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Bacterial Profile Associated with Dental Caries in Jos, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AEJO designed the study and wrote the protocol, author JAAO managed the literature searches and wrote the first draft of the manuscript. Author OJO managed the data analysis, author GM managed the experimental process, author OA managed the data collection and interpretation. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was aimed at determining bacterial prevalence and their susceptibility to commonly used antibiotics.
Study Design: The research was a cross sectional study which cuts across all age group and gender.
Place and Duration of Study: The study was carried out at the Bacteriology Laboratory of

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Department of Medical Microbiology, Federal College of Veterinary and Medical Laboratory Technology, Vom, Jos, Nigeria, between July and September 2014.

Methodology: We included 150 patients (30 men, 120 women; age range 11-70 years) who gave consent to be included in the study. Swabs were taken from dental plaque and inoculated on basal salt medium containing yeast and bacterial colonies obtained were identified biochemically according Bergey's Manual of Systematic Bacteriology. Antibiotic susceptibility test was then conducted on the isolates identified.

Results: Ninety-five (63.3%) patients had cavities located at the molars, forty-five (30%) between molar and premolar and ten (6.7%) located at the gingival margin. One hundred and twenty (80%) samples were obtained from female patients and thirty (20%) from male patients. *Lactobacillus* species had the highest occurrence (28.8%). The least was *Fusobacterium* species (0.7%). The antibiotic susceptibility test revealed that *Enterobacter* species was resistant to all the three antibiotics used in this study. *Bacillus subtilis* and *Staphylococcus* were resistant to erythromycin and vancomycin but sensitive to all the three antibiotics.

Conclusion: A few common acidogenic bacteria known to colonize dental plaque were identified. Susceptibility of the various bacterial isolates to the selected antibiotics varied. Further studies on susceptibility of these bacteria to commonly used antibiotics, antibacterial mouth washes and toothpastes are necessary in order to understand their epidemiology, to limit the spread of resistant bacteria. Larger sample size studies with better isolation, identification and characterization methods are needed. These studies could lead to the identification of strategies for effective biological interventions in the caries process and thereby contribute to improved prevention and treatment.

Keywords: Teeth; dental caries; dental plaque; cariogenic bacteria.

1. INTRODUCTION

Dental caries is reported to be one of the most chronic oral infections in the world and more than 90% of adults experience the disease, however, the depth of that experience varies extensively in individuals [1]. Dental caries is a biofilm-dependent oral disease, and fermentable dietary carbohydrates are the risk factors in its initiation and development. Caries results from the interaction of some specific bacteria with the dietary constituents of biofilm known as "dental plaque". Bacterial plaques gathered over time on dental surfaces are composed of native oral flora, the primary etiologic agents of dental caries [2]. Sucrose is considered the most cariogenic dietary carbohydrate because of its fermentable nature, and it also serves as a substrate for the synthesis of extracellular and intracellular polysaccharides in dental plaque [3]. The integrity of enamel is often disrupted due to formation of dental biofilm and the caries proceed along the interface between the dental biofilm and the enamel surfaces [4]. The exposure to sucrose concentration has been associated with frequency of cario-genicity [5]. Acids produced by bacteria, particularly *Streptococcus mutans, Streptococcus sobrinus* and *lactobacilli* ferment dietary carbohydrates thereby causing imbalance in the oral micro-flora ecology and leading to de-mineralization [6]. The activities of these organisms occur within dental plaque; a bacteria-laden gelatinous material that adheres to the surfaces of teeth. It is a dynamic process; periods of demineralization alternate with periods of re-mineralization [7, 8]. An earlier study has reported *Streptococcus sanguinis* to be associated with oral health, while others such as *S. mutans*, *Veillonella* spp, *Lactobacillus fermentum*, *Actinomyces* spp and *Bifidobacterium* spp were found to be associated with caries [9]. It has also been reported that apart from these few organisms, the overall activities of the total plaque micro-flora are responsible for outcome of carries [10].

The use of molecular techniques to demonstrate the presence of less common group of organisms (*Lactobacillus, Prevotella, elenomonas, Dialister, Fusobacterium, Eubacterium, Olsenella, Bifidobacterium, Propionibacterium*, and *Pseudoramibacter)* associated with caries have been reported [11]. In contrast, bacterial species, including *Streptococcus parasanguinis, Streptococcus mitis, Streptococcus oralis, Abiotrophia* defectiva, and *S. sanguinis*, have been known to predominate in the indigenous bacterial flora of caries [12]. These earlier findings suggested that there was a distinctive microbiota of the healthy oral cavity from those that are recently reported to be associated with oral diseases.

The bactericidal activity of chlorophenol derivative (triclosan) and that of fluorinated products on germs are well documented by way of interfering with the enzymes required for fatty acid synthesis. These biochemical activities reduce cariogenic bacterial impacts on development of caries [13]. The use of caries-risk assessment models is a combination of factors such as diet, fluoride exposure, micro-flora and susceptible host interplay with a variety of socio-cultural and behavioral factors. The ability of healthcare workers to detect caries at early stage is vital to prevention of cavitation. One of the ways to predict future dental caries development in adult is through their past eating habits and dental care history. However, this method is not very useful in predicting caries in children, though plague accumulation is strongly associated with caries in children [14]. It is also important to understand the disease process which will inform management strategies of dental caries for possible interventions in order to reverse the demineralization process that characterizes the development of carious lesion [15]. The purpose of this study was to assess the prevalence of dental caries associated pathogen and selected antibiotic susceptibility to inform strategies that will improve oral health.

2. MATERIALS AND METHODS

2.1 Population and Collection of Samples

The study was carried out at three different hospitals within Bukuru Jos Metropolis, Plateau State, Nigeria. The hospitals care for patients in and from outside the city of Jos for dental care. These were patients who had some complaints about their oral health and had to visit the hospitals. Patients without tooth ache complaint were excluded. The volunteered participants aged 11 years and were consented with a written informed consent and consecutively recruited at the point of visit after explanation of the purpose of the study. Ethical approval for the study was granted by the ECWA Evangel Hospital, Jos and Vom Christian Hospital, Vom, Plateau state. The samples collected were dental plaque of all participants using forceps and by swabbing the caries lesions and or enamel decay of the tooth [16]. The swabs were immediately placed in 5ml of Robertson cooked meat medium and stored at 4°C. The samples were processed within 24 hours of collection at the Bacteriology Laboratory of Federal College of Veterinary and Medical Laboratory Technology, Vom.

2.2 Isolation of Bacteria from Dental Plaque

Basal salt medium containing yeast (BSMY I) extract was used for the isolation of bacteria from dental plaque samples contained; 1.0 g yeast extract, 0.3 g $(NH_4)_2SO_4$, 0.05 g KH_2PO_4, 0.14 g, 0.1 g NaCl, $MgSO_4 \cdot 7H_2O$, 0.2 g $CaCl_2 \cdot 2H_2O$, 0.05 g K_2HPO_4, 0.17 mg $CoCl_2 \cdot 6H_2O$, 0.6 mg H_3BO_3, 0.09 mg $CuCl_2 \cdot 2H_2O$, 0.22 mg $ZnCl_2$, 0.1 mg $MnCl_2 \cdot 4H_2O$, 10 g glucose in 1 liter of Tris-HCl buffer (pH 8.0) [17,18].

The sample in various tubes were inoculated into 25 ml of BSMY I broth. The inoculated flasks incubated at 35ºC±1ºC for 48 hours. The freshly grown culture of 1 ml from each dental plaque were diluted serially (10-5) with distilled water. One hundred microliters (100 µl) diluted samples were spread over agar plates and incubated at 37ºC for 3 days aerobically. The isolated colonies were picked and streaked on slant of BSMY I for pure culture preservation [19].

2.3 Identification of Bacteria

2.3.1 Cultural and morphological characteristics

The cultural characteristics observed were: size, shape, pigmentation, elevation and margin of colony are recorded by culturing them on BSMY I medium and incubated for 24-48 hours at 37ºC. Colonies were observed under reflected light for optical properties (diameter, irregular, white, convex/flat, raised, smooth, translucent colony under light). The bacteria were Gram stained and observed under light microscope for the following characteristics: coccid, bacilli, single, paired, chain and dense clusters observed. Biochemical properties of the isolates were tested according to Bergey's Manual of Systematic Bacteriology. The following properties were determined: Catalase test, acid production from carbohydrates glucose [20,21]

2.4 Antibiotic Susceptible Test

The susceptibility of the bacterial isolates to antimicrobial agents was determined using disk diffusion method [22] and interpreted according to Clinical and Laboratory Standards Institute [23]. The following antimicrobial agents were obtained as standard reference disks for their known potency in laboratory use: vancomycin (V) 10 µg, erythromycin (E) 15 µg, chloramphenicol

(C) 10 µg (Himedia). All of these tests were performed on plates of BSMY I. A 0.5 ml. Suspension of tested bacterial isolates was spread on BSMY I plates. Antimicrobial disks were placed on the agar with sterile forceps. The agar plates were incubated inverted at 35ºC for 24 hours. Results were recorded by measuring the inhibition zone. Out of nine isolates, 4 were resistance to erythromycin and three resistant to vancomycin, and one isolate was resistant to the three antibiotics while all except one were sensitive to chloramphenicol.

3. RESULTS

A total of 150 samples were collected from the patients as shown in Table 1. Of 150 samples 95 (63.3%) had dental caries located at the molar while 10 (6.7%) had dental caries located at the gingival margins. Table 2 depicts the age and sex distribution of patients attending dental clinics in Jos. Higher percentage (80%) of females where seen to be affected with dental caries. The least percentage (6.7%) was found to be among the male patients in the age group of 51-60, while the highest was among those in the age group of 31-40 which cut across the gender. Table 3 showed the frequency of occurrence of bacteria, whereby *Lactobacillus* species was frequently isolated (28.4%). This was followed by *Streptococcus mutans* (17.7%). The least frequency observed was with *Fusobacterium* species (0.7%). Table 4 showed the antibiotics sensitivity test using disk diffusion method, and the result revealed that most bacterial isolates were acidogenic, and were found to be more sensitive (5/9) to the selected antibiotics tested. Out of 9 bacteria isolates, three were resistant to vancomycin, 1 to chloramphenicol, and 4 to erythromycin. Of the observed isolates, only *Enterobacter* species was resistant to all the three selected antibiotics. Observed isolates; *Bacillus subtilis (*BS) and *Staphylococcus aureus* (SA) were resistant to Erythromycin and vancomycin.

Table 1. Occurrence of dental caries taken from different Infection sites in Jos

Sites of infection	Number	Frequency (%)
Cavity at the molar	95	63.3
Cavity between molar and premolar	45	30
Cavity at the gingival margin	10	6.7
Total	150	

Table 2. Age and sex distribution of patients attending dental clinics in Jos

Age groups (years)	Number of cases (%)		
	Male; n = 30(20.0)	Female; n = 120(80.0)	Total; N = 150
11-20	5(16.7)	10(8.3)	15(10.0)
21-30	6(20.0)	12(10.0)	18(12.0)
31-40	9(30.0)	60(50.0)	69(46.0)
41-50	5(16.6)	20(16.7)	25(16.7)
51-60	2(6.7)	12(10.0)	14(9.3)
61-70	3(10.0)	6(5.0)	9(6.0)

Table 3. Frequency of bacterial isolates associated with dental caries from dental plague

Bacterial isolates	Number of organisms isolated	Frequency (%)
Bacillus subtilis (BS)	38	13.5
Staphylococcus aureus(SA)	40	14.2
Streptococcus mutans(SM)	50	18.0
Lactobacillus species (LS)	80	28.4
Klebsiella species (KS)	30	11.0
Escherichia coli (EC)	20	7.1
Fusobacterium species (FS)	2	0.7
Proteus mirabilis (PM)	15	5.3
Enterobacter species (ES)	7	2.5
Total	282	

Table 4. Antimicrobial activity of selected common antibiotics [erythromycin (E), chloramphenicol (C) and vancomycin (V)] against bacterial isolates from dental plaque

Bacterial isolates coded	Antibiotics		
	Erythromycin	Chloramphenicol	Vancomycin
Bacillus subtilis	R	S	R
Staphylococcus aureus	R	S	R
Streptococcus mutans	R	S	S
Lactobacillus species	S	S	S
Klebsiella species	S	S	S
Escherichia coli	S	S	S
Fusobacterium species	S	S	S
Proteus mirabilis	S	S	S
Enterobacter species	R	R	R

R= resistance, S= sensitivity

4. DISCUSSION

Dental caries is one of the most common and less attended diseases in the developing countries. There are vast diversities of human bacteria pathogen that are present in the mouth, and each individual has his or her own unique oral flora; but the composition of the oral flora determines the susceptibility of tooth decay.

The Study observed that most of the patients that visited the dental clinics had their tooth decay at the molar (63.3%). This could be attributed to the position of these teeth where food particles are easily retained. The least frequency was found among the age category of 51-60 (6.7%) male, while in the female age group 61-70 was 5.0%. We also observed that females had the highest percentage (80.0%) of respondents. It was not surprising because females are known to have more tendencies for snacking and drinks of sugared beverages or eat mints to combat mouth odor during the day than males [2,13]. Similarly the result showed highest number of cases among the age group of 31-40 having a percentage of 30% (males) and 50% (females) respectively. The age group 61-70 had the frequency of (6.0%). This finding is at variance with earlier study [23] that stated the prevalence of dental caries increases with advance in age. However, the bacteria species isolated corroborate earlier reported cases in Nigeria [24], and other parts of the world that documented *Lactobacillus* spp and *Streptococcus mutans* as the commonest bacteria associated with dental caries [6,25]. We identified few *Fusobacterium* spp in some samples, but *Streptococcus mutans* and *Lactobacillus* species predominated. An earlier study reported that some bacteria can become dominant in plaque community with attendant demineralization and formation of caries lesion due to process of selection, succession and dominance [26]. However, we do not have evidence to substantiate this assertion with regard to bacterial isolates observed. Dental caries in majority of individuals is characterized by interplay of interactions and succession within the plaque biofilm due to organisms' population responses to pH changes resulting to succession in lesion [27,28]. These interactions in population are complex, and apart from the commonly known mutual associations, competitions could also emanate from stress responses and adaptations due to genetic variations which are vital to the selection of bacteria species that survived the oral ecological imbalance [29]. The use of simple approaches such as oral health hygiene plays a significant role in modulation of the microbial ecology of caries for healthy living [30]. This suggests that the most prevalent organisms; *Lactobacillus* species (LS) and *Streptococcus mutans* (SM) were responsible for the development of caries observed in the patients with gingivitis [31].

The result of disk diffusion method revealed that most isolates were found to be sensitive to the antibiotics tested. Our result does not agree with earlier reported findings by Maripandi et al. [13] that dental caries pathogens were resistant to penicillin, bacitracin, streptomycin, vancomycin and chloramphenicol. This showed that the use of available antibiotics could still be effective in the treatment of dental caries, though chloramphenicol and vancomycin were found to be more sensitive compared to erythromycin. It suggests that the use of vancomycin and chloramphenicol in treating caries should be encouraged because of their bactericidal effects on the isolates considering the high level of sensitivity observed.

5. CONCLUSION

We observed few common acidogenic bacteria isolates known to colonize the dental plaque. Almost all bacteria isolates found in the oral cavity have sufficient pathogenic potential to induce enamel decay which suggests the need for good oral hygiene practices. More importantly, the isolated bacteria species were sensitive to the tested antibiotics. Further studies are needed to investigate the possibility that previously unsuspected species may play important roles in caries development. Larger sample size studies with better isolation, identification and characterization methods are needed. These studies could lead to the identification of strategies for effective biological interventions in the caries process and thereby contribute to improved prevention and treatment of caries.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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