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ORIGINAL RESEARCH

Salivary Bacterial Count and its Implications on the Prevalence of Oral Conditions

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ABSTRACT

Aim: To determine the relationship between salivary bacterial count and some oral lesions.

Materials and methods: This research was designed as a cross-sectional study assessing the mean bacterial count in the saliva of Nigerians in Ile-Ife who has no history of medical illness. Subjects were randomly selected from consenting staffs and students of Obafemi Awolowo University, Ile-Ife, Nigeria. Oral examinations were done and their saliva collected using spitting method. The bacterial count was determined in the laboratory by culture the bacteria after which the counting was done using colony counter. Data analysis was done using STATA 13 software.

Results: A total of sixty participants were recruited for the study, 41 males and 19 females, their mean age was 23.12+3.8 years. The mean salivary bacteria count among the participants was 8.41 x 108 + 1.06x 109 per mL. The salivary bacterial count is highest among those with poor oral hygiene (1.89 x 109) and also increases with age. Males have a higher bacterial load compared to female. Subjects with periodontitis have the highest bacterial load and are significantly higher in patients with periodontitis in comparison with those who do not have periodontitis p = 0.03

Conclusion: Periodontitis is associated with the significantly higher salivary bacterial load. Male sex and increasing age were also associated with increased bacterial load in saliva.

Clinical significance: This study showed another potential role of saliva as a reliable diagnostic tool to monitor the severity of oral infections. It also showed the association between salivary bacterial count and some oral lesions further substantiating the possibility of using saliva to monitor disease progression and treatment responses.

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INTRODUCTION

Saliva is a colorless biological fluid which reflects systemic health, and it is secreted into the oral cavity by major and minor salivary glands in oral and perioral tissues.[1] Whole mouth saliva, in addition to the primary secretion from the glands, contains crevicular fluids from the gingivae.[2] The major salivary glands are the submandibular gland, sublingual gland, and parotid gland while the minor glands are widely distributed in the oral mucosa such as palate, buccal mucosa, and tongue. A healthy adult produces 500 to 1500 mL of saliva daily at a rate of 0.5 mL/min, the rate can rise to above 1 mL/min following stimulation.[3] The relative contribution of each type of gland to the total unstimulated saliva secreted are 65%, 23%, 8%, to 4% for submandibular, parotid, Von Ebner and sublingual gland. However, with nervous stimulation, there is an alteration in the relative contribution of each of the glands to the whole saliva, with parotid glands contributing over 50% to the whole saliva.[2]

Saliva contains both organic and inorganic component which are physically combined together. Water constitutes 99.5% of the constituents of saliva; the other components include glycoprotein, mucin, proteins, enzymes, and electrolytes. The quantity and the quality of these constituents reflect the state of health of both systemic and oral health.[1] The Saliva is thus able to perform several roles due to its content, these roles are; digestion (due to mucin, digestive enzyme, water), taste perception

(water), deglutition (mucin) and oral mucosa immunity (immunoglobulin, protein). Primary saliva is produced directly from the cells and is essentially an ultrafiltrate of the plasma. Following modifications such as ionic exchange at the ductal cells, secondary saliva is produced. The saliva from a non-infected gland is essentially bacteria free, but as soon as the saliva gets to the mouth, it mixes with oral microflora; hence the saliva in the mouth contains a certain number of bacterial which is measurable in the laboratory. The quantity of salivary bacteria is thus a reflection of bacterial load in the mouth; this, in turn, is greatly influenced by the immune status of the individuals and severity of ongoing local or systemic infection.[4]

When there is no active infection affecting the glands, the primary saliva is free of bacteria. However, on getting to the mouth, several additions are included from plaque, calculus, inflammatory transudates, and some other oral products. These tend to increase the bacterial content of the saliva, and there appears to be a correlation between salivary bacteria and oral hygiene, oral infection systemic health of the patients. Some of the bacteria in the saliva that are being reported in scientific studies are *streptococcus mutans, porphyromonas gingivalis, and bacteroidis spp*. These bacteria usually get entrance into the saliva through inflamed gingiva and other oral tissues. These organisms also play some role in the development of periodontitis, osteomyelitis, dental caries, halitosis and so many other odontogenic and non-odontogenic infections that manifest in the mouth.

Most of the highly pathologic oral bacteria reside in the pocket where the oxygen tension is lower, hence the majority of these organisms are obligate anaerobes grand positive organisms, being able to survive in an environment of lower oxygen tension. These organisms are also known for their ability to produce toxic substances that can destroy oral connective tissues.

Several studies have reported a correlation between the severity of oral disease conditions and salivary bacterial load, but studies on the actual bacterial count in the saliva of subjects with no history of underlying systemic diseases are very few. Data from this study would serve as a baseline value of bacterial count in the mouth of apparently healthy subjects which can be used as a comparison with those subjects with specific oral or systemic diseases in future studies. The present study determined the actual mean salivary bacterial count of apparently healthy Nigerian adult population with or without oral lesions and compare the salivary bacterial count based on age, sex and presence of oral lesions.

MATERIALS AND METHODS

- *Study design:* Cross-sectional study of the bacterial count among the participants
- *Study location:* Obafemi Awolowo University, Ile-Ife University Community

Subjects

Subjects were volunteers from Obafemi Awolowo University, Ile-Ife campus community comprising the students, staff, and traders. Participants were chosen using simple random sampling method among the team of individuals who presented for oral health screening at the dental hospital following an Oral Health awareness talk given by researches to the members of the University community. Inclusion criteria include the absence of debilitating systemic diseases or any established case of salivary gland diseases.

Ethical Consideration

Ethical approval was sought from the Research and Ethics Committee of the Institute of Public Health OAU Ile-Ife. Permission to carry on the study was also sought and obtained from all participants. All information was treated with the utmost confidentiality.

Study Protocol

Patients who met the inclusion criteria were informed about the study after which a signed consent of those willing to participate was obtained. The questionnaire was administered to each patient by the researcher. Section A of the questionnaire elicited information related to the patients' biodata such as name, age, gender, address, ethnicity, marital status, and occupation. Section B records the findings from oral examination. The presence or the absence of halitosis was assessed by using Organoleptic method reported by Miyazaki and colleagues at the November 2001 ADA conference on the diagnosis and management of oral malodor [5] with the researcher as the observer. Those with organoleptic scores 0 and 1 (indicating those with no perceivable oral malodor) were marked absent for halitosis while those from score 2 and above were marked present. Gingival status was assessed using gingival index Loe and Silness, as reported by Benamghar[6] A tooth is said to have developed periodontitis when there is associated established pocket of more than 3 mm depth.[7,8] For the purpose of this study, teeth with established pocket more than 3 mm were taken as having periodontitis. Tooth mobility was assessed using bimanual assessment, and caries assessment was done using visual examination and explorer.

Saliva Collection

Findings from the salivary collection were recorded in section C of the questionnaire. Saliva collection was done using spitting method for all participants between 10 am to 12 am to minimize the effects of diurnal variation on salivary constituents. The participants were earlier informed to abstain from food one hour before the procedure. They were also told not to use perfumes or other ornaments that can modify saliva secretion. The spitting method was used to collect the saliva. The participants were told to relax on the dental chair in a recumbent position and were told to remain motionless as much as they could do as they spit the saliva into the salivary jar given them for five minutes. After the collection, the saliva samples were transported to the laboratory for storage at –10° C prior to analysis.

Laboratory Procedure (Total Coliform Count in cfu/mL)

The total numbers of living bacteria in the samples were estimated by culturing method. Prior to the culture of the samples, a serial dilution of the 1mL of each salivary sample was done in a ratio ranging from 10^1 to 10^{10}. A serial solution of 10 different concentrations from 10^1 to 10^{10} was obtained. The solution with 10^{10} contains the least concentration of the bacteria. The samples at 10^{10} dilution were cultured on nutrient Agar medium and incubated at 37°C for 24 hours. Following incubation, the total number of viable colonies on each cultured plates was counted using colony counter in cfu/unit.

Table 1: Sociodemographics of participants

Variable	*Frequency (%)*
Age category	
<20	15 (25.0)
21–30	41 (68.4)
31–40	4 (6.6)
Sex	
Male	41 (68.3)
Female	19 (31.7)
Marital Status	
Single	56 (93.3)
Married	4 (6.7)
Ethnicity	
Yoruba	47 (78.3)
Hausa	7 (11.7)
Ibo	1 (1.6)
Others	7 (8.3)
Occupation	
Schooling	5.5 (91.7)
Trading	2 (3.3)
Civil servants	3 (5.0)

Data Analysis

Data were analyzed using STATA 11 statistical software (StataCorp, College Station, Texas). Percentages and proportion were used to describe the distribution of participants with oral lesion while descriptive statistics were used to characterize sociodemographic variables such as age and sex. Likewise, analyzing salivary bacterial count include checking for mean, median, mode, and range and after subjecting to normality tests, comparison of the mean values of the salivary bacterial count at the various age groups and sex were done using Mann–Whitney rank sum test and Kruskal–Wallis test since the data showed a non-parametric distribution. Statistical significance will be set at $p < 0.05$.

RESULTS

Sociodemographic of Participants

A total of sixty participants were recruited for the study, 41 males and 19 females. Their mean age was 23.12 ± 3.8. More than two-thirds of the participants fall within the age group of 21–30 years. The majority were single, schooling and of Yoruba ethnicity (Table 1).

Distribution of Oral Signs and Symptoms among the Participants

Dental caries was the most prevalent seen, in 11 (18.3%) participants. Other oral findings are bleeding gum, pain, mobile tooth and swollen gum (Table 2).

Table 2: Distribution of oral signs and symptoms among the participants

Variable	*Frequency (%)*
Pain	
Present	7 (11.7)
Absent	53 (88.3)
Bleeding gum	
Present	9 (15)
Absent	51 (85)
Swollen gum	
Present	5 (8.3)
Absent	55 (91.7)
Gingival recession	
Present	7 (11.7)
Absent	53 (88.3)
Mobile teeth	
Present	5 (8.3)
Absent	55 (91.7)
Malodor	
Present	7 (11.7)
Absent	53 (88.3)
Caries	
Present	11 (18.3)
Absent	49 (81.6)

Salivary Bacteria Count and Oral Hygiene Status

The mean salivary bacteria count among the participants was $8.41 \times 10^8 \pm 1.06 \times 10^9$ per mL. The salivary bacterial count is highest among those with poor oral hygiene (1.89×10^9) and lowest among those with good oral hygiene 2.17×10^8. The difference is statistically significant $p = 0.04$ (Graph 1).

$p = 0.0001$ (Kruskal–Wallis test was used for the comparison).

Age Changes and Salivary Bacterial Count

The salivary bacterial count increases with age: <20 years was $7.78 \times 10^8 \pm 1.26 \times 10^9$, 21–30 years was $8.38 \times 10^8 \pm 1.01 + 10^8$ and at ages of 31–40 years $1.0 \times 10^9 \pm 8.01 \times 10^8$. The difference was statistically significant, $p = 0.04$ (Graph 2).

Sex and Salivary Bacterial Count

Salivary bacterial count was higher among males ($9.3 \times 10^8 \pm 1.11 \times 10^9$) when compared to females ($6.34 \times 10^8 \pm 9.2 \times 10^8$) but the differences were not statistically significant, $p = 0.152$, t-test.

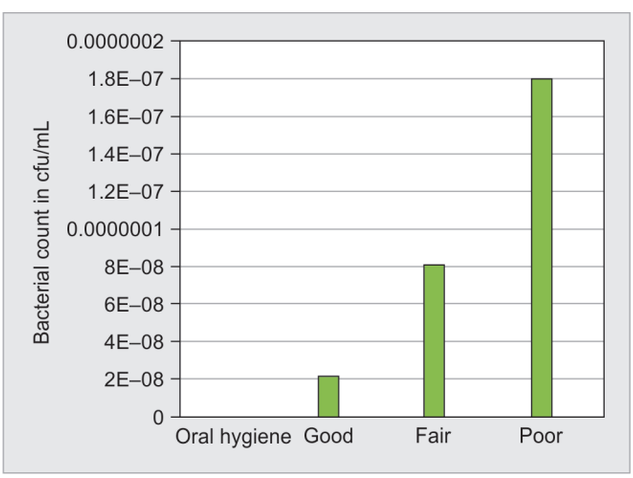
Relationship between Oral Lesions and Salivary Bacterial Count

The highest salivary bacterial count was found among participants with dental pain. The bacterial count among participants with periodontitis was significantly greater than those without periodontitis, p = 0.03. Other oral lesions and habits studied showed no statistically significant difference between those with and without the lesions as shown in Table 3.

DISCUSSION

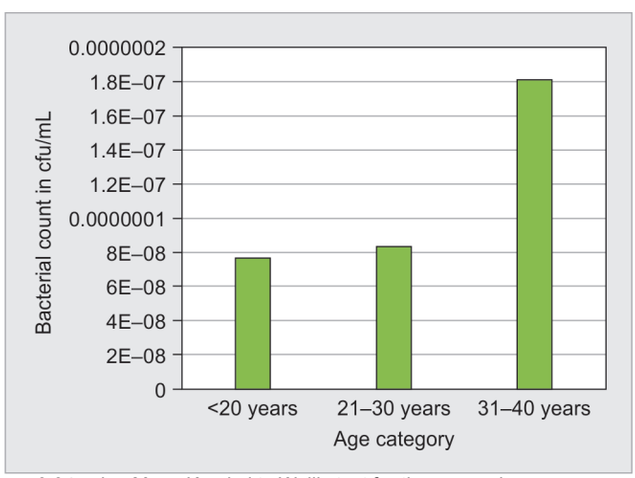
The mouth represents a perfect ecological niche that encourages microbial growth. About 100–200 species of bacteria reside in saliva at any given time. In addition to bacterial; fungi and virus are also grown in the saliva. Bacterial often get into the saliva through the dental plaque, dental calculus, foreign body introduced into the mouth, infected lesions in the mouth or perioral structures, and the infection spread from the surrounding cavities such as gastrointestinal tract, nasal cavity, and respiratory system. Oral bacterial therefore be an indicator of the oral hygiene status, oral infection or infections from the surrounding cavities.[9]

Most studies on oral bacterial focused on the number of different species of bacteria present in the oral cavity or tooth surfaces the general report is that the number of oral bacteria is higher in neglected/dirty mouth as well as patients with increased pathogenicity of oral bacterial due to impaired immunity and those with increased plaque and calculus accumulation.[10-12] This study, aimed



p = 0.0001 (Kruskal–Wallis test was used for the comparison)

Graph 1: Salivary bacteria count and oral hygiene status



p = 0.04 using Mann Kruskal to Wallis test for the comparison

Graph 2: Age changes and salivary bacterial count

Table 3: Relationship between oral lesions/habits and salivary bacterial count

Variables	*Present (cfu/mL)*	*Absent (cfu/mL)*	*p value*
Dental pain	9 x 108 ± 1.2 x 109	8.3 x 108 ± 1.1 x 109	0.431
Gum bleeding	7.4 x 108 ± 2.7 x 108	8.5 x 108 ± 1.4 x 108	0.612
Gum swellings	6.56 x 108 ± 9.9 x 108	8.56 x 108 ± 1.07 x 109	0.65
Gingival recession	3.44 x 108 ± 9.09 x 108	1.48 x 108 ± 1.08 x 107	0.35
Caries	8.08 x 108 ± 1.17 x 109	8.83 x 108 ± 1.06 x 109	0.43
Periodontitis	1.12 x 109 ± 1.22 x 109	6.39 x 108 ± 8.8 x 109	0.03*
Smoking	1.00 x 109 ± 1.12 x 109	8.36 x 108 ± 1.06 x 109	0.22
Alcohol	1.20 x 109 ± 1.2 x 109	7.7 x 108 ± 1.04 x 109	0.14

*$p < 0.05$

at determining the salivary bacterial count in apparently healthy individuals, showed that the mean bacterial count of apparently healthy Nigerian was $8.41 \times 108 + 1.06 \times 10^9$ per mL of saliva. A progressively increasing salivary bacterial count progressively was also observed as oral hygiene tends towards becoming poor. Mantilla and colleagues 13 in a study conducted in Netherland reported the mean salivary bacterial count of healthy subjects as 8.6×09. The value obtained by Mantilla and colleagues was higher than the result of the present study, which was done among Nigerians. The reason for the higher bacterial count may be due to the effects of tongue scrapping which was done before taking saliva samples in the Mantilla and colleagues' study, and tongue scrapping was not done in this study. It should be noted that tongue scrapping before saliva collection might have exaggerated the salivary bacterial count in saliva since the majority of oral bacterial resides on the tongue deposits.[13]

The increase in salivary bacterial count as the oral hygiene status becomes worse another conspicuous finding in this study which is in agreement with some scientific reports.[14,15] Oral hygiene status is largely determined by the amount of plaque and calculus accumulation. Higher accumulation of debris (plaque and calculus) are seen in patients with poor oral hygiene and this justified the use of chlorhexidine mouth wash which reduces bacterial count in the management of neglected mouth,[16] this becomes more evident as the present study showed a significant reduction in bacterial count in subjects with good oral hygiene when compared with subjects with poor oral hygiene. Some earlier reports had shown that salivary bacterial load is affected by the sex of the subjects.[17,18] This study showed a higher bacterial count in the saliva of male subjects when compared with female. These findings are in agreement with the reports of Kudirkaite et al. in a study among adolescents, and they reported higher bacterial load in the mouth of male subjects. They attributed their findings to the fact that females tend to brush their teeth more than males; hence females tend to have a cleaner mouth.[18] In another study, Ferraro et al., however, reported that female has a higher bacterial load due to oral health challenges during menstruation and pregnancy.[17]

Consistent with the reports from the scientific pieces of literature,[18,19] this study showed an increasing salivary bacterial count with increasing age. As age increases the immunity reduces and that might have been responsible for the increased proliferation of bacteria in the saliva.[20] The fact that oral hygiene practices tend to decrease with increasing age towards elderly can also be explained to be due to the loss of dexterity and muscular control at this age group, and dexterity is highly needed for effective tooth brushing. Powered toothbrushes which appear may, in part, be a solution to these problems is not readily available in many parts of Africa.

Although the participants of this study have normal systemic health, some oral problems were seen. The most frequent oral lesion seen was dental caries followed by bleeding gum and periodontitis. These findings are in agreement with reports that dental caries and periodontitis were the common oral disease in the population. This study showed variations in oral bacterial load with respect to different oral lesions in the subjects' mouth. The highest number of the bacterial count was seen among the subjects who had periodontitis, followed by subjects that smoke. Periodontitis is a common oral infection associated with the gram-negative anaerobic bacteria resulting in inflammation of the periodontium due to the effects of C reactive protein and inflammatory cytokines released by the microorganisms. The primary etiologic agent of periodontitis is a dental plaque, which is about 80% of oral bacteria. The periodontal destruction is brought about by the toxins released by the periodontopathic bacterial. Umoh et al. had earlier reported a higher bacterial load in the saliva of patients with the neglected mouth, a condition that is closely associated with poor oral hygiene and periodontitis.[21] Consistent with the findings of Petrusic et al., 2015, this study showed a positive association between smoking and increase the oral bacterial count. Smokers generally pay less attention to their oral health practices that may encourage plaque accumulation in the mouth. In addition, the destructive effects of smoking on periodontal health encourage bacterial proliferation. Smoking causes a lack of oxygen in the bloodstream such that the infected periodontal tissue will not heal as well as enhancing the proliferation of the oral bacterial due to the impaired oral mucosa immunity and reduced salivation.[22]

Bacterial count in saliva will not only reflect the ongoing pathogenic processes in the mouth but also show the integrity of systemic health. Its evaluation thus could be a quick and cheap means of monitoring the severity of oral infections, especially in resource-limited environments.

CONCLUSION

There was a significant relationship between salivary bacterial load and oral diseases. Periodontitis and smoking were associated with the significantly higher salivary bacterial load, unlike in subjects with; gingiva recession, gum bleeding and gum swelling which showed relatively lowered salivary bacterial count. Male sex and increasing age were also associated with increased bacterial load in the saliva of apparently healthy individuals.

CLINICAL SIGNIFICANCE

This study showed another possible clinical application of saliva as a reliable diagnostic tool. This study showed the association between salivary bacterial and some specific oral lesions as well as age and sex.

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