



# Functional Exposed Amino Acids of *CarO* Analysis as a Potential Vaccine Candidate in *Acinetobacter Baumannii*

Maryam Tohidinia<sup>1</sup> · Seyed Mohammad Moshtaghioun<sup>1</sup> · Fatemeh Sefid<sup>2,3</sup> · Ali Falahati<sup>1</sup>

Accepted: 21 August 2019 / Published online: 28 August 2019  
© Springer Nature B.V. 2019

## Abstract

*Acinetobacter baumannii* is a well-recognized cause of nosocomial infections. This organism is recognized to be among the most difficult antimicrobial-resistant gram-negative bacilli to control and treat. One of the main challenges we face is Carbapenem resistance in *A. baumannii*. Carbapenem resistance in *A. baumannii* associated with the loss of an outer membrane protein designated *CarO* (Carbapenem resistance outer membrane protein). This protein is a membrane porin of *A. baumannii*. Using specific antibodies against this protein exert a bacteriostatic or bactericidal effect in vitro. Attempts should be made to discover peptides that could mimic protein epitopes and possess the same immunogenicity as the complete protein. Subsequently, bioinformatics methods for epitope prediction have been developed leading to synthesis of such peptides that are important for development of vaccine. This study provides a basis for the design of pathogen specifically, B cell epitope-based vaccine that is targeted to diseases caused by *A. baumannii* in the global human population. A combination of available bioinformatics tools are used to understand and characterize the *Baumannii Acinetobactin* utilization structure of *A. baumannii* and appropriate selection regions as effective B cell epitopes and functional exposed amino acids. In conclusion, amino acids 19–158 were selected as vaccine candidate.

**Keywords** *Acinetobacter Baumannii* · *CarO* · B cell epitope prediction · Vaccines

## Introduction

*Acinetobacter baumannii* is an aerobic, pleomorphic, rod-shaped and Gram-negative bacterium. The organism has low virulence but is capable of causing infection in organ transplantation and febrile neutropenia (Blue-Hnidy and Allen 2006; Weinstein et al. 2005). *A. baumannii* has known as an

important and common pathogen creating nosocomial pneumonia and bacteremia among patients who admitted in the intensive care unit (ICU) worldwide, followed by skin, soft tissue, and urinary tract infection, and secondary meningitis over the past few decades (Kempf et al. 2012; Longo 2006; Peleg et al. 2008; Sánchez-Encinales et al. 2017; Wong et al. 2017). The resistance of *A. baumannii* to the commonly used antimicrobial drugs such as Aminopenicillins, Ureidopenicillins, Cephalosporins and Cephamycins has observed for the last 25 years (García-Patiño et al. 2017; Kang et al. 2017).

Since then, strains of *A. baumannii* have gained resistance to new antimicrobial drugs (Knezevic et al. 2016). Multidrug-resistant (MDR) *A. baumannii* is an emerging pathogen in healthcare and nosocomial infection rapidly. (Dijkshoorn et al. 2007; Pagdepanichkit et al. 2016). Carbapenem-resistant *A. baumannii* (CRAB) presently is a serious infection control challenge (Higgins et al. 2009; Livermore et al. 2016).

Carbapenem are regarded as the most powerful antibiotics because of its extremely effective antibacterial activity and low toxicity, but the emergence of Carbapenem resistance

✉ Seyed Mohammad Moshtaghioun  
moshtaghiun@yazd.ac.ir

Maryam Tohidinia  
Maryamtohidini90@yahoo.com

Fatemeh Sefid  
sefid.fateme@yahoo.com

Ali Falahati  
Ali.falahati@yazd.ac.ir

<sup>1</sup> Department of Biology, Faculty of Science, Yazd University, Safaeih, Yazd, Yazd Province, Iran

<sup>2</sup> Department of Medical Genetics, Shahid Sadoughi University of Medical Science, Yazd, Iran

<sup>3</sup> Department of Biology, Science, Art University, Yazd, Iran

in *A. baumannii* has become a global concern recently. Non-enzymatic molecular mechanisms act in synergy with Carbapenem-hydrolyzing *b*-lactamases to provide high-level resistance, but they have been barely described (Shaker and Shaaban 2017; Wong et al. 2017). The Carbapenem-associated outer membrane protein, also called *CarO*, is the most characterized porin of *A. baumannii* (Mussi et al. 2005; Poirel and Nordmann 2006). *CarO* allows the selective uptake of the ornithine, other basic amino acids, and uptake of Carbapenem based on structural homologies (Gordon and Wareham 2010). MDR strains showed disruptions in the *CarO* gene by the various insertion elements (Catel-Ferreira et al. 2011).

This paper briefly discusses and explores bioinformatics tools in vaccine design. The best antigenic region of *CarO* has been determined as a novel construct for vaccine or diagnostics. That could be used as peptide vaccines against *A. Baumannii*.

## Methods

### Sequence Availability, Homology Alignment and Template Search

The *CarO* protein sequence was obtained from NCBI at <http://www.ncbi.nlm.nih.gov/protein> and saved in FASTA format for further analyses; then Protein BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) against reference sequence on the database was performed using Blosum80 matrix to collect homologous sequences among different bacteria. For Template Search, the protein sequence of *CarO* was used as an input data for the PSI-BLAST against protein data bank (PDB) at <http://blast.ncbi.nlm.nih.gov/Blast.cgi> to identify its homologous structures. In order to choose the best template, lower E value, higher query coverage and maximum identity was considered. The best result of PSI-BLAST was selected as template (Altschul et al. 1990).

T-COFFEE at <http://tcoffee.crg.cat/apps/tcoffee/do:regul> and PRALINE (Notredame et al. 2000) at <http://www.ibi.vu.nl/programs/pralinewww/> (Pirovano et al. 2008) were used for generating of alignments. Alignments can demonstrate the conservancy of the protein residues among various strains. In this regard, suitable vaccine candidates are those effective against all strains of a given pathogen. Conservancy of the amino acids among bacteria other than *Acinetobacter* implies probable cross-reactivity levels.

### Protein Topology and Signal Peptide Prediction

Topology prediction servers predict the transmembrane and outer membrane region of proteins. Transmembrane helix and signal peptides are not suitable regions as B cell epitope.

PRED-TMBB2 at <http://www.compgen.org/tools/PRED-TMBB2> (Bagos et al. 2004) was employed to predict the transmembrane beta-strands of the gram-negative bacteria outer membrane proteins, and discriminating such proteins from water-soluble ones when screening large datasets. The transmembrane regions was also determined by SPOCTOPUS at <http://octopus.cbr.su.se/> (Viklund et al. 2008).

SignalP 4.1 server at <http://www.cbs.dtu.dk> (Petersen et al. 2011) used to determine the presence and location of signal peptide cleavage sites in amino acid sequences. This property was also predicted by SOSUI at <http://harrier.nagahama-i-bio.ac.jp/sosui/> (Hirokawa et al. 1998).

### Functional Annotation of Protein Domain

Recognition of protein domains helps us recognize functionally important domains within a protein. Antibodies appointed against such domains could impair *CarO* functions and result in virulence reduction.

DomPred at <http://bioinf.cs.ucl.ac.uk/psipred/dompred> (Bryson et al. 2007) was used to predict Protein domains and detect homolog domains. InterProScan at <http://www.ebi.ac.uk/interpro/about.html> (Zdobnov and Apweiler 2001) was used to detect functional analysis of protein sequences by classifying them into families and predict the presence of domains and important sites.

### Orientation of the Protein 3D Structure in Membrane

Rotational and translational positions of transmembrane and peripheral proteins in membranes using their 3D structure (PDB coordinate file) as input were calculated by the PPM server at <http://opm.phar.umich.edu/server.php>. It can be applied to newly determine experimental protein structures or theoretical models. Many membrane-associated proteins from the PDB have already been pre-calculated and can be found in the OPM database.

### Predicting Functionally and Structurally Important Residues

Functional conserved amino acids allow more realism and robustness in the description of epitope prediction and protein binding surfaces. There are different ways to predict functionally and structurally important residues. In this study, conserved functional and structural amino acids were determined by ConSurf at <http://consurf.tau.ac.il> (Berezin et al. 2004). ConSurf predicts functional residues according to the neural-network algorithm. InterProSurf at <http://curie.utmb.edu/pattest9.html> (Negi et al. 2007) used to predict functional sites on protein surface using patch analysis.

## Physicochemical Properties of Protein

Parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity of polypeptides chains have been correlated with the location of B cell epitopes. This has led to a search for empirical rules that would allow the position of B cell epitopes to be predicted from certain features of the protein sequence. Bcepred at <http://www.imtech.res.in/raghava/bcepred/> (Saha and Raghava 2004) was used to predict linear B-cell epitopes in a protein sequence. Average score of physico-chemical properties (hydrophobicity, flexibility/mobility, accessibility, polarity, exposed surface and turns) of proteins were predicted with IEDB at <http://tools.iedb.org/bcell/> (Vita et al. 2014).

## Prediction of Linear B Cell Epitope

The identification and characterization of B-cell epitopes play an important role in vaccine design, immunodiagnostic tests, and antibody production. There are many tools act as B cell epitope predictor. Since each software uses special algorithm and individual methods in epitope prediction. Therefore, usage of several tools to predict linear B-cell epitopes in protein sequences are highly desirable.

BepiPred 1.0 server at <http://www.cbs.dtu> (Jespersen et al. 2017) was used to predict linear B-cell epitopes based on hidden Markov model. Bcepred server at <http://ailab.ist.psu.edu> (EL-Manzalawy et al. 2008) with 90% specificity was also employed with report of overlapping epitopes. Bcepred explores two machine learning approaches for predicting flexible length linear B-cell epitopes. antigenic epitopes using support vector machine to integrate tri-peptide similarity and propensity scores (SVMTriP) in order to achieve the higher accuracy and specificity were predicted by Svmtrip at <http://sysbio.unl.edu/SVMTriP/prediction.php> (Yao et al. 2012).

## Prediction of Conformational B Cell Epitope

Ellipro at <http://tools.immuneepitope.org> (Ponomarenko et al. 2008) predicted linear and discontinuous antibody epitopes based on a protein antigen's 3D structure. DiscoTope at <http://cbs.dtu.dk/services/DiscoTope/> (Kringelum et al. 2012) determined discontinuous B cell epitopes from protein 3D structures. The method utilizes calculation of surface accessibility and a novel epitope propensity amino

acid score. The final scores are calculated by combining the propensity scores of residues in spatial proximity and the contact numbers.

Epitopia at <http://epitopia.tau.ac.il/> (Rubinstein et al. 2009) used to detect immunogenic regions in a given protein structure or sequence. The input is analyzed with regards to its physico-chemical and structural-geometrical properties.

## Pocket and Binding Site Detection

GHECOM (Grid-based HECOMi finder) at <http://strcomp.protein.osaka-u.ac.jp/ghecom/> (Zhang et al. 2011) was used to find multi-scale pockets on protein surfaces. In addition, Depth (<http://mspc.bii.a-star.edu.sg/tankp/help.html>) was employed to calculate/predict depth, cavity sizes, ligand binding sites and PKA. Depth measures the closest distance of a residue/atom to bulk solvent (Tan et al. 2011).

## Immunogenic Regions Selection

A region with the largest gatherings of linear and conformational epitopes could be selected as vaccine candidates. This region should be qualified as single-scale amino acid properties assay. Some specifications such as probability of antigenicity, physiochemical properties average, PI and etc. should also be considered in region selection. Hence, one region was selected as appropriate antigenic candidates. Further analyses were performed on the selected regions to validate the selection.

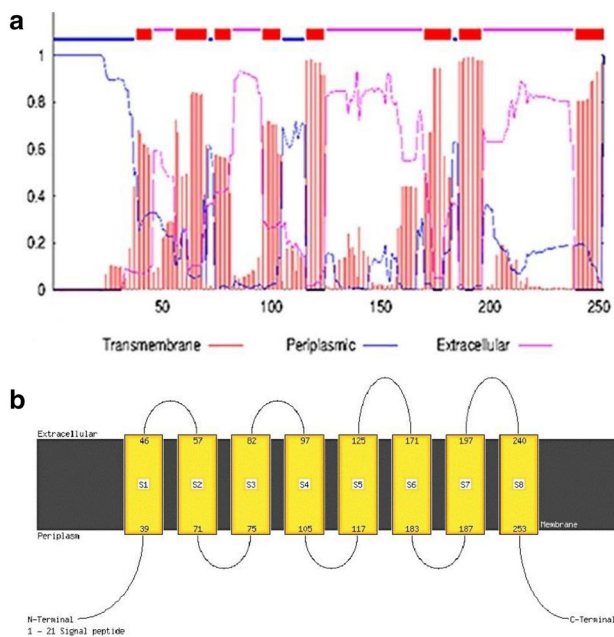
The flow chart of experimental procedure which summarizes an overview of the methodology used in this study is shown in Fig. 1.

## Results

### Protein Sequencing and Alignments

*CarO* protein sequence with accession No. AKL79738.1 is available as a query for protein BLAST against Acinetobacter producing a set of sequences containing various species of Acinetobacter. Out of 100 alignments, 50 sequences with the highest similarity (identity  $\geq$  90%, query coverage: 100% and E value: 0) were selected as PRALINE and T-COFFEE alignment input. BLAST search of Acinetobacter revealed numerous sequences of *CarO*. These hits showed that the *CarO* protein is specific for Acinetobacter species. The

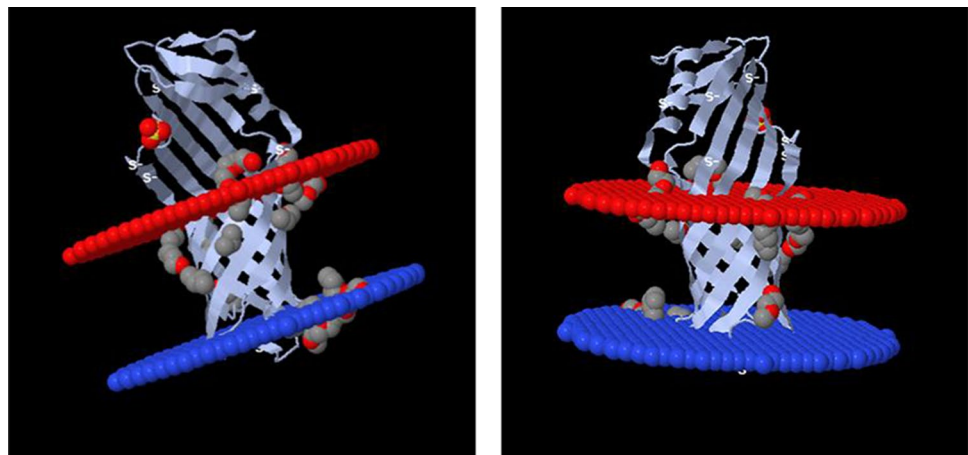




**Fig. 3** Topology prediction and detection of beta-barrel outer membrane proteins by PRED-TMBB 2 server. The diagram shows (a) the estimated preference of a particular residue to be located either on the transmembrane (red) or on the periplasmic (blue) or extracellular (pink). Schematic picture (b) shows residues from 39–46, 53–59, 61–69, 96–104, 116–124, 171–181, 185–195 and 237–249 are transmembrane regions (Color figure online)

of individual subunits or domains. The precision of calculated hydrophobic thicknesses and tilt angles are  $\sim 1 \text{ \AA}$  and  $2^\circ$ , respectively, as judged from their deviations in different crystal forms of the same proteins. The fluctuations of these parameters calculated within 1 kcal/mol around the global minimum of transfer energy are usually smaller than  $2 \text{ \AA}$  and  $4^\circ$ , respectively ( $\pm$  values in all Tables of the database). The calculated tilt angles in homologous proteins differ by  $2\text{--}16^\circ$

**Fig. 4** Orientation of protein in cell membrane. The origin of coordinates corresponds to the center of lipid bilayer. Z axis coincides with membrane normal; atoms with the positive sign of Z coordinate are arranged in the “outer” leaflet as defined by the user-specified topology. Positions of DUMMY atoms correspond to locations of lipid carbonyl groups



depending on the size of the protein, its oligomeric state and percentage of sequence identity (Fig. 4).

### Functionally and Structurally Important Residues

ConSurf and interproSurf annotated functional residues on the 3D structure of *CarO*. Results are shown in Figs. 5 and 6, respectively. In ConSurf server Protein structure shows in ribbon. Functional residues which were predicted by ConSurf at the protein structure differentiated with Purple filling space model. InterproSurf revealed residues number 95, 152, 154, 156, 166, 19, 161, 223, 224, 225, 82, 83, 84, 91, 92, 93 by auto patch analysis.

### Physicochemical Properties of Protein

IEDB and Bcepred servers predict properties such as hydrophilicity, accessibility, antigenicity, flexibility and beta turn secondary structure in the protein sequence. Although single-scale amino acid properties were acquirable in all sequence length, most salient regions of higher probability were located in regions 45–60, 100–120 and 145–160. Peaks in the plot indicate putative susceptible epitope boundaries (Fig. 7).

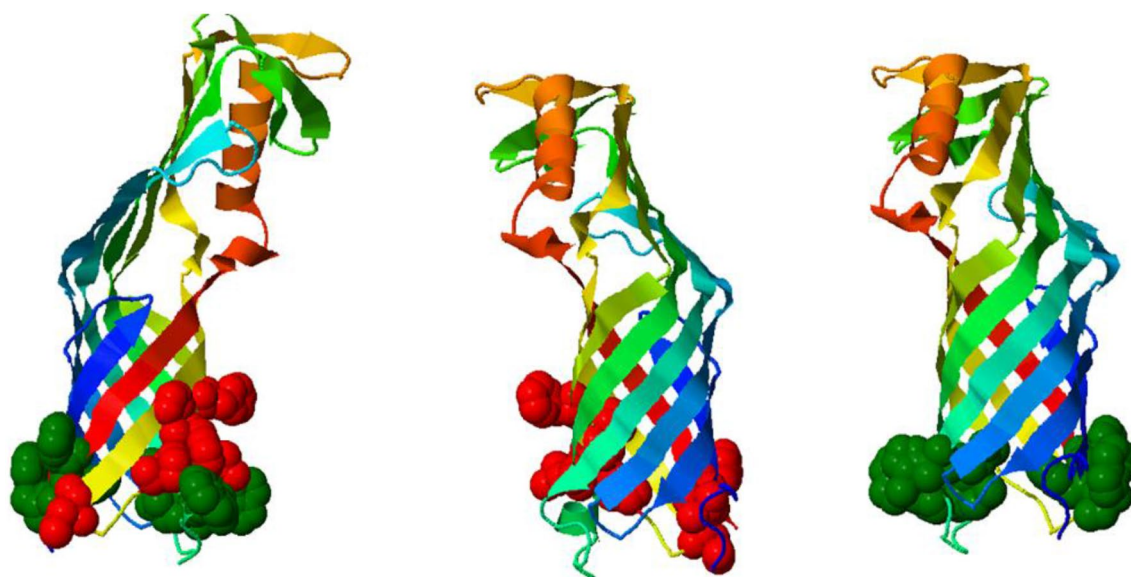
### Linear and Conformational B Cell Epitope Prediction

Linear B cell epitopes predicted by Bepipred server are more concentrated in the regions of 45–50, 74–82, 148–159 and 212–229. A region at region 19–38 shows the presence of a high density of linear epitopes. The highest score is related to “MKVLRVLT TALLAAGAAMADEAV-VHDSYAFDKNQLIP” sequence at region 1–39 (Fig. 7). ABCpred result shows 20 hits of 16 meric peptide sequences as B-cell epitopes ranking based on scores (Fig. 7).

BCpred predicted 6 linear B-cell epitopes in *CarO* sequence ranking based on scores (Fig. 7). The most



**Fig. 5** ConSurf, identification of functionally and structurally important residues results. Functional residues which were predicted by ConSurf at the protein structure differentiated with Purple filling space model (Color figure online)

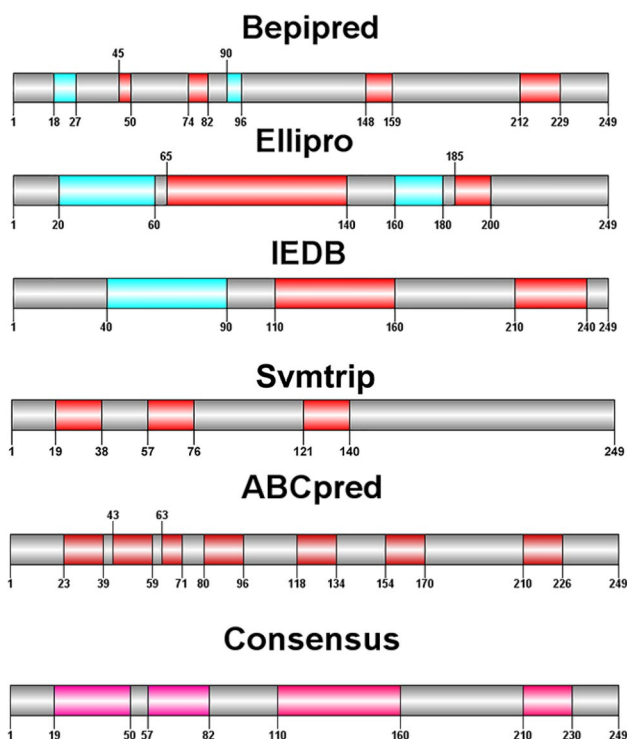


**Fig. 6** Functional residues at the protein structure surface predicted by Interprosurf. Protein structure shows in ribbon. Functional residues which were predicted by InterProSurf at the protein structure differentiated with red and green filling space model (Color figure online)

reliable linear epitopes predicted by BCPred (score = 0.994 and 0.987) are “SIDGKNYQQAVPGQEGGVRG” and “LNAEIRPWGASTNPWAQGLY” started at position 142 and 98 respectively. Svmtrip predicted 3 linear B cell epitopes ranking based on score (Fig. 7). The best epitope recommended by this server is “AMADEAVVHDSYAFDKNQLI” at region 19–38. “GAAYLDNDYDLAKRIGNGDT” at region 121–140 is the second best

epitope predicted by Svmtrip. Six linear along with 2 discontinuous B cell epitopes were predicted by ElliPro software (Tables 1, 2). Two linear and 2 discontinuous epitopes with the highest PI (protrusion index) are shown in Fig. 8a, b.

Discontinuous B cell epitopes predicted from the 3D structure of protein by Discotope are shown in Fig. 9. All these servers highlighted outer membrane loops as conformational B cell epitope. Epitopia predicted 5 immunogenic



**Fig. 7** Graphical display of linear epitopes predicted by various software. The length and position of linear epitopes are graphically illustrated on a gray rod. The epitopes with the highest score are shown in red and the remainders are in blue. The consensus predictions of more or all servers shown in violet (Color figure online)

**Table 1** Linear epitopes predicted by ellipro

No	Start	End	Peptide	Number of residues	Score
1	13	19	KNQLIPV	7	0.792
2	81	95	IRPWGASTNRWAQGL	15	0.79
3	113	134	NVDATRSFRVNNQDFIA-GADGV	22	0.736
4	154	164	FAPKINKNWGV	11	0.724
5	183	200	SGSAVTTGDSLEEVNA	18	0.725
6	52	65	DISWSDDVKVNGST	14	0.663

regions. These regions are located at positions between 417 and 436, 287 and 307, 470 and 482, 554 and 570 and 609 and 619 respectively ranking based on scores.

**Table 2** Discontinuous epitopes predicted by ellipro

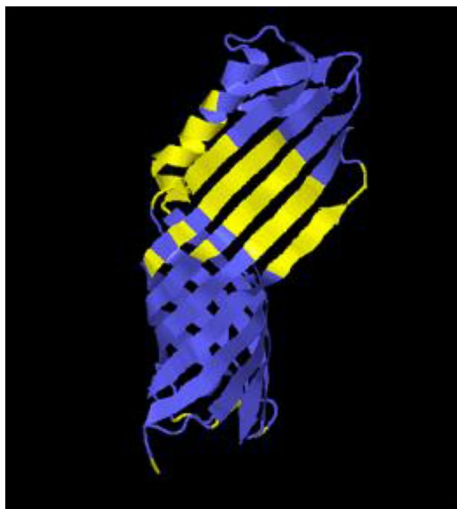
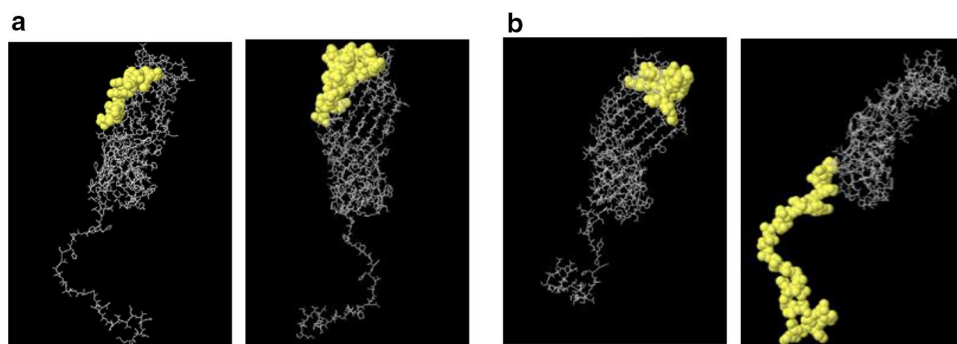
No	Residues	Number of residues	Score
1	A:K13, A:N14, A:Q15, A:L16, A:I17, A:P18, A:V19, A:W36, A:A38, A:N39, A:P40, A:Y41, A:V42, A:R82, A:P83, A:W84, A:G85, A:A86, A:S87, A:T88, A:N89, A:R90, A:W91, A:A92, A:Q93, A:L95, A:F154, A:A155, A:P156, A:K157, A:I158, A:N159, A:K160, A:N161, A:W162, A:G163, A:V164, A:F224, A:W225	39	0.762
2	A:S56, A:D57, A:D58, A:V59, A:K60, A:V61, A:N62, A:G63, A:S64, A:T65, A:Y103, A:N113, A:V114, A:D115, A:A116, A:T117, A:R118, A:S119, A:F120, A:R121, A:N123, A:N124, A:Q125, A:D126, A:F127, A:I128, A:A129, A:G130, A:A131, A:D132, A:G133, A:V134, A:K143, A:N144, A:D145, A:I146, A:Y172, A:T173, A:G174, A:N175, A:P176, A:S183, A:G184, A:S185, A:A186, A:V187, A:T188, A:T189, A:G190, A:D191, A:Q192, A:S193, A:L194, A:E195, A:E196, A:A197, A:N199, A:A200, A:R203, A:K204, A:A206, A:N207, A:D208, A:D209, A:K212	65	0.705

**Protein Pocket Detection**

GHECOM server found 11 pockets on protein surfaces using mathematical morphology. A residue in a deeper and larger pocket has a greater chance to be a true pocket. The pockets of small-molecule binding sites and active sites were higher than the average value; specifically, the values for the active sites were much higher. This suggests that pockets contribute to the formation of binding sites and active sites of protein. GHECOM results are shown in Fig. 10a, b.

The potential binding sites (PBS) of proteins are part of residue or pocket which bind to ligands directly on protein surface, they are near to the ligand binding sites. Binding cavity is a protein sub-structure of conserved geometrical and chemical properties complimentary to its bound ligand. The algorithm estimates the probability value of forming part of a binding cavity for every residue of the protein. The plot shows both mean and standard deviation of depth

**Fig. 8** Epitope mapping on 3D models. Discovery Studio Visualizer 2.5.5 software was used. From left to right, 2 linear (a) and 2 discontinuous epitopes (b) with the highest PI score predicted by Ellipro server are shown



**Fig. 9** Discontinuous B cell epitopes predicted from protein 3D structures by Discotope. Protein 3D structure is showing in ribbon in gray. Discontinuous B cell epitopes at the protein structure differentiated with yellow (Color figure online)

values. Probability of residue forming a binding site and residue depth plot and a 3D rendition of the cavity prediction is shown in Fig. 11a, b.

### Immunogenic Region Selection

One region covering residues 19–158 was selected as vaccine candidate by various software (Fig. 12) and several properties were compared to her parent protein (*CarO*). Vaxijen antigenicity score, PI, instability index, solubility, hydrophilicity, accessibility, flexibility and secondary structure

properties calculated for vaccine candidate. All results for candidate and *CarO* protein were summarized in Table 3. Parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity of polypeptides chains have been correlated with the location of continuous epitopes. This has led to a search for empirical rules that would allow the position of continuous epitopes to be predicted from certain features of the protein sequence. All prediction calculations are based on propensity scales for each of the 20 amino acids. Each scale consists of 20 values assigned to each of the amino acid residues on the basis of their relative propensity to possess the property described by the scale.

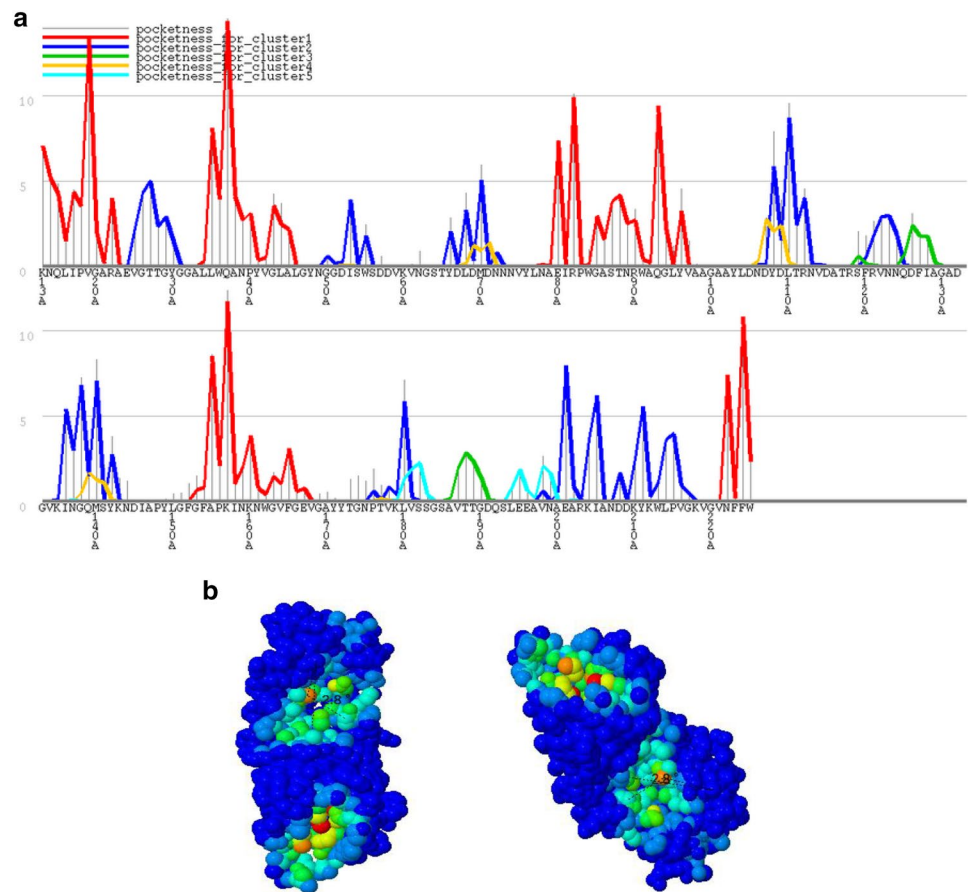
### Discussion

Nosocomial infections caused by strains *A. baumannii* are a serious clinical problem. *CarO* from *A. baumannii* is a small membrane porin with a monomeric eight-stranded barrel lacking an open channel and a large extracellular domain (Longo 2006). *CarO* functions as an uptake channel for small molecules such as Carbapenem (Zahn et al. 2015).

A proteomic study was performed on outer membrane vesicles (OMVs) of *A. baumannii* ATCC 19606. Immunization with OMVs induces protective immunity against challenge with *A. baumannii*. Among OMV proteins, six proteins were nominated as highly immunogenic, one of which was *CarO* (McConnell et al. 2011). Using several criteria to assess immunogenicity and B epitope densities *CarO* was selected as an immunogen in *A. baumannii* which contributes to siderophore mediated iron uptake (Bazmara et al. 2019).



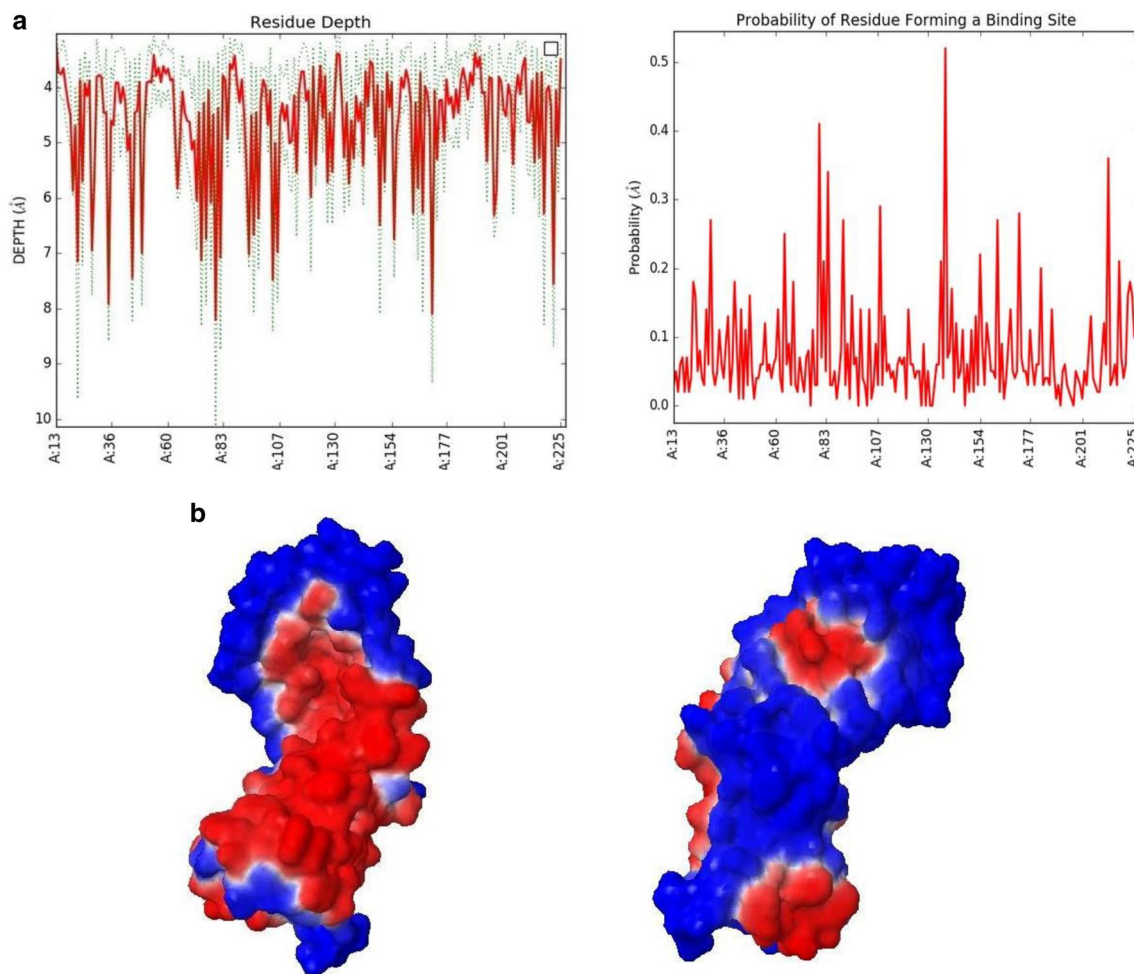
**Fig. 10** Pocket detection of *CarO* protein by GHECOM server. **a** Graph residue-based pocketness. The height of the bar shows the value of pocketness [%] for each residue. The color of pocketness bar indicates cluster number of pocket (red: cluster 1, blue: cluster 2, green: cluster 3, yellow: cluster 4, cyan: cluster 5). **b** Jmol view of pocket structure based on pocketness color (Color figure online)



This protein participates in the selective uptake of L-ornithine, Carbapenem, and other basic amino acids in *A. baumannii* (Mussi et al. 2007). Thus, employment of bioinformatics tools to select appropriate region as a vaccine candidate seems logical. *CarO* exists in all pathogenic and clinical strains of *A. baumannii* (Siroy et al. 2005). Vaccine developed based on this protein could be effective against all pathogenic strains of *A. baumannii*. Alignment of sequences from various strains suggests that such vaccines could trigger antibodies with complete specificity for *A. baumannii*. *CarO* is an acidic outer membrane protein with pI about 4.66. Acidic nature of *CarO* as well as its localization are stimulating for B-cell responses (Rahbar et al. 2012a). Tertiary structure of *CarO* is like other outer membrane transport proteins with determined structure. Topology predictions illustrated that 21 amino acids at the N-terminus of the protein were predicted as signal peptide. This region

could not be exposed and suitable to participate in B cell epitopes. InterProSurf result shows that most of the functional conserved amino acids are located in selected region (Ashkenazy et al. 2010). Therefore, it could be inferred that this region is the most important functional site of the protein. Thus, antibodies raised against these domains could block Acinetobactin uptake by *CarO*.

New genome analysis tools based on bioinformatics and immunoinformatics approaches help us select suitable antigens or epitopes directly from the genomes of pathogens in order to design a vaccine. These tools could be employed for epitope selection and vaccine design. Moreover, prediction of protein structures is one of their wide applications (Jahangiri et al. 2017; Rahbar et al. 2012b; Sefid et al. 2013, 2015). Some of these designed vaccine candidates validate with experimental analyses. In vivo analyses confirmed in



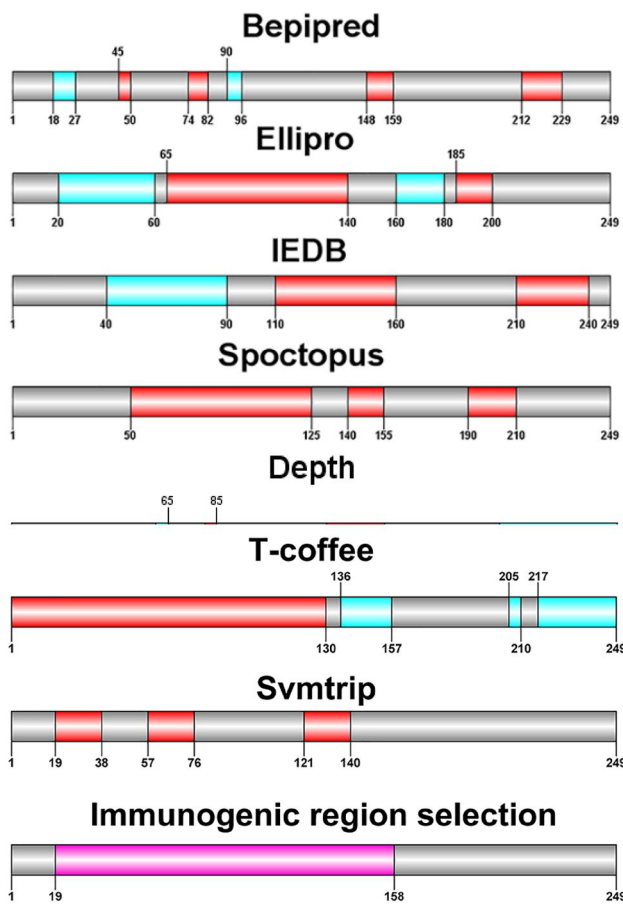
**Fig. 11** Prediction of Probability of residue forming a binding site and residue depth plot and a 3D rendition of the cavity by depth server. **a** Probability of residue forming a binding site and residue

depth plot. **b** A 3D rendition of the cavity prediction is shown using Jmol. Residues of the predicted binding cavity are colored red and the rest of the protein is colored blue (Color figure online)

silico predictions (Esmailkhani et al. 2019; Fattahian et al. 2011; Noori et al. 2014; SANGROODI et al. 2015).

Propensity scale methods assign a propensity value to each amino acid which measures the tendency of an amino acid to be part of a B-cell epitope (as compared to the background). To reduce fluctuations, the score for each target amino acid residue in a query sequence is computed as the average of the propensity values of the amino acids in a sliding window centered at the target residue. Hydrophilicity,

accessibility, antigenicity, flexibility and secondary structure properties have fundamental role in B cell epitope prediction (Rubinstein et al. 2008). Relying on just one of these properties, reliable results could not be achieved. Amino acid pair (AAP) antigenicity scale is based on the finding that B-cell epitopes favor particular AAPs. Using SVM classifier, the AAP antigenicity scale approach has much better performance than the scales based on the single amino acid propensity (Sefid et al. 2015). Thus, we combined all the


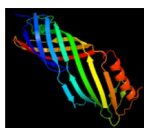


**Fig. 12** Graphical display of immunogenic regions predicted by various software. The region with the highest score are shown in red and the remainders are in blue. The consensus predictions of more or all servers shown in violet (Color figure online)

data obtained from various servers and software to predict the B-cell epitopes. Region suggested by single-scale amino acid properties assay (50–240) includes our selected region. This region could efficiently induce B cell responses. B-cell epitopes are regions on the surface of antigens recognized by B-cell receptors or specific antibodies. These epitopes can be categorized into two types: linear and conformational epitopes. Linear epitope is recognized by its linear sequence of amino acids, In contrast conformational epitope recognized by specific three-dimensional shape and its protein structure (Ansari and Raghava 2010). Linear and conformational epitopes in *CarO* protein predict by several software and various algorithms to achieve consensus epitopes. Consensus epitopes obtained from various algorithms are more reliable for selection. The highest scored linear B cell epitopes are located in region 19–38. The second best linear B cell epitope introduced by Svmtrip is located in region 121–140. Fifteen of 20 hits (\*75%) predicted as B cell epitopes were within amino acids 19–160 (selected region) by ABCpred (Saha and Raghava 2007). Linear and conformational B cell epitopes predicted by Ellipro were located within selected region (Dehghani and Sefid 2016). Depth server result shows that higher possibility of residue forming a binding site are located in region 80–85 and 130–154. All servers predicting conformational B cell epitopes based on 3D structure such as Discotope demonstrated that residue (70, 108, 138, 140, 178, 176, 201, 202, and 205) have the most important role to form discontinues epitopes (Reimer 2009).

The humoral immune system response is based on the interactions between antibodies and antigens for the clearance of pathogens and foreign molecules (Parkin and Cohen 2001). An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune

**Table 3** Average physicochemical properties of vaccine candidate and *CarO*

Candidate number	Residue number	3D structure	Weight	Vaxigen antigenicity score	Instability index	
1	25		15079.48	0.5793	11.53	
<i>CarO</i>	46		26871.94	0.6212	15.25	
Candidate number	B cell epitope	Hydropathicity	Felexibility	Accessibility	Beta turn	Isoelectric PH
1	0.824	−0.312	0.509	7.222	1.31	4.15
<i>CarO</i>	0.52	−0.391	0.492	7.167	1.279	4.66

system. The epitope is the specific piece of the antigen to which an antibody binds (Peters et al. 2005). Epitopia server can predict immunogenic regions on protein structure. Fifty percent of immunogenic regions predicted by the server are located in region 107–225 of *CarO*. All these analyses reveal that majorities as well as the best B cell epitopes are located in 20–160 region. So, this region was selected as vaccine candidate. Average of each single scale amino acid propensity (hydrophilicity, accessibility, flexibility, beta turn and B cell epitope averages) was increased in the candidate vaccines than their parent protein, *CarO*. The present study was designed to in silico resolving the major obstacles in the control or in prevention of the Infection caused by *A. baumannii*. We exploited bioinformatics tools for better understanding and characterizing the *CarO* structure of *A. baumannii* and selection of appropriate region as effective B cell epitopes.

**Acknowledgements** We thank Yazd University for granting us a permission to use its web server.

**Funding** This study was supported by an unrestricted free access to Yazd University web site for data collection. The authors have received no financial support for the elaboration of this manuscript.

## Compliance with Ethical Standards

**Conflict of interest** Yazd University did not play any decision-making role in the study analysis or writing of the manuscript. All authors declare no Potential Conflicts of Interest.

**Ethical Approval** As the present study involved no experimental with animals or human, hence there was no need for approval by the Ethics Committee of Yazd University.

## References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410
- Ansari HR, Raghava GP (2010) Identification of conformational B-cell epitopes in an antigen from its primary sequence. *Immunome Res* 6:6
- Ashkenazy H, Erez E, Martz E, Pupko T, Ben-Tal N (2010) ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. *Nucleic Acids Res* 38:W529–W533
- Bagos PG, Liakopoulos TD, Spyropoulos IC, Hamodrakas SJ (2004) PRED-TMBB: a web server for predicting the topology of  $\beta$ -barrel outer membrane proteins. *Nucleic Acids Res* 32:W400–W404
- Bazmara H, Rasooli I, Jahangiri A, Sefid F, Astaneh SDA, Payandeh Z (2019) Antigenic properties of iron regulated proteins in *Acinetobacter baumannii*: an in silico approach. *Int J Pept Res Ther* 25:205–213
- Berezin C et al (2004) ConSeq: the identification of functionally and structurally important residues in protein sequences. *Bioinformatics* 20:1322–1324
- Blue-Hnidy DE, Allen SD (2006) Microbiology for the surgical pathologist. In: Essentials of anatomic pathology. Springer, New York, pp 155–229
- Bryson K, Cozzetto D, Jones DT (2007) Computer-assisted protein domain boundary prediction using the Dom-Pred server. *Curr Protein Pept Sci* 8:181–188
- Catel-Ferreira M et al (2011) Structure–function relationships of CarO, the carbapenem resistance-associated outer membrane protein of *Acinetobacter baumannii*. *J Antimicrob Chemother* 66:2053–2056
- Dehghani E, Sefid F (2016) Evaluation of lamb linear and conformational epitopes on the vibrio Harveyi. *Int J Adv Biotechnol Res* 7(3):667–671
- Dijkshoorn L, Nemec A, Seifert H (2007) An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* 5:939–951
- El-Manzalawy Y, Dobbs D, Honavar V (2008) Predicting linear B-cell epitopes using string kernels. *J Mol Recognit* 21:243–255
- Esmailkhani H, Rasooli I, Hashemi M, Nazarian S, Sefid F (2019) Immunogenicity of cork and loop domains of recombinant *Baumannii acinetobactin* utilization protein in murine model. *Avicenna J Med Biotechnol* 11:180
- Fattahian Y, Rasooli I, Gargari SLM, Rahbar MR, Astaneh SDA, Amani J (2011) Protection against *Acinetobacter baumannii* infection via its functional deprivation of biofilm associated protein (Bap). *Microb Pathog* 51:402–406
- García-Patiño MG, García-Contreras R, Licona-Limón P (2017) The immune response against *Acinetobacter baumannii*, an emerging pathogen in nosocomial infections. *Front Immunol* 8:441
- Gordon NC, Wareham DW (2010) Multidrug-resistant *Acinetobacter baumannii*: mechanisms of virulence and resistance. *Int J Antimicrob Agents* 35:219–226
- Higgins PG, Damhayan C, Hackel M, Seifert H (2009) Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 65:233–238
- Hirokawa T, Boon-Chiang S, Mitaku S (1998) SOSUI: classification and secondary structure prediction system for membrane proteins. *Bioinformatics* 14:378–379
- Jahangiri A, Rasooli I, Owlia P, Fooladi AAI, Salimian J (2017) In silico design of an immunogen against *Acinetobacter baumannii* based on a novel model for native structure of outer membrane protein A. *Microb Pathog* 105:201–210
- Jespersen MC, Peters B, Nielsen M, Marcatili P (2017) BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic Acids Res* 45(W1):W24–W29
- Kang AD, Smith KP, Eliopoulos GM, Berg AH, McCoy C, Kirby JE (2017) In vitro apramycin activity against multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Diagn Microbiol Infect Dis* 88:188–191
- Kempf M et al (2012) Cell surface properties of two differently virulent strains of *Acinetobacter baumannii* isolated from a patient. *Can J Microbiol* 58:311–317
- Knezevic P, Aleksic V, Simin N, Svircev E, Petrovic A, Mimica-Dukic N (2016) Antimicrobial activity of Eucalyptus camaldulensis essential oils and their interactions with conventional antimicrobial agents against multi-drug resistant *Acinetobacter baumannii*. *J Ethnopharmacol* 178:125–136
- Kringelum JV, Lundegaard C, Lund O, Nielsen M (2012) Reliable B cell epitope predictions: impacts of method development and improved benchmarking. *PLoS Comput Biol* 8:e1002829
- Livermore DM, Mushtaq S, Warner M, Woodford N (2016) In-vitro activity of eravacycline against carbapenem-resistant Enterobacteriaceae and *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 60(6):3840–3844
- Longo DL (2006) Idiotype vaccination in follicular lymphoma: knocking on the doorway to cure. Oxford University Press, Oxford

- McConnell MJ, Domínguez-Herrera J, Smani Y, López-Rojas R, Docobo-Pérez F, Pachón JJI (2011) Vaccination with outer membrane complexes elicits rapid protective immunity to multidrug-resistant *Acinetobacter baumannii*. *Infect Immunity* 79:518–526
- Mussi MA, Limansky AS, Viale AM (2005) Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of  $\beta$ -barrel outer membrane proteins. *Antimicrob Agents Chemother* 49:1432–1440
- Mussi MA, Relling VM, Limansky AS, Viale AM (2007) CarO, an *Acinetobacter baumannii* outer membrane protein involved in carbapenem resistance, is essential for L-ornithine uptake. *FEBS Lett* 581:5573–5578
- Negi SS, Schein CH, Oezguen N, Power TD, Braun W (2007) InterProSurf: a web server for predicting interacting sites on protein surfaces. *Bioinformatics* 23:3397–3399
- Noori E, Rasooli I, Owlia P, Gargari SLM, Ebrahimizadeh W (2014) A conserved region from biofilm associated protein as a biomarker for detection of *Acinetobacter baumannii*. *Microb Pathog* 77:84–88
- Notredame C, Higgins DG, Heringa J (2000) T-Coffee: a novel method for fast and accurate multiple sequence alignment. *J Mol Biol* 302:205–217
- Pagepanichkit S, Tribuddharat C, Chuanchuen RJ (2016) Distribution and expression of the Ade multidrug efflux systems in *Acinetobacter baumannii* clinical isolates. *Can J Microbiol* 62:794–801
- Parkin J, Cohen B (2001) An overview of the immune system. *The Lancet* 357:1777–1789
- Peleg AY, Seifert H, Paterson DL (2008) *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 21:538–582
- Peters B et al (2005) The immune epitope database and analysis resource: from vision to blueprint. *PLoS Biol* 3:e91
- Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat Methods* 8:785–786
- Pirovano W, Feenstra KA, Heringa J (2008) PRALINE™: a strategy for improved multiple alignment of transmembrane proteins. *Bioinformatics* 24:492–497
- Poirel L, Nordmann P (2006) Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin Microbiol Infect* 12:826–836
- Ponomarenko J, Bui H-H, Li W, Füsseder N, Bourne PE, Sette A, Peters B (2008) ElliPro: a new structure-based tool for the prediction of antibody epitopes. *BMC Bioinform* 9:514
- Rahbar MR et al (2012) A potential in silico antibody–antigen based diagnostic test for precise identification of *Acinetobacter baumannii*. *J Theoret Biol* 294:29–39
- Reimer U (2009) Prediction of linear B-cell epitopes epitope mapping protocols. *Methods Mol Biol* 524:335–344
- Rubinstein ND, Mayrose I, Halperin D, Yekutieli D, Gershoni JM, Pupko T (2008) Computational characterization of B-cell epitopes. *Mol Immunol* 45:3477–3489
- Rubinstein ND, Mayrose I, Martz E, Pupko T (2009) Epitepia: a web-server for predicting B-cell epitopes. *BMC Bioinform* 10:287
- Saha S, Raghava G (2004) BcePred: prediction of continuous B-cell epitopes in antigenic sequences using physico-chemical properties. In: Nicosia G, Cutello V, Bentley PJ, Timmis J (eds) *Artificial immune systems*. ICARIS 2004. Lecture notes in computer science, vol 3239. Springer, Berlin, Heidelberg, pp 197–204
- Saha S, Raghava GP (2007) Prediction methods for B-cell epitopes. *Methods Mol Biol* 409:387–394
- Sánchez-Encinales V et al (2017) Overproduction of outer membrane protein A by *Acinetobacter baumannii* as a risk factor for nosocomial pneumonia, bacteremia, and mortality rate increase. *J Infect Dis* 215:966–974
- Sangroodi YH, Rasooli I, Nazarian S, Ebrahimizadeh W, Sefid F (2015) Immunogenicity of conserved cork and  $\beta$ -barrel domains of baumannii acinetobactin utilization protein in an animal model. *Turkish J Med Sci* 45:1396–1402
- Sefid F, Rasooli I, Jahangiri A (2013) In silico determination and validation of baumannii acinetobactin utilization a structure and ligand binding site. *BioMed Res Int* 2013:172784
- Sefid F, Rasooli I, Jahangiri A, Bazmara H (2015) Functional exposed amino acids of BauA as potential immunogen against *Acinetobacter baumannii*. *Acta Biotheor* 63:129–149
- Shaker MA, Shaaban MI (2017) Formulation of carbapenems loaded gold nanoparticles to combat multi-antibiotic bacterial resistance: in vitro antibacterial study. *Int J Pharm* 525:71–84
- Siroy A et al (2005) Channel formation by CarO, the carbapenem resistance-associated outer membrane protein of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 49:4876–4883
- Tan KP, Varadarajan R, Madhusudhan MS (2011) DEPTH: a web server to compute depth and predict small-molecule binding cavities in proteins. *Nucleic Acids Res* 39:W242–W248
- Viklund H, Bernsel A, Skwark M, Elofsson A (2008) SPOCTOPUS: a combined predictor of signal peptides and membrane protein topology. *Bioinformatics* 24:2928–2929
- Vita R et al (2014) The immune epitope database (IEDB) 3.0. *Nucleic Acids Res* 43:D405–D412
- Weinstein RA, Gaynes R, Edwards JR (2005) Overview of nosocomial infections caused by gram-negative bacilli. *Clin Infect Dis* 41:848–854
- Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B (2017) Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges. *Clin Microbiol Rev* 30:409–447
- Yao B, Zhang L, Liang S, Zhang C (2012) SVMTriP: a method to predict antigenic epitopes using support vector machine to integrate tri-peptide similarity and propensity. *PLoS ONE* 7:e45152
- Zahn M, D’Agostino T, Eren E, Baslé A, Ceccarelli M, van den Berg BJ (2015) Small-molecule transport by CarO, an abundant eight-stranded  $\beta$ -barrel outer membrane protein from *Acinetobacter baumannii*. *J Mol Biol* 427:2329–2339
- Zdobnov EM, Apweiler R (2001) InterProScan—an integration platform for the signature-recognition methods in InterPro. *Bioinformatics* 17:847–848
- Zhang Z, Li Y, Lin B, Schroeder M, Huang BJB (2011) Identification of cavities on protein surface using multiple computational approaches for drug binding site prediction. *Bioinformatics* 27:2083–2088