JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Biofilm-associated genes and their role in antibiotic resistance among clinical *Staphylococcus aureus* isolates

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Abstract

Introduction: *Staphylococcus aureus* is a significant pathogen known for its ability to form biofilms, which enhances survival under adverse conditions. This study investigated the molecular mechanisms underlying biofilm formation in clinical *S. aureus* isolates, focusing on key biofilm-associated genes and their correlation with antibiotic resistance (ABR).

Methodology: A total of 40 clinical *S. aureus* isolates were collected from Medical City Teaching Hospital, Baghdad. Biofilm production was assessed using the microtiter plate assay. Biofilm-related genes (*icaB*, *icaC*, *crtM*, *crtN*) were examined by polymerase chain reaction (PCR). Antimicrobial susceptibility was evaluated using the Kirby-Bauer disk diffusion method, and genes was correlated with resistance profiles.

Results: A significant variation in biofilm production was observed, with 80% of isolates classified as non-biofilm formers, 12.5% as weak, 5% as moderate, and 2.5% as strong biofilm producers. Genetic testing revealed that all investigated isolates had a high percentage of *icaB* and *icaC*, with 95 % possessing *crtM*. A significant prevalence of *mecA* (97.5 %) was observed; however, an increased prevalence of *mecA* did not correlate significantly with enhanced biofilm formation.

Conclusions: This is the first report from Baghdad demonstrating the prevalence of these biofilm-associated genes and their link to ABR, offering potential targets for future therapeutic interventions. These results highlight the importance of biofilm-associated genes in the persistence and reinfection potential of *S. aureus* within clinical settings. Additionally, the common occurrence of *icaB* with *icaC* suggests that both genes could serve as new targets for developing treatments aimed at preventing biofilm formation and enhancing therapy effectiveness.

Key words: *Staphylococcus aureus*; biofilm; genes; antibiotic; resistance.

J Infect Dev Ctries 2026; 20(3):416-424. doi:10.3855/jidc.22026

(Received 02 July 2025 – Accepted 18 September 2025)

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Introduction

Staphylococcus aureus is a major human pathogen. It is a Gram-positive, globular bacterium often found in clusters resembling bunches of grapes. It can be seen on the skin and mucous membranes of humans in its non-infectious form; but when it crosses into the pathogenic form, it can cause a wide range of infections, ranging from relatively benign skin infections to severe conditions like sepsis, endocarditis, and pneumonia [1].

The unique factor about *S. aureus* that contributes to its clinical persistence is the wide array of virulence factors it possesses; biofilm formation is one of the most important factors. These enable the bacteria to develop a protective mechanism against environmental factors, antibiotics, and the immune systems of infected hosts [2]. Biofilms are populations of microbial cells that adhere to surfaces and are enclosed within a self-produced extracellular matrix. This sticky outer matrix provides structural stability and enhances adherence to biological and non-biological surfaces, such as catheters, open sore sites, and deep tissues. As a result, biofilm formation contributes to therapeutic resistance [3,4].

One of the major determinants of biofilm formation in these bacteria is a gene cluster known as *icaADBC*, which regulates the synthesis of a sticky material called polysaccharide intercellular adhesin (PIA) [3]. These genes act synergistically: *icaC* elongates the polymer, *icaB* modifies the chains for better stability, and *icaA* enhances adhesion properties. The expression of this gene cluster provides the biosynthetic capacity of the bacteria to establish a cohesive biofilm, which is associated with chronic and recalcitrant infections [5].

Additionally, the accessory gene regulator (*Agr*) system plays a specific role in modulating bacteria-host interactions in vivo. This system is functionally equivalent to quorum sensing, wherein gene expression depends on the density of the cells [6]. Under high-density conditions, *agr* represses genes associated with adhesion, while it induces potential virulence factors that disrupt the biofilm and promote bacterial spread. Dysfunction of this system has been linked to biofilm tolerance and chronic infection [3].

The *pvl* gene encodes the Panton-Valentine leukocidin toxin, which aggressively attacks white blood cells and causes extensive tissue damage. The presence of this gene is strongly associated with severe infections such as skin abscesses and necrotizing pneumonia, particularly in community-acquired virulent strains [7].

The situation worsens when specific strains of *S. aureus* carry the *mecA* gene. This gene codes for an altered penicillin-binding protein (PBP2a), conferring resistance to beta-lactam antibiotics, especially methicillin. These strains, known as methicillin-resistant *Staphylococcus aureus* (MRSA), in combination with other biofilm-related genes, pose a major challenge that is difficult to eradicate with conventional therapies [8].

Therefore, the present study aimed to investigate biofilm formation and antibiotic resistance in clinical isolates of *S. aureus*, with a particular focus on the molecular mechanisms underlying biofilm formation. This study also highlights the dissemination of biofilm-associated genes and their correlation with antimicrobial resistance (AMR). To our knowledge, this represents one of the few reports from Baghdad addressing this critical issue.

Methodology

Isolation and identification of Staphylococcus aureus clinical isolates

Clinical specimens were collected from patients with various infections at the Medical City Teaching Hospital between January and June 2024. The specimens were then transported under complete aseptic conditions to the Al-Mustansiriya Department of Microbiology Laboratory.

Staphylococcus aureus isolates were obtained using selective and non-selective media, including mannitol salt agar, nutrient agar, and blood agar. Identification was based on colony morphology, Gram staining, catalase activity, and additional biochemical tests. Confirmation was performed using the Vitek® 2 system (bioMérieux, Marcy l′Etoile, France) and polymerase chain reaction (PCR) targeting the 16S rRNA gene. The isolates were stored in glycerol at – 80 °C for further analyses [9].

Quantitative evaluation of biofilm production

Biofilm formation was assessed using the microtiter plate assay. Each well of a 96-well plate was inoculated with 100 µL of bacterial culture in nutrient broth and incubated at 37 °C for 24–48 hours. After incubation, non-adherent cells were carefully removed, and the remaining biofilm was stained with crystal violet. Biofilm biomass was quantified by measuring absorbance at 570 nm.

The isolates were categorized into four biofilm formation groups based on the optical density (OD) cutoff (ODc), calculated as 3 standard deviations above the mean OD of the negative control [10]:

Non-producer: OD ≤ ODc
Weak producer: ODc < OD ≤ 2 × ODc
Moderate producer: 2 × ODc < OD ≤ 4 × ODc
Strong producer: OD > 4 × ODc

All assays were performed in triplicate to ensure reproducibility. This methodology provided a standardized approach to quantify and compare biofilm production among isolates.

Antimicrobial susceptibility assessment

The antimicrobial susceptibility of *S. aureus* isolates was determined using the Kirby-Bauer disk

Table 1. Oligonucleotide sequences of primers used in this study.

Genes		Nucleotide Sequence (5̀→ 3̀)	Amplicon Size (Bp)	Reference
icaB	*Fw*	ACCCAACGCTAAAATCATCG	**141**	[14]
	Rv	GCGAAAATGCCCATAGTTTC		
icaC	*Fw*	CTTGCTGGCGCAGTCAATAC	**209**	[15]
	Rv	GTAGCCAACGTCGACAACTG		
crtN	*Fw*	TCAACGCATGGTTTTATGTCCTT	100	[16]
	Rv	AGTCACTCGGTTTGCGATACG		
crtM	Fw	GGTGTTGCTGGTACAGTAGGTGAA	**91**	
	Rv	CACCAAGTCTTCTTGCGACATC		
mecA	*Fw*	GTAGAAATGACTGAACGTCCGATAA	310	[17]
	Rv	CCAATTCCACATTGTTTCGGTCTAA		
pvI	*Fw*	ATCATTAGGTAAAATGTCTGGACATGATCA	433	
	Rv	GCATCAAGTGTATTGGATAGCAAAAGC		
AgrI	*Fw*	ATGCACATGGTGCACATGC	441	
	Rv	GTCACAAGTACTATAAGCTG CGAT		
AgrII	*Fw*	ATGCACATGGTGCACATGC	575	
	Rv	TATTACTAATTGAAAAGTGCCATAGC		
AgrIII	*Fw*	ATGCACATGGTGCACATGC	323	
	Rv	GTAATGTAATAGCTTGTATAATAATACCCG		
16SrRNA	*Fw*	CTACAATGGACAATACAAAGGGC	141	[18]
	Rv	TCACCGTAGCATGCTGATCT		

Fw: forward; *Rv*: reverse; *bp*: base pair.

diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. The tested antibiotics included gentamicin (10 µg), moxifloxacin (5 µg), clindamycin (2 µg), tigecycline (15 µg), erythromycin (15 µg), doxycycline (30 µg), ceftaroline (30 µg), rifampicin (5 µg), fusidic acid (10 µg), vancomycin (30 µg), teicoplanin (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), co-trimoxazole (25 µg), chloramphenicol (30 µg), oxacillin (1 µg), and linezolid (30 µg). The isolates were classified as multidrug-resistant (MDR; resistant to ≥ 1 agent in ≥ 3 antimicrobial classes), extensively drug-resistant (XDR; resistant to all but 1–2 classes), or multidrug-sensitive (MDS; susceptible to most tested antibiotics) [12].

Extraction of genomic DNA by boiling method

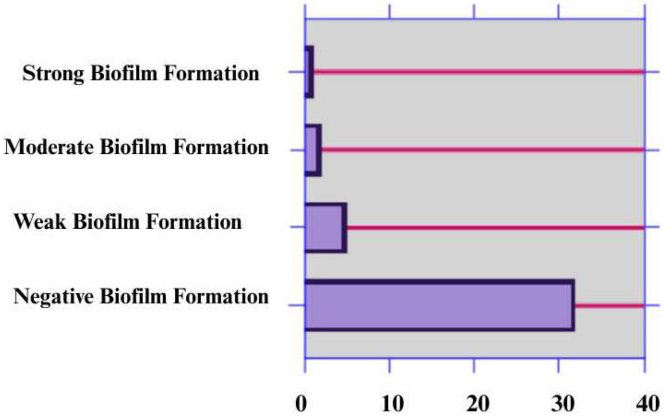
Genomic DNA was extracted using the boiling method to obtain DNA suitable for molecular analyses, including PCR. Bacterial colonies from overnight cultures were re-suspended in 1 mL of sterile filtered water or TE buffer and subjected to boiling at 100 °C for 5–10 minutes in a water bath. Following boiling, the suspension was centrifuged at 12,000 rpm for 5 min to remove cellular debris, and the resulting supernatant containing genomic DNA was collected. The extracted DNA was stored at − 20 °C for future molecular analyses [13].

Molecular detection of quorum sensing and virulence-associated genes by PCR

PCR was performed to detect quorum sensing and virulence-related genes in *S. aureus* isolates. The reaction mixture contained 12.5 µL of GoTaq® Green Master Mix (2X, Promega,Madison, USA), 1.5 µL each of forward and reverse primers (10 pmol/µL), 5 µL of template DNA, and nuclease-free water to a final volume of 25 µL. PCR amplification conditions are presented in Table 1 [14–18]. The PCR products were separated on a 1 % agarose gel at 50 V for 1 hour, stained with ethidium bromide, and visualized under UV light using a transilluminator.

Statistical analysis

Figure 1. Distribution of biofilm formation phenotypes in *Staphylococcus aureus* isolates.



The y-axis represents the biofilm formation categories (negative, weak, moderate, strong), and the x-axis represents the number of *S. aureus* isolates in each category.

All experiments were performed in triplicate. Statistical analyses were conducted using GraphPad Prism 10. Differences in biofilm formation, antibiotic susceptibility, and gene prevalence were evaluated using one-way analysis of variance (ANOVA) and Student's *t*-test, with $p < 0.05$ considered statistically significant. The relationships between biofilm-associated genes and antibiotic resistance, as well as their distribution across resistance patterns and biofilm categories, were analyzed. Phylogenetic trees illustrating genetic relationships among isolates were generated using PAST software.

Results

Isolation and identification of isolates

A total of 40 clinical *S. aureus* isolates were obtained from 150 clinical samples. The isolates were distributed as follows: 17 (42.5%) from urine, 4 (10%) from blood, 14 (35%) from wound infections, 2 (5%) from skin boils, 2 (5%) from swabs, and 1 (2.5%) from other clinical fluids, as shown in Table 2.

Characterization of biofilm phenotypes and antibiotic resistance

Biofilm formation was assessed among the 40 isolates. A total of 8 isolates (20%) demonstrated

Table 2. Distribution of clinical *Staphylococcus aureus* isolates according to sample source.

Source of isolates	n (%)	Isolate ID
Urine	17 (42.5%)	Sa1, Sa2, Sa3, Sa5, Sa8, Sa19, Sa22, Sa23, Sa25, Sa27, Sa28, Sa29, Sa30, Sa31, Sa32, Sa33,S a34
Blood	4 (10%)	Sa4, Sa11, Sa14, Sa15
Wound infections	14 (35%)	Sa6, Sa7, Sa10, Sa12, Sa16, Sa17, Sa18, Sa20, Sa21, Sa26, Sa35, Sa36, Sa39, Sa40
Skin boils	2 (5 %)	Sa9, Sa13
Swabs	2 (5 %)	Sa37, Sa24
Fluids	1 (2.5%)	Sa38

Table 3. Antimicrobial susceptibility profiles and resistance patterns of *Staphylococcus aureus* isolates.

Antimicrobial agents	**Susceptibility pattern of the clinical isolates, (N = 40)**		
	Sensitive patterns count (proportion, *P*)	**Intermediate patterns count (proportion, *P*)**	**Resistant patterns count (proportion, *P*) ***
Gentamicin	40 (1.000, < 0.001)	-	-
Moxifloxacin	36 (0.900, < 0.001)	1 (0.025, < 0.001)	3 (0.075, < 0.001)
Clindamycin	30 (0.750, 0.002)	7 (0.175, < 0.001)	3 (0.075, < 0.001)
Tigecycline	38 (0.950, < 0.001)	-	2 (0.050, < 0.001)
Erythromycin	4 (0.100, < 0.001)	33 (0.825, < 0.001)	2 (0.050, < 0.001)
Doxycycline	39 (0.975, < 0.001)	-	1 (0.025, < 0.001)
Ceftaroline	4 (0.100, < 0.001)	-	36 (0.900, < 0.001)
Rifampin	4 (0.100, < 0.001)	2 (0.050, < 0.001)	34 (0.850, < 0.001)
Fusidic acid	12 (0.300, 0.017)	18 (0.450, 0.636)	10 (0.250, 0.002)
Vancomycin	33 (0.825, < 0.001)	4 (0.100, < 0.001)	3 (0.075, < 0.001)
Teicoplanin	37 (0.925, <0.001)	3 (0.075, < 0.001)	-
Ciprofloxacin	37 (0.925, < 0.001)	3 (0.075, < 0.001)	-
Tetracycline	36 (0.900, < 0.001)	2 (0.050, < 0.001)	2 (0.050, < 0.001)
Co-trimoxazole	35 (0.875, < 0.001)	3 (0.075, < 0.001)	2 (0.050, < 0.001)
Chloramphenicol	39 (0.975, < 0.001)	1 (0.025, < 0.001)	-
Oxacillin	2 (0.050, < 0.001)	-	38 (0.950, < 0.001)
Linezolid	35 (0.875, < 0.001)	3 (0.075, < 0.001)	2 (0.050, < 0.001)

*Proportions tested against value: 0.5.

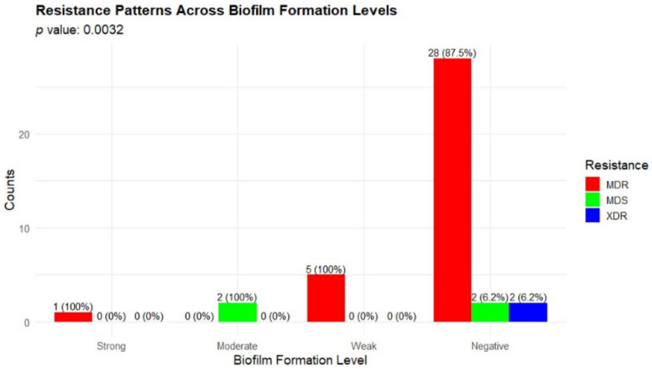
biofilm-forming ability, including 5 weak (12.5%), 2 moderate (5%), and 1 strong producer (2.5%); while the remaining 32 isolates (80%) were non-biofilm formers, as depicted in Figure 1.

Antimicrobial susceptibility testing was performed for all isolates. Gentamicin and doxycycline showed 100% sensitivity. Oxacillin exhibited the highest resistance (95%), while moxifloxacin and vancomycin showed resistance in 7.5–10% of isolates, as illustrated in Table 3. Statistical significance was considered at $p < 0.05$.

Resistance profiles indicated a predominance of MDR *S. aureus* isolates: 34 isolates (85%) were MDR, 2 isolates (5%) were XDR, and 4 isolates (10%) were MDS, as shown by Table 4.

Biofilm formation correlated significantly with antibiotic resistance ($p = 0.0032$). Most biofilm-negative isolates (87.5 %, 28/32) were MDR, whereas only 6.2% was XDR or MDS. All weak and strong biofilm-forming isolates were MDR, while moderate biofilm-forming isolates were MDS (100%), as depicted in Figure 2.

Figure 2. Distribution of antibiotic resistance patterns according to biofilm formation levels.



The bar chart shows how different antibiotic resistance types—MDR (red), MDS (green), and XDR (blue)—are spread out based on the levels of biofilm formation: strong, moderate, weak, and none. Most of the isolates that did not form biofilms showed multidrug resistance (MDR), with 87.5% (28 isolates) falling into this category, while 6.2% were classified as multidrug-susceptible (MDS) and another 6.2% as extensively drug-resistant (XDR). All weak biofilm formers exhibited MDR, while all intermediate biofilm formers displayed MDS. A solitary, robust, biofilm-forming isolate was also MDR. The statistically significant p value (0.0032) shows a strong link between the ability to form biofilms and patterns of antibiotic resistance, suggesting that isolates that do not form biofilms usually have higher levels of MDR in this dataset.

Table 4. Distribution of resistance patterns among *Staphylococcus aureus* isolates.

Resistance patterns	**Out of 40 isolates**			
	Isolates	**n**	**%**	***p****
Multi-drug resistant (MDR)	Sa1, Sa2, Sa3, Sa4, Sa5, Sa6, Sa7, Sa8, Sa9, Sa10, Sa13, Sa15, Sa16, Sa17, Sa18, Sa19, Sa20, Sa21, Sa22, Sa23, Sa24, Sa25, Sa26, Sa27, Sa28, Sa29, Sa30, Sa31, Sa32, Sa33, Sa34, Sa38, Sa39, Sa40	34	85%	$p < 0.001$
Extensively drug resistant (XDR)	Sa35, Sa36	2	5%	$p < 0.001$
Multidrug-sensitive (MDS)	Sa11, Sa37, Sa12, Sa14	4	10%	$p < 0.001$

*Proportions tested against value: 0.5.

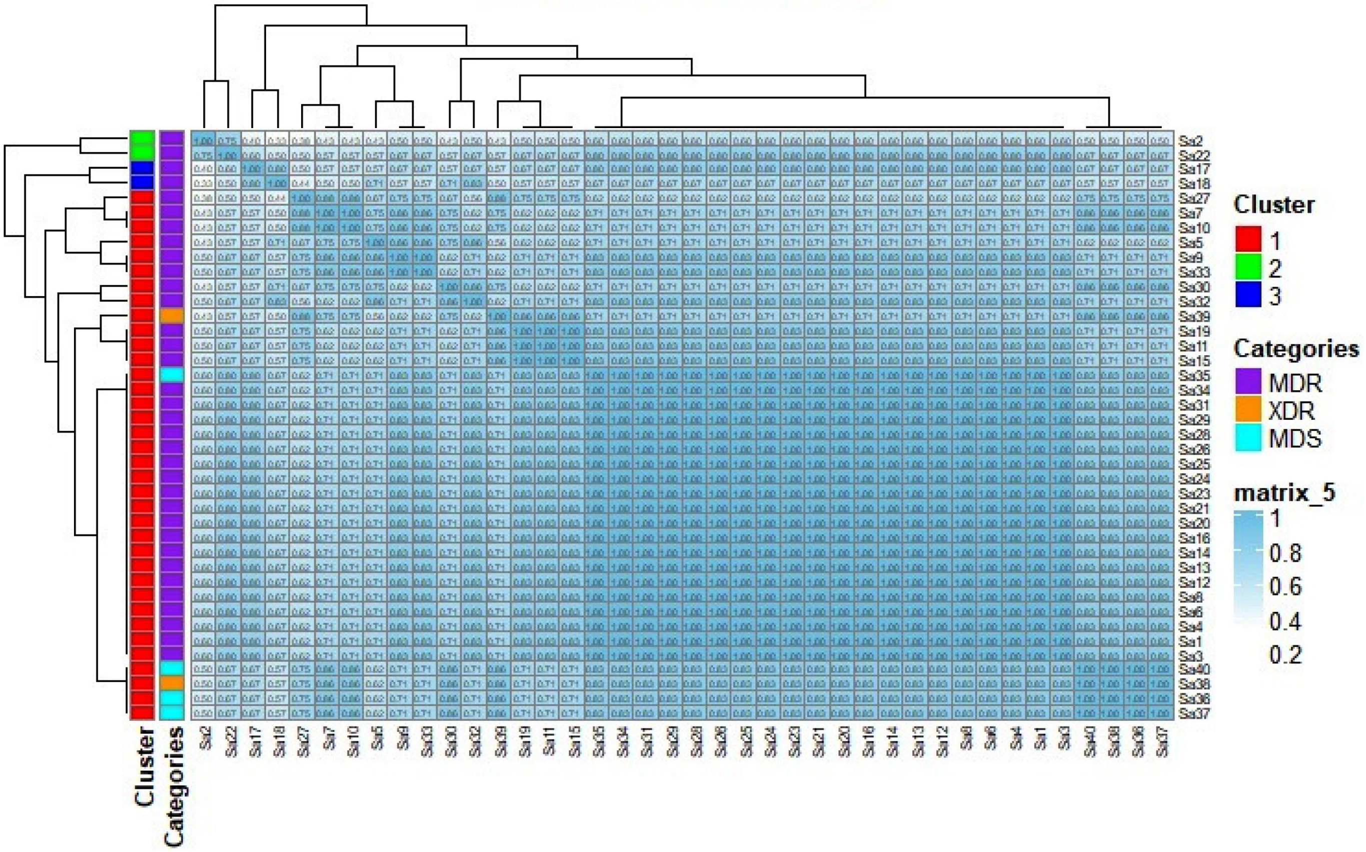
Table 5. Prevalence of biofilm-associated genes among Staphylococcus aureus isolates.

Genes	Prevalence of biofilm genes among isolates (n = 40)				95% CI for proportion	
	Prevalence	Counts	Proportion	p	Lower	Upper
crtM	Absent	2	0.050	$p < 0.001$	0.000	0.149
	Present	38	0.950	$p = 1.000$	0.000	0.991
icaB	Present	40	1.000	$p = 1.000$	0.000	1.000
icaC	Present	40	1.000	$p = 1.000$	0.000	1.000
crtN	Absent	2	0.050	$p < 0.001$	0.000	0.149
	Present	38	0.950	$p = 1.000$	0.000	0.991
mecA	Absent	1	0.025	$p < 0.001$	0.000	0.113
	Present	39	0.975	$p = 1.000$	0.000	0.999
PvI	Absent	31	0.775	$p = 1.000$	0.000	0.877
	Present	9	0.225	$p < 0.001$	0.000	0.360
Agr1	Absent	35	0.875	$p = 1.000$	0.000	0.949
	Present	5	0.125	$p < 0.001$	0.000	0.245
Agr2	Absent	34	0.850	$p = 1.000$	0.000	0.933
	Present	6	0.150	$p < 0.001$	0.000	0.275
Agr3	Absent	36	0.900	$p = 1.000$	0.000	0.965
	Present	4	0.100	$p < 0.001$	0.000	0.214

Genomic basis of biofilm-related genes

The distribution of biofilm- and resistance-related genes among *S. aureus* isolates was as follows: *crtM* was detected in 95% of the isolates, *icaB* and *icaC* in 100%, *crtN* in 95%, and *mecA* in 97.5%. The *pvl* gene was identified in 22.5% of the isolates; while *Agr1*, *Agr2*, and Agr3 were detected in 12.5%, 15%, and 10%, respectively, as depicted in Table 5.

Figure 3. Heatmap depicting the similarity of bacterial isolates and their correlation with antibiotic resistance patterns.



The x-axis denotes 40 isolates labeled S1 to S40, and the y-axis represents the clusters and categories of the isolates. This heat map illustrates the similarity associations among bacterial isolates based on genetic or behavioral characteristics, with similarity values from 0.4 to 1.0 represented by a blue color gradient. Hierarchical clustering was employed to categorize the isolates into 3 discrete clusters—cluster 1 (red), cluster 2 (green), and cluster 3 (blue)—demonstrating patterns of relatedness among the isolates. The isolates are classified based on their antibiotic resistance profiles: MDR (multidrug-resistant) indicated in purple, XDR (extensively drug-resistant) in orange, and MDS (multi-drug-sensitive) in cyan. This integrated image elucidates the relationship between isolate similarity and resistance patterns, enhancing comprehension of microbial epidemiology and informing efficient antibacterial tactics.

Table 6. Distribution of virulence and resistance genes among multi-drug resistant (MDR), extensively drug resistant (XDR), and multidrug-sensitive (MDS) *Staphylococcus aureus* isolates.

Genes	MDR isolates (34)		XDR isolates (2)		MDS isolates (4)	
	n	%	n	%	n	%
CrtM	33	94.3%	2	100%	3	100%
IcaB	35	100%	2	100%	3	100%
IcaC	35	100%	2	100%	3	100%
CrtN	33	94.3%	2	100%	3	100%
MecA	34	97.1%	2	100%	3	100%
Pvl	9	25.7%	-	-	1	33.3%
Agr1	4	11.4%	-	-	1	33.3%
Agr2	6	17.1%	-	-	-	-
Agr3	4	11.4%	-	-	-	-

Correlation between virulence and resistance genes in various S. aureus resistance patterns

The distribution of genes among different resistance patterns was as follows: *icaB* and *icaC* were detected in 100% of all isolates. *crtM* and *crtN* were identified in 94.3% of MDR isolates and in all XDR and MDS isolates. *mecA* was detected in 97.1% of MDR isolates and in 100% of XDR and MDS isolates. The *pvl* gene was identified in 25.7% of MDR isolates and 33.3% of MDS isolates, but was absent in XDR isolates. *Agr1*, *Agr2*, and *Agr3* were detected in a small proportion (25.7%) of MDR isolates and were not found in XDR isolates, as shown in Table 6 and Figure 3.

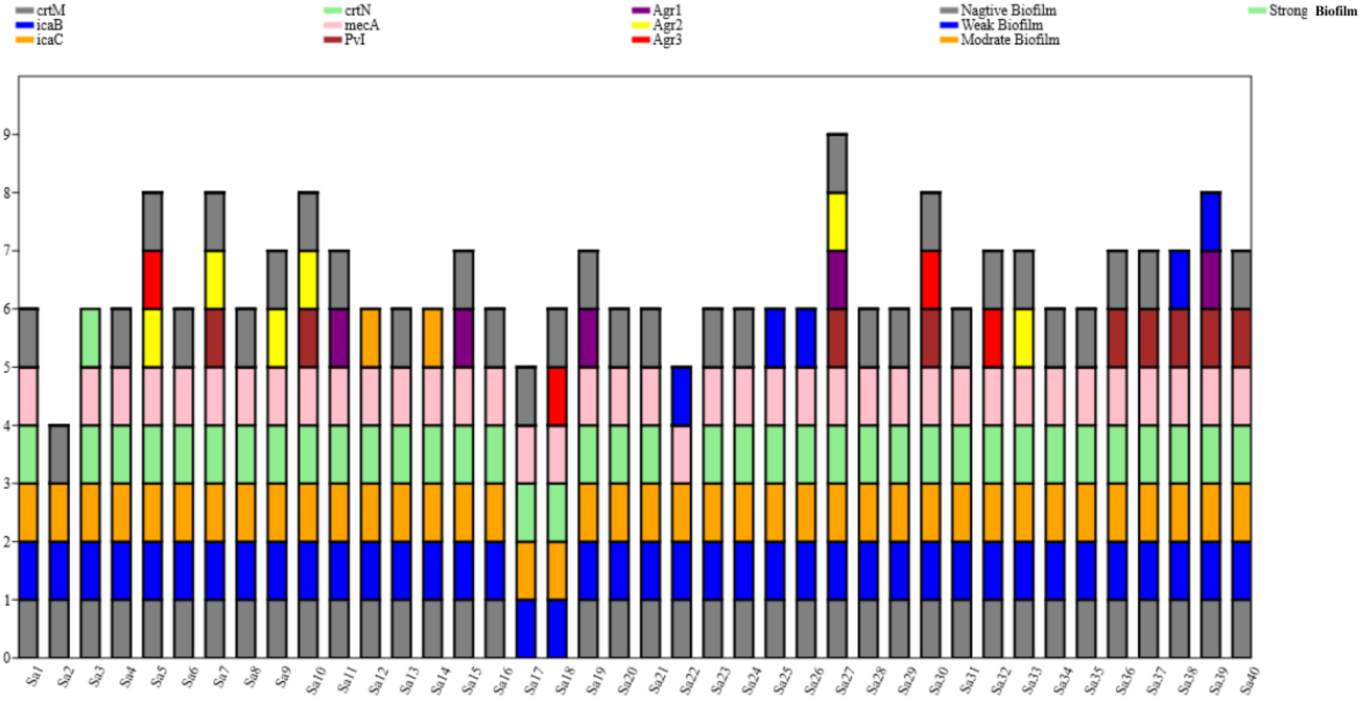
Prevalence and linkage of virulence and resistance genes in Staphylococcus aureus isolates according to biofilm formation phenotypes

The prevalence of the tested genes among biofilm-negative isolates, was as follows: *crtM* 90.9 %, *crtN* 90.9 %, *icaB* 97 %, *icaC* 97 %, *mecA* 93.9 %, *pvl* 27.3 %, *Agr1* 15.2 %, *Agr2* 18.2 %, and *Agr3* 12.1 %.

All weak biofilm-forming isolates (100%) were positive for *crtM*, *crtN*, *icaB*, *icaC*, and *mecA*; while *pvl*, *Agr1*, *Agr2*, and *Agr3* were absent.

All moderate and strong biofilm-forming isolates (100%) were positive for *crtM*, *crtN*, *icaB*, *icaC*, and *mecA;* while *pvl*, *Agr1*, *Agr2*, and *Agr3* were not

Figure 4. Stacked bar chart illustrating the distribution of virulence and resistance genes among *Staphylococcus aureus* isolates according to biofilm formation patterns.



The x-axis denotes 40 isolates labeled S1 to S40, whilst the y-axis signifies the quantity of identified genes or biological features for each isolate. The various colors in the bars signify virulence and resistance genes, including crtM, icaB, icaC, mecA, and pvl, as well as the classification of biofilm formation: dark blue denotes weak biofilm formation, orange indicates moderate biofilm formation, and light green represents strong biofilm formation.

Table 7. Distribution of virulence and resistance genes across various biofilm-forming phenotypes of *Staphylococcus aureus* isolates across all biofilm classifications.

Gene	Negative biofilm (33)		Weak biofilm (2)		Moderate biofilm (5)		Strong biofilm (1)	
	n	%	n	%	n	%	n	%
crtM	30	90.9%	2	100%	5	100%	1	100%
icaB	32	97%	2	100%	5	100%	1	100%
icaC	32	97%	2	100%	5	100%	1	100%
crtN	30	90.9%	2	100%	5	100%	1	100%
mecA	31	93.9%	2	100%	5	100%	1	100%
pvl	9	27.3%	0	0%	0	0%	0	0%
Agr1	5	15.2%	0	0%	0	0%	0	0%
Agr2	6	18.2%	0	0%	0	0%	0	0%
Agr3	4	12.1%	0	0%	0	0%	0	0%

detected, as illustrated in Table 7 and Figure 4.

Discussion

In the present study, only 20% of *S. aureus* isolates demonstrated biofilm formation. This relatively low prevalence may reflect strain-specific differences in biofilm-forming capacity or the influence of environmental and clinical pressures on gene expression. This finding is supported by Murthy *et al.* [19], who reported lower biofilm production among clinical isolates. However, these results contrast with Lavoie *et al.* [20], who observed a much higher prevalence of biofilm formation (60–70%) in hospital-acquired *S. aureus* isolates. Such discrepancies could be due to differences in sample sources, patient demographics, or the sensitivity of the biofilm detection methods used, as well as variations in antibiotic stewardship and infection control practices. Although only a subset of isolates formed biofilms, these strains universally carried biofilm-associated genes, indicating that genetic potential exists even in non-biofilm-forming isolates, but is only expressed under favorable conditions. The combination of biofilm-associated genes and oxidative stress-protective genes likely provides a synergistic advantage, enabling survival under both host immune attack and antimicrobial pressure.

Antibiotic susceptibility testing revealed that oxacillin resistance was the most prevalent among the isolates, whereas gentamicin and doxycycline showed complete susceptibility, with moderate resistance observed for moxifloxacin and vancomycin. These patterns suggest that certain antibiotics remain effective despite high resistance levels to others, reflecting different selective pressures in clinical environments. These findings are partially consistent with Tang *et al.* [21], who reported similar susceptibility patterns among *S. aureus* isolates.

Further analysis showed a high prevalence of multidrug-resistant (MDR) isolates, with 34 of 40 isolates (85%) classified as MDR, 2 isolates as XDR, and 4 as MDS. The persistence of high MDR rates highlights the role of hospitals as reservoirs for resistant strains and emphasizes the selective advantage conferred by mobile genetic elements and biofilm formation. These results align with Adhikari *et al.* [22], who reported a 94.05% prevalence of MDR among MRSA isolates.

Molecular analysis revealed the presence of *icaB* and *icaC* in all isolates, reinforcing their central role in polysaccharide intercellular adhesin synthesis and biofilm stability. Similarly, Hong *et al.* [23] reported a high prevalence of these genes in clinical *S. aureus* isolates. The *crtM* and *crtN* genes were present in 95% of isolates, contributing to staphyloxanthin production, which enhances oxidative stress resistance and bacterial survival, consistent with Siems *et al.* [24]. This indicates that staphyloxanthin biosynthesis is a widely conserved mechanism aiding persistence under oxidative and immune pressures. Additionally, *mecA* was detected in 97.5% of isolates, aligning with findings reported by Anwar *et al.* [25]. The widespread presence of these genes likely reflects strong selective pressures in clinical settings, allowing resistant strains to withstand both antibiotics and host defenses.

In contrast, virulence genes such as *pvl* and *Agr* were detected at low frequencies among our isolates, similar to Najafi Olya *et al.* [26]. It appears that highly resistant strains rely more on biofilm-associated and oxidative stress-protective genes, while classical virulence factors play a secondary role, reflecting an evolutionary trade-off between resistance and virulence. Conversely, non-resistant or non-biofilm-forming isolates may retain some of these traditional virulence factors but are less equipped to survive under high antibiotic pressure.

A clear dynamic association was observed between virulence genes, resistance patterns, and biofilm formation. All MDR, XDR, and MDS isolates consistently carried *icaB* and *icaC*, highlighting their indispensable role in biofilm-related survival. The *crtM* and *crtN* genes were highly prevalent in MDR isolates

(94.3%) and present in all XDR and MDS isolates, while *mecA* was detected in 97.1% of MDR and 100% of XDR and MDS isolates. This pattern reflects the reliance of resistant strains on biofilm-associated and oxidative stress-protective genes rather than traditional virulence factors. Conversely, *pvl* and *Agr* genes were generally absent in XDR isolates and infrequent in MDR and MDS strains, indicating that extreme antibiotic resistance reduces the dependency on classical virulence traits.

When examined in relation to biofilm formation, all isolates forming weak, moderate, or strong biofilms were positive for *crtM*, *crtN*, *icaB*, *icaC*, and *mecA*; while *pvl* and *Agr* genes were absent. Biofilm-negative isolates showed lower prevalence of these genes (*pvl* 27.3%, *Agr1* 15.2%, *Agr2* 18.2%, *Agr3* 12.1%), suggesting that these strains may retain some traditional virulence determinants but are less equipped for antibiotic resistance or oxidative stress [27]. Overall, these findings demonstrate a balanced adaptive strategy in clinical *S. aureus* isolates: highly resistant strains primarily rely on biofilm-associated and oxidative stress-protective genes, while classical virulence factors play a supporting role, reflecting the bacterium's ability to survive in challenging hospital environments.

Conclusions

This study demonstrates the critical role of biofilm-associated genes (*icaB*, *icaC*, *crtM*, and *mecA*) in the survival and antibiotic resistance of clinical *S. aureus* isolates. Their high prevalence, combined with alarming rates of MDR and XDR, highlight the contribution of biofilm formation to bacterial persistence, particularly in hospital settings and on medical devices. These findings emphasize the importance of developing targeted strategies to disrupt biofilm formation and associated resistance mechanisms, which could guide more effective therapeutic and infection control interventions against *S. aureus* infections.

Acknowledgements

This study has been made possible thanks to the contributions of all individuals and institutions involved in it; among them, we particularly appreciate all the technical and moral support from the Microbiology Laboratory team at Al-Mustansiriya University. In addition, we are thankful to those patients who permitted us to take the required samples for this research.

Ethical considerations

The study complied with the ethical standards prevailing in medical and scientific research. Clinical *S. aureus* isolates were collected from Medical City Teaching Hospital Baghdad, administered by the official authority of the hospital. Data and sample information about the patients were also kept confidential. The study followed the principles of scientific research regarding rights to participation, no harm, and fairness in data collection and analysis. In this study, chemicals or techniques injurious to the environment or public health were not used. Safety precautions were in place during sample collections and handling of biological materials, and in conformity with local and international safety regulations. The study was also reviewed by the Research Ethics Committee at Mustansiriyah University to ensure compliance with ethical principles in medical and microbiological research.

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Conflict of interest

No conflict of interest is declared.

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