International Journal of Multidisciplinary Research and Development
www.allsubjectjournal.com
ISSN Online: 2349-4182
Received: 18-07-2026, Accepted: 17-08-2026, Published: 01-09-2026
Volume 13, Issue 3, 2026, Page No. 208-212

Molecular and phenotypic characterization of mannitol-fermenting and non-mannitol-fermenting *Staphylococcus aureus* clinical isolates

Mowafaq Saleh Hussain*, Essra Ghanim Alsammak
Department of Biology, Collage of Science, University of Mosul, Mosul, Iraq
Corresponding Author: Mowafaq Saleh Hussain

Abstract
Staphylococcus aureus is an important opportunistic pathogen involved in healthcare and community-associated infections. Mannitol fermentation and coagulase positivity are standard criteria for phenotypic identification, but non-mannitol-fermenting strains can be misidentified. This study aimed to characterize mannitol fermenting and non-mannitol fermenting, coagulase positive *Staphylococcus aureus* isolates for antimicrobial resistance, biofilm formation and selected resistance- and virulence-associated genes. The total of 262 clinical and non-clinical specimens were examined, isolates were characterized on the basis of growth and fermentation on Mannitol Salt Agar (MSA) and confirmed by tube coagulase test. The susceptibility to 10 different antibiotics was determined and the ability to form biofilm was determined by microtiter plate assay. Six representative isolates (3 mannitol-fermenting and 3 non-mannitol-fermenting) were further subjected to polymerase chain reaction (PCR) to detect the presence of the *femA*, *mecA*, *icaD*, *tst-1* and *eta* genes. Bacterial growth was detected in 212 of 262 specimens. Of 82 Gram-positive isolates that grew on MSA, 55 (67.1%) were coagulase-positive *Staphylococcus aureus*, including 51 (92.7%) mannitol-fermenting and 4 (7.3%) non-mannitol-fermenting isolates. Resistance rates to cefoxitin and oxacillin were high in both groups (72.5% and 75.0%, respectively), while biofilm production was observed in 84.3% of mannitol-fermenting isolates and 75.0% of non-mannitol-fermenting isolates. Of the 6 isolates analyzed by PCR, all (100%, 6/6) were positive for *femA*, 66.7% (4/6) were positive for *mecA* and *icaD*, 33.3% (2/6) for *tst-1*, and 16.7% (1/6) for *eta*. To summarize, despite non-mannitol-fermenting, coagulase-positive *Staphylococcus aureus* isolates showed significant antimicrobial resistance, biofilm-forming capacity and virulence-associated genes, which highlights the phenotypic diversity of *Staphylococcus aureus* and emphasizes the importance of combining mannitol fermentation, coagulase testing and molecular characterization to avoid misidentification of atypical isolates.

Keywords: *Staphylococcus aureus*, non-mannitol fermentation, Mannitol Salt Agar, *femA*, *mecA*, *tst-1*, *eta* and *icaD* biofilm, antimicrobial resistance

Introduction

Staphylococcus aureus is an important opportunistic pathogen that causes a broad spectrum of infections in healthcare and community settings. It is a facultatively anaerobic gram-positive bacterium that normally resides on the skin and mucous membranes of humans. It has been found to express several virulence factors such as toxins, enzymes, adhesion factors and biofilm formation that contribute to its ability to persist and evade host immune defenses. The ongoing emergence of antimicrobial resistance, in particular methicillin-resistance, continues to be an important challenge in the clinical management of *Staphylococcus aureus* infections (Brdová *et al.*, 2024; Touaitia *et al.*, 2025) [1, 2].

Phenotypic variation in *Staphylococcus aureus* is routinely observed through mannitol fermentation on Mannitol Salt Agar (MSA), where typical strains produce acid, changing the medium's color to yellow. However, non-mannitol-fermenting variants are increasingly reported. Recent evidence describes the emergence of mannitol-utilization-deficient *Staphylococcus aureus* strains, demonstrating that the absence of mannitol fermentation does not exclude the species nor indicate diminished pathogenic potential (Tille, 2021; Schlievert *et al.*, 2026) [3, 4].

Among the conventional phenotypic markers, coagulase production is still the most important diagnostic characteristic of *Staphylococcus aureus*. Coagulase is involved in virulence by catalyzing the formation of fibrin which helps in bacterial survival and protection against host immune mechanisms (Wang *et al.*, 2025) [5].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious clinical problem because it is resistant to almost all β-lactam antibiotics. Resistance is primarily mediated by the *mecA* gene which encodes an altered penicillin-binding protein (PBP2a) with low affinity for β-lactams allowing cell wall synthesis to continue. The *mecA* gene is generally located in the staphylococcal cassette chromosome mec (SCCmec) that confers acquisition and dissemination (Brdová *et al.*, 2024; Maree *et al.*, 2024) [1, 6].

Another important mechanism of persistence in *Staphylococcus aureus* is biofilm formation, which results in increased adherence of the cells, long-term survival, tolerance to antimicrobials and resistance to host defenses. The ica operon, particularly the *icaD* gene, plays a critical role in biofilm maturation thru the synthesis of enzymes for polysaccharide intercellular adhesin (PIA) (Peng *et al.*, 2023; Armoon *et al.*, 2024) [7, 8].

Beside determinants of antimicrobial resistance and biofilm, *Staphylococcus aureus* carries a number of toxin-encoding genes. The *tst-1* gene encodes toxic shock syndrome toxin-1 (TSST-1) and *eta* encodes exfoliative toxin A, the cause of staphylococcal scalded skin syndrome (SSSS). The existence of these toxin genes points to their importance in severe systemic and cutaneous manifestations. (Touaitia *et al.*, 2025; Monecke *et al.*, 2025) [2, 9].

Conversely, we found that the *femA* gene was a highly conserved molecular marker for the species and a reliable control for *Staphylococcus aureus* identification (Neamah *et al.,* 2019) [10]. Hence, the present study was designed to investigate the molecular characteristics of atypical non-mannitol-fermenting, coagulase-positive *Staphylococcus aureus* isolates identified by detection of *femA, mecA, icaD, tst-1*, and *eta* genes and to compare their antimicrobial resistance and biofilm-producing properties with typical mannitol-fermentation cultures

Materials and method

Sample Collection

This study was conducted from December 2025 to March 2026. It was a cross-sectional and laboratory based study. A total of 262 clinical specimens and nasal swabs were collected from patients and healthy persons in Al-Salam Teaching Hospital, Nineveh Province, Iraq. These included urine (n=57), abscesses (n=43), wound swabs (n=55), sputum (n=28), nasal swabs (n=50), blood (n=24), cerebrospinal fluid (CSF) (n=30) and diabetic foot swabs (n=2). Nasal swabs were also taken from healthy persons to determine the asymptomatic carriage of *Staphylococcus aureus.*

Isolation and Identification of *Staphylococcus aureus*

The samples were inoculated on Blood Agar and Mannitol Salt Agar (MSA) and incubated at 37 °C for 18-24 h. Suspected *Staphylococcus aureus* colonies were confirmed by standard criteria for colony morphology, Gram stain, catalase production and tube coagulase tests. Confirmation was performed automatically using VITEK 2 Compact system (bioMérieux, France).

DNA extraction

Genomic DNA was extracted from the selected *Staphylococcus aureus* isolates using the FavorPrep™ Bacterial Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp., Taiwan) following the manufacturer's instructions. The eluted DNA was quantified and stored at −20 °C until further PCR processing.

Primer Preparation

Lyophilized primers were reconstituted in nuclease free water to prepare a stock solution of a final concentration of 100 pmol/µL. Stock solutions were kept at −20°C until utilized. Working primer solutions were prepared at a concentration of 10 pmol/µL by diluting 10 µL of the 100 pmol/µL stock solution with 90 µL nuclease-free water to a final volume of 100 µL. Primer used in the study in Table 1.

Table 1: Primers used for amplification of the, *femA mecA, Tst-1, eta and icaD* genes in the present study

Target gene	Primer	Sequence (5′–3′)	Amplicon size (bp)	Reference
femA	F	CTTACTTACTGCTGTACCTG	685	(Moazen *et al.,* 2022) [12]
	R	ATCTCGCTTGTTGTGTGC		
mecA	F	TCAGGTACTGCTATCCACCCT	816	(Burhannuddin *et al.,* 2025) [11]
	R	AAACCACCCAATTTGTCTGCC		
Tst-1	F	TTATCGTAAGCCCTTTGTTG	398	(Havaei *et al.,* 2013) [13]
	R	TAAAGGTAGTTCTATTGGAGTAGG		
eta	F	CTATTTACTGTAGGAGCTAG	741	(Sakurai *et al.,* 1995) [14]
	R	ATTTATTTGATGCTCTCTAT		
icaD	F	ACCCAACGCTAAAATCATCG	270	(Lafta *et al.,* 2020) [15]
	R	TTCCTCTCTGCCATTTTTGAA		

Detection of the *femA* Gene by PCR

Detection of *femA* gene of selected isolates of *Staphylococcus aureus* by polymerase chain reaction (PCR). The forward primer sequence was 5′-CTTACTTACTGCTGTACCTG-3′ and the reverse primer sequence was 5′-ATCTCGCTTGTTGTGTGC-3′ and the expected amplicon was 685 bp. The amplification by PCR was performed according to the same conditions described by (Moazen *et al.,* 2022) [12]. PCR products were electrophoretically analyzed on 1.5% agarose gel and visualized under UV light. The *femA* gene was confirmed by the presence of a DNA band of the expected size.

Detection of the *mecA* Gene by PCR

The *mecA* gene was detected by PCR using the forward primer 5′-TCAGGTACTGCTATCCACCCT-3′ and the reverse primer 5′-AAACCACCCAATTTGTCTGCC-3′ with an expected amplicon size of 816 bp (Burhannuddin *et al.,* 2025) [11]. The PCR was performed for 30 cycles with denaturation at 95°C for 30 s, annealing at 55.5°C for 30 s and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The initial denaturation was done at 95°C for 5 min. The PCR products were run on 1.5% agarose gel and visualized under UV light.

Detection of the *tst-1* Gene by PCR

The *tst-1* gene was amplified by PCR with the forward primer 5′-TTATCGTAAGCCCTTTGTTG-3′ and reverse primer 5′-TAAAGGTAGTTCTATTGGAGTAGG-3′ to produce the expected amplicon of 398 bp (Havaei *et al.,* 2013) [14]. PCR amplification was performed at 94°C for 5 min, then 40 cycles of denaturation at 94°C for 40 s, annealing at 60°C for 40 s and extension at 72°C for 1 min, with a final extension of 72°C for 5 min. PCR products were separated by electrophoresis on 1.5% agarose gel and visualized by UV illumination. A DNA band at 398 bp was observed confirming the presence of tst-1 gene.

Detection of the *eta* Gene by PCR

The primers used to detect the eta gene by polymerase chain reaction (PCR) were those reported by (Sakurai *et al.,* 1995) [13]. The forward primer was 5′CTATTTACTGTAGGAGCTAG3′ and reverse primer was 5′ATTTATTTGATGCTCTCTAT3′ with expected amplicon of 741 bp. PCR amplification was initiated with denaturation at 95°C for 4 min, followed by 20 cycles of denaturation at 94°C for 1.5 min, annealing at 50°C for 1.5 min and extension at 73°C for 1.5 min. The PCR products were subjected to electrophoresis on a 1.5% agarose gel.

Detection of the *icaD* Gene by PCR

The *icaD* gene was detected by PCR using the forward primer 5′-ACCCAACGCTAAAATCATCG-3′ and reverse primer 5′-TTCCTCTCTGCCATTTTTGAA-3′, generating an expected amplicon of 270 bp (Lafta *et al.*, 2020) [15]. PCR amplification was performed with an initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 35 s, annealing at 52°C for 35 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 4 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel and visualized under ultraviolet illumination. The presence of the icaD gene was confirmed by the appearance of a DNA band at the expected size of 270 bp.

Results and Discussions

Phenotypic characterization of isolates of *Staphylococcus aureus*

For the present study, 262 clinical specimens and nasal swabs were examined. Of these, 212 (80.9%) had bacterial growth on Blood Agar . Gram stain revealed 138 (65.0%) Gram-positive and 74 (35.0%) Gram-negative isolates. Among the Gram-positive isolates, 82 (59.4%) grew on Mannitol Salt Agar (MSA). Based on the colony morphology and Gram staining, 55 (67.1%) of the isolates were confirmed as *Staphylococcus aureus* by phenotypic identification, MSA, catalase and coagulase tests. Of these 51 (92.7%) were mannitol fermentors and 4 (7.3%) were coagulase positive, non-mannitol fermentors. 32.9% (27 isolates) were coagulase-negative and were excluded from further analysis as described in Table 2

Table 2: Isolation and identification steps of *Staphylococcus aureus* clinical isolates.

Identification step	Number of isolates	Percentage	Denominator
Clinical specimens collected	262	100	262
Growth on blood agar	212	80.9	262
Gram-positive isolates	138	65.0	212
Growth on Mannitol Salt Agar	82	59.4	138
Coagulase-positive *Staphylococcus aureus*	55	67.1	82
Mannitol-fermenting *Staphylococcus aureus*	51	92.7	55
Non-Mannitol fermenting *Staphylococcus aureus*	4	7.3	55

Notes: Percentages were computed using the prior identification stage as a denominator. The number of isolates examined were 262 on Blood Agar, 212 Gram positive isolates, 138 MSA positive isolates and 82 coagulase positives isolates. Among the 55 confirmed isolates, *Staphylococcus aureus* mannitol fermentors and non-mannitol fermentors were calculated. Non-mannitol-fermenting coagulase-positive isolates indicate atypical *Staphylococcus aureus* that may be missed by mannitol-based screening.

Antimicrobial Susceptibility Test of Mannitol-Fermenting and Non-Mannitol-Fermenting *Staphylococcus aureus* Isolates

Resistance rates were highest for cefoxitin and oxacillin, with both mannitol-fermenting *Staphylococcus aureus* isolates (51) having 72.5% resistance to each antibiotic. It was followed by erythromycin and clindamycin (50.9% each), trimethoprim/sulfamethoxazole (41.1%), levofloxacin and tetracycline (19.6% each) and gentamicin (15.6%). The lowest resistance rates were observed for vancomycin and fusidic acid (3.9%, respectively). Among the four *Staphylococcus aureus* isolates that do not ferment mannitol, the highest rates of resistance were observed for cefoxitin, oxacillin and clindamycin (75.0% each). Fifty percent of the strains were resistant to erythromycin and 25.0 % to gentamicin, levofloxacin, tetracycline and trimethoprim/sulfamethoxazole.

Resistance to vancomycin and fusidic acid was not detected among the non-mannitol-fermenting isolates. Phenotypic susceptibility profiles to cefoxitin and oxacillin were used to classify 55 *Staphylococcus aureus* isolates into 38 (69.1%) methicillin resistant *Staphylococcus aureus* (MRSA) and 15 (27.3%) methicillin susceptible *Staphylococcus aureus* (MSSA). Two isolates (3.6%) showed a vancomycin resistant phenotype as determined by the susceptibility testing method employed. Mannitol-fermenting isolates were found to have both vancomycin-resistant phenotypes, whereas, no vancomycin resistance was observed among the non-mannitol-fermentation isolates. The predominant phenotype among *Staphylococcus aureus* isolates in the present study was MRSA (69.1%), followed by MSSA (27.3%) and VRSA (3.6%). The findings are consistent with the results of (Yaseen *et al.*, 2013) [16] in Mosul who found a high prevalence of MRSA in *clinical Staphylococcus aureus* isolates (88%) and the emergence of VRSA strains.

The predominance of MRSA isolates (69.1%) in the present study suggests a high prevalence of methicillin resistance among *Staphylococcus aureus* isolates. This may be due to the extensive use and misuse of β-lactam antibiotics that favors selection and spread of resistant strains carrying the *mecA* gene. are shown in Table 3.

Table 3: Antibiotics sensitivity of mannitol and mannitol fermenters *Staphylococcus aureus*

Antibiotics	Mannitol Fermenters		Non-mannitol fermenters	
	R (%)	S(%)	R (%)	S (%)
Cefoxitin	37(72.5%)	14(27.5%)	3(75%)	1(25%)
Oxacillin	37(72.5%)	14(27.5%)	3(75%)	1(25%)
Gentamicin	8(15.6%)	43(84.4%)	1(25%)	3(75%)
Levofloxacin	10(19.6%)	41(80.4%)	1(25%)	3(75%)
Erythromycin	26(50.9%)	25(49.1%)	2(50%)	2(50%)
Clindamycin	26(50.9%)	25(49.1%)	3(75%)	1(25%)
Vancomycin	2(3.9%)	53(96.1%)	0(0%)	0(0%)
Tetracycline	10(19.6%)	41(80.4%)	1(25%)	3(75%)
Fusidic acid	2(3.9%)	53(96.1%)	0(0%)	0(0%)
Trimethoprim/ Sulfamehoxazole	21(41.1%)	34(58.9%)	1(25%)	3(75%)

Biofilm Formation

The capacity of biofilm formation was determined by the microtiter plate assay in the 55 *Staphylococcus aureus* isolates. Of the 51 isolates that fermented mannitol, 43 isolates (84.3%) were biofilm producers and 8 isolates (15.7%) were non-producers. Of the four non-mannitol-fermenting isolates, three (75.0%) showed biofilm formation and one (25.0%) was classified as a non-producer. Biofilm production was detected in 46 (83.6%) of 55 *Staphylococcus aureus* isolates. This finding are comparable to the study that repored by (Raoof *et al.*, 2013) [17] among mannitol fermented *Staphylococcus aureus* who

obsereved biofilm formation in 87.5% of *Staphylococcus aureus* and slightly lower than among non-mannitol fermentor. The results showed that the loss of mannitol the fermentation ability did not lead to the loss of the biofilm-forming capacity.

Genotypic Detection of Resistance and Virulence Associated Genes

Molecular analysis was done on six representative isolates of *Staphylococcus aureus* including three mannitol fermenting and non-mannitol fermenting isolates. The *femA* gene was found in all the six isolates (100%) including 3/3 (100%) mannitol fermenting and 3/3 (100%) non-mannitol fermenting isolates. This result is consistent with (Kareem *et al.,* 2020) [18] and (Neamah *et al.,* 2019) [10] who indicated the usefulness of *femA* as a reliable molecular marker for identification of *Staphylococcus aureus*. Full detection of *femA* in both phenotypic groups is in agreement with the loss of mannitol fermentation does not change the genetic identity of the tested isolates.

The mecA gene was detected in 4/6 (66.7%) isolates, 2/3 (66.7%) mannitol-fermenting and 2/3 (66.7%) non-mannitol-fermenting isolates. Similar detection rates were observed in both groups, suggesting that the presence of *mecA* and the ability to ferment mannitol were not correlated among the isolates tested. In the current study, the prevalence was slightly higher than the 60% reported in isolates of *Staphylococcus aureus* from patients with boils by (Al-Halaq and Utba 2023) [19]. The presence of *mecA* in non-mannitol-fermenting isolates indicates that atypical phenotypic features may not exclude important methicillin-resistance determinants. However, the molecular analysis was limited to six isolates and the results should be interpreted with caution. The *tst-1* gene was found in 2/6 (33.3%) isolates, one positive isolate among the mannitol-fermenting group (33.3%) and 1 in the non-mannitol-fermenting group (33.3%). This prevalence was less than that reported by (Omar and Mohammed 2021) [20] which was 46.7%. The difference may be due to differences in geographical distribution, clinical sources, sample size or characteristics of the isolates studied. Detection of *tst-1* in both phenotypic groups suggests that the absence of mannitol fermentation does not necessarily correlate with the absence of this virulence-associated gene.

The *eta* gene was detected in 1/6 (16.7%) of isolates. It was detected in 1/3 (33.3%) of the mannitol fermenting isolates. It was not detected in all the three non mannitol fermenting isolates. The overall prevalence was lower than the 23% reported by (Neamah 2024) [21]. Although eta was only detected in the mannitol-fermenting group, the low number of isolates examined does not permit definitive association of mannitol fermentation with the presence of this gene. However, this finding suggests that the non-mannitol-fermenting phenotype does not necessarily involve the loss of all virulence-associated determinants.

The *icaD* gene was found in 4/6 (66.7%) isolates, in 2/3 (66.7%) mannitol fermenting and in 2/3 (66.7%) non-mannitol fermenting isolates. The prevalence in this study was higher than that reported by (Sulaiman and Abdulla 2018) [22] (42.8%) but lower than the prevalence reported by (Hashim and Al-Khafaji 2022) [23] (78%). All four *icaD*-positive strains were biofilm formers, as shown in Table 4-9, while the two *icaD*-negative strains were non-biofilm producers. Therefore, 4/4 (100%) of *icaD*-positive isolates were biofilm-forming. Phenotypic level: Biofilm formation was detected in 43/51 (84.3%) isolates of mannitol fermentors and 3/4 (75.0%) isolates of non-mannitol fermentors. The results indicate that the capacity to form biofilm is maintained in both phenotypic groups the fact that *icaD* was present in both mannitol fermenting and non-mannitol fermenting isolates, and that *icaD* positivity correlated with biofilm formation, indicates that the loss of mannitol This biofilm-associated virulence factor may not necessarily be lost upon fermentation.

Conclusion

The results of this study indicate that isolates of *Staphylococcus aureus* which are atypical coagulase-positive and non-mannitol-fermenting may be missed when mannitol fermentation is the sole criterion used for identification. These isolates showed significant antimicrobial resistance and biofilm-forming capacity, indicating that lack of mannitol fermentation does not necessarily equate to a lower pathogenic potential. The clinical relevance of these isolates is further supported by the detection of *femA* in all isolates studied and the presence of *mecA*, *icaD*, *tst-1* and *eta* in a subset of isolates. Overall, the use of complementary biochemical and molecular techniques is recommended for proper identification of atypical *Staphylococcus aureus*, particularly when coagulase-positive isolates do not ferment mannitol.

References

1. Brdová D, Ruml T, Viktorová J. Mechanism of staphylococcal resistance to clinically relevant antibiotics. Drug Resist Updat,2024:77:101147.
2. Touaitia R, Mairi A, Ibrahim NA, Basher NS, Idres T, Touati A. *Staphylococcus aureus*: a review of the pathogenesis and virulence mechanisms. Antibiotics (Basel),2025:14(5):470.
3. Tille PM. Bailey & Scott's diagnostic microbiology. 15th ed. Elsevier, 2021.
4. Schlievert PM, Kilgore SH, Yoshida T, Beck LA, Leung DYM. Observations on emergence of mannitol-use-deficient *Staphylococcus aureus*. Microbiol Spectr,2026:14(2):e03091-25.
5. Wang D, Wang L, Liu Q, Zhao Y. Virulence factors in biofilm formation and therapeutic strategies for *Staphylococcus aureus*: a review. Animals and Zoonoses,2025:1(2):188-202.
6. Maree M, Ushijima Y, Fernandes PB, Higashide M, Morikawa K. SCCmec transformation requires living donor cells in mixed biofilms. Biofilm,2024:7:100184.
7. Peng Q, Tang X, Dong W, Sun N, Yuan W. A review of biofilm formation of *Staphylococcus aureus* and its regulation mechanism. Antibiotics (Basel),2023:12(1):12.
8. Armoon M, Babapour E, Mirnejad R, Babapour M, Taati Moghadam M. Evaluation of icaA and icaD genes involved in biofilm formation in *Staphylococcus aureus* isolates from clinical sources using reverse transcriptase PCR. Arch Razi Inst,2024:79(6):1329-1335.
9. Monecke S, Burgold-Voigt S, Braun SD, Diezel C, Müller E, Reinicke M, et al. Emergence of livestock-associated MRSA in the Egyptian Nile Delta that carry the exfoliative toxin gene eta: a case for enhanced

surveillance. Eur J Clin Microbiol Infect Dis,2025:44(10):2383-2400.
10. Neamah AJ, Ayyez HN, Klaif SF, Khudhair YI, Hussain MH. Molecular and phylogenetic study of *Staphylococcus aureus* isolated from human and cattle of Al-Qadisiyah Governorate, Iraq. Vet World,2019:12(9):1378-1384.
11. Burhannuddin B, Prameswari IAEA, Suyasa IBO, Putra IGND. Primer design and optimization of polymerase chain reaction method for detection of the mecA gene in methicillin-resistant *Staphylococcus aureus*. In: International Conference on Multidisciplinary Approaches in Health Science,2025:3(1):771-790.
12. Moazen J, Zaniani FR, Asghar BH. Characterization of virulence genes and antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates in intensive care unit (ICU) and non-ICU wards. Trends Med Sci,2022:2(2):e129037.
13. Havaei SA, Azimian A, Fazeli H, Naderi M, Ghazvini K, Samiee SM, et al. Isolation of Asian endemic and livestock associated clones of methicillin resistant *Staphylococcus aureus* from ocular samples in Northeastern Iran. Iran J Microbiol,2013:5(3):227.
14. Sakurai S, Suzuki H, Machida K. Rapid identification by polymerase chain reaction of staphylococcal exfoliative toxin serotype A and B genes. Microbiol Immunol,1995:39(6):379-386.
15. Lafta IJ, Najem MJ. Characterization of mannitol fermenter and salt tolerant staphylococci from breast tumor biopsies of Iraqi women. Baghdad Sci J,2020:17(2):415.
16. Yaseen IH, Shareef AY, Daoud AS. High prevalence of multidrug-resistance MRSA and VRSA of different infections from Al-Jumhuory Teaching Hospital patients in Mosul. J Life Sci,2013:7(12):1255-1259.
17. Raoof WM, Mohamad AM, Al-Omari AW. Detection of biofilm formation in some pathogenic bacteria using tube and Congo red agar methods. Rafidain J Sci,2013:24(12):55-65.
18. Kareem SM, Aljubori SS, Ali MR. Novel determination of spa gene diversity and its molecular typing among *Staphylococcus aureus* Iraqi isolates obtained from different clinical samples. New Microbes New Infect,2020:34:100653.
19. Al-Halaq AAI, Utba NM. Prevalence of methicillin-resistant *Staphylococcus aureus* carrying lukS-lukF gene in Iraqi patients with furunculosis. Iraqi J Sci,2023:64(7):3323-3329.
20. Omar NN, Mohammed RK. A molecular study of toxic shock syndrome toxin gene (tsst-1) in β-lactam resistant *Staphylococcus aureus* clinical isolates. Iraqi J Sci,2021:62(3):825-837.
21. Neamah MM. Prevalence of exfoliative toxin genes (eta and etb) in *Staphylococcus aureus* isolates from tonsillitis, wound, and urinary tract infections in Iraq. J Curr Med Res Opin,2024:7(11):3724-3728.
22. Sulaiman AI, Abdulla BA. Detection of Biofilm Genes (IcaA and IcaD) in *Staphylococcus* spp. Rafidain J Sci,2018:27(4):60-63.
23. Hashim MH, AlKhafaji MH. Molecular detection of biofilm coding genes in *Staphylococcus aureus*. Int J Health Sci,2022:6(S4):4672-4681.