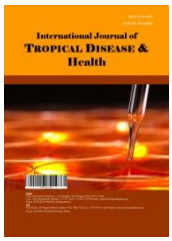

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Bacterial Profile and Risk Factors Associated with Dental Caries in Enugu Metropolis, Southeast Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author UCM conducted the study and performed the statistical analysis. Author NFO wrote the protocol and the first draft of the manuscript. Author MBCC managed the analysis and arranged the manuscript. Author SNM designed and managed the literature. Author IPU assisted in literature, and in the laboratory investigation. All the authors read and approved the final manuscript.

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ABSTRACT

Dental caries is one of the most prevalent and costly diseases that are a challenge to health care providers. There is limited data on the bacterial profile and risks associated with dental caries in Nigeria. The study aimed to isolate and identify bacteria and risk factors associated with dental caries in Enugu. It was a case-controlled study. A total of 336 samples were randomly collected comprising of 125 healthy subjects and 211 patients with dental caries attending various hospitals and dental clinics in Enugu, Nigeria. Samples were subjected to standard microbiological and biochemical techniques. Standardized questionnaires were used to record demographic variables and risk factors. A total of 635 and 254 microorganisms were isolated from carious lesions and healthy subjects respectively. Of these 635 isolates, 218 (34.4%) Gram-positive bacilli, 210 (33.0%)

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Gram-positive cocci, 46 (7.2%) Gram-negative cocci, and 43 (6.8%) yeasts were recovered. Two hundred and seven samples were (98.1%) polymicrobial while 4 samples (1.9%) were monomicrobial. Among the organisms recovered, anaerobic *Lactobacillus spp* (141, 66.8%) was the most prevalent followed by *Streptococcus mutans* (104, 49.2%), and the least were *Actinomyces israelii* and *Capnocytophaga spp* (1, 0.05%) respectively. Of the 254 isolates from apparently healthy subjects, *Streptococcus sanguis* ranked highest and the least was *Porphyromonas gingivalis*. The factors that were found to be significantly associated with dental caries were level of education P=0.005, feeding habit P= 0.001, Favourite snack P=0.004, sweet consumption P< 0.0001, type of gum P < 0.0001, history of toothache P < 0.0001, visit dentist P< 0.0001, and the bleeding gum P < 0.0001. This research highlighted the polymicrobial nature of carious lesions and associated predictors of dental caries. Hence public enlightenment and the implementation of oral health education in schools are crucial.

Keywords: Dental caries; Bacterial profile; Streptococcus mutans; Lactobacillus spp; Risk factors; Enugu-Nigeria.

1. INTRODUCTION

Dental caries is one of the most prevalent chronic diseases of people globally [1,2]. It is painful, expensive to treat, and can disturb nutrition and overall health [3]. Caries is caused by a complex interaction of factors including bacteria, host predisposition, diet, and time [4]. There is also strong evidence for genetic components in the etiology of this disease [5]. Dental caries is the localized degradation by bacterial action of the tooth tissues [6]. These tissues progressively breakdown producing dental cavities (holes in the teeth) and if left untreated, leads to pain, tooth loss, infection, and other complications like Ludwig angina [4,7]. Disease process may involve the enamel, dentin, and cementum, causing decalcification of these tissues and disintegration of the organic substances [8]. Caries has a high financial burden and a major problem for health service providers [9]. The prevalence of dental caries has been studied in the United States [10], in the United Kingdom[11]**,** in China [12], in Philipines [13], and India [14], and many other countries of the world. In Nigeria, reports by several researchers showed the prevalence to be between 4- 35.5% [3, 15-18]. In the 20th century, the mutans streptococci (MS) group attracted the greatest attention of all possible etiological species in dental caries. Researchers initially isolated *Streptococcus mutans* from the human carious lesion, but it was not until much later, when works were done on animal studies that the bacterial etiology was established [19]. Caries is a microbial disease in which etiologic bacteria are normal oral flora that cause disease only when their proportions and pathogenicity respond to changes in environmental conditions [20]. Although *S. mutans* is one of the most researched cariogenic microorganisms, it is only one of more than 500 species found in dental plaque [19,21].

Increasing utilization of sweet/sugary foods, poor tooth brushing habits, poor oral hygiene, and low level of enlightenment are some of the factors that increase the prevalence of dental caries [19,22]. Furthermore, lifestyles, eating habits, social status, and socio-demographic factors also contribute to the development of caries. Caries can be prevented by decreasing sugar intake and brushing teeth after every meal using appropriate techniques and regular check-ups [22]

In developing countries of the world, including Nigeria, information available on the probable factors responsible for dental caries is scarce. Few reported works dwelt on prevalence. The first study on microbial profile in Nigeria was done in Jos [23], Minna [24], and Imo state [7]. *Streptococcus mutans, Staph. aureus, Peptostreptococcus,* and Lactobacillus spp. and *Actinomyces species* have been recovered in caries [7,23-25]. The microbial populations involved in dental caries are known to be highly complex and variable and have not yet been fully identified, although key organisms are generally recognized. However not much is known about bacterial agents in carious lesions and associated risk factors in Enugu Metropolis, hence this study.

2. MATERIALS AND METHODS

2.1 Study Design

This was a case-controlled hospital-based study conducted in the microbiology laboratory of the

University of Nigeria (UNTH) Enugu. All samples were drawn from patients attending the out-patient department of dental clinics of University of Nigeria Teaching Hospital Ituku-Ozalla, Federal School of Dental Technology and Therapy, Enugu, Nigeria, and some private dental clinics in Enugu Metropolis. The healthy controls were drawn from subjects that escorted patients to the hospital and dental health was defined as the complete absence of cavity. None of the patients have received antibiotics within the period of study.

2.2 Sample Collection

Information on the general, personal, and other relevant data were recorded on standardized questionnaires. Samples were collected by a collaborative dentist who understood the nature of the research after being briefed to maintain consistency. The cheesy part of the carious lesion was excavated with the help of a sterile excavator and then swabbed with a sterile swab and inoculated into Robertson's cooked meat (RCM), (Himedia) and carried to the laboratory. Samples were taken with sterile swabs from the plaque of caries-free subjects from the healthy subjects.

2.3 Culture of the Specimen

Samples inoculated into Robertson's cooked meat (RCM) medium were incubated at 37^0C for 48 hours [2]. Subcultures were made from RCM into different media using a standard wire loop. For isolation of aerobic bacteria, subcultures were made from the top of RCM into sheep blood agar (Columbia agar, (Oxoid) and MacConkey agar (Oxoid) and incubated at 37^0C for 24 hours. Facultative anaerobes were sub-cultured into chocolate agar, mitis salivarius agar, and brain heart infusion agar from the middle of RCM and incubated at 5-10% carbon dioxide at 37^0c for 24-48 hours. For the isolation of anaerobes, subcultures were made from near meat particles into Rogosa agar and Anaerobic basal agar (Oxoid) supplemented with 5% horse blood (Oxoid) and placed inside the anaerobic jar and incubated at 37^0C for 72 hours- 7days. The anaerobic condition was achieved using AnaeroGen Atmosphere Generation System (AN0025). Anaerobiosis was monitored with the chemical indicator (methylene blue) and biological indicator (*Pseudomonas aeruginosa*)

2.4 Identification of isolated organisms:

Identification of isolates was done following standard microbiological techniques which involved the morphological study of colonies, Gram staining reactions, and biochemical tests including indole, citrate, oxidase, coagulase, Rapid ID 32 Strept, for streptococcal species, and Rapid ANA 11 for anaerobes (Oxoids).

2.5 Statistical Analysis

All statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad, San Diego, CA, USA). Categorical variables were described using descriptive statistics (frequencies and percentages). Chi-square test and Fisher's exact test (at 95% confidence intervals) were used to test for significant differences in the proportions/prevalence of dental caries in comparison to healthy controls, concerning the socio-demographic variables and predisposing risk factors. P-value ≤ 0.05 was considered statistically significant. Multivariate/binary logistic regression analysis was used to measure the strength of the association.

3. RESULTS

All 211 samples yielded micro-organisms, 97(46.0%) yielded three different types of isolates, 53(25.1%) yielded two different types of isolates, 49(23.2%) four, 8(3.8%) yielded five, and only 4 (1.9%) yielded one type of isolate. While in 125 healthy subjects 54(42.85) yielded two different types of isolates, 50 (40.0%) yielded three, 9 (7.2%) yielded four, one yielded five (0.8%) and 11 yielded only one (8.8%). As seen in Table 1.

Table 2 shows that of 635 different microorganisms isolated, 218 (34.3%)were Gram positive bacilli Anaerobic lactobacillus spp, *Actinomyces viscosus, Actinomyces naeslundii, Actinomyces odontolyticus, Actinomyces israelii,* 210 (33.0%) were Gram positive cocci, 118 (18.6%) were Gram negative bacilli *Aggregatibacter actinomycetecomitans, Prevotella intermedia, Prevotella melaninogenica, Fusobacterium nucleatum, Porphyromonas gingivalis, Klebsiella spp, Pseudomonas aeruginosa*, *Capnocytophaga spp*), 46 (7.2%) were Gram negative cocci (*Veillonella spp*), and 43 (6.8%) were yeast (*Candida albicans* and other yeasts). Anaerobic

lactobacilli had the highest isolation frequency of 66.8%.

Table 3 shows that 336 participated (134 males and 202 females) aged 0-100 years with a mean (SD) of 27.5(14.1) years. The sociodemographic variables show that the majority of participants were females and many of the participants fell within the age range of 16-31; 174 (51.6%) and their level of education showed that the majority of the respondents had or were in a tertiary institution 215 (64.0%). Gender distribution showed that caries were more prevalent in females than males in the ratio of 1.6:1 although this was statistically insignificant P=0.350. Age distribution showed that children >15 years had the highest prevalence 83% followed by 16-31 years 60.3%. Prevalence decreased with increasing age and there was a shoot up again at age 64^{+} to 75%. The level of education showed that prevalence decreases with increasing levels of education. Age and level of education of participants were found to be statistically significant P< 0.05.

Table 1. Analysis based on the number of isolates

No of Isolates	No of samples in Caries	%	No of samples in healthy subjects	%
One Isolate	4	1.9	11	8.8
Two Isolates	53	25.1	54	42.8
Three Isolates	97	46.0	50	40.0
Four Isolates	49	23.2	9	7.2
Five Isolates	8	3.8	1	0.8
Total	211	100	125	100

Table 2. Distribution of isolates based on their Gram reaction, number and percentage occurrence

Name of Organisms	Caries N=211 No (%)	Healthy subjects N=125 No (%)
Gram Positive Cocci		
Streptococcus mutans	104(49.2)	21(16.8)
Streptococcus sanguis	18(8.5)	41(32.8)
Streptococcus mitis	14(6.6)	0(0)
Enterococcus faecalis	7(3.3)	4(3.2)
Staphylococcus aureus	18(8.5)	0(0)
Peptostreptococcus micros	11(5.2)	25(20.0)
Peptostreptococcus anaerobius	38(18.0)	17(13.6)
Gram Positive Bacilli		
Aerobic Lactobacilli spp	1(0.5)	0(0)
Anaerobic Lactobacilli spp	141(66.8)	0(0)
Actinomyces viscosus	33(15.6)	8(6.4)
Actinomyces naeslundii	34(16.1)	15(12.0)
Actinomyces odontolyticus	8(3.8)	0(0)
Actinomyces israelii	1(0.5)	0(0)
Gram Negative Cocci		
Veillonella spp	46(21.8)	28(22.4
Gram Negative Bacilli		
Aggregatibacter actinomycetecomitans	8(3.8)	25(20)
Klebsiella spp	19(9.0)	33(26.4)
Pseudomonas aeruginosa	19(9.0)	7(5.6)
Prevotella intermidia	19(9.0)	2(1.6)
Prevotella melaninogenica	!6(7.6)	0(0)
Porphyromonas gingivalis	13(6.2)	1(0.8)
Fusobacterium nucleatum	23(10.9)	13(10.4)
Capnocytophaga species	1(0.5)	0(0)
Fungi		
Candida albicans	38(18.0)	6(4.8)
Other yeasts	5(2.8)	7(5.6)

The distribution of dental caries according to dietary habits participants were is shown in Table 4. Feeding habits, type of food, favourite snacks, frequencies of snacking, sweet consumption, soft drink consumption, chewing gum, and type of gum were factors that significantly associated with dental caries when compared with healthy groups.

Table 3. Sociodemographic characteristics of Patients and Control subjects

Variables	Control No (%)	Dental Caries No (%)	Total (%)	P-value
Gender				
Male	54(40.2)	80(59.7)	134(40.0)	0.350
Female	71(35.3)	131(65.1)	202(60.1)	
Age Group				
<15	9(16.1)	47(83.9)	56(16.6)	
16-31	69(39.7)	105(60.3)	174(51.8)	0.0001
32-47	35(44.3)	44(55.7)	79(23.5)	
48-63	11(47.8)	12(52.2)	23(6.8)	
64^{+}	1(25.0)	3(75.0)	4(1.2)	
Level of Education				
Illiterate	0(0)	1(100)	1(0.3)	0.002
Primary	4(12.5)	28(87.5)	32(9.5)	
Secondary	18(20.5)	70(79.5)	88(26.2)	
Tertiary	103(47.9)	112(52.1)	215(64.0)	

Table 4. Dietary habits of patients and controls

Variables	Control No (%)	Dental Caries No (%)	Total (%)	P-value
Feeding habit				
Once/day	2(33.3)	4(66.7)	6(10.7)	0.0005
Twice/day	5(12.2)	36(87.8)	41(12.2)	
Thrice/day	109(46.4)	126(53.6)	235(69.9)	
>3	9(16.7)	45(83.3)	54(16.1)	
Type of food				
Homemade/	12(24.0)	38(76,0)	50(14.9)	0.0397
Homemade/Snack	106(38.7)	168(68.0)	274(81.5)	
Snacks alone	7(5.9)	5(41.7)	12(3.6)	
Favorite snack				
Biscuits	23(20.4)	90(79.6)	113(33.6)	0.0017
Chocolates	89(52.4)	81(47.6)	170(50.6)	
Cake	49(22.2)	14(77.8)	18(5.3)	
Doughnut	7(23.3)	23(76.7)	30(8.9)	
None	2(4.0)	3(8.0)	5(1.5)	
Snacking Frequency				
Sometimes	92(48.7)	97(51.3)	189(56.3)	0.0001
N.A	2(40.0)	3(60)	5(1.5)	
As many times	1(8.3)	11(91.7)	12(3.6)	
Once/day	13(44.8)	16(51.2)	29(8.6)	
Twice/ day	17(19.8)	69(80.2)	86(25.6)	
Thrice/day	0(0.0)	15(100)	(15(4.5)	
Soft drink Consumption frequency				
Once/day	122(51.3)	116(48.7)	238(70.8)	0.0001
Twice/day	1(2.6)	37(97.3)	38(11.3)	
Thrice/day	1(1.9)	50(98.0)	51(15.2)	
4-or more/day	0(0)	4(100)	4(1.2)	
Not at all	1(50)	1(50)	2(0.6)	
Sometimes	0(0)	3(1.4)	3(0.9)	
Bottle/day				

Variables	Control No (%)	Dental Caries No (%)	Total (%)	P-value
0-1	122(40.0)	183(60.0)	305(90.8)	0.0001
2-3	2(6.9)	27(93.1)	29(8.6)	
NA	1(50.0)	1(50.0)	2(0.3)	
Sweet Consumption				
Yes	67(30.3)	154(69.7)	221(65.8)	0.0001
No	58(50.4)	57(49.6)	115(34.2)	
Frequency of Consumption				
NA	58(46.8)	57(27.0)	115(34.2)	0.0001
Everyday	7(17.9)	32(80.1)	39(11.6)	
Sometimes	60(37.7)	108(64.3)	168(50.0)	
Always	0(0)	14(100)	14(4.2)	
Chewing Gum Consumption				
No	82(52.2)	75(47.8)	157(46.7)	0.0001
Yes	43(20.0)	136(75.9)	179(53.3)	
Type of Gum				
Non-Sweetened	23(44.2	29(55.8)	52(15.5)	0.0001
Sweetened	20(15.7)	107(84.3)	127(37.8)	
NA	82(52.2)	75(47.8)	157(46.7)	

Table 5 summarizes the distribution of oral hygiene and other dental experiences. Method of oral hygiene, previous history of toothache and at what age it was experienced, visit the dentists, and bleeding gums were factors that significantly associated with caries $P < 0.05$.

Table 6 shows the multivariate analysis. The factors that are significantly associated with dental caries are level of education, feeding habits, favorite snacks, sweets consumption, gum and type of gum, history of toothache, and a visit to the dentist. Respondents who attended primary education or lower had increased odds of having caries 4.6 times compared to those who had higher education. Feeding >3 times per day was associated with increased odds of having caries almost 4 times compared to ≤ 2 times per day. The odds of having dental caries increased 6.6 times among subjects who take snack against those who do not take. A person who takes sweet has 2.3 times chance of developing caries than those people who do not take. Those who reported consuming gum were associated with an increased odds of having caries by 3.5 times compared with those who do not. The odds of having caries increased 4.2 times among those that chew sweetened gum against those that take non-sweetened gum. The subject who reported a history of toothache had 58.7 times higher odds of having caries than those who reported negative. A visit to the dentist is also a very significant risk factor.

4. DISCUSSION

The study assessed the bacterial profile in dental caries and associated risk factors. There are vast diversities of bacterial pathogens present in the mouth and each individual has his or her unique percentage mix of oral flora, but the composition determines the susceptibility of tooth decay [21]. All samples in this study yielded microorganisms, polymicrobial growth was evident in 207(98.1%) of the patient samples and 4(1.9%) were monomicrobial. This study has portrayed the polymicrobial nature of the carious lesion. The observation that more than one causative agent may be involved in a patient has been demonstrated in several studies [2, 21, 23-26] and this is evident in this work.

Gram-positive bacilli accounted for 34.3% of all microorganisms isolated. The most common was anaerobic Lactobacillus species, not only among the Gram-positive bacilli but also among the total isolates 141(66.8%). Lactobacilli were also common in other studies in India 13(17.6%) [2], and in Jos, Plateau State Nigeria 80(28.4%) [23] but at variance with the work done in Imo State [7] and Niger State [24] Nigeria where *Streptococcus mutans* was the most predominant microorganism. Lactobacillus has been reported as the second most cariogenic bacteria of oral flora, although not the caries initiator but plays a vital role in caries progression [27]. The high isolation rate of lactobacilli in this study may have been due to selective media used (Rogosa agar). Of 210 Gram-positive cocci,

104(49.2%) were *Streptococcus mutans*. This organism has been implicated in the initiation of caries. The virulence traits include acidogenesis and acid tolerance, intracellular polysaccharides storage, and extracellular glucan formation which promote Mutan streptococcus attachment and increases plaque pH-lowering ability [20]. Other survival strategies are the ability to produce bacteriocin and the ability to coaggregate with previously adherent bacteria [1]. Among 210 Gram-positive cocci, besides *Streptococcus mutans* other significant cocci were *Streptococcus mitis* 14 (6.6%) *Staphylococcus aureus* 18(8.5%), and *Enterococcus faecalis* 7(3.3%). This corroborates with that of other researchers but with different isolation frequencies [2, 7, 23]. The differences in the recovering rates may be due to varying criteria of patient selection, differences in sampling sites and cultural methods employed, geographically based differences, and the presence of the organism below the threshold of detection [2].

Of the 118 Gram-negative bacilli, the significant ones were *Prevotella intermidia* 19(9.0%), *Prevotella melaninogenica* 16(7.6%), *Porphyromonas gingivalis* 13(6.2%). Other studies recovered few or no anaerobic Gram-negative rods or did not investigate these bacteria; this may be due to difficulties in the isolation of anaerobes or the use of an inadequate anaerobic environment. Synergistic anaerobic infection in animal models has demonstrated the pathogenic potentials of both *Prevotella* and *Porphyromonas* particularly when they are associated with *Fusobacterium nucleatum* and *P. micros* [25]. *Candida albicans* was significantly isolated 38(18.0%). Evidence has supported the role of *Candida albicans* as a member of the mixed oral microbiota involved in caries causation [28]. Researchers reported that a symbiotic relationship exists between *Strept. mutans* and *Candida albicans*. It reported that cells of *C. albicans* were frequently detected along with heavy infections of *S. mutans* in plaque-biofilm from early childhood caries-affected children. They showed that the ability of these organisms to form biofilms is enhanced in vitro and in vivo. The presence of *C. albicans* according to them accrues more biomass and habour more viable *S. mutans* cells than single species [29]. Whether this occurs in an adult is yet to be elucidated.

Table 5. Method of oral hygiene/ experience of Patients and Control subjects

Method of Oral hygiene				
Brush & paste	90(36.9)	154(63.1)	244(72.6)	0.007
Chewing Stick only	12(37.5)	20(62.5)	32(9.5)	
Both	23(38.3)	37(61.7)	60(17.9)	
Frequency of Oral hygiene				
Once/day	81(39.3)	125(60.7)	206(61.3)	0.53
Twice/day	41(34.5)	78(65.5)	119(35.4)	
After each meal	3(27.3)	8(72.7)	11(3.3)	
History of toothache				
No	116(75.3)	38(24.7)	154(45.8)	0.0001
Yes	9(4.9)	173(95.1)	182(54.2)	
At what age				
<20	6(4.7)	123(95.3)	129(38.4)	0.0001
21-40	2(4.2)	44(93.6)	47(14.0)	
41-60	0(0)	10(100)	10(3.0)	
60^{+}	0(0)	1(100)	1(0.3)	
NA	116(77.9)	33(22.1)	149(44.3)	
Visit the Dentist				
No	116(100)	0(0)	116(52.7)	0.0001
Yes	9(4.9)	211(94.3)	220(65.5)	
Bleeding Gum				
No	122(50.0)	122(50.0)	244(72.6)	0.0001
Yes	3(33.3)	89(96.7)	92(27.3)	

Table 6. Multivariate Analysis of factors associated with dental caries

Variables	Odds Ratio	P-value
Gender		
Female	0.8029	0.3579
Male	1	
Level of Education		
≤ Primary	4.57	0.005
> Post Primary	1	
Feeding habit		
≤ 2times	3.9	0.001
≥3 times	1	
Type of food		
Snack and Homemade	0.5005	0.0503
Homemade	1	
Favorite snack		
All kinds	6.6	0.004
No snack	1	
Snacking frequency		
≤ once/day	0.674	0.261
≥ once/day	1	
Soft drink consumption		
Various kinds	1.69	0.710
No soft drinks	1	
Frequency of soft drink		
≥ 3/day	0.111	0.003
≤ 3/day	1	
Sweet consumption		
Yes	2.33	0.0003
No	1	
Gum consumption		
Yes	3.46	< 0.0001
No	1	
Type of Gum		
Sweetened	4.243	0.0001
Non-sweetened	1	
Method of oral hygiene		
Brush and Paste	1.755	0.147
Chewing stick	1	
Frequency of oral hygiene		
Once/day	0.789	0.312
>once/day	1	
History of toothache		
Yes	58.678	< 0.0001
No	1	
Age of 1st toothache		
< 20 yrs	0.6989	0.6191
20 yrs and above	1	
Visit the Dentist		
Yes	5187.315	< 0.0001
No	1	

Another reason for the significant isolation of *Candida albicans* may also be due to abuse and inappropriate use of antibiotics that are common in Nigeria. Almost all bacteria isolates found in the oral cavity have sufficient pathogenic potential to induce enamel decay and hence need for good hygiene practice [19]. The factors that were significantly associated with dental caries were level of education, feeding habits, favorite snacks, sweets consumption, gum and type of gum, history of toothache, and a visit to the dentist. The study found that dental caries was higher among those that had a lower level of education and this study is consistent with those

done in North West Ethiopia [22,30]. Many researchers have also reported a significant association between consumption of sweets and processed snacks and caries which agrees with this study. They deduced that frequent consumption of sweets and processed snacks may be hazardous to the oral health of children of all ages and that sugar-sweetened beverages, as well as non-nutritious snacks, may be particularly hazardous to children's oral health in early childhood [31]. Our study also found that respondents with dental aches were 58.7 times likely to have dental caries, This is consistent with works done in Ethiopia [4,32]. Although our figure is higher when compared with their work probably because this is a case-controlled study and theirs was a cross-sectional study. Non-utilization of dental services is evident in our study. Table 5 shows that 99.1% of healthy controls have not visited the dental clinic revealing the negative attitude of people towards dental/oral health. This agrees with what was reported in South West Nigeria [33]. Preventive oral health is widely practiced in advanced countries such as the United States and the United Kingdom, whereas in developing regions of the World, the purpose of visiting a dental clinic is to ease the pain. It could be that lack of awareness of the necessity of oral health care, as well as widespread poverty in developing countries, have both contributed to poor dental visiting habits observed in this study [33].

5. CONCLUSION

The present study has highlighted the diversity of microorganisms associated with dental caries. Some of these pathogens are also responsible for endodontic treatment failures. The factors that were significantly associated with dental caries were level of education, feeding habits, favorite snacks, sweets consumption, gum and type of gum, history of toothache, and a visit to the dentist. Public awareness, the introduction of oral health education in schools, and the use of good fluoridated toothpaste are advocated.

STUDY LIMITATIONS

Although the study has several important findings, it has some flaws. Self-reported variables were used in the study, which may have been erroneous; the respondents may have exaggerated the data. The use of microscopy may have underrated the microbial proportions, molecular analyses like PCR would have provided more sensitive and specific data.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Informed and written consent was sought and obtained from the patients

ETHICAL APPROVAL

The work was approved by the ethics committee of the University of Nigeria Teaching Hospital Ituku-Ozalla. The work was also performed following the ethical standard laid down in the 1964 declaration of Helsinki

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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