

Bacteriological Profile of Tonsillitis with Emphasis on *Staphylococcus aureus*, MRSA, and Biofilm Formation in Wasit Province

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Abstract

The study investigates the antibiotic resistance of *Staphylococcus aureus* and its ability to form biofilm formation. In addition to the identification of causative agents *Streptococcus pyogenes* and other bacteria in tonsillitis patients at Al-Numaniya, and Al-Zahra Teaching Hospitals between September 2024 and May 2025. A total of 130 tonsil specimens (swabs and tissue samples) were collected, of which showed positive microbial growth 110. The identification of these isolates was done based on cultural, microscopic tests, biochemical tests and the VITEK 2 system identification kits. Results indicate that 38 isolates (34.5%) were positive for *S. aureus* with the high prevalence observed in recurrent tonsillitis, accounting for 65.7%. Moreover, Methicillin-resistant *S. aureus* (MRSA) was identified in 44.7% of cases. Additionally, *Streptococcus pyogenes* was detected in (20.9%) of the cases. Findings underscore the significance of *S. aureus* as a major pathogen in Tonsillitis and highlight the need for effective monitoring and treatment strategies to combat antibiotic resistance.

Keywords: Methicillin-resistant *S. aureus* (MRSA), Tonsillitis.

1. Introduction

Tonsillitis, defined as the inflammation of the pharyngeal tonsils, predominantly affects the pediatric demographic, with a notably elevated incidence observed in children [1].

Tonsillitis is caused by a viral infection in 70% to 95% of cases. The principal bacteria in tonsillitis are Group A beta hemolytic *Streptococcus pyogenes* GABHS, but other bacterial pathogens isolated in such cases are *S. aureus*,

Streptococcus pneumoniae, and *Haemophilus influenzae* [2]. *S. aureus* is the most common cause of RT and can acquire broad antibacterial resistance. *S. aureus* strains isolated from patients with RT were considered multidrug-resistant and methicillin-resistant (MRSA). The formation of multilayered, mature biofilms by *S. aureus* may facilitate its persistence within tonsillar tissue and contribute significantly to the chronicity of infection [3]. *S. aureus* is a non-motile, fermentative, and non-spore-forming bacterium that is facultative anaerobic and it is a desiccation and high osmotic condition [4].

Microbial biofilms are organized bacterial populations that stick to surfaces and are encased in an extracellular polymeric substance (EPS) [5]. Self-produced extracellular polymeric matrix that encases microorganisms promotes survival in hostile environments, including tolerance to antibiotics, and provides structure to the biofilm [6].

2. Materials and Methods

2.1 Samples Collection

The study was conducted from September 2024 to May 2025, during which 130 samples from patients with tonsillitis from different age groups and both genders

were collected under the supervision of a physician at ear, nose, throat clinic (E.N.T) in Al-Numaniya, and Al-Zahra Teaching Hospital. Sterile swabs were used and the swabs were transported to the laboratory within two hours. Samples were cultured on selective media (MSA) for *S. aureus*, and the isolates were then stained and subjected to biochemical tests for identification.

2.2 Preparation of Culture Media

2.2.1 Blood Agar

Forty grams of the medium (in powder form) were dissolved in distilled water to prepare one liter of solution to make blood agar. The mixture was then heated to a boil with continuous agitation to ensure complete dissolution of the components. After sterilizing, cool the medium to 45 °C and add 5% human blood and pour into petri dish.

2.2.2 Mannitol salt agar

Clinical samples were swabbed and subsequently cultured on a selective medium, Mannitol Salt Agar (MSA). This medium serves to differentiate *S. aureus* based on its capacity to ferment mannitol, resulting in the production of acidic by products that lower the pH of the medium. Therefore, the phenol red indicator in the

agar turns yellow [7]. Samples were subcultured onto blood agar and subsequently onto Mannitol Salt Agar.

2.2.3 Congo Red Agar

The reagent was prepared by addition of 0.8 g of Congo red and 36 g of saccharose to 1 L of BHI. Plates were incubated for 24 hours at 37 °C and overnight at room temperature. The production of rough black colonies by slime producing strains was used to differentiate them from non-slime producing *S. aureus* strains (red smooth colonies) [8].

2.2.4 Gram Stain Method

Gram staining process was initiated by preparing a smear, which involved suspending a small bacterial colony in normal saline on a glass slide. Then, heat-fixed and stained, crystal violet, followed by iodine as a mordant, decolorization with alcohol, and finally, counterstaining with safranin. The slide was viewed under a 100 × oil immersion objective [9]

3. Results and Discussion

3.1. Descriptive Data on Study Subject

This study included a total of one hundred and thirty (130) tonsil specimens

(swabs and from the tissue of tonsils after tonsillectomy) had been collected from patients with tonsillitis. Included Acute Tonsillitis 29 (22.3%), Chronic Tonsillitis 42 (32.3%), and Recurrent Tonsillitis 59 (45.3%). Patients from Al-Numaniya hospital and Al-Zahra a teaching hospital in Waist province during the period from September 2024 to May 2025. The culture investigation showed positive microbial growth 110 (84.6%).

Table1: Prevalence of Bacteria species among various types of tonsilitis.

Microorganism spp.	No.	%
Gram positive bacteria	75	68.1%
<i>S. aureus</i>	38	34.5%
<i>Streptococcus pyogenes</i>	23	20.9%
<i>Streptococcus pneumonia</i>	8	7.27%
<i>Streptococcus viridans</i>	4	3.6%
<i>Streptococcus pasteurianus</i> <i>Group D</i>	2	1.81%
Gram negative bacteria	30	27.3%
<i>K. pneumonia</i>	13	11.8%
<i>H. influenza</i>	9	8.2%
<i>E. coli</i>	8	7.3%
Yeast	5	4.5%
<i>Candida</i>	5	4.5

Results in the present study were agreed with another study done by Al-Akwa

[9] that found that *S. aureus* was 35% *S. mitis* 6.3%, *S. agalactia* 5.6 %. Also agreed with Al-Amara [10] that found *S. aureus* percentage was 22.9%.

3.2 Prevalence of *S. aureus* Among Type of Tonsillitis

The highest percentage of *S. aureus* infections was found in recurrent tonsillitis 25 out of 38 (65.7%) followed by chronic tonsillitis was 12 (31.7%), While the lower incidence was 1 (2.6%) in acute tonsillitis. The study noticed that the large proportion of *S. aureus* isolates were from sample tissue of tonsils [11].

3.3 Streptococcus Diagnoses

Stained bacterial smears were gram positive cells. Depending on cultural and microscopic characteristics, Beta hemolysis, alpha and gamma hemolysis were observed after 24 hours of incubation. Samples were cultured on blood agar after incubation at 37 °C for 24 hours. A catalase test was used to distinguish *Streptococci* from *Staphylococci*, and all were negative [12].

3.4 Bacitracin Sensitivity test for streptococcus species.

Bacitracin test is used to determine the effect of bacitracin on an organism.

Streptococcus is inhibited by a small number of bacteria (0.04 U) [13].

3.5 Identification of Methicillin Resistant *S. aureus* (MRSA)

Out of 38 *S. aureus* 17 (44.7%) isolates identified Methicillin Resistant *S. aureus* (MRSA). Isolates were identified as *S. aureus* were cultured on HiCrome MeReSa Agar Base medium, a selective medium for MRSA isolation by combining it with cefoxitin supplement (FD259) and MeReSa Selective Supplement (FD229). Positive colonies are detected by their bluish green color and identified as MRSA [14] as illustrated in figure 1.



Figure 1: Methicillin-Resistant *S. aureus* (MRSA) on HiCrome MeReSa Agar Base medium.

3.6 Prevalence of (MRSA) Among Type of Tonsillitis

The prevalence of MRSA among various types of tonsillitis was a variant rate. Acute Tonsillitis 5.8% followed by Chronic Tonsillitis 5/17 (29.4%), and the Recurrent Tonsillitis were 11/17 (64.7%).

This study agreed with study found the amplification of the staph 16S rRNA and *mecA* genes confirmed that 34 (60.71%) were *S.aureus* and resistant to methicillin (MRSA). In addition, the prevalence of MRSA in recurrent tonsillitis was 11/17 (64.7%) was consistent with a previous study which found 58.97% of *S. aureus* isolates were MRSA [15].

Table 2: Prevalence of (MRSA) among types of tonsillitis.

Type of tonsillitis	Number	Percentage
Chronic Tonsillitis	5	29.4%
Recurrent Tonsillitis	11	64.7%
Acute Tonsillitis	1	5.8%
Total	17	100%

The prevalence of MRSA is high, and the emergence of MRSA isolates resistance to cefoxitin. Other broad-spectrum antibiotics have exacerbated the multi-drug resistance of MRSA in oral infections, The prevalence is influenced by factors such as previous antibiotic usage,

hospitalization, chronic illnesses, and catheterization, which contribute to an increased risk of MRSA in oral samples [16].

3.7 Biofilm Formation of *S. aureus*

Biofilm formation of isolates was also evaluated on Congo Red Agar (CRA) plates. Based on the previously described protocol CRA plate test uses Congo red agar and allows for the direct analysis of the colonies and the identification of slime-forming strains. Black colonies and non-slime-forming strains red-coloured colonies [17].

In the current study out of 38 *S. aureus* (17) isolated from tonsils swab and from tissue were biofilm producer, changing the color of Congo red agar from red color to black or dark as shown in the figures 2, 3 and listed table 3.



Figure 2. Phenotypic biofilm producer of *S. aureus* on Congo red agar shows the black colonies (positive result).

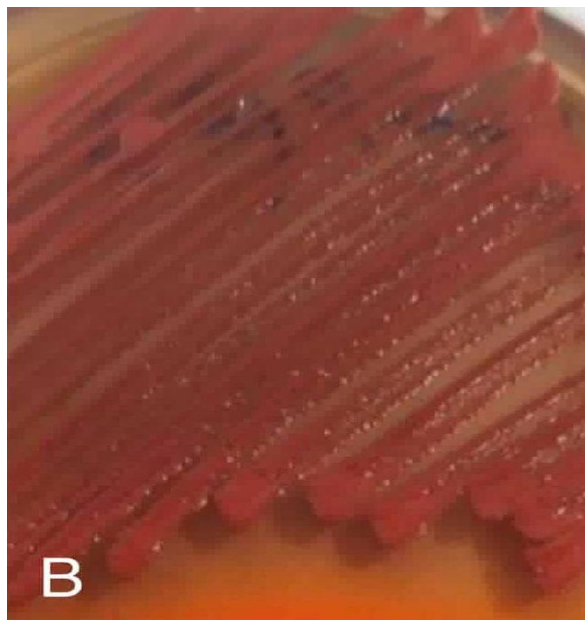


Figure 3: Phenotypic biofilm producer of *S. aureus* on Congo red agar with red colonies (negative result).

Table3: Biofilm formation of *S.aureus* in type of Tonsillitis.

Type of Tonsillitis	No.	%
Recurrent Tonsillitis	11	44%
Chronic Tonsillitis	6	50%
Total	17	44.7%

In current study 11 out of 25 (44%) *S. aureus* bacteria produce biofilm in recurrent tonsillitis. Six of of 12 (50%) in chronic tonsillitis. This conclusion was consistent with the findings of conducted by 18. Klagisa [18]. Another study in Iraq found that 40-50 % of *staphylococcus* produce biofilm [19]. Bacterial biofilms are recognized as the main factor involved in the chronicity of infections and resistance to antibiotic treatment. Studies by Feng and co-workers [20] discovered that PNPs inhibited bacterial adhesion, extracellular polysaccharide synthesis and prevented biofilm formation.

4. Discussion

The current paper demonstrates that *S. aureus* is the predominant bacterial pathogen in tonsillitis in Wasit Province. A high prevalence in recurrent cases of MRSA was detected in 44.7% of *S. aureus* isolates.

The presence of *Streptococcus pyogenes* and other Gram-positive and Gram-negative organisms confirms the

polymicrobial nature of tonsillar infections. Nearly half of the *S. aureus* isolates were biofilm producers, especially in recurrent and chronic tonsillitis. Overall, the study emphasizes the need for careful antibiotic selection and consideration of biofilm-associated infections.

5. Conclusion

The present findings indicated that *S. aureus* is the most prevalent bacterial pathogen in tonsillitis among patients in Wasit Governorate especially recurrent cases. A high MRSA (44.7%) prevalence among *S. aureus* isolates suggesting increasing antimicrobial resistance. The high rate of biofilm-producing isolates, in recurrent and chronic tonsillitis, could indicate that biofilm formation plays an important role in the bacterial persistence and failure of treatment. These results reinforce the necessity of ongoing monitoring of antibiotic resistance trends, reasonable use of antibiotics in treatment strategies.

6. References

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