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*Full Length Research Paper*

# Oral hygiene practices and microbial assessment of used toothbrushes by undergraduates in a tertiary institution in Benin City, Nigeria

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**Poor oral hygiene practices which involve inappropriate use and storage of manual toothbrushes among others could significantly increase the prevalence of oral diseases. In this study, personal oral hygiene practices and microbial contaminants present in used toothbrushes by undergraduates resident in the hostels were ascertained. A close-ended questionnaire was issued to twenty (20) subjects randomly selected. New toothbrushes were distributed to the participants and were instructed to maintain standard oral hygiene practice for a period of four (4) weeks. A new toothbrush served as the control. All the toothbrushes were analysed using Standard microbiological methods. Our finding shows that majority of the students brushed their teeth twice daily, changed their toothbrushes monthly, store them inside lockers and fail to use plastic cap to cover their toothbrushes. The total heterotrophic bacterial count (THBC) of the toothbrush bristles and handles were within the range of 0-6.70 and 6.07-6.71 $\log_{10}$ CFU/mL, whereas the equivalent values for total fungal count (TFC) were 0-6.74 and 0-6.85 $\log_{10}$ CFU/mL, respectively. Meanwhile, THBC of the handle and bristles of the new toothbrush were 6.0 and 6.70 $\log_{10}$ CFU/mL, while the values for TFC were 6.30 and 6.0 $\log_{10}$ CFU/mL, respectively. Forty-six (46) bacterial isolates and thirty-three (33) fungal isolates were encountered in the toothbrush bristles and handles. The bacterial isolates identified were *Streptococcus mutans, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus* sp.*, Lactobacillus* sp.*, Klebsiella* sp.*, Proteus* sp., *Enterobacter* sp. And *Citrobacter* sp. while the fungal isolates were *Saccharomyces* sp.*, Penicilliumchrysogenum, P. notatum, Candida albicans, Fusariumoxysporum, Blastomycesdermatitidis, Microsporiumcanis, Aspergillusniger, A. Clavatus* and *A. Flavus.* Only *Klebsiella* sp. and *Candida albicans* were present in the new toothbrush. Since all the toothbrushes were contaminated with pathogenic microorganisms, the students are at risk of manifesting oral diseases. Consequently, the use of approved antimicrobial solutions to decontaminate toothbrushes, plastic covers for the toothbrushes, storage of the toothbrushes inside clean dry containers and implementing good oral hygiene practices are recommended as preventive measures.**

**Keywords:** *Used toothbrushes, oral hygiene, microbial assessment, oral diseases*

## INTRODUCTION

Oral health is an integral part of the general health of every individual (Olusile *et al*., 2014). Enjoying oral health implies that an individual is not experiencing oral and throat cancer, oral infections and sores, mouth and facial pain, periodontal disease, tooth loss, tooth decay and other disease conditions which poses a big challenge for the individual to speak, bite, chew, and smile. Two predominant dental conditions are periodontal diseases and dental caries (Olusile *et al*., 2014; Naseem *et al*., 2017).

The human oral cavity is home to diverse microorganisms namely viruses, bacteria, fungi, protozoa, archaea, and yeast (Rouabhia, 2002; Montelongo-Jauregui and Lopez-Ribot, 2018). Many microorganisms found in the oral cavity are commensals while others are pathogenic (Avila *et al.,* 2009). Currently, up to 700bacterial species in the oral cavity have been identified (Susheela and Raha, 2016).Over the years, toothbrush has been a useful tool used by most persons to maintain personal oral hygiene and remove plaques effectively (Saini and Kulkarni, 2013; Kim *et al*., 2018). Studies have shown that majority of the microorganisms found in toothbrushes constitute part of the oral microbiota (Mobin *et al*., 2011).

Oral hygiene practices are essentially preventive measures against negative impact caused by oral diseases (Olusile *et al*., 2014). It involves the use of toothbrushes and toothpaste which is the most widely used oral hygiene aid without compromising other oral hygiene practices. Less commonly used oral hygiene aids are wood stick, dental floss, interproximal brush and interspace brush. Toothbrush is a small-sized brush which has a long handle used for cleaning the teeth (Paul *et al*., 2014; Arthur *et al.,* 2016). As far back as 1498, the Chinese Emperor Hongzhi invented the first toothbrush made of wild boar-like bristles attached to a handle made of bamboo or bone which resemble the toothbrushes in use today (Kaveri *et al.,* 2017). In 1780, modern toothbrush made of natural bristles and bone handle was first manufactured in England by William Addis (Mobin *et al.,* 2011; Kaveri *et al.,* 2017). In 1844, Dr. Mayer L. Rhein was the first person to patent a toothbrush which is a three-row brush of serrated bristles that has large tufts (Karibasappa *et al*., 2011). Since then till date, toothbrush has undergone several modifications based on handle shape, head design, bristle type, length, and width (Kaveri *et al.,* 2017). Recent improvement in manual toothbrushes resulted in the development of battery-powered and sonic-powered toothbrushes (Dörfer *et al.,* 2016; Kaveri *et al.,* 2017; Ng *et al*., 2020).

To maintain good oral hygiene, regular tooth brushing at least twice a day with the use of toothpaste containing fluoride is recommended (Umanah and Braimoh, 2017).

Toothpaste contains ingredients such as lauryl sulphate, sodium fluoride, *Menthe spicata, Curcuma longa* etc which has the ability to reduce microbial load. Therefore, toothpaste is regarded as a drug, not a cosmetic (Gautam, 2017; Hujoel, 2019). Although the general public is familiar with the use of toothbrushes, many are not properly enlightened about microbial contamination of toothbrushes resulting from improper storage and handling (Kim *et al*., 2018). In many homes, it is a common practice to store toothbrushes still in use inside bathrooms or toilets attached to bathrooms without using a plastic cap to cover it. Several studies have reported that toothbrushes used regularly are heavily contaminated with microorganisms (Taji and Rogers, 1998; Al-Talib *et al*., 2008). Without adequate oral hygiene practice, the use of toothbrush could reintroduce microorganisms which is part of the oral microbiota and microorganisms from other sources into the oral cavity (Karibasappa *et al*., 2011; Arthur *et al*., 2016).The oral cavity, environment, hands, aerosols and storage containers are possible sources of contamination of toothbrushes (Michelle and Cindy, 2011; Samuel and Ifeanyi, 2015).

The prevalence of poor oral hygiene, irregular tooth brushing, and lack of awareness of oral health among Nigerians have been reported (Olusile *et al*., 2014). Poor oral hygienic practices worsened by overcrowded hostels and dilapidated infrastructure are some of the factors that predisposes toothbrushes used by undergraduate students resident in the hostels to microbial contamination which increases the risk of manifesting oral diseases. Available data estimate that 51 million school hours per year is lost due to illnesses associated with the dental region (Okafor *et al*., 2016). Currently, limited studies have been carried out to identify and enumerate microorganisms that contaminate toothbrushes used by students at various levels of education as well as ascertain common oral hygiene practices among them. Therefore, the present study is aimed at ascertaining the oral hygiene practices and microbial assessment of toothbrushes used by undergraduate students resident in the hostel in a tertiary institution in Benin City, Nigeria.

## MATERIALS AND METHODS

### Study subjects

The study population was randomly selected which comprise of twenty (20) undergraduate students (10 males and 10 females) between the ages of 16-25 years. All the undergraduate students used for the study were resident in the hostel of Wellspring University, Benin City.

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**Criteria for selection**

The undergraduates selected for the study had healthy periodontium without gingival inflammation, absence of systemic disease, gingival or periodontal diseases. Also, they have not received antibiotic treatment three (3) months prior to the study. The volunteers were requested to sign a written consent to participate in the study on the condition that all the information generated is strictly for research purpose. Thereafter, a close-ended questionnaire aimed at obtaining personal information on dental care and oral hygiene of the study subjects was administered.

**Sample preparation and collection**

All the participants used for the study were issued a new manual toothbrush (Oral B) and Oral B brand of toothpaste. They were requested to maintain oral hygiene in the standard way (obligatory tooth brushing in the morning and in the evening) for a period of four (4) weeks. The toothbrushes were retrieved from the participants using a transparent sterile plastic bag for each sample and properly labelled. A new tooth brush (Oral B) served as the control. All the samples were taken to the Microbiology laboratory, Wellspring University not later than 6 h from the time the last sample was collected for microbiological analysis.

**Serial dilution**

The procedure described by Sammons *et al.* (2004) with slight modification was adopted. Aseptically, each of the used toothbrushes properly labelled were decapitated and the head bearing the bristles aseptically transferred into a test tube containing ten millilitres (10 ml) of sterile phosphate buffer and left to stand for 30 min. followed by vortex mixing for 2 min. to dislodge microorganisms stuck in the bristles into the solution. Nine millilitres (9 ml) of sterile normal saline was dispensed in ten (10) sterilized test tubes. One millilitre (1 ml) of phosphate buffer with toothbrush bristles immersed in it was transferred into the first test tube containing 9 ml of normal saline. Tenfold serial dilution was carried out using a sterile 1 ml pipette for each

transfer until dilution $10^{-6}$was reached. A brand new toothbrush which served as the control was subjected to the same procedure applied to the used toothbrushes.

**Microbiological analysis**

**Total heterotrophic bacterial count**

Total heterotrophic bacteria count (TBC) of each toothbrush was determined using nutrient agar (NA) culture media and pour plate inoculating method. Aliquot of 1 ml of the $10^{-4}$ and $10^{-5}$ dilutions of the samples were inoculated in well-labeled sterile Petri dishes in duplicates. Immediately, the autoclaved nutrient agar medium which was allowed to cool to about 45 $^{o}$C was poured on the Petri dishes, gently stirred and allowed to solidify. The plates were incubated at 37 °C for 48 h. Thereafter, the colonies observed on the culture plates were counted and results obtained were recorded. The mean of the number of colonies for each sample in duplicate was calculated and the bacterial population for the sample was also calculated using the formula stated below and the result is expressed in CFU/mL (colony forming unit per millilter).

$$\text{CFU/mL} = \text{no. of colonies} \times \frac{\quad}{\quad} \times \frac{\quad}{\quad}$$

**Isolation and maintenance of pure culture**

Single colonies were identified and streaked as a primary inoculant on the surface of a nutrient agar plate to obtain pure culture after repeated subculturing. After achieving a pure culture, the same colony was streaked onto a nutrient agar slant and incubated at 37 °C for 24 h. The slant maintained inside Bijoux bottles were kept inside a refrigerator at 4 $^{o}$C as pure culture until the isolates were identified.

**Characterization and identification of bacterial isolates**

Bacterial isolates were characterized and presumptively identified based on their cultural, morphological characteristics, Gram staining, motility test and series of biochemical tests namely catalase, oxidase, urease, indole, citrate, sugar fermentation test using the method described by Cheesbrough (2000). Identification of the bacterial isolates was accomplished by comparing the characteristics of each isolate with that of known characteristics using the determinative schemes.

**Total fungal count**

An aliquot (1 ml) of dilutions $10^{-4}$ and $10^{-5}$ of the samples were transferred aseptically into freshly prepared potato dextrose agar (PDA) in duplicates in well labelled Petri dishes and were incubated at room temperature (28±2 $^{o}$C) for 5 days. After incubation, the fungal colonies on the plates were counted and the results expressed in colony forming units per millilitre (CFU/ml) using the formula below.

$$\text{CFU/mL} = \text{no. of colonies} \times \frac{\quad}{\quad} \times \frac{\quad}{\quad}$$

**Purification of the fungal isolates**

A sterile inoculating needle was used to cut out a discrete colony on the fungal plates and transferred to the edge of a freshly prepared potato dextrose agar (PDA) plates. The plates were incubated at room temperature (28±2 $^{o}$C) for 5 days to obtain pure isolates. Repeated subculturing of the fungal isolates was done until pure cultures was obtained.

**Table 1:** Summarised responses from questionnaires regarding oral hygiene practices of undergraduate students resident in the hostel

| S/N. | Questions | Close-ended responses of the participants | | | |
|---|---|---|---|---|---|
| 1a. | Age | 16-20 years | ≥ 21 years | | |
| 1b | Female participants | 3 (30 %) | 7 (70 %) | | |
| | Male participants | 2 (20 %) | 8 (80 %) | | |
| 2a. | How many times have you visited a dentist? | 1-2 times | 3-4 times | Above 4 times | No visit |
| 2b | Female participants | 2 (20 %) | 0 | 0 | 8 (80 %) |
| | Male participants | 4 (40 %) | 2 (20 %) | 0 | 4 (40 %) |
| 3a | If Yes to Q2a, what was your reason for visiting the dentist? | Check-up | Bleeding gum | Loose tooth trauma | Others |
| 3b | Female participants | 2 (100 %) | 0 | 0 | 0 |
| | Male participants | 3 (50 %) | 2 (33.33 %) | 1 (16.67 %) | 0 |
| 4a | How many times do you clean your teeth daily? | Once | Twice | Thrice | |
| 4b | Female participants | 1 (10 %) | 7 (70 %) | 2 (20 %) | |
| | Male participants | 2 (20 %) | 5 (50 %) | 3 (30 %) | |
| 5a | Which tools do you use regularly to clean your teeth? | Manual tooth brush and tooth paste | Chewing sticks | | |
| 5b | Female participants | 10 (100 %) | 0 | | - |
| | Male participants | 10 (100 %) | 0 | | |
| 6a | Which additional tool do you use occasionally to clean your teeth? | Dental floss | Oral irrigators | Tongue scrappers and mouthwash | None |
| 6b | Female participants | 2 (20 %) | 0 | 1(10 %) | 7 (70 %) |
| | Male participants | 5 (50 %) | 0 | 5 (50 %) | 0 |
| 7a | Where do you store the tools used for cleaning your teeth? | Toilet counter | Bathroom sink | Locker | Wallmounted toothbrush holder |
| 7b | Female participants | 0 | 0 | 7 (70 %) | 3 (30 %) |
| | Male participants | 0 | 0 | 9 (90 %) | 1 (10 %) |
| 8a | How often do you change the tools used for cleaning your teeth? | Weekly | Monthly | Every 2-3 months | Yearly |
| 8b | Female participants | 0 | 8 (80 %) | 0 (0 %) | 2 (20 %) |
| | Male participants | 0 | 10 (100 %) | 0 (0 %) | 0(0%) |
| 9a | Why do you change the tools used for cleaning your teeth? | Worn out cleaning tools | Fed up using it | | |
| 9b | Female participants | 4 (40 %) | 6 (60 %) | | |
| | Male participants | 6 (60 %) | 4 (40 %) | | |
| 10a | Do the cleaning tool used for your teeth have a primary cover after use? | Yes | No | | |

| | | | |
|---|---|---|---|
| 10b | Female participants | 6 (60 %) | 4 (40 %) |
| | Male participants | 1 (10 %) | 9 (90 %) |
| 11a | If Yes for Q10a, do you use it always? | Yes | No |
| 11b | Female participants | 4 (66.67 %) | 2 (33.33 %) |
| | Male participants | 1 (100 %) | 0 (0 %) |
| 12a | Do you consider oral health care as a priority? | Yes | No |
| 12b | Female participants | 5 (50 %) | 5 (50 %) |
| | Male participants | 8 (80 %) | 2 (20 %) |
| 13a | Do you consider treatment in the oral cavity as much importance as treatment in other parts of the body? | Yes | No |
| 13b | Female participants | 7 (70 %) | 3 (30 %) |
| | Male participants | 6 (60 %) | 4 (40 %) |

**Table 2:** Cultural and morphological characteristics of the bacterial isolates

| Parameters / Isolate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Colour | Yellow | Greyish-white | Greyish-white | Cream | Whitish | Whitish-Creamy | Red | Grey | Pale white | Grey-white |
| Elevation | Raised | Low convex | Convex | Flat | Convex | Flat | Convex | Convex | Flat | Slightly convex |
| Surface appearance | Glistening | Smooth | Mucoid | Smooth | Smooth | Smooth | Smooth and shiny | Mucoid | Smooth | Rough |
| Shape form | Circular | Circular | Circular | Circular | Circular | Circular | Circular | Circular | Irregular | Irregular |
| Edge type/ margin | Entire | Entire | Entire | Irregular | Entire | Entire | Entire | Entire | Entire | Curled |
| Transparency | Opaque | Opaque | Opaque | Translucent-opaque | Semi-transparent | Opaque | Translucent - Opaque | Opaque | Opaque | Opaque |
| Gram staining | + | - | - | - | + | - | - | + | - | + |
| Cell type | Cocci | Rods | Rods | Rods | Rods | Rods | Rods | Rods | Rods | Rods |
| Cell arrangement | Cluster | Singly | Singly | Singly | Chains | Singly | Singly | Chains | Singly | Chains |
| Probable isolates | *Staphylococcus* sp. | *Escherichia coli* | *Klebsiella* sp. | *Pseudomonas aeruginosa* | *Streptococcus* sp. | *Enterobacter* sp. | *Citrobacter* sp. | *Lactobacillus* sp. | *Proteus* sp. | *Bacillus* sp. |

**Table 3:** Biochemical tests for identification of the bacterial isolates

| Isolate code | Oxidase | Catalase | Indole | Urease | Citrate | Motility | Glucose | Probable isolates |
|---|---|---|---|---|---|---|---|---|
| 1 | - | + | - | + | + | - | A(+) | *Staphylococcus* sp. |
| 2 | - | + | + | - | - | + | AG | *Escherichia coli* |
| 3 | - | + | - | + | - | - | AG | *Klebsiella* sp. |
| 4 | + | + | - | - | + | + | A(+) | *Pseudomonas aeruginosa* |
| 5 | - | - | - | - | + | - | A | *Streptococcus* sp. |
| 6 | - | + | - | - | + | + | AG | *Enterobacter* sp. |
| 7 | - | + | - | - | + | + | A(+) | *Citrobacter* sp. |
| 8 | - | - | - | - | - | - | A(+) | *Lactobacillus* sp. |
| 9 | - | + | - | + | + | + | A(+) | *Proteus* sp. |
| 10 | - | + | - | - | + | + | A(+) | *Bacillus* sp. |

**Identification of the fungal isolates**

The fungal isolates were characterized and identified based on colonial morphology and microscopic characteristics. The microscopic morphology of the fungal isolates were determined by viewing their mycelia under the microscope at x40 objective lens with lactophenol cotton blue stain. The morphology of the fungal isolates under the microscope was compared with reference standards as described by Geo *et al.* (2013) and Ellis *et al.* (2007).

**RESULTS**

Presented in Table 1 is the summary report of self-administered questionnaires retrieved from ten (10) male and ten (10) female undergraduate students resident in the hostels located in Wellspring University.

Fig. 1 and Fig. 2 shows the total heterotrophic bacterial count (THBC) of the used toothbrushes obtained from the male and female subjects, respectively. Total heterotrophic bacterial count of bristles and handles of the toothbrushes used by the males were within the range of 0-6.75 and 6.07-6.70 CFU/mL, respectively. As for the females, THBC of the bristles and handles of their toothbrushes were within the range of 0-6.73 and 6.41-6.72 CFU/mL, respectively. There is no significant difference ($P<0.05$) between THBC of the bristles of toothbrushes used by the males and the female students. Similarly, there is no significant difference ($p<0.05$) between THBC of toothbrush handles used by the males and the female students.

Shown in Fig. 3 and 4 is the total fungal count (TFC) of the used toothbrushes from the male and female subjects, respectively. The total fungal count of bristles and handles of toothbrushes obtained from the males is within the range of 0-6.74 and 6.48-6.75 CFU/mL, respectively. The bristles and handles of toothbrushes obtained from the females had a TFC ranging from 0-6.69 and 0-6.85 CFU/mL, respectively. There is no significant difference ($P<0.05$) between TFC of the toothbrush bristles used by the male and the female students. In contrast, a significant difference ($p>0.05$) exist between TFC of the toothbrush handles obtained from the male and female students.

Presented in Fig. 5 is the total heterotrophic bacterial count and total fungal count of the new toothbrush which served as the control. The bristles of the toothbrush had a THBC and TFC of 6.0 and 6.3 CFU/mL, respectively. Meanwhile, THBC and TFC of the handle of the toothbrush is 6.7 and 6.04 CFU/mL, respectively.

Table 2 depicts the cultural and morphological characteristics of the bacterial isolates from the used toothbrushes. A total of ten (10) probable bacterial species identified were *Klebsiella* sp., *Citrobacter* sp., *Bacillus* sp., *Proteus* sp., *Citrobacter* sp., *Escherichia coli*, *Pseudomonas* sp., *Lactobacillus* sp., *Enterobacter* sp., and *Streptococcus* sp. Presented in Table 3 is the biochemical tests for further identification of the bacterial isolates.

Table 4 depicts the macroscopy and microscopical identification of the fungal isolates. A total of ten (10) fungal species identified were *Blastomycesdermatitidis, Microsporumcanis*, *Candida albicans*, *Saccharomyces* sp., *Penicilliumnotatum, P. chrysogenum, Aspergillusniger ,A. flavus*, *A. clavatus*, and *Fusariumoxysporum.*

Presented in Fig. 6 is the frequency of occurrence of bacteria isolated from the handle of the toothbrushes. Among the bacterial isolates, *Klebsiella* sp. and *Proteus* sp. had the highest (8) and lowest (1) frequency of occurrence, respectively. Other bacterial isolates were *Escherichia coli* (4), *Pseudomonas* sp. (3), *Streptococcus* sp. (3). *Lactobacillus* sp. (2), *Enterobacter* sp. (2), *Citrobacter* sp. (2), and *Staphylococcus* sp. (2).

The frequency of occurrence of bacteria species isolated from the toothbrush bristles is presented in Fig. 7. The

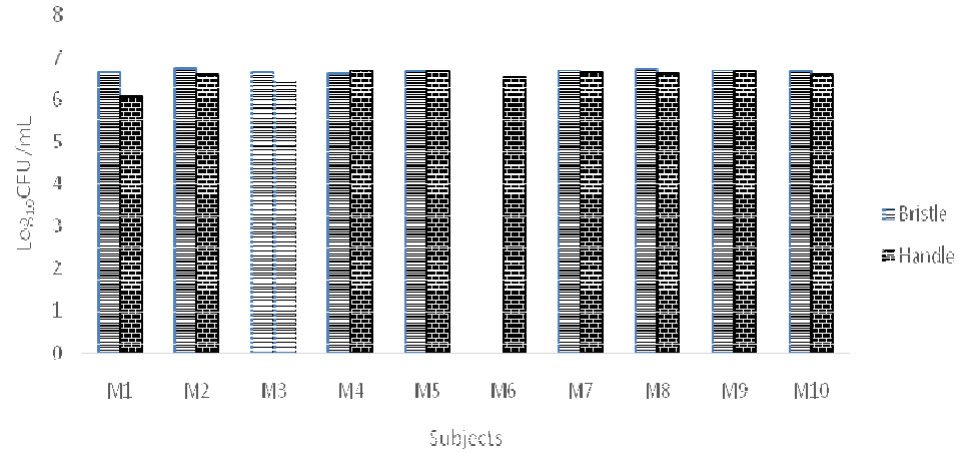


**Figure 1:** Total heterotrophic bacterial count of the used toothbrushes from the male subjectts.
Key: $F_1B$-$F_{10}B$represents the handle of the toothbrushes
$F_1B$-$F_{10}B$represents the brisstles of the toothbrushes

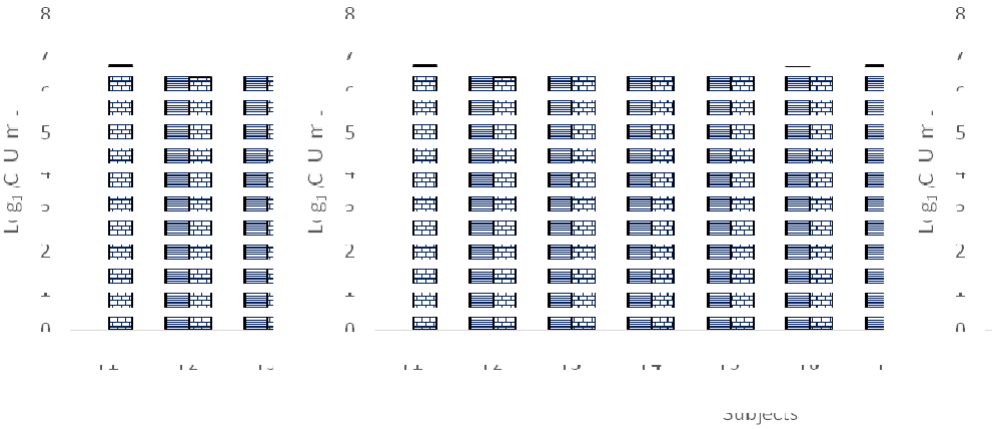


**Figure 2:** Total heterotrophicc bacterial count of the used toothbrushes from the female subjeccts.
Key: $F_1H$ – $F_{10}H$ represent thhe handle of the toothbrushes
$F_1B$ – $F_{10}B$ represent the brisstles of the toothbrushes

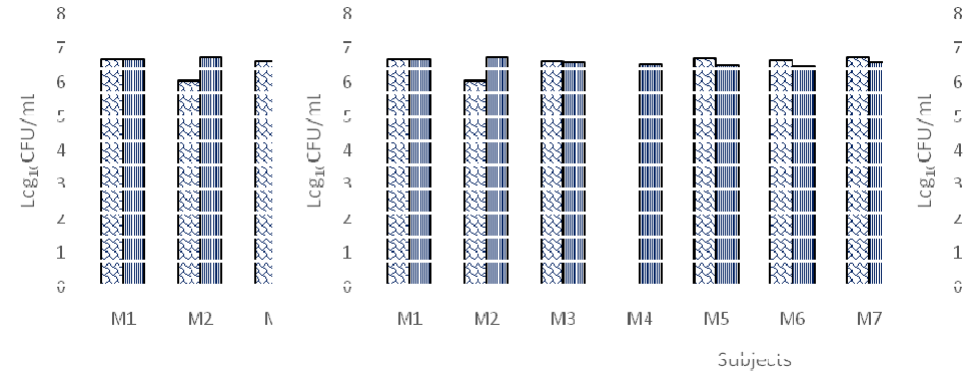


**Figure 3:** Total fungal counnt of the used toothbrushes from the male subjects.
Key: $M_1H$ – $M_{10}H$ represent the handle of the toothbrushes
$M_1B$ – $M_{10}B$ representt the bristles of the toothbrushes

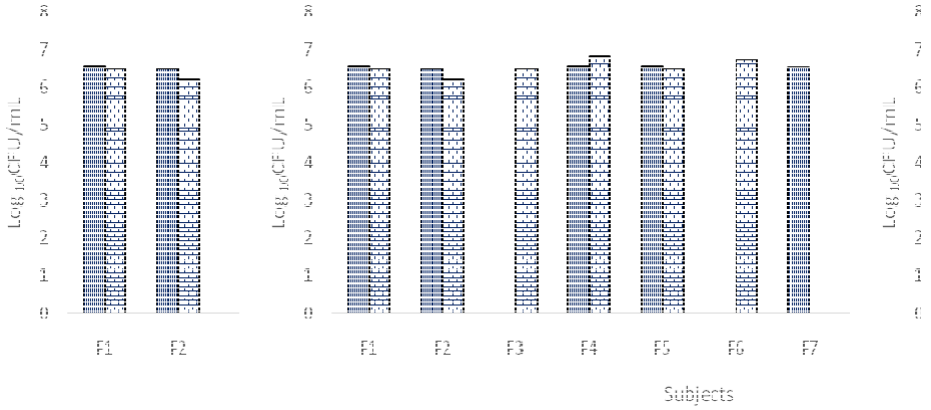


Figure 4: Total fungal couunt of the used toothbrushes from the female subjects.
Key: $F_1B$-$F_{10}B$represent the handle of the toothbrushes
$F_1B$-$F_{10}B$represent the bristles of the toothbrushes

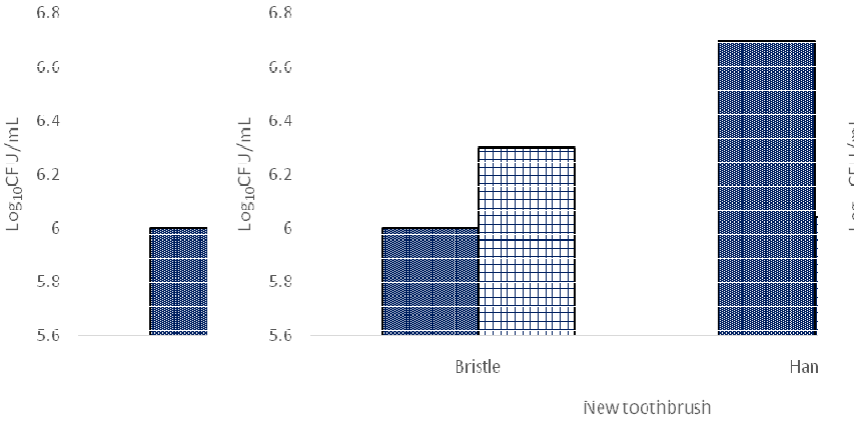


**Figure 5:** Bacterial and fungal count of the new toothbrush (Control)
Key: THBC-Total heeterotrophic bacterial count (THBC)
TFC-Total fungal count (TFC)

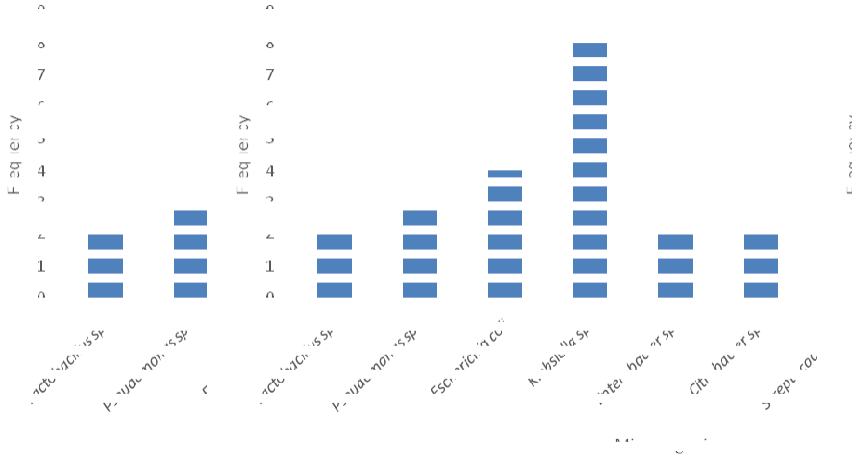


**Figure 6:** Frequency of occurrence of bacterial isolates from the toothbrush handles

**Table 4:** Macroscopy and microscopical identification of the fungal isollates

| Parameter / Isolate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Colour | Creamy | Blue-green | White-creamy | Yellow-green | Whitish | Yellow-white | Olive- green | White | Dark brown | Blue-green |
| Elevation | Flat | Craterform | Flat | Flat | Flat | Flat | Flat | Flat | Flat | Raised |
| Surface | Smooth and moist | Rough and cottony | Smooth and rough | Smooth | Rough and cottony | Wrinkled and waxy | Smooth and powdery | Cottony | Powdery | Smooth |
| Margin | Entire | Curled | Filiform | Entire | Filiform | Filiform | Entire | Filiform | Filiform | Entire |
| Form | Circular | Slightly irregular | Circular | Circular | Irregular | Filamentous | Circular | Filamentous | Irregular | Circular |
| Hyphae | Absent | Septate | Pseudohyphae | Septate | Septate | Septate | Septate | Septate | Septate | Septate |
| Spore | Ascospores | Conidia spores | Chlamydospores | Blastospore | Chlamydospore | Conidia spores | Conidia spores | Macrocoonidia | Conidial | Candiophore |
| Cells | Spherical | Spherical | Spherical | Spherical | Spherical | Spherical | Spherical | Rod | | |
| Probable isolates | *Saccharomyces* sp. | *Penicilliumchrysogenum* | *Candida albicans* | *Aspergillusflavus* | *Fusarium oxysporum* | *Blastomycesdermatitidis* | *Penicilliumnotatum* | *Microsporiumcanis* | *Aspergillusniger* | *Aspergillusclavatus* |

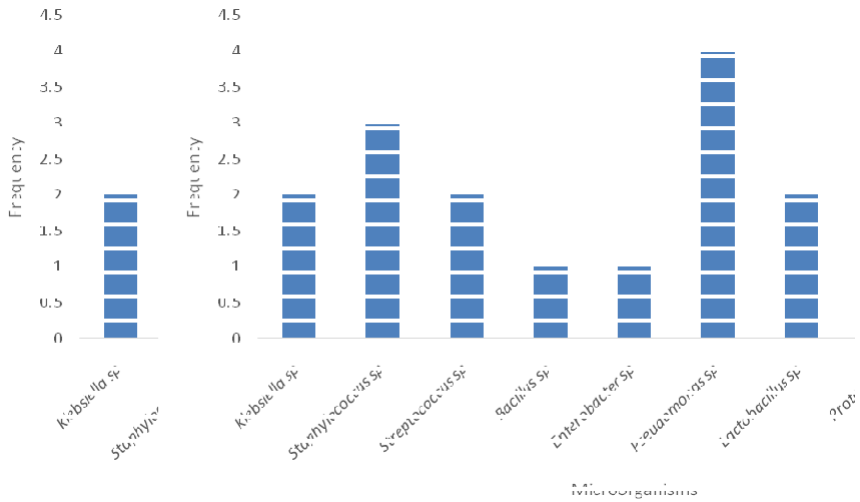


**Figure 7:** Frequency off occurrence of bacterial isolates from the toothbrush bristles

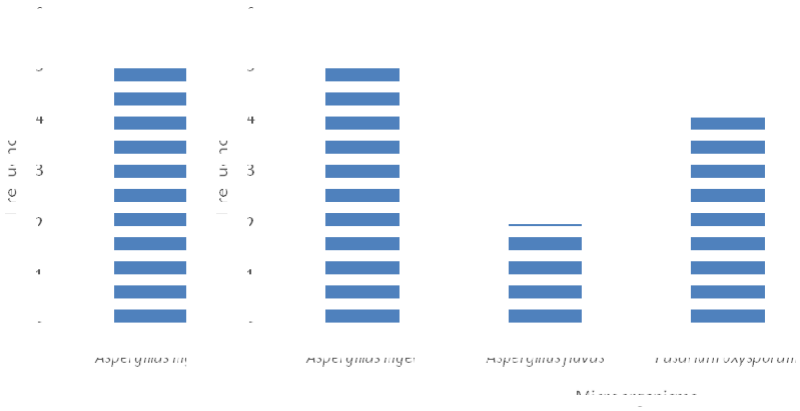

**Figure 8:** Frequency of occurrence of fungal isolates from the toothbrush bristles

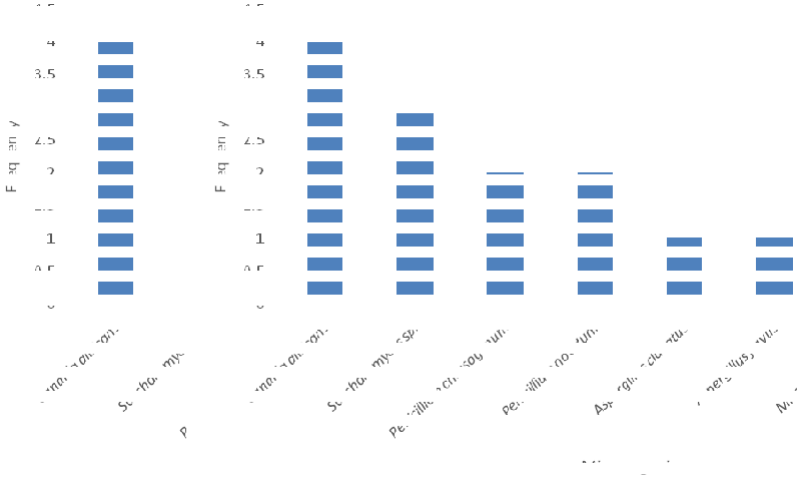

**Figure 9:** Frequenncy of occurrence of fungal isolates from the toothbrush handles

bacterium that had the highest frequency of occurrence was *Pseudomonas* sp. (4) whereas the lowest involved *Bacillus* sp. (1) and *Enterobacter* sp. (1). *Staphyloc occus* sp. and *Proteus* sp. had the same frequency of occurrence of three (3), while *Klebsiella* sp., *Streptococcus* sp. *Laactobacillus* sp. and *Escherichia coli*had two (2).

Presented in Fig.8 is the frequency of occurrence of fungal isolates from the bristles of the toothbrushes. Among the fungal isolates, *Aspergillusniger* hadd the highest frequency of occurrence (5), followed by *Fusariumoxysporum* (4). The fungal isolate which had the least frequency of occurrence of twwo (2) were *Aspergillusflavus and Candida albicans.*

The frequency of occurrence of fungal sppecies isolated from the handle of the toothbrushes is preseented in Fig. 9. The fungal isolates which had the highest frequency of occurrence were *Candida albicans* (4) and *Microsporumcanis* (4), whereas the lo west involved *Aspergillusclavatus* (2) and *A. flavus* (2). The frequency of occurrence of other fungal species isolated from the toothbrushes were *Saccharomyces* sp. (3), *Blastomycesdermatitidis* (3).

## DISCUSSION

The open-ended questionnaires administered to randomly selected male and female undeergraduate students resident in the hostels revealed their attitude and personal opinions towards oral hygiene practices.

### Oral health service utilization by the participants

Information obtained from the questionnaires revealed that majority of the female participan ts (80 %) have not visited a

dentist in their lifetime, whereas 40 % of the male participants have done so. It is an indication that the female participants enjoyed better oral health than their male counterparts. This report is in agreement with research findings by Umanah and Braimoh (2017) from a related study. Among the female participants (20 %)and male participants (60 %) that visited the dentist, 100 % of the females compared with 50 % of the males went for a regular check-up. Other reasons for the remaining 33.33 % and 16.67 % of the male participants to visit the dentist were bleeding gum and trauma, respectively. According to Olusile *et al.* (2014), more educated persons are more likely to embark on frequent dental visits when compared with those that are less educated.

**Oral hygiene practices of the participants**

Findings from this study revealed that majority of the female participants (70 %) compared with the males (50 %) brush their teeth twice daily as recommended by the American Dental Association (ADA), respectively. This report is in agreement with a similar study carried out by Umanah and Braimoh (2017) which found that 80 % for female and 44.3 % for male undergraduate students in University of Port Harcourt, Nigeria brushed their teeth at least twice daily. The ability of larger percentage of the female participants to adhere to ADA recommendation could be one of the reasons a larger percentage (80 %) of the females had not visited the dentist compared with 40 % for the males. However, 30 % of the males and 20 % of the females brush their teeth thrice daily, whereas 20 % of the males and 10 % of the females perform the same activity once a day which is against the ADA recommendation. Kim *et al*. (2018) carried out a related study and reported that toothbrushes used three times daily had a higher population of general bacteria and coliforms than toothbrushes used twice a day. Also, the population of *Staphylococcusaureus* in the toothbrushes used thrice a day is four times higher than the values reported for toothbrushes used twice a day.

All the male and female participants reported that they used manual toothbrushes for cleaning their teeth. Several studies have reported that toothbrushes and toothpaste is the most common oral hygiene aid among people of different age groups, religion, race, gender, and social status (Karibasappa *et al*., 2011; Susheela and Radha, 2016). The popularity of toothbrush and toothpaste among the students could be associated with adverts, literacy level, social status and unique properties such as flavour, taste, colour and appearance of the toothpaste (Umanah and Braimoh, 2017).Findings from this study shows that chewing stick is totally unacceptable among the students for cleaning their teeth. Meanwhile, majority of the female participants (70 %) do not use oral hygiene aid such as dental floss, oral irrigators, tongue scrappers or mouthwash in addition to toothbrush and toothpaste to clean their teeth. Only 20 % of the female participants and 50 % of the male participants use dental floss. In a similar study, Umanah and Braimoh (2017) reported that undergraduate students are not familiar with interdental cleaning aids such as dental floss etc. According to the researchers, flossing once a day and cleaning the teeth twice daily promotes good oral health. The remaining male participants (50 %) use mouthwash and tongue scrappers in addition to toothbrush and toothpaste to clean their teeth when compared with 10 % for the female participants. The use of dental floss, mouthwash and tongue scrappers to clean the teeth in addition to toothbrush and toothpaste by higher percentage of the male participants(100 %) compared with the females (30 %) could be as a result of bad habits such as smoking peculiar with the males which affects the teeth as well as produce mouth odour associated with smokers. In a related study carried out by Naseem *et al.* (2017) to ascertain the oral hygiene practices and teeth cleaning techniques among medical students, they reported that 21.6 % and 16. 9 % of the participants smoked cigarettes and 'shisha', respectively while 1.4 % ate 'paan' (betel leaf with areca nut combined with tobacco).

Information extracted from the questionnaires shows that higher percentage of the participants (70 % for the females and 90 % for the males) store their toothbrushes inside lockers located in their hostels. The remaining percentage of the females (30 %) and males (10 %) store their toothbrushes inside a wall-mounted toothbrush holder. In a similar study, Okafor *et al.* (2016) reported that larger portion of students (53.75 %) that participated in the study prefer storing their toothbrushes in the bedroom probably inside lockers. According to the researchers, there is no relationship between the location where toothbrushes are stored and oral health. However, storage location could influence the level of microbial contamination of toothbrushes. Kim *et al*. (2018) reported that a humid environment favour bacteria growth in the used toothbrushes unlike a dry environment. Therefore, wall-mounted toothbrush holder is better than lockers for storage of toothbrushes. Proper storage of toothbrushes used in cleaning the teeth is very important because it is a fomite for transmission of diseases especially among individuals who are immunocompromised (Okafor *et al.,* 2016).

All the male participants (100 %) reported that they changed their toothbrushes monthly compared with 80 % for the females. Shockingly, 20 % of the female participants reported that they changed their toothbrushes once a year. A study conducted in 1997 reported that half of the population of Brazilians do not have a toothbrush due to low per capita. On average, Brazilians buy new toothbrush every 17 months (Ferreira *et al*., 2012). Findings from a recent study carried out by Kim *et al*. (2018) revealed that population of *Staphylococcus aureus*, coliform bacteria and general bacteria in toothbrushes used for 1 month was 36.15, 201.54 and 2489.23 CFU/mL which increased to 504.23, 561.54, and 5096.54 CFU/mL when the toothbrushes were used for 2 months, respectively. After 3

months of using the toothbrushes, the population of *S. aureus*, coliform bacteria and general bacteria further increased to 2386.67, 874.00 and 5028.67 CFU/mL, respectively. The oral health society recommend that healthy individuals should change their toothbrushes every three months. As for patients undergoing chemotherapy, they should do it every three days(Ferreira *et al*., 2012).

Worn out toothbrush is the reason majority of the males (60 %) changed their toothbrushes compared with 40 % for the females. Easily worn toothbrushes could be attributed to rough handling by the males during brushing of their teeth. The bristles of toothbrushes is made of nylon synthetic resins which vary in thickness. Over time, the bristles of toothbrushes become worn out due to usage. Consequently, the user need to replace it with a new toothbrush for effective cleaning of the teeth. According to a study carried out by Ferreira *et al*. (2012), the greater the wear of toothbrushes, the more microorganisms will accumulate in the bristles.

Majority of the female participants (60 %) reported that their toothbrushes had a plastic cap provided by the manufacturer to cover the toothbrushes when they are not in use especially the bristles, but only 66.67 % of them use it regularly. Meanwhile, only 10 % of the male participants reported that their toothbrushes have plastic caps and all of them (100 %) regularly use it. According to Kim *et al*. (2018), microorganisms present in the air is likely to contaminate toothbrushes exposed to the environment in addition to microorganisms inhabiting the oral cavity. Studies carried out by Al-Talib *et al*. (2008) reported the presence of aerobic microorganisms such as *Staphylococcus epidermidis, Lactobacillus* sp., *Moraxella catarrhalis*, α haemolytic streptococcus, *Escherichia coli, Bacillus subtilis, Corynebacterium* sp., *Candida albicans*, *Proteus* sp. and *Klebsiella* sp. while anaerobic microorganisms reported were *Bacteroides* sp., *Peptococcus* sp. and *Peptostreptococcus* sp. in toothbrushes which were exposed to the air.

In conclusion, half of the female participants consider oral health as a priority whereas the remaining half did not. However, majority of the male participants (80 %) consider oral health as a priority while the remaining 20 % did not. In a related study, Olusile *et al*. (2014) reported that adult Nigerians are concerned about their oral health, but they usually engage in poor oral hygiene practices. Higher priority oral health by the male participants selected for this study when compared with their female counterparts could be attributed to bad habits such as smoking peculiar with males which negatively impact oral health. This could also be one of the reasons majority of the male participants have visited the dentist when compared with their female counterparts.

**Microbial population in the toothbrush bristles and handles**

The total heterotrophic bacterial count (THBC) of the toothbrush bristles ranging from 0-6.75 $\log_{10}$CFU/mL is lower than 6.07-6.72 $\log_{10}$CFU/mL for the toothbrush handles. It was a similar trend for the total fungal count (TFC) of the toothbrush bristles and handles which were within the range of 0-6.74 and 0-6.85 $\log_{10}$CFU/mL, respectively. The toothbrush bristles having a lower THBC and TFC when compared with the toothbrush handles could be as a result of antimicrobial properties of toothpaste applied on the bristles, not on the handles. Higher microbial count in the toothbrush handles when compared with the bristles could be as a result of large population of microorganisms present in the fingers which forms part of the normal floral of the skin. Meanwhile, the THBC of the new toothbrush handle (6.7 $\log_{10}$ CFU/mL) is higher than the result obtained for the bristles (6.0 $\log_{10}$ CFU/mL). In contrast, the TFC of the handle (6.04 $\log_{10}$ CFU/mL) is lower than what was obtainable for the bristles (6.3 $\log_{10}$ CFU/mL). Poor storage conditions and improper handling of the new toothbrush in the shops could be responsible for microbial contamination of the new toothbrush. Lack of strict implementation of good manufacturing practices (GMPs) could also be a contributory factor. The microbial population encountered in the bristles and handles of used toothbrushes is a confirmation that toothbrushes can support microbial growth (Rodrigues *et al*., 2012). According to Mobin *et al*. (2011), storage of wet toothbrushes in a closed environment could favour the survival and multiplication of fungal species. This study revealed that majority of the students keep wet toothbrushes inside their lockers which favours the growth of microorganisms. This oral hygiene practice, though not acceptable could be responsible for high microbial count encountered in the toothbrush bristles and handles.

**Microorganisms found in the toothbrush bristles and handles**

Bacterial genera found in the toothbrush bristles and handles are *Klebsiella* sp., *Proteus* sp., *Staphylococcus aureus, Escherichia coli*, *Pseudomonas* sp., *Lactobacillus* sp., *Enterobacter* sp., and *Streptococcus* sp. Also isolated from the toothbrush bristles and handles are *Bacillus* sp. and *Citrobacte*r sp., respectively. In a related study, Okafor *et al*. (2016) isolated *Lactobacillus* sp., *Staphylococcus* sp., *Streptococcus* sp. and *Bacillus* sp. from the used toothbrushes from university students. According to a study carried out by Samuel and Ifeanyi (2015) which involved assessment of bacterial contamination of used toothbrushes obtained from undergraduate students, they reported the presence of *Streptococcus mutans, Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermidis,*

*Escherichia coli*, and *Enterobacteraerogenes*. The findings from both studies is in agreement with our study.

In the oral cavity, *Lactobacillus* sp. and *Streptococcus* sp. have been identified as commensals. However, the presence of *Lactobacillus* sp. in the oral cavity could enhance dental caries (Karibasappa *et al.,* 2011). *Pseudomonas aeruginosa* is a common opportunistic pathogen which is ubiquitous in nature. It is implicated in nosocomial infections. The source of this bacterium in the toothbrushes could be from tap water used in rinsing toothbrushes after brushing the teeth.It could also be from the humid environment where the toothbrushes were stored. The presence of *Staphylococcus* sp. in the toothbrushes could be attributed to contamination from the fingers during brushing of the teeth. *Staphylococcus* sp. is a common bacterium that inhabit the human skin as part of the normal flora (Samuel and Ifeanyi, 2015). According to Rodrigues *et al*. (2012), the presence of *Staphylococcus aureus* in toothbrushes should be taken serious since the bacterium could cause many oral infectious diseases. *Enterobacter* sp. isolated from the used toothbrushes could be attributed to improper storage where aerosols from the toilet could reach the toothbrushes and use of untreated water to rinse the toothbrushes after brushing the teeth. The presence of *E. coli* on the toothbrushes is an indication of fecal contamination which could be attributed to untreated tap water used for rinsing the toothbrushes. Both *Enterobacteraerogenes* and *Escherichia coli* are coliforms that belong to the family *Enterobacteriaceae* (Samuel and Ifeanyi, 2015).

*Aspergillusniger, A. flavus*, *Fusariumoxysporum* and *Candida albicans* were found in the handle of the used toothbrushes while *Blastomycesdermatitidis, Microsporumcanis*, *Candida albicans*, *Saccharomyces* sp., *Penicilliumnotatum, P. chrysogenum, Aspergillusclavatus* and *A. flavus* were found in the bristles.This result substantially agrees with the fungal isolates reported by Mobin *et al.* (2011) which contaminated toothbrushes. Studies have shown that apart from infected animals especially cats and dogs, *Microsporumcanis* is present in dust which could be the source of contaminating exposed toothbrushes in the hostels. It is important to note that arthrospores of *M. canis* in the environment remains infectious for 12-24 months (Mancianti *et al.,* 2003). *M. canis* is a known cause of dermatophytes among other fungi species. Poor hygiene, high temperature and high humidity among others are predisposing factors for dermatophytosis (Wisal *et al.,* 2018). *Blastomycesdermatitidis* is a dimorphic fungus responsible for blastomycosis. This fungus produces spores deposited in the soil. Inhalation of the spores could result in a disseminated disease which could also affect the oral cavity (Muzyka and Epifanio, 2013).

Studies have shown that *Aspergillus* species are widely distributed in nature. They are commonly found in indoor environment (Mousavi *et al*., 2016). Since *Aspergillus* sp. are present in soil, water, air, organic waste, surface of human beings etc., exposure of toothbrushes to the environment is a likely source of contamination. According to Rogawansamy *et al*. (2015), the population of *Aspergillus* sp. and *Penicillium* sp. is usually higher indoors than outdoors. Entry of fungi into buildings is through the windows, doors, air conditioning, heating and ventilation systems. These are probable sources of contamination of exposed toothbrushes used by undergraduate students resident in the hostels. In a related study, Rodrigues *et al*. (2012) reported the presence of yeast in toothbrushes in use. According to Rouabhia (2002), *Candida* sp. and *Saccharomyces* sp. constitute part of the oral microbial community. Therefore, the oral cavity could be the source of both fungal genera isolated from the toothbrush bristles. Studies have shown that *Candidaalbicans* is the most frequently isolated fungi implicated in oral infection (Rouabhia, 2002). *Candida albicans* isolated from the toothbrush bristles and handles had the highest frequency of occurrence among the fungal isolates. This result is in agreement with a similar study reported by Mobin *et al.* (2011).

Surprisingly, *Klebsiella* sp. and *Candida albicans* were found in the new toothbrush. This result is an indication that good manufacturing practices (GMPs) was not strictly implemented by the manufacturer. Although, the new toothbrush was originally sealed, poor storage condition and handling of the item by traders might have exposed the toothbrush to microbial contamination. According to Karibasappa *et al.* (2011), *Klebsiella* sp. is responsible for septicaemia, diarrhea, pneumonia, urinary tract infections and pyogenic infections while *Candida* sp. cause candidiasis. In a related study, Susheela and Radha (2016) reported absence of microorganisms in four new packed toothbrushes which contradicts the result obtained from this study.

In order to reduce the risk of oral diseases due to the use of contaminated toothbrushes, Rodrigues *et al.* (2011) recommended that toothbrushes used daily for three days should be soaked in 0.12 % chlorhexidine. Another study has also shown that bacteria present in toothbrushes were eliminated after they were soaked in phenolic compounds (Listerine) for a period of 20 minutes. In a related study involving dental students, Al-Talib *et al.* (2008) reported that toothbrushes separately soaked in 0.2 % chlorhexidinegluconate and 1 % sodium hypochlorite after daily brushing of their teeth for a period of four weeks had a lower microbial load when compared with toothbrushes rinsed with tap water and kept in the air. Due to lack of awareness, the most common hygienic practice among those that use toothbrush to clean their teeth is to rinse it with tap water and keep it in the air after brushing. Generally, chlorhexidine is regarded as the gold standard antimicrobial solution to decontaminate toothbrushes.

## CONCLUSION

This study revealed that all the undergraduate students resident in the hostel make use of manual toothbrushes in maintaining oral hygiene. Also, majority of the female participants in this study brush their teeth twice daily, never visited the dentist but, few that visited went for medical check-up when compared with their male counterparts. However, majority of the female participants do not use other oral hygiene aids in addition to toothbrushes and toothpaste when compared with the male participants. Most of those that participated in the study consider oral health important as their general health, change their toothbrushes monthly, and store them inside a locker after each use. Total heterotrophic bacterial count (THBC) of bristles and handles of the toothbrushes were within the range of 0-6.75 and 6.07-6.72 $\log_{10}$ CFU/mL, respectively. Meanwhile, the total fungal count (TFC) of the bristles and handles were within the range of 0-6.74 and 0-6.85 $\log_{10}$ CFU/mL, respectively. Bacterial species isolated from the used toothbrushes were *Klebsiella* sp., *Citrobacter* sp., *Bacillus* sp., *Proteus* sp., *Citrobacter* sp., *Escherchia coli*, *Pseudomonas* sp., *Lactobacillus* sp., *Enterobacter* sp., and *Streptococcus* sp. while the fungal species were *Blastomycesdermatitidis, Microsporumcanis*, *Candida albicans*, *Saccharomyces* sp., *Penicilliumnotatum, P. chrysogenum, Aspergillusniger, A. flavus*, *A. clavatus*, and *Fusariumoxysporum.* Meanwhile, *Klebsiella* sp. and *Candida albicans* were isolated from the new toothbrush which had a THBC and TFC of 6.0 and 6.3 $\log_{10}$ CFU/mL for the bristles, THBC of 6.7 and TFC of 6.04 $\log_{10}$ CFU/mL for the handle, respectively. What is considered as the major limitation in this study is the reliance on self-reported information from the participants which is subject to bias. However, the findings from this study have provided some useful and reliable information concerning oral hygiene practices among undergraduate students in a privately-owned tertiary institution as well as the level of microbial contamination of toothbrushes used by them.

## CONFLICT OF INTEREST

The product used in this study is commonly seen in the market and widely used across the country where this study was conducted. There is absolutely no conflict of interest between the authors and the product manufacturer because there is no conflict intention whatsoever from the authors to initiate any litigation with the products, but for the sales and purpose of the product.

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