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Triclosan Resistance and icaA-Associated Biofilm Formation in *Staphylococcus aureus* from Urinary Tract Infections

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ABSTRACT

Staphylococcus aureus, a widely disseminated human pathogen causes various infections of all ages and sexes. The development and spread of antimicrobial-resistant S. aureus strains represent a substantial global concern for the effective treatment of these infections. Staphylococcus aureus: a significant human pathogen Notwithstanding its virulence, antibiotic resistance and biofilm-forming ability Correct identification and characterization of its phenotypic and genotypic features are vital to control of infection and targeted treatment strategies. Staphylococcus aureus is a prominent human pathogen associated with diverse infections including urinary tract infections (UTIs). Antibiotic resistance and biofilm formation complicate treatment and increase its pathogenicity. The objective of this study was to investigate the resistance to triclosan and the correlation between icaA gene presence and biofilm production in S. aureus strains that originated from women with urinary tract infections (UTIs). Quantitative Crystal Violet (CV) assay was used to measure biofilm formation. 54.2% of icaA-positive isolates and 76.5% of icaA-negative isolates were biofilm positive, confirming that development of biofilms can occur in the absence of an icaA gene (7). A proportion of isolates had resistance to triclosan, possibly suggesting that biofilm-forming bacteria is under selective pressure. These results reveal the intricate S. aureus biofilm formation process and its role in chronic infections. Effective treatment and infection control methods depend on an understanding of the interaction between biofilm-associated virulence and antibiotic resistance. "Accordingly, the icaA gene may contribute to triclosan resistance in some S. aureus Iraqi isolates through increased expression. In contrast, additional antiseptic-resistance genes may play a role in triclosan resistance in biofilm-negative S. aureus isolates. Furthermore, triclosan appears to influence the expression of genes involved in biofilm formation, leading to up- or downregulation in specific Iraqi isolates (A5 and A7).

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1. Introduction

Triclosan mainly inhibits the enoyl-acyl carrier protein reductase (FabI), which prevents the synthesis of fatty acids. It has a bacteriostatic effect at low concentrations and a bactericidal effect at higher quantities. Research indicates that triclosan exposure may increase efflux pump activity and promote antibiotic resistance, including in *S. aureus* (Wang et al., 2023). Notwithstanding the paucity of direct research on triclosan resistance in *S. aureus* from pregnant patients with UTIs, the mechanism upregulated efflux pumps, such as NorA and MepA is widely known in MRSA and other resistant strains. This highlights a tenable molecular explanation for uropathogenic *S. aureus* decreased triclosan susceptibility (Dashtbani-Roozbehani & Brown, 2021). The icaADBC operon, specifically the icaA and icaD components, is essential for *S. aureus* biofilm accumulation and polysaccharide intercellular adhesin (PIA) synthesis. Studies in different clinical settings (burns, wounds and UTIs) repeatedly demonstrate a high prevalence of icaA among biofilm-forming

CONTACT Ammar Adnan Tuama

isolates, between approximately 77% to nearly complete presence (François et al., 2023). Biofilm formation is clearly related to elevated antimicrobial resistance and persistence in UTI isolates. In all 69.2% of *S. aureus* UTI isolates that biofilm production was also positive for the presence of icaA, fnbA and clfA genes. A separate systematic review revealed that the icaA, clfA, and fnbpA genes were 100% present in UTI-derived S. aureus, with approximately 48% of them producing strong biofilms and 35% moderate biofilms (Del Fatti, 2023). Within the genus Staphylococcus, coagulase-negative staphylococci (CoNS) constitute a large and clinically relevant group. These are commonly found on human and animal skin, as well as mucous membranes. Hospital setting are conductive conditions for opportunistic CoNS infections, namely due to indwelling catheters or other medical devices. As CoNS can form biofilms on surfaces, knowing that during the insertion of clinical instruments, they spread from skin to normally sterile sites (Becker et al., 2020). *Staphylococcus aureus i*s a coccoid gram-positive microorganism that exists as a commensal on human skin surfaces and nasal passages. It is an opportunistic organism and causes a wide variety of infections, from superficial skin ulcers to severe bloodstream infections and toxic shock syndrome. It is a major worldwide health concern due to its rapid development of antibiotic resistance (Gor et al., 2019). Because of the ability of bacterium to form biofilm through the generation of polysaccharide matrix *S. aureus* could adhere to surfaces by using adhesins. When compared to their planktonic counterparts, bacterial cells in biofilms have shown to be more resistant to the environmental stressors, and antimicrobial agents (Torlak et al., 2017). The accessory gene regulator (Agr) system is an important part of QS as well. Agr systems can be found in Gram-positive bacteria, with *S. aureus* being a great example. *RNAII* and *RNAIII* are the two transcripts that make up the Agr locus. *RNAII* is transcribed into the *agr* operon, but *RNAIII* reduces the expression of surface adhesins while increasing the production of capsules, poisons, and proteases (Vinodhini & Kavitha, 2024). Because *agrA* activity increases the expression of many important virulence factors in *S. aureus*, inhibition of this system has caught the interest of researchers as a non-bactericidal, or "anti-virulence" strategy for preventing infections (Liu et al., 2024). Quorum sensing is a critical bacterial communication system that coordinates gene expression in response to increased cell density. This system is involved in controlling the formation of biofilm, a major virulence factor. The major quorum-sensing system in Staphylococcus species is the accessory gene regulator (agr) system, which comprises four key components: two signal transduction proteins, AgrA and AgrC; a precursor pheromone, AgrD; and a membrane-associated protein, AgrB, that processes and exports the peptide signal. Collectively, these components regulate the synthesis of virulence factors via transcriptional and translational control (Preda & Săndulescu, 2019). This study was designed to assess the influence of antiseptics on the expression of antiseptic resistance and quorum-sensing genes in Staphylococcus aureus, their effect on biofilm formation, as well as the correlation between MIC values for antiseptics and prevalence of antiseptic resistance genes.

2. Material and Method

2.1 *Staphylococcus aureus* Isolation and Identification

A total of 150 midstream urine samples were collected from pregnant women clinically diagnosed with urinary tract infections (UTIs) who attended Al-Batool Teaching Hospital in Baqubah, Diyala, Iraq, between January and March 2025. The samples were cultured on blood agar and mannitol salt agar (MSA) plates and incubated at 37°C for 24 hours. Colonies obtained from primary cultures were sub-cultured on brain heart infusion agar and re-inoculated on MSA to obtain pure bacterial strains, which were incubated at 37°C for 24 hours. Colonies displaying morphological characteristics of Staphylococcus aureus were further identified using Gram staining, catalase, coagulase, DNase, and mannitol fermentation tests. Final confirmation was performed using the Vitek 2 system (Al-Awadi et al., 2025).

2.2 Biofilm Before Treatment with Triclosan

In the microtiter biofilm assay, biofilms were grown on polystyrene well surfaces containing static media. This widely used, high-throughput model is suitable for assessing bacterial biofilm-forming ability and testing anti-biofilm compounds. The capacity of *S. aureus* to form biofilms on abiotic surfaces has been reported previously (Ahmed et al., 2025). The *S. aureus* strains were activated by growing in double-strength brain heart infusion (BHI) broth supplemented with 0.5% glucose at 37°C for 24 hours. In sterile 96-well U-shaped polystyrene microplates, 200 µL of an overnight BHI bacterial culture (adjusted to 0.5 McFarland standard) was dispensed per well. Plates were covered and incubated aerobically at 37°C for 24 hours. Each isolate was tested in triplicate, and bacteria-free BHI wells were included as controls.

To visualize biofilms, the contents of each well were aspirated, and wells were washed three times with 200 µL of distilled water. Biofilms were fixed with 200 µL of methanol for 15 minutes, air-dried, and then stained with 200 µL of 0.1% crystal violet solution at room temperature for 5 minutes. Wells were washed as before, air-dried at 37°C for 30 minutes, and destained with 200 µL of absolute

ethanol for 10 minutes. The ethanol containing the released stain was transferred to another microplate, and the optical density of each well was measured at 600 nm using a microplate reader.

2.3 Estimation of Triclosan Minimum Inhibitory Concentration (MIC)

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2015), the minimum inhibitory concentrations (MICs) of Triclosan were determined using a modified agar dilution method.

A. Mueller-Hinton agar was prepared at double strength, melted, and cooled to approximately 50°C.

B. Double serial concentrations of Triclosan (0.09–50%) were prepared in glass tubes and labeled. The 4.85% concentration in the antiseptic reagent bottle was considered 100%.

C. Equal volumes of the double-strength agar and Triclosan solution were mixed gently to achieve final concentrations ranging from 0.09% to 50%.

D. The mixture was poured into labeled, sterile Petri dishes.

E. After agar solidification, the plates were inverted and incubated overnight to confirm sterility.

2.4 Biofilm after Treatment with Triclosan

The *S. aureus* strains were activated by growing in double-strength brain heart infusion (BHI) broth supplemented with 0.5% glucose at 37°C for 24 hours. An aliquot of 200 µL of the overnight BHI bacterial culture (adjusted to 0.5 McFarland standard) was added to each well of a 24-well polystyrene microtiter plate Figure 1. To reach a final volume of 400 µL, 200 µL of Triclosan at the desired sub-MIC concentration for each isolate was added. Each isolate was tested in triplicate. Positive controls contained 400 µL of bacterial culture (0.5 McFarland) without Triclosan, while negative controls contained 400 µL of sterile BHI broth. The plates were incubated at 37°C for 24 hours to allow biofilm formation. Biofilms were subsequently assessed by optical density measurement and calculation of the cutoff value (Ricart et al., 2010).

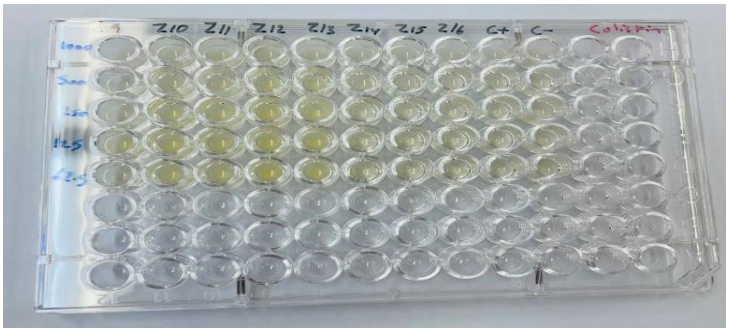


Figure 1: Preparation Biofilm after Treatment with Triclosan

2.5 DNA Extraction

Genomic DNA was extracted from confirmed *S. aureus* isolates using the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) according to the manufacturer's instructions. DNA quality and concentration were verified by spectrophotometry. genes, for DNA extraction used kit manufacturer's instructions (Geneaid, Korea), the total DNA genomic was extracted using a particular (+)ve bacteria (proteinase K) technique. Conventional PCR was employed to detect resistance genes (icaA) in Coagulase Negative Staphylococcus isolates (Intron, Korea) Table (1-2-3 and 4).

Electrophoresis was defining DNA bands after the extraction or to detect exposes the result of PCR through the attend of the standard DNA to differentiate the bundle size of PCR outcome on the Agarose gel

2.6 PCR Detection of the icaA Gene

The icaA gene was amplified using specific primers described by Kot, Sytykiewicz and Sprawka (2018)Kot et al. (2018)

Table 1: Primer of PCR Detection of the *icaA* Gene

Primer name	Sequence (5' – 3')	Amplicon size (bp)	Reference
*Ica*A	R- TACACATATGTTCTTCCCTAATAA	102	(Kot et al., 2018)
	F-CAATACTATTTCGGGTGTCTTCACTCT		
tpi	R-TTT GCA CCT TCT AAC AAT TGT AC	475	MLST Database

	F-TCG TTC ATT CTG AAC GTC GTG AA

Table 2: Mixture of PCR Worker Solution

Component	Concentration	Volume µl
Taq PCR PreMix	1X	5
Primer F.	10 pm/ µl	1
Primer R.	10 pm/ µl	1
DNA Template		1.5
Deionized D.W.	-	16.5
Total volume		25

Table 3: Mixture of PCR Worker Solution

Component	Concentration	Volume µl
Taq PCR PreMix	1X	5
Primer F.	10 pm/ µl	1
Primer R.	10 pm/ µl	1
DNA Template		1.5
Deionized D.W.	-	16.5
Total volume		25

Table 4: PCR Program for Amplification of icaA Resistance Gene by Thermal Cycler

No.	Steps	Temperature	Time	No. of Cycles
1	Initial denaturation	94˚C	5 min.	1
2	Denaturation	94˚C	30 sec.	
3	Annealing	56˚C	30 sec.	35
4	Elongation	72 ˚C	30 sec.	
5	Final elongation	72 ˚C	7 minutes	1

2.7 Gene Expression for Biofilm Produced by *S. aureus* Before and after Triclosan Treatment

Two isolates no. (5 and 7) have four of the five investigated genes in this study; that evaluated for gene expression by qPCR. biofilm of previous two *S. aureus* isolates was assayed and each isolate tested before and after treatment with Triclosan Bacterial cells were pelleted from 1.4 ml of cell culture by centrifugation for 2 min at 13000 rpm and the supernatant was discarded. Thereafter, TRIzol™ Reagent (0.75 ml) was added per 0.25 ml of bacterial suspension (1 × CFU/ml).

2.7.1 qPCR Protocol

The PCR protocol was carried out as described in Table 2-10, following the method of Lewis and Rice (2015). Real-time PCR was performed using the MicqPCR Cycler. Dissociation curves were generated to ensure the specificity and efficiency of the reactions. As a control, cDNA reactions without reverse transcriptase were routinely included to confirm the absence of genomic DNA contamination.

2.7.2 Detection of *icaA Gene* Expression

The gene expression analysis using livak method performed to find out how much the expression levels were raised; relative quantitation was used. The difference in the threshold for cycling (¦CT) was also calculated, and the expression levels changed between the treated groups and their controls were written down (Rahman et al., 2018). The expression of the fbp gene was employed as a housekeeping gene to demonstrate the results in a typical manner, as illustrated below: The concentration of RNA following conversion to cDNA determines the analysis and computation of gene expression levels for one or more genes. We achieved these results by estimating the expression of genes treated with SeNPs using the Real-Time PCR technique. The diagram displays the reaction setup (Livak & Schmittgen, 2001).

2.8 Statistical Analysis

Data was analyzed using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). The association between biofilm production categories and the presence of the icaA gene was evaluated using the Chi-square (χ^2) test. A p-value < 0.05 was considered statistically significant.

3. Result

3.1 Isolation and Identification of *Staphylococcus aureus*

S. aureus isolates were collected from patients attending hospitals, private clinics, and laboratories in Diyala, Iraq. Samples were cultured on blood agar and mannitol salt agar (MSA) for preliminary identification. Colonies exhibiting a clear, translucent zone on blood agar indicated beta-hemolysis due to complete red blood cell lysis. On MSA, *S. aureus* ferments mannitol, producing acid that changes the phenol red indicator from red to yellow. Of 150 urine samples collected from pregnant women with clinically confirmed UTIs, 136 (90.7%) yielded bacterial growth, and 93 isolates (68.4%) were identified as *S. aureus* based on colony morphology on MSA (smooth, creamy, yellow-pigmented), Gram staining (Gram-positive cocci in clusters), positive catalase and coagulase reactions, DNase positivity, and mannitol fermentation. Fig. (2) and Table 5.

Table 5: Biochemical and Microscopical Properties of *S. aureus*

Biochemical and Microscopical Properties of *S. aureus* Id	Test	Results
1	Hemolysin production	100% β- hemolysis of red Blood cells
1	Mannitol fermentation	100% positive with yellow colonies
2	Gram stain	100% positive cocci
3	Catalase	100% positive
4	Oxidase	100% negative
5	Coagulase	100% positive
6	Acetoin production	100% positive



(a) (b)

Figure 2: *Staphylococcus aureus* Isolates: a) Blood Agar, b) Mannitol Agar

3.2 Biofilm Formation Assay

The microtiter plate assay revealed that all *S. aureus* isolates produced biofilms of varying intensities. Based on OD570 measurements, 44 isolates (47.31%) were strong biofilm producers, 44 isolates (47.31%) were moderate producers, and 5 isolates (5.38%) were weak producers. No non-biofilm-producing isolates were detected. The majority of isolates, 88 (94.62%) demonstrated moderate to strong biofilm formation, suggesting a high prevalence of biofilm-associated virulence among UTI-associated *S. aureus* strains. Fig (3) and Table 6.

Table 6: Biochemical and Microscopical Properties of *S. aureus*

Biofilm Formation Category	Number of Isolates (n)	Percentage (%)
Strong	44	47.31%
Moderate	44	47.31%
Weak	5	5.38%
None	0	0.0 %



Figure 3: Classification of *S. aureus* Isolates into Biofilm-Forming Capacity (Strong, Intermediate, Weak)

3.3 Triclosan Minimum Inhibitory Concentration Against *S. aureus* Isolates

The Dettol solution with 4.85% triclosan was used in this study. The antibacterial activity was determined using the microdilution method. Triclosan was found to inhibit *S. aureus* isolates at different concentrations. Results: The MIC values were 0.303 for one isolate, 0.152 (14 isolates), 0.076 (10 isolates), and 0.038 (19 isolates). The varying strains of *S. aureus* and the 3rdMICs of triclosan reflect this in the current study. Antiseptics are generally assessed for their in vivo effectiveness based on activity against planktonic bacteria. However, it is widely known that bacteria in biofilms possess considerably higher resistance to them than their planktonic counterparts, making these infections notoriously difficult to clear. Staphylococcus aureus is a well-known strong biofilm former and can form excellent biofilms on biotic and abiotic surfaces. Biofilm formation was inhibited in most *S. aureus* isolates when exposed to triclosan. A total of 47.31% were categorized as strong biofilm producers before treatment. However, 2 (2.4%) of the isolates sustained a capacity for strong biofilm formation following triclosan treatment. Conversely, most of the isolates showed a decreased ability to form biofilms: 47.31% turned into moderate biofilm-formers, 5.38% produced weak biofilms and 4 isolates (4.8%) were unable to produce biofilms at all.

3.4 Molecular Study

3.4.1 Detection of the icaA Gene

DNA was extracted using the ABIOpure™ Total DNA Kit, and its concentration was measured with a Quantus Fluorometer. The concentrations ranged between 12–20 ng/µl, with purity values between 1.88 and 1.99. A ratio of 1.8–2.0 is generally accepted as "pure" DNA. Gel electrophoresis was subsequently performed to confirm DNA integrity, and the results showed single, distinct bands, indicating intact DNA. PCR analysis of 13 representative *S. aureus* isolates revealed that 11 (84.6%) carried the icaA gene, producing the expected amplicon size of 102 bp, while 2 isolates (15.4%) were negative for the gene. The presence of icaA showed a strong correlation with strong and moderate biofilm formation Fig (4). There was a clear association between the presence of the icaA gene and the biofilm-forming capacity, with the strongest biofilm producers carrying the icaA gene.

Table 7: icaA gene–biofilm Association

Biofilm Strength	*ica*A Positive (n)	*ica*A Negative (n)
Strong	7(50%)	0 (0%)
Moderate	5(35%)	0(0%)
Weak	0(0%)	2(15%)
Total	12(85%)	2(15%)

Statistical Analysis Confirmed a Significant Association between biofilm Strength and icaA Gene Presence (χ^2 test, $p < 0.05$).

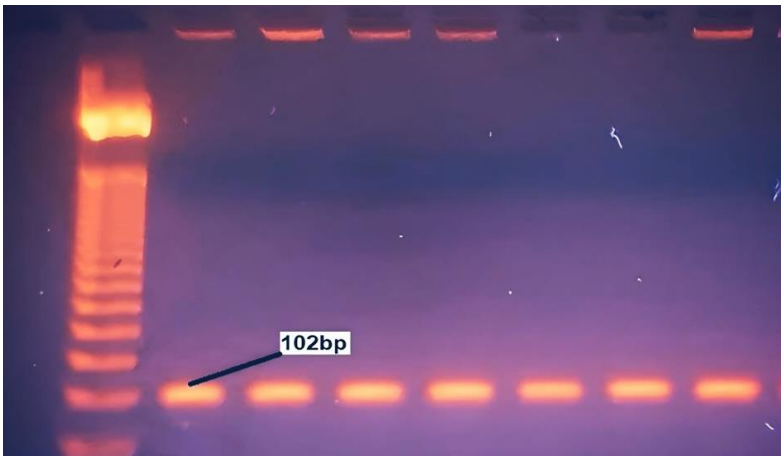


Figure 4: Amplification of icaA Genes of Bacterial Species were Fractionated on 1.5% Agarose Gel Electrophoresis Stained with Eth. Br. M: 100bp Ladder Marker (A). Lanes A1-A13 Resemble 102 bp PCR Products and the Negative Control is N. C.

3.4.2 Gene Expression

Using PCR, the genes icaA was present in all" of the isolates that had a band that could be evaluated at the concentration. These strains were demonstrated to have a significant amount of biofilm formation. The RT-qPCR method was employed "to investigate the expression of the *icaA genes*" in two *S. aureus* strains. When compared to the typical gene expression in bacteria, this methodology demonstrates "a significant decrease in expression and an increase in the expression of the *ica*A genes" as a result of the exposure. Triclosan. Two isolates (code 5 and 7) out of 25 isolates were chosen because each isolate harboured the previous three genes, and all these isolates were treated with Triclosan at sub-MIC (Table 8).

Table 8: Triclosan Sub-MIC of Selected Isolates in the Current Study

S. aureus Isolate	Sub-MIC* %
5	0.075
7	0.303

3.4.3 icaA genes

Selenium nanoparticles affected the *icaA gene* expression variably (Table 9). A down-regulation in gene expression was noticed in isolates A5 and A7.

Table 9: *icaA* Gene Expression Changes Before and After Treatment with Triclosan

Sample	Tpi	icaA	dct	duct	Folding
A5 (after)	15.089	21.130	6.042	0.000	1.000
A5 (before)	11.651	22.142	10.490	4.449	0.046
A7 (after)	16.705	20.078	3.373	0.000	1.000
A7 (before)	12.794	24.087	11.293	7.920	0.004

4. Discussion

Urinary tract infections (UTIs) are among the most common infections affecting women at different stages of life. This study aimed to identify the uropathogens responsible for UTIs in women and to evaluate their antibiotic susceptibility patterns. A high prevalence of Staphylococcus aureus was detected among UTI cases in pregnant women, with 68.4% of isolates exhibiting classical morphological and biochemical characteristics. All isolates demonstrated biofilm production, with the majority classified as strong or moderate producers, findings that are consistent with previous reports from Iraq (Farhood & Hamim, 2025). Coagulase-negative staphylococci (CoNS) represent a subgroup of staphylococci that can be differentiated from *Staphylococcus aureus* and *Staphylococcus intermedius* by their inability to produce coagulase. Since coagulase secretion is a major virulence factor contributing to the pathogenesis and persistence of staphylococcal infections, it has long served as a key criterion for distinguishing *S. aureus* from other staphylococcal species (De Buck et al., 2021). Staphylococci were classified into two groups: *Staphylococcus aureus*, regarded as the more pathogenic

species and considered a 'major pathogen,' and all other staphylococci, collectively designated as 'minor pathogens' and termed coagulase-negative staphylococci (CoNS) (Wang et al., 2023). More recently, an alternative classification has been proposed in which all staphylococcal species other than *S. aureus* are grouped under the term non-*aureus* staphylococci (NAS) (Okada et al., 2000).

Biofilms are complex biological matrices composed of aggregated bacterial communities embedded within secreted exopolysaccharides (EPS), proteins, lipids, surfactants, extracellular DNA, and water. In staphylococci, biofilm production is primarily regulated by the staphylococcal accessory regulator (*sarA*), which influences major pathways and genes involved in biofilm formation. These include the *icaABCD* genes located within the intracellular adhesion (*ica*) operon, whose product is polysaccharide intercellular adhesin (PIA; also known as poly-β-1,6-N-acetylglucosamine [PNAG]); the accessory gene regulator (*agr*) pathway; the biofilm-associated protein (Bap, encoded by the *bap* gene); and fibronectin-binding protein (FnbA, encoded by the *fnbA* gene). Bap is thought to play a critical role in the initial attachment of bacteria to biotic or abiotic surfaces, whereas PIA and FnbA are more relevant during the accumulation and maturation phases of biofilm development (Leroy et al., 2021). "One possible explanation may be the induction of biofilm production by sublethal concentrations of antibiotics. This phenomenon has been particularly described for the MLS group of antibiotics and rifampicin (RIF), but not for β-lactams, vancomycin, aminoglycosides, or tetracycline derivatives. For example, Lima-e-Silva et al. demonstrated that sublethal concentrations of rifampicin (25% and 50% of the MIC) strongly induced biofilm formation in S. aureus, a response attributed to gene expression changes in biofilm-associated genes (Rodríguez-López et al., 2020). The present study demonstrated an association between the *icaA* gene and biofilm activity. Biofilm production was observed in 53.8% of isolates carrying the *icaA* gene and in 76.5% of *icaA*-negative isolates. These findings are in agreement with a study conducted in France (Zhao et al., 2020), which reported that 60.5% of *S. epidermidis* isolates were *icaA*-positive and produced biofilm. Similarly, concordant results were observed in India (Gajdács et al., 2021), where 41.8% of *S. epidermidis* isolates were *icaA*-positive and biofilm-producing, as well as in Germany (Azizi et al., 2016), which also confirmed this association. A subgroup of Staphylococcus aureus isolates in this investigation carried the icaA gene and exhibited both triclosan resistance and biofilm production. Evidence of bacterial tolerance and cross-resistance to a wide range of important antibiotics has surmounted global concerns over triclosan, a broad-spectrum biocide commonly used in medical devices and personal care products (Yazdankhah et al., 2006). Our findings are in line with previous studies showing that *S. aureus* is able to modify its gene expression and enhance its survival under sublethal concentrations of triclosan (Carey & McNamara, 2015). Formation of biofilms is crucial for *S. aureus* to withstand environmental and antimicrobial stressors. N-acetylglucosaminyltransferase, a protein encoded by the icaA gene that belongs to the icaADBC operon, is responsible for synthesising polysaccharide intercellular adhesin (PIA) and maturing biofilms (Arciola et al., 2012). Both icaA-positive and icaA-negative strains of our isolates, presented biofilm activity, indicating that even though the icaA contributes to biofilm formation, other mechanisms, such as the sarA and agr regulators, are very important for biofilm in these strains, besides fibronectin-binding proteins (fnbA) and biofilm-associated protein (bap) (Wang et al., 2025). Notably, a much higher proportion of isolates that were negative for icaA also exhibited indications of biofilm production. This is consistent with research that indicates biofilm formation might be mediated by proteinaceous and extracellular DNA-dependent pathways even when the ica operon is not present. This demonstrates the complexity of staphylococcal biofilm formation and the drawbacks of relying solely on icaA as a biomarker for biofilm potential (Liu et al., 2020). Numerous research have highlighted the connection between biofilm formation and triclosan resistance. It has been demonstrated that triclosan at sublethal concentrations causes *S. aureus* to develop biofilms by upregulating genes linked to biofilms as a result of stress reactions (Wang et al., 2023). Such adaptive reactions could make eradication efforts more difficult by increasing bacterial persistence in clinical settings. Additionally, the co-occurrence of biofilm formation and triclosan resistance raises concerns for infections linked to medical devices and urinary tract infections, where biofilm-mediated protection dramatically lowers the effectiveness of antibiotics (Li et al., 2019). When combined, our findings lend credence to the mounting evidence that *S. aureus* uses both biofilm formation and triclosan resistance as associated adaptation mechanisms. This emphasizes the need for more molecular research to identify the mechanisms relating triclosan tolerance to biofilm-associated virulence, as well as for sensible regulation of triclosan use in consumer goods and healthcare.

5. Conclusion

According to this study, Staphylococcus aureus plays a major role in causing UTIs in expectant mothers and is capable of developing resistance to antibiotics. Although triclosan demonstrated antibacterial action against *S. aureus* isolates, strain-to-strain variability in MIC values suggested varying susceptibilities. After being exposed to Triclosan, the majority of isolates showed a significant decrease in their capacity to form biofilms, indicating that the antiseptic may hinder biofilm formation at sub-MIC levels. Its significance as a major predictor of biofilm-related pathogenicity was confirmed by molecular analysis, which showed a high prevalence of the icaA gene and a strong correlation with strong and moderate biofilm development. Overall, the results highlight the clinical importance of

tracking biofilm-associated genes and antiseptic resistance in *S. aureus* since these variables affect infection duration and treatment difficulties. In order to improve infection prevention in clinical settings, future research should concentrate on investigating alternative or combined therapeutic approaches to overcome antiseptic resistance and interfere with biofilm formation.

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