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Research Article

# Antibiotic Susceptibility Profile of the Bacteria Associated With Dental Caries Among Patients Attending Dental Clinic at Ahmad Sani Yariman Bakura Specialist Hospital, Gusau.

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**Abstract:** Dental caries is one of the most prevalent oral health problems worldwide and is primarily associated with bacterial colonization of the tooth surface. This study was conducted to determine the antibiotic susceptibility profile of bacteria associated with dental caries among patients attending the Dental Clinic of Ahmad Sani Yariman Bakura Specialist Hospital, Gusau, Zamfara State, Nigeria. A total of ten (10) dental caries samples were collected using sterile swab sticks and analyzed using standard microbiological techniques. The isolates were cultured, and identified based on their biochemical properties. Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. The results revealed the presence of two bacterial species associated with dental caries, namely Lactobacillus spp. and Streptococcus mutans. Lactobacillus spp. was the predominant isolate, accounting for 60.0% of the total isolates, while Streptococcus mutans accounted for 40.0%. Antibiotic susceptibility testing showed that both isolates were highly susceptible to ciprofloxacin, with inhibition zone diameters of 36 mm and 39 mm respectively. The isolates also demonstrated susceptibility to septrin and streptomycin. However, resistance to ampicillin was observed in both isolates, while varying responses were recorded against augmentin and azithromycin. The study concluded that Lactobacillus spp. and Streptococcus mutans are important bacterial agents associated with dental caries among patients attending Ahmad Sani Yariman Bakura Specialist Hospital, Gusau. Ciprofloxacin was found to be the most effective antibiotic against the isolates, whereas resistance to ampicillin suggests the emergence of antimicrobial resistance. The study recommends routine bacteriological examination and antibiotic susceptibility testing to ensure effective management of dental infections and to minimize the development of antibiotic-resistant strains.

***Keywords:*** *Dental caries, Lactobacillus spp., Streptococcus mutans, antibiotic susceptibility, ciprofloxacin, antimicrobial resistance.*

## 1. Introduction

Dental caries is a chronic, infectious disease of the teeth characterized by the demineralization and destruction of the hard tooth tissues (enamel, dentin, and cementum) due to acids produced by bacteria acting on fermentable carbohydrates (Boyd *et al*., 2020).

The **tooth** is a small, hard, calcified, whitish structure found in the jaws or mouth of many vertebrates. It functions primarily in the mechanical breakdown of food. Structurally, a tooth consists of the **crown**, which is the visible part covered by hard enamel; the **neck**, which is the junction between the crown and root, covered by the gums; and the **root**, which anchors the tooth to the jawbone. Human adults generally possess 32 teeth, distributed across four quadrants. Each quadrant contains incisors for cutting, canines for tearing, premolars for tearing and grinding, and molars for grinding food.

Over a million of years ago, hominids such as Australopithecus suffered from cavities. The largest



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increase in the prevalence of caries has been associated with dietary changes. Archeological evidence shows that tooth decay is an ancient disease dating far into prehistory. The increase in caries during the Neolithic period (a period in the development of human technology) may be attributed to the increased consumption of food containing carbohydrate. Periodontal disease:- the word periodontal literally means around the tooth is the chronic bacterial infection that affect the gums supporting the teeth.

**Objectives**

1. To isolate and identify the bacterial species present in dental caries from patients attending Ahmad Sani Yariman Bakura specialist hospital Gusau.
2. To determine the antibiotic susceptibility patterns of the bacteria isolated from dental caries.

## 2. Materials and Methods

2.1 Study Area

The study was conducted at Ahmad Sani Yariman Bakura Specialist Hospital, located in Gusau, the capital city of Zamfara State, North-Western Nigeria. Gusau lie**s** within the Sudan savannah ecological zone and experiences a tropical climate characterized by distinct rainy and dry seasons (Olaniyi *et al*., 2021). The area has a predominantly urban population with diverse socio-economic and cultural backgrounds, making it suitable for dental health studies.

2.2 Sample Collection

Samples were collected from infected teeth using sterile swabs from patients attending the dental clinic at Ahmad Sani Yariman Bakura Specialist Hospital, Gusau, Zamfara State. A total of ten (10) samples were obtained using sterile gloves and swab sticks. Each swab stick were placed into a separate sterile universal bottle containing peptone water to maintain sample integrity. The collected samples were transported to the Microbiology Laboratory of the Federal University Gusau for further analysis, following standard microbiological protocols (Cheesbrough, 2021).

2.2 Preparation of Media

2.2.1 Blood Agar Medium

Blood agar medium was prepared according to the manufacturer's instructions. Twenty-eight grams (28 g) of the powdered medium will be weighed using a precision weighing balance and transferred into a conical flask containing 1000 ml (1 liter) of deionized water. The mixture **was stirred** to dissolve completely and then sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to approximately 47°C, after which 1.5 ml of blood was aseptically added, mixed gently, and poured into sterile Petri dishes under aseptic conditions Cheesbrough, 2021).

2.2.2 Nutrient Agar Medium

Nutrient agar was prepared by weighing 28 g of powdered medium, which was transferred into a conical flask containing 1000 ml of distilled water. The mixture was allowed to soak for 18 minutes and then sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the medium **was poured** into sterile Petri dishes and allowed to solidify before use Cheesbrough, 2021).

2.3 Bacteriological Analysis of the Samples

2.3.1 Inoculation and Cultivation of Bacteria

The swab samples were collected from infected teeth was processed immediately in the laboratory. Each sample was aseptically inoculated onto prepared Chocolate Agar and MRS Agar plates using the streak plate technique. The inoculated plates were properly labelled and incubated at 37°C for 24–48 hours under appropriate growth conditions (Cheesbrough, 2021).

After incubation, the culture plates were examined for bacterial growth. Distinct bacterial growths observed on the media were aseptically picked using a sterile inoculating loop and sub-cultured onto freshly prepared media to obtain pure cultures. The pure isolates obtained were subsequently used for Gram staining, biochemical characterization, and antibiotic susceptibility testing.

**2.4 Gram Staining**

A drop of distilled water was placed on a clean glass slide. Using a sterile inoculating wire loop, a small portion of the bacterial colony isolated was placed on the slide and mixed with distilled water to form a thin smear. The smear was allowed to air dry completely, and then heat-fixed by passing it through a Bunsen burner flame. The smear was covered with crystal violet stain and allowed to stand for 1 minute, ensuring the smear is fully covered. The slide was then rinsed gently with distilled water. Iodine solution was applied as a mordant and allowed to stand for another 1 minute. The slide was rinsed again with distilled water. The smear was carefully decolorized by adding ethanol until the runoff became clear for 30 seconds after which the slide was immediately rinsed with distilled water. Safranin was added as a counterstain and allowed to stand for 1 minute. The slide was then rinsed with distilled water and allowed to air dry before viewing under the microscope using the oil immersion objective lens. Gram-positive bacteria appeared purple due to the retention of crystal violet, while Gram-negative bacteria appeared pink or red after taking up the safranin counterstain (Cheesbrough, 2006).

### 2.5 Biochemical Identification

The following biochemical tests were carried out to identify the bacterial isolates obtained from the patients with dental cavity.

#### 2.5.1 Indole Test

The indole test was carried out by inoculating the bacterial isolate into a test tube containing sterile tryptone broth and incubating it at 37°C for 24–48 hours. After incubation, about 0.5 mL of Kovac's reagent will be gently added to the culture. The development of a red layer at the surface of the broth will indicate a positive indole test, while the absence of color change will indicate a negative result (Cheesbrough, 2016).

#### 2.5.2 Methyl Red (MR) Test

The methyl red test was performed by inoculating the bacterial isolate into glucose phosphate broth and incubating it at 37°C for 48 hours. After incubation, five drops of methyl red indicator will be added to the culture. The appearance of a red color will indicate a positive test, whereas a yellow or orange color will indicate a negative result (Cheesbrough, 2016).

#### 2.5.3 Voges–Proskauer (VP) Test

The VP test was carried out using the same culture used for the MR test but in a separate tube. After incubation at 37°C for 48 hours, 0.6 mL of alpha-naphthol and 0.2 mL of potassium hydroxide (KOH) solution will be added to the broth. The tube will be shaken gently and allowed to stand for 15–30 minutes. The development of a red or pink color will indicate a positive result, while the absence of color change will indicate a negative reaction (Cheesbrough, 2016).

#### 2.5.4 Citrate Utilization Test

Citrate utilization test was carried out by inoculating the bacterial isolate onto Simon's citrate agar slant using a sterile wire loop. The inoculated slant will be incubated at 37°C for 24–48 hours. A visible growth accompanied by a change in the color of the medium will indicate a positive citrate utilization test, while no growth or color change will indicate a negative result (Cheesbrough, 2016).

#### 2.5.5 Triple Sugar Iron (TSI) Test

The triple sugar iron (TSI) test was conducted by inoculating the bacterial isolate into TSI agar slants by first stabbing the butt and then streaking the slant surface using a sterile inoculating needle. The inoculated tubes will be incubated at 37°C for 24 hours. After incubation, the color reactions in both the slant and butt will be observed for carbohydrate fermentation, gas production and hydrogen sulfide ($H_2S$) formation (Cheesbrough, 2016).

2.6 Antibiotic Susceptibility Testing

2.6.1 Media Preparation

A Mueller Hinton medium was prepared according to the manufacturer's instructions. The media will be heated and autoclaved at 121°C for 15 minutes. It will be allowed to cool to 45°C and the media will be dispensed into Petri dishes.

2.6.2 Antibiotic Discs Used

The antibiotics that will be used in this test include the following: Augmentin (30 µg), Ampicillin (30 µg) azithromycin (10 µg), Ciprofloxacin (10 µg), Septrin (30 µg), and Streptomycin (30 µg), (Firesbhat *et al*., 2021)**.**

Using a sterile 5 ml pipette, 5 ml of sterile saline will be added to a sterile test tube. An inoculating loop will be used to transfer several colonies from the subculture plate to the tube of sterile saline. The organism will be diluted until a turbidity equivalent to the 0.5 McFarland standards is obtained. The diluted tube and the 0.5 McFarland standards will be held against the black-lined McFarland reference card to accurately compare the turbidity.

Within 15 minutes of preparing the diluted organism, a sterile swab will be dipped into the properly adjusted inoculum. The swab will be lifted slightly out of the suspension and rotated firmly several times against the upper inside wall of the tube to remove excess fluid. The entire surface of the agar will then be streaked three times with the swab, turning the plate 60 degrees between streaking to obtain an even inoculation. The lid will be closed and the plate will be allowed to sit for 3 to 5 minutes before applying the drug-impregnated disks.

The disks will be applied using a dispenser under aseptic conditions. Each disk will be lightly pressed down with a sterile swab to ensure proper contact with the agar surface. The agar plates will be incubated in an inverted position at 37°C for 24 hours (Firesbhat *et al*., 2021).

## 3. RESULTS

**Table 3.1 Morphological and Biochemical Characteristics of Bacteria Isolates**

Table 4.1 presents the biochemical characteristics of the bacterial isolates recovered from dental caries samples. The isolates were subjected to various biochemical tests, including citrate utilization, indole production, urease activity, carbohydrate fermentation (glucose, sucrose, and lactose), and methyl red (MR), Voges-Proskauer (VP), gas production, hydrogen sulphide ($H_2S$) production, and motility tests. Based on the reactions obtained, two bacterial species were identified, namely *Lactobacillus* spp. and *Streptococcus mutans*. The isolates showed varying biochemical responses which were useful in differentiating and confirming their identities. These organisms are recognized as important

contributors to the development and progression of dental caries.

### 3.2 Frequency of Occurrence of Bacterial Isolates

Table 4.2 shows the frequency distribution of the bacterial isolates identified from dental caries samples. A total of 10 isolates were recovered, comprising two bacterial species. *Lactobacillus* spp. recorded the highest occurrence with 6 isolates, representing 60.0% of the total isolates, while *Streptococcus mutans* accounted for 4 isolates, representing 40.0%. The predominance of *Lactobacillus* spp. suggests its significant involvement in the progression of dental caries among the patients examined, while the occurrence of *S. mutans* further confirms its established role as a major cariogenic bacterium.

### 3.3 Antibiotic Susceptibility Patterns of the Bacterial Isolates

Table 4.3 presents the antibiotic susceptibility profiles of the bacterial isolates against six commonly used antibiotics: ampicillin, streptomycin, augmentin, ciprofloxacin, septrin, and azithromycin. The results indicate varying levels of susceptibility and resistance among the isolates. The *Lactobacillus* spp. isolate showed susceptibility to streptomycin, ciprofloxacin, septrin, and azithromycin but was resistant to ampicillin and augmentin. Similarly, the *Streptococcus* spp. isolate was susceptible to ciprofloxacin and septrin but resistant to ampicillin and azithromycin. Ciprofloxacin produced the largest zones of inhibition for both isolates, indicating the highest antibacterial activity. The findings suggest that ciprofloxacin and septrin may be more effective therapeutic options against the bacterial isolates recovered, whereas resistance to ampicillin highlights the potential challenge of antimicrobial resistance among bacterial pathogens associated with oral infections.

**Table 3.1 Morphological and Biochemical Characteristics of Bacterial Isolates**

| S/N | Sample Code | Gram reaction | Cit | Ind | Ure | Glu | Suc | Lac | MR | VP | GAS | $H_2S$ | Mot | probable Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | +ve Rod | − | − | − | + | + | + | + | + | − | − | − | *Lactobacillus* |
| 2 | B | +ve Cocci | − | − | + | + | + | + | + | − | + | + | + | *S. mutan* |

**KEY: CIT= Citrate IND=Indole URE=Urea GLU=Glucose, SUC= Sucrose LAC=Lactose MR= Methylred VP=Vorges proskauer, MOT=Motility**

- **=absence + = presence**

**Table 3. 2 Frequency of Occurrence of Bacterial Isolates**

| Bacterial Isolates | Frequency of occurrence | Percentage of occurrence (%) |
|---|---|---|
| ***Lactobacillus*** spp. | 6 | 60.0 |
| ***Streptococcus mutans*** | 4 | 40.0 |
| Total | **10** | **100.0** |

**Table 3.3: Antibiotics Susceptibility Patterns of Bacterial Isolates**

| Sample Code | Bacterial Isolates | Ampicillin (30 µg) | Streptomycin (30 µg) | Augumentin (30 µg) | Ciprofloxacin (10 µg) | Septrin (30 µg) | Azithromycin (10 µg) |
|---|---|---|---|---|---|---|---|
| S1 | *Lactobacillus* spp. | 12mm(R) | 29mm (S) | 14mm (R) | 36mm (S) | 30mm (S) | 21mm(S) |
| S2 | *Streptococcus* spp. | 10mm (R) | 21mm (S) | 22mm (S) | 39mm (S) | 29mm (S) | 13mm (R) |

**KEY:** 0–14 = Resistant (R), 15-20 = Intermediate (I), 21 above = Susceptibility (CLSI, 2021).

## 4. Discussion, Conclusion and Recommendations

### 4.1 Discussion

The present study was conducted to determine the antibiotic susceptibility profile of bacteria associated with dental caries among patients attending the Dental Clinic of Ahmad Sani Yariman Bakura Specialist Hospital, Gusau. The findings revealed the presence of two major bacterial isolates, namely *Lactobacillus* spp. and *Streptococcus mutans*, with *Lactobacillus* spp. accounting for 60.0% of the isolates and *S. mutans* accounting for 40.0%. The predominance of these organisms is consistent with their established roles in the initiation and progression of dental caries. *Streptococcus mutans* is widely recognized as a primary cariogenic bacterium due to its ability to adhere to tooth surfaces, produce extracellular polysaccharides, and generate organic acids that demineralize dental enamel. Similarly, *Lactobacillus* species are associated with the progression of dental caries because of their acidogenic and aciduric properties, which enable them to thrive in acidic oral environments (Abranches *et al*., 2018; Tanner *et al*., 2018).

The frequency of distribution obtained in this study showed that *Lactobacillus* spp. Were more prevalent than *S. mutans*. This finding agrees with the work of Badet and Thebaud (2018), who reported a high prevalence of *Lactobacillus* species in active carious lesions due to their ability to survive and proliferate under low pH conditions. Likewise, a study conducted by Fakhruddin *et al*. (2020) identified both *Streptococcus mutans* and *Lactobacillus* species as dominant microorganisms associated with dental caries, emphasizing their synergistic role in disease progression. The higher occurrence of *Lactobacillus* spp. observed in the present study may indicate advanced stages of carious lesions among the patients sampled, as these organisms are often associated with deep dentinal caries rather than the initiation stage of the disease.

The antibiotics susceptibility results demonstrated varying responses of the bacterial isolates to the tested antibacterial agents. The bacterial isolate identified as *Streptococcus* spp. showed resistance to ampicillin and augmentin but was susceptible to streptomycin, ciprofloxacin, septrin, and azithromycin. The high susceptibility observed with ciprofloxacin, which produced the largest inhibition zone diameter (36 mm), suggests that this antibiotic remains highly effective against the isolate. Similar findings were reported by Ibrahim *et al*. (2021), who observed substantial susceptibility of oral bacterial isolates to fluoroquinolones, particularly ciprofloxacin, owing to their broad-spectrum activity and ability to inhibit bacterial DNA gyrase (Tanner *et al*., 2018).

The *Streptococcus* spp. isolate exhibited resistance to ampicillin and azithromycin but demonstrated susceptibility to ciprofloxacin and septrin, with inhibition zone diameters of 39 mm and 29 mm, respectively. The observed resistance to ampicillin may be attributed to the widespread alteration of penicillin binding protein or reduce permeable cell wall, which confer resistance to β-lactam antibiotics. This finding is consistent with the reports of Prestinaci *et al.* (2019), who highlighted increasing resistance of bacteria due to using of similar antibiotics due to prolonged and indiscriminate antibiotic use.

The susceptibility of both isolates to ciprofloxacin is noteworthy and agrees with findings reported by Ahmed *et al*. (2022), who found ciprofloxacin to be among the most effective antibiotics against oral bacterial pathogens. The effectiveness of ciprofloxacin may be linked to its mechanism of action, which involves inhibition of bacterial DNA replication and transcription, thereby preventing bacterial proliferation. Similarly, the susceptibility of the isolates to septrin observed in this study aligns with previous investigations that reported good antimicrobial activity of cotrimoxazole-containing formulations against oral and enteric bacterial pathogens (Auta *et al*., 2020).

The resistance observed against ampicillin and augmentin may indicate the emergence of antibiotic-resistant strains within the study population. Such

resistance patterns have become a global public health concern due to the misuse and overuse of antibiotics in both clinical and community settings. According to the World Health Organization (WHO, 2023), antimicrobial resistance continues to threaten the effectiveness of commonly prescribed antibiotics, necessitating continuous surveillance and susceptibility testing to guide appropriate antimicrobial therapy.

The findings of this study indicate that *Lactobacillus* spp. and *Streptococcus mutans* are important bacteria associated with dental caries among patients attending Ahmad Sani Yariman Bakura Specialist Hospital, Gusau. Furthermore, ciprofloxacin, streptomycin, and septrin exhibited substantial antibacterial activity against the tested isolates, whereas ampicillin showed poor efficacy. These results underscore the importance of routine antimicrobial susceptibility testing to support effective management of oral infections and to reduce the spread of antibiotic-resistant bacterial strains.

### 4.2 Conclusion

This study was carried out to determine the antibiotic susceptibility profile of bacteria associated with dental caries among patients attending the Dental Clinic of Ahmad Sani Yariman Bakura Specialist Hospital, Gusau. The findings revealed the presence of two major cariogenic bacteria, namely *Lactobacillus* spp. and *Streptococcus mutans*.

Among the isolates identified, *Lactobacillus* spp. had the highest occurrence, accounting for 60.0% of the total isolates, while *Streptococcus mutans* constituted 40.0%.

The antibiotics susceptibility testing demonstrated varying responses of the bacterial isolates to the antibiotics evaluated. Ciprofloxacin exhibited the highest antibacterial activity against the isolates, producing the largest zones of inhibition, while septrin and streptomycin also showed considerable effectiveness. In contrast, resistance to ampicillin and augmentin was observed among some of the isolates, indicating the presence of bacteria resistance. These findings suggest that ciprofloxacin remains a highly effective antibiotic against bacterial pathogens associated with dental caries in the study area.

The study confirms that dental caries is associated with specific bacterial species capable of exhibiting varying susceptibility patterns to commonly used antibiotics. Therefore, routine bacteriological examination and antibiotic susceptibility testing are essential for effective treatment, prevention of complications, and reduction of antimicrobial resistance.

### 4.3 Recommendations

Based on the findings of this study, the following recommendations are made:

1. Dental clinics should conduct antibiotic susceptibility testing whenever possible before prescribing antibiotics for the treatment of dental infections to ensure effective therapy.
2. Healthcare professionals should promote the rational use of antibiotics and avoid indiscriminate prescription practices that may contribute to the development of antibiotic-resistant bacterial strains.
3. Individuals should be encouraged to undergo regular dental examinations for early detection and management of dental caries before complications arise.
4. Additional studies involving a larger sample size and a wider range of bacterial isolates should be conducted to provide more comprehensive information on the microbial profile and antibiotic resistance patterns associated with dental caries in the study area.
5. Continuous monitoring of bacteria susceptibility patterns among oral pathogens should be established to guide evidence-based treatment and help combat the growing challenge of antibiotic resistance.

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