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# Drug Sensitivity Pattern of Bacteria from Dental Extraction: A Microbiological Study

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Introduction:** Teeth are hard structures in the mouth and pharynx of vertebrates, used for eating, defense, and specialized purposes. Bacterial infections often cause purulent inflammations in the head and neck, due to diverse oral microbiota and dental lesions. This study underscores the need for antibacterial mouth rinses and systemic antibiotics, while noting that misuse of antibiotics has led to resistant bacteria, reducing some antibiotics' efficacy.
**Materials:**
**Equipment**: Weighing balance, Petri dishes, Autoclave, Wire loop, Bunsen burner, Incubator, Beakers, Conical flask, Measuring cylinders, Pipettes, Test tubes, Glass slides.
**Specimen Collection:** Swabs from 10 areas of extracted teeth were collected and transported to Benue State University, Makurdi, within 2-3 hours.
**Antibiotics:** Gentamycin, Ofloxacin, Erythromycin, Vancomycin, Ciprofloxacin, Amoxicillin.
**Reagents**: Kovac's reagent, normal saline, tetramethyl-p-phenylenedamine, bromothymol blue, hydrogen peroxide.
**Sterilization**: Glassware was autoclaved at 121 °C for 15 minutes.
**Methods:**
**Media Preparation:** Nutrient Agar, MacConkey Agar, and Peptone Water were prepared, sterilized, and incubated to check sterility.
**Inoculation:** Swabs were used to inoculate media, incubated at 37 °C for 24 hours.
**Purification:** Isolates were sub-cultured, gram-stained, and preserved for biochemical tests.
**Identification:** Based on colony appearance, microscopic examination, and biochemical tests (catalase, citrate, oxidase, indole, coagulase, gram staining).
**Antibiotic Susceptibility**: Kirby-Bauer disc diffusion method was used to test antibiotics, with results interpreted using NCCLS criteria.
**Results:**
**Isolates:** Staphylococcus aureus, Lactobacillus spp., and Streptococcus spp. were identified from 10 patients.
**Antibiotic Susceptibility:** Ciprofloxacin, Ofloxacin, and Gentamycin were highly effective, while resistance to Vancomycin and Amoxicillin was noted.
**Conclusion:** Staphylococcus aureus, Lactobacillus spp., and Streptococcus spp. were the primary bacteria in dental caries. Ciprofloxacin, Ofloxacin, and Gentamycin were effective, while Vancomycin and Amoxicillin resistance is concerning. Proper antibiotic use and routine susceptibility testing are essential for managing dental infections.


*Keywords: Drug sensitivity; dental extraction; bacterial infections; antibiotic susceptibility.*

## 1. INTRODUCTION

Teeth, as hard, resistant structures in or around the mouth and pharynx areas of vertebrates, play critical roles in catching and masticating food, defense, and various specialized functions [1]. The human oral cavity, a complex biological system, is home to a myriad of microorganisms. These microorganisms, under normal circumstances, exist in a balanced state. However, when they penetrate deeper tissues or if the host's immune system is compromised, they can cause infections [2].

Bacterial infections are a major cause of purulent soft tissue inflammations in the head and neck regions [3]. This is facilitated by the diverse oral microbiota and the presence of lesions in dental tissues and the periodontium, which provide entry points for bacteria [4,5] Infections in the oral cavity can be classified into odontogenic and nonodontogenic origins, with a significant majority (70-90%) being odontogenic. Common causes of odontogenic infections include gangrenous teeth, complicated third molar eruptions, infected dental cysts, residual tooth roots, and complications post-endodontic treatment [6-8].

Pathogens such as Streptococcus mutans and Streptococcus sobrinus are frequently identified in dental cavities [9]. When these bacteria invade deeper tissues, they can cause odontogenic infections, especially in the presence of a high bacterial load and a weakened immune response. Such infections can spread to various spaces within the oral cavity, leading to severe complications [10 and 11]. Effective management

of these infections involves the use of antibacterial mouth rinses and systemic antibiotics to control and prevent their spread [12]. Early diagnosis and identification of the causative microorganisms through culture and antibiotic sensitivity testing, coupled with prompt antibiotic treatment and removal of the infection source, are essential to prevent complications and ensure early recovery. This approach necessitates both surgical and supportive therapy [13].

Studies have shown that transient bacteria, which is the presence of bacteria in the bloodstream following tooth extraction, occurs in 30-60% of adults and 33-80% in children [14] and [15]. To mitigate this risk, the use of antibacterial mouth rinses and systemic antibiotics is recommended. The cornerstone of treating these infections is the selection of a high-efficacy antibiotic. However, the inappropriate and excessive use of antibiotics has led to the elimination of sensitive microorganisms, creating an environment that allows resistant bacteria to thrive. This resistance significantly reduces the efficacy of some antibiotics.

Throughout history, human beings and their ancestors have been plagued by various diseases. The development of modern or allopathic medicine marked a shift from plant-based remedies to synthetic drugs. While these synthetic preparations have advanced medical treatment, the adverse side effects of many modern drugs and the rise of drug-resistant organisms have renewed interest in ethno medicinal studies. Ethno medicine focuses on traditional medical practices and natural remedies, providing potential alternatives or complements to modern pharmacological treatments. This resurgence highlights the need for a balanced approach that integrates the benefits of both modern and traditional medicine to address the challenges of antibiotic resistance and improve patient outcomes.

## 2. MATERIALS

### 2.1 Equipment Used

Weighing balance, Petri dishes, autoclave, wire loop, Bunsen burner, incubator, beakers, conical flask, measuring cylinders, pipettes, test tubes and glass slides.

### 2.2 Collection of Specimens

The specimens were collected using sterile commercial swab sticks, properly labeled accordingly by swabbing the 10 areas of extracted teeth of different patients and the swab sticks were carefully placed back into the swab jacket. The specimens were transported to the Microbiology Laboratory of Benue State University, Makurdi for the analysis within 2- 3 hours of collection.

### 2.3 Antibiotics Used

The antibiotics used include; Gentamycin (10mcg), Ofloxacin Erythromycin (15mcg), Vancomycin (30mcg), Ciprofloxacin (5mcg), Amoxicillin (10mcg).

### 2.4 Reagents Used

The reagents used include; Kovac's reagent, normal saline, dye tetramethyl-p-phenylenedamine, bromothymol blue and hydrogen peroxide.

### 2.5 Antibiotics and Dosages

**Ciprofloxacin (5mcg) and Ofloxacin (5mcg): C**iprofloxacin was administered at 500 mg twice daily, while ofloxacin was administered at 200-400 mg twice daily.

**Gentamycin (10mcg):** Gentamicin was administered intramuscularly at 500 mg twice daily.

**Vancomycin (30mcg):** Vancomycin was administered at 125-500 mg taken orally 3-4 times daily.

**Amoxicillin (10mcg) and Ampicillin (10mcg):** Amoxicillin and Ampicillin were administered at 500 mg orally 3 times daily.

**Dosage Adjustment:** The dosage was administered based on the patient's age, weight and severity of infection.

**Duration of Treatment:** Antibiotic treatment for dental infections was for 7 days

## 3. METHODOLOGY

### 3.1 Sterilization of Materials

All the glass wares via pipettes, test tubes, beakers, conical flasks, petri dishes etc, were

properly foiled and sterilized at 121 °C for 15 minutes in the autoclave.

#### 3.1.1 Preparation of nutrients agar

The nutrient agar was prepared by dissolving 8.4 g into 300 ml of distilled water. The conical flask containing the medium was adequately labeled and properly shaken for it to dissolve completely. The medium was sterilized in the autoclave for 15 minutes at 121 °C. It was allowed to solidify and plates were then labeled properly. The plates were incubated for 18-24 hours for sterility of plates.

#### 3.1.2 Preparation of macconkey agar

The MacConkey agar was prepared by dissolving 15.6 g of the powder in 300 ml of distilled water. The conical ask containing the medium was shaken for the agar to dissolve properly. The conical flask was plugged with cotton wool and then sterilized in the autoclave for 15 minutes at 121 °C. It was allowed to cool to a temperature of 45 °C - 50 °C and was dispensed into sterile petri dishes which were labeled appropriately after solidification. The plates were incubated for 15-24 hours for sterility of plates.

#### 3.1.3 Preparation of peptone water

The peptone water was prepared by dissolving 4 g of peptone powder in 250 ml of distilled water. The conical flask containing the medium was adequately labeled and properly shaken for it to dissolve completely. The flask was then plugged with cotton wool and then sterilized in the autoclave for 15minutes at 121 °C. It was allowed to cool to a temperature of 45 °C - 50 °C and then poured into test tubes. The test tubes were covered with foil.

### 3.2 Inoculation of Sample/ Specimens

Swab sticks were used to make a smear that is inoculating the organisms into the media and then incubated at 37 °C for 24 hours.

#### 3.2.1 Purification of isolates

Each of the isolates was aseptically subculture from the plates and inoculated by streaking into a freshly prepared sterile nutrient agar plates and then incubated at 37 °C for 24 hours. After the duration of incubation period, discrete lonies were picked using a sterile wire loop and gram stained to confirm their purity. Isolates were either gram positive or gram negative and was preserved as stock cultures for biochemical tests.

#### 3.2.2 Identification of bacteria isolates

The pure isolates were identified using their colonies appearance, microscopic examinations and biochemical test. The biochemical test includes catalase test, citrate test, oxidase test, indole test coagulase test, and gram stain.

#### 3.2.3 Gram staining

Smears of the isolates were prepared on clean grease free slides. The smear was allowed to air dry, heat fixed by passing the slide over flame. The slide was placed on a staining rack and flooded with crystal violet (the primary dye) for 1 minute, it was rinsed with water. The smear was flooded with lugol's iodine (mordant) left for 1minute and then rinsed off immediately with water. The smear was flooded with acetone and rinsed off immediately with The smear was flooded with safranin for 1minute, rinsed with water and then allowed for 1minute, rinsed with water and then allowed to dry. It was examined under an oil immersion lens 200 Organisms that retained the colour of the primary dye purple) were gram positive while those that retained secondary our (pink) were gram negative.

#### 3.2.4 Catalase test

The enzyme catalase catalyzes the degradation of hydrogen peroxides to water and molecular oxygen 2 $H_2O_2$ >>>>> 2 $H_2O$ + $O_2$. Catalase positive organisms rapidly produce bubbles when exposed a solution containing hydrogen peroxide.

A drop of 3% hydrogen peroxide diluted with 7ml of water was placed on a clean grease free slide using a Pasteur pipette. A colony of test organism was collected using an inoculating wire loop and placed on the slide with hydrogen peroxide. The reaction observed was gas bubbles confirmed the presence of catalase positive while catalase negative organisms do not produce gas bubbles.

#### 3.2.5 Citrate test

This test is based on the ability of an organism to utilize citrate as the only source of carbon. The organism was cultured to medium containing sodium citrate ammonium salt and an indicator

(Bromothymol blue) which changes to blue if the organism is positive.

The method involves using a sterile wire loop to collect a clony of the test organisms and stab into the Simon's citrate agar slant. This was incubated for 24 hours at 37 °C. A blue colouration indicates that citrate has been utilized.

#### 3.2.6 Oxidase test

The oxidase reagent which contains dye tetramethyl-p- phenylenediamine was prepared by dissolving 0.1 g in 100 ml of distilled water. For each organism, a drop of the reagent was placed on the top of the drop.

Purple colour developed between 3-5 seconds for oxidase positive organisms. This is due to the possession of the cytochrome oxides and no colour change for oxidase negative organisms.

#### 3.2.7 Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acids, tryptophan to indole which accumulates the medium. For each isolate, peptone water was prepared; 5 ml were dispensed into test tubes and autoclave at 121 °C for 15 minutes. A sterile wire loop charged with the test organism inoculated into the medium and incubated for 7 days at 37 °C. Then 0.5 ml of kovac's reagent was added to each test tube.

A deep red colour was observed in the reagent layer indicates positive result and no color indicates negative result colour indicates.

#### 3.2.8 Coagulase test

The enzyme coagulase causes plasma to clot converting fibrogen to fabrin. It was done by dropping a normal saline to emulsify colonies of the test organism to form a smooth emulsion. Two drops of human plasma was placed on it and the slide was rocked for one minute. A clumping of the indicates presence of a coagulase organism.

### 3.3 Antibiotic Susceptibility Testing

The antibiotics susceptibility tests were performed using the Kirby Bauer method (disc diffusion techniques). The antibiotics disc was designed and contained appropriate centration of the antibiotics, which include: Gentamycin (10mcg), Ofloxacin (5mcg), Erythromycin (15mcg), Vancomycin (30mcg), Ampicillin (10mcg), amoxillin (10mcg), Ciprofloxacine (5mcg).

### 3.4 Preparation of Mcfarland Turbidity

1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated suphuric acid to 99 ml of water. The 1% w/v solution of barium chloride was also prepared by dissolving 0.5 g of dehydrate barium chloride into 50 ml distilled water. 0.5 ml of the barium chloride solution was added into 99.5 ml of sulphuric acid solution. Then 10 ml of the turbid solution was transferred into a capped test tube.

For each isolate, 5 ml of distilled water was pipette into test tubes and autoclave at 121 °C for 15 minutes. A sterile wire loop charged with test organisms were inoculated into the tubes marking it with McFarland turbidity standard of 0.5 concentrations.

Muller Hinton agar was prepared, poured into Petri dishes and sterility test carried out. The test organisms were inoculated from the test tubes using the spread technique. The sensitivity discs containing the antibiotics were placed aseptically into plates using a sterile forceps. A sterile forceps was used to gently lap each disc to ensure even contact with the agar surfaces the plates were incubated at 37 °C for 18-24 hours.

The different inhibition zones sizes were measured and recorded in millimeter (mm). Then the zone size interpretation criteria or the National Committee for Clinical Laboratory Standards (NCCLs) were used to interpret the zone sizes.

## 4. RESULTS

### 4.1 Physical and Biochemical Observation

A total of 10 (TEN) swab specimen from extracted teeth examined. The most probable organisms (bacteria) isolated from 10 patients with dental caries Staphylococus aureus, Lactobacillus spp. and Streptococcus spp. Also biochemical test such as catalase, indole oxidase, coagulase citrate were done to characterize the isolate.

**Table 1. Cell morphology, gram reaction, biochemistry characteristics and identification of bacteria**

| S/No. | Colonial Characteristics | Grams Characteristics | Catalyst Test | Oxidase Test | Citrate Test | Coagulase Test | Indole Test | Motility Test | Glucose Test | Most portable Organisms |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Golden yellow pigment smooth, entire, raised and convex elevation on nutrients ager. | Gram positive cocci in clusters. | + | - | + | + | - | + | A | *Staphylococcus aureus* |
| 2. | Colonies are pontiform convex with an entire margin. | Gram positive rods (bacilli) | + | - | + | - | - | + | A | *Lactobacillus spp.* |
| 3. | Colonies are pontiform,Grey and smooth on nutrient ager pontiform, Entire and convex lactose fermenting colonies (Pinkish MacConkey ager). | Gram positive cocci in chains | + | - | + | + | - | + | A | *Staphylococcus spp.* |

*KEY: + Positive; - Negative; A Acid only*

**Table 2. Antibiotics susceptibility of bacteria isolate**

| Plate No. | Ery | Amp | Van | Amx | Cpr | Ofl | Gen | Portable Organisms |
|---|---|---|---|---|---|---|---|---|
| 1 | S | S | R | R | S | S | S | *Staphylococcus spp.* |
| 2 | R | R | R | R | S | S | S | *Staphylococcus aureus* |
| 3 | I | R | R | R | S | S | S | *Staphylococcus aureus* |
| 4 | S | R | R | R | S | S | S | *Staphylococcus aureus* |
| 5 | S | T | R | R | S | S | S | *Lactobacillus spp.* |
| 6 | S | S | R | R | S | S | S | *Staphylococcus spp.* |
| 7 | I | R | R | R | S | S | S | *Staphylococcus aureus* |
| 8 | S | I | R | R | S | S | S | *Staphylococcus spp.* |
| 9 | I | S | R | R | S | S | S | *Staphylococcus spp.* |
| 10 | R | R | R | R | A | S | S | *Staphylococcus aureus* |

*KEY: S = Sensitive ≥ 16mm; R = Resistance ≤ 12mm; I = Intermediate 12 - 15mm; Erythromycin (Ery) =15mcg; Gentamycin (Gen) =10mcg; Ofloxacin (Ofl) =5mcg; Vancomycin (Van) =30mcg; Ciprofloxacin; (Cpr) =5mcg; Amoxycillin (Amx) =10mcg; Ampicillin (Amp)=30mcg*

**Table 3. Frequency of Antibiotics susceptibility pattern of bacteria isolate**

| S/No. | Antibiotics (Conc.) | Sensitivity (%) | Intermediate (%) | Resistance (%) |
|---|---|---|---|---|
| 1. | Erythromycin (15mcg) | 5 (50.00) | 3 (30) | 2 (20) |
| 2. | Ampicillin (30mcg) | 8 (30.00) | 1(10) | 6 (60) |
| 3. | Vancomycin (30mcg) | 0 (0) | 0 (0) | 10 (100) |
| 4. | Amoxycillin (10mcg) | 0 (0) | 0 (0) | 10 (100) |
| 5. | Ciprofloxacin(5mcg) | 10 (100) | 0 (0) | 0 (0) |
| 6. | Ofloxacin(5mcg) | 10 (100) | 0 (0) | 0 (0) |
| 7. | Gentamycin(10mcg) | 10 (100) | 0 (0) | 0 (0) |

**Table 4. Index ratio of antibiotics susceptibility pattern of isolates**

| S/No. | Antibiotics (Conc.) | Index |
|---|---|---|
| 1. | Erythromycin (15mcg) | 1.00 |
| 2. | Ampicillin (30mcg) | 0.50 |
| 3. | Vancomycin (30mcg) | 0.09 |
| 4. | Amoxycillin (10mcg) | 0.09 |
| 5. | Ciprofloxacin(5mcg) | 11.00 |
| 6. | Ofloxacin(5mcg) | 11.00 |
| 7. | Gentamycin(10mcg) | 11.00 |

*KEY: Formula= S + 1 ÷ Rn + 1 = n*
*Where: S = Sensitivity; Rn = Resistance + Intermediate; n = Index Ratio*
*Note: Value ≥ 1 is significant; ≤ 1 is not significant*

### 4.2 Antibiotics Susceptibility of Bacteria Isolated

The antibiotics susceptibility of bacteria isolated were listed in Table 2 and were also grouped into sensitive (S), intermediate (I) and resistant (R).

### 4.3 Antibiotics Susceptibility Pattern of Bacteria Isolation

The frequency of Antibiotics susceptibility pattern of bacteria isolated were in Table 3 from Benue State University, Makurdi.

### 4.4 Statistical Analysis

Calculated the proportions (%) of isolates that were sensitive, intermediate, or resistant to each antibiotic for each species (Staphylococcus aureus, Lactobacillus spp., Streptococcus spp and Chi-square Test of Independence was used to determine whether there is a significant association between the antibiotic susceptibility patterns (sensitive, intermediate, resistant) and the bacterial species.

### 4.5 Efficacy Index Ratio of Antibiotics Susceptibility

The efficiency index ratio of antibiotics susceptibility pattern of isolates shown in (Table 4).

## 5. DISCUSSION

The study examined swab specimens from extracted teeth of ten patients with dental caries to identify the bacterial isolates and their antibiotic susceptibility patterns. The organisms identified included Staphylococcus aureus, *Lactobacillus spp*., and *Streptococcus spp.*

### 5.1 Morphological and Biochemical Characterization

***Staphylococcus aureus*:**

- ★ Colonial characteristics: Golden yellow pigment, smooth, entire, raised, and convex elevation.
- ★ Gram characteristics: Gram-positive cocci in clusters.
- ★ Biochemical tests: Catalase positive, oxidase negative, citrate positive, coagulase positive, indole negative, motility positive, glucose fermentation positive (acid only).

***Lactobacillus spp*.:**

- ★ Colonial characteristics: Pontiform convex colonies with an entire margin.
- ★ Gram characteristics: Gram-positive rods (bacilli).
- ★ Biochemical tests: Catalase positive, oxidase negative, citrate positive, coagulase negative, indole negative, motility positive, glucose fermentation positive (acid only).

***Streptococcus spp*. (listed as *Staphylococcus spp.* in some entries):**

- ★ Colonial characteristics: Grey, smooth colonies on nutrient agar, lactose fermenting colonies (pinkish on MacConkey agar).
- ★ Gram characteristics: Gram-positive cocci in chains.
- ★ Biochemical tests: Catalase positive, oxidase negative, citrate positive, coagulase positive, indole negative, motility positive, glucose fermentation positive (acid only).

These identifications are consistent with known characteristics of these organisms. Staphylococcus aureus and other *Staphylococcus spp*. are common in dental infections due to their presence in the oral cavity and their ability to form biofilms [16 and 17]. Lactobacillus spp. are also frequently found in carious lesions because of their role in acid production and enamel demineralization [18].

### 5.2 Antibiotic Susceptibility Patterns

The study tested various antibiotics against the bacterial isolates, with results categorized as sensitive (S), intermediate (I), and resistant (R).

**Erythromycin (15 mcg):**

- ★ 50% sensitivity, 30% intermediate, 20% resistance.

**Ampicillin (30 mcg):**

- ★ 30% sensitivity, 10% intermediate, 60% resistance.

**Vancomycin (30 mcg):**

★ 0% sensitivity, 0% intermediate, 100% resistance.

**Amoxicillin (10 mcg):**

★ 0% sensitivity, 0% intermediate, 100% resistance.

**Ciprofloxacin (5 mcg):**

★ 100% sensitivity, 0% intermediate, 0% resistance.

**Ofloxacin (5 mcg):**

★ 100% sensitivity, 0% intermediate, 0% resistance.

**Gentamycin (10 mcg):**

★ 100% sensitivity, 0% intermediate, 0% resistance.

These results indicate a worrying level of resistance to some commonly used antibiotics such as Vancomycin and Amoxicillin, with complete resistance noted for all tested isolates which is in control which is same as the studies of [19]. In contrast, Ciprofloxacin, Ofloxacin, and Gentamycin exhibited 100% sensitivity, making them highly effective against the tested isolates.

## 5.3 Efficacy Index Ratio

The efficacy index ratio calculated for each antibiotic indicates their effectiveness:

★ Ciprofloxacin, Ofloxacin, and Gentamycin had an index of 11.00, indicating high efficacy.
★ Erythromycin had an index of 1.00, which is marginally significant.
★ Ampicillin had an index of 0.50, indicating low efficacy.
★ Vancomycin and Amoxicillin had indices of 0.09, indicating poor efficacy.

Antibiotics with an index value ≥ 1 are considered significant. Thus, Ciprofloxacin, Ofloxacin, and Gentamycin show significant efficacy against the isolated bacteria, making them preferred choices for treatment [20].

The study's results provide insight into the antibiotic resistance patterns among dental caries pathogens. The high resistance observed for Vancomycin and Amoxicillin is concerning, as these antibiotics are commonly used to treat infections [21]. The high resistance rates to Vancomycin and Amoxicillin are concerning, given the widespread use of these antibiotics in clinical practice. The 100% sensitivity to Ciprofloxacin, Ofloxacin, and Gentamycin suggests these antibiotics should be considered for treating dental infections, especially in cases where resistance to other antibiotics is observed [17].

Ciprofloxacin, Ofloxacin, and Gentamycin show promising efficacy, with all isolates being sensitive to these antibiotics. This suggests that these antibiotics may be effective choices for treating dental caries infections caused by the identified bacteria.

The resistance pattern observed aligns with broader concerns about antibiotic resistance in microbial populations, particularly in oral pathogens which can exchange resistance genes easily within the biofilm environment. This underscores the importance of routine susceptibility testing and cautious antibiotic use to mitigate the development and spread of resistant strains.

The data presented in the study highlight the importance of ongoing surveillance and tailored antibiotic therapy based on susceptibility patterns. The results also emphasize the necessity for proper dental hygiene and preventive measures to reduce the incidence of bacterial infections leading to dental caries.

## 6. CONCLUSION

The findings on *Staphylococcus aureus*, particularly its resistance patterns, align with broader trends seen in clinical settings where methicillin-resistant Staphylococcus aureus (MRSA) is a known concern. The sensitivity patterns suggest that fluoroquinolones (Ciprofloxacin, Ofloxacin) and aminoglycosides (Gentamycin) could be viable treatment options.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image

generators have been used during writing or editing of manuscripts.

## CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

## COMPETING INTEREST

Authors have declared that no competing interests exist.

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