Title: **Antimicrobial Susceptibility Pattern of Streptococcus pyogenes Isolated from Patients with Respiratory Tract Infections in ESUT Teaching Hospital Parklane**

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ABSTRACT

Respiratory infections are the leading cause of heavy burden to public health.The commonly known respiratory bacteria pathogens are Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas species, Klebsiella species, Haemophilus influenzae ,streptococcus pyogene. The increasing prevalence of respiratory tract infections (RTIs) caused by Streptococcus pyogenes has highlighted the need for comprehensive data on its antimicrobial susceptibility patterns. To isolate and characterize streptococcus pyogenes from patients with respiratory tract infections in ESUTH .To determine the demographic factors associated with streptococcus pyogenes from patients with respiratory tract infections in ESUTH .This is a cross-sectional investigation into the isolation and identification of streptococcus pyogen obtained from individuals with respiratory tract infection at the Enugu State University Teaching hospital parklane.

Sample collection method: the sample was collected in a sterile container.sample preparation:macroscopic examination,culture method.Identification test:gram staining,bacitracin test.antimicrobial sensitivity test:Disk diffusion method.Total number of sputum cultured was 150 samples according to crochan method ..Result:No bacteria growth:55%,respiratory infection:45%,Streptococcuspyogene:20.4%.sensitive to ciprofloxacin:72:3%,Ofloxacin:81.8%,cefotraxime:81.8%etc.resistant too amoxicillin:90.9%,pefloxacin:90.9%,cefotraxone:81.8%,Cefuroxime90.9%, Chloramphenicol:90.9%etc.Male:41.7%,female:58.3%. The antimicrobial susceptibility patterns of Streptococcus pyogenes isolated from respiratory tract infections reveal critical insights into the management of these infections. Despite the consistent susceptibility of S. pyogenes to beta-lactam antibiotics, such as penicillin and amoxicillin, the emergence of

resistance to alternative treatments like macrolides and clindamycin is a growing concern, particularly in regions with high antibiotic misuse. Penicillin and ofloxacin are the gold standards for treating GAS respiratory infections

Alternatives for Penicillin-Allergic Patients For patients with documented beta-lactam allergies:Macrolides (e.g., erythromycin, azithromycin, clarithromycin):

Introduction

Streptococcus pyogenes, also known as Group A Streptococcus (GAS), is a common pathogen responsible for a wide range of infections, including upper respiratory tract infections (URIs) such as pharyngitis and tonsillitis. S. pyogenes is typically sensitive to penicillin; however, the rise of antimicrobial resistance is a growing concern. Inappropriate use of antibiotics contributes to the emergence of resistant strains. Understanding the local antimicrobial resistance patterns of S. pyogenes is crucial to optimizing treatment and reducing the burden of infections. This study seeks to investigate the antimicrobial susceptibility profile of S. pyogenes isolates from patients with RTIs at ESUT Teaching Hospital Parklane, Enugu, Nigeria.Respiratory tract infections are termed as the infectious diseases of the respiratory tract and are the leading illnesses globally(Mirsaeidi, M et al., 2016).These infections AAare classified as upper and lower respiratory tract infections and are the leading cause of morbidity and mortality especially in developing countries. Respiratory infections are the leading cause of heavy burden to public health(Prajapati, B.,et al., 2011).The commonly known respiratory bacteria pathogens are Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas species, Klebsiella species, Haemophilus influenzae ,streptococcus pyogene(Guclu, A.U et al., 2021). Resistance to antibiotics is a global challenge to the health sectors especially in Kenya. This has been attributed to the emergence of mutant bacteria strains. Respiratory bacterial pathogens that are associated with reduced susceptibility to multiple classes of antibiotics include Pseudomonas aeruginosa, Streptoococcus pneumoniae and Mycobacterium tuberculosis(Shah S, Ullah B et al., 2016).

Streptococcus pyogenes is a significant bacterial pathogen

responsible for a variety of infections, including respiratory tract infections(Berwal, A. et al., 2019). The antimicrobial susceptibility pattern of this bacterium is crucial for managing infections effectively, particularly as resistance to commonly used antibiotics becomes an increasing concern. In respiratory infections, Streptococcus pyogenes is often isolated from sputum samples.

Streptococcus pyogenes, a Group A Streptococcus (GAS), is a major pathogen responsible for both mild and severe infections(Brouwer, S et al., 2023). Although it primarily causes upper respiratory tract infections like pharyngitis, it can be isolated from sputum in cases of more severe infections such as pneumonia. Understanding the antimicrobial susceptibility pattern of S. pyogenes is essential for effective treatment, as this bacterium can exhibit resistance to certain antibiotics (Beres, S.B.et al., 2022). Global and Regional Susceptibility Trends Penicillin remains the most effective antibiotic against S. pyogenes, with virtually no reported resistance globally, maintaining its position as the first-line treatment(Muteeb, G et al., 2023).,.Understanding the antimicrobial susceptibility patterns of Streptococcus pyogenes (Group A Streptococcus) isolated from sputum samples is critical for several

reasons:

1. Rising Antimicrobial Resistance Although S. pyogenes remains largely susceptible to penicillin, the rise in resistance to other antibiotics, especially macrolides (e.g., erythromycin), clindamycin, and tetracyclines, is a growing concern in many parts of the world (Cattoir, V.et al.,, 2022).. Studies have shown increased macrolide resistance, driven by the widespread use of these antibiotics in both clinical settings and over-the-counter purchases.

2. Guiding Empirical Therapy In many healthcare settings, particularly in developing countries, empirical treatment is often initiated before the causative pathogen is confirmed through laboratory testing (Mapala, L.et al.,2022). If local antimicrobial resistance patterns are unknown, empirical therapy may be less effective, resulting in prolonged infections, higher treatment costs, and increased morbidity. Thus, updating susceptibility patterns regularly can guide the appropriate use of antibiotics and improve treatment outcomes (Bassetti, S.et al., 2022)

3. Preventing Treatment Failures Accurate knowledge of the local resistance patterns of S. pyogenes is essential to prevent treatment failures, especially in lower respiratory infections, which are more severe than upper respiratory tract infections(Efstratiou, A et al.,2022). The inappropriate use of antibiotics can not only lead to treatment failure but also promote the development and spread of resistant strains

Streptococcus pyogenes, a Gram-positive bacterium, is a common cause of respiratory tract infections (RTIs) such as pharyngitis, tonsillitis, and sinusitis. The proper treatment of these infections heavily relies on effective antimicrobial therapy. However, in recent years, there has been growing concern about the antimicrobial resistance patterns emerging in S. pyogenes isolates, complicating the management of these infections. The antimicrobial susceptibility patterns of S. pyogenes play a crucial role in guiding treatment decisions. Historically, penicillin and other β -lactam antibiotics have been the mainstay of treatment for S. pyogenes infections. However, increasing resistance to macrolides, tetracyclines, and other antibiotic classes has been reported in various regions, making empirical treatment challenging and raising concerns about the potential future resistance to frontline therapies. The lack of updated and region-specific data on the susceptibility patterns of S. pyogenes isolates from patients with respiratory tract infections limits clinicians' ability to prescribe the most effective antibiotics, leading to the potential for treatment failure, longer recovery times, and higher healthcare costs. Additionally, inappropriate antibiotic usage contributes to the development of further resistance.

Materials and Methods

Study Design:

This was a cross-sectional, laboratory-based study conducted from 1st August to 3rd November 2024 at ESUT Teaching Hospital Parklane, Enugu.This is a cross-sectional investigation into the isolation and identification of streptococcus pyogen obtained from individuals with respiratory tract infection at the Enugu State University Teaching hospital parklane. Enugu State Teaching Hospital (ESUTH), commonly referred to as Parklane, is a prominent medical institution located in Enugu, the capital city of Enugu State, Nigeria. It serves as a center for medical education, healthcare delivery, and research. Below is an overview of ESUTH Parklane:

History and Background

- Origin: The hospital started as a general hospital and was later upgraded to a teaching hospital to support the medical training of students from the Enugu State University College of Medicine.
- Affiliation: ESUTH Parklane is affiliated with the Enugu State University of Science and Technology (ESUT), particularly its Faculty of Medicine.
- Location: Situated in the heart of Enugu city, it is accessible to residents of Enugu and neighboring states, making it a referral center for the southeastern region of Nigeria.

INCLUSION CRITERIA;

- Patients with symptoms of respiratory tract infection (RTI) such as sore throat, cough, runny nose, and fever.
- Patients who are willing to provide a sputum sample.

3**EXCULSION CRITERA**

- Patients who have recently taken antibiotics may be excluded because antibiotics can interfere with the isolation
- Patients with a history of allergic reactions to the reagents or culture media used for isolation.

Sample Collection:

A total of 150 sputum were collected from patients aged 5 to 65 years presenting with symptoms of RTIs, including sore throat, fever, and cough. Informed consent was obtained from each participant, and ethical approval was granted by the hospital ' s institutional review board.Sputum samples will be collected from patients with respiratory tract infections

Sample Collection

- Collection Method:
- Sputum is collected in a sterile, leak-proof container. The patient should be instructed to provide a deep cough sample, not saliva.
- Ensure aseptic technique during collection.
- Transportation:
- Transport the sample to the laboratory promptly, ideally within 2 hours, to avoid overgrowth of contaminants.
- If delayed, refrigerate at 4°C.

Sample Preparation

- Macroscopic Examination:
- Assess the sample for color, consistency, and presence of pus or blood. Sputum should be thick and purulent for optimal processing.
- Microscopic Examination (Gram Stain):
- Perform a Gram stain to detect gram-positive cocci in chains, which is characteristic of S. pyogenes.
- Also assess for leukocytes (indicating infection) and epithelial cells (indicating contamination).

Primary Culture

- Media:
- Inoculate the sputum sample on:
- Blood Agar (BA): Detects beta-hemolysis (complete hemolysis) characteristic of S. pyogenes.
- Chocolate Agar: For enhanced growth if other pathogens are suspected.
- Inoculation Technique:
- Use a sterile loop to streak the sample onto the agar plates.
- Incubation Conditions:
- Incubate plates at 35–37°C in a 5% CO_2 -enriched environment for 18–24 hours.

Colony Morphology

- Observation:
- On blood agar, S. pyogenes forms small (1–2 mm), round, translucent colonies with a clear beta-hemolysis zone.

Identification Tests

- Gram Staining:

- Confirm gram-positive cocci in chains.
- Catalase Test:
- Negative (differentiates Streptococcus from catalase-positive Staphylococcus).
- Bacitracin Sensitivity Test:
- S. pyogenes is sensitive (zone of inhibition around bacitracin disk).

Antimicrobial Susceptibility Testing .

- Disk Diffusion Method:
- Use Mueller-Hinton agar with 5% sheep blood.
- Test susceptibility to penicillin, erythromycin, clindamycin, and other relevant antibiotics.
- Interpretation:
- Follow CLSI or EUCAST guidelines for zone size interpretation.

Reporting Results

- Report as Streptococcus pyogenes based on the following criteria:
- Beta-hemolysis on Blood Agar.
- Gram-positive cocci in chains.
- Negative catalase test.
- Positive bacitracin sensitivity and/or PYR test.

GRAM STAINING

Gram staining is a differential staining technique used to classify bacteria into two major groups: Gram-positive and Gram-negative, based on the characteristics of their cell walls. Below is the step-by-step procedure for Gram staining:

Materials Needed

1. Clean microscope slides.
2. Inoculating loop.
3. Bacterial sample (e.g., sputum, culture).
4. Reagents:
- Crystal violet (primary stain).
- Gram's iodine (mordant).
- 95% ethanol or acetone-alcohol (decolorizer).
- Safranin (counterstain).
5. Bunsen burner or heat source.
6. Distilled water.
7. Blotting paper.
8. Microscope.

Procedure

1. Preparing the Smear

1. Place a small drop of sterile distilled water in the center of a clean glass slide (if using a culture sample).
2. Use a sterile inoculating loop to collect a small amount of the bacterial sample and mix it with the water on the slide. For clinical samples like sputum, spread a thin layer directly onto the slide.
3. Spread the suspension into a thin, even smear.
4. Air-dry the smear completely.
5. Heat-fix the smear by passing the slide quickly through a Bunsen burner flame 2 –3 times. This step kills the bacteria and fixes them to the slide.

2. Applying the Stains

1. Crystal Violet (Primary Stain):
- Cover the smear with crystal violet.
- Allow it to sit for 1 minute.
- Rinse the slide gently with distilled water to remove excess stain.
2. Gram's Iodine (Mordant):
- Flood the smear with Gram's iodine solution.
- Let it sit for 1 minute.
- Rinse with distilled water.

(Gram's iodine forms a complex with crystal violet inside the cell walls.)

3. Decolorization:
- Hold the slide at an angle and add a few drops of ethanol or acetone-alcohol until no more purple runs off (approximately 10–15 seconds).
- Immediately rinse with distilled water to stop the decolorization process.

(Gram-positive bacteria retain the crystal

violet-iodine complex, while Gram-negative bacteria lose it due to thinner peptidoglycan layers.)

4. Safranin (Counterstain):

- Flood the smear with safranin.
- Let it sit for 30–60 seconds.
- Rinse with distilled water and gently blot dry with blotting paper.

3. Microscopic Examination

1. Place the slide under the microscope, starting with the lowest objective lens (10x) to focus.
2. Switch to the oil immersion lens (100x) for detailed examination. Use immersion oil for clarity.

Interpretation of Results

- Gram-positive bacteria: Appear purple or blue (retain crystal violet).
- Thick peptidoglycan layer traps the stain.
- Gram-negative bacteria: Appear pink or red (stained by safranin).
- Thin peptidoglycan layer allows the crystal violet to be washed out.

BACITRACIN SENSITIVITY TEST

The bacitracin sensitivity test is a straightforward and reliable method for identifying Streptococcus pyogenes (Group A streptococci) from sputum or other clinical samples. This test takes advantage of the fact that S. pyogenes is typically sensitive to bacitracin, whereas most other streptococcal species are resistant.

Materials Needed

1. Blood Agar (BA) plate.
2. Isolated colonies of the bacterial culture from the sputum sample.
3. Bacitracin (0.04 units) disk.
4. Sterile forceps.
5. Incubator (35–37°C with 5% CO_2 atmosphere).

Procedure

1. Preparation of the Plate:

- Use an inoculating loop to pick a colony of the suspected streptococci from the Blood Agar plate.
- Streak the colony on a new Blood Agar plate to create a lawn or streak pattern for confluent growth.

2. Placement of the Bacitracin Disk:

- Using sterile forceps, place a bacitracin disk in the center or near the streaked area on the Blood Agar plate.
- Ensure the disk is firmly in contact with the agar surface.

3. Incubation:

- Incubate the plate at 35–37°C in a 5% CO_2 atmosphere for 18–24 hours.

4. Observation:

- After incubation, observe the area around the bacitracin disk.

Interpretation of Results

- Sensitive (Positive Result):
- A clear zone of inhibition ($\geq$10 mm) around the bacitracin disk indicates bacitracin sensitivity. This strongly suggests Streptococcus pyogenes.
- Resistant (Negative Result):
- Growth up to the edge of the bacitracin disk indicates resistance. This result suggests that the organism is not S. pyogenes.

Controls

- Positive Control: Use a known S. pyogenes strain to validate sensitivity.
- Negative Control: Use a strain of another streptococcal species (e.g., Streptococcus agalactiae) to confirm resistance.

Limitations

1. Bacitracin sensitivity is not entirely specific to S. pyogenes; some other beta-hemolytic streptococci may occasionally show sensitivity.
2. For definitive identification, use additional tests like the PYR test, Lancefield

grouping, or molecular methods.

Isolation of Streptococcus pyogenes:

The sputum were cultured on 5% sheep blood agar and incubated at 37 ° C for 24-48 hours. Identification of S. pyogenes was performed based on colony morphology, Gram stain, catalase test, and a positive result for bacitracin sensitivity.

Patients at ESUTH, Parklane (n=150)

Prevalence.	N	%
No Bacteria Growth	86	55.0
Respiratory Tract Infections.	64	45.0
Streptococcus pyogenes	11	20.4

Antimicrobial Susceptibility Testing:

The antimicrobial susceptibility of S. pyogenes isolates was tested using the Kirby-Bauer disk

Antibiotic susceptibility of Streptococcus pyogenes from patients with respiratory tract infections in ESUTH, Parklane.

Antibiotics	Antibiotic susceptibility patterns	
	Sensitive	Resistance
N (%)		
Ciprofloxacin	8 (72.7).	3 (27.3)
Ofloxacin	9 (81.8)	2 (18.2)
Ceftriaxone	2 (18.2)	9 (81.8)
Gentamicin	7 (63.6).	4 (36.4)
Cefotaxime	9 (81.8)	2 (18.2)
Chloramphenicol	2 (18.2)	9 (81.8)
Pefloxacin	1 (9.1)	10 (90.9)
Streptomycin	2 (18.2)	9 (81.8)
Amoxicillin	1 (9.1)	10 (90.9)
Clindamycin	3 (27.3)	8 (72.7)
Erythromycin	3 (27.3)	8 (72.7)
Levofloxacin	2 (18.2)	9 (81.8)
Cefuroxime	1 (9.1)	10 (90.9)
Cephalexin	2 (18.2)	9 (81.8)

This outlines the antibiotic susceptibility and resistance patterns of Streptococcus pyogenes isolated from patients with respiratory tract infections at ESUTH, Parklane. The data show substantial variations in the effectiveness of the antibiotics tested. Ofloxacin demonstrated the highest sensitivity at 81.8%, with a resistance rate of 18.2%, closely followed by Cefotaxime, which also showed 81.8% sensitivity and 18.2% resistance. Ciprofloxacin ranked next with a sensitivity rate of 72.7% and resistance at 27.3%. Gentamicin showed moderate effectiveness, with a sensitivity of 63.6% and resistance of 36.4%.

In contrast, several antibiotics had low sensitivity rates and alarmingly high resistance. Pefloxacin and Amoxicillin both had the lowest sensitivity at 9.1%, accompanied by a resistance rate of 90.9%. Similarly, Cefuroxime, Streptomycin, Chloramphenicol, Ceftriaxone, Cephalexin, and Levofloxacin all exhibited poor sensitivity (ranging between 9.1% and 18.2%) and high resistance rates (81.8% to 90.9%). Clindamycin and Erythromycin, with sensitivity rates of 27.3% each, also demonstrated high resistance at 72.7%.

Results

Demographic Data:

The study included 150 patients (30males and 70 females), with a mean age of 25.3 ± 12.4 years. The majority of cases were in children aged 5–14 years (50), followed by adults aged 15–24 years .

Characteristics	
N (%)	
Age	Less than 15years 23 (19.2)
	15-30years 42 (35.0)
	31-65years

22 (15.4)

More than 65years

33 (27.5)

Gender Male

50 (41.7)

Female

70 (58.3)

Martial status Single

45 (38.8)

Married

71 (61.2)

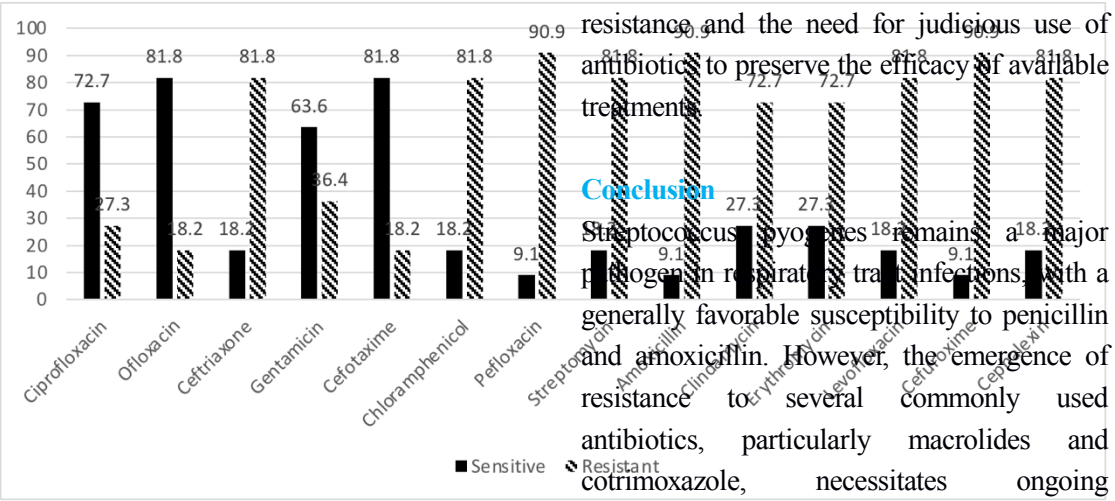
Geographical location Rural

13 (10.8)

Urban

107 (89.2)

PREVALENCE OF STREPTOCOCCUS PYOGENES



Multidrug Resistance (MDR):

A total of 10 isolates (15.4%) showed resistance to three or more classes of antibiotics, indicating the presence of multidrug-resistant strains.

Discussion

This study highlights the antimicrobial susceptibility pattern of S. pyogenes in patients with respiratory tract infections at ESUT Teaching Hospital Parklane. The majority of isolates were still susceptible to penicillin and amoxicillin, which are commonly used to treat S. pyogenes infections. However, the increasing resistance to erythromycin, clindamycin, and cotrimoxazole raises concerns, especially in the context of empiric treatment for RTIs.

The high resistance rate to Pefloxacin (90.9%) and Amoxicillin (90.9%) is consistent with reports from other regions, where macrolide resistance in S. pyogenes is becoming more common due to the overuse and misuse of antibiotics. Resistance to fluoroquinolones (ciprofloxacin) and gentamicin, while lower, also signals potential issues in treatment options. The prevalence of multidrug-resistant strains in this study suggests that alternative therapies may be needed for some patients.

The findings underscore the importance of continued surveillance of antimicrobial resistance and the need for judicious use of antibiotics to preserve the efficacy of available treatments.

Conclusion

Streptococcus pyogenes remains a major pathogen in respiratory tract infections, with a generally favorable susceptibility to penicillin and amoxicillin. However, the emergence of resistance to several commonly used antibiotics, particularly macrolides and cotrimoxazole, necessitates ongoing antimicrobial surveillance and responsible antibiotic stewardship. Clinicians should consider local susceptibility patterns when selecting empirical therapy for respiratory tract infections.

Recommendations

1. Surveillance: Ongoing surveillance of antimicrobial susceptibility of S. pyogenes should be conducted to monitor resistance

trends.

2. Antibiotic Stewardship: Encourage rational use of antibiotics, particularly in the treatment of upper respiratory tract infections.

3. Alternative Therapies: Exploration of alternative treatment regimens for resistant strains, including the use of higher-generation antibiotics or combination therapies, may be warranted.

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