Journal of Cell and Molecular Research (2014) 6 (2), 57-64

Research Article

In silico analysis for identifying potential vaccine candidates against *Staphylococcus aureus*

Jafar Amani[1], Somayeh Delfani [2], Abbas Ali Imani Fooladi[1]*

1) *Applied Microbiology Research center, Baqiyatallah University of Medical Sciences, Tehran, Iran.*
2) *Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Ira.*

Received 26 Jan 2014 Accepted 12 Mar 2014

Abstract

Staphylococcus aureus is a salient nosocomial infectious agent. The increasing incidence of multiple antibiotic-resistant *S. aureus* strains and the emergence of vancomycin-resistant *S.aureus* have placed renewed interest on alternative means of prevention and control of infection. *S. aureus* produce variety of virulence factors, so a multivalent vaccine may have a greater chance of preventing *S. aureus* infections than a monovalent vaccine. We selected three important virulence factors of *S. aureus*, clumping factorA (clfA), iron-regulated surface determinant (isdB) and gamma hemolysinare (hlg) that are potential candidate genes for vaccine development. We designed synthetic genes encoding the *isdB*, *clfA* and *hlg.* The structure of the synthetic construct and their stabilities were analyzed by bioinformatic tools. VaxiJen analysis of the protein showed high antigenicity. Linear and conformational B-cell epitopes were identified. The proteins encoded by these genes were useful as vaccine candidates against *Staphylococcus aureus* infections. *In silico* tools are highly suited to study, design and evaluate vaccine strategies

***Keywords*:** *In silico* analysis, *Staphylococcus aureus*, chimeric vaccine, virulence factor

Introduction

Staphylococcus aureus is a major cause of a variety of nosocomial infections, including ventilator-associated pneumonia, intravenous catheter-associated infections, postsurgical wound infections, as well as invasive infections(Spellberg and Daum, 2012). *S. aureus* strains exhibiting multiple antibiotic resistances are isolated in 60% of community and up to 80% of hospital infections(Yukiko et al., 2006). The dramatic increase in methicillin resistant bacteria, coupled with the recent emergence of vancomycin resistant isolates has accelerated and broadened the interest in developing novel therapeutics against *S.aureus*.

S. aureus produce variety of virulence factors, so efforts to develop effective vaccines against this organism have been largely unsuccessful (Adhikari et al., 2012). In this regard, many antigens have been evaluated in the search to find a vaccine with the potential to protect against staphylococcal diseases.

Clumping factor A (ClfA) proteins provide an excellent target for immunological attack by antibodies (Hall et al., 2003). ClfA is the major virulence factor responsible for the observed clumping of *S. aureus* in blood plasma and has been implicated as a virulence factor in a mouse model of septic arthritis and in rabbit and rat models of infective endocarditis. ClfA generates strong immune responses and has shown potential as a vaccine component in active and passive immunization studies (Ganesh et al., 2008) .

There is a signal sequence for Sec-dependent secretion at the N terminus of ClfA structure, whereas the C terminus contains an LP XTG motif. Previous studies have localized the Fg-binding activity of ClfA to the N-terminal region of this protein (Hartford et al., 2001).

Iron surface determinant B (IsdB) is an iron-sequestering protein that is conserved in diverse *S. aureus* clinical isolates. IsdB expressed only under conditions of limiting iron (Kuklin et al., 2006). The biological role of IsdB has been demonstrated in numerous studies and suggests that
IsdB is a major virulence factor of *S. aureus*(Kuklin et al., 2006).

Corresponding authors E-mail:
* Imanifouladi.a@gmail.com

Previous studies have identified IsdB as a candidate vaccine antigen *S. aureus* infections(Schaffer and Lee, 2008). An evaluation of binding regions by mutein analysis determined that amino acids residues 130 to 454 of IsdB protein are required for binding, and MAbs bind to this portion effectively (Brown et al., 2009).

Staphylococcus aureus also produces the gamma-hemolysin toxin, which function as two component toxin in the disruption and lysis of erythrocytes and leukocytes(Gouaux et al., 1997). gamma-hemolysin locus (hlg) contains three open reading frames, *h1gA*, *hlgB* and *hlgc* genes.The three encoded proteins are all translated with a single sequence(Gouaux et al., 1997). IgG levels directed to g hemolysin B (HlgB) was higher in S. aureus–infected patients than in control subjects (Verkaik et al., 2010). Gamma-hemolysin is produced by more than 99% of S. aureus strains (Prevost et al., 1995). As elucidated above, ClfA, IsdB and Hlg play key roles in the pathogenesis of *S. aureus* infections. So in the present study, we designed a novel multi subunit antigen that provides a suitable and safe vaccine candidate against *Staphylococcus aureus* infections. Advanced technologies for vaccine development, such as bioinformatics database tools and computational vaccinology can be applied for vaccine development of several diseases (Nazarian et al., 2012).

Briefly, a chimeric protein containing three putative antigenic determinants of ClfA ,IsdB and Hlg, fused together by hydrophobic linkers. Then, structure of the chimeric protein was analyzed through an *in silico* approach. The results are discussed in the following paragraphs.

Methods

Sequences, databases and construct design

Related sequences for *clfA*, *isdB* and *hlg* were obtained from publicly available sequence databases, primarily from the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov). Multiple sequence alignments were performed using ClustalW software of the European Bioinformatics Institute website (http://www.ebi.ac.uk/Tools/clustalw2), in order to identify a fragment common to all the sequences. An antigenic sequence was constructed by fusing three putative antigenic determinants of *clfA* ,*isdB* and *hlg* together by hydrophobic linkers. The *in silico* gene optimization of the synthetic chimera gene was performed using online gene optimization data bases (http://www.jcat.de/) (Grote et al., 2005). The chimeric Gene was designed for cloning and expression in *E. coli*. VaxiJen server was used to predict the immunogenicity of the whole antigen and its subunit vaccine (www.jenner.ac.uk/VaxiJen) (Doytchinova and Flower, 2007).

Prediction of mRNA secondary structure

The messenger RNA secondary structure of the chimeric gene was analyzed by the program mfold. The portal for the mfold web server is (www.bioinfo.rpi.edu/applications/mfold) (Zuker, 2003).

The physico-chemical parameters

The physico-chemical parameters, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, half-life, instability index, aliphatic index and grand average hydropathy (GRAVY) were computed using the Expasy's ProtParam(http://us.expasy.org/tools/protparam.html) (Wilkins et al., 1999).

Secondary and Tertiary structure prediction

The protein secondary structure prediction was performed by GOR secondary structure prediction method(Sen et al., 2005). For 3D structure prediction, I-TASSER ab initio online software was used (Zhang, 2008). The structure was validated to see the quality of the resulting stereochemistry of structure by ramachndran plot in PROCHECK software.

Prediction of antigenic B-cell epitopes

For prediction of B-cell epitopes, the amino acid sequence was analyzed using web based B-cell epitope Prediction algorithms. The first, was analyzed chimeric protein for continuous B-cell epitopes using Bcepred (http://www.imtech.res.in/raghava/bcepred/) (El-Manzalawy et al., 2008). Then we used Discotope server (http://www.cbs.dtu.dk/services/DiscoTope) (Kringelum et al., 2012) to predict discontinuous B cell epitopes from three-dimensional protein structures.

Results

Sequences, databases and construct design

The residues 500–559 within the N-terminal A region of ClfA, have been reported to be involved in Polyclonal antibodies generated against a recombinant protein ClfA .The amino acids residues 130 to 454 of IsdB protein are required for binding, and MAbs bind to this portion effectively. For the third fragment, the amino acids residues 26

to 76 of Hlg protein was selected. Upon Sequence comparison by ClustalW, N-terminal A region of ClfA (59 amino acids) and IsdB (324 amino acids) and Hlg (50 amino acids) showed that these three parts were highly conserved among different strains of *Staphylococcus aureus*. In order to separate the different domains, linkers consisting of EAAAKEAAAKEAAAK repeats and DPRVPSS repeats were designed. It has been shown that the salt bridge Glu--Lys+ between repeated Ala can stabilize helix formation. The *Bam*HI and *Hin*dIII restriction sites for cloning in prokaryotic vectors were successfully introduced at the N and C-terminal of sequence respectively. Schematic diagram of protein domain structures with linker's sites designed is shown in (Fig. 1).

Fig. 1 Schematic representation of *Staphylococcus aureus* antigenic construct consists of clfA, isdB and hlg genes bound together by appropriate linkers for expression in *E. coli*

Both the wild type and the synthetic chimera were analyzed for their codon bias (Fig. 2) and GC content (Fig. 2). Codon adaptation index (CAI) on the chimeric gene was 0.99 compared to that of the wild type gene, which was only 0.60 (Fig. 2). The overall GC content was reduced from 45.56 to 45.06%, which should increase the overall stability of mRNA from the synthetic gene. Antigen index by VaxiJen server for ClfA, IsdB, Hlg and chaimeric gene was 0.60, 0.47, 0.58 and 0.58 respectively.

mRNA structure prediction

The minimum free energy for secondary structures formed by RNA molecules was predicted. All 28 structural elements obtained in this analysis revealed folding of the RNA construct. ΔG of the best-predicted structure was -336.80 kcal/mol (Fig. 3A) and the first nucleotides at 5′ did not have a hairpin or pseudoknot (Fig. 3B), in native mRNA, first nucleotides did not also formed pseudoknot and the structure ΔG was -279.30 kcal/mol (Table 1).

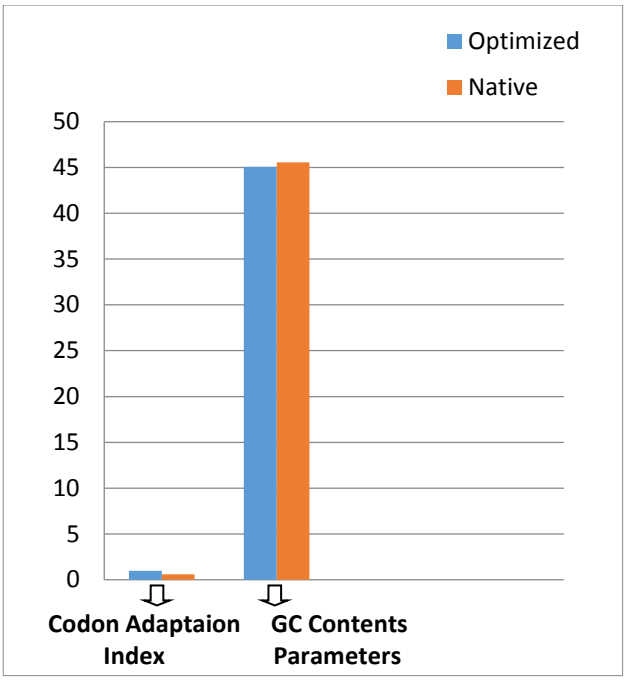

Fig. 2 Codon usage analysis of wild type and optimized chimeric gene.

Table 1. Statistical values of candidates reference genes

Helix	-6.70	4 base pairs.
Hairpin loop	4.20	Closing pair is G_{834}-C_{846}
Stack	-2.40	External closing pair is U_4-A_{809}
Stack	-3.30	External closing pair is C_5-G_{808}
Stack	-2.10	External closing pair is C_6-G_{807}

The physico-chemical parameters

The average molecular weight of chimeric protein calculated was 53.2 kDa. Isoelectric point (pI) was defined as the pH at which the surface of protein is covered with charge but net charge of the protein is zero. Acidity of the protein was indicated by the pI value pI 7.75.

Extinction coefficient of chimeric protein at 280 nm was 59,250 M^{-1} cm^{-1}. The biocomputed half-life was greater than 10 h. On the basis of instability index, Expasy's ProtParam classifies the chimeric protein as stable (Instability index 30.11). Aliphatic index and Grand average of hydropathicity (GRAVY) of chimeric protein was 67.94 and -0.806.

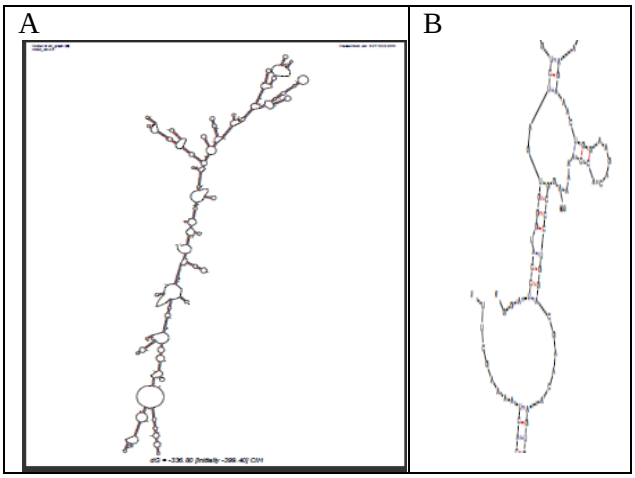

Fig. 3 A: Prediction of RNA secondary structure of chimeric gene using Mfold algorithm. B: Predicted structure has no hairpin and pseudo knot at 5′ site of mRNA

Secondary and Tertiary structure prediction

The secondary structure of the chimeric protein was predicted by online software (applying Mfold algorithm). In order to validate our method of secondary structure prediction, first the clfA, isdB and hlg were used as test sequences.

Using the software GOR IV, we obtained predicted structural elements. The results show that random coil, extended strand and alpha helix are structural contents of protein. Composition of predicted secondary structure for chimeric protein was 29.25% alpha helix, 25.81% extended strand and 44.95% random coil (Table 2). Analysis of the amino acid composition demonstrated two regions with a low sequence complexity.

Table 2. Percentage of secondary structure elements of chimeric and single proteins.

Seguence	Alpha helix	Extended strand	random coil
clfA	0.00%	37.29%	62.71%
isdB	31.23%	26.73%	42.04%
hlg	20.00%	26.00%	54.00%
chimeric gene	29.25%	25.81%	44.95%

These regions have linker sequences. The secondary structure prediction of the protein is shown in (Fig. 4).

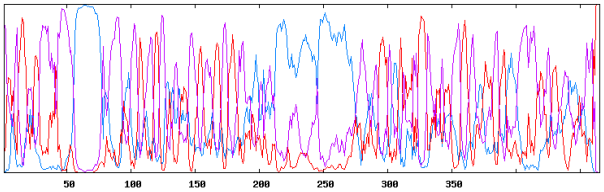

Fig.4 Graphical results for secondary structure prediction of chimeric protein. Extended strand: purple, Coil: red, Helix: blue.

Comparative and ab initio modeling of the synthetic sequence was exploited to produce 3D models of the chimeric protein. The 3D modeled structure for protein was generated by I-TASSER software. Results of tertiary structure of the fusion protein construction using I-TASSER showed a protein with three main domains linked together with linkers (Fig. 5).

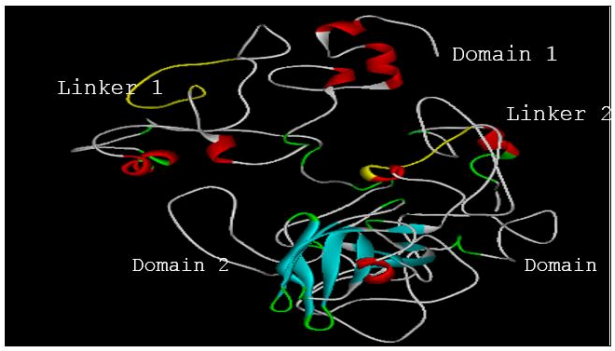

Fig. 5. Modeled structure of chimeric protein by I-TASSER software. The 3D modeled structure generated by I-TASSER software showed a protein with three main domains linked together with linkers

The confidence score (C-score) for estimating the quality of predicted models by I-TASSER was -0.22 C-score is typically in the range of [−5 to 2], where a C-score of higher value signifies the model with a high confidence. In addition, the expected TM-score for this model was 0.68±0.12. The expected RMSD was 7.7±4.3Å.

The Ramachandran plot analysis revealed that 0.98%of amino acid residues from modeled structure generated by I-TASSER were incorporated in the favored regions (A, B, and L) of the plot. Apart from that 2%of residues were in allowed regions (a, b, l,and p) of the plo (Fig. 6).

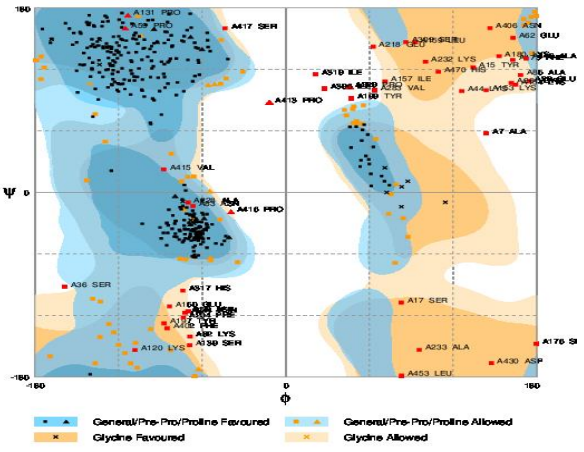

Fig.6 Validation of protein structure using Ramchandran plot. The Ramachandran plot revealed that 98% of amino acid residues from modeled structure were incorporated in the favored regions of the plot. 2% of the residues were in allowed regions of the plot.

Prediction of B-cell epitopes

BcePred software was used to predict the chimeric protein epitopes. All predicted B-cell epitopes (20 mers) having a BCPreds cutoff score >0.8 were selecte (Table 3,4).

Table 3. B-cell epitopes from full length proteins using BCPred.

Positions	Epitopes	BcePred Score
46	EQPDEPGEIEPIPEEAAAKE	1
25	NEVAFNNGSGSGDGIDKPVV	0.999
349	TISKDAKNNTRTIIFPYVEG	0.997
99	EMKKKDGTQQFYHYASSVKP	0.985
405	DKSHDPRVPSSGKITPVSVK	0.984
292	DTKYVVYESVENNESMMDTF	0.972
78	IKNPAIKDKDHSAPNSRPID	0.948
430	VTLYKTTATADSDKFKISQI	0.947
327	MVMETTNDDYWKDFMVEGQR	0.924
181	IVSSTHFNNKEEKYDYTLME	0.912
225	APYKKAKTLERQVYELNKIQ	0.863
202	AQPIYNSADKFKTEEDYKAE	0.764

BcePred software was also used to determine the continuous B cell epitope based on single characters including hydrophilicity, antigenicity, flexibility, accessibility, polarity and exposed surface (Table 3). As shown in Table 3, linkers between different domains (aa 60 to 75 and aa 410 to 417) contained no epitope sites. The results of computational analysis included peptides and their corresponding threshold scores. The higher threshold score means higher specificity and binding affinity. The conformational epitopes for B cells were predicted by the Discotope server. 90 B-Cell epitope residues out of 113 total residues identified (Table 4).

Table 4. Epitopes predicted in chimeric protein by different parameters based on BcePred software.

Prediction parameters	Epitope sequence
Hydrophilicity	NNGSGSGDGIDK,VPEQPDEPGEIE.EEAAAKEAAAKEAAAKRE.IKDKDHSAPNS.EMKKKDGTQQ,DSKPEIE,EVYEGDK,SYDTVKD,SVSNGTK,FNNKEEKYDYT.DKFKTEEDYKAEK,DEQVKSA,NVQPTNEKMTDLQDTK,ESVENNES,METTNDDY.SKDAKNNTRT,TKANTDKSHDPR,KTTATADSDK,KDKSYDKDT
Flexibility	VAFNNGSGSG,PAIKDKDHSAPN,IDFEMKKKDGT,VIFTDSK,IRFSVSNGT,THFNNKE.AEYKKKL,QNVQPTN,RTISKDAKNNT,AFTKANTDKSHDPRVPSSGK,VSVKKVD,ATADSDK
Accessibility	RSMSWDN,DKPVVPEQPDEPGEIEPIPEEAAAKEAAAKEAAAKREAIKNPAIKDKDHSAPNSRPIDFEMKKKDGTQQFYHY,SSVKPAR,FTDSKPEIEL,SGQFWRKFEVYEGDKKLPIKL,SYDTVKDYAYIR,NGTKAVK,STHFNNKEEKYDYTLM,IYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLKAEYKKKLEDTKKALDEQVKSA,TEFQNVQPTNEKMTDLQDTKYV,YESVENNESMMD,KHPIKTGMLNGKKYMVMETTNDDYWKDF,VEGQRVRTISKDAKNNTRTI,PYVEGKTLYD,KTIDYDGQYHVR,DKEAFTKANTDKSHDPRVPSSGK,SVKKVDDKVTLYKTTATADSDKFKISQ,NFIKDKSYDKDTL
Turns	GYNSNII,VAFNNGSG,SSTHFNNKE,ETTNDDYW
Exposed Surface	PEQPDEP,KEAAAKREAIKNPAIKDKDHSA,DFEMKKKDGTQQ,VYEGDKKLPIKL,HFNNKEEKYDYT,DKFKTEEDYKAEKL,APYKKAKTLER,LNKIQDKLPEKLKAEYKKKLEDTKKALDEQ,VQPTNEKM,NDDYWKD,SKDAKNNTR,TKANTDKSHDPR,VKKVDDKV,IKDKSYDKDT
Polarity	DEPGEIEPIPEEAAAKEAAAKEAAAKREAIKNPAIKDKDHSAP,IDFEMKKKDGTQ,DSKPEIEL,QFWRKFEVYEGDKKLPIK,THFNNKEEKYDYT,ADKFKTEEDYKAEKL,PYKKAKTLERQVYEL,QDKLPEKLKAEYKKKLEDTKKALDEQ,ESVENNE,FVKHPIKTG,KKYMVME,NDDYWKDF,VEGQRVRTISK,VKVHVKTI,HVRIVDKEAFTK,NTDKSHDPRV,SVKKVDDKVT,ADSDKFKI,IKDKSYDKDT
Antigenic Propensity	GIDKPVVPEQPD,KLPIKLVSYDTVK,IRFSVS,VKIVSSTHF,QDTKYVVYESVE,TFVKHPIK,TIIFPYVE,IVKVHVKTID,QYHVRIVDK,KITPVSVKKVD

Discussion

A major challenge for healthcare in the 21st century is the increasing levels of resistance to antimicrobial compounds of *S. aureus*. Therefore the search for an efficacious vaccine or immunoglobulin (Ig) preparation to prevent invasive disease due to *S. aureus* is urgently needed (Kuklin et al., 2006), and development of *S. aureus* vaccine to aid in the treatment of hospitalized individuals, as well as to reduce the economic burden on the health care system, is well established. Over the past several decades live, heat-killed, and formalin fixed preparations of S. *aureus* cells have been tested as vaccines to prevent staphylococcal infections. Numerous virulence factors have been targeted by vaccination, including alpha hemolysin, PVL,clumping factor A, fibrinogen binding protein, enolase (laminin-binding protein), and protein A. But in many studies show that a multivalent vaccine may have a greater chance of preventing *S. aureus* infections at multiple anatomical sites than a monovalent vaccine(Schaffer and Lee, 2009).

However, choosing appropriate antigens to include in a multicomponent vaccine is a major challenge. Candidate antigens should be surface exposed, expressed by the majority of clinical *S.*

aureus isolates belonging to diverse lineages, and show minimal serological variability among strains (Schaffer and Lee, 2008).

In present study we have selected surface proteins Gamma-hemolysin, Clumping factor A and Iron surface determinant B, that play important role in *S. aureus* infection and produced by more than 99% of *S. aureus* strains.

For designing our chimeric vaccine as chimeric protein, we selected epitopes from residues 500 – 559 of the A region Clumping factor A, amino acids residues 130 to 454 of IsdB protein and first 50 amino acids residues after signal peptide Hlg protein. Because Polyclonal antibodies generated against these regions. Successfully constructed fusion proteins require not only the desired component proteins, but also suitable linkers to connect the protein domains.

In order to separate different domains of our chimeric protein, linkers consisting of EAAAK repeats and DPRVPSS repeats expected to form a monomeric hydrophobic α-helix and random coil, were designed. Our successful experience of using these linkers in chimeric gene has shown that it could lead to logically acceptable results (Amani et al., 2009). The result clearly suggested the ability of the helical linker to control the distance and reduce the interference between the domins.

Codon optimization has applications in designing synthetic genes and DNA vaccines. We were performed Codon optimization to enhance recombinant protein production in *Escherichia coli*. codon adaptation index (CAI) on the optimized gene sequence was 0.99 compared to that of the wild type gene, which was only 0.60, indicating that the optimized gene sequence could be expressed well. VaxiJen server was also showed that chaimeric gene was immunogenic.

Secondary structure of messenger RNA plays an important role in the bio-synthesis of proteins For instance, regulation of gene expression is highly dependent on the formation of stable structures by nucleotide pairing in the the 5' terminus(Gaspar et al., 2013). We used mfold software for prediction of RNA secondary structure.The data from mRNA prediction showed that the mRNA was stable enough for efficient translation in the host. mRNAs with weakly folded 5′-UTRs have higher translation rates, higher abundances of the corresponding proteins, longer half-lives, and higher numbers of transcripts, and are upregulated after heat shock. Furthermore, 5′-UTRs have significantly higher folding free energies than other genomic regions and randomized sequences. When we were analyzed, the physic-chemical parameters of our chimeric gene with protpram software, the pI value (pI < 7.75) showed acidic nature of the protein. Extinction coefficient of chimeric protein at 280 nm was high. On the basis of instability index, Expasy's ProtParam classifies the chimeric protein as stable (Instability index 30.11). Functional characterization of a protein sequence is one of the most frequent problems in biology. This task is usually facilitated by accurate three-dimensional structure of the protein in question. Our result showed that *ab initio* I-TASSER software could predict the folds and good resolution model for our chimeric protein. For the evaluation of the predicted models, both RMSD and TM-score were used. Expected TM-score of 0.68±0.12.validates the accuracy of then model. A TM-score >0.5 indicates a model of correct topology. Other scores including z-score and C-score suggest its confidence. The z-score indicates overall model quality and measures the deviation of the total energy of the structure with respect to an energy distribution derived from random conformations(Zhang, 2008). Z-scores outside a range characteristic for native proteins indicate erroneous structures. Our chimeric structure showed desirable protein stability based on Ramachandran plot predictions. In Ramachandran plot analysis, nearly, a negligible 2% of the residues were to be in outliner region that could probably be due to the presence of chimeric junctions.

A crucial step in vaccine design, immunodiagnostic tests, and antibody production is identification of B-cell epitopes. B-cell epitopes are defined by a specific surface region of an antigenic protein, and may be divided into two different types of epitopes: continuous (linear) epitopes and discontinuous (conformational) epitopes. Therefore, computational tools for reliably predicting linear B-cell epitopes are highly desirable. In this study, we used several distinct methods such as Hydrophilicity method, Accessibility method, Antigenicity method, flexibility method and secondary structure analysis for prediction of B-cell epitopes of our chimeric protein. Applying just one of these methods is not enough for obtaining results good enough to predict the B-cell epitope. The epitopes located on the surface of the protein could interact easily with antibodies, and they were generally flexible.

Another crucial step for the rational design of novel vaccines is identification of conformational epitopes in antibody-antigen interaction. DiscoTope software recognized 90 conformational epitopes for B cells. DiscoTope uses tertiary structure which seems to be more reliable than another software.

Table 5. Conformational B-cell epitopes from full length proteins using DiscoTope server.

Start & End position	Start & End position	Start & End position	Start & End position	Start & End position	Start & End position	Start & End position
288-4	305-12	320-5	334-19	348-12	361-25	381-3
289-10	307-10	321-17	335-10	349-15	362-20	382-3
290-11	309-23	322-10	336-22	350-18	364-8	383-8
191-14	310-2	323-0	337-33	351-11	366-10	384-0
292-6	311-22	324-11	338-13	352-10	367-4	385-14
294-10	312-30	325-14	339-14	353-11	368-1	386-1
296-17	313-19	326-21	340-31	354-0	370-7	387-13
299-6	314-16	328-26	341-9	355-0	373-8	388-4
300-7	315-10	329-32	343-12	356-12	375-7	389-23
301-1	316-31	330-15	344-0	357-9	377-8	390-9
302-8	317-17	331-33	345-10	358-11	378-23	397-8
303-8	318-21	332-11	346-7	359-19	379-5	400-11
304-2	319-16	333-15	347-30	360-13	380-9	

Conclusion

In recent years, the *in silico* epitopes prediction tools have facilitated the progress of vaccines development significantly and many have been applied to predict epitopes in bacteria successfully.

In this study, we have combined several bioinformatics techniques for immonogenicity prediction of chimeric protein. Our data indicates that epitopes of our chimeric protein, designed from clumping factor A, iron surface determinant B and gamma-hemolysin of *S.aureus* could induce B-cell mediated immune responses successfully. Therefore, our chimeric protein is suggested as a vaccine candidate against *S.aureus* infections. Further in vitro synthesis of the determined peptide and in vivo experimental study to test the efficacy are the recommended as the future steps to this preliminary study for vaccine development.

Conflict of interest

The authors declare that they have no competing interest.

References:

1- Adhikari R. P., Karauzum H., Sarwar J., Abaandou L., Mahmoudieh M., Boroun A. R., Vu H., Nguyen T., Devi V. S., Shulenin S., Warfield K. L. and Aman M. J. (2012) Novel structurally designed vaccine for S. aureus alpha-hemolysin: protection against bacteremia and pneumonia. PloS one 7:e38567.

2- Amani J., Mousavi S. L., Rafati S. and Salmanian A. H. (2009) In silico analysis of chimeric espA, eae and tir fragments of Escherichia coli O157:H7 for oral immunogenic applications. Theoretical biology & medical modelling 6:28.

3- Brown M., Kowalski R., Zorman J., Wang X. M., Towne V., Zhao Q., Secore S., Finnefrock A. C., Ebert T., Pancari G., Isett K., Zhang Y., Anderson A. S., Montgomery D., Cope L. and McNeely T. (2009) Selection and characterization of murine monoclonal antibodies to Staphylococcus aureus iron-regulated surface determinant B with functional activity in vitro and in vivo. Clinical and vaccine immunology : CVI 16:1095-1104.

4- Doytchinova I. A. and Flower D. R. (2007) VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC bioinformatics 8:4.

5- El-Manzalawy Y., Dobbs D. and Honavar V. (2008) Predicting flexible length linear B-cell epitopes. Computational systems bioinformatics / Life Sciences Society. Computational Systems Bioinformatics Conference 7:121-132.

6- Ganesh V. K., Rivera J. J., Smeds E., Ko Y. P., Bowden M. G., Wann E. R., Gurusiddappa S., Fitzgerald J. R. and Hook M. (2008) A structural model of the Staphylococcus aureus ClfA-fibrinogen interaction opens new avenues for the design of anti-staphylococcal therapeutics. PLoS pathogens 4:e1000226.

7- Gaspar P., Moura G., Santos M. A. and Oliveira J. L. (2013) mRNA secondary structure optimization using a correlated stem-loop prediction. Nucleic acids research 41:e73.

8- Gouaux E., Hobaugh M. and Song L. (1997) alpha-Hemolysin, gamma-hemolysin, and leukocidin from Staphylococcus aureus: distant in sequence but similar in structure. Protein science : a publication of the Protein Society 6:2631-2635.

9- Grote A., Hiller K., Scheer M., Munch R., Nortemann B., Hempel D. C. and Jahn D. (2005) JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. Nucleic acids research 33:W526-531.

10- Hall A. E., Domanski P. J., Patel P. R., Vernachio J. H., Syribeys P. J., Gorovits E. L., Johnson M. A., Ross J. M., Hutchins J. T. and Patti J. M. (2003) Characterization of a protective monoclonal antibody recognizing Staphylococcus aureus MSCRAMM protein clumping factor A. Infection and immunity 71:6864-6870.

11- Hartford O. M., Wann E. R., Hook M. and Foster T. J. (2001) Identification of residues in the Staphylococcus aureus fibrinogen-binding MSCRAMM clumping factor A (ClfA) that are important for ligand binding. The Journal of biological chemistry 276:2466-2473.
12- Kringelum J. V., Lundegaard C., Lund O. and Nielsen M. (2012) Reliable B cell epitope predictions: impacts of method development and improved benchmarking. PLoS computational biology 8:e1002829.
13- Kuklin N. A., Clark D. J., Secore S., Cook J., Cope L. D., McNeely T., Noble L., Brown M. J., Zorman J. K., Wang X. M., Pancari G., Fan H., Isett K., Burgess B., Bryan J., Brownlow M., George H., Meinz M., Liddell M. E., Kelly R., Schultz L., Montgomery D., Onishi J., Losada M., Martin M., Ebert T., Tan C. Y., Schofield T. L., Nagy E., Meineke A., Joyce J. G., Kurtz M. B., Caulfield M. J., Jansen K. U., McClements W. and Anderson A. S. (2006) A novel Staphylococcus aureus vaccine: iron surface determinant B induces rapid antibody responses in rhesus macaques and specific increased survival in a murine S. aureus sepsis model. Infection and immunity 74:2215-2223.
14- Nazarian S., Mousavi Gargari S. L., Rasooli I., Amani J., Bagheri S. and Alerasool M. (2012) An in silico chimeric multi subunit vaccine targeting virulence factors of enterotoxigenic Escherichia coli (ETEC) with its bacterial inbuilt adjuvant. Journal of microbiological methods 90:36-45.
15- Prevost G., Cribier B., Couppie P., Petiau P., Supersac G., Finck-Barbancon V., Monteil H. and Piemont Y. (1995) Panton-Valentine leucocidin and gamma-hemolysin from Staphylococcus aureus ATCC 49775 are encoded by distinct genetic loci and have different biological activities. Infection and immunity 63:4121-4129.
16- Schaffer A. C. and Lee J. C. (2008) Vaccination and passive immunisation against Staphylococcus aureus. International journal of antimicrobial agents 32 Suppl 1:S71-78.
17- Schaffer A. C. and Lee J. C. (2009) Staphylococcal vaccines and immunotherapies. Infectious disease clinics of North America 23:153-171.
18- Sen T. Z., Jernigan R. L., Garnier J. and Kloczkowski A. (2005) GOR V server for protein secondary structure prediction. Bioinformatics 21:2787-2788.
19- Spellberg B. and Daum R. (2012) Development of a vaccine against Staphylococcus aureus. Seminars in immunopathology 34:335-348.
20- Verkaik N. J., Dauwalder O., Antri K., Boubekri I., de Vogel C. P., Badiou C., Bes M., Vandenesch F., Tazir M., Hooijkaas H., Verbrugh H. A., van Belkum A., Etienne J., Lina G., Ramdani-Bouguessa N. and van Wamel W. J. (2010) Immunogenicity of toxins during Staphylococcus aureus infection. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 50:61-68.
21- Wilkins M. R., Gasteiger E., Bairoch A., Sanchez J. C., Williams K. L., Appel R. D. and Hochstrasser D. F. (1999) Protein identification and analysis tools in the ExPASy server. Methods in molecular biology 112:531-552.
22- Yukiko K., Jansen K., Stranger U., Taeok B. and Schneewind O. (2006) Vaccine assembly from surface proteins of Staphylococcus aureus. pnas.
23- Zhang Y. (2008) I-TASSER server for protein 3D structure prediction. BMC bioinformatics 9:40.
24- Zuker M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic acids research 31:3406-3415.