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Original Article

Genotypic and Phenotypic Characterization of *Staphylococcus aureus* in Recurrent Tonsillitis Patients and Molecular Detection of Resistance Gene (*mecA*) and Biofilm Formation Gene (*icA*) in Sulaimani Province

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) demonstrates resistance to several frequently used antibiotics. Recurrent tonsillitis (RT) is a common inflammation of the palatine tonsils, typically induced by bacterial infection. This study aimed to identify MRSA in patients with recurrent tonsillitis by utilizing both phenotypic and genotypic techniques, including molecular detection of 16S rRNA and the mecA resistance gene, and biofilm formation screening by a 96-well microtiter plate test with detection of icaA and icaD genes. A total of 69 superficial throat swabs were collected from individuals diagnosed with recurrent tonsillitis infections. Among them, 36 (52.17%) tested positive for S. aureus, including 14 (38.88%) MRSA and 22 (61.11%) methicillin-sensitive S. aureus (MSSA). Fifty percent of MRSA isolates were multidrug-resistant (MDR), and 72.72% of MSSA isolates also had MDR characteristics. All isolates exhibited complete resistance to penicillin (100%) while demonstrating full susceptibility to vancomycin (100%). Most MRSA isolates (92.85%) showed susceptibility to ciprofloxacin, except for one strain (P11), whereas only 45.45% of MSSA isolates demonstrated susceptibility. Biofilm generation was observed in 97.22% of isolates, signifying a robust ability for persistence and antibiotic resistance. These data underscore the necessity of employing culture-guided antibiotic treatment for recurrent tonsillitis to guarantee suitable antimicrobial selection. Fluoroquinolones had significant efficacy against S. aureus isolates, indicating their potential use in the culture-based treatment of recurrent tonsillitis. The significant occurrence of biofilm-forming multidrug-resistant strains highlights the necessity for biofilm-targeting techniques to improve treatment efficacy and diminish infection recurrence.

1. Introduction

Staphylococcus aureus is one of the most prevalent and pathogenic bacteria in the world that can be found in different parts of the body, such as the skin, nose, and oral cavity. Methicillin-resistant Staphylococcus aureus is a highly pathogenic strain that is resistant to most of the antibiotics available (Elkady et al., 2022; Ferreira et al., 2021). Methicillin-resistant S. aureus strains have obtained the mecA gene, which encodes for a modified penicillin-binding protein, leading to their global spread. This modified protein exhibits a decreased propensity for binding to all β-lactams, hence reducing its therapeutic efficacy in managing hospital- and community-acquired MRSA infections

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(Elkady et al., 2022; Ferreira et al., 2021). In MRSA, resistance linked to the production of the new PBP2a protein is of paramount significance. Genes that encode supplementary penicillin-binding proteins (PBPs) are situated within the SCCmec chromosomal cassettes and are disseminated by conjugation or transduction (Mlynarczyk-Bonikowska et al., 2022). Strains exhibiting PBP2a demonstrate resistance to all therapeutic beta-lactam antibiotics, except for ceftobiprole and ceftaroline.

Tonsillitis is the inflammation of the tonsils that happens quickly. Tonsillitis can occur as a result of reactions to viruses, germs, allergens, and respiratory conditions. (O'Handley et al., 2020). Recurrent tonsillitis (RT) is the recurring inflammation of the palatine tonsils, which is mostly or only caused by a bacterial infection. S. aureus is one of the most prevalent bacteria that causes RT because it is resistant to medications and can live in the tissues inside the tonsils (Buname et al., 2021). RT is characterized by repeated episodes, during which the patient exhibits symptoms including fever, odynophagia, sore throat, cervical lymphadenopathy, and congested tonsils (Klagisa et al., 2022). One reason the illness keeps coming back is that the antibiotics weren't given properly. According to Kardooni et al. (2020), tonsillectomy is the final option for this issue. Antimicrobial resistance in S. aureus has been increasing due to the extensive use of antibiotics, making infections by this bacterium increasingly hazardous and lethal in recent times. Infections by antibiotic-resistant strains were pricier and more likely to kill people than infections from strains that were sensitive to antibiotics (Abdulrahim et al., 2019). S. aureus infections can start in the mouth and nose and then move to other areas of the body, where they can cause systemic infections.

Many researchers assert that S. aureus is the principal etiological agent of RT. Still, it is not clear how this infection causes RT to get worse, abscesses to form, and resistance to antibiotic treatment. Since S. aureus does not show significant resistance to antibiotics in RT, it is crucial to investigate alternative protective mechanisms, such as biofilm formation. Biofilm development is thought to be associated with antibiotic resistance (Klagisa et al., 2022). Using scanning electron microscopy, researchers discovered biofilms in 80% (16/20) of patients with recurrent tonsillitis and in 45% (9/20) of healthy controls. Patients with RT had a higher prevalence of biofilms, indicating a potential association between the two illnesses (Klagisa et al., 2022).

Biofilm production is essential for bacterial survival and antibiotic resistance, recognized as a principal virulence factor in severe chronic diseases (Suresh et al., 2019). To enhance our comprehension of the molecular pathogenesis of S. aureus, it is essential to examine not just the bacterium's antibiotic resistance but also its capacity to create biofilm, a multi-layered cellular matrix that is both architecturally intricate and dynamic. This opens new ways to treat and stop the disease. Extracellular toxins, surface characteristics, and biofilm creation are just a few of the many virulence factors that help infections start and stay in hosts (Abdulrahim et al., 2019). Biofilm production is crucial for MRSA survival and persistence, as it shields bacteria from the host immune response and ultimately leads to treatment resistance. It is widely recognized as the principal microbiological factor responsible for chronic infections. (Cavalcanti et al., 2019; Kostić et al., 2022). Due to their cryptic structure and frequent direct exposure to respiratory bacterial pathogens, tonsillar and adenoidal tissues are at risk for biofilm formation (Kostić et al., 2022).

A prior investigation revealed that S. aureus and Streptococcus pyogenes can establish biofilms in the palatine tonsils of children, influencing local immune cells in unique ways. A significant decrease in dendritic cells (CD1a) and neutrophils (CD15) was noted, hindering phagocytosis, antigen processing, and presentation. These results corroborate the correlation between biofilm production and the

chronicity of tonsillar infections (Hussein & Rasheed, 2025).

Despite the acknowledged importance of S. aureus in RT infection and its capacity for biofilm formation, there is a paucity of evidence from Iraq—especially from the Sulaymaniyah region—concerning the genetic correlation between antibiotic resistance and biofilm production. Limited research has concurrently investigated the mecA and ica genes in clinical isolates from RT patients.

This study aimed to identify S. aureus in patients with recurrent tonsillitis through both phenotypic and genotypic methods, including the detection of the mecA resistance gene and biofilm-associated genes (icaA and icaD), to enhance understanding of their association with antimicrobial resistance and biofilm formation in the local population.

2. Material and Methods

2.1. Ethics Approval

Approval was obtained from the Ethics Committee of the College of Science/University of Sulaymaniyah (NO.24, 21/09/2025).

2.2. Inclusion criteria

Patients of all age groups, both male and female, with recurrent tonsillitis were included in this study.

2.3. Exclusion criteria

Cases other than recurrent tonsillitis, such as chronic tonsillitis, acute tonsillitis, tonsillar hypertrophy, and obstructive sleep apnea, were excluded from this study.

2.4. Statistical Analysis

Statistical analysis was conducted utilizing GraphPad Prism version 10.4.1 (GraphPad Software, San Diego, CA, USA). An exact binomial test was utilized to determine if the proportion of S. aureus-positive samples substantially deviated from the hypothesized prevalence value. The chi-square test was utilized to assess the relationships between age groups and S. aureus positive, gender and MRSA/MSSA distribution, and age groups and MRSA/MSSA strains, Chi-square test also utilized to assess the relationships between antibiotic resistance and MSSA/MRSA strains, A p-value of less than 0.05 was deemed statistically significant.

2.5. Sample collection and processing

A total of 69 superficial swabs were taken from recurrent tonsillitis patients. All cases were diagnosed as (RT) by an (ENT) physician in Baxshin private hospital; the collection date began in November 2024 and ended in February 2025, and one swab was taken from each patient. When the swab was taken from tonsillitis patients, the question was a requested form patent, which included some history and information such as age, sex, location of residence, and empirical treatment. The samples were processed at both the microbiology laboratory department of the same hospital and the Advanced Research Laboratory of Microbiology and Molecular Biology in the Biology Department at the University of Sulaymaniyah. Swabs were cultured on blood agar and mannitol salt agar (Liofilchem Diagnostic, Italy) for 18 hours at 37°C (Rajeswarie et al., 2022). Antibiotic susceptibility testing was conducted using the Kirby–Bauer method on Mueller–Hinton agar (Liofilchem Diagnostics, Italy). The bacterial inoculum was calibrated to a 0.5 McFarland standard, and the plates were incubated at 35 ± 2 °C for 18 hours, in accordance with the Clinical and Laboratory Standards Institute((CLSI), 2024). Gram staining and coagulase tests, including both the slide and tube methods, were done for all samples (Vorobieva et al., 2008).

2.6. Detection of MRSA strains

All isolates (36) of S. aureus were subjected to the VITEK2 COMPACT System by preparing (0.5) McFarland and the disc diffusion method using a cefoxitin disc (30µg) and culturing on Muller Hinton agar (Liofilchem Diagnostic-Italy). CLSI guidelines were used for the interpretation of results accordingly((CLSI), 2024).

2.7. DNA extraction of isolates

DNA was extracted from all 36 clinical isolates using the Bacteria Genomic DNA Extraction Kit (Gene Sand-China), and the Nano-Drop 2000 spectrophotometer (Thermo-Scientific, USA) was utilized to evaluate the concentration and purity of nucleic acid.

2.8. Molecular confirmation of S. aureus

A set of primers (Sina clone, Iran) was used against 16S r RNA gene by PCR (Applied Biosystems, USA). PCR reactions were carried out in a final volume of (20µl): 10µl of Add Taq Master (2x Conc., KOREA), 1 µl of each primer (10pmol), 6µl of nuclease-free water and 2µl of DNA template were utilized. PCR conditions and primer sequences are summarized in Table 2. The PCR products were stained with ethidium bromide and analyzed using 1.5% agarose gel, 5µl of the product was run through electrophoresis at 80 volts for one hour. A DNA ladder of 100 bp was utilized for verification of the product and visualized using the Bio-Rad Gel Doc XR+ imaging system (Bio-Rad Laboratories, USA) (Lee et al., 2012).

2.9. Genotypic detection of MRSA

A set of primers (Sina clone, Iran) specific for the mecA gene was utilized by PCR, 10µl of Add Taq Master (2x Conc. KOREA) was used in a final volume of 20µl, 1µl of each primer, 2 µl of DNA template, 6µl of nuclease-free water. Details of PCR conditions are illustrated in Table 2. PCR products were stained with ethidium bromide and analyzed using 1.5% agarose gel, 5µl of PCR product was run through electrophoresis at 80 volts for 1 hour.

2.10. PCR detection of icaA and icaD genes

Two sets of primers (Sina clone, Iran) were utilized targeting intercellular adhesion protein A and intercellular adhesion protein D using PCR (Applied Biosystem, USA). The final reaction volume was 20µl, 2µl of each primer was utilized, 10 µl of Add Taq Master (2x Conc. KOREA), 2 µl of DNA template, and 6µl of nuclease-free water. The details of the PCR reaction conditions are mentioned in Table 5. 1.5% of agarose was used and the PCR product was run through electrophoresis at 90 volts for 1 hour.

2.11. Biofilm determination using Crystal Violet Assay

A slightly modified version of the microtiter biofilm formation test using 96 flat-bottom wells was carried out, with thirty-six isolates tested following the methodology suggested by (Stepanović et al., 2007).

2.11.1. Inoculum preparation

The bacteria were cultured in Tryptic Soy Broth at 37 OC for 18 hours. After that, the suspension was adjusted to 0.5 McFarland Standard and then diluted 1:100 with new sterile TSB, which was made by mixing 30µl of adjusted growth culture with 3 ml of TSB. 200 microliters of each tested isolate's diluted growth culture were added to the designated wells of a sterile, flat-bottomed 96-well polystyrene microtiter plate. Only TSB was used as a sterility and negative control in the last row of wells, which were left uninoculated. The plates were placed in an incubator set at 37O C with no shaking for 24 hours after being covered with their lids.

2.11.2. Biofilm Fixation and Staining

Using a microtiter plate reader set to OD600, the plate was examined after incubation to ensure sterility in the uninoculated wells and to verify that all isolates had grown at the same rate in the biofilm wells. Afterwards, 250 µl of 1X Phosphate-Buffered Saline (PBS) PH 7.2 was gently pipetted twice into the wells to wash them, and the medium and planktonic cells were aspirated. Once the biofilms had been fixed for 60 minutes in an incubator at 80 OC, they were cooled to room temperature. To stain them, 200 microliters of a 0.1% (W/V) crystal violet solution was carefully added to each well, including the negative control wells, without causing any splashing. The plate was left for 20 minutes before being carefully immersed in D.W. multiple times to remove the unbound crystal violet. After the final washing, the washing solution should have been clear. The water was drained by tapping onto paper

Table 2. Shows the targeted genes, primer sequences, conditions, and product size.

Name of Gene	Name of Primer	Primer Sequences (5' -------- › 3')	Primer conditions	Amplicon Size (bp)	Reference
16S rRNA	**16S F**	AACTCTGTTATTAGGGAAGAACA	Denaturation 95°C 5min, Annealing 56°C 45 s, No. of cycles 35, Final extension 72°C 5 min.	756	(Naji & Arif, 2024)
	16S R	CCACCTTCCTCCGGTTTGTCACC			
Methicillin Resistant	***mec*A F**	CCTAGTAAAGCTCCGGAA	Denaturation 95°C 5min, Annealing 55°C 30 s, No. of cycles 35, Final extension 72°C 7 min.	310	(Naji & Arif, 2024)
	***mec*A R**	CTAGTCCATTCGGTCCA			
Intercellular adhesion protein A	***icaA* F**	CCTAACTAACGAAAGGTAG	Denaturation 95°C 5min, Annealing 56°C 30 s, No. of cycles 35 cycles, Final extension 72°C 5min.	1351	(Khoramrooz *et al.*, 2016)
	***icaA* R**	AAGATATAGCGATAAGTGC			
Intercellular adhesion protein D	***icaD* F**	AAACGTAAGAGAGGTGG	Denaturation 95°C 5min, Annealing 49°C 30 s, No. of cycles 35 cycles, Final extension 72°C 5min.	381	(Khoramrooz *et al.*, 2016)
	***icaD* R**	GGCAATATGATCAAGATA			

towels, and the plate was left inverted until the wells dried and were free of water.

2.11.3. Spectrophotometry biofilm mass quantification

After adding 200 µl of a decolorizing solution (80:20% absolute ethanol to acetone) (Tomaras et al., 2003) to the biofilm-bound stain, the plate was covered with a lid to prevent evaporation. It was then left at room temperature for 30 minutes. The plates were then read at optical density (OD) 570 using a Multiskan EX plate reader from Thermo-Fisher Scientific. This assay was done in three wells for each sample and replicated at least three times.

2.11.4. Biofilm Assessment

A cut-off value (ODc) was established at three standard deviations (SD) above the mean OD of the negative control to evaluate a biofilm formation for different strains relative to the mean (average) of triplicate OD readings, since each assay was performed in triplicate (Stepanović et al., 2007).

$$ODc = Average\ OD\ of\ Negative\ Control + (3 \times OD\ of\ Negative\ Control).$$

After subtracting the ODc value from the average OD value, the final OD value of each tested isolate was reported.

$$OD = Average\ OD\ of\ a\ strain - ODc$$

and individual ODc values were determined for each microtiter plate. Any positive value indicated biofilm production, while negative values were presented as zero.

In accordance with the conventional equations, they were signed as follows:

Table 1. Classifying isolates according to their biofilm-forming potential as measured by optical density (OD)

Categories		
No biofilm producer	-	$OD \leq ODc$
Weak biofilm producer	+	$ODc < OD \leq 2 \times ODc$
Moderate biofilm producer	++	$2 \times ODc < OD \leq 4 \times ODc$
Strong biofilm producer	+++	$OD \geq 4 \times ODc$

Table 3. Social demographic and clinical data for 36 studied isolates (patients).

Variable	Total (n=36)		Range	Percentage %
		Age		
Small Age	**24**		**(3-11)**	**66.66**
Adolescence	**3**		**(12-17)**	**8.33**
Adult	**9**		**(23-75)**	**25**
		Sex		
Male	**18**		**(3-55)**	**50**
Female	**18**		**(4-75)**	**50**
		Place of Residence		
Urban	**36**		**(3-75)**	**100**
Rural	**0**		**-**	**0.0**

3. Results

A total of 69 superficial throat swabs were obtained from patients diagnosed with recurrent tonsillitis at the ENT clinic. Among the samples, 36 (52.17%) tested positive for *Staphylococcus aureus*. The measured positive rate was significantly higher than the expected prevalence of 25% (exact binomial test, $p = 1.87 \times 10^{-6}$; 95% CI = 39.8–64.4%). The proportion of male and female patients was the same, each constituting 50% of the total. Most patients were children aged 3 to 11 years (66.66%), whereas teenagers were the lowest demographic (8.33%). Statistical analysis for 69 patients employing the chi-square test indicated no significant correlation between age group and *S. aureus* isolation (p = 0.602); also, statistical analysis found no significant correlation between age group and MRSA and MSSA strains (p = 0.889); moreover, MRSA and MSSA were equally distributed among genders and statistical analysis shows no significance (p = >0.999). Table 3.

Out of 36 isolates, 14 isolates (38.88%) were identified as MRSA isolates, as they show resistance to the cefoxitin test; 22 isolates (61.1%) were identified as MSSA isolates; all strains (100%) were susceptible to vancomycin except one strain of MSSA (P49), which was intermediate for vancomycin; all 36 isolates (100%) are resistant to penicillin; only 3 isolates (8.0%) were resistant to trimethoprim/sulfamethoxazole; and furthermore, 69.44% of isolates were resistant to erythromycin. Surprisingly, all MRSA isolates (92.85%) were susceptible to ciprofloxacin except one isolate (P11), which was resistant. On the other hand, only 45.45% of MSSA isolates were susceptible to ciprofloxacin (Table 4).

Table 4. Antibiotic resistance among both MSSA and MRSA isolates.

Antibiotics	MSSA N=22	MRSA N=14	Total	P-value
Penicillin	22 (100%)	14 (100%)	36 (100%)	>0.999
Cefoxitin (30 µg)	-	14 (100%)	14 (38.88)	0.0001****
Erythromycin (15 µg)	17 (77.27%)	8 (57%)	25 (69.44%)	0.147
Clindamycin (2 µg)	15 (68.18)	7 (50%)	22 (61.11%)	0.273
Teicoplanin (30 µg)	4 (18.18%)	3 (21.42%)	7 (19.44%)	0.999
Vancomycin (30 µg)	0.0	0.0	0.0	-
Ciprofloxacin (30 µg)	12 (54.54%)	1 (7.14%)	13 (36.11%)	0.0048**
Tetracycline (30 µg)	13 (59%)	0.0	13 (36.11%)	0.0003***
Trimethoprim/Sulfamethoxazole (1.25 µg/ 23.7 µg)	2 (9%)	0.0	2 (5.5%)	0.511

Table 5. Screening of the isolates for biofilm formation by microtiter plate assay.

Patient ID	(OD) of Strains	Categories	Patient ID	(OD) of strains	Categories
P3	0.180	Non-Biofilm	P50	0.376	Weak
P4	0.485	Moderate	P51	0.406	Moderate
P7	0.471	Moderate	P54	0.276	Weak
P8	0.354	Weak	P55	0.427	Moderate
P10	0.469	Moderate	P56	0.624	Moderate
P11	0.359	Weak	P58	0.300	Weak
P13	0.303	Weak	P59	0.418	Moderate
P14	0.462	Moderate	P60	0.707	Moderate
P17	0.325	Weak	P62	1.023	Strong
P18	0.343	Weak	P64	0.590	Moderate
P21	0.268	Weak	P67	0.474	Moderate
P27	0.440	Moderate	P68	0.609	Moderate
P32	0.665	Moderate	P69	0.401	Moderate
P34	0.292	Weak			
P35	0.347	Weak			
P37	0.410	Moderate			
P39	0.662	Moderate			
P40	0.896	Strong			
P41	0.417	Moderate			
P43	0.271	Weak			
P45	0.495	Moderate			
P47	0.450	Moderate			
P49	0.288	Weak			

*Strong: OD570 (>0.240), Moderate: OD570 (0.120-0.240), Weak: OD570 < 0.120

Biofilm formation was assessed using the 96-well microtiter plate method with crystal violet staining, as previously described by (Stepanović et al., 2007). The experiment showed that 97.22% of the isolates could form biofilms, and only strain P3 was found to be a non-biofilm producer. 55.55% of the biofilm-positive isolates were identified as moderate biofilm producers, 36% as weak biofilm producers, and only two bacteria showed robust biofilm production. Interestingly, the biggest group of MRSA isolates (57%) and a similar group of MSSA isolates (54.54%) were both classified as moderate biofilm producers (Table 5).

All 36 isolates were subjected to polymerase chain reaction (PCR) targeting *16S rRNA* to confirm bacterial identity. All isolates tested positive for this gene (100%), confirming their classification as *S. aureus* (Figure 1).

Fourteen isolates were positive for *the mecA* gene (38.88%), which confirms their identity as MRSA (Figure 1).

Summary of Biofilm formation categories

Biofilm category	Number of isolates (n)	Percentage (%)
Strong (+++)	2	5.55
Moderate (++)	20	55.55
Weak (+)	13	36.11
Non-Biofilm (-)	1	2.77
Total	36	100

Further molecular characterization was performed to assess the presence of intercellular adhesion protein A *icaA* and *icaD* genes, which encode intercellular adhesion proteins associated with biofilm production. All isolates (97.2%), except one (P3), were positive for both *icaA* and *icaD* (Figure 2).

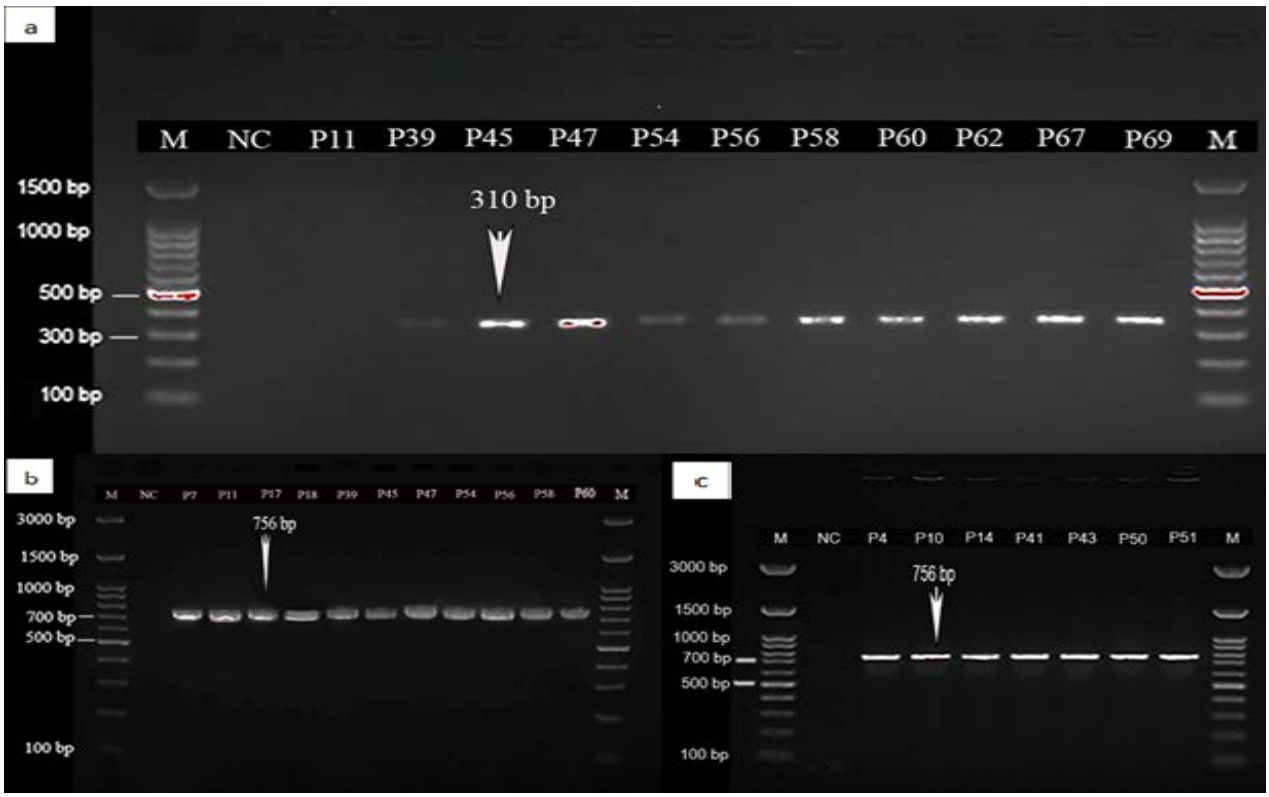

Figure 1. (a) Agarose gel electrophoresis of the mecA gene of approximately 310bp, Lanes M: 100 bp DNA ladder; NC is the negative control; P11 to P69 are strains of MRSA that are positive for this gene. (b) Agarose gel electrophoresis of 16S rRNA gene product of approximately 756 bp. Lanes: M 100 bp DNA ladder. NC is negative control, and P7 to P60 are some strains of MRSA that are positive for this gene. (c) Agarose gel electrophoresis of 16S rRNA gene product of approximately 756bp.Lanes: M 100 bp DNA ladder. Lane NC is the negative control, and Lanes P4 to P51 are some strains of MSSA, which are positive for this gene.

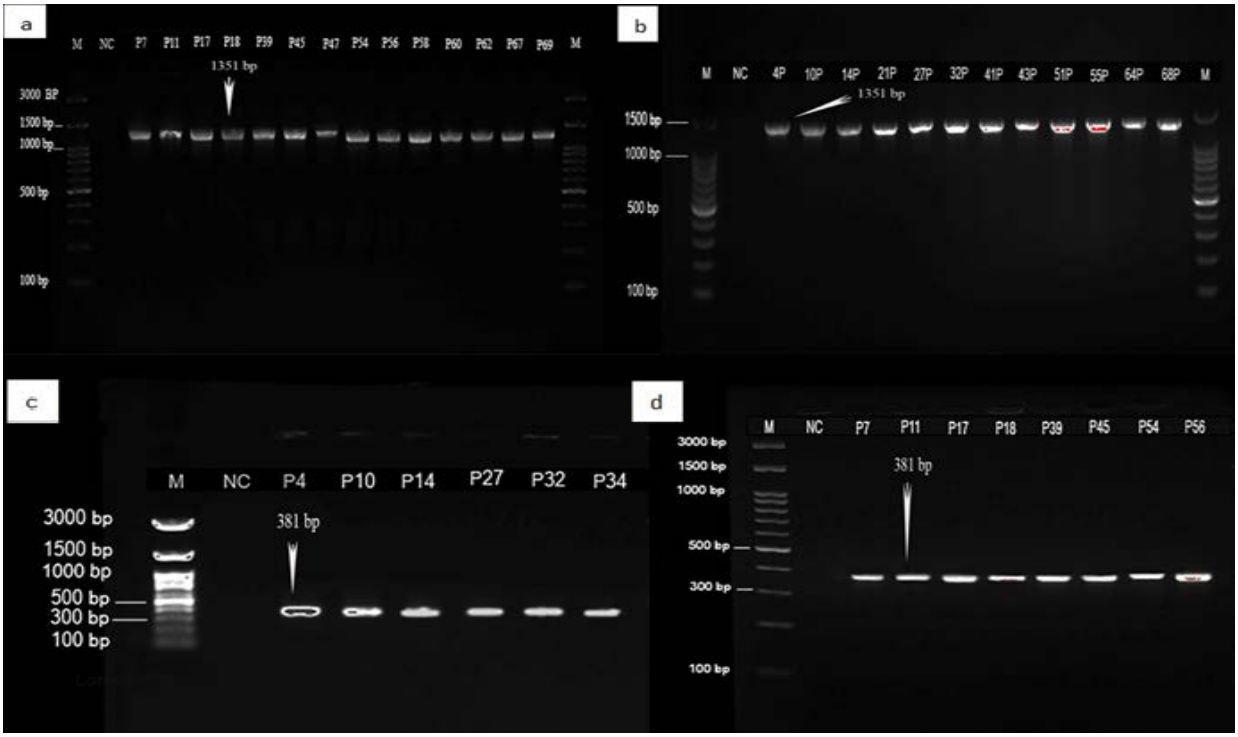

Figure 2. (a) Agarose gel electrophoresis of ica A gene of approximately bp, Lanes M: 100bp DNA ladder, NC is negative control, P7 to P69 are MRSA strains that carry this gene. (b) Agarose gel electrophoresis of icaA gene of approximately 1351bp, Lanes M: 100 bp DNA ladder, NC is negative control, P4 to P68 are MSSA strains that carry this gene. (c) Agarose gel electrophoresis of icaD gene of approximately 381bp, Lanes: M 100 bp DNA ladder, NC is negative control, P4 to P34 are MSSA isolates that carry this gene. (d) Agarose gel electrophoresis of icaD gene of approximately 381 bp, Lanes: M 100 bp DNA ladder, NC is negative control, P7 to P56 are MRSA isolates that carry this gene.

4. Discussion

This study collected 69 superficial throat swabs from individuals with recurrent tonsillitis, isolating *Staphylococcus aureus* from 52.17% of the samples. This finding supports that *S. aureus* continues to be one of the most common bacterial pathogens associated with chronic and recurring tonsillar infections. (Fadhil & Mohammed, 2022) observed a less prevalence rate of 25.64%, demonstrating that *S. aureus* may remain intracellularly inside tonsillar epithelial cells, enabling its evasion of host immune responses and leading to recurrent infections.

among the 36 *S. aureus* isolates, 14 (38.88%) were classified as methicillin-resistant *S. aureus* (MRSA) using both phenotypic and molecular characterization. The chi-square test demonstrated a highly significant correlation between cefoxitin resistance and strain type ($p = 0.0001$). This prevalence aligns with the data of (Buname et al., 2021), suggesting a similar distribution of MRSA strains, who similarly identified the *mecA* gene in a comparable percentage of isolates in Iraq. The identification of the *mecA* gene, responsible for encoding the modified penicillin-binding protein PBP2a, validates the molecular foundation of methicillin resistance in these isolates. All MRSA isolates exhibited sensitivity to vancomycin, except for one MSSA sample (P49), which had lower susceptibility. This may result from the limited clinical application of vancomycin in the area, as it is designated as a last-resort treatment for severe Gram-positive infections and is hardly administered for tonsillitis.

The study demonstrated that 13 out of 14 MRSA isolates (92.86%) exhibited susceptibility to ciprofloxacin, indicating that fluoroquinolones may remain effective against specific MRSA strains present in Sulaymaniyah. The chi-square test revealed a significant correlation between ciprofloxacin resistance and strain type ($p =$

0.0048**), suggesting that sensitivity to this antibiotic may differ between MRSA and MSSA isolates.

All isolates, irrespective of methicillin resistance, exhibited resistance to penicillin, suggesting a substantial prevalence of β-lactam resistance within this community. These findings correspond with those of (Fadhil & Mohammed, 2022), who documented significant penicillin resistance in *S. aureus* isolates from tonsillitis patients in Iraq. This prevalent resistance presumably indicates the empirical and occasionally improper application of penicillin-class medicines in regional clinical practice.

Fifty percent of the MRSA isolates in this investigation demonstrated multidrug resistance (MDR), consistent with findings by (Ferreira *et al*., 2021), who highlighted that MRSA strains often possess several resistance characteristics that augment their survival under antimicrobial pressure. Notably, the MDR incidence among MSSA isolates was elevated at 72.72%, indicating that methicillin susceptibility does not inherently align with comprehensive antimicrobial sensitivity. A similar observation was reported by (Momtaz *et al*., 2013), who stated that detection of multidrug-resistant patterns among methicillin-susceptible *S. aureus* isolates indicates that the spread of resistance genes is not limited to MRSA strains. The increased MDR rates noted in both groups may result from excessive empirical antibiotic use, insufficient treatment regimens, and potential horizontal gene transfer among coexisting bacterial populations in the tonsillar niche.

Molecular screening for biofilm-associated genes (*icaA* and *icaD*) indicated that virtually all isolates possessed both genes, with detection rates of 100% for MRSA and 95.45% for MSSA isolates. Only one isolate (P3) tested negative for both genes. Gel electrophoresis revealed that isolate P47 generated amplicons for both *icaA* and *icaD* that exceeded anticipated sizes, indicating potential insertions or genetic changes within these loci. The optical density measurements in the microtiter plate experiment indicated a modest capacity for biofilm formation in this isolate. The elevated biofilm production rate (97%) observed in the phenotypic experiment corroborates the concept that *S. aureus* in recurrent tonsillitis has an augmented potential for biofilm formation. The results align with the findings of (Almayali *et al*., 2018), which indicated the prevalent occurrence of *icaA* and *icaD* genes in *S. aureus* clinical isolates in Iraq.

The prevalence of biofilm-forming isolates is due to the distinctive conditions of the tonsillar crypts, which offer a sheltered home for bacterial adhesion and survival. (Zautner *et al*., 2010) emphasized that *S. aureus* may endure intracellularly and within biofilm matrices in tonsillar tissues, facilitating recurring infections despite antibiotic treatment. This persistence mechanism may elucidate why conventional antibiotic therapies frequently fail to eliminate the bacterium.

These data highlight the combined involvement of antimicrobial resistance and biofilm development in perpetuating *S. aureus* infections in recurrent tonsillitis. The concurrent existence of *mecA*, *icaA*, and *icaD* genes in most isolates indicates a significant adaptive benefit for colonization and survival in the tonsillar environment. The results suggest that traditional antibiotic treatment may be inadequate for eliminating biofilm-associated *S. aureus* infections, underscoring the necessity of antimicrobial stewardship and, in certain instances, surgical procedures like tonsillectomy for successful management. Subsequent research should incorporate molecular characterization of SCCmec elements and other virulence determinants to further comprehension of strain diversity and transmission dynamics in this area.

This research possesses many limitations. The sample size was modest, and the study was performed at a single location, thereby constraining the generalizability of the results. The research also lacked a healthy control group, limiting direct comparisons with the broader population. The limitations were mostly attributable to restrictions in time and available resources. Nonetheless, the study

offers significant insights into the frequency and antibiotic resistance patterns of *S. aureus* in recurrent tonsillitis.

5. Conclusions

This study concluded that most of the *Staphylococcus aureus* strains isolated from cases of recurrent tonsillitis are multidrug-resistant (MDR) and exhibit resistance to the most of antibiotics commonly used in treatment. All strains demonstrated the ability to produce biofilms, which may protect them from the host immune response and reduce antibiotic efficacy.

Due to the potential polymicrobial characteristics of recurrent tonsillitis, empirical antibiotic selection must be supplemented with culture and sensitivity testing to enhance treatment efficacy. Fluoroquinolones demonstrate potential efficacy against *S. aureus* isolates, warranting their inclusion in culture-guided therapeutic approaches for recurrent tonsillar infections.

Tonsillectomy remains a feasible option for people with chronic or persistent infections. The integration of culture-based treatment with biofilm-targeting techniques may diminish recurrence rates and enhance the overall management of recurrent tonsillitis.

Conflict of interest.

I affirm that there are no conflicts of interest pertaining to the publication of this manuscript. No one, not even a person, group, or business, has given money to support this work. I have not received help from or been associated with any institution while writing this paper. The research was done on its own, and the manuscript is my own original work.

CRediT authorship contribution statement.

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References

Abdulrahim, U., Kachallah, M., Rabiu, M., Usman, N., Adeshina, G., & Olayinka, B. (2019). Molecular detection of biofilm-producing Staphylococcus aureus isolates from national orthopaedic Hospital Dala, Kano State, Nigeria. Open Journal of Medical Microbiology, 9(03), 116. https://doi.org/https://doi.org/10.4236/ojmm.2019.93012

(CLSI), C. a. L. S. I. (2024). Performance Standards for Antimicrobial Susceptibility Testing (34th edition ed.). Clinical and Laboratory Standards Institute. https://doi.org/https://clsi.org/standards/products/microbiology/documents/m100

Almayali, E. J. B., AL-Kraety, I. A. A., & Al-Muhanna, A. S. (2018). Genotypic and phenotypic detection of biofilm production by icaa and icad genes in staphylococcus aureus isolated from tonsillitis. Biochem Cell Arch, 18(2), 1017–1021.

Buname, G., Kiwale, G. A., Mushi, M. F., Silago, V., Rambau, P., & Mshana, S. E. (2021). Bacteria patterns on tonsillar surface and tonsillar core tissue among patients scheduled for tonsillectomy at Bugando Medical Centre, Mwanza, Tanzania. Pathogens, 10(12), 1560. https://doi.org/https://doi.org/10.3390/pathogens10121560

Cavalcanti, V. P., Camargo, L. A. d., Moura, F. S., Melo Fernandes, E. J. d., Lamaro-Cardoso, J., Braga, C. A. d. S. B., & André, M. C. P. (2019). Staphylococcus aureus in tonsils of patients with recurrent tonsillitis: prevalence, susceptibility profile, and genotypic characterization. Brazilian Journal of Infectious Diseases, 23, 8–14. https://doi.org/https://doi.org/10.1016/j.bjid.2018.12.003

Elkady, F. M., Al-Askar, A. A., Tawab, A. A., Alkherkhisy, M. M., Arishi, A. A., & Hashem, A. H. (2022). Comparative genotypic analysis of RAPD and RFLP markers for molecular variation detection of methicillin-resistant Staphylococcus aureus clinical isolates. Medicina, 58(9), 1245. https://doi.org/https://doi.org/10.3390/medicina58091245

Fadhil, H., & Mohammed, B. J. (2022). Molecular and classical identification of Staphylococcus aureus, isolated from Iraqi patients with recurrent tonsillitis. Medico-legal Update, 22(1), 23.

Ferreira, C., Costa, S. S., Serrano, M., Oliveira, K., Trigueiro, G., Pomba, C., & Couto, I. (2021). Clonal lineages, antimicrobial resistance, and PVL carriage of Staphylococcus aureus associated to skin and soft-tissue infections from ambulatory patients in Portugal. Antibiotics, 10(4), 345. https://doi.org/https://doi.org/10.3390/antibiotics10040345

Hussein, S. A., & Rasheed, T. K. (2025). Role of Staphylococcus aureus and Streptococcus pyogenes Biofilms on the Alternation of Cellular Immunity in Pediat-ric Tonsillitis Patients. Karbala International Journal of Modern Science, 11(3), 9. https://doi.org/https://doi.org/10.33640/2405-609X.3418

Kardooni, M., Mosavian, S. M., Lotfinia, M., Eghbalnejad, A. M., Mofrad, M. S., Saki, N., & Abshirini, H. (2020). Study of common bacterial agents and antibiotic susceptibility in patients with chronic and repeated tonsillitis. Journal of Advanced Pharmacy Education & Research| Apr-Jun, 10(S2), 91.

Khoramrooz, S. S., Mansouri, F., Marashifard, M., Hosseini, S. A. A. M., Chenarestane-Olia, F. A., Ganavehei, B., Gharibpour, F., Shahbazi, A., Mirzaii, M., & Darban-Sarokhalil, D. (2016). Detection of biofilm related genes, classical enterotoxin genes and agr typing among Staphylococcus aureus isolated from bovine with subclinical mastitis in southwest of Iran. Microbial pathogenesis, 97, 45–51. https://doi.org/https://doi.org/10.1016/j.micpath.2016.05.022

Klagisa, R., Racenis, K., Broks, R., Balode, A. O., Kise, L., & Kroica, J. (2022). Analysis of microorganism colonization, biofilm production, and antibacterial susceptibility in recurrent tonsillitis and peritonsillar abscess patients. International Journal of Molecular Sciences, 23(18), 10273. https://doi.org/10.3390/ijms231810273

Kostić, M., Ivanov, M., Babić, S. S., Tepavčević, Z., Radanović, O., Soković, M., & Ćirić, A. (2022). Analysis of tonsil tissues from patients diagnosed with chronic tonsillitis—microbiological profile, biofilm-forming capacity and histology. Antibiotics, 11(12), 1747. https://doi.org/https://doi.org/10.3390/antibiotics11121747

Lee, P. Y., Costumbrado, J., Hsu, C.-Y., & Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. Journal of visualized experiments: JoVE(62), 3923. https://doi.org/https://doi.org/10.3791/3923

Zautner, A. E., Krause, M., Stropahl, G., Holtfreter, S., Frickmann, H., Maletzki, C., Kreikemeyer, B., Pau, H. W., & Podbielski, A. (2010). Intracellular persisting Staphylococcus aureus is the major pathogen in recurrent tonsillitis. PloS one, 5(3), e9452. https://doi.org/https://doi.org/10.1371/journal.pone.0009452.

Mlynarczyk-Bonikowska, B., Kowalewski, C., Krolak-Ulinska, A., & Marusza, W. (2022). Molecular mechanisms of drug resistance in Staphylococcus aureus. International Journal of Molecular Sciences, 23(15), 8088.

Momtaz, H., Dehkordi, F. S., Rahimi, E., Asgarifar, A., & Momeni, M. (2013). Virulence genes and antimicrobial resistance profiles of Staphylococcus aureus isolated from chicken meat in Isfahan province, Iran. Journal of Applied Poultry Research, 22(4), 913–921.

Naji, D. E., & Arif, S. K. (2024). Genotyping of Methicillin Resistant Staphylococcus aureus Clinical Isolates in Sulaimani City using Pulsed -Field Gel Electrophoresis. Tikrit Journal of Pure Science, 29(1), 1–15. https://doi.org/10.25130/tjps.v29i1.1397

O'Handley, J. G., Tobin, E. J., & Shah, A. R. (2020). Otorhinolaryngology. Textbook of family medicine, 300.

Rajeswarie, S., Pradha, V., & Annet Olinda, D. S. (2022). Comparison of phenotypic and genotypic characterization methods for the detection of methicillin-resistant Staphylococcus aureus. Cureus, 14(3).

Stepanović, S., Vuković, D., Hola, V., Bonaventura, G. D., Djukić, S., Ćirković, I., & Ruzicka, F. (2007). Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Apmis, 115(8), 891–899. https://doi.org/https://doi.org/10.1111/j.1600-0463.2007.apm_630.x

Suresh, M. K., Biswas, R., & Biswas, L. (2019). An update on recent developments in the prevention and treatment of Staphylococcus aureus biofilms. International Journal of Medical Microbiology, 309(1), 1–12.

Tomaras, A. P., Dorsey, C. W., Edelmann, R. E., & Actis, L. A. (2003). Attachment to and biofilm formation on abiotic surfaces by Acinetobacter baumannii: involvement of a novel chaperone-usher pili assembly system. Microbiology, 149(12), 3473–3484. https://doi.org/https://doi.org/10.1099/mic.0.26541-0

Vorobieva, V., Bazhukova, T., Hanssen, A.-M., Caugant, D., Semenova, N., Haldorsen, B. C., Simonsen, G. S., & Sundsfjord, A. (2008). Clinical isolates of Staphylococcus aureus from the Arkhangelsk region, Russia: antimicrobial susceptibility, molecular epidemiology, and distribution of Panton-Valentine leucocidin genes. Apmis, 116(10), 877–887. https://doi.org/ https://doi.org/10.1111/j.1600-0463.2008.01092.x