



The Incidence of Bacterial Isolates Among Dental Caries Patients Attending Clinic At Irrua Specialist Teaching Hospital, Irrua, Nigeria

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Abstract

Despite the global public health importance of dental caries, it is greatly understudied. This study investigates the incidence and prevalent bacteria species in dental carries among patients attending dental clinic in Irrua Specialist Teaching Hospital, Irrua, Nigeria. Patients presenting with signs, symptoms and suspected cases of dental caries attending dental clinic in the hospital between May and July 2017 were recruited. Following standard laboratory procedures, swabs were obtained, morphologically and biochemically analyzed and data subjected to simple statistical analysis. Of the 340 suspected cases, 65.59% were positive for bacteria (25.11% in children and 74.89% in adult). Females were more likely (1.23 times) to be affected and 2.98 times more common in adults than children. The prevalent bacteria isolated was *Streptococcus mutans* (151; 67.7%), *Streptococcus sobrinus* (36; 16.4%), *Lactobacillus acidophilus* (22; 9.9%), *Streptococcus salivarius* (10; 4.5%) and lastly *Streptococcus mitis* (4; 1.8%). Biochemical characterization showed isolated *Streptococcus* and *Lactobacillus* species were non-motile and negative to catalase, citrate, oxidase, indole and urease tests. This study showed the incidence of dental caries is high in the study area and that *Streptococcus mutans* is the most causative bacteria.

Keywords: Incidence; Dental caries; Bacteria; Ekpoma.

1. Introduction

Dental caries known also as tooth decay or rottenness, is a breakdown of teeth due to microorganisms activities [1] and associated with pain in teeth and gum following chewing [2] with complications such as inflammation of the tissue around the tooth, tooth loss, infection or abscess formation [3, 4]. It has become more common in both children and adults in recent years [3] with approximately 2.43 billion people (36% of the population) worldwide present with dental caries in their permanent teeth [5]. In fact, WHO estimated that nearly all adults have dental caries at some point in time [6, 7] and affects 620 million baby teeth worldwide [5].

Oral diseases are public health problems worldwide [8] and according to [9], a person experiencing caries may not be aware of the disease. The impact of dental caries on individuals and communities in terms of pain, suffering, functional impairment and reduced quality of life is immense and are the fourth most expensive to treat in most industrialized countries [9]. According to Southern and Southam and Soames [10], four things are necessary for caries formation: a tooth surface (enamel or dentin), caries causing bacteria, fermentable carbohydrates (such as sucrose) and time. Individual's susceptibility varies depending on the teeth shape, oral hygiene, and the buffering capacity of saliva [11].

Many people wrongly think that worms causes tooth decay. The chemoparasitic caries theory explains that bacteria inhabits the mouth and produced acids that dissolves tooth structures when in the presence of fermentable carbohydrates [12]. About 300 oral bacteria species colonized the mouth but only a few specific species cause dental caries with *Streptococcus mutans* and *Lactobacillus* species forming the two principal species [1, 13-15]. Following fermentation of dietary sugars, these organisms overgrowth produced high levels of lactic acid and are resistant to the low pH; a condition essential for cariogenic activity [16]. These bacteria along with others colonize tooth surfaces as plague recognize as biofilm [17] which is a well-organized cooperating community of microorganisms [18].

Worrisome, there are very few publications on national dental caries prevalence in Nigeria, with only one national study on the caries in children in Nigeria conducted in 1995 [19]. The survey showed that 30% and 43% of 12 and 15 year old children had caries [19]. Other publications on prevalence of caries in Nigeria are limited to

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specific towns and cities and reported prevalence ranging from 13.5 – 35.5% between 2004 and 2011 [20, 21]. It is therefore the aim of this study to investigate the incidence and assess the bacteria species in dental carries among patients attending clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria.

2. Materials and Method

2.1. Location of Study Site

The study was carried out in Irrua Specialist Teaching Hospital (I.S.T.H), Irrua in Esan Central Local Government Area of Edo State, Nigeria. This hospital is a tertiary health facility in the administrative headquarters of this local government which lies at Latitude 6.45°N and Longitude 6.15°E. It has a population of about 10,000 people whose major occupation is farming and trading [22].

2.2. Materials/Instrument and Techniques

The equipment used in this study include the following: hot air oven, fridge, incubator, anaerobic jars, petri dishes, measuring cylinder, beakers, weighing balance, autoclave, centrifuge, light microscope, racks, hand lens, cotton wool, test tubes, bijou bottles, hand gloves, acridine orange/sodium dodecyl sulphate, flasks, glass slides, cover slips, stains and media. Media were prepared according to the manufacturer's instruction. Cultural characteristics were observed using standard microbiological techniques. Pure cultures were isolated, Gram stained, followed by biochemical tests to identify the isolates.

2.3. Sterilization of Equipment

All glass wares were washed with detergent, rinsed in distilled water and sterilized at 100°C in the hot air oven for 1 hour before use, while wire loops, and straight wire were sterilized by passing them through a bunsen burner flame until it was red hot before use.

2.4. Study Population

The subjects for this study included all patients attending clinic for dental problems at Irrua Specialist Teaching Hospital, Irrua.

2.5. Duration of Study

The duration of the study was five months (April 2017 – August 2017) and this was divided in three phases. In the 1st phase ethical approval from institution concerned, and purchase of reagents/working materials (April, 2017), the 2nd phase include sample collection, processing and analysis (May – July, 2017) while the 3rd phase involves result summary, statistical analysis, discussion and writing (August, 2017).

2.6. Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee of Ambrose Alli University, Ekpoma, Edo State.

2.7. Sample Collection and Processing

Three hundred and forty samples of suspected cases of dental caries were collected from patients attending dental clinic at Irrua Specialist Teaching Hospital, Irrua between the month of May and July 2017. Samples were collected from patients presenting with signs and symptoms of dental problems. In accordance with the recommendation of *Dragica, et al.* [23], sterile dental forceps and sterile swab sticks, were used aseptically to collect samples, by scrapping or swabbing the suspected caries lesion. Samples on the swab and dental forceps were inoculated aseptically into sterile McCartney bottles containing 2ml of phosphate buffer as a transport medium, and also into Robertson cooked meat medium. Specimen from the transport media were cultured aseptically in duplicates onto blood agar, chocolate agar, mitissalivarius bacitracin agar, lactobacilli (MRS) agar, Sabouraud dextrose agar, chocolate sucrose agar, nutrients agar, brain heat infusion agar and brain heat infusion broth.

A set of plate were incubated anaerobically, and the other set aerobically, both at 37°C for 24hr to 72hrs. All samples were cultured within 1 hour of collection in the Medical Microbiology Laboratory of Irrua Specialist Teaching Hospital.

2.8. Morphological and Biochemical Identification of Isolates

The culture characteristics of isolated bacteria such as size, shape, haemolysis, pigmentation and consistency were noted according to *Cheesbrough* [24]. The bacteria were gram stained and observed under the light microscope with the oil immersion objective (100x). The biochemical properties of the isolates were studied according to *Bergey's Manual of Determinative Buchanan and Gibbons* [25]. The following test were done to identify the isolates; Gram stain, catalase, coagulase, citrate, urease, oxidase, indole (tryptophanase) production, H₂S production, motility, starch hydrolysis, growth at different temperature, growth in sodium chloride and sugar fermentation test.

3. Results

Figure 1 shows the number of positive samples and the incidence of bacteria colonization of dental caries among patients attending dental caries clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria. Of the 340 examined samples suspected with dental caries at the time of the study, 223 were positive for bacteria isolates; giving an incidence of 65.59%. Table 1 contains the age and sex distribution of the positive bacteria culture from the patients attending dental caries clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria. The distribution showed females (55.16%) are more likely (1.23 times) to be affected with dental caries than men (44.84%) and dental caries is 2.98 times more common in adults (74.89%) than children (25.11%).

Figure-1. Number of positive samples and incidence of bacteria colonization of dental caries among patients attending dental caries clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria

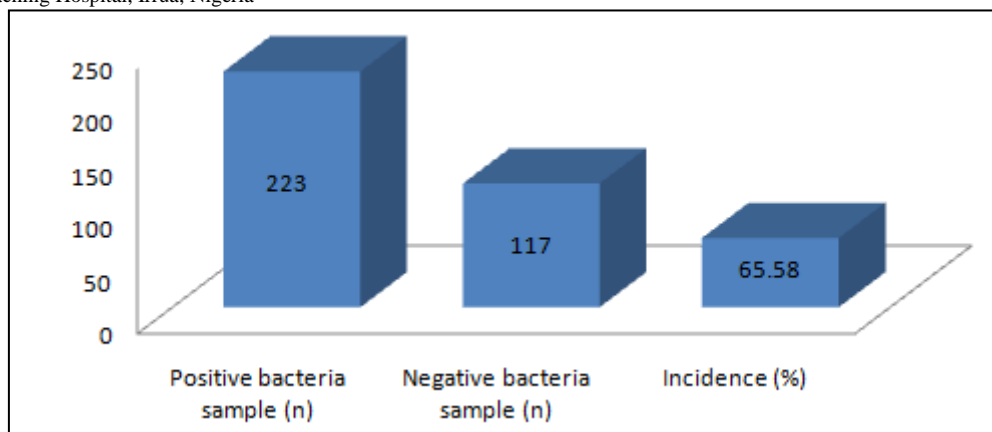


Table-1. Age and sex distribution of positive bacterial culture among patients attending dental caries clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria

Sex	Age		Total (%)
	< 18 years	> 18 years	
Male	24	76	100 (44.84)
Female	32	91	123 (55.16)
Total (%)	56 (25.11)	167 (74.89)	223 (100)

Figure 2 shows the bacterial isolated from dental caries among patients attending dental caries clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria. The bacterial isolates included *Streptococcus mutans* (151; 67.7%), *Streptococcus sobrinus* (36; 16.4%) *Lactobacillus acidophilus* (22; 9.9%), *Streptococcus salivarius* (10; 4.5%) and *Streptococcus mitis* (4; 1.8%).

Figure-2. Bacterial isolated among patients attending dental caries clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria

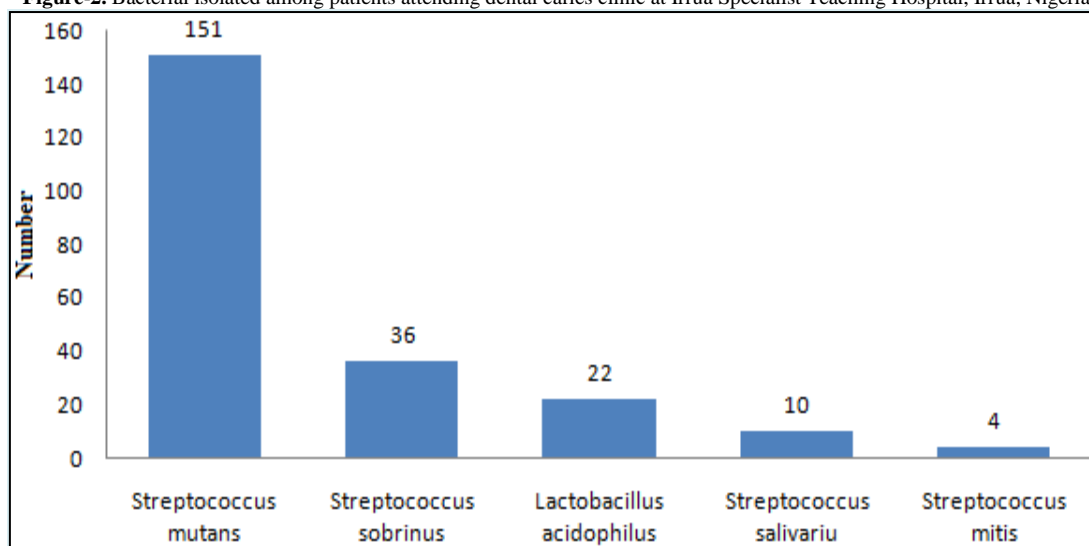


Table 2 and 3 show the biochemical characterization of the isolated *Streptococcus* and *Lactobacillus* species from the samples of the patients. Both the *Streptococcus* species and *L. acidophil* were negative to the following tests; catalase, citrate, oxidase, indole and urease and were non-motile. *L. acidophil* was further negative to triple sugar iron and sorbitol tests and did not showed growth at 15⁰C but showed growth at 37⁰C and 45⁰C.

Table-2. Biochemical characterization of the isolated *Streptococcus* species

Organism	Gram reaction	Haemolysis	Motility	Optochin test	Bacitracin test	Glucose	Lactose	Sucrose	Mannitol	Arginine	Sorbitol	Bile solubility	Growth in 4% NaCl	Growth in 6.5% NaCl
<i>Streptococcus mutans</i>	C	α	-	R	R	+	+	+	+	-	+	-	+	-
<i>Streptococcus salivarius</i>	C	α	-	R	R	+	+	+	-	-	-	-	-	-
<i>Streptococcus mitis</i>	C	α	-	R	R	+	+	+	-	-	-	-	-	-
<i>Streptococcus sobrinus</i>	C	α	-	R	R	+	+	+	-	-	-	-	-	-

KEY: + = Positive; - = Negative; C = Gram positive cocci in chains, α = Alpha haemolysis, S = Sensitive, R = Resistance

Table-3. Biochemical characterization of the isolated *Lactobacillus* species

Lactobacillus Species	Growth at 15 ^o c only	Growth at 45 ^o c only	Growth at 37 ^o c only	Gram stain	Acid from glucose	GAS from glucose	Arginehydrolysis	Arabinose	Cellulose	Mannitol	D – Mannose	Melbiose	Raffinose	Ribose	Salicin	Ramnose	Melezitose	Xylose	galactose
<i>L. acidophilus</i>	-	+	+	P	+	-	-	-	+	-	+	+	+	-	+	-	-	+	+

Key: + = Positive, - = Negative, P = Gram positive rods

4. Discussion

Evidence continues to suggest that oral health is linked to overall health, and dental care utilization may lead to health care cost savings [26, 27]. Yet, dental health is still regarded as optional especially in Africa where it is understudied. Globally, dental caries are a common chronic condition among people of all ages [28]. In fact, it is one of the most common and less attended to diseases in the developing countries. In this study, the overall incidence of dental caries was 65.58% of patients visiting dental clinic with a 25.11% in children and 74.89% in adult. This is comparable with the overall caries prevalence of 32.5% in children and 66.7% in adults among children and adults in selected districts in Uganda Kutesa, *et al.* [29]. The overall incidence of 65.58% in this study is an indication of wide spread of dental caries among the population.

Also in this study, it was observed that dental caries have an association with age and gender. Specifically, the study revealed that dental caries is 2.98 times more likely to affects adults compared to the young and 1.23 times more likely to affect female compare to male population. In line with these findings, other studies have reported dental caries to increase with age in other African countries [29-31]. Studies in 26 non Africa countries including USA, UK, Australia, Canada, Brazil, Japan and Spain have reported a vastly increased problem of caries existed in adults than in children [32]. Also in line with the findings of this study, Enweani, *et al.* [33] and Lukacs and Largaespada [34] have reported association between dental caries and gender with more female affected than males. The higher percentage of dental caries in adult as compared to children may be due to differences in activities between adults and children. For example, the use of tobacco may increase the risk for caries formation as some brands of smokeless tobacco contain high sugar content, increasing susceptibility to caries [35]. Also, evidence has been provided to demonstrate that caries risk factors for women include a different salivary composition and flow rate, hormonal fluctuations, dietary habits, genetic variations, and particular social roles among their family [36].

Dental caries usually demolish the enamel and dentin by bacterial activity [37-40] and it is now avowed that the formation of bacterial biofilm is responsible for a variety of human diseases including dental caries [41]. The dynamic breakdown of tissues enhanced by *Streptococcus mutans*, *Staphylococcus aureus* and *Lactobacillus* spp. produce dental caries [42, 43]. In the present study, 223 bacterial isolates distributed as *Streptococcus mutans* (67.7%), *Streptococcus sobrinus* (16.4%), *Lactobacillus acidophilus* (9.9%), *Streptococcus salivarius* (4.5%), and *Streptococcus mitis* (1.8%) were the isolates from dental caries positive patients in the study area. These bacterial species isolated corroborate earlier reported cases in Nigeria [44] and other part of the world that reported *Streptococcus mutans* and *Lactobacillus* species as some of the commonest bacteria associated with dental caries [45, 46]. The most predominant bacteria organism isolated were the *Streptococcus* species which accounted for 90.1% while the least was *Lactobacillus species* which accounted for 9.9%. This is similar to the report of Jubair [47], who obtained 102 positive cultures of dental caries with *Streptococcus* species accounted for 84 (70%) of the 102 positive culture, and within the *Streptococcus* group, *Streptococcus mutans* accounted for 60 (71.4%) as the highest number of isolate in the report. These findings from this study is also in conformity with the report of Enweani, *et al.* [33], who reported *Streptococcus mutans* as the highest number of isolates 36 (72%) from 50 extracted carious specimens and scrapings.

5. Conclusions

Based on the findings of this study, dental caries is prevalent in the study area and affects more female than male and adults than children. The causative bacteria species are of the *Streptococcus* and *Lactobacillus* species with the *S. mutans*, *S. sobrinus* and *Lactobacillus acidophilus* mostly colonized in patients.

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Conflict of Interest

None to declare.

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