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Presence of Bacteria With Pathogenic Potential Among Already-Used Toothbrushes From University Students

Arthur C Okafor [1,*], Serah N Igwesi [1], Elijah E David [2], Vitus K Okolo [3], Kingsley Agu [4]

[1] *Department of Microbiology, University of Renaissance, Ugbawka, Nigeria*

[2] *Department of Chemistry/Biochemistry/Molecular Biology, University of Federal, Ndufu-Alike Ikwo, Nigeria*

[3] *Department of Pharmaceutical Biology and Traditional Medicine, University of Nnamdi Azikiwe, Awka, Nigeria*

[4] *Department of Applied Microbiology and Brewing, University of Nnamdi Azikiwe, Awka, Nigeria*

* *Corresponding authors: Arthur C Okafor, Department of Microbiology, University of Renaissance, P.M.B. 01183, Ugbawka, Nigeria. E-mail: okaforac1@gmail.com*

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Abstract

Introduction: The most common oral hygiene aid used to improve the oral health of an individual is the toothbrush. Hence, it could play a role in initiation and transmission of infections and diseases. This study aimed at investigating the presence of haemolytic bacteria on used toothbrushes.

Methods: A close-ended questionnaire was designed and distributed to a hundred Nigerian students. This was employed to ascertain the attitudes of students towards their toothbrush. Twenty samples of already-used toothbrush (1-3 months) were randomly collected from students. The heads containing bristles were detached soaked in peptone water and vortexed. Serial dilution was done and appropriate dilution factor was plated out on Nutrient agar medium. The isolates were tentatively identified with morphological and biochemical properties. They were also assayed for haemolytic activity and antibiotics susceptibility.

Results: Results indicated that 80% of the students responded to the questionnaire, out of which > 60% agreed to have sometime injured themselves while brushing their teeth. A little above half of the participants do not care about the purity of the water they use in brushing. Majority (70%) saw no use submitting their toothbrush for microbiological assessment. However, 81.25% replace their toothbrush within 5 months of use. Seventeen out of the twenty samples examined had aerobic bacterial count of > 10^4 cfu/toothbrush. The 24 isolates obtained were found to belong to *Lactobacillus, Staphylococcus, Streptococcus*, and *Bacillus* genera with 55%, 30%, 20%, and 15% occurrence, respectively. Moreover, 40% of the isolates were found to be either alpha or beta haemolytic indicating their potential to cause disease.

Conclusions: Generally, all isolates were susceptible to most of the commonly used antibiotics. It is envisaged that the results of this study would chaperone oral hygiene practice among individuals such as the immunocompromised.

INTRODUCTION

Toothbrush is a small brush with a long handle for cleaning the teeth. It plays a pertinent role in oral hygiene and commonly found in homes and hospitals. It could play a significant role in disease transmission since it can serve as reservoir for microorganisms in healthy and medically ill adults [1]. Oral diseases can be greatly controlled by reducing the microbial load in the oral cavity and this can be achieved by maintaining proper oral hygiene [2].

Toothbrushes are most commonly located near the bathroom sink which is very conducive for microbial growth. A new toothbrush is usually not a favourable habitat for bacteria, but in some cases, it is already slightly contaminated

because of the absence of regulations that ensure its sterility when packaged for sale [3]. Because the mouth is a hospitable niche for all kinds of microbes, the toothbrush will always be contaminated through brushing [4].

Bacteria are the most obvious inhabitants of the oral cavity [5]. *Neisseria* and *Streptococcus* species are early colonizers of the tooth surface. The growth and metabolism of these pioneer species alter the environmental conditions such that more fastidious microbes are able to further colonize, forming dental plaque [6].

Prolonged use of toothbrush facilitates contamination by various microorganisms such as *Streptococcus*, *Staphylococcus* and lactobacilli [7]. These bacteria are implicated as causative agents of diseases that affect both oral and general health [8]. It has been estimated that 51 million school hours per year are lost due to dental related illnesses alone [9].

Therefore, the need to appraise the attitude of a cross section of students towards their toothbrush cannot be over emphasized. Reports on the pathogenic potential of microbes isolated from toothbrush are rarely found in literature. Such study is imperative since toothbrush, as a neglected fomite, is likely to initiate and transmit disease.

The objective of the study was to isolate haemolytic bacteria from used toothbrushes and to assess the level of oral hygienic practices of university students regarding their toothbrush.

METHODS

Distribution of Questionnaires

A short close-ended questionnaire was designed and distributed to a hundred University students. The questions posed bordered on personal oral hygiene practices. All the participants provided consent and showed willingness to participate.

Collection of Samples

Twenty samples of toothbrushes already in use for 1-3 months were aseptically collected from students. The students agreed to release their toothbrush upon receiving new ones. The samples were labeled appropriately and quickly taken to the laboratory for analysis.

Preparation of Samples

The head regions of the toothbrushes were detached with sterile scissors and soaked in test tubes containing 10 mL of peptone water for 60 minutes. After which they were vortexed for a minute to dislodge bacteria from the samples.

Enumeration and Identification of Isolates

Serial dilution was done with the soaked samples and 1 mL of the appropriate dilution factor from each sample was pour plated with molten Nutrient agar medium. Plates were incubated aerobically at 37°C for 24 hours for enumeration of bacteria.

The bacterial isolates were tentatively identified by morphological properties and biochemical tests.

Test for Haemolysis

The blood agar medium was prepared according to the manufacturer's instructions and a loopful of the isolates were streaked on the medium. Plates were incubated aerobically at 37°C for 24 hours and examined for zones of haemolysis.

Antibiotics Susceptibility Test

The following antibiotics were used: cefalexin, ciprofloxacin, clindamycin, cloxacillin, cotrimoxazole, erythromycin, tetracycline, and Amoxyclav. Normal saline (3-4 mL) was poured into sterile test tubes, after which an aliquot of an isolate was emulsified in this tube in such a way to make it as clear as 0.5 MacFarland standards. Then a sterile swab stick that was dipped into this test tube was swabbed all over the surfaces of plates containing Mueller Hinton agar medium, and plates were allowed to stand for 10 minutes. Then, the antibiotics discs were placed on the surface of the medium and incubated at 37°C for 24 hours. Zones of inhibition were measured and recorded in millimeter.

RESULTS

Out of a hundred questionnaires that were distributed to hundred students, eighty students responded: 41.25% male and 58.75% female. Majority of the respondents (56.25%) agreed to brush twice daily. The participants (61.25%) preferred a particular brand of toothpaste. Table 1 summarizes the response of the students. Moreover, >60% had sometime injured themselves while brushing their teeth. More than half of the respondents do not care about the potability of the water they use in brushing.

Table 1: Responses of Students to Questionnaire on Oral Hygiene

Questions Posed	Percentage Affirmation, %
I Keep My Toothbrush in the Bathroom	10.00
I Keep My Toothbrush in the Toilet	12.50
I Keep My Toothbrush in My Travelling Bag	23.75
I Keep My Toothbrush in the Bedroom	53.75
I Use My Toothbrush for < 5 Months Before Replacement	81.25
I Once Shared My Toothbrush With a Colleague	3.75
I Sometimes Injure Myself While Brushing	61.25
I Do not Care About the Purity of Water I Use in Brushing	52.50
I Think My Toothbrush Often Makes Me Sick	11.25
I Sometimes Brush Without Applying Toothpaste	3.23
It Is of no Use Submitting My Toothbrush for Microbiological Analysis	70.00

The total aerobic bacterial count ranged from 1.16×10^4 cfu/toothbrush to 1.00×10^6 cfu/toothbrush (Table 2).

Table 2: Total Viable Count of Bacteria from Samples

Samples	Bacterial Count, cfu/Sample
T_1	1.16×10^4
T_2	7.20×10^4
T_3	1.20×10^5
T_4	ND
T_5	8.40×10^4
T_6	9.20×10^4
T_7	1.80×10^5
T_8	ND
T_9	1.28×10^4
T_{10}	1.00×10^6
T_{11}	7.20×10^4
T_{12}	1.28×10^4
T_{13}	ND
T_{14}	2.88×10^4
T_{15}	4.00×10^5
T_{16}	1.92×10^4
T_{17}	7.20×10^4
T_{18}	1.20×10^5
T_{19}	5.20×10^4
T_{20}	8.40×10^4

Abbreviations: cfu, colony forming unit; ND, not determined.

Twenty four isolates were obtained and all were found to be gram positive bacteria with dorminance being: *Lactobacillus* > *Staphylococcus* > *Streptococcus* > *Bacillus* (Table 3).

Table 3: Percentage of Occurrence of the Isolates Among the Samples

Probable Genera	Percentage, %
Lactobacillus	55
Staphylococcus	30
Streptococcus	20
Bacillus	15

Fourteen of the isolates tested negative to test for haemolysis (Table 4), while about 40% had haemolytic activity.

Table 4: Results of the Haemolytic Test of the Isolates

Isolate	Haemolytic Activity
S_1	Negative
S_2	Positive (A)
S_3	Negative
S_4	Negative
S_5	Negative
S_6	Negative
SR_7	Positive (B)
SR_8	Positive (B)
SR_9	Positive (A)
SR_{10}	Positive (B)
LB_{11}	Positive (A)
LB_{12}	Negative
LB_{13}	Negative
LB_{14}	Negative
LB_{15}	Negative
LB_{16}	Negative
LB_{17}	Negative
LB_{18}	Positive (A)
LB_{19}	Negative
LB_{20}	Negative
LB_{21}	Negative
BA_{22}	Positive (B)
BA_{23}	Positive (B)
BA_{24}	Positive (A)

Abbreviations: S, *Staphylococcus*; SR, *Streptococcus*; LB, *Lactobacillus*; BA, *Bacillus*; A, alpha haemolysis; B, beta haemolysis.

Almost all the isolates were susceptible to the antibiotics used in this study as shown in Table 5
Abbreviations: S, *Staphylococcus*; SR, *Streptococcus*; LB, *Lactobacillus*; BA, *Bacillus*; AMC, amoxyclav; CN, cefalexin; CIP, ciprofloxacin; CD, clindamycin; COX, cloxacillin; COT, cotrimoxazole; E, erythromycin; TE, tetracycline.

Table 5: Antibiotics Susceptibility Profile of the Isolates

Isolate	AMC, mm	CN, mm	CIP, mm	CD, mm	COX, mm	COT, mm	E, mm	TE, mm
S_1	15	2	22	2	5	13	10	12
S_2	18	0	24	3	11	10	17	19
S_3	16	5	19	3	13	10	22	24
S_4	15	0	16	4	9	22	15	19
S_5	15	12	20	18	20	22	17	22
S_6	19	8	21	0	21	15	10	17
SR_7	8	0	19	5	10	21	3	18
SR_8	20	15	16	0	13	16	19	15
SR_9	11	0	22	10	10	20	18	16
SR_{10}	19	2	24	20	8	10	15	21
LB_{11}	15	4	19	7	10	18	16	18
LB_{12}	17	8	13	2	15	13	18	19
LB_{13}	18	10	15	3	12	11	19	21
LB_{14}	17	12	18	5	13	10	16	15
LB_{15}	16	0	15	10	7	19	10	18
LB_{16}	15	19	22	18	10	10	18	22
LB_{17}	18	15	20	6	11	19	9	24
LB_{18}	15	1	18	6	9	8	15	20
LB_{19}	20	0	18	17	15	18	9	12
LB_{20}	15	3	16	10	10	15	18	15
LB_{21}	19	5	20	12	10	18	3	19
BA_{22}	15	6	19	19	13	11	18	15
BA_{23}	17	7	17	8	16	12	17	15
BA_{24}	24	6	19	0	15	14	19	18

DISCUSSION

This study employed questionnaires to ascertain the attitudes of students towards their toothbrush. This is important because oral health knowledge is considered an essential requirement for general wellbeing [10]. Some studies have established a link between knowledge and improved oral health [11, 12]. From Table 1, majority of the students (53.75%) preferred storing their toothbrushes in the bedroom to anywhere else. This does not tally with the findings of a similar study that indicated 53.1% of Turkish dentists preferring to store their oral hygiene aid in the bathroom to anywhere else [13]. This may depend on whether the individual takes his/her breakfast before or after bath. Storage location of toothbrush is expected to be among the factors that affect oral health and general wellbeing. However, a particular study did not find any relationship between storage location and oral health [14]. More than 80% of the respondents agreed they replace their toothbrush in < 5 months of use. This should indicate good oral hygiene practice among these students since the American dental association (ADA) recommends such replacement should be made once in 3-4 months based on the deterioration of the bristles [15]. It is shocking that many of the respondents (61.25%) have sometime injured themselves while brushing their teeth. This observation supports the result of a recent study [10]. Such injury could lead to odontogenic infections. There are statistics to show oral infections cost about $200 million annually to treat in the United States [16]. Moreover, about half of the respondents do not care about the purity of the water they use for oral hygiene. The center for disease control and prevention (CDC) recommends the use of fluoridated water to protect the teeth [17]. Seventy percent of the respondents saw no need to submit their toothbrushes for microbiological assessment even though 11.25% think their toothbrush makes them sick. This betrays their poor knowledge of the presence of microbes in the oral cavity. Several studies [18-20] have reported that many individuals brush twice daily, which corroborates with the present study (Table 1). Several respondents (61.25%) expressed preference to a specific brand of toothpaste. The active ingredients, that distinguish this brand from the rest, are stannous chloride and a polychelation technology. There are reports to show that applying toothpaste significantly reduces microbial contamination of toothbrush [4, 21].

All the toothbrushes, examined in the present study, contained total aerobic count > 10^4 cfu/toothbrush (Table 2) except for three samples whose bacterial load could not be determined. Taji et al. [14] found 10^4–10^5 cfu/toothbrush, while Verran et al. [22] reported 10^8 cfu/toothbrush in their studies. These findings lend weight to the possible role of toothbrush as a fomite in disease transmission especially among immunocompromised individuals.

The 24 isolates obtained from the present study were tentatively found to belong to the following genera: *Staphylococ-*

cus, *Streptococcus*, *Lactobacillus* and *Bacillus* (Table 3). Except for *Bacillus*, all other three genera have also been reported to be present on toothbrushes in other studies [2, 23]. *Lactobacillus* species and *Streptococcus* species are known commensals of the oral cavity. In fact, the levels of *Streptococcus* mutants and lactobacilli are being proposed as useful tool for detecting approximal and secondary dental caries [24]. *Bacillus* species could be accidental contaminants given their low percentage of occurrence (15%).

Ten of the isolates were positive for haemolysis (Table 4). Haemolysis is an important trait that indicates the pathogenicity potential of an organism. It involves the partial or complete lyses of erythrocytes [25].

Generally, the attitude of Nigerian students towards their toothbrush appeared relatively poor, but they are able to make good choices of toothpaste. Simple enlightenment campaign is likely to improve their oral hygiene.

About 40% of the isolates obtained from the samples were found to be haemolytic. This could be considered as an important trait for these bacterial isolates to initiate and sustain disease. In as much as this is worrisome, most of these isolates were susceptible to commonly used antibiotics.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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