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In Silico Predictions of Thr136Arg Missense Variant Shows a Remarkable Negative Impact on the Biological Activity of Enterotoxin Type A

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ABSTRACT

Background: *S. aureus* can secrete enterotoxin. In this study, one non-synonymous single nucleotide polymorphism (nsSNP) that exhibits a missense impact on the enterotoxin type A protein was investigated, namely Thr136Arg.
Objectives: The current investigation was carried out to predict the final consequences of this missense SNP on the enterotoxin type A protein structure, function, stability and binding affinity with antibiotics directed against this toxin.
Materials and methods: Isolation and identification of *S. aureus* and genetic detection of enterotoxin A gene (*sea*) in this *S. aureus* isolates. After sequencing the sea gene, the 3-dimensional structure of the enterotoxin type A protein was generated, and this nsSNP was highlighted in the generated 3-dimensional structure of enterotoxin type A. Several *in silico* tools were used to study the effect of missense SNP on this protein's structure and function. Subsequently, a set of five *in silico* tools was also implemented to evaluate the effect of this SNP on the stability of enterotoxin type A upon mutation.
Results: The cumulative results of structure-function *in silico* tools indicated clear deleterious consequences of Thr136Arg on the protein structure and function of the enterotoxin type A protein. Further deleterious consequences of Thr136Arg were evolutionary confirmed, and the highly conserved region of the investigated SNP was validated in enterotoxin type A. In addition to structure-function predictions, all tools that utilized stability-prediction showed that this SNP exhibited a remarkable reduction in protein stability with a noticeable negative effect on the stability of enterotoxin type A. Docking experiments showed a noticeable alteration in the binding of enterotoxin type A with nafcillin, with a considerable alteration in the conformation of its 3D dimension.
Conclusion: It has been found from cumulative *in silico* predictions that the Thr136Arg SNP may be involved in inducing a noticeable damaging and destabilizing role on enterotoxin type A, with several consequent negative impacts on the biological activities in which this protein is involved. The binding affinity of the destabilized enterotoxin type A with the antibiotic nafcillin would be altered accordingly. This study suggests that nafcillin would bind more effectively with the Thr136Arg-damaged enterotoxin type A, which may implying that the strains having this protein may exhibit altered susceptibility to antibiotic treatment.

Keywords: *In silico*; Enterotoxin type A; Single nucleotide polymorphisms; Docking.

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INTRODUCTION

S. aureus is isolated from different animal-origin foods and identified as the world's third most common food-borne disease source [1,2]. The concept "*in silico*" refers to silicon, a component of computers. *In silico* methods use computer tools to forecast effects

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before developing laboratory procedures. In vitro, research needs various supplies, specialized lab equipment, and lengthy optimizations. Computational modeling is a powerful tool for managing the explosion of bioinformatics data. Computational analysis is now the fastest and cheapest approach to determine if an SNP will cause illness [3].

It is important to evaluate a variety of factors, including clinical, populational, structural, and bioinformatics; when interpreting data. *In silico*, modeling has become an accurate supplementary, and in some cases, critical prediction approach as the number of computer tools and crystal structures accessible grows exponentially. Different computer systems take into account many factors to varied degrees, including the fundamentals of protein chemistry, three-dimensional structure, and homologies of amino acids among various species or related proteins [4].

SNPs (single nucleotide polymorphisms), one of the most frequent forms of genomic sequence variations, might influence illness outcomes. There are more than a million SNPs known, and most of them are found in DNA coding sections or inside introns and intergenic regions that do not directly encoded/translated into amino acids. As a single nucleotide alteration code for different amino acids, missense non-synonymous SNPs (nsSNPs) are of special interest since they can influence the encoded protein's function and illness outcome, as well [5]. A growing number of *in silico* approaches have been developed to investigate the association between genetic sequence variation and protein structure and function. *In silico* approaches can be utilized as preliminary tools to investigate the impact of nsSNPs because experimental procedures to determine the effect of many nsSNPs are expensive, arduous, and time-consuming [6].

Therefore, our aim was to predict how the missense SNP will ultimately affect the enterotoxin type A protein's structure, function, stability, and affinity for binding antibiotics that are designed to block this toxin.

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF *S. AUREUS*

Sample collection and bacterial identification

This experimental study was conducted at the Biology Department, College of Science, Babylon University, Babil, Iraq. Five hundred different samples were randomly collected from different clinical and non-clinical samples between January and April 2021. A loop containing meat suspension (by adding 1 g of meat to the 10 ml of normal saline) was streaked on mannitol salt agar and incubated at 37 °C for 24 hours [7].

Genetic detection of *sea* gene

The bacterial DNA was extracted by the phenol-chloroform DNA extraction method. F (CGGCACTTTTTTCTCTTCGG), R: (GGTTATCAATGTGC GGGT G G) primer. The amplicon size (102 pb) of *the sea* gene [8]was used in this study. The polymerase chain reaction (PCR) was conducted in three major phases denaturation: 94/30 sec, annealing: 57/30 sec, extension: 72/30 sec, and number of cycles:35 [9]. BioEdit Sequence Alignment Editor Version 7.1 software (DNASTAR, Madison, WI, USA), used to edit, align, and analyze the sequencing results of PCR products.

Designing protein structures

The partial amino acid sequences of the enterotoxin type A protein were not available online from the protein data bank (http://www.ncbi.nlm.nih.gov). The three-dimensional structure of enterotoxin type A was constructed by Phyre2 (protein homology/analogY recognition engine), an online three-dimensional model prediction software [10]. PyMOL-v1,7.0.1 software was used to accomplish the proposed virtual alterations inside its related mutations (www.shrodinger.com).

Evaluating the functionality of the observed nsSNP using PROVEAN

PROVEAN, or protein variation effect analyzer, is a program that predicts the probable impact of an amino acid substitution on the protein structure and function to annotate coding nonsynonymous SNPs [11]. The method can provide a high throughput prediction tool for the nsSNP source in the query. PROVEAN predicts –2.5 for the default threshold of each studied variation. If the variation are less than –2.5, it is likely to be harmful.

SIFT to predict the functional impact of a detected nsSNP

The effect of the nsSNP on the target protein was confirmed using the SIFT (Sorting Intolerant from Tolerant SNPs) programme [12]. The SIFT approach may be used to predict how an amino acid substitution might affect a protein's biological activity. The hazardous amino acids may be separated from their damaging counterparts by using this service. Places with tolerance indices below 0.05 were expected to have detrimental or intolerant replacements, while places with tolerance indices above 0.05 were projected to have "tolerated" substitutions.

Predicting the functional effect of nsSNPs using PolyPhen-2

Using basic physical and comparative variables, PolyPhenotyping predicts the effect of amino acid changes on protein structure and function. PolyPhen-2 is a new version of the PolyPhen SNP annotation tool [13]. Prediction results might be classified as destructive or benign based on a score of 0–1.

Identifying the negative impact of the observed nsSNP by using PhD-SNP

Researchers may investigate the effect of missense variations on the protein they are researching using PhD-SNP (or Predictor of Human Deleterious Single Nucleotide Polymorphisms) [14]. The PhD-SNP algorithm's main objectives are to predict polymorphisms that are detrimental and are associated with illness. When a variant's prediction score is more than 0.5, there are repercussions.

Using SNAP predict the severity effect of an observed nsSNP

SNAP is a technical method for differentiating between beneficial and harmful nsSNP [15], which ranged from 100 strong detrimental predictions to +100 strong effect predictions, may be related to the analytical prediction scores, which varied from 100 strong negative predictions to +100 strong effect predictions.

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Characterizing the pathogenicity of the observed nsSNP using Meta-SNP

Meta-SNP is a website service that uses the support vector machine method to predict the effects of SNPs in enterotoxin type A by computing functional information from the Gene Ontology (GO) database, for example, biological activity, molecular function, and cellular components. The impact of polymorphisms can be predicted using this data [16]. Native enterotoxin types A protein and the variant that was of interest were both given as input. There was a chance of more than 50% of each variant being a disease.

Analyzing the evolutionary conservation status of the observed nsSNP using ConSurf

The online computer programme ConSurf was used to identify the evolutionarily conserved areas of enterotoxin type A [17]. The Consurf tool was used to align the homologs of the enterotoxin type A sequence and determine position-specific scores. There are nine grades of predictions, starting from 1 (highly variable) to 9 (highly conserved), the numbers between 1 and 9 indicate the severity of the conservation of a particular SNP.

Investigating the effect of the observed nsSNP on the protein stability using I-MUTANT2

To get a better idea of how mutations affect the protein's stability, I-MUTANT2 was used to look at the places where the protein changed [18]. In this case, the study of the effect of a mutation could change the stability of what we want to learn more about, which could change its main characteristics. There is a web server called I-MUTANT2 that uses a support vector machine to make predictions about how the stability of proteins changes when they have one-site mutations. There was an analysis of DDG value (kcal/mol) that took into account the whole protein sequence and changes in its residues. Calculations were made at a temperature of 25°C and a pH of 7.0. This is how it works: If the DDG value is greater than 0, the stability of proteins goes up. If the DDG value is less than 0, the stability of proteins goes down.

The effect of the observed nsSNP on the protein stability by mCSM

Assessment of the nsSNP effect on protein stability by means of mCSM can give a better assessment of the stability of the investigated enterotoxin type A protein impacted by the observed missense mutation [19]. Accordingly, the effect of the targeted amino acid substitution on the analyzed protein stability was predicted using mCSM [20]. The input data were protein data bank (PDB) sequences of the referring human enterotoxin type A protein (4WMQ). The submitted PDB file of the enterotoxin type A was computed along with its amino acid substitution and analyzed in terms of free energy change (ΔΔG) values (kcal/mol). Negative values of ΔΔGwere destabilizing, while positive values of ΔΔG were stabilizing to the 3D structure of the altered proteins.

The outcome of the observed nsSNP on the protein stability DynaMut

The DynaMut tool is a web server that can be used to assess the impact of missense mutations on protein stability and dynamics. The input data for this web server is the 3D structure of the enterotoxin type A protein in PDB format. As in the case of the other stability prediction tools utilized, the output data are DDG values [21].

The effect of the observed nsSNP on the protein stability by the CUPSAT tool

CUPSAT (Cologne University Protein Stability Analysis Tool) is one of the protein stability prediction servers that calculates the change of the protein stability induced by mutations (ΔΔG) utilizing specific learning machines to predict the effect of the observed SNP on the protein stability. The CUPSAT server can be utilized for a wider range of proteins with PDB or FASTA format input files [22].

The effect of the observed nsSNP on the protein stability by the Mupro server

Mupro is a set of machine learning software to predict how amino acid substitution affects protein stability. Both PDB and FASTA sequences of the targeted protein were provided as input files and the accuracy of the predicted free energy of this tool was validated and confirmed [23].

Docking

A noticeable toxic activity of enterotoxin type A was validated and confirmed on the UniProt server (https://www.uniprot.org/uniprot/P0A0L2). Since entA toxin is usually targeted by several antibiotics, its potential drug was predicted using the drug bank server (https://go.drugbank.com/). Several candidates for antibiotics were identified, which were found to bind with several microbiological toxins with high affinity. Within these antibiotics, nafcillin was chosen to bind with both wild-type entA and mutant entA forms. Nafcillin is one of the penicillin members that has beta-lactamase-resistant characteristics. It is used to treat infections caused by Gram-positive bacteria, especially staphylococci, that are resistant to other penicillin structures [24]. Nafcillin was retrieved from the drug bank server in PDB format. The rened PDB format of normal entA, as well as its mutant form, were subjected to molecular docking with the nafcillin substrate using Hex 8.0.0 [20]. The default procedure of docking was used, in which the maximum rotational increments for the entA receptor and nafcillin ligand were enabled by setting the angle to 180°.

Ethics approval

The ethical approval was obtained from the College of Science, University of Babylon, (reference number 7/17/6034 on 5-9-2019).

RESULTS

The result findings revealed that 30 (13.6%) of the bacterial isolates are *S. aureus* related. The findings detected by agarose gel electrophoresis showed that the prevalence of *S. aureus* enterotoxin A gene (*sea*) was 30 (100%)(Figure 1).

Only one nucleic acid variation (G44C) was found in the 102 bp samples when compared to the appropriate *S. aureus* reference sequences. It was found that the detected nucleic acid substitution was found in the amino acid glutamine (Tyr or T) in position 136 within the amplified sea locus.

Comparing the 102 bp samples' alignment findings to the relevant *S. aureus* reference sequences, just one nucleic acid variant (G44C) was found (Figure 2 and Table 1).

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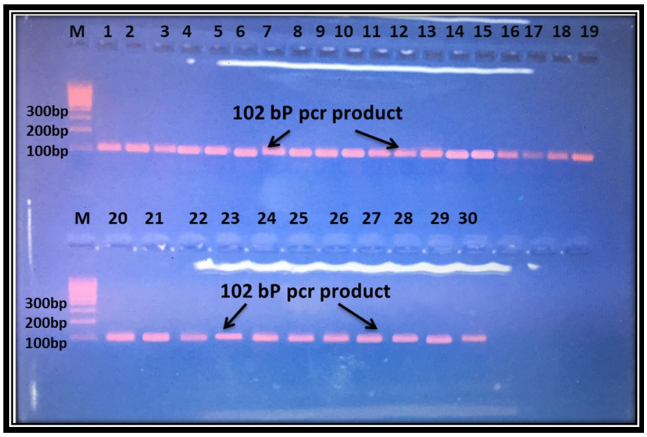

Figure 1. Ethidium Bromide stains agarose gel electrophoresis for polymerase chain reaction product for *S. aureus*. Enterotoxin A (*sea*) gene product (amplified size 102 bp).

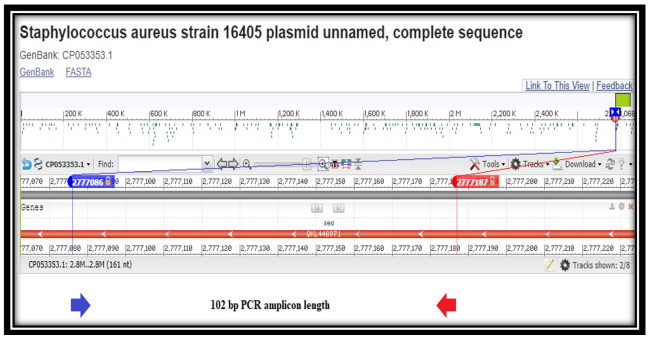

Figure 2. Exact position of the retrieved 102 bp (GenBank acc. no. CP053353.1).

The cytosine was substituted for a guanine in position 44 of the PCR amplicons in thirteen of the investigated samples (Figure 3).

The detected variance was subsequently examined to determine whether the nucleic acid substitution would result in a change to the amino acids that are encoded for the corresponding location in the sea. It was found that the detected nucleic acid substitution was found in the amino acid threonine (Thr or T) in position 136 within the amplified sea locus. An amino acid substitution was observed in this locus, represented by the substitution of Thr with Arg (Thr136Arg) in the sea-encoded staphylococcal enterotoxin type A protein (Figure 4).

The sites and annotations of the discovered nucleic acid substitution mutation were documented in the NCBI reference sequences as displayed in Table 2 to provide a summary of all the findings from the sequenced marine gene fragments.

Table 1. The amplified sequences were extended from 2777086 to 2777187 of the NCBI reference sequence (GenBank accession no. CP053353.1).

Amplicon	Reference locus sequences (5' – 3')	Length
Sea gene sequences	CGGCACTTTTTTCTCTTCGGTCAAT CGATTATTATCATGTAACGTTACACC ACCATACATACAAGCTGTTTTGTTTG GTGTACCACCCGCACATTGATAACC	102 bp

Figure 3. Alignment of thirty bacterial samples' nucleic acid sequences with their respective reference sequences of the sea gene within the *S. aureus* genomic DNA sequences.

The observed Thr136Arg variant was analyzed further to determine its effects on its corresponding position in the modified enterotoxin type A. It was found that the uncharged amino acid Threonine (Thr or T) was changed into a positively charged amino acid, which is Arginine (Arg or R). Upon retrieving the primary amino acid sequences of the enterotoxin type A protein from NCBI, the position of the observed amino acid substitution was highlighted (Table 3).

The consequences of this SNP was evaluated using different publicly available computational algorithms, namely PROVEAN, SIFT, PolyPhen-2, PhD-SNP, SNAP, meta-SNP, and ConSurf bioinformatics tools. These computational tools that were utilized in the present study have not shown any discrepancy in results in such a way that all of the *in silico* tools utilized have revealed a deleterious effect of Thr136Arg (Table 4).

To add another layer of confirmation for structure-function prediction, conserved and variable regions in enterotoxin type A were added using the ConSurf tool. It was discovered that the reported nsSNP was located inside a highly conserved region of the enterotoxin type A protein (Figure 5). The results of the ConSurf tools can also corroborate the severely harmful effect of this variation on the structure and function of the protein.

The construction of a virtual three-dimensional structure of enterotoxin type A gave more in-depth computational data than previously available. A 3-D model of this protein was created using the Phyre2 protein modeling program and then displayed in PyMOL using the data from the experiment. The discovered Thr136Arg SNP was introduced into the protein's native sequence, and the effects of this substitution was investigated using a variety of computational methods. PyMOL was used to locate the Thr136Arg SNP on the protein 3-dimensional structure and to analyze both native and mutant structures in enterotoxin type A, which is made of 257

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Table 2. Pattern of the observed SNPs in the 102 bp amplicons of the sea genetic sequences of the *S. aureus* compared to the NCBI referring sequences (GenBank acc. no. CP053353.1). The symbol "S" followed by a number represented the sample numbers under investigated sample numbers.

Sample No.	Native	Allele	Position in the PCR fragment	Position in the reference genome	AA position	SNP summary
S1,S2,S5,S6,S7,S8,S11,S12, S17,S18,S21,S22,S23	C	G	44	2777129	Thr136 Arg	CP053353.1;g.2777129C>G (QKL44697.1;p.Thr136Arg)

Table 3. The missense Thr136Arg mutations positioning within the encoded enterotoxin type A. The red-colored amino acid residue in the wild-type and mutant protein refers to the site at which the mutation occurred.

Protein status	Staphylococcal enterotoxin type A amino acids sequences
Wild-type staphylococcal enterotoxin type A	MKKTAFTLLLFIALTLTTSPLVNGSEKSEEINEKDLRKKSELQGTALGNLKQIYYYNEKAKTENKESH DQFLQHTILFKGFFTDHSWYNDLLVDFDSKDIVDKYKGKKVDLYGAYYGYQCAGGTPNKTACMYG GVTLHDNNRLTEEKKVPINLWLDGKQNTVPLETVKTNKKNVTVQELDLQARRYLQEKYNLYNSDV FDGKVQRGLIVFHTSTEPSVNYDLFGAQGQYSNTLLRYRDNKTINSENMHIDIYLYTS
Mutant staphylococcal enterotoxin type A	MKKTAFTLLLFIALTLTTSPLVNGSEKSEEINEKDLRKKSELQGTALGNLKQIYYYNEKAKTENKESH DQFLQHTILFKGFFTDHSWYNDLLVDFDSKDIVDKYKGKKVDLYGAYYGYQCAGGTPNKTACMYG GVRLHDNNRLTEEKKVPINLWLDGKQNTVPLETVKTNKKNVTVQELDLQARRYLQEKYNLYNSDV FDGKVQRGLIVFHTSTEPSVNYDLFGAQGQYSNTLLRIYRDNKTINSENMHIDIYLYTS

Table 4. *In silico* analysis of the observed nonsynonymous SNP (Tyr136Arg) on the structure and function of enterotoxin type A using several bioinformatics tools.

Enterotoxin type A 1 (T136R)	Score	Prediction
PROVENANCE	-5.519	Deleterious
SIFT	0.00	Disease
PolyPhen-2	1.000	Damaging
PhD-SNP	0.779	Disease
SNAP	0.845	Disease
Meta-SNP	0.805	Disease

Table 5. *In silico* analysis of the observed nonsynonymous SNP (Tyr136Arg) on the structure and function of enterotoxin type A using several bioinformatics tools.

Enterotoxin type A1(Thr136Arg)	Score	Prediction
I-Mutant2	-0.15	Destabilizing
mCSM	-0.082	Destabilizing
DynaMut	-0.067	Destabilizing
CUPSAT	-2.64	Destabilizing
Mupro	-0.37	Destabilizing

amino acid residues. Therefore, the Thr136Arg amino acid substitution was visualized in the 3-D structure of enterotoxin type A in both native proteins as well as its mutant counterpart (Figure 6). The amino acids before and after mutation are characterized by different charge properties, since the original wild-type (uncharged) Thr136, and the newly introduced (positively-charged) Arg136 mutant residue differ in their properties. This is because the mutant residue (Arg136) is larger than the wild-type (Thr136) residue in the overall diameter. These differences may involve introducing the destabilized properties of the altered enterotoxin type A.

It was necessary to investigate the effect of the discovered nsSNP because of the changes in charge and size between the wild-type and mutant residues to assess the potential effect of this SNP on stability using a set of five *in silico* tools, namely I-Mutant2, mCSM, DynaMut, CUPSAT, and Mupro, which give results in the form of free energy values to exert the effect of this mutation on the stability of the analyzed enterotoxin type A protein [18]. All these tools were based on the 3D structures of enterotoxin type A, as they were provided as PDB files. All the stability prediction tools utilized revealed that the Thr136Arg SNP decreased the stability of the studied protein upon mutation (Table 5). These binding differences were explained by the observed changes in the binding energies in each case, with an obvious tilt in the binding of nafcillin with protein upon mutation with Arg136 (Figure 7).

I-Mutant2: If the DDG value (kcal/mol) is < 0, protein stability decreases, **mCSM:** negative DDG values decrease stability, **DynaMut:** negative DDG values decrease stability, **CUPSAT**; if the DDG value is negative, protein stability decreases, **Mupro:** the negative change in the binding free energy means that the mutation reduces stability upon mutation.

DISCUSSION

The present study emphasizes the analysis of the genetic polymorphism consequences of the Thr136Arg variant on the enterotoxin type A protein. This protein may be essential to *S. aureus* pathogenicity and serve as an antibiotic target (https://www.uniprot.org/UniProt/P0A0L2). The PCR-sequencing strategy was used to detect polymorphisms of the enterotoxin type A gene. The in vitro results indicated that the observation of the amino acid substitutions of Thr136Arg. The sequencing interpretation tools found that this SNP exerts a missense effect on the final protein structure. Due to the change of the uncharged amino acid (Thr) with a positively charged amino acid (Arg), the altered protein may exhibit a considerable impact on structure, function, or stability. To study the role of this mutation in the subsequent

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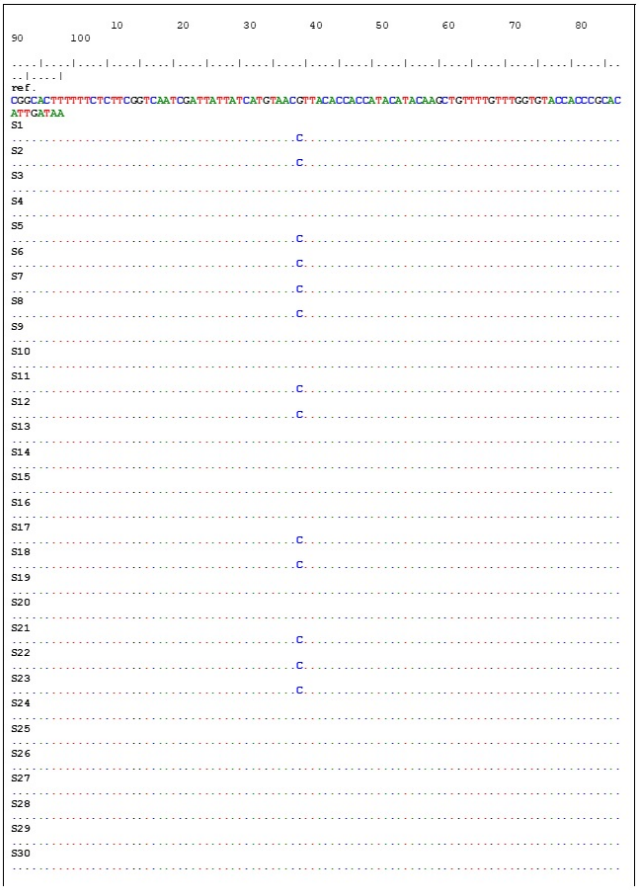

Figure 4. Alignment of amino acid residues of the detected variations within the investigated samples of S. aureus sequences. A) refers to the location of the detected missense variant in the polymerase chain reaction amplicon, and B) refers to the location of the identical variant in the mature total staphylococcal enterotoxin type A protein.

potential alteration in the resulting protein to validate this observation, some *in silico* tools were used The different publicly available computational algorithms (PROVEAN, SIFT, PolyPhen-2, PhD-SNP, SNAP, meta-SNP, and ConSurf) and bioinformatics tools were employed to assess the impact of this SNP. Thus, the validation of the predicted damaging effect of the observed Thr136Arg mutation on the structure and function of enterotoxin type A was provided through all these tools collectively [11, 13, 15, 16].

To add another layer of confirmation for structure-function prediction, conserved and variable regions in enterotoxin type A were added using the ConSurf tool. The ConSurf tool computed the conservation scores in an evolutionary pattern, with certain places evolving slowly and being referred to as "conserved," while others evolved quickly and were referred to as "variable" [17].

In addition to the highly deleterious effect of Thr136Arg predicted on both structure and function, this SNP caused a considerable reduction in the stability of enterotoxin type A. As a result of this reduced stability, the destabilized enterotoxin type A may not exhibit its biological roles properly.

Experiments involving molecular docking were conducted

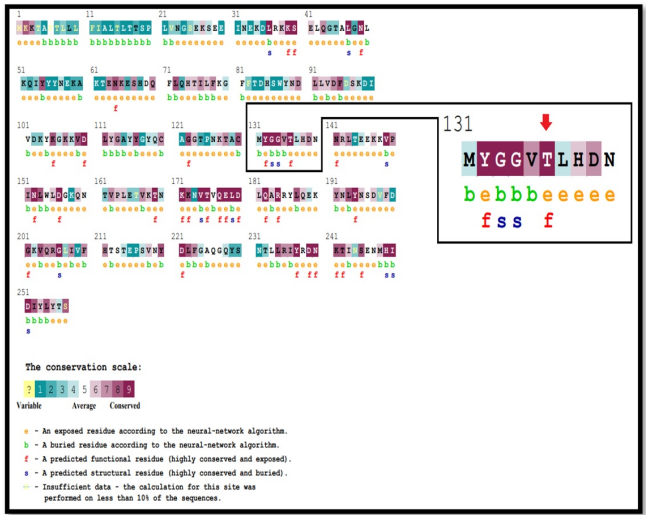

Figure 5. The primary structure with the evolutionary variable and conserved regions in enterotoxin type A. Amino acids were ranked on a conservation scale of 1–9 and highlighted with a range of color intensities to show the conservative grade of each amino acid residue. The red arrow refers to the position of the highlighted amino acid Thr-136.

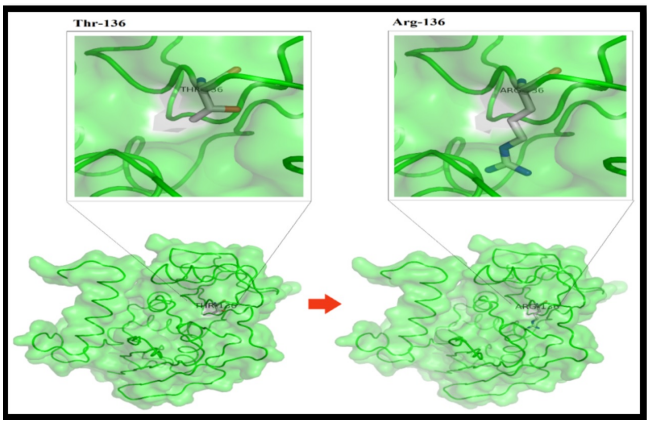

Figure 6. 3-D structure of Staphylococcal enterotoxin type A in which the Thr136 amino acid was substituted with Arg136. Each 3D structure was visualized as a cartoon loop using PyMol software.

between normal enterotoxin type A and the harmful mutant to determine the difference in the total interaction energy with nafcillin before and after mutation. Similar to penicillin, nafcillin is a semisynthetic beta-lactam antibiotic. Following a mutation. Nafcillin is a beta-lactam antibiotic that is semisynthetic in nature and bears resemblance to penicillin. The aforementioned beta-lactamase-resistant penicillin is utilised for the treatment of specific Staphylococcal infections that are caused by strains that are resistant to other penicillin molecules [25, 26]. However, the docking of this specied antibiotic with enterotoxin type A protein indicated two types of interactions with wild-type and mutant forms of enterotoxin type A. The observed variations in binding energies between two cases were attributed to the differences in binding particularly the binding of nafcillin with protein following mutation

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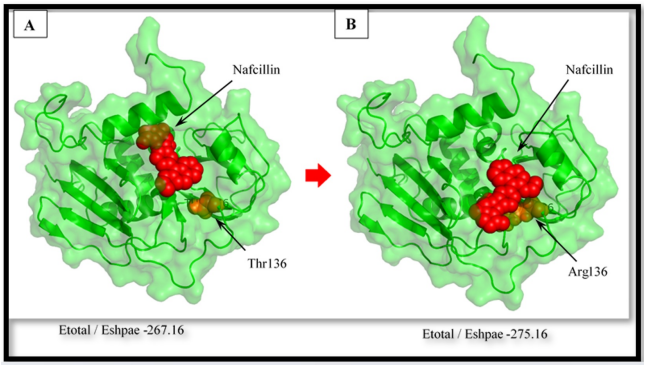

Figure 7. Comparative docking parameters of native enterotoxin type A (Panel A) and its mutant forms (Panel B) in terms of their binding with the antibiotic nafcillin, respectively. The antibiotic nafcillin was highlighted in red spheres, while the 3D protein was shown as a green surface.

with Arg136 [27].

There are three limitations to the current study: the difficulty of obtaining a sufficient number of pathological samples, the unavailability of the sequencing technique at the University of Babylon, which increases the cost of the current investigation, and the high cost of completing some other aspects related to the topic of the study.

CONCLUSION

After exposing observed Thr136Arg nsSNP of enterotoxin type A to several *in silico* tools, it was revealed that this mutation has highly deleterious effects on the protein structure, function, and stability. Similarly, this SNP exerts a noticeable effect on the stability of the enterotoxin type A, in which proteins having this SNP would exert considerable loss of stability. Thr136Arg was found to be contributed to modulating the binding activity of mutant enterotoxin type A with its corresponding nafcillin. This remarkable modulation signies a more dramatic role driven by this amino acid substitution in damaging the main characteristics of this toxin. Consequently, the mechanism of the intervention of the antibiotic nafcillin in changing the conformation of enterotoxin type A is revealed. This study provides in-depth interpretation for clinicians to assess the effect of antibiotic treatment of this type of staphylococcal toxin upon mutation.

ETHICAL DECLARATIONS

Acknoweldgements

We appreciate the laboratory personnel of the Biology Department.

Ethics Approval and Consent to Participate

The study was approved by the College of Science, University of Babylon, Babil, Iraq.

Consent for Publication

None.

Availability of Data and Material

All data are available on request from the corresponding author for reasonable reasons.

Competing Interests

The authors declare that there is no conflict of interest.

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Authors' Contributions

Jabuk SIA and Jaralla EM designed the research, wrote, and analyzed the data. Jabuk SIA was responsible for the literature review, its revision, and manuscript drafting . Both authors have viewed and authorized the final manuscript version.

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