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ORIGINAL ARTICLE

GENETIC POLYMORPHISM OF ARCC, AROE, TPIA AND YQIL IN STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM BAQUBAH PATIENTS

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ABSTRACT

Background: *Staphylococcus aureus* is a significant human pathogen with high adaptability, causing diverse infections. Genotyping is vital for understanding its epidemiology. MLST, analyzing housekeeping gene sequences, is a robust method for strain characterization. The present study aims to investigate the genetic polymorphism of the *arcC*, *aroE*, *tpiA*, and *yqiL* housekeeping genes in *Staphylococcus aureus* strains isolated from patients in Baqubah, Iraq, using MLST, in order to determine the genetic diversity, clonal distribution, and potential epidemiological relationships of these strains.

Materials & Methods: The Methods section describes a molecular observational study on *Staphylococcus aureus* isolates from burn wound infections at Baquba Teaching Hospital between December 2023 and February 2024. Thirty-two *S. aureus* isolates from Baqubah burn patients were analyzed. Genomic DNA was extracted and four housekeeping genes (*arcC, aroE, tpiA, yqiL*) were amplified by PCR. Selected PCR products were sequenced. Sequence data were analyzed using bioinformatics tools for alignment, mutation detection, and phylogenetic tree construction.

Results: Analysis of *arcC, aroE, tpiA*, and *yqiL* genes confirmed all isolates as *S. aureus* with high sequence similarity to reference strains (99%). Mutations were identified across the genes, particularly in isolate S2, which consistently showed a distinct but still *S. aureus* phylogenetic position.

Conclusion: Phylogenetic analysis using *arcC, aroE, tpiA*, and *yqiL* genes confirmed all isolates were *S. aureus*. While intra-species variation was observed, no divergence beyond the species level was indicated. This molecular approach proved effective for accurate identification and genetic characterization of *S. aureus* isolates, aiding epidemiological understanding.

KEY WORDS: *arc; aroE;* Genetic diversity; Genetic mutations; Genotyping; Housekeeping gene; *Staphylococcus aureus; tpiA; yqiL.*

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INTRODUCTION:

Staphylococcus aureus is a common and versatile human pathogen.[1,2] Bacteria are carried asymptomatically by 20-30% of the population. Since the bacteria are highly adaptable, a single strain could cause a dangerous epidemic or pandemic.[3] Staphylococcus is one of the most prevalent bacteria in nature, existing in soil, air, on the skin, mucous membranes, and the upper respiratory tract.[4,5] These bacteria are naturally found in the teeth, nose, and throat. They can cause serious health problems, including deep skin infections, blood infections, and heart valve inflammation.[6]

Genotyping methods quickly and accurately determine the genetic relationships among clinical isolates. This is crucial for understanding how outbreaks spread, helping with epidemiological investigations, and improving surveillance of bacterial strains.[7] Bacterial typing systems differ in effort, cost, accuracy, and ability to distinguish strains. Genotyping methods fall into two categories: Multi-locus sequence typing (MLST) and staphylococcal protein A (spa)

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typing. Pulsed-field gel electrophoresis (PFGE) and PCR restriction fragment length polymorphism (RFLP).[8] MLST characterizes isolates by analyzing the DNA sequences of internal fragments of housekeeping genes, which are approximately 450–500 base pairs in length.[9]

Several studies worldwide have demonstrated the value of MLST in understanding the genetic diversity of S. aureus. For example[10] identified that MLST could distinguish more than 40 clonal complexes of S. aureus, aiding in global epidemiological mapping. In a Malaysian study[11], reported a predominance of ST239 and ST1 among clinical MRSA isolates, reflecting clonal spread within healthcare settings. Locally, an Iraqi study by[12] found that MLST revealed significant diversity among S. aureus strains isolated from Baghdad hospitals, with several novel allelic profiles detected. Regionally, studies in neighboring Iran demonstrated high variability in *arcC and yqiL* loci, suggesting ongoing genetic evolution in circulating strains.[13]

The present study aimed to investigate the genetic polymorphism of the *arcC*, *aroE*, *tpiA*, and *yqiL* housekeeping genes in *Staphylococcus aureus* strains isolated from patients in Baqubah, Iraq, using MLST, in order to determine the genetic diversity, clonal distribution, and potential epidemiological relationships of these strains.

MATERIALS AND METHODS

The Methods section describes a molecular observational study on *Staphylococcus aureus* isolates from burn wound infections at Baquba Teaching Hospital between December 2023 and February 2024. Thirty-two clinical samples were collected from burn wound patients. These samples were cultured on blood agar and MacConkey agar for initial isolation, then on Mannitol Salt Agar (MSA) to presumptively identify *S. aureus*. Genomic DNA was extracted from the bacterial samples using a Geneaid Kit. The concentration, purity, and integrity of the DNA were checked using a Nanodrop spectrophotometer and 0.8% agarose gel electrophoresis.

Four specific genes-*arcC, aroE, tpiA, and yqiL*-were targeted using PCR to generate DNA fragments of different sizes (570 bp, 536 bp, 475 bp, and 598 bp). The PCR was performed using AccuPower PCR premix with a thermal cycling program that included denaturation at 94°C, annealing at 55°C, and elongation at 72°C. Successful amplification was confirmed by running the PCR products on a 1.5% agarose gel. The resulting bands were sharp and specific, indicating the samples were suitable for subsequent sequencing and phylogenetic analysis.

The PCR products were sent to Macrogen Inc. for commercial sequencing. The resulting sequences were then edited and aligned using BioEdit software.

Variations in the sequences were translated into amino acids using the ExPASy online program, and a multiple amino acid alignment was performed using Clustal Omega. A bacterial phylogenetic tree was built by comparing the local sequences with reference sequences from the NCBI-BLAST server. The final tree, showing the relationships between the different strains, was visualized as a polar cladogram using the Figtree program.

Table 1. Primers were used to amplify three loci in *S. aureus* genomic DNA, with bold letters marking amplicon ends and (+)/(-) indicating strand orientation.

Primer	Sequence (5′-3′)	Amplicon size	GenBank Accession Number	Annealing temperature
arcC-F	TTGATTCACCAGCGCGTATTGTC	570 bp	AP018923.1 (2768906 – **2768928**) -	55°C
arcC-R	AGGTATCTGCTTCAATCAGCG		(**2768359** – 2768379) +	
aroE-F	ATCGGAAATCCTATTTCACATTC	536 bp	AP018923.1 (1710156–**1710178**) -	55°C
aroE-R	GGTGTTGTATTAATAACGATATC		(**1709643**–1709665) +	
tpiA -F	TCGTTCATTCTGAACGTCGTGAA	475 bp	AP018923.1 (**828405**–828428) +	55°C
tpiA -R	TTTGCACCTTCTAACAATTGTAC		(828858–**828880**) -	
yqiL-F	CAGCATACAGGACACCTATTGGC	598 bp	AP018923.1 (**378277**–378299) +	55°C
yqiL-R	CGTTGAGGAATCGATACTGGAAC		(378852–**378874**) -	

RESULTS

Five samples were analyzed within this locus, each exhibiting an amplicon length of exactly 570 base pairs. Before sending the *acr*C amplicons for sequencing, we ensured that all amplified products displayed sharp, specific, and clear bands (Fig. 1).

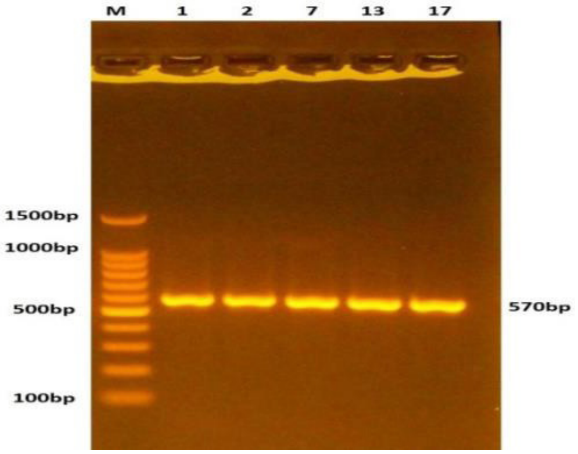


Fig. 1. Agarose gel showing 570 bp *arcC* PCR products.

The sequencing reactions confirmed the identity of the amplified products using the NCBI BLAST tool.[14] The 570 bp PCR amplicons of the *arcC* gene, which encodes carbamate kinase, showed 99% homology with the target sequence. Comparison with GenBank sequences (accession number: AP018923.1) identified the exact positions of the PCR fragment. The alignment of these sequences with the *arcC* gene is summarized in **Table 2**.

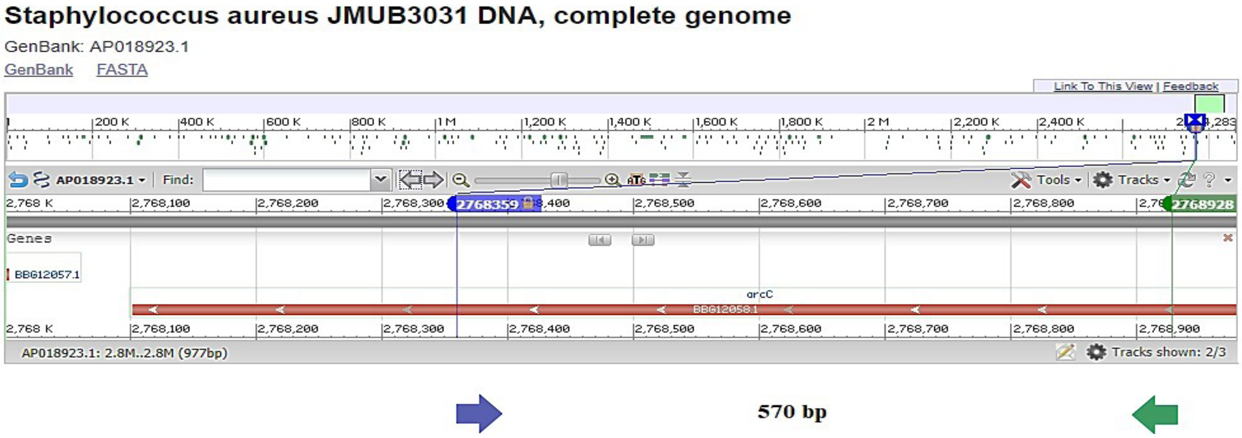
Two mutations were detected in samples No. 2 and No. 4 when aligned against the reference *arcC* sequences. These mutations and their positions are visualized in **Fig. 3**, alongside the sequencing chromatograms. **Table 3** summarizes the 570 bp fragment and the mutation locations relative to the NCBI reference sequences.

Analysis of 100 aligned nucleic acid sequences from various bacterial isolates revealed the presence of a single species: *Staphylococcus aureus*. The phylogenetic tree indicated that all *arcC* variants belonged to *S. aureus* with slight variations. The S2 isolate showed slight genetic divergence but was still classified within *S. aureus*.

Table 2. The 570 bp PCR amplicons cover the *arcC* gene region from position 2,768,359 to 2,768,928 (GenBank acc. no. AP018923.1).

Amplicon	Referring Locus Sequences (5′ - 3′)	Length
arcC DNA sequences	AGGTATCTGCTTCAATCAGCGTTGCTAATTTCTCACTAGCAAAATCTTTATC- TATAACCGCTTCAACACCT TCATAGGTATTTTCTTTTTTTATAACTGGAATACCG- CCACCACCGCATGCAATGACAATATTTTTACCGTC TGCTAAAGTTCGAATTA- ACTGGTGTTCTAGTATAGATTGAGGTAGTGGTGACGCAACTACTTTTCTATAAC CACGTCCTGCATCTTCTTTAAAGACTGAGTCTGGCTGTTCTTTTTGTAAT- TCTTCAACTTCTTCTTTCGTA TAAAAAGGACCAATTGGTTTGGTTGGGTTATT- GAATCGTGGATCATCTTTATCTACTTCCACACGTGTAAC GATTGTGCCTACAGTTC- TATCACTATTCATTTCAGTTAAAATGCGATTGATTTCAGTTTCCAACCAATAGC CTATCATACCCTGTGACATTGCACCACAAGTATCCAATGGCATTGCCGGCGTTGT- GTCACTGTTCGATTTA GCTTGTTGGATTAATAAACTTCCAATTTGTGGGCCATTAC- CATGTGAAATGACAATACGCGCTGGTGAATC AA	570 bp

*[1] *Notice that The forward primer was set to reverse complement mode*.



Staphylococcus aureus JMUB3031 DNA, complete genome

GenBank: AP018923.1

GenBank FASTA

Fig. 2. The 570 bp amplicon covers a segment of the *arcC* gene (acc. no. AP018923.1), marked by the green (start) and cyan (end) arrows.

Table 3. The studied specimens exhibited mutations in the 570 bp *arcC* DNA sequences.

No.	Reference nucleotide	Mutant nucleo-tide	Position in the PCR fragment	Position in the genome	Position in the protein	Type of mutation	Observed in mutation summary
1	A	T	137	2768495	G183	Silent	S2, S17 g.2768495A>T
2	A	G	368	2768726	T106	(G183G) Silent (T106T)	S2 g.2768726A>G

A phylogenetic tree was constructed to display variant positions. Group 1 included the S1 and S7 amplicons, closely matching NCBI genomic sequences. Isolates S2 and S17 shared related positions with one to two mutations. Despite alignment differences, close association was observed between groups, as indicated by the 570 bp *arcC* amplicons (Fig. 4).

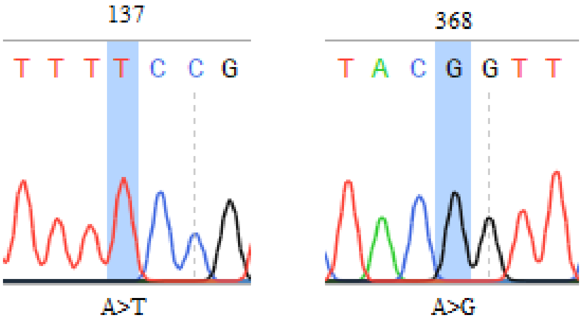


Fig. 3. The chromatogram profile of *arcC* gene variants in local *Staphylococcus aureus*

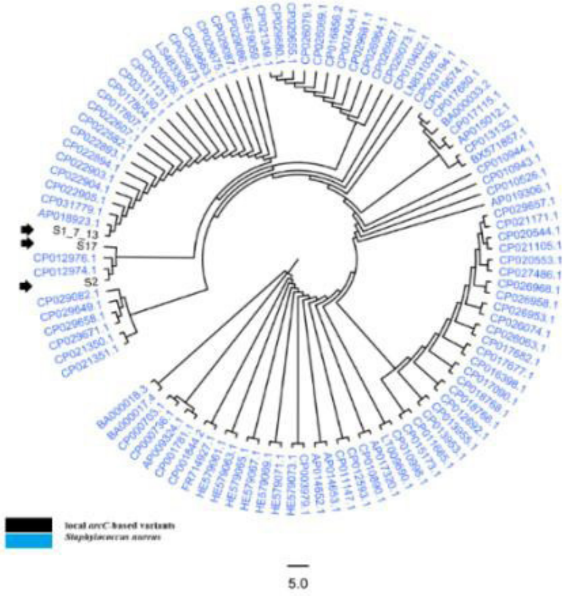


Fig. 4. The phylogenetic tree shows *arcC* gene variants from local *Staphylococcus aureus* isolates.

Five samples were included at this locus, all producing 536 bp amplicons. Before sequencing the *aroE* amplicons, clear and specific bands were confirmed (Fig. 5).

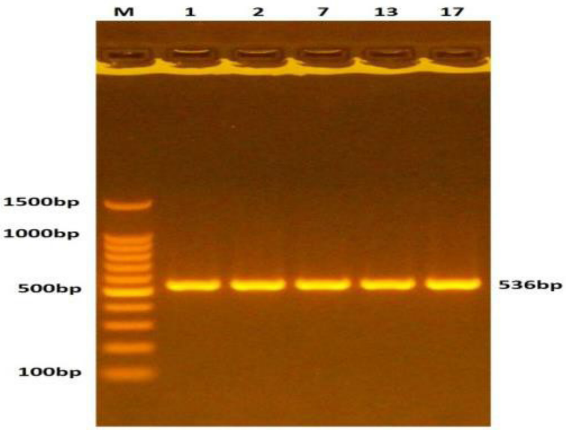
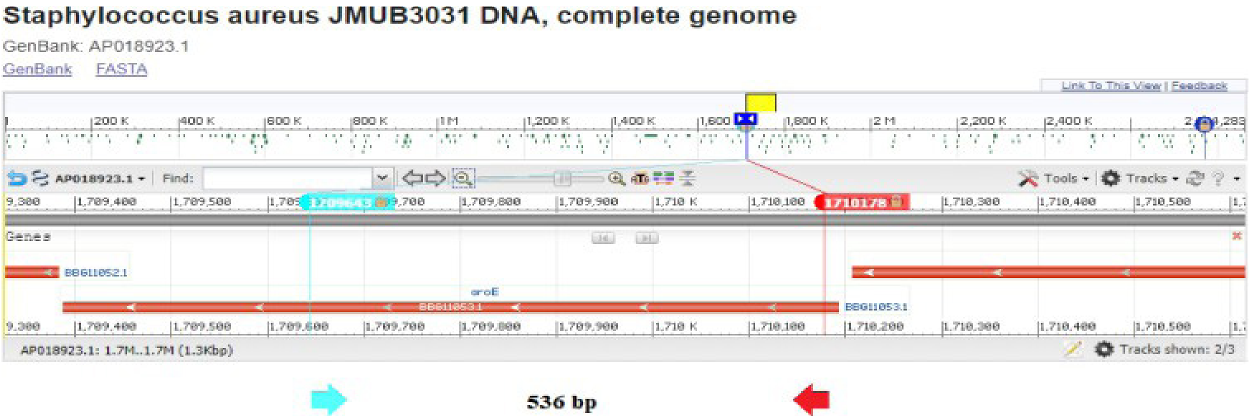


Fig. 5: Agarose gel electrophoresis of 536 bp *aroE* PCR amplicons.

Sequencing confirmed the identity of the amplified products using the NCBI BLASTn tool[14]. The 536 bp PCR amplicons of the *aroE* gene, encoding shikimate dehydrogenase, showed 99% homology with the target. Comparison with GenBank sequences (accession no. AP018923.1) identified the precise fragment locations (Fig. 6). Alignment details are summarized in Table 4.

Three unique mutations were detected in the S2 strain compared to the reference *aroE* sequences . The sequencing chromatogram for S2 and detailed mutation positions are shown in Fig. 7 and Table 5. Analysis of 100 sequences revealed only *Staphylococcus aureus* species. The S2 isolate was slightly distinct but remained within *S. aureus*.

The phylogenetic tree grouped the variants into two clusters: S1, S7, S13, and S17 matched the reference sequences, while S2 had three mutations but was closely related. The *aroE* primers effectively distinguished these groups.



Staphylococcus aureus JMUB3031 DNA, complete genome

GenBank: AP018923.1

Fig. 6: Location of the 536 bp *aroE* amplicon (acc. no. AP018923.1).

Table 4: Details of the 536 bp PCR amplicons amplifying a portion of the *aroE* gene. The NCBI reference sequence spans positions 1,700,643 to 1,710,178 (GenBank acc. no. AP018923.1).

Amplicon	Referring Locus Sequences (5′ - 3′)	Length
aroE DNA sequences	GGTGTTGTATTAATAACGATATCGAATTCAGCTAAATACTTTTCAGCATCTGCTAATGAAATT TGGTTTATATTTAAATTCCAAGATTCAAAACGAGCCATCGTTCTATTCGCAACAGTTAATTTG GGCTTTACAAATTTTGCTAATTCATAAGCAATACCTTTACTTGCACCACCTGCGCCCAAAATT AAAATGTATGCATTTTCTAAATCTGGATAAACGCTGTGCAATCCTTTAACATAACCAATACCA TCTGTATTATACCCTATCCACTTGTCATCTTTTATCAAAACAGTGTTAACTGCACCTGCATTA ATCGCTTGTTCATCAACATGATCTAAATACGGTATGATACGTTCTTTATGAGGAATTGTGATA TTAAAGCCATCTAATTCTTTTTTCGAAATAATTTCTTTAATTAAATGAAAATCTTCAATTGGA ATATTTAAAGCTTCATAAGTATCATCTAATCCTAAAGAATTAAAATTTGCTCTATGCATAACG GGCGACAAGGAATGTGAAATAGGATTTCCGAT	536 bp

*[1]*Notice that The forward primer was configured in reverse complement orientation.*

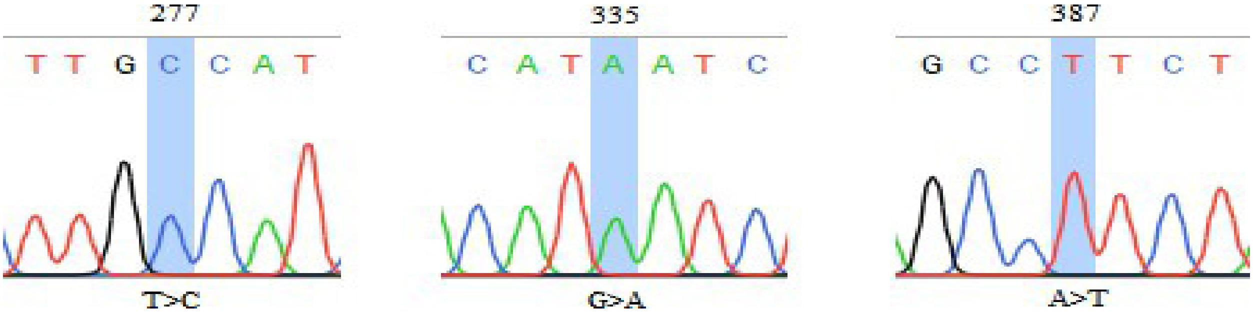


Fig. 7: Chromatogram showing single nucleotide substitutions in the *aroE* gene of local *Staphylococcus aureus.*

Table 5: Observed mutations in the 536 bp aroE sequences of all studied specimens.

No.	Reference nucleotide	Mutant nucleotide	Position in the PCR fragment	Position in the genome	Position in the protein	Type of mutation	Observed in	mutation summary
1	T	C	277	1709919	D92	Missense (D92G)	S2	g.1709919T>C
2	G	A	335	1709977	H73	Missense (H73Y)	S2	g.1709977G>A
3	A	T	387	1710029	D55	Missense (D55E)	S2	g.1710029A>T

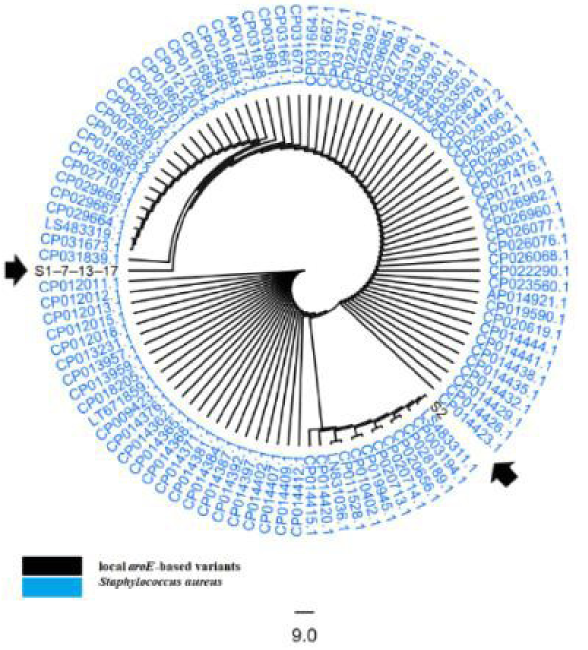


Fig. 8: Phylogenetic tree of *aroE* variants from local *Staphylococcus aureus*

Only five samples were analyzed, resulting in an amplicon length of 475 base pairs. Before sequencing the *tpiA* amplicons, all amplified products were verified to have sharp, specific bands (Fig. 9), ensuring reliable results.

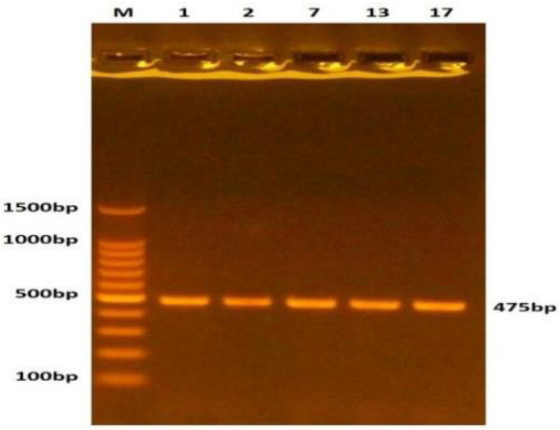
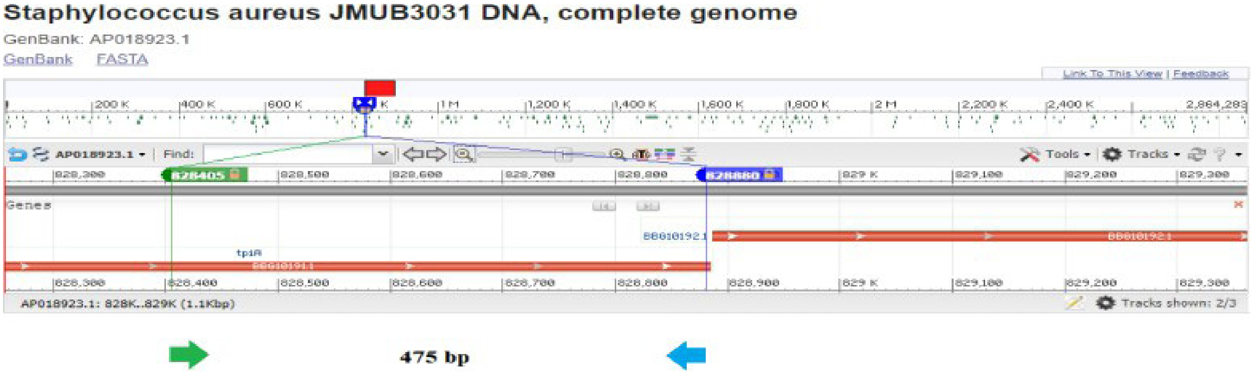


Fig. 9: Agarose gel electrophoresis of 475 bp *tpiA* PCR amplicons.

Sequencing confirmed the identity of the amplified products using NCBI [14]. The 475 bp PCR amplicons of the *tpiA* gene, encoding phosphate acetyltransferase, showed 99% similarity with the target.

Comparison with GenBank sequences (acc. AP018923.1) identified precise fragment locations (Fig. 10). Alignment details are in Table 6.

Nine substitution mutations were detected in all strains compared to the reference . Mutation positions within the 475 bp fragment are summarized in Table 7.



Staphylococcus aureus JMUB3031 DNA, complete genome

GenBank: AP018923.1

GenBank FASTA

Fig. 10: Location of the 475 bp *tpiA* amplicon (acc. no. AP018923.1).

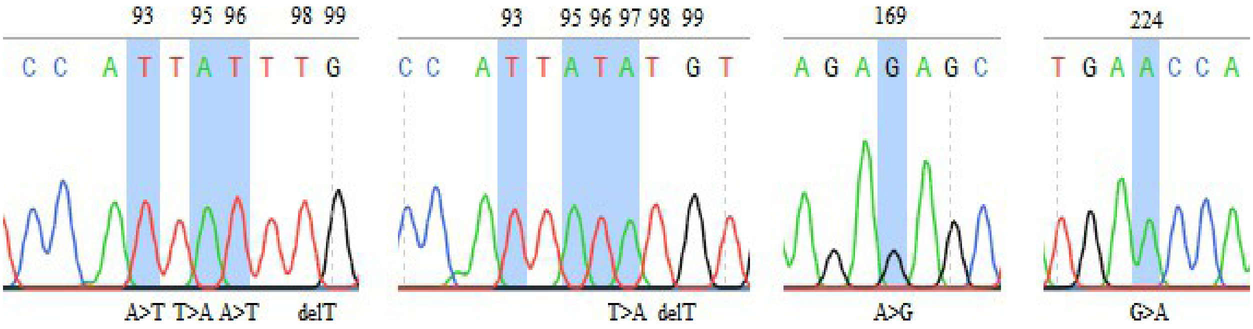


Fig. 11: Chromatogram showing nucleotide substitutions in the *tpiA* gene of local *Staphylococcus aureus*.

Table 6. The 475 bp PCR amplicons amplified a portion of the *tpiA* fragment, extending from 828405 to 828880 (GenBank acc. no. AP018923.1).

Amplicon	Referring Locus Sequences (5′ - 3′)	Length
tpiA DNA sequences	TCGGTCATTCTGAACGTCGTGAATTATTCCACGAAACAGATGAAGAAATTAA- CAAAAAAGCGCACGCTATT TTCAAACATGGAATGACTCCAATTATTTGTGTTG- GTGAAACAGACGAAGAGCGTGAAAGTGGTAAAGCTAA CGATGTTGTAGGTGAG- CAAGTTAAGAAAGCTGTTGCAGGTTTATCTGAAGATCAACTTAAATCAGTTGTAA TTGCTTATGAGCCAATCTGGGCAATCGGAACTGGTAAATCATCAACATCTGAAGATG- CAAATGAAATGTGT GCATTTGTACGTCAAACTATTGCTGACTTATCAAGCAAAGAAG- TATCAGAAGCAACTCGTATTCAATATGG TGGTAGTGTTAAACCTAACAACATTA- AAGAATACATGGCACAAACTGATATTGATGGGGCATTAGTAGGTG GCGCATCACT- TAAAGTTGAAGATTTCGTACAATTGTTAGAAGGTGCAAA	475 bp

*[1] ***Notice:*** *In reverse complement mode, the reverse primer was positioned.*

Table 7: Observed mutations in the 475 bp *tpiA* sequences of all studied specimens.

No.	Reference nucleotide	Mutant nucleotide	Position in the PCR fragment	Position in the genome	Position in the protein	Type of mutations	Observed in	Mutation summary
1	A	T	93	828498	I126	Missense	S1,S2,S7,S1	g.828498A>T
						(I126L)	3,S17	
2	T	A	95	828500	I126	Missense (I126L)	S1,S2,S7,S1 3,S17	g.828500T>A
3	A	T	96	828501	I127	Missense	S1,S2,S7,S1	g.828501A>T
						(I127F)	3,S17	
4	T	A	97	828502	I127	Missense	S2	g.828502T>A
5	T	-	98-99	828503-	I127	(I127Y) Missense	S1,S2,S7,S1	g.828503-
				828504		(I127C)	3,S17	828504delT
6	A	G	169	828574	K151	Missense	S2	g.828574A>G
						(K151R)		
7	G	A	224	828629	E169	Silent (E169E)	S2	g.828629G>A

Phylogenetic analysis of 100 sequences identified only *S. aureus* species. Variants formed two clusters; S2 showed slight divergence but remained within *S. aureus*.

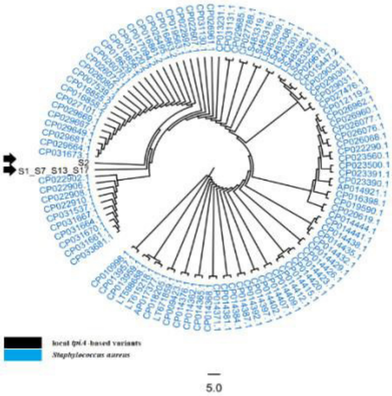


Fig. 12: Phylogenetic tree of *tpiA* variants from local *Staphylococcus aureus* isolates.

Only five samples with 475 bp amplicons were included in this locus. Before sequencing the *yqiL* amplicons, we confirmed that all showed sharp, specific, and clear bands (Fig. 13).

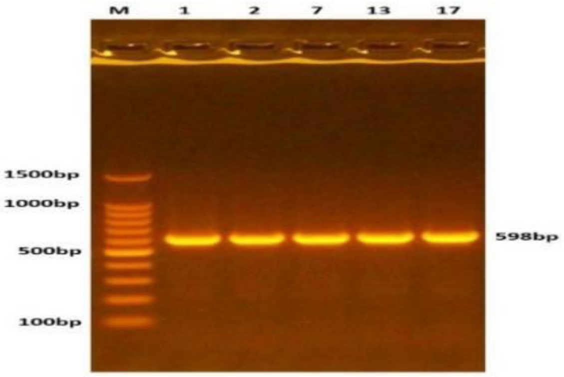


Fig. 13. The agarose gel electrophoresis profile shows PCR amplicons of the 598 bp yqiL fragment.

Sequencing confirmed the identity of the amplified products using NCBI BLASTn [14]. The 598 bp PCR amplicons of the *yqiL* gene, encoding a probable acetyl-CoA acyltransferase, showed 99% homology with the target sequence. Comparison with GenBank sequences (acc. AP018923.1) identified the exact fragment positions. The aligned 598 bp *yqiL* sequences are detailed in Table 14.

Alignment revealed nine substitution mutations present in all analyzed strains compared to the reference (Fig. 14). Sequencing chromatograms and detailed annotations illustrate the mutation positions within the PCR amplicons. These mutations and their precise locations relative to NCBI reference sequences are summarized in Table 9.

Phylogenetic analysis of 100 aligned sequences identified only *S. aureus*. The S2 isolate showed slight divergence but remained within the *S. aureus* clade. Seven substitution variants observed among *yqiL* sequences did not affect species classification.

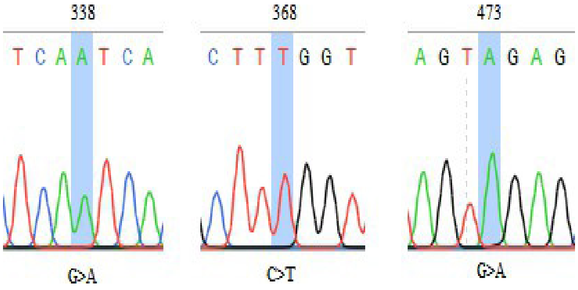


Fig. 15: Chromatogram showing nucleotide substitutions in the *yqiL* gene of local *Staphylococcus aureus*

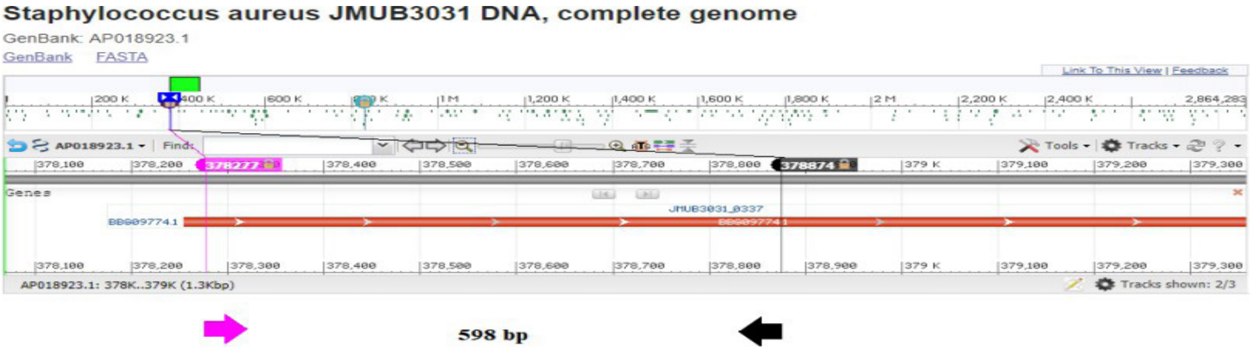


Fig. 14. Location of the 598 bp *yqiL* amplicon (acc. no. AP018923.1).

Table 8: Position and length of the 598 bp PCR amplicons used to amplify a portion of the *yqiL* fragment, spanning nucleotides 378277 to 378874 of the NCBI reference sequence (GenBank acc. no. AP018923.1).

Amplicon	Referring Locus Sequences (5′ - 3′)	Length
yqiL DNA sequences	CAGCATACAGGACACCTATTGGCGTTTTTGGAGGTGCGTTTAAAGACGTGCCAGCCTATGATTTAGGTGCG ACTTTAATAGAACATATTATTAAAGAGACGGGTTTGAATCCAAGTGAGATTGATGAAGTTATCATCGGTAA CGTACTACAAGCAGGACAAGGACAAAATCCAGCACGAATTGCTGCTATGAAAGGTGGCTTGCCAGAAACAG TACCTGCATTTACAGTGAATAAAGTATGTGGTTCTGGGTTAAAGTCGATTCAATTAGCATATCAATCTATT GTGACTGGTGAAAATGACATCGTGCTAGCTGGCGGTATGGAGAATATGTCTCAGTCACCAATGCTTGTCAA CAACAGTCGCTTCGGTTTTAAAATGGGACATCAATCAATGGTTGATAGCATGGTATATGATGGTTTAACAG ATGTATTTAATCAATATCATATGGGTATTACTGCTGAAAATTTAGTGGAGCAATATGGTATTTCAAGAGAA GAACAAGATACATTTGCTGTAAACTCACAACAAAAAGCAGTACGTGCACAGCAAAATGGTGAATTTGATAG TGAAATAGTTCCAGTATCGATTCCTCAACG	598 bp

*[1] **Notice;** *the reverse primer was placed in a reverse complement mode.*

Table 9. The observed mutations in all of the studied specimens of 598 bp in the *ypiL* DNA sequences.

Reference nucleotide	mutant nucleotide	Position in the PCR fragment	Position in the genome	Position in the protein	Type of mutations	Observed in	Mutation summary
G	A	338	378614	Q120	Silent (Q120Q)	S2	g.378614G>A
C	A	368	378644	F130	Silent (F130F)	S2	g.378644C>A
G	A	473	378749	V165	Silent (V165V)	S2	g.378749G>A

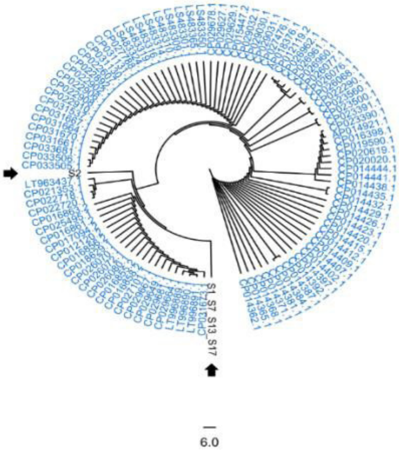


Fig. 16. Phylogenetic tree of *yqiL* variants from local *Staphylococcus aureus* isolates.

DISCUSSION

This study examined *S. aureus* isolates from Baqubah patients using four housekeeping genes, confirming >99% identity with *S. aureus*. Nucleotide changes, especially in isolate S2, indicated genetic diversity. Phylogenetic analysis placed S2 in a distinct branch, suggesting possible divergence. Future integration of molecular, phenotypic, and clinical data could clarify links between genetic variation, pathogenicity, and treatment outcomes.

All *arcC* amplicons matched *Staphylococcus aureus* with ≥99% identity and clustered within intraspecies clades, consistent with regional studies showing minor housekeeping gene variation without species-level divergence. [15] The limited polymorphism observed-two SNPs and the slightly divergent S2 isolate- reflects subtle local diversification, similar to findings in Iran and Pakistan. Differences with studies reporting multiple novel alleles likely result from smaller sample size and localized sampling. These results confirm that housekeeping gene analysis can distinguish lineages within *S. aureus* and suggest that expanded MLST or whole-genome sequencing could reveal additional allelic diversity. These results are consistent with recent studies showing housekeeping gene polymorphism without species-level changes. Khan M et al[16] reported novel *aroE* alleles in Pakistani isolates, while Liu Y et al[17] identified multiple *aroE* mutations among invasive *S. aureus* strains in Southwest China, revealing high local genetic diversity. The slight divergence of S2 in our study aligns with these observations, likely reflecting regional microevolution or limited sampling.

Recent studies have confirmed the high specificity of *tpiA*-based phylogenetic analysis for identifying *Staphylococcus aureus*. For example, *tpiA* was part of a 248-gene phylogenomic tree used to differentiate bovine and human isolates, revealing virulence patterns linked to mastitis [18]. Similarly, whole-genome sequencing highlighted *tpiA*'s role in distinguishing clinical mastitis strains [19]. These findings support *tpiA* as a valuable marker for strain differentiation and epidemiological surveillance. These findings align with recent studies demonstrating intraspecies variation in *yqiL* without species-level divergence. For example, a study by Smith J et al [20] reported multiple *yqiL* mutations among invasive *S. aureus* strains in the United States, revealing high local genetic diversity. Similarly, a study by Shammer K et al [21] observed novel *yqiL* alleles in Iraqi isolates, contributing to the genetic diversity within *S. aureus*. The slight divergence of the S2 isolate in our study is consistent with these observations, likely reflecting regional microevolution or limited sampling.

CONCLUSION

Phylogenetic analysis of four genes confirmed all isolates as *S. aureus*, showing genetic diversity without species divergence. PCR was specific and effective for identification and subspecies differentiation. Isolate S2 occupied a distinct position but remained within *S. aureus*. These gene- based phylogenies provide reliable strain identification and highlight local genetic variability.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design: ASA, ASA
Acquisition, Analysis or Interpretation of Data: ASA, ASA, NKS
Manuscript Writing & Approval: ASA, ASA, NKS

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.