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# Bacteriological study of in Kirkuk City

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## Abstract

Tonsillitis is one of the most widespread diseases; for the period from November 1/11/2023 to January 18/1/2024, 36 smear samples were taken from persons with tonsillitis of both sexes, within the age range of (4-60) year. They had performed their source diagnostic tests on basis of cultural, microscopic, and biochemical characteristics of 24 different aerobic bacterial isolates from 36 samples of persons suffering from tonsillitis. According to the findings made by this study, there are high chances of contracting tonsillitis infections from Gram-positive bacteria than from the Gram-negative bacteria. Gram-positive bacterial isolates in this study was found to be (21) isolates, 87.5% while Gram-negative bacteria was found to be (3) isolates, 12.5%. A sensitivity test for some of the antibiotics and reagents was carried out and the result of the test for *Staph. aureus* isolates showed high resistance to the specific antibiotics Azithromycin, Cloxacillin, which were 93%, 80% respectively while 73% for each of Ceftazidime and Amoxicillin. The findings also confirmed that the *Streptococcus spp* isolates had no resistance to the Amoxicillin, which was 100% resistant and Cloxacillin which was 83%, while moderate sensitivity was recorded to the Azithromycin, which was 83% sensitive and moderate resistance to the Ceftazidime, which was 33% resistant. Concerning *Klebsiella spp* isolates, they were all resistant to Azithromycin, moderates to Cloxacillin and Amoxicillin at 67% each. Also, the *Klebsiella spp* isolates proved highly sensitive to Ceftazidime at a 100% level of sensitivity.

**Keywords:** Tonsillitis; Bacteriological; Kirkuk; Antibiotics

## 1. Introduction

They are one of the organs of upper respiratory system as well as second lymphoid organs in a very sensitive place at both sides of the oral throat in the back (Abd-AL-Kareem, 2013). These two tonsils on this ring in pharynx are mucosal associated lymphoid tissues (MALT) and are under the first line of defense against many types of bacterial and viral infections as they are located in subepithelial layer of the pharynx. The tonsils help make antibodies — like immunoglobulin A (IgA) — when bacteria or viruses are inhaled or swallowed. Immune tissues are that first line of defense of the immune system to foreign pathogenic agents that are ingested or inhaled (Mahajan, & Ingale, 2017; Smith *et al*., 2023).

The tonsils are part of the Secondary Lymphoid Organs and their primary function is to create immunity against common pathogens and protect the throat and lungs from bacterial infections. The tonsils are relatively small in the first years of a child's life and increase in size as the child grows (Wang *et al*., 2010).

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**Figure 1** Tonsillitis (Smith et al., 2023)

Tonsillitis can be caused by *Staphylococcus aureus* indicated as the most common and main causative agent of tonsillitis by the researcher (Altamimi *et al*., 2012) or by viruses that infect the respiratory system. According to the researcher (Nada , 2008) the outcome of the infection depends on the severity and degree of resistance or susceptibility of the pathogen to antimicrobial chemotherapy, as the prevalence of resistant bacteria maybe attributed to inappropriate use of antibiotics.

Tonsillitis is a very serious infection. Increasing incidence of the disease makes it a major oral health problem, as the disease can attack the same disease several times within one year (Kalairasi *et al*., 2018). Tonsillitis was found to affect most age groups, however it particularly affected people in the age range from 3 to 10 years (Mitchell *et al*., 2019).

If tonsil tissue is repeatedly infected with recurrent infections, mostly bacteria and viruses, the tonsils lose their effectiveness for helping the immune system and actually become a source of recurrent infections (Bhargava *et al*., 2011). Group A beta hemolytic streptococcai and S. aureus are the garden most important bacterial causes of tonsillitis, and other bacterial species found in chronic, acute, recurrent tonsillitis (Abidali, 2014).

Pharyngitis or other inflammation of lymphatic tissue in the pharyngeal region is associated with tonsillitis. The inflammation causes clear clinical symptoms, including: Sore throat fever, tonsils swelling and redness, headache, nasal congestion, difficulty swallowing, the whites and yellow spots, swollen lymph nodes, and tonsil adenoid swelling of third tonsil (EChalabi, 2010). The concept of tonsillitis as a medical one has been known only at the end of the nineteenth century and it is believed to be a common disease in various countries of the world. The term shows the interaction between factors causing inflammations with the lymphatic tissue of the tonsils (Abbood, H.A.R & Alabden, S.S.Z. 2019). Incidence rates are higher in children than adults and the disease is known as tonsillitis, referring to an inflammation in the palatine tonsils (Alotaibi, 2017). Pathogenic bacteria is responsible for the second most tonsil infection, and *Streptococcus* is the first of those pathogens (Alasmari *et al*., 2017). The *Streptococcus* bacteria. pyogenes infection is often associated not only with tonsillitis, but also with otitis media (Di Pierro *et al*., 2012). In kids with chronic or recurrent tonsillitis, complications, such as dehydration and kidney failure caused by inflammation and spread of infection, are rare. Rarely, however, complications such as rheumatic fever or glomerulonephritis occur. In poor countries, these complications are a major problem (Chandra *et al*., 2017; Danchin *et al*. 2002).

The microorganisms penetrate and attach themselves to epithelial cells, producing cytokines and activation of complement, with this causing stimulation of an inflammatory reaction of the tonsillar mucosa (Mahajan *et al*., 2017), when the virulence factors and activity of the pathogen in the tonsillar lymphoid tissue exceeds its protective capacity on the activated lymphocytes and immunoglobulin producing cells. Although most cases are of bacterial origin (Utsunomiya *et al*., 1998), viruses are the main cause of tonsillitis. The forefathers of these are *Streptococcus* bacteria such as *S.pyogenes*, and *Staphylococcus* bacteria such as *Staphylococcus aureus*, the latter being the most virulent pathogen in tonsil pathology (Zhou & Li, 2021)

## 2. Material and methods

### 2.1. Ready-made culture media

Blood agar, MacConkey agar, mannitol agar were prepared as per the instructions of the company supplier and all culture media were used. For all culture media, they were all sterilized in an autoclave at 121°C, 15 pounds/inch2 for 15 minutes. After cooling to (45-50)°C they were poured into sterile Petri dishes. The dishes were sterilized, incubated 37°C for 24 h to ensure that it was not contaminated then incubated at 4°C until used (Tille, 2014).

**Table 1** Laboratory culture media used in the study

| Purpose of use | Manufacturer | media | ت |
|---|---|---|---|
| growth of bacterial species for diagnosis | LAB (England) | Blood base agar | 1 |
| Growing Gram-negative bacteria and identifying lactose fermenting species | LAB (England) | MacConkey agar | 2 |
| Antibiotic sensitivity test | LAB (England) | Muller Hinton agar | 3 |
| Distinguishing mannitol-fermenting and non-fermenting staphylococci | LAB (England) | Mannitol salt agar | 4 |

### 2.2. Diagnostic kit

Microscopic differentiation of Gram-positive and Gram negative bacteria was performed using Gram stain solutions.

### 2.3. Specimens collection

The 36 samples collected in this study were from patients suffering tonsillitis of both sex in the period from 11/1/2023 to 18/1/2024. People with tonsillitis' samples were taken and the patient's information were recorded. Sterile cotton swabs (Transport cotton swabs) were used to take the swabs with nutrient medium to sustain samples until they are delivered to the Microbiology Laboratory at the College of Education for Pure Sciences for culutation.

### 2.4. Sample culture

Blood agar, MacConkey medium and mannitol medium were used to culture tonsil swabs purpositively and corresponding isolates were purified from streaks. All plates were incubated aerobically in an incubator at 37°C for 24 h. Samples that grew were then examined for diagnostic, morphological and biochemical features.

### 2.5. Diagnosis of bacterial isolates

#### *2.5.1. Morphology diagnosis*

We identified what was mentioned in (Bergey, 1994) to identify bacterial isolates based on their cultural characteristics by colony size, color.

#### *2.5.2. Microscopic diagnosis*

A single colony was taken in a sterile loop (Full Loop) and placed on a glass slide with drops of sterile distilled water, and Gram staining method was used. We then spread the sample and left to dry and fix it by passing it across the flame and staining it using Gram stain. Next cells were shaped and color was observed under light microscope by means of 100X oil lens (Benson, 2002).

### 2.6. Biochemical test

#### *2.6.1. Catalase test*

This test was done by taking a portion of a bacterial colony with a sterilized wooden stick into a clean glass slide, then adding a drop of catalase $H_2O_2$ (3%) reagent and gently mixing it. It indicated the test was positive as gas bubbles were formed. The ability of the bacteria to produce the catalase enzyme, which decomposes toxic hydrogen peroxide, was determined by this test; the catalyst enzyme releases oxigen $O_2$ gas and water $H_2O$ (Atlas, 2010).

*2.6.2. Oxidase test*

A wooden stick was used to transfer a portion of a 24-hour old pure growing colony to a filter paper saturated with a few drops of Tetramethyl p-phenylene diamin dihyrochloride reagent. A positive test is shown by the colony becoming purple in less than 10 seconds, as it does (Tille & Bailey, 2014).

*2.6.3. Coagulase test*

To do this, we mixed a single young bacterial colony with a drop of normal saline solution on the surface of a clean glass slide and added another drop of blood plasma and mixed it well. The possibility of aggregation within 15 seconds became a positive result (Muktha et al., 2015).

*2.6.4. Antibiotic resistance testing*

The response of the isolates under study to antibiotics was investigated based on (Vandamme et al., 1996).

- Prepare the bacterial suspension by taking 4-5 young colonies, 16-24 hours old, growing on a blood agar plate, to a test tube containing 5 ml of physiological solution, with good shaking.
- The density of the bacterial suspension was compared with the standard turbidity constant McFarland (0.5 Standard turbidity McFarland), which is equivalent to the number of bacteria in it x1.5 (108) cells/ml.
- The bacterial suspension was spread on the surface of the dishes containing the previously prepared Mueller-Hinton agar medium using a cotton swab by dipping the swab in the suspension after shaking it well and rotating the swab at the top of the tube to remove the excess solution and the dishes were left to dry for 5-10 minutes at room temperature.
- Then, the antibiotic discs were placed on the surfaces of the cultured dishes using sterile forceps at a rate of 5-6 discs per dish, taking into account sterilizing the forceps with a flame after taking each disc.
- The dishes were incubated directly at a temperature of 37 °C for 24 hours, after which the diameter of the growth inhibition zone around each disc was measured, after which the isolates were identified as sensitive or resistant to these antibiotics. The table shows the diameters of the standard inhibition zones for the antibiotics.

**Table 2** Antibiotics used in the study

| **origin** | **Manufacturer** | **concentration** | **symbol** | **Antibiotic** | |
|---|---|---|---|---|---|
| Turkey | Bioanalyse | 30 | CX | Cloxacillin | 1 |
| Turkey | Bioanalyse | 15 | AZM | Azithromycin | 2 |
| Turkey | Bioanalyse | 10 | AX | Amoxicllin | 3 |
| Turkey | Bioanalyse | 30 | CAZ | Ceftazidime | 4 |

## 3. Results and discussion

### 3.1. Isolation

From November 1/11/2023 to January 18/1/2024, bacterial growth appeared on culture media of 36 samples from 24 different bacterial isolates of patients with tonsillitis and those who attended Al-Daqouq Clinic, patients. The results were shown that the percentage of tonsillitis infections brought by Gram positive bacteria were more than those caused by Gram negative bacteria, with the number of Gram positive bacterial isolates were (21) isolates with 87.5% and Gram negative bacteria were (3) isolates and 12.5%. According to the researcher (Alghamdi *et al*., 2023) , (Abbood & A. H. S, 2020) isolated bacteria included most commonly are *Streptococcus pyogenes* of group A beta-hemolytic, *Staphylococcus aureus*, and *Klebsiella pneumoniae.*

### 3.2. Diagnosis

*3.2.1. Culture diagnosis*

The isolates were initially diagnosed based on their cultural characteristics after growing them on culture media, and the morphological characteristics of the growing colonies, their sizes and the type of lysis were studied. The results showed that out of 21 isolates positive for Gram stain, 15 of them were *Staph. aureus*, as their colonies appeared yellow

with a creamy texture on mannitol salt agar medium as a result of their fermentation of mannitol sugar (Kateete *et al*., 2011; Shrestha *et al*., 2018), and 6 of them were *Streptococcus spp*, as they appeared in the form of small spherical colonies that were hemolytic of the beta type on blood agar medium. All isolates negative for Gram stain were shown to be *Klebsiella spp*, as they showed growth on MacConkey medium and their colonies appeared in a light pink color because they ferment lactose sugar, as shown in Figure (3-1).

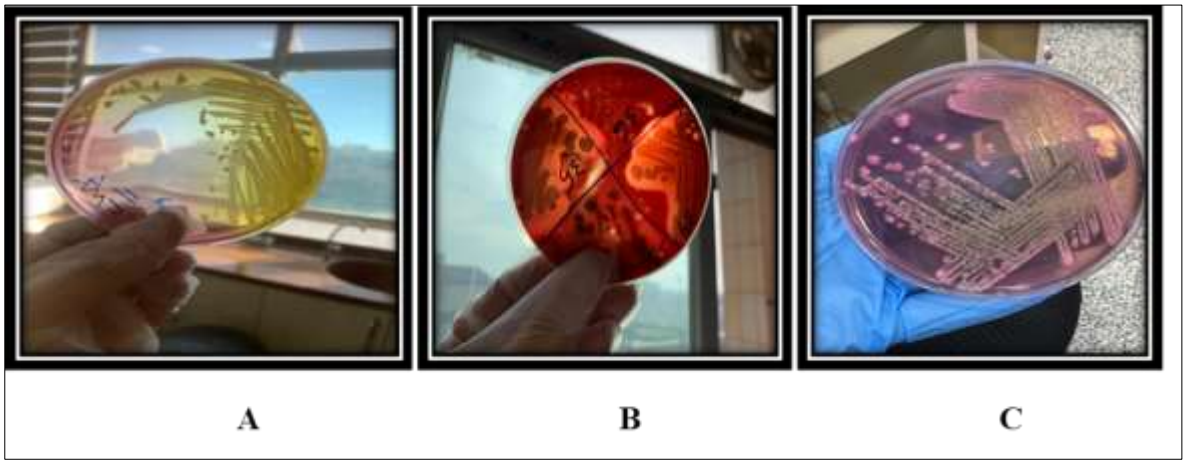


**Figure 2** A.Growth of Gram negative bacteria *Klebsiella spp* on MacConkey medium; B. *Streptococcus spp* grown on blood agar media; C. Gram positive bacteria *Staph. aureus* growth on mannitol agar medium

*3.2.2. Microscopic diagnosis*

Microscopic examination diagnosed the bacterial species. Gram positive and Gram negative stained smears of bacteria were shown to be spherical, clustered and rod shaped cells. These single cells are Gram positive, spherical or oval and appear as individuals or in pairs. They were arranged together to form long chains of them, primarily in shape and *Streptococcus spp*. Similarly, the bacteria of *Staph. aureus* were Gram positive, and were shown as purple clusters (Alkhafaji & Al-Saimary, 2020). Regarding *Klebsiella spp*., they were Gram-negative and were of the shape (Rod Brooks *et al*., 2013).

### 3.3. Biochemical test

*3.3.1. Gram-positive bacteria*

The Gram positive bacteria had some biochemical tests done and it was found that the all *Staphylococcus aureus* isolates were 100% positive for the catalase test and 100% negative for the oxidase test. Variation in the percentage of the Coagulase blood plasma coagulation test performed by the slide method of isolates *Staphylococcus aureus* was 72%. This Coagulase test is one of the most important tests for separating out the *staphylococci*, as coagulase enzyme causes blood plasma to clot by converting fibrinogen to fibrin. it has two types of Coagulase enzyme that include the aggregation factor which, in the bacterial suspension in plasma, clumps in the wall of the bacterial cell when plasma is added (Rakotovao-Ravahatra *et al*., 2019). For *Streptococcus spp* bacteria, which were on the other side of the scale to *Staphylococcus aureus* bacteria, all isolates did not show a positive result for the catalase test at a rate of 100% (Figure (3-2).

*3.3.2. Gram-negative bacteria*

*Klebsiella spp*. isolates displayed 100% negativity for oxidase and catalase tests.

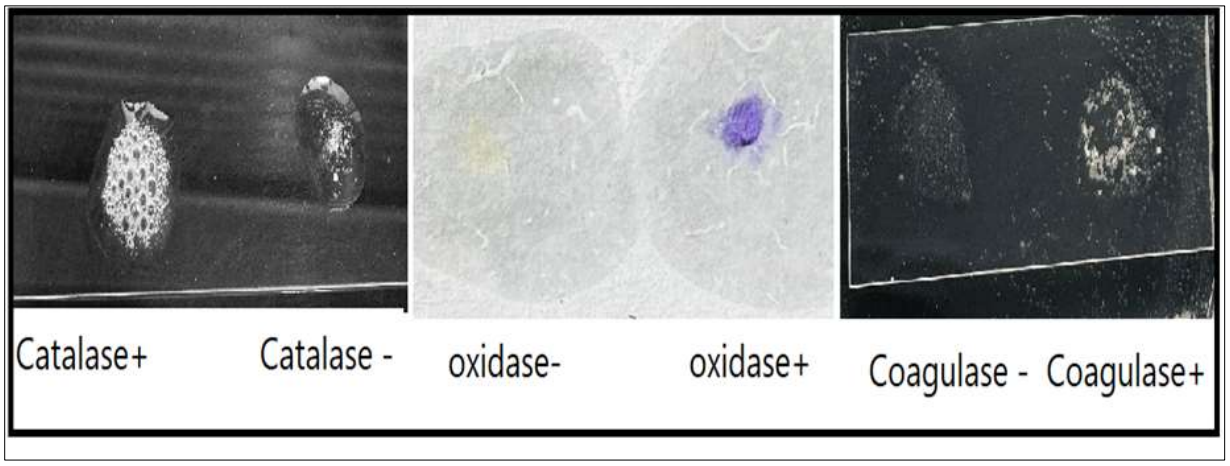


**Figure 3** Biochemical tests

### 3.4. Sensitivity and resistance of bacterial isolates to some antibiotics (disc method)

As in paragraph (2-6-4) of the materials and methods of work, sensitivity was tested against (4) antibiotics for the (24) isolates under study and the diameter of inhibition was measured. Antibiotic resistance showed variation among the isolates, and such results are presented in Table (3-1), and Figure (3-3). Results revealed that the *Staph. aureus* isolates were highly resistant to the antibiotics Azithromycin, Cloxacillin 93%, 80%; for Ceftazidime, Amoxicillin 73%, 73%; respectively. A comparison of (Naimi *et al*. 2017) with the current study showed that *Staph. aureus* mechanism of acquired resistance to antibiotics was through efflux pumps, enzymes that inactivate antibiotics coded by genes (ermC, ermA, blaZ, ermB, aac-apD,), resistance to antibiotics β-Lactam penicillin through penicillin-binding proteins (PBPS) coded by the gene cassette mecA SCCmec (Yok-Al Que and Moreillon, 2009). The isolates of *Streptococcus spp*. bacteria were found to be absolutely resistant (100%) to Amoxicillin antibiotics; separately resistant (83%) to Cloxacillin antibiotics; and sensitive (83%) and slightly resistant (67%) to Azithromycin antibiotics, and to Ceftazidime antibiotics (67%). For the *Klebsiella spp* isolates they were all resistant to Azithromycin at 100%, Cloxacillin and Amoxicillin at 67% each respectively. In addition, the *Klebsiella ssp*. isolates were 100% sensitive to Ceftazidime.

**Table 3** Antibiotic sensitivity of bacterial as a result of different inhibition zone

| ***Klebsiella spp* 3** | | | | ***Streptococcus spp* 6** | | | | ***Staph.aureus* 15** | | | | **Bacteria** / **Antibiotic** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sensitivity | | resistance | | Sensitivity | | resistance | | Sensitivity | | resistance | | |
| % | N | % | N | % | N | % | N | % | N | % | N | |
| 33 | 1 | 67 | 2 | 17 | 1 | 83 | 5 | 20 | 3 | 80 | 12 | Cloxacillin |
| 0 | 0 | 100 | 3 | 83 | 5 | 17 | 1 | 7 | 1 | 93 | 14 | Azithromycin |
| 33 | 1 | 67 | 2 | 0 | 0 | 100 | 6 | 27 | 4 | 73 | 11 | Amoxicllin |
| 100 | 3 | 0 | 0 | 67 | 4 | 33 | 2 | 27 | 4 | 73 | 11 | Ceftazidime |


**Figure 4** Sensitivity and resistance of bacterial isolates to antibiotics

## 4. Conclusion

The research concludes that the pathogenic bacterial isolates taken from tonsils, *Klebsiella spp*., are *Staphylococcus aureus* and *Streptococcus spp*., resistant to many groups of the antibiotics. It is this, how antibiotics work and are used, and even if new drugs are developed, antibiotic resistance will continue to be a major threat unless behavior changes around the way these drugs are used. The first change has also to include measures to diminish the contagion of infections from vaccination, hand washing, safe sex and proper food hygiene.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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