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Antigenic Properties of Iron Regulated Proteins in *Acinetobacter baumannii*: An In Silico Approach

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Abstract

Acinetobacter baumannii, an emerging nosocomial pathogen, causes multi-drug and pan-drug resistant infections. This phenomenon necessitates the development of new treatment strategies or vaccines against this pathogen. Iron acquisition systems are important factors for the virulence of pathogenic organisms. Antibodies against iron regulated outer membrane proteins (IROMPs) showed bactericidal and opsonizing activities against *A. baumannii* in vitro. Data obtained from proteomic studies could be valuable for vaccine design. Comparative proteomic analysis of total lysate and outer membrane fractions isolated from *A. baumannii* ATCC 19606 cells cultured under iron-rich and chelated conditions resulted in the identification of protein spots differentially produced. Need for bioinformatic tools is imperative for analysis of these data as well as to design novel proteins bearing desired properties. This study undertakes in silico identification of the most effective immunogenic target amongst these iron regulated proteins in *A. baumannii* which can be employed as a vaccine candidate. Here a screening was carried out on iron- regulated *A. baumannii* OMPs, based on hydrophilicity, flexibility, beta-turns, solubility and overall antigenic probability. 3D structure of the proteins was modeled. Linear and conformational B cell epitopes predicted and their densities were used for comparison of their immunogenicity. CarO was selected as an efficient immunogenic IROMP in *A. baumannii* which contributes to sidrophore mediated iron uptake.

Keywords *A. baumanni* · IROMPs · Immunogenicity · Epitope density · Bioinformatics · 3D structure

Introduction

Acinetobacter baumannii, an aerobic Gram-negative non motile bacterium is an emerging nosocomial pathogen. It causes mild-to-severe various types of diseases like post-surgical urinary tract and respiratory tract infections, nosocomial pneumonia, skin and wound infections and bacteremia; some of which can be fatal with mortality rates as high as 75%. The escalating number of infections caused by multi-drug and pan-drug resistant strains necessitates the development of new treatment options against this emerging pathogen (Dallo et al. 2010; Jin et al. 2011; Mortensen and Skaar 2012). Several virulence determinants, such as biofilm formation, adherence and ability to invade host cells, as well as iron acquisition and host cell death, have been assessed in previous studies (Jin et al. 2011; Antunes et al. 2011; Islam et al. 2011). Iron is an essential element for almost all organisms, including bacteria. Upon entry into the mammalian host, bacterial pathogens have to acquire iron from tissues to survive. Almost all bacterial pathogens require iron to

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be infectious (Tong and Guo 2009). Free iron is a limited micronutrient in hosts where it is typically tightly bound within a range of biomolecules, such as heme. Hence, iron acquisition systems are important factors for the virulence of pathogenic organisms. *A. baumannii* expresses high-affinity iron acquisition functions needed for growth under iron-limiting laboratory conditions (Eijkelkamp et al. 2011). Its growth under iron limited conditions resulted in major transcriptional changes of not only many iron acquisition related genes, but also genes involved in other processes such as motility. Precise analysis of the microarray data has shown significantly differential expression of 1207 genes under iron limited conditions as compared to iron replete conditions (Eijkelkamp et al. 2011). Proteomic methods could be used, usually in high-throughput mode, for analyzing the complete protein profile of a microorganism, including protein localization, protein–protein interactions, post translation modifications, and differential expression in specified conditions. Data obtained from proteomic studies could be valuable for vaccine design (Rinaudo et al. 2009). Recently, a comparative analysis of total lysate and outer membrane fractions isolated from *A. baumannii* ATCC 19606 cells cultured under iron-rich and chelated conditions using 2D gel electrophoresis- mass spectrometry resulted in the identification of 58 differentially produced protein spots. Among the introduced spots, 19 and 35 of them represent iron-repressed and iron-induced protein spots respectively, while four other spots represent a metal chelation response unrelated to iron. Most of the iron-repressed protein spots represent outer membrane siderophore receptors. The iron-induced protein spots represent a wide range of proteins including those involved in iron storage, metabolic and energy processes (Nwugo et al. 2011). Antibodies against iron regulated outer membrane proteins (IROMPs) showed bactericidal and opsonizing activities against *A. baumannii* in vitro. These antibodies also blocked siderophore mediated iron uptake via IROMPs in bacteria (Goel and Kapil 2001). An unprecedented increase in new vaccine development has occurred over the past three decades. New approaches for screening and discovery, such as reverse vaccinology, structural vaccinology and systems biology invoked by bioinformatics and immunoinformatics tools, help select suitable antigens or epitopes directly from the genomes of pathogens in order to design novel vaccines (Negahdaripour et al. 2017; Nezafat et al. 2017). Bioinformatic tools could be employed for epitope selection and vaccine design (Jahangiri et al. 2011, 2012, 2017a, b; Sefid et al. 2015; Khalili et al. 2015). Moreover, prediction of protein structures is one of their wide applications (Rahbar et al. 2010, 2012; Sefid et al. 2013; Khalili et al. 2017; Mohammadpour et al. 2016). Availability of *A. baumannii* genome and bioinformatics tools make an in silico approach to identifying potentially immunogenic antigens from *A. baumannii* proteins possible (Ni et al. 2017; Singh et al. 2016; Hassan et al. 2016; Chiang et al. 2015; Moriel et al. 2013). Such an approach allows systematic antigen selection and provides a basis for laboratory experiments that may reduce the high cost of cloning and subsequent evaluation.

The present study made use of in silico approaches to analyze a total of 58 proteins previously identified as up regulated upon exposure of the organism to the presence or absence of iron condition (Nwugo et al. 2011). The study undertakes identification of the most effective immunogenic target amongst iron regulated proteins in *A. baumannii* which can be employed as a vaccine candidate.

Methods

Selection of Iron-Regulated *A. baumannii* OMPs and Sequence Availability

Between iron-regulated proteins of *A. baumannii*, twelve outer membrane proteins were selected for analysis. The amino acid sequences of these proteins were obtained from NCBI at http://www.ncbi.nlm.nih.gov/protein and swiss prot at http://us.expasy.org/sport were saved in FASTA format for further analyses.

Antigenicity Prediction

Average score of hydrophilicity, flexibility and Beta turns of proteins were predicted with IEDB at http://tools.immuneepitope.org (Zhang et al. 2008). Antigenic probability was predicted by VaxiJen at http://www.darrenflower.info/VaxiJen/, the first server for alignment-independent prediction of protective antigens. It was developed to allow antigen classification solely based on the physicochemical properties of proteins without recourse to sequence alignment (Doytchinova and Flower 2007). Antigenicity of proteins also predicted with ANTIGENpro at http://scratch.proteomics.ics.uci.edu/ a sequence-based and alignment-free predictor of protein antigenicity, predictor of the whole protein antigenicity trained using reactivity data obtained by protein microarray analysis for five pathogens (Cheng et al. 2005). Proteins with the lowest scores of antigenicity were ignored for next step.

Solubility Prediction

Antigenic proteins were selected from previous step. Solubility of these proteins was predicted by SOLpro at http://scratch.proteomics.ics.uci.edu. SOLpro uses a sequence-based prediction method able to accurately predict the propensity of a protein to be soluble on over expression using a two-stage SVM architecture based on multiple

representations of the primary sequence (Magnan et al. 2009). This property was also predicted by PROSO at http://webclu.bio.wzw.tum.de:8080/proso. a machine-learning approach called PROSO to assess the chance of a protein to be soluble upon heterologous expression in *Escherichia coli* based on its amino acid composition (Smialowski et al. 2007). Proteins predicted as insoluble by both software were omitted and other proteins selected for next step.

Linear B cell Epitopes were Prediction

Linear B cell epitopes were predicted by ABCpred at http://www.imtech.res.in/raghava/abcpred. This server uses recurrent neural networks (RNN) and was trained with a dataset of 700 experimentally detected B-cell epitopes from the Bcipep database and 700 random peptides from the Swiss-Prot database for which no antibody binding is reported as a negative data set. The best results were achieved using a window length of 16 residues (Reimer 2009). Threshold was set as 0.85 for predictions without epitope overlapping filter. BCpreds at http://ailab.cs.iastate.edu/bcpreds/predict.html, with 90% specificity was also employed with report of overlapping epitopes. BCpreds explores two machine learning approaches for predicting flexible length linear B-cell epitopes (Yasser et al. 2008). BepiPred located at http://www.cbs.dtu.dk/services/BepiPred combines Hidden Markov models (HMM) and the propensity scale methods of Parker et al. and Levitt (Reimer 2009). ElliPro at http://tools.immuneepitope.org/tools/ElliPro/iedb input also was used for prediction of both linear and conformational B cell epitopes. A web-tool that implements Thornton's method and, together with a residue clustering algorithm, the MODELLER program and the Jmol viewer, allows the prediction and visualization of antibody epitopes in a given protein sequence or structure. ElliPro has been tested on a benchmark dataset of discontinuous epitopes inferred from 3D structures of antibody–protein complexes (Ponomarenko et al. 2008). Linear B cell epitope densities of proteins (ratio of epitope residue number into protein length) after signal peptide omission was determined and top two proteins with high densities were selected.

Three Dimentional Structures of Proteins

Since conformational epitope prediction servers use a 3D model in PDB format for analysis, we carried out 3D structure prediction for proteins with Esypred3D at http://www.fundp.ac.be/sciences/biologie/urbm/bioinfo/esypred/ a new automated homology modeling program (Lambert et al. 2002). The Phyre and Phyre2 at http://www.sbg.bio.ic.ac.uk/ servers predict the three-dimensional structure of a protein sequence using the principles and techniques of fold recognition and ab initio (Kelley and Sternberg 2009) and I-TASSER server at http://zhanglab.ccmb.med.umich.edu/I-TASSER/ predicts protein structures and functions and 3D models are built based on multiple-threading alignments as well as ab initio (Roy et al. 2010). Best models were selected based on Qmean score from Qmean server at http://swissmodel.expasy.org/qmean which provides an estimate of the "degree of nativeness" of the structural features observed in a model and describes the likelihood that a given model is of comparable quality to experimental structures (Benkert et al. 2008).

Conformational B cell Epitopes

Conformational B-cell epitopes were predicted by DiscoTope at http://www.cbs.dtu.dk/services/DiscoTope/ a novel method for discontinuous epitope prediction that uses protein three-dimensional structural data. The method is based on amino acid statistics, spatial information, and surface accessibility in a compiled data set of discontinuous epitopes determined by X-ray crystallography of antibody/antigen protein complexes. DiscoTope is the first method to focus explicitly on discontinuous epitopes (Kringelum et al. 2012). SEPPA http://lifecenter.sgst.cn/seppa/index.php, uses a concept of "unit patch of residue triangle" to better describe the local spatial context in protein surface. Besides that, SEPPA incorporated clustering coefficient to describe the spatial compactness of surface residues (Sun et al. 2009). Conformational epitopes also was predicted by Ellipro. Conformational B-cell epitope densities of proteins also were determined for proteins and the top two proteins with high densities were selected.

Data Validation

All bioinformatic procedures performed for the queries were carried out on *A. baumannii* OmpA protein as a positive control. Pseudomonas aeruginosa glycosyltransferase alg8 protein was chosen as a negative control. This task was accomplished to compare the results obtained from the servers and to validate the results on the query proteins.

Results

Selection of Iron-Regulated *A. baumannii* OMPs

Twelve outer membrane iron regulated proteins of *A. baumannii* were selected for analysis. These included iron repressed ferrienterochelin and colicins receptor, FepA (YP_001845601.1), Ferric aerobactin receptor protein (ZP_05827344.1), Ferric anguibactin receptor, BauA (AAT52186.1), TonB-dependent vitamin B12 receptor, BtuB (ZP_05829364.1), FhuE receptor (ZP_05827326.1),

a protein annotated as an outer membrane siderophore receptor (OMR) with accession number ZP_05829585.1, Carbapenem-associated resistance protein, CarO (ZP_05828783.1), F0F1 ATP synthase a subunit, AtpA (YP_001083238.2) and iron induced Putative outer membrane protein W, OmpW (ZP_05828557.1), Bacterioferritin, Bfr (EEX03909.1), Hypothetical protein A1S_1295 (ABO11723.2), Outer membrane protein A, OmpA (ABO30515.1) that its trans-membrane domain used for immunogenicity and Pseudomonas aeruginosa Glycosyltransferase alg8 (Q52463).

Antigenicity Prediction

Based on average scores of Hydrophilicity, flexibility and Beta turns and antigenicity of these twelve proteins, four of them viz., AtpA, OmpW, Bfr and hypothetical protein A1S_1295 bearing the lowest scores were omitted (Table 1).

Solubility Prediction

Aerobactin receptor and BauA predicted as insoluble by SOLpro and PROSO servers were omitted and the other six proteins were selected for the next step (Table 2).

Table 2 Solubility of proteins

Proteins	SOLpro S: soluble; I: insoluble (probability)	PROSO S: soluble; I: insoluble
Aerobactin receptor	I (0.818995)	I; 0.722
BauA	I (0.671559)	I; 0.561
BtuB	I (0.715596)	S; 0.516
CarO	S (0.632658)	S; 0.695
FepA	S (0.623812)	S; 0.598
FhuE	I (0.577281)	S; 0.696
OMR	S (0.802982)	S; 0.645
OmpA (contro l+)	S (0.835207)	S; 0.543
Glycosyltransferase alg8 (control −)	I (0.749880)	I; 0.982

Aerobactin receptor and BauA were predicted as insoluble

Linear B cell Epitopes were Prediction

The number of residues predicted as epitope with ABCpred proportional to all residues showed CarO and FepA with high epitope densities. ElliPro predicted CarO and FhuE with high epitope densities. BCPREDS predicted CarO and OmpA to have high epitope densities and Bepipred predicted CarO and FhuE to have high linear B cell epitope densities as compared to other proteins (Table 3).

Three Dimentional Structures of Proteins

The best 3D models were proposed by phyre for BtuB and OmpA (trans-membrane domain), phyre2 for FhuE and ESyPred3D for FepA and OMR and I-TASSER for

Table 1 Antigenic and immunogenic properties of proteins

	Proteins	IEDB			VaxiJen	ANTIGENPro
		Hydrophilicity	Flexibility	Beta turn		
	Iron-repressed proteins					
1	FepA	2.202	1.016	1.036	0.8136	0.918599
2	Aerobactin receptor	2.033	1.005	1.013	0.6083	0.924555
3	BauA	1.948	1.005	1.023	0.6071	0.915486
4	BtuB	1.861	1.00	1.005	0.6481	0.924984
5	FhuE	2.178	1.011	1.045	0.6523	0.931725
6	OMR	1.044	1.006	1.023	0.6791	0.858810
7	CarO	1.868	0. 997	1.038	0.6223	0.898285
8	AtpA	1.590	0.995	0.950	0.4084	0.653519
	Iron-induced proteins					
9	OmpW	0.891	0.989	0.967	0.6625	0.545317
10	Bfr	1.239	0.985	0.930	0.4087	0.454328
11	Hypothetical protein A1S 1295	1.520	0.995	0.958	0.3773	0.748460
12	OmpA (control +)	1.878	1.005	1.035	0.9582	0.798376
13	Glycosyltransferase alg8 (control -)	0.032	0.975	0.925	0.2915	0.101030

Average scores of hydrophilicity, flexibility and beta turns predicted with IEDB and score of probable antigenicity of proteins predicted with VaxiJen and ANTIGENPro. For each scale four proteins with the lowest scores were determined. AtpA, OmpW, Bfr and hypothetical protein A1S_1295 in consensus had the lowest scores of antigenicity and immunogenicity

Table 3 Linear B-cell epitope densities of proteins

Proteins	Length	ABCpred		ElliPro		BCPREDS		Bepipred	
		EN	ED	EN	ED	EN	ED	EN	ED
BtuB	586	193	0.33	247	0.42	104	0.18	251	0.43
CarO	228	124	0.54	136	0.59	107	0.47	129	0.56
FepA	730	302	0.41	336	0.46	232	0.32	381	0.52
FhuE	686	271	0.39	350	0.51	212	0.31	398	0.58
OMR	681	269	0.39	202	0.30	280	0.41	308	0.45
OmpA (control +)	178	48	0.30	72	0.40	92	0.52	82	0.46
Glycosyltransferase alg8 (control −)	459	160	0.35	155	0.34	0	0	87	0.19

Number of linear B cell epitope residues and protein amino acid length without signal peptide used in protein epitope density determination

EN epitope residue number, *ED* epitope density

CarO (Fig. 1). A 3D model was predicted for Glycosyltransferase alg8 with phyre2 (Fig. 2).

Conformational B cell Epitope

Based on predicted conformational epitopes in protein sequence, DiscoTope and Ellipro demonstrated CarO and FepA with high epitope densities and SEPPA demonstrated CarO and OmpA as having high densities of conformational B cell epitopes as compared to others (Table 4).

Discussion

Emergence of nosocomial diseases driven by multidrug resistant bacteria is a major issue for healthcare system (Prasad et al. 2006). *A. baumannii* is able to persist in the hospital environment and in particular intensive care units, due to its wide variety of resistance mechanisms and high survival rate on abiotic surfaces (Eijkelkamp et al. 2011). Proteins with iron regulated production in *A. baumannii* were selected for immunogenicity analysis in vaccine and drug design because one strategy explored to bypass the bacterial adaptation to drugs is to target the iron metabolism of bacteria, since iron is critical for all bacteria to grow (Ballouche et al. 2009). Surface-exposed proteins

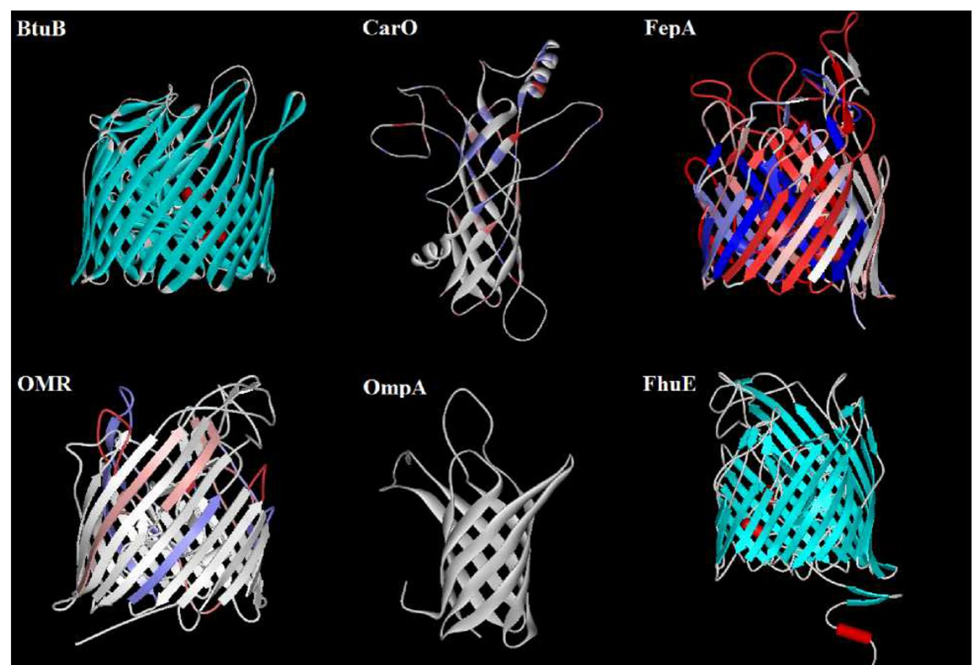

Fig. 1 3D models of proteins in *Acinetobacter baumannii*

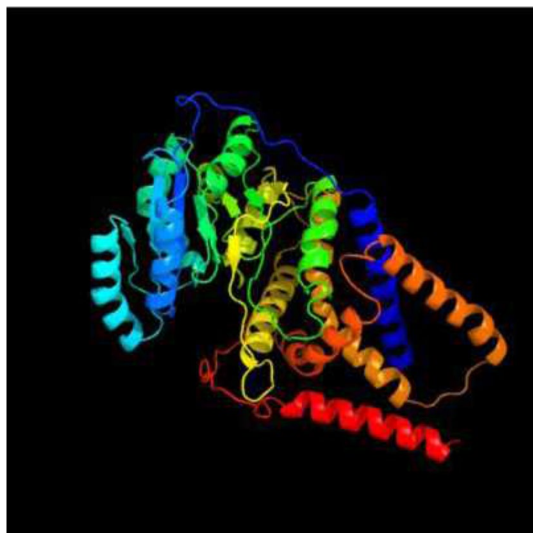

Fig. 2 3D model of *Pseudomonas aeruginosa* Glycosyltransferase alg8

such as OMPs, are ideal targets for vaccine development (Vivona et al. 2006). In addition to accessibility of antibodies to OMPs, these proteins of Gramnegative bacteria are known as strong immunogens (Toobak et al. 2013). Strong immunogenicity of Gram-negative bacteria OMPs is also demonstrated for *A. baumannii* OMPs by several studies (Moriel et al. 2013; McConnell et al. 2011; McConnell and Rumbo 2011). Antibodies directed against IROMPs of *A. baumannii* ATCC 19606 exert a bacteriostatic or bactericidal effect by blocking siderophore mediated iron uptake pathways (Goel and Kapil 2001). Moreover, these antibodies showed opsonising activity against *A. baumannii* in vitro (Goel and Kapil 2001). IROMPs localization engages them to be exposed to host immune system. It could be deduced that higher immunogenicity could provoke higher antibody titer and consequently higher protection probability. In the current study, IROMPs in *A. baumannii* were selected to predict their immunogenicity for vaccine and drug design. *A. baumannii* proteins for our analysis were selected based on the prior demonstration of an up regulated proteomic response to iron-rich and chelated conditions resulted in the identification of 58 protein spots differentially produced. 19 and 35 of them represent iron-repressed and iron-induced protein spots respectively. Four other spots representing a metal chelation response unrelated to iron (Nwugo et al. 2011) were omitted and then 30 total cell proteins were excluded. Several characteristics contribute to desired immunogenicity of a certain antigen. Hydrophilicity, mobility of backbone atoms, accessibility, topology and protein flexibility are involved in antigenicity of a protein (Kim et al. 2013). Also in proteins, immunogenic determinants that can induce protein-reactive antipeptide antibodies reside mostly in those parts of the molecule that have a high tendency to form beta-turns (Krchnak et al. 1987). IEDB provides an average score for hydrophilicity, beta turns and flexibility for proteins. These scales used to assess antigenicity and proteins that have the lowest scores and therefore low antigenic probability were omitted. Antigenic probability of proteins was estimated with Vaxijen and ANTIGENpro. Vaxijen is an appropriate server for prediction of protective antigens and can be used for in silico screening of genome information for vaccine development (Doytchinova and Flower 2007). ANTIGENpro also predicts the likelihood that a protein is a protective antigen (Cheng et al. 2005). These antigenicity predictors were also used for screening and elimination of the proteins with low scores. All data obtained from IEDB, ANTIGENpro and Vaxijen were consensus on the lowest antigenicity scores of AtpA, OmpW, Bfr and hypothetical protein A1S_1295. Hence, these proteins were omitted in the next step analyses. Protein solubility was another factor used for immunogenicity screening. Based on this fact,

Table 4 Conformational B cell epitope densities of proteins predicted with DiscoTope, SEPPA and ElliPro

Proteins	DiscoTope		SEPPA		ElliPro	
	EN	ED	EN	ED	EN	ED
BtuB	201	0.35	103	0.18	283	0.48
CarO	118	0.51	108	0.43	135	0.59
FepA	372	0.52	173	0.24	389	0.53
FhuE	350	0.49	132	0.18	353	0.51
OMR	265	0.38	163	0.24	199	0.29
OmpA (control +)	8	0.04	84	0.52	83	0.47
Glycosyltransferase alg8 (control −)	25	0.06	Not done	Not done	105	0.24

Number of conformational B cell epitope residues and protein length used for protein epitope density prediction

EN epitope residue number, *ED* epitope density

insoluble proteins were omitted. Solubility prediction was carried out with SOLpro and PROSO to assess the chance of solubility upon heterologous expression based on amino acid composition (Magnan et al. 2009; Smialowski et al. 2007). Two proteins predicted as insoluble were omitted and 6 proteins were selected for epitope mapping analysis. As density of B cell epitopes is important in immunogenicity of the proteins, hence B cell epitopes in the remaining six proteins were determined. A major task in vaccine design is to select and design proteins containing antibody-binding epitopes (B-cell epitopes) able to induce an efficient immune response (Haste Andersen et al. 2006). Because the experimental determination of B-cell epitopes is time-consuming and expensive, there is an urgent need for computational methods for reliable identification of putative B-cell epitopes from antigenic sequences (EL-Manzalawy et al. 2008). B-cell epitopes typically belong to one of two classes: linear (continuous or sequential) or conformational (discontinuous) epitopes. Linear epitopes are short peptides that correspond to a contiguous amino acid sequence fragment of a protein. Conformational epitopes are composed of amino acids that, although not contiguous in primary sequence, are brought into close proximity within the folded 3-dimensional protein structure (Yasser and Honavar 2010). Linear and conformational B cell epitopes within the proteins were predicted with several servers to elevate accuracy and confidence of prediction results. All these servers predicted CarO to have high contents of linear and conformational B cell epitopes compared to other proteins. Recent studies have shown a close correlation between epitope density and epitope-specific humoral immune responses. Comparison of the antigenic and immunogenic differences between protein molecules bearing low or high degrees of epitope density, show clearly that fusion proteins bearing higher epitope density resulted in higher average avidity for epitope specific mAb and polyclonal antibodies (pAb) with enhanced an average affinity constant (KA) for epitope peptide compared to fusion proteins bearing low epitope density. Thus, high epitope density in a single protein molecule significantly enhances antigenicity and immunogenicity (Liu and Chen 2005). Relationship between epitope density and protective immunity might provide a novel strategy for vaccine development that could induce efficient protective immunity. By lethal challenge assay in mice, it was observed that recombinant proteins of higher epitope densities resulted in higher survival rates. The survival rate was directly related to the degree of epitope density in the single recombinant protein (Liu et al. 2004). *A. baumannii* OmpA was used as control positive for analysis. OmpA has been identified as a primary target of humoral immune response after intravenous infection by *A. baumannii* in mice. Vaccination of mice with recombinant OmpA (rOmpA) markedly improved survival and reduced tissue bacterial burden in mice infected intravenously. Vaccination induced high titers of anti-OmpA antibodies, the levels of which correlated with survival in mice. Passive transfer with immune sera recapitulated protection (Luo et al. 2012). Bacterial, viral and tumour protein datasets were used to derive models for prediction of whole protein antigenicity in VaxiJen server. Every set consisted of 100 known antigens and 100 non-antigens. The derived models were tested by internal leave-one-out cross-validation and external validation using test sets. An additional five training sets for each class of antigens were used to test the stability of the discrimination between antigens and non-antigens. The models performed well in both validations showing prediction accuracy of 70–89% (Doytchinova and Flower 2007). Pseudomonas aeruginosa Glycosyltransferase alg8 a Multi-pass membrane protein is a bacterial non antigen that this server introduces and in this used as control negative. BlastP of this protein against non-redundant database showed 82% coverage and 22% identity with *N*-glycosyltransferase of *A. baumannii*. Using several criteria to assess immunogenicity and B epitope densities CarO was selected as an immunogen in *A. baumannii*. Recently, 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 and Rumbo 2011). However, additional experimental evidence is needed to confirm the validity of these predictions. This protein participates in the selective uptake of L-ornithine, carbapenems, and other basic amino acids in *A. baumannii* (Mussi et al. 2007) and whose loss was concomitant with increased carbapenem resistance among clonally related nosocomial isolates of this opportunistic pathogen (Mussi et al. 2011). Carbapenem resistance in *A. baumannii* has mostly been ascribed to plasmid and chromosome-encoded carbapenemases. Various studies have shown that loss of the CarO OMP had only a minor effect on carbapenem resistance in *A. baumannii* (Lee et al. 2011), and this factor has little dispersion and less important than other factors. In a study, 44 *A. baumannii* clinical isolates obtained in the period from 1990 to 2005 from patients hospitalized in 12 public nosocomial institutions of major urban centers of Argentina, 40 isolates presented MDR phenotypes, from which 28 showed additional carbapenem-resistant phenotypes. All these isolates had the *carO* gene (Mussi et al. 2011). CarO may contribute in siderophore mediated iron uptake. The positive effect of DIP (iron chelator) on the production of CarO, supports the possibility of the

expression of alternative siderophore-mediated utilization systems (Nwugo et al. 2011).

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Compliance with Ethical Standards

Conflict of interest We declare no conflict of interests.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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