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Antibiogram Analysis of Pathogenic Bacteria Isolated from Throat Swabs in District Peshawar.

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Abstract

Background: Throat infections, such as pharyngitis and tonsillitis, are common and usually cause symptoms such as sore throat, fever, and discomfort. This study aimed to identify the bacterial pathogens responsible for these infections and analyze their antibiotic resistance patterns that area.

Methods: A total of 40 throat swabs were randomly collected between September 15, 2024, and February 15, 2025, from private clinics in District Peshawar and inoculated onto MacConkey agar, Blood and Nutrient agar at 37^{o}C for 24 hrs. Bacterial pathogens were identified using microscopy, biochemical tests, and sugar fermentation. The antibiotic susceptibility of the isolates was assessed according to CLSI and relevant guidelines.

Results: The bacterial isolates identified in this study included *Neisseria meningitidis* **(**22.86%), *Corynebacterium spp.* (20%), *Staphylococcus aureus* **(**17.14%), and *Moraxella catarrhalis* (14.28%). Other organisms detected were *Streptococcus pyogenes* **(**8.57%), *Listeria spp.* (5.71%), Bacillus *spp.* (5.71%), *and Micrococcus spp.* (5.71%). Antibiotic sensitivity testing was performed using the disc diffusion method with commonly prescribed antibiotics which included azithromycin (15µg), doxycycline (30µg), penicillin (10µg), ceftriaxone (30µg), and amoxicillin (25µg). Azithromycin was the most effective antibiotic, with very little resistance observed, and followed by doxycycline. In contrast, ceftriaxone showed limited effectiveness. A high level of resistance was also observed against penicillin and amoxicillin in this study.

Conclusion: Throat infections require careful monitoring because of the variability in antibiotic resistance among different pathogens. Regular review of resistance patterns is

crucial to ensure proper and effective treatment. Conducting Large-scale studies is necessary to better track and understand antibiotic resistance in the area.

Introduction

Infectious diseases cause numerous illnesses and deaths, accounting for approximately 20% of all health issues (Murray & Lopez, 1997). Nearly 25% of people suffer from two to three sore throat infections yearly, making it the most widespread illness (Fry, 1979). Throat discomfort, also referred to as throat pain or inflammation of the throat, and is commonly used to describe inflammation of the pharynx or tonsils (Oxford *et al*., 2013). It may result in severe pain, fever, headache (Azeez, 2000), continuous nasal discharge, and a sensation of fullness in the ear (Elaine *et al*., 1987). Throat infections are among the top ten reasons people visit emergency rooms for treatment (Bhatia & Khutpale, 2011).

Tonsillitis or Inflammation of the tonsils, occurs rapidly within a short period (Ferri, 2015). According to Wang *et al*. (2017), *Group A beta-hemolytic streptococcus* is the most common bacterium causing tonsillitis. Babaiwa & JO. (2013) investigated from Nigeria, and Bakir and Ali. (2016), investigated from Iraq showed that *Staphylococcus aureus* was the major bacterium isolated from tonsillitis at the rates of (30.5%) and (70%). Pharyngitis is characterized by inflammation of the pharynx, which causes an itchy and painful throat (Kliegman *et al*., 2020). It is sometimes, symptom of another disease, such as the common flu or cold (Idowu *et al*., 2020). Aalbers *et al*. (2011) found that the majority of throat infections are caused by viral pharyngitis, accounting for approximately 80% of the total cases. Bacterial infections account for approximately 30% to 40% of acute pharyngotonsillitis cases, highlighting their significant role in this condition (Brook & Dohar, 2007).

Researchers have concluded that common pathogenic bacteria of the throat include *Streptococcus pyogenes, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae* (Murray *et al*., 2003), and *Neisseria meningitidis* (Fry, 1979). *S. pyogenes* can cause pharyngitis, commonly in school-aged people and children from low socioeconomic backgrounds (World Health Organization, 1981). Kumar *et al.* (2018) stated that *S. pyogenes* is gram-positive, catalase-negative, and β-hemolytic, with positive bacitracin sensitivity (Patel *et al*., 2019). According to Kumar *et al*. (2019), *M. catarrhalis* is catalase and oxidase positive, while gram staining, indole and urease negative bacteria. Bhattacharya *et al*. (2019), Stated that *N. meningitidis* is gram negative, indole negative, catalase, oxidase positive and, maltose fermenting bacteria. In certain parts of the world, *S. aureus* is an infectious agent of the throat. It is a gram positive, coagulase-positive, catalase-positive, and oxidase-negative bacterium (Hasary & Kareem, 2022).

Antibiotics are antimicrobial chemotherapeutic agents (Neu & Gootz, 2014). The mechanism of action of such antibiotics is the inhibition of cell wall synthesis, interference with protein, cell membrane, nucleic acid, and inhibition of any metabolic pathway (Strohl, 1997). The use of antibiotics has efficiently reduced the mortality rate of bacterial infections (Blair *et al*., 2015). However, due to the overuse or misuse of antibiotics, bacterial resistance is continuously increasing (Verma *et al*., 2021). The World Health Organization has stated that the misuse of antibiotics is one of the main reasons for the development of bacterial resistance (Harbarth *et al*., 2015).

Other factors responsible for antibiotic resistance include biofilm production and the synthesis of enzymes that degrade the antibiotics (Wilke *et al*., 2005). According to the CDC, approximately 2 million infections are due to antibiotic resistance, resulting in more than 20,000 deaths and a loss of $55 billion annually in the United States of America (Reta *et al*., 2019). Despite efforts to reduce the excessive consumption of antibiotics in hospitals and communities, this trend continues to grow (Raveh *et al.*, 2001).

Throat infections are common and affect many people every year, but surprisingly little is known about which bacteria are causing them in our local communities or how resistant these bacteria are to antibiotics. Understanding this is important, because antibiotic misuse is a growing problem that can make infections harder to treat. Therefore this study aimed identify the bacterial strains responsible for throat infections in the area and investigate their antibiotic resistance, providing insights that could help guide better treatment and prevention strategies.

Materials and methods

Experimental Design: The study was conducted in Peshawar, which belongs to the province of Khyber Pakhtunkhwa, Pakistan, during the period of 15 September 2024 to 15 February 2025. Forty throat swab samples were collected from patients randomly in private clinics. The samples were transported according to standard protocols (Collee *et al.*, 2007).

Isolation and Identification:

Samples were processed in the Microbiology Laboratory of Abasyn University Peshawar and cultured on blood agar, MacConkey agar, and nutrient agar, then incubated at 37°C for 24 h to observe the bacterial isolates (Murray *et al*., 2003). For the microscopic properties, the procedure described by Chaskes and Austin (2021) was followed. Standard biochemical tests were performed, including the catalase test (Khatoon *et al*., 2022), coagulase test (Hartline, 2023), indole test (MacWilliams, 2009), citrate test (Hartline, 2023), urease test (BD Diagnostics, 2009), and oxidase test (Reynolds, 2024). Sugar fermentation tests for glucose, maltose, mannitol, sucrose, and lactose, as well as additional tests, were performed for precise identification.

Antibiotic Susceptibility:

Antibiotic susceptibility was tested using the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). Mueller-Hinton agar plates were prepared, and the bacterial isolates were spread onto the agar. Antibiotic discs were applied, including doxycycline (30 µg), azithromycin (15 µg), ceftriaxone (30 µg), penicillin (10 µg), and amoxicillin (25 µg), were applied. The plates were incubated at 37°C for 24 h. Following incubation, the results were interpreted according to the CLSI 2024 guidelines. For bacteria with missing CLSI breakpoints then guidelines and relevant literature were used.

Results

Prevalence of bacteria: A total of 40 clinical throat swabs were collected for this study. Of these, 35 (87.5%) showed bacterial growth, whereas five samples (12.5%) showed no growth (Figure 1).

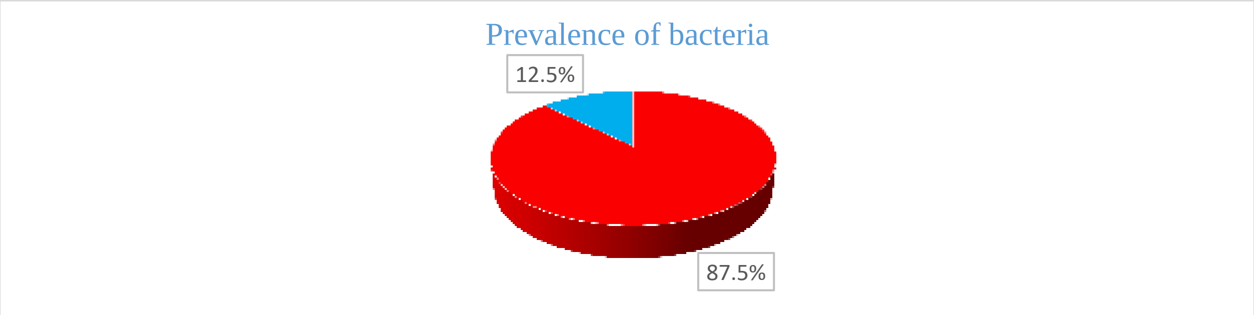

Figure 1-Prevalence of bacterial isolates in throat swabs.

Gender wise distribution of samples: Of the 40 swab samples, 27 (67.5%) were collected from male patients, with 24 (88.9%) tested positive and 3 (11.1%) tested negative. Thirteen samples (32.5%) were from female patients, of which 11 (84.6%) were positive and 2 (15.4%) were negative (Figure 2).

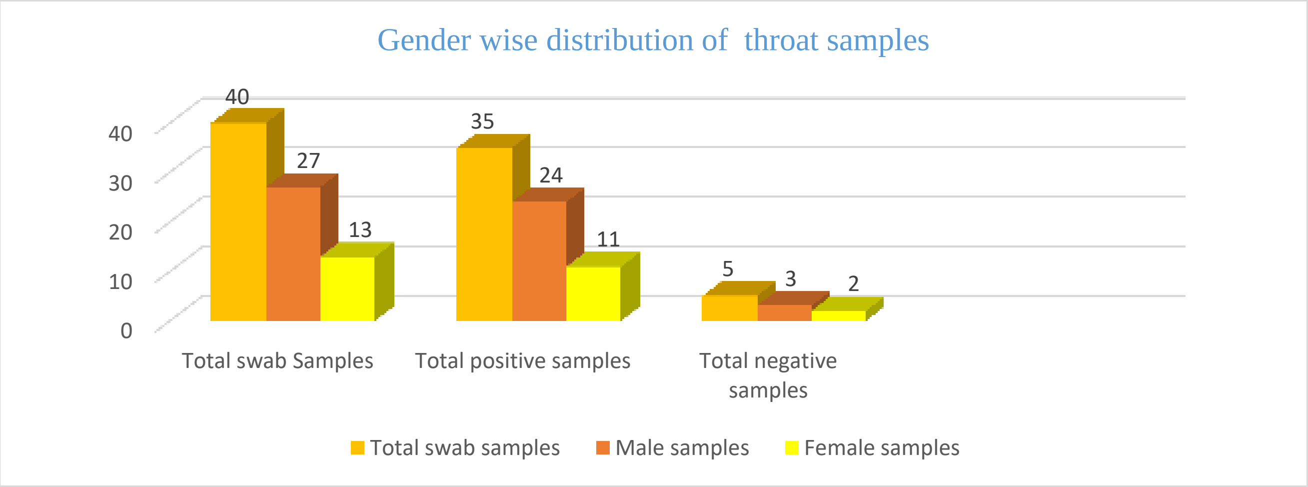

Figure 2- Shows gender wise distribution of throat samples.

Bacterial Isolates: Bacterial isolates were identified using differential staining, biochemical tests, and sugar fermentation assays. The identified bacterial isolates are summarized below.

Table 1- below Shows the differential, biochemical and sugar fermentation test conducted for bacterial identification.

NO	Name of bacteria	G Staining	Shape	Catalase	Oxidase	Urease	Coagulase	Citrate	Heamolysis	MSA	Indole	Maltose Fermentation	Glucose Fermentation	Lactose Fermentation	Sucrose Fermentation	Additional Test 1. H_2S production 2. Galactose and Fructose 3. Fermentation 4. Growth on salt media
1	C. species	+	Rod	+	–	–	–	–	+/-	–	–	+	+/-	–	–	1. H_2S + 2. Galactose + 3. Fructose +
2	N. meningitidis	–	Cocci pairs	+	+	–	–	–	–	–	–	+	+	–	–	Not performed
3	S. aureus	+	Cocci Grapes	+	–	w +	+	+	+ β	+	–	+	+	+	+	1. H_2S – 2. Glucose and Fructose fermentation +
4	M. catarrhalis	–	Cocci Pairs	+	+	+	–	–	–	–	–	–	–	–	–	Not performed
5	S.	+	Coc	–	–	–	–	–	+	–	–	+	+	–	+	1. G and F

	pyogenes		**ci Chains**						**β**							**fermentation +**
6	**Listeria spp**	+	**Coccobacillus**	+	–	–	–	–	+ β	–	–	+	+	**w+**	–	**7.5 % salt medium, -ive growth**
7	**Bacillus spp**	+	**Rod**	+	–	–	–	+	+/-	-/+	–	+	+	–		**7.5 % salt medium, +ive growth**
8	**Micrococcus species**	+	**Cocci Tetrad**	+	+	**w+**	–	–	–	–	–	**w+**	+	–	–	**Not performed**

β+, Shows beta hemolysis. H2S -, shows no H2S production. w+ shows weak positive reaction. +/- , species show variable results.

Table 2- Shows bacterial isolates from throat swab samples

S.no	**Name of bacteria**	**Total Number**	**Percentage**
1	*Corynebacterium spp*	7	20%
2	*Neisseria meningitidis*	8	22.857%
3	*Staphylococcus aureus*	6	17.14%
4	*Moraxella catarrhalis*	5	14.283%
5	*Streptococcus pyogenes*	3	8.57%
6	*Listeria spp.*	2	5.71%
7	*Bacillus spp.*	2	5.71%
8	*Micrococcus spp.*	2	5.71%
		Total= (35)	Total 100%

Resistance of Bacterial Isolates to Antibiotics: Bacterial isolates exhibited high resistance to penicillin, followed by amoxicillin. Some isolates also showed high resistance to Ceftriaxone as shown in Fig, 3.

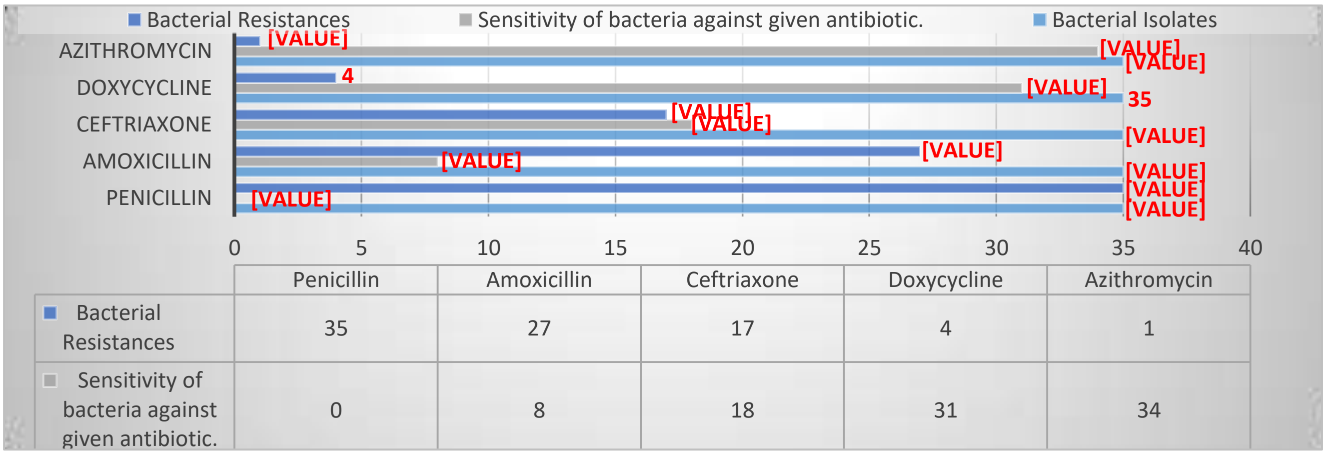

Figure 3- Shows the sensitivity and resistance of bacterial isolates against antibiotics.

Discussion

Research conducted by Rahman *et al*. (2022), involved collection of 111 throat samples. Of these, 27 samples 24% showed bacterial growth, whereas 84 samples (76%), did not yield any pathogenic isolates. The most common organism identified was *S. aureus,* accounting for 55.55%. Following that, *Streptococcus spp.* made up 18.51%, *K. pneumoniae* represented 14.81%, and *Pseudomonas spp.* comprised 11.11%. According to our findings, a total of 40 samples were collected, of which 35 (87.5%) tested positive, while 5 (12.5%) were negative for bacterial isolates. *N. meningitidis* was the most commonly detected bacterium, accounting for 22.86% of the total isolates, followed by *Corynebacterium spp.* at 20% and *S. aureus* at 17.14%. Other bacterial species identified in smaller amounts included *M. catarrhalis* (14.28%), *S. pyogenes* (8.57%), and *Listeria spp* (5.71%). *Micrococcus and Bacillus spp.* accounted for (5.71%) of the isolates.

Regarding *S. pyogenes*, previous studies reported resistance rates of 50% to penicillin (Chandra *et al.*, 2017), resistance to ceftriaxone was 86.7% (Al-Badaii *et al*., 2021) and 50% (Sayyahfar *et al.,* 2015), amoxicillin 16.6% (Atia *et al.,* 2020) and 100% (Akinterinwa *et al*., 2023), and 44% to doxycycline (Wikipedia contributors, 2024).In contrast, our observations revealed that *S. pyogenes* was fully resistant to penicillin and amoxicillin but fully susceptible to ceftriaxone, azithromycin, and doxycycline.

The resistance of *S. aureus* to azithromycin was reported to be 20% (Al-Badaii *et al*., 2021). Resistance to doxycycline was 33.33% (Rahman *et al*., 2022) and resistance to amoxicillin was 35% (Atia *et al.*, 2020) and 100% (Akinterinwa *et al*., 2023). Bakir and Ali (2016) from Iraq and Agrawal *et al.* (2014) from India reported that *S. aureus* resistance to β-lactam antibiotics was 92.5% and 75%, respectively. In contrast, our results indicated that all *S. aureus* isolates were 100% resistance to penicillin, 83.33% resistance to amoxicillin, 50% resistance to ceftriaxone, and fully sensitive to doxycycline and azithromycin

Hatami *et al*. (2024) found that *M. catarrhalis* showed complete resistance to penicillin and 81.2% resistance to amoxicillin. Additionally, resistance rates were reported as 73.3% for ceftriaxone (Al-Badaii *et al*., 2021), 75% for azithromycin (Raveendran *et al*., 2020), and 28.10% (Zhao *et al*., 2022). Notably, macrolides are the most effective antibiotics against *M. catarrhalis,* with 0% resistance (Hatami *et al*., 2024). Our study indicated that all *M. catarrhalis* isolates were completely resistant to penicillin, with 60% resistance to amoxicillin and 40% to ceftriaxone, while being fully sensitive to azithromycin and doxycycline.

N. meningitidis showed 100% resistance to penicillin, 66.7% resistance to ceftriaxone (Fenta *et al*., 2020), 33.3% to amoxicillin, and 30% to penicillin (Khademi & Sahebkar, 2019). Furthermore, 5% of the isolates demonstrated resistance to azithromycin (Mulatu, 2019). Conversely, our analysis revealed that all *N. meningitidis* isolates were entirely resistant to penicillin and amoxicillin, with resistance rates of 87.5% for ceftriaxone, 37.5% for doxycycline, and 12.5% for azithromycin.

The resistance rates of *Corynebacterium spp.* were 91.2% for penicillin, 45.2% for tetracycline (Ozdemir *et al*., 2021), and 72% for ceftriaxone (Yahia sami, 2021). A study conducted by Arcari *et al.* (2023) reported that no resistance gene for amoxicillin was found in *Corynebacterium diphtheriae*. However, they reported that certain species of *diphtheriae* have genes that alter the target site, resulting in resistance to macrolides such as azithromycin. Conversely, this study indicated that *Corynebacterium spp.* exhibited complete resistance to penicillin, with resistance rates of 57.14% for amoxicillin, 42.85% for ceftriaxone, and 14.28% for doxycycline. Notably, no resistance to azithromycin was observed.

Bacillus species are commonly found foodborne pathogens that can cause illness, with *Bacillus cereus* being a well-known example. In the European Union, approximately 500-700 confirmed cases of foodborne diseases caused by *B. cereus* are reported annually (Bottone, 2010). Earlier studies have reported that *Bacillus species* may be resistant to antibiotics such as penicillin, ceftriaxone, and azithromycin (Cheung & Chan, 2022). In contrast, our study revealed that *Bacillus spp.* exhibited complete resistance to penicillin and amoxicillin but showed no resistance to ceftriaxone, doxycycline, and azithromycin.

In typical clinical presentations, *Listeria* infections do not primarily cause pharyngitis or throat infections in healthy individuals (Schlech, 2019). *Listeria spp*., particularly *L. monocytogenes*, are well-known bacteria that can spread through food consumption. Because they are widely present in the environment, they often contaminate food products, making them a common concern for food safety (Gandhi & Chikindas, 2007). The isolates were completely sensitive to macrolides (Tirziu *et al.,* 2021), and were fully resistant to penicillin (Duma *et al*., 2024). *L. monocytogenes* showed 67% resistance to amoxicillin/clavulanic acid (Bouymajane *et al.,* 2021). Our study indicated that all isolates of *Listeria spp* were completely resistant to penicillin and ceftriaxone. Additionally, 50% showed resistance to amoxicillin, while there was no resistance observed to doxycycline and azithromycin.

Raheem and Said (2023) determined that *M. luteus* showed completely resistant to penicillin and ceftriaxone and exhibited 60% resistance to azithromycin. However, the present study found that all isolates of *Micrococcus spp.* probably *M. luteus* were completely resistant to penicillin, 50% resistant to amoxicillin, and showed no resistance to ceftriaxone, doxycycline, or azithromycin.

In many countries, antibiotics contain 30-50% of prescription drugs among therapeutic agents. Although antibiotic prescription is essential in most bacterial infections, and avoiding it may endanger the patient's life. Most studies have shown that 30-60% of the prescribed drugs are inappropriate. Indeed, the physicians might make the wrong prescription, or people may use a kind of self-medication (Markotic *et al*,. 2019).

Conclusion

These findings highlight the growing concern of antibiotic resistance among common throat pathogens and emphasize the importance of selecting appropriate antimicrobial therapy. The results contribute valuable insight into the local epidemiology of throat infections and can support evidence-based treatment strategies. Furthermore, continuous monitoring of resistance patterns is essential to ensure effective clinical management. Future large-scale studies are recommended to better understand and control the spread of antibiotic-resistant bacteria in the region.

Limitations of the study

This study has a few important limitations. First, the sample size was quite small, with only 40 samples included, making it difficult to generalize the findings to a larger population. Also, the study was carried out only in Peshawar, so the results may not reflect the situation in other regions. Another limitation is that samples were collected only from private clinics, which may not represent all types of patients. In addition, bacterial identification relied on conventional culture and biochemical methods rather than molecular techniques. Lastly, only a limited number of antibiotics were tested, so the overall resistance pattern may not be fully understood.

Recommendations

Reduce the use of penicillin and amoxicillin due to significant resistance, prioritize azithromycin and doxycycline as primary treatment options.

Conduct ongoing research into the effectiveness of new antibiotics.

Promote research on natural antimicrobial agents and bacteriophage therapy.
Increase public awareness about the risks associated with antibiotic misuse.

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Conflict of interest: None declared

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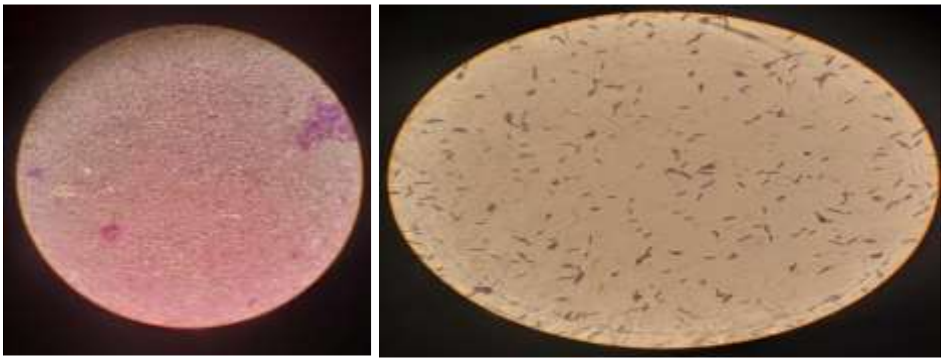
Annexure

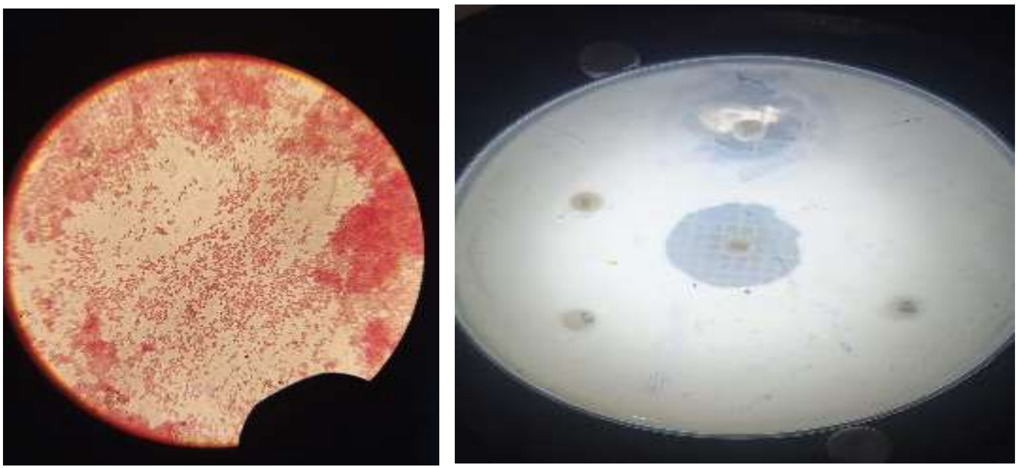


Gram positive *staphylococcus aureus* and Gram positive *corynobacterium spp*

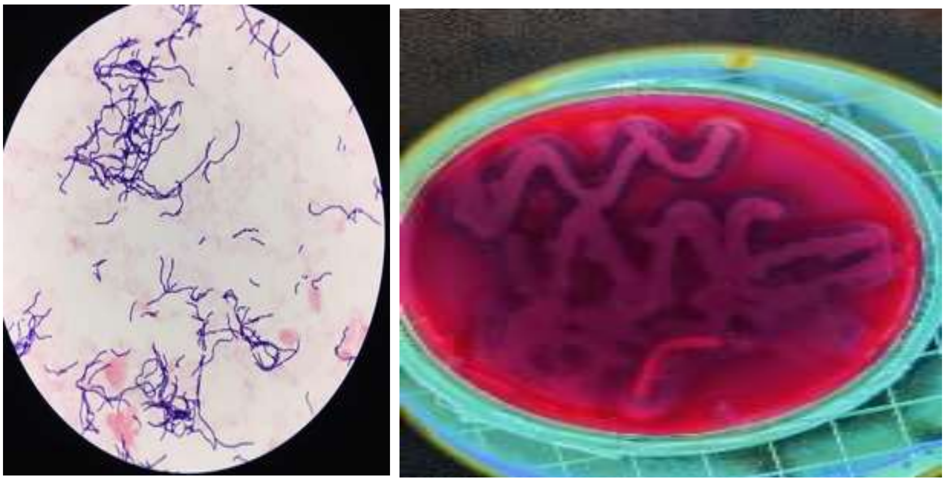
Catalase positive and oxidase positive vs negative result**.**

Maltose positive fermentation of *Neisseria meningitidis* and Negative fermentation by *Moraxella catarrhalis*.

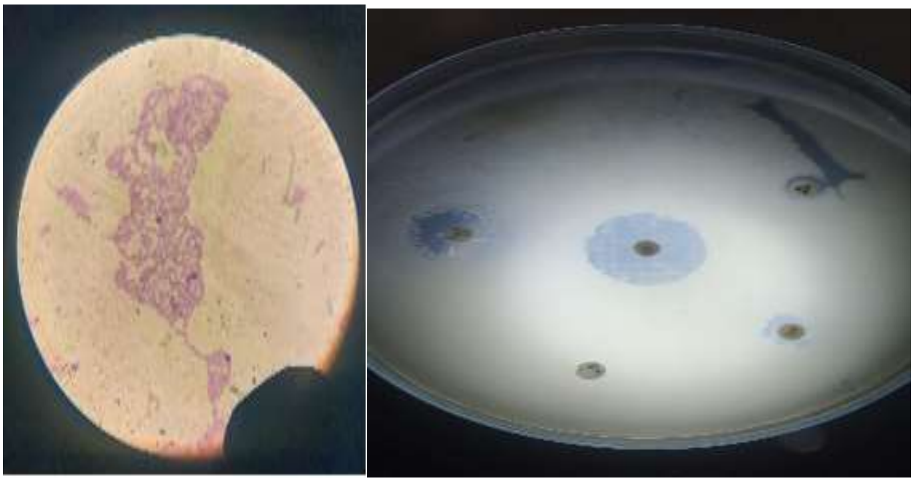
Citrate positive results vs citrate negative, Urease positive vs urease negative results



Moraxella catarrhalis and Culture Susceptibility



Streptococcus pyogenes and its hemolysis



Neisseria meningitidis and Culture susceptibility testing

Coagulase positive result and Culture sensitivity

No Growth on MacConkey agar by *Moraxella catarrhalis* while positive mannitol fermentation by *S. aureus.*