a scaffold. Capacity of the scaffold is potentially defined as position of loops, turns, N-terminal and C-terminal of the OMP. Mature Omp34 was predicted to be an insoluble protein upon overexpression, with probability of 0.79. In contrast, Omp34–4 was predicted to be soluble one, with probability of 0.58, upon overexpression. The predicted solubility of the obtained construct resolved one disadvantage (insolubility) of Omp34 recombinant protein. Properties involved in B-cell epitope assignment, have overlap with those affecting protein solubility. So, removal of the unfavorable residues enhanced predicted solubility in addition to increasing antigenicity and epitope density.

As an innovative approach, no artificial linker was employed in this design and transmembrane β-strands, which are assigned as undesirable with regard to B-cell epitopes, served as linkers. To our knowledge, this is the first sequence-based linear B-cell epitope-based design in which existing transmembrane β-strands are utilized as natural linkers.

To date, OmpA is nominated as the most promising protein antigen of this pathogen (reviewed in (Chen, 2015)). However, multicomponent and multiple subunit antigens are currently proposed to overcome *A. baumannii*-associated infections (Ahmad et al., 2016; Chen, 2015; Pachón and McConnell, 2014). Recently, based on "structural vaccinology" and "high epitope density", we designed a novel OmpA-derived antigen with higher antigenicity and lower cytotoxicity (Jahangiri et al., 2017). We presume that co-administration of this OmpA-derived antigen and Omp34-4 construct could confer sufficient protection against the successful nosocomial pathogen, *A. baumannii*.

4. Conclusion

The designed construct is predicted to be a soluble antigen which could trigger antibody responses with higher avidity than mature Omp34. The designed construct could also serve as an appropriate candidate in diagnostic kits as well as an immunotherapeutic molecule against *A. baumannii*. Administration of Omp34-4 construct and the previously described OmpA-derived antigen could confer sufficient protection against *A. baumannii*. However, *in vitro* and *in vivo* evaluations are needed to support the obtained *in silico* results. The innovative approach could also theoretically be applied to most if not all vaccine designs for OMPs.

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Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2017.11.008.

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