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ORIGINAL RESEARCH

Molecular characterization and epitope-based vaccine predictions for *ompA* gene associated with biofilm formation in multidrug-resistant strains of *A.baumannii*

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Abstract
The present study was conducted to molecularly characterize the biofilm associated *ompA* gene from the drug resistant strains of *A. baumannii* and its immuno-dominant vaccine epitope predictions through immuno-informatic approach. *ompA* was amplified by PCR from the genomic DNA and was sequenced. Using the ORF, ompA protein sequence was retrieved and was subjected for IEDB T cell and B cell epitope analysis for the selection of the epitope peptides. Selected peptides were evaluated using appropriate servers and tools to assess the propensity for its antigenicity, solubility, physico-chemical property, toxigenicity and class-I immunogenicity. MHC class I and II restriction of HLA alleles was also performed. 48% (n = 24) of the strains possessed *ompA* gene. Protein structure was successfully retrieved with the selection of two epitopes viz., E1- FDGVNRGTRGTSEEGTLGNA and E2-KLSEYPNATARIEGHTDNTGPRKL. Final docking with TLR-2, showed E2 as the best epitope candidate predicted with the highest number of hydrogen bonds.

Keywords *A. baumannii* · Biofilm · ompA · Epitope vaccines · In-silico

Introduction

Acinetobacter baumannii is an opportunistic, aerobic, non-motile, pleomorphic gram-negative bacteria and is commonly associated with nosocomial infections (Howard et al. 2012). Over the last 15 years, it has been considered as the most burdensome gaining incredible attention due to its ability to acquire resistance determinants, threatening the current era of antibiotics (Peleg et al. 2008). *A. baumannii* is a saprophyte growing luxuriously in aquatic habitat while in the in-vitro cultures, it is mostly characterized from urine samples, respiratory secretions, and wounds and thus seems to be associated with a range of infections like urinary tract infection, meningitis, bacteremia, and pneumonia (Dexter et al. 2015). Having been declared as a priority pathogen by WHO (2017), the high-risk group are usually the patients in hospitals because *A. baumannii* is known to colonize on the in-built devices through its potent biofilm formation (Fournier et al. 2006).

Acinetobacter baumannii establishes the infection in hospitalized host mainly through the biofilm formation on both biotic and abiotic surfaces and thus the same fact is considered as a potent virulent factor (Eze et al. 2018). Formation of biofilms in *A.baumannii* is associated with various genes such as *PER1, bap, csu, csgA*, *KpsMT,* etc., amidst which *ompA* gene takes a huge role in biofilm and porin formation (Gaddy et al. 2009). A review by Muhammed Asif et al. (Asif et al. 2018), has documented that *ompA* in *A.baumannii* aids in improving adhesion to the respiratory tract, over-expression resulting in cell death and modulation in the host immune response. In addition, studies have also documented the prevalence of *ompA* due to its overexpression and up-regulation of the same, stimulating the formation and development of biofilm layers (Cassin and Tseng, 2019; Orme et al. 2006). The role of *ompA* and its proteomic

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chemistry in biofilm formation was detailed in *B. fragilis* and in *Sodalis* commensal bacteria (Cassin and Tseng, 2019). Studies also report the failure of the biofilm formation in the mutant strains of *A. baumannii* and in the strains that do not express the *ompA* production (Maltz et al. 2012). It is exciting to note that the ability of *A. baumannii* to survive in dry, harsh environment is highly associated with *ompA* based biofilms leading to a mortality rate ranging from 26 to 68% (Vijayakumar et al. 2016).

In this context, assessing the correlation of *ompA* in MDR strains and targeting the *ompA* gene/protein associated with virulence would be a promising alternating strategy to combat the menace of drug resistance. Conventional vaccine models yielding partial protection, the prediction of immuno-dominant peptides, and its antigenic potential by applying the bioinformatics tools and databases is a fascinating approach leading to peptide synthesis and further vaccine development. In this context, the present investigation is undertaken to molecularly characterize the *ompA* gene among the drug-resistant *A. baumannii* strains and to predict the immuno-dominant vaccine candidates by immune-informatic approach.

Methods

Extraction of genomic DNA

Fresh cultures of 50 multidrug-resistant strains of *A. baumannii* which were maintained at – 80 °C in 80%/20% (v/v) glycerol in LB medium from our repertoire used in our earlier studies (Smiline et al. 2019) was retrieved on Mac Conkey agar with incubation at 37 °C for 24 h. Genomic DNA was extracted using the Qiagen DNA extraction kit conforming to the instructions provided by the manufacturer. The procured DNA was stored at − 20 °C up to the time of further use.

PCR amplification of ompA

7.8 µl of 2 × master mix [Taraka, Japan] in 5.6 µl of double-distilled water, in addition to 0.31 µl of 100 pmol/ml concentration of the specific F'primer and R'primer [Eurofins Genomic India Pvt Ltd, Bangalore] of *ompA* gene was used to formulate 15 µl of the PCR reaction mixture. 1 µl of the extracted genomic DNA was combined with the master mix and PCR was performed for amplification. F: GCGCCA CAACCAAGCAATTA and R: GCGCCACAACCAAGC AATTA primers were used for the same. Using the reaction condition of annealing temperature as 58 °C for 35 cycles, PCR amplification for *ompA*was carried out in Eppendorfthermocycler, Germany. 1% agarose gel electrophoresis containing ethidium bromide was used to examine the resulting PCR amplicons. The results were visualized by the gel documentation system. The PCR amplicon size was assessed using the 100 bp DNA ladder.

Sequencing of the ompA for amplicon confirmation:

The using Big-Dye terminator cycle sequencing kit and 3730XL Genetic Analyzer is used to bi-directionally sequence the amplicon product of *ompA*. Bio-Edit Sequence Alignment Editor v7.2.5 was used to align the sequences from forward and reverse primers. For nucleotide similarity search it was subjected to BLAST (Basic Local Alignment Search Tool). ClustalW software version 1.83 was used to align the sequences using default parameters.

ORF and Blast-p similarity analysis

The genomic sequence of *ompA* obtained by molecular sequencing of the amplicon was further subjected to open reading frame (ORF) analysis in ORF finder and programmed for the protein detection. The obtained protein sequenced was then predicted for the specific *ompA* protein with further multiple sequence alignment for the protein and its phylogenetic evaluation with BLASTp. A BLASTp analysis was performed on the sequence against the non-redundant protein sequence database and a non-human homologous protein sequence database of *Homosapiens taxid 9606* was interpreted and recorded. Phylogenetic similarity analysis was done using the protein sequence of the gene as evaluated using the gene sequence obtained from the sequence analysis.

Analysis of druggability, physico-chemical properties, and antigenicity

The protein of choice (*ompA*) was evaluated using the Drug bank database to check for potential drug targets. It was evaluated against all the drug targets. Using online tools such as PSORTb and CELLO v2.5 analysis of the predictions based on the concurrent location of the biological orientation of the druggable protein was performed. The prediction using these tools will render the location of the epitopes that can be applied during drug development. The Protparam expasy server was used to analyze the physicochemical properties, such as molecular weight, instability index, theoretical PI, aliphatic index, and GRAVY. To confirm whether the protein selected possesses any potent epitopes, VaxiJen webserver was used. A threshold value of 0.5 was set against

which *ompA* was subjected to analysis for potent antigenicity predictions.

Prediction of T-cell epitopes and MHC class restrictions

NetCTL server [Predictions of MHC class I epitopes (Prediction threshold value 0.75 was set for epitope identification IEDB AR (T-cell epitopes were subjected to MHC-I binding prediction, using the immune epitope database analysis resource (IEDB AR). MHC molecules represent the antigenic peptides which are recognized by the T-cells. IEDB is recommended as a default prediction method and uses the consensus method consisting of ANN32, SMM33, and CombLib34 and NetMHCpan35. The tools provide the high combinatorial scores for on peptide's intrinsic potential. It also integrates several predictions on various biological factors such as proteosomal cleavage mechanism, TAP transporter efficiency involved in antigen processing, affinity scores of MHC-I predictions. Identified T-cell epitopes with HLA alleles were selected by IC50 values and percentile rank. Lower the percentile rank the higher the interaction shown between the peptide and MHC molecules. IC_{50} values divided in three different categories: high binding affinity, IC_{50} value < 50 nM; intermediate binding affinity, IC_{50} value < 500 nM; and low binding affinity, IC_{50} value < 5000 nM36).

Cluster analysis of the MHC restricted alleles

For the further strengthening of the predictions done, the restricted MHC alleles were subjected to MHC clusterV2.0 server to identify the clusters to interpret the functional relationship between the selected epitope peptides and the HLA alleles. The interactions were obtained in the form of the graphical tree and static heat maps.

B-cell epitope mapping with antigenicity predictions

IEDB linear epitope B cell prediction tests can be performed to check if the epitopes can bind to B cell receptors and stimulate a humoral immune response. This prediction server predicts the peptide epitopes based on various biochemical properties involving the composition of amino-acid, affinity to water and secondary protein structures. The obtained score is termed as Protrusion Index, which is an average of overall epitope residues, and 3D structure of each protein is approximated through some ellipsoids and the structures yielding larger scores shows the solvent accessibility of the proteins. The *ompA* sequence is subjected to various standardized tests like BepiPred linear epitope prediction (Larsen et al. 2006), Karplus-Schulz flexibility prediction (Karplus and Schulz 1985), Chou-Fasman beta-turn prediction (Yang 1996), Kolaskar Tongaonkar antigenicity (As and Pc 1990), Emini surface accessibility prediction (Emini et al. 1985), and Parker hydrophilicity prediction (Jm et al. 1986). As the name implies each prediction tool evaluates the properties of the epitopes and further its antigenicity as observed by yellow peaks in the graphs. MHC class I and II predictions were made in the same way as that of T-cell epitope predictions in the IEDB server and the common epitopes were selected for further docking analysis. All the selected epitopes were subjected for the analysis of antigenicity (Magnan et al. 2010) and solubility by VaxiJenV2.0, and physico-chemical property by Protparam servers.

Interaction between protein and TLR2 receptor

The selected epitope peptides were finally subjected to interact with the TLR-2 receptor. TLR-2 is selected due to the fact that it can interact with huge number of non-TLR molecules and can recognize a wide variety of complex molecules from all microbial phyla. It is ubiquitous in most of the immune cells, endothelial and epithelial cells with vast roles and functions (Flo et al. 2001). Galaxy web server (Afgan et al. 2018) was used for analyzing possible interactions of the *ompA* predicted epitopes with the TLR2 receptor. Based on the energy obtained on the interpretation of the number of hydrogen bonds formed between the complexes, it will facilitate the designing of an efficient *ompA* vaccine.

Results

Molecular characterization of the ompA gene and its correlation with multi-drug resistance

From the screened 50 genomes of MDR *A.baumanni*i, 48% showed positive amplicons for the *ompA* gene associated

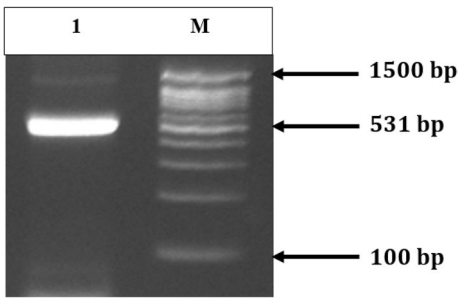

Fig. 1 Electrophoregram of ompA gene product of size 531 bp in lane 1 and 2 with marker ladderof size 1.5Kbp (M)

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0220_247_8_PCR_OMP_OMPF      ATTACTAAAAACTACGACAGCAAAATCAAGCCGTACGTATTATTAGGTGCTGGTCACTAT      60
CP042931.1:A.baumannii       ATTACTAAAAACTACGACAGCAAAATCAAGCCGTACGTATTATTAGGTGCTGGTCACTAT      60
CP045528.1:A.baumannii       ATTACTAAAAACTACGACAGCAAAATCAAGCCGTACGTATTATTAGGTGCTGGTCACTAT      60
CP032743.1:A.baumannii       ATTACTAAAAACTACGACAGCAAAATCAAGCCGTACGTATTATTAGGTGCTGGTCACTAT      60
                             ************************************************************

0220_247_8_PCR_OMP_OMPF      AAATATGACTTTGATGGCGTAAATCGTGGTACACGTGGTACTTCTGAAGAAGGTACTTTA      120
CP042931.1:A.baumannii       AAATATGACTTTGATGGCGTAAATCGTGGTACACGTGGTACTTCTGAAGAAGGTACTTTA      120
CP045528.1:A.baumannii       AAATATGACTTTGATGGCGTAAATCGTGGTACACGTGGTACTTCTGAAGAAGGTACTTTA      120
CP032743.1:A.baumannii       AAATATGACTTTGATGGCGTAAATCGTGGTACACGTGGTACTTCTGAAGAAGGTACTTTA      120
                             ************************************************************

0220_247_8_PCR_OMP_OMPF      GGTAACGCTGGTGTTGGTGCTTTCTGGCGCTTAAACGACGCTTTATCTCTTCGTACTGAA      180
CP042931.1:A.baumannii       GGTAACGCTGGTGTTGGTGCTTTCTGGCGCTTAAACGACGCTTTATCTCTTCGTACTGAA      180
CP045528.1:A.baumannii       GGTAACGCTGGTGTTGGTGCTTTCTGGCGCTTAAACGACGCTTTATCTCTTCGTACTGAA      180
CP032743.1:A.baumannii       GGTAACGCTGGTGTTGGTGCTTTCTGGCGCTTAAACGACGCTTTATCTCTTCGTACTGAA      180
                             ************************************************************

0220_247_8_PCR_OMP_OMPF      GCTCGTGCTACTTATAATGCTGATGAAGAGTTCTGGAACTATACAGCTCTTGCTGGCTTA      240
CP042931.1:A.baumannii       GCTCGTGCTACTTATAATGCTGATGAAGAGTTCTGGAACTATACAGCTCTTGCTGGCTTA      240
CP045528.1:A.baumannii       GCTCGTGCTACTTATAATGCTGATGAAGAGTTCTGGAACTATACAGCTCTTGCTGGCTTA      240
CP032743.1:A.baumannii       GCTCGTGCTACTTATAATGCTGATGAAGAGTTCTGGAACTATACAGCTCTTGCTGGCTTA      240
                             ************************************************************

0220_247_8_PCR_OMP_OMPF      AACGTAGTTCTTGGTGGTCACTTGAAGCCTGCTGCTCCTGTAGTAGAAGTTGCTCCAGTT      300
CP042931.1:A.baumannii       AACGTAGTTCTTGGTGGTCACTTGAAGCCTGCTGCTCCTGTAGTAGAAGTTGCTCCAGTT      300
CP045528.1:A.baumannii       AACGTAGTTCTTGGTGGTCACTTGAAGCCTGCTGCTCCTGTAGTAGAAGTTGCTCCAGTT      300
CP032743.1:A.baumannii       AACGTAGTTCTTGGTGGTCACTTGAAGCCTGCTGCTCCTGTAGTAGAAGTTGCTCCAGTT      300
                             ************************************************************

0220_247_8_PCR_OMP_OMPF      GAACCAACTCCAGTTGCTCCACAACCACAAGAGTTAACTGAAGACCTTAACATGGAACTT      360
CP042931.1:A.baumannii       GAACCAACTCCAGTTGCTCCACAACCACAAGAGTTAACTGAAGACCTTAACATGGAACTT      360
CP045528.1:A.baumannii       GAACCAACTCCAGTTGCTCCACAACCACAAGAGTTAACTGAAGACCTTAACATGGAACTT      360
CP032743.1:A.baumannii       GAACCAACTCCAGTTGCTCCACAACCACAAGAGTTAACTGAAGACCTTAACATGGAACTT      360
                             ************************************************************

0220_247_8_PCR_OMP_OMPF      CGTGTGTTCTTTGATACTAACAAATCAAACATCAAAGACCAATACAAGCCAGAAATCGCT      420
CP042931.1:A.baumannii       CGTGTGTTCTTTGATACTAACAAATCAAACATCAAAGACCAATACAAGCCAGAAATCGCT      420
CP045528.1:A.baumannii       CGTGTGTTCTTTGATACTAACAAATCAAACATCAAAGACCAATACAAGCCAGAAATCGCT      420
CP032743.1:A.baumannii       CGTGTGTTCTTTGATACTAACAAATCAAACATCAAAGACCAATACAAGCCAGAAATCGCT      420
                             ************************************************************

0220_247_8_PCR_OMP_OMPF      AAAGTTGCTGAAAAATTATCTGAATACCCTAACGCTACCTGCCACGAA   468
CP042931.1:A.baumannii       AAAGTTGCTGAAAAATTATCTGAATACCCTAACGCTACTGCACG----   464
CP045528.1:A.baumannii       AAAGTTGCTGAAAAATTATCTGAATACCCTAACGCTACTGCACG----   464
CP032743.1:A.baumannii       AAAGTTGCTGAAAAATTATCTGAATACCCTAACGCTACTGCACG----   464
                             ************************************    *
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Fig. 2 Partial sequence alignment of *ompA* gene from the present study (Ab) with reference sequences available in the database. The deleted regions are depicted as dashes (–), mismatch as gap () and conserved sequences as star (*)

with biofilm and porin formation with 531 bp as amplicon size (Fig. 1). The gene sequence obtained is shown in Fig. 2. Correlation of its occurrence was high in carbapenem-resistant strains, followed by aminoglycoside, fluoroquinolone, and efflux pumps. The correlation of *ompA* was high (100%; n = 24) in the resistant groups of beta-lactam inhibitors such as piperacillin and tazobactam; cephalosporins such as ceftazidime, cefepime, ceftriaxone; carbapenems such as doripenem, meropenem, and imipenem; and folates. It was followed by an occurrence of 83.3%, 50% and 33.3% among aminoglycosides, fluoroquinolones and efflux pumps mediated resistant strains respectively.

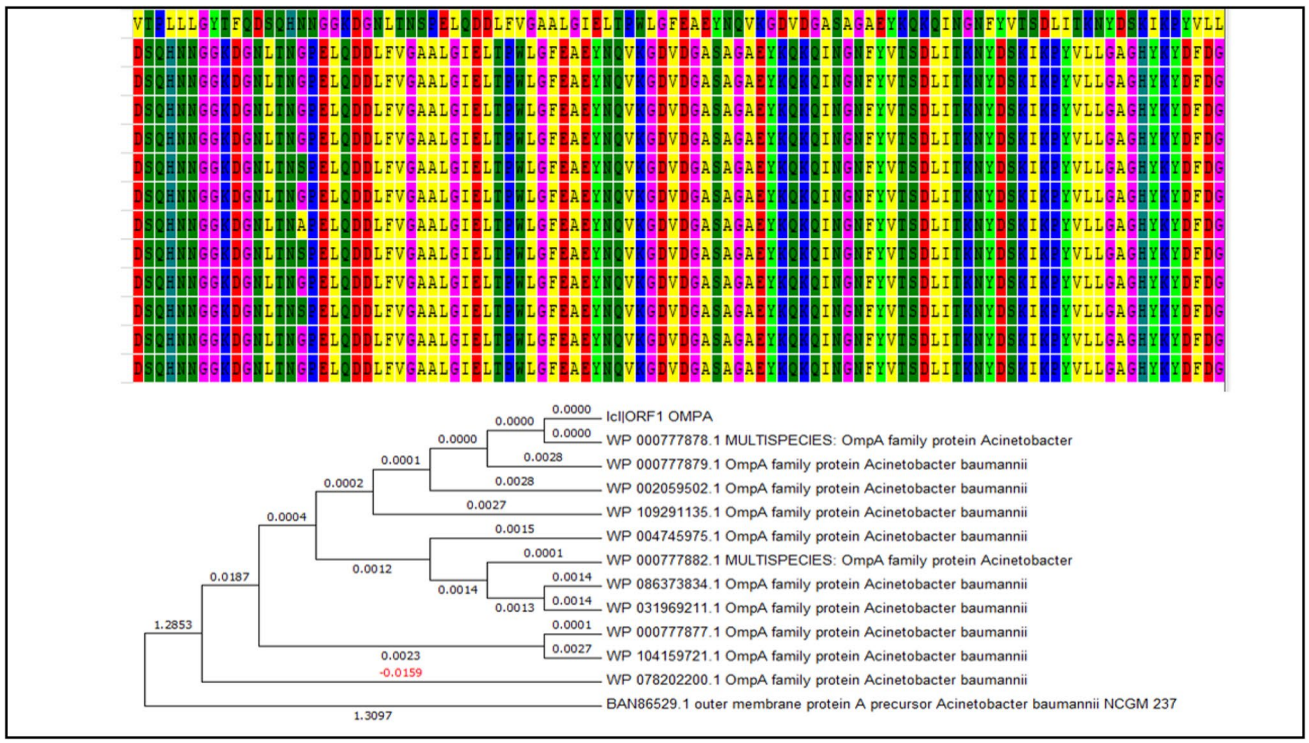

Fig. 3 Phylogenetic similarity of the *ompA*protein sequence obtained through the ORF mega search results, obtained from the input sequence of the *ompA*gene of *A.baumannii*

Preliminary evaluation of ompA epitopes

Using the ORF finder the obtained *ompA* sequence yielded potential protein encoding segments which was further confirmed with the sequence alignment (Fig. 3) and similarity through the phylogenetic tree. The obtained sequence of *ompA* of *A.baumannii* Q6RYW5 upon human similarity search by BLASTp did not yield any significant similar proteins. The selected protein had a 355aa length and was found to be located in the outer membrane by PSORTb and CELLO servers. GRAVY values were negative (− 0.388) with a high aliphatic index (80.93) and the protein was found to be stable with the predicted value < 40 (Table 1). Overall prediction on antigenicity was observed using VaxiJen server and was found to be a probable antigen with a score of 0.8524 (at a threshold value of 0.5).

Table 1 Prediction of MHC class I epitopes *ompA* of A. baumannii proteins using NetCTL

Residue number	Peptide sequence	Aff	Aff rescale	cleavage	TAP	Prediction score
176	NADEEFWNY	0.7323	3.1091	0.9308	2.7860	3.3880
107	TSDLITKNY	0.6866	2.9153	0.9470	2.8150	3.1981
222	LTEDLNMEL	0.3464	1.4710	0.9172	0.6260	1.6398
184	YTALAGLNV	0.3077	1.3065	0.5209	0.4310	1.4062
25	TVTPLLLGY	0.2219	0.9420	0.9574	3.0530	1.2382
251	KVAEKLSEY	0.2132	0.9052	0.8816	3.3410	1.2045
122	YVLLGAGHY	0.1905	0.8087	0.9754	2.9160	1.1008
124	LLGAGHYKY	0.1816	0.7709	0.9761	2.8320	1.0589
269	HTDNTGPRK	0.2151	0.9131	0.8370	0.1460	1.0460
290	VKSALVNEY	0.1431	0.6075	0.9167	3.0860	0.8994

Table 2 Class I immunogenicity predictions from *ompA* of *A.baumannii*

Peptide	Length	Class I Immunogenicity
NADEEFWNY	9	0.49881
YVLLGAGHY	9	0.10257
HTDNTGPRK	9	0.09127
VKSALVNEY	9	0.06677
YTALAGLNV	9	0.0584
TVTPLLLGY	9	– 0.00936
LLGAGHYKY	9	– 0.0153
TSDLITKNY	9	– 0.0236
LTEDLNMEL	9	– 0.05177
KVAEKLSEY	9	– 0.18811

T-cell dominant epitopes and MHC class-1 and class – II restrictions

Using the NetCTL server, the T-cell epitopes and its MHC class-I peptides were retrieved (Table 1). T cell class-I immunogenicity predictions were assessed using the scores obtained and the peptides of higher scores were considered as the probable antigens to elicit an immune response. Thus the top 5 epitopes with the higher scores were selected for further analysis (Table 2). Out of 5 epitopes, 2 epitopes were predicted as probable antigens with the antigenic score of > 0.5, SVM scores as non-toxins and yielded 100% conservancy (Table 3). Using the IEDB server, the MHC class-II restricted alleles were also predicted from where 5 epitopes were selected and were considered to elicit a proper humoral immune response. This selection was based on the lowest percentile ranks.

Table 3 Toxigenicity, antigenicity and Class I conservancy of T cell epitopes from *ompA* of *A.baumannii*

Peptide	Toxicity (SVM score)	Antigenicity [Vaxijen v2.0 (Threshold 0.5)]	Conservancy (%)
NADEEFWNY	– 0.48	– 0.0679	100
YVLLGAGHY	– 1.03	– 0.1289	100
HTDNTGPRK	– 0.57	**1.6471**	100
VKSALVNEY	– 0.90	0.3883	100
YTALAGLNV	– 1.27	**0.6177**	100

Selection of B-cell epitopes and their antigenic properties

B-cell epitope prediction is crucial to peptide driven vaccine design in concert with the predictions on the MHC class II epitopes (Table 4). The recognition of epitopes by B cells is based on features such as hydrophilicity, surface accessibility, flexibility, linearity and presence of beta turns. Bepipred tool aids in the determination of single scale amino acid propensity profiles. B cell epitopes were thus determined by the highest peak yellow region (threshold value) which corresponds to beta turns of the epitope. Further, using the IEDB linear epitope predictions, 8 epitopes were totally selected towards the immuno-dominant B-cell epitope selection (Table 5). Further analysis and selection of the B-cell dominant epitopes done based on the biochemical parameters under the IEDB B-cell epitope analysis showed that the selected epitopes possessed good biochemical properties of flexibility, beta turns, antigenicity, accessibility, and hydrophilicity. The propensity of the antigenicity was measured above the threshold values and was observed as yellow coloured peaks. Figure 4 shows the graphical peaks with yellow color as interpreted as the B-cell antigenic epitopes. Based on the physico-chemical property, immunogenicity and antigenicity scores, (Table 5), two epitopes viz., E1- FDGVNRGTRGTSEEGTLGNA and E2- KLSEYPNATARIEGHTDNTGPRKL were selected for further docking with TLR-2.

Table 4 Prediction of MHC class II epitopes by IEDB server from *ompA* of *A. baumannii*

Epitope (Percentile Rank)	MHC-II HLA ALLELES
RRVFATITGSRTVVV(0.10)	HLA-DRB1*01:05 HLA-DRB1*01:09 HLA-DRB1*01:01 HLA-DRB1*01:03
NRRVFATITGSRTVV (0.17)	HLA-DRB1*01:05 HLA-DRB1*01:09 HLA-DRB1*01:01 HLA-DRB1*01:03
ERLSLARANSVKSAL (0.24)	HLA-DRB1*01:05 HLA-DRB1*01:09 HLA-DRB1*01:03 HLA-DRB1*01:01
NERLSLARANSVKSA (0.24)	HLA-DRB1*01:05 HLA-DRB1*01:09 HLA-DRB1*01:03 HLA-DRB1*01:01
RVFATITGSRTVVVQ (0.27)	HLA-DRB1*01:05 HLA-DRB1*01:09 HLA-DRB1*01:03 HLA-DRB1*01:01

Table 5 Predicted B cell epitopes of protein using IEDB Linear Epitope prediction from *ompA* of *A.baumannii*

Position		Epitope	Length	Protaparam(stability) < 40—stable	Immunogenicity	Antigenicity [Vaxijen v2.0 (Threshold > 0.5)]
36–56	E1	QDSQHNNGGKDGNLTNGPELQ	21	48.43	− 0.12578	2.1704
78–100	E2	EYNQVKGDVDGASAGAEYKQKQI	23	4.74	− 0.62768	1.4468
134–153	E3	FDGVNRGTRGTSEEGTLGNA	20	16.13	0.40772	1.3390
172–181	E4	RATYNADEEF	10	33.11	0.20456	0.2758
198–223	E5	LKPAAPVVEVAPVEPTPVAPQPQELT	26	92.34	0.37525	0.5815
238–257	E6	KSNIKDQYKPEIAK	14	50.01	− 0.16435	0.6970
255–278	E7	KLSEYPNATARIEGHTDNTGPRKL	24	20.62	0.51349	0.9870
310–326	E8	FAWDQPIADNKTKEGRA	17	29.68	− 0.07549	1.2577

Protein-peptide interactions

Using the Galaxy-web server, the ability of the epitopes to evoke an immune response is assessed by the docking of the epitopes with the TLR-2 epitopes. E2 was considered to be the best epitope predicted from the ompA protein of *A.baumannii* with a highest number of hydrogen bonds (n = 16) followed by 13 hydrogen bonds by E-1 (Fig. 5). The possible interactions between E1 and E2 with the TLR-2 receptor was observed and was promising (Table 6). However, both the epitopes showed a similarity score of − 10.0 kcal/mol.

Discussion

The multi-drug resistant *A. baumannii* is considered as a serious threat especially among hospitalized patients. Being a potent nosocomial pathogen, it requires novel treatment strategies to be implemented promptly and the best possible option currently is apt prophylaxis. With the advent of bioinformatics, in-silico experimentation and molecular analysis is integrated into this study to additionally analyze the immuno-dominant epitopes against the *ompA* of *A. baumannii* to cease the formation of biofilms. The vital step in the construction of vaccine is the selection of B cell and T cell dominant epitopes which will bind significantly with the immunological receptors. This evokes and elicits the humoral and cell mediated immune responses. With the help of immuno informatics, promising antigenic epitopes have been successfully identified corresponding to the ompA protein. This was possible by utilizing the genomic and proteomic reservoirs available in a single computational platform consisting of various tools and databases.

The specific target under study, the ompA protein and its relevant predictions on the antigenic epitope structures was timely to curb the biofilm formation. The present study, documents the co-occurrence of *ompA* gene with different groups of drug resistant strains. 48% of the strains showed the presence of *ompA* gene substantiating its virulence in the establishment of infection among nosocomial patients. The *ompA* gene is known to be present in both gram positive and gram-negative organisms on the outer membrane, but most predominantly on the latter (Achouak et al. 1998). It accounts for its propensity of multi-drug resistance (Handal et al. 2017), biofilm formation (Gaddy and Actis 2009; Shin and Eom 2020) and the aggressiveness of virulence attributed to *ompA* based biofilms. The present study aims to assess the same at a molecular level and find the correlation of its occurrence with its property of multidrug resistance which is known to worsen the conditions among nosocomial patients. A similar study conducted in Iran suggested that the multidrug-resistant property of the bacteria will prove to be more virulent and thus causes nosocomial infections. The preventive measure to control such infection contains the inception and spread of the extremely drug-resistant (XDR) *A. baumannii* (Zeighami et al. 2019). Multiplex PCR assay of the strains was carried out in clonal lineage analysis. This was done to document the role of the *ompA* gene in *A. baumannnii* strains resistant to tetracycline. This interpretation of the results from this study documents *33.3%* of the tetracycline-resistant strains of the bacteria. Some studies state that the upregulation of the *ompA*gene is associated with tetracycline mediated RND pumps (Soto, 2013). These pumps are considered to directly or indirectly influence the formation of outer membrane porin which eventually leads to the ejection of the drug, leading to resistance (Fernández and Hancock 2013). The percentage of reduction of *ompA* occurrence in this study might be due to the effect of the *ompA* gene on the porin formation. A previous study contradicts this by stating that in other gram-negative bacteria such as *E.coli*, there is decreased tetracycline susceptibility with increased ompA homologue expression (Smani et al. 2014).

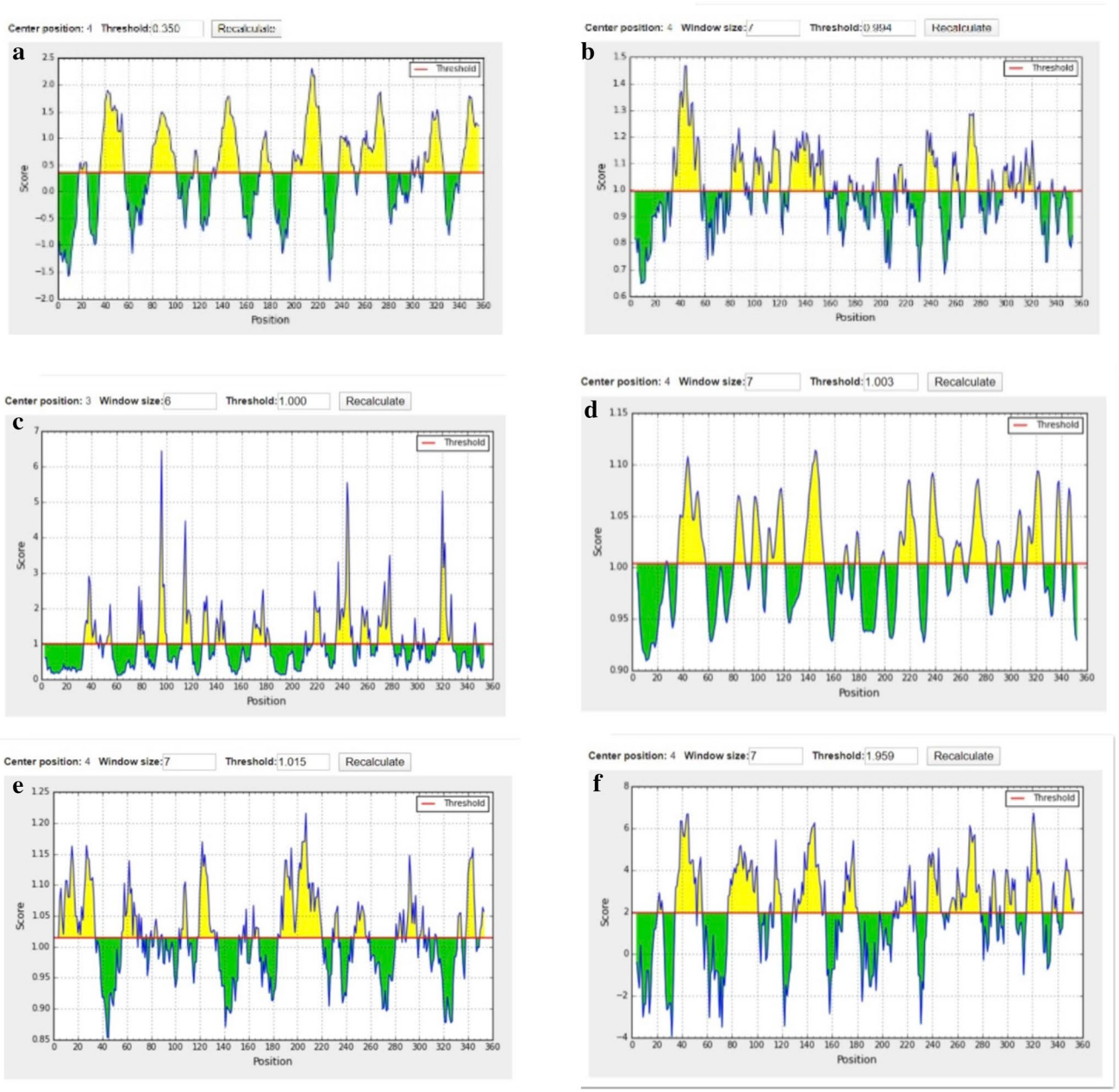

Fig. 4 B cell epitope antigenicity predictions: **a** Bepipred Linear Epitope, **b** Chou &Fasman Beta-TurnPrediction, **c.**Emini Surface Accessibility Prediction, **d** Karplus& Schulz Flexibility Prediction, **e** Kolaskar&Tongaonkar Antigenicity, **f** Parker Hydrophilicity Prediction. The X-axis and Y-axis denote the sequence position and antigenic propensity respectively. The regions above the threshold value are antigenic, and are depicted in yellow

In a similar study conducted in Korea, they proved that the ompA mutant was more susceptible to trimethoprim with > 5.33-fold, but showed a decrease in MIC for colistin, imipenem and tigecycline showed a < twofold decrease for the same mutant ompA gene. They also suggested that there is a reduction of susceptibility to beta-lactam drugs due to the carriage of beta-lactamase. They finally suggested that the ompA gene is responsible for the intrinsic resistance of the bacteria to the antibiotic stressful condition (Kwon et al. 2017).The purified form of *ompA* in multidrug-resistant strains of *A.baumannii* is said to be associated and documented to have an influence over apoptosis and internalization of the bacteria which causes disruption to the epithelial cells of the host tissue (Mj 2011). Thus, the

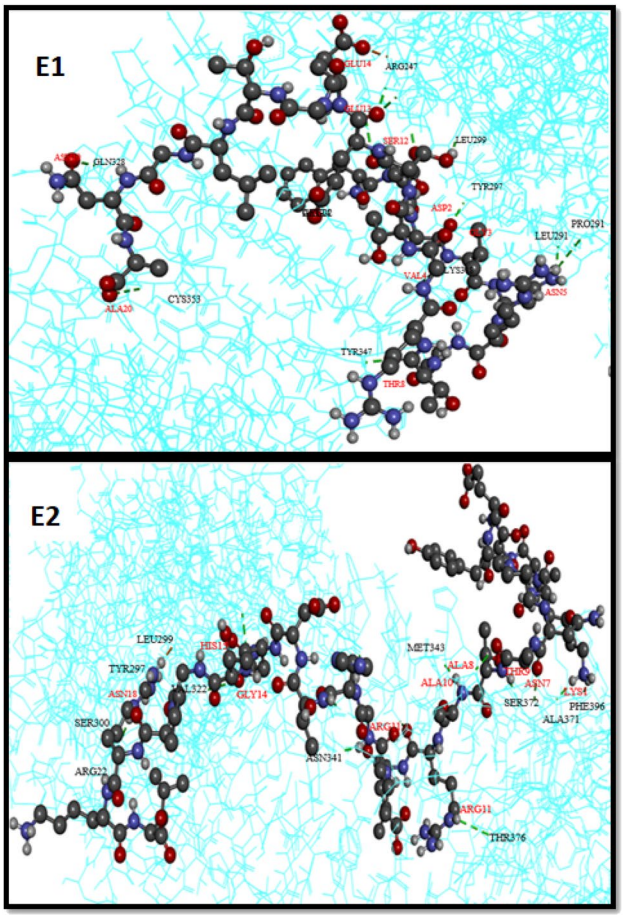

Fig. 5 Docking of the final epitopes from *ompA* of *A.baumannii* E1 (13 hydrogen bonds) and E2 (16 hydrogen bonds) with TLR-2. (Key residues forming interaction with epitope 1 and 2 (scaled ball and stick model colored according to atoms—labelled in red color) and amino acid residues in Human TLR-2 (line model in cyan blue color—labelled in black color) (Detailed interactions documented in Table 6)

present study aims to target the ompA protein for the predictions of antigenic epitopes and thus selected for vaccine peptide construction analysis, and was achieved with the help of immuno-informatics. The first step in predicting vaccine peptides being the selection of specific proteins, it was done based on the non-redundant protein similarity under the human BLASTp. The occurrence of *ompA* amidst ESBL producers was 100% in the present study.

The study also aims to predict the possible B-cell dominant epitopes from the ompA of *A. baumannii*. The results suggested that there were many possible epitopes based on in-silico analysis. The recognition of these B-cell dominant epitopes from the partial sequence of *ompA* gene from the multidrug-resistant *A. baumannii* has proven to be very reinforcing in identifying the potential and putative vaccine candidates. PSORTb tool was used to identify the location of ompA for filtration and localization of ompA as an outer membrane and cytosolic protein. Thus it can be considered for small molecule drug development which is a variant of antimicrobials. The physico-chemical analysis of ompA protein was highly promising as the results showed a negative GRAVY value which on interpretation signifies a hydrophilic property. The instability index test was used to analyze the stability of the epitope. The results showed − 9.88, 36.69, 29.29 and 33.89 which is < 40 suggesting that the epitopes are stable. The aliphatic index was used to check the thermo-stability of the epitopes and was calculated with a relative volume occupied by the aliphatic side chains.

For an ideal vaccine peptide, it is important that a humoral response is stimulated. Thus, from the B-cell epitope evaluation tests, it is suggested that two epitopes are the final shortlisted epitopes for vaccine prediction from the *ompA* gene. The protein turns predicted was based on the standard tools that can assess the antigenic epitopes with 80% confidence and 70% of accurate predictions (Pellequer et al. 1993). The propensity scales measured above the set threshold value was used to assess the antigenic epitopes and was interpreted as the yellow peaks. Most of the predictions yielded promising peaks with good scales of measures, however, Kolaskar predictions showed a less score. MHC class-II restrictions with frequent alleles yielded 5 epitope peptides with only 2 peptides restricted to MHC class-I restrictions. Based on the percentile ranks and IC_{50} values upon ANN, SMM, COMBLib combinatorial ranks, and scores HLA/MHC allele restrictions were interpreted. A final interaction of the selected E1 and E2 epitopes were docked with the TLR-2 receptor and was found to possess an effective docking with multiple interactions with good distance. However further experimental validations related to immunological response and memory of the predicted peptides, have to be initiated and assessed using suitable in-vivo studies.

Conclusion

An attempt on characterizing the *ompA* gene from the drug resistant strains of *A. baumannii* and screening of few putative antigenic epitope peptides using immune-informatics approach suggests promising immunodominant peptides in the present study. The study has also documented the correlation of *ompA* gene occurrence among the drug resistant strains. However, it is the need of the hour to experimentally validate the predicted vaccine peptides with further studies for their suitable immunological response and memory.

Table 6 Interactions of the predicted epitopes E1 and E2 with the human TLR-2 receptor with the distance of binding

Epitope 1	Human TLR2	Distance (Å)
GLU14 (OE2)	ARG247 (HH11)	2.07
SER12 (HG)	TYR297 (O)	1.99
ASN5 (HD21)	ILE290 (O)	2.20
ASN5 (HD21)	PRO291 (O)	3.04
GLY3 (O)	TYR297 (HH)	2.03
ASP2 (O)	LEU299 (H)	1.96
GLU13 (O)	ARG297 (HH22)	2.04
VAL4 (H)	LYS318 (O)	2.93
SER12 (O)	SER300 (HG)	3.04
GLU13 (O)	ARG267 (HH12)	2.01
THR8 (O)	TYR347 (HH)	2.02
ALA20 (OXT)	CYS353 (HG)	3.18
ASN19 (OD1)	GLN328 (HE22)	2.08
Epitope 2	Human TLR2	Distance (Å)
ARG11(HE)	THR376(O)	3.08
LYS1(HZ2)	PHE396(O)	1.91
LYS1(HZ1)	ALA371(O)	2.00
ASN7(O)	SER372(H)	2.57
THR9(OG1)	ALA371(H)	2.28
ALA8(O)	GLU345(H)	2.34
ALA10(H)	MET343(O)	1.91
HIS15(H)	PHE320(O)	2.47
ARG11(O)	ASN341(HD21)	2.01
HIS15(O)	VAL322(H)	2.83
GLY14(H)	VAL322(O)	1.99
ASN18(HD21)	TYR297(O)	2.31
ASN18(OD1)	LEU299(H)	2.24
ASN18(OD1)	SER300(H)	2.93
ARG22(HE)	ASP272(O)	1.57
ARG22(HH11)	ASP298(OD1)	1.86

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Compliance with ethical standards

Conflict of interest None to declare.

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