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Prevalence of *Streptococcus mutans* among Patients Presenting Tooth Decay Symptoms in Murtala Muhammed Specialist Hospital, Kano State, Nigeria

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Abstract

Dental caries remains among the most common oral health problems globally, with Streptococcus mutans being a key etiological agent. Understanding the prevalence of this bacterium among symptomatic patients is crucial for developing targeted preventive and therapeutic strategies. This study aimed to determine the prevalence of Streptococcus mutans among patients presenting with tooth decay symptoms at Murtala Muhammed Specialist Hospital, Kano State, Nigeria. A cross-sectional study was conducted involving 400 patients. Oral dental plaque samples were aseptically collected from dental caries patients at the Dental Clinic, Murtala Muhammed Specialist Hospital, Kano, transported in sterile peptone water, cultured on chocolate agar and incubated in a candle jar at 37°C for 24 hours. Bacterial identification was via colony morphology, Gram staining, catalase, optochin sensitivity tests, and confirmation using the API 20 Strep system. Antibiotic susceptibility testing was performed via the disk diffusion method, using five antibiotics and interpreted based on CLSI 2020 guidelines. Out of 400 samples processed, 351 (87.8%) yielded positive cultures, all of which were Gram-positive cocci. Among the 116 suspected viridans streptococci, 87 (75.0%) were confirmed as Streptococcus mutans. Antibiotic susceptibility testing results indicated that the isolates exhibited the highest resistance to tetracycline (67.8%), followed by erythromycin (28.7%) and ciprofloxacin (27.6%). The study found a high prevalence of Streptococcus mutans (75.0%) among patients with dental caries, with significant resistance observed to tetracycline (67.8%). These findings underscore the need for targeted oral health interventions and prudent antibiotic use in dental practice.

Keyword: Antibiotics, Dental caries, Linezolid, Streptococcus mutans, Tooth decay

INTRODUCTION

Oral diseases are an emerging major global public health threat; though preventable, they affect about 3.5 billion people, according to the Global Burden of Disease Study 2019 (Baldi *et al.*, 2023). Dental caries, commonly known as tooth decay, is one of the most prevalent oral health issues affecting people of all ages (Chaurasiya and Verma, 2024). It is caused by cariogenic bacteria that stick to teeth and are capable of metabolizing sugars from dietary carbohydrates to generate acid, which gradually demineralizes the structure of teeth (Abbas, 2024). Notably, *Streptococcus mutans* is the major bacterial agent responsible for dental caries (Daboor *et al.*, 2015).

Streptococcus mutans is a facultative anaerobic Gram-positive coccus bacterium that commonly inhibits the human oral cavity (Abranches *et al.*, 2018). *S. mutans* belongs to the mutans streptococci group, which also includes *S. sobrinus*, *S. rattus*, *S. ferus*, *S. cricetus*, and *S. macacae* (Ahmed *et al.*, 2023). These closely related species share similar characteristics and collectively play a significant role in the development of dental caries. *S. mutans* are lactic acid bacteria that depend completely on glycolysis for energy production (Washio *et al.*, 2024). Primarily, *S. mutans* are inhabited in the mouth, pharynx, and intestine as normal flora (Ahmed *et al.*, 2023). As an agent to cause dental caries, it exists as biofilms on the human tooth surface (Cai and Kim, 2023). Several factors, such as adherence to enamel surfaces, production of acidic metabolites, the capacity to build up glycogen reserves, and the ability to synthesize extracellular polysaccharides, allowed the bacterial organism to be present in dental caries (Forssten *et al.*, 2010).

The glucose moiety of sucrose is used as the substrate by strains of S. mutans to manufacture up to three different glucosyltransferases (GTFs), GtfB, -C, and -D, which create glucose polymers of glucans (also called mutans) (Lemos *et al.*, 2019).

MATERIALS AND METHODS

Study Area

The study was conducted at the Murtala Muhammad Specialist Hospital (MMSH) in the Kano metropolis, Kano State, Nigeria. Kano State is located in the Northwest geopolitical zone of Nigeria. It comprises 44 Local Government Areas with an estimated population of over 13 million and 20,760 km^2. It lies between latitudes 10o 33N to 11º 15N and longitudes 34ºCE to 8º20CE (NBS, 2018).

Ethical Approval

Ethical approval was obtained from the Kano State Ministry of Health before the commencement of the research.

Informed Consent

Volunteering subjects were recruited after obtaining their informed, conscious and autonomous consent. No patient identifier or personal information was collected as part of the study. The research was designed and executed in compliance with the World Health Organization (WHO) standards and operational guidance for ethics review of health-related research with human participants (WHO, 2011).

Sample Size Determination

Lwanga and Lemeshow (1991) determined the sample size using standard epidemiological formula forward.

$$n = \frac{Z^2 Pq}{d^2}$$

Where: n= number of minimum samples
Z = statistic for level of confidence at 95% = 1.96
d = allowable error of 5%, (0.05)
q = 1 - P
P = Previous prevalence
A prevalence of 30.4% based on a previous study in Kano by Yahaya and Ramatu (2016) was used to determine the sample size.

$$n = \frac{(1.96)^2 0.304\ (1-0.304)}{(0.05)^2}$$

n= 325.1
The sample size was rounded up to 400.

Sample Collection

Four hundred (400) oral dental plaque (DP) samples were aseptically collected with sterile cotton rolls from different patients with dental caries by the trained dental technician in the MMSH, Kano dental clinic. All the collected samples were transferred into a sterile, wide-mouthed screw-capped universal bottle containing 10ml of sterile peptone water. The samples were kept on ice and processed within 2 hours.

Sample Processing

The sample was processed in the Murtala Muhammad Specialist Hospital Microbiology Department, Kano. Using a sterile wire loop, a loop full of the inoculated peptone water was aseptically taken after a vigorous mix by vortexes and inoculated onto a chocolate agar plate. All the inoculated plates were incubated for 24 hours at 37ºC in a candle jar.

Bacterial Identification

All plates showed growth after 24 hours of incubation at 37oC, with the presence of small, circular, and raised colonies usually opaque with a smooth surface considered positive.

Biochemical Tests

The biochemical tests such as Gram stain reaction, Catalase test, and optochin sensitive test and further confirmed by Analytical Profile Index 20 Strep (API 20 Strep) Strep (Patterson, 1996 and Cheesbrough, 2010).

Gram Staining

Gram staining of the isolated colonies was carried out to identify the Gram reaction of the isolates. The species of *Streptococcus* appeared to be Gram-positive cocci in chain form (Tripathi and Sapra, 2021).

Catalase Test

This test differentiates bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase-producing bacteria, such as streptococci. The species of *Streptococcus* appeared to be catalase-negative by showing no evidence of bubble formation (O_2) by breaking hydrogen peroxide (H_2O_2) into hydrogen as gas and water (Sanda and Idris, 2021).

Optocin Sensitivity Test

A disposable sterile wire loop was used to remove a colony from an overnight culture, after which it was streaked onto a Blood agar plate. An optocin (P) disc (6mm, 5µg) was placed within the streak area of the plate and incubated overnight at 37°C in a carbondioxide enriched atmosphere (or in a candle-jar). Growth on the Blood agar plate near the P disc was observed, and the zone of inhibition was measured with a ruler. A 14mm or greater zone of inhibition indicates sensitivity and allows for presumptive identification of Pneumococci. A zone of inhibition <14mm or no zone of inhibition indicates that the bile solubility test is required, though Pneumococci are sometimes optochin-resistant.

Streptococcus pneumoniae strain ATCC (American-type culture collection) inhibited by optochin was used as the positive control (CDC, 2014).

Analytical Profile Index 20 Strep (API 20 Strep)
All the suspected Streptococcus species were further confirmed by the API 20 Strep multi-test system (Biomeniuex, France). These tests were used according to the manufacturer's protocol for the identification of Streptococcus species. The biochemical test was inoculated with bacterial suspension made from fresh bacterial culture. The suspension matched 0.5 McFarland turbidity standards and incubated at 37ºC for 24 hours.

Interpretation
The generated code obtained with numerical values was separated into 3, and a value 1, 2, or 4 is indicated for each on the interpretation result sheet. By adding together the value of each group, a 7-digit profile number was obtained. This code was interred into the API-web TM identification software for species identification (API 20 Strip, Biomerieux, France, 2021).

Preparation of Turbidity Standard (0.5 McFarland Standard)
Barium (1%v/v) standard suspension was used as turbidity standard, which was prepared in accordance with the procedure explained by Cheesbrough (2006). One percentage (1%) solution of sulphuric acid was prepared by adding 1ml of concentrated sulfuric acid (H_2SO_4) to 99ml of water. One percentage (1%) weight per volume solution of barium chloride in 1000ml distilled water. About 0.6ml of barium chloride solution was combined with 99.4ml sulphuric acid to yield 1.0% v/v sulphuric sulphate suspension. The turbid solution formed was transferred into a test tube for comparison, as standard, which matched with 0.5 McFarland standards (Cheesbrough, 2006).

Antibiotics Susceptibility Test
The susceptibility testing of isolates to 5 antibiotics was carried out by the disk diffusion method according to Clinical Laboratory Standards Institute (CLSI, 2020). Using a sterile loop, a few morphologically pure colonies were picked from the agar plate of the test organism and inoculated into a test tube containing sterile saline. The suspension was adjusted until its turbidity matched the 0.5 McFarland standard. A loopful of the suspension was aseptically transferred to the center of the chocolate agar (Blood Agar Base supplement with 5% sheep blood agar) (Oxoid, UK) medium, which was prepared according to the manufacturer's instructions, and a sterile cotton swab was used to streak the entire surface of the plate by rotating the plate approximately 60 degrees each time to ensure an even distribution of the inoculums. The moisture is allowed to be absorbed for at least 5 – 10 minutes, and sterile forceps were used to apply the antibiotic discs to the surface of the agar plate while ensuring the discs were placed equidistant from each other. Single disc antimicrobial agents (Oxoid, Basingstoke United Kingdom) Ciprofloxacin (CIP 5µg), Tetracycline (TET 30µg), Clindamycin (DA 2µg), Linezolid (LNZ 30µg), and Erythromycin (ERY 15µg) were dispensed onto the surface of the agar plates. All the plates were aerobically incubated at 37ºC overnight, after which the plates were held a few inches above a black, non-reflective surface illuminated with reflected light; a ruler was used to measure each zone with the unaided eye while viewing the back of the Petri dish. The result was recorded and interpreted as resistant, susceptible or intermediate using the CLSI (2020) interpretation guideline (CLSI, 2020).

RESULTS

Out of the 400 samples processed, 351 (87.8%) yielded positive cultures, given an overall prevalence of 87.8% (Table 1). Among these, 286 (81.5%) were alpha-haemolytic on chocolate agar, while 28 (8.0%) were beta-haemolytic on both chocolate and blood agar. Sixty-five (18.5%) were catalase-positive, and 116 (40.6%) were resistant to the optochin screen test (Table 2). Of the 116 suspected *Viridans Streptococci*, 87 (75.0%) were confirmed as *S. mutans*, 19 (16.4%) as *S. mitis*, 7 (6.0%) as *S. sanguinis*, and 3 (2.6%) as *S. gordonii* (Table 3). The antibiotic susceptibility testing revealed the highest resistance to tetracycline (67.8%), followed by erythromycin (28.7%) and ciprofloxacin (27.6%) (Table 4).

Table 1: Outcomes of Dental Plaque Cultures

Culture Result	Frequency	Percentages
Growth	351	87.8
No growth	49	12.2
Total	400	100.0

Table 2: Biochemical Properties of Positive Isolates

Biochemical Test	Frequency	Percentages
Blood Agar		
Beta Haemolytic	28	8.0
Non haemolytic	323	92.0
Total	**351**	**100.0**
Chocolate Agar		
Alpha haemolytic	286	81.5
Non-Haemolytic	37	18.5
Total	**351**	**100.0**
Gram Status		
Gram Positive Cocci	351	100.0
Gram Negative	0	0.0
Total	**351**	**100.0**
Catalase Test		
Positive	65	18.5
Negative	286	81.5
Total	**351**	**100.0**
Optocin Test		
Resistance	116	**40.6**
Sensitive	170	**59.4**
Total	**286**	**100.0**

Table 3: Distribution of the isolated *viridans streptococci*

Isolates	Frequency	Percentages
S. gordonii	3	2.6
S. mitis	19	16.4
S. mutans	87	75.0
S. sanguis	7	6.0
Total	**116**	**100.0**

Table 4 Susceptibility Profile of the Isolated *Streptococci mutans*

Antibiotics	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	62 (71.3)	1 (1.2)	24 (27.6)
Tetracycline	3 (3.5)	25 (28.7)	59 (67.8)
Linezolid	86 (98.9)	0 (0.0)	1 (1.2)
Clindamycin	63 (72.4)	9 (10.4)	15 (17.2)
Erythromycin	49 (56.3)	13 (14.9)	25 (28.7)

DISCUSSION

Streptococcus mutans is one of the primary bacteria responsible for tooth decay and enamel deterioration, typically inhabiting most surfaces of teeth, particularly in regions like pits and fissures that are challenging to clean (Abidalhussein and Taj-Aldeen, 2023). Findings from this study indicate that S. mutans was responsible for 75.0% of dental caries cases, consistent with the 73.0% prevalence rate cited in a study by Zubaidah *et al.* (2022). A study by Babaeekhou *et al.* (2020) found that the highest percentage recorded was 77.1%, while a study by Oda *et al.* (2015) recorded 96.6%. A lower prevalence rate of 49.2% was reported by Maduakor *et al.* (2021) in their study.

Antibiotics play a vital role in the treatment of several diseases (Gao *et al.*, 2020). However, for over a decade, these synthetic antibiotics have continued to be becoming less effective against most of the disease-causing microorganisms (Uddin *et al.*, 2021).

In this study, we observed a significant level of tetracycline resistance (67.8%) in the isolated oral *Streptococcal mutans* followed by erythromycin and ciprofloxacin, 28.7% and 27.6%, respectively.

Linezolid was the most active drug, followed by clindamycin. It has been reported that the penicillin class of antibiotics is widely used in prophylactic treatment to reduce dental infections, but long-term use of this group of antibiotics could be compromised by the emergence of resistant strains (Salh *et al.*, 2022). Erythromycin and clindamycin have been recommended as alternative options and are also widely used for antibiotic prophylaxis of endocarditis associated with dental procedures (Salh *et al.*, 2022). Clindamycin is less frequent use due to its bitter taste and its price cost. This study agreed with the report of Alhasani *et al.* (2020), who all reported the activity of clindamycin in their study. Salh *et al.* (2022) recorded high resistance to erythromycin, which is in line with what was obtained in the present study.

CONCLUSION

This study found out that *S. mutans* is the most frequent (75.0%) pathogenic microorganism associated with dental cavities. Likewise, linezolid and clindamycin are the most effective antibiotics against *S. mutans*.

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