# Microbial contamination of tooth brushes and tooth brush keeping places among apparently healthy individuals in Egor LGA, Benin City.

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**ABSTRACT**

***Background***: Toothbrushes may become contaminated with microorganisms as they are often kept in unsanitary conditions such as the bathroom and toilets which facilitate bacteria growth.

***Methods***: This study was conducted to evaluate the microbial contamination of toothbrushes and tooth brush keeping places among apparently healthy individuals in Egor LGA, Benin City. A total of sixty (60) apparently healthy individuals that consented to participate were recruited for this study. Toothbrush was given to each participant for normal use and keep and collected after a period of 4months. Pour plate technique using nutrient agar was used for primary isolation. Culture on Mac Conkey and chocolate agar plates was used for differentiation of organisms. Macroscopic, microscopic and biochemical examination were further carried out for identification of each organism isolated.

***Results:*** A predorminance of 58.33% microorganism was obtained from the toothbrush of apparently healthy individuals in Egor LGA. Of the microorganism isolated, *E. coli* (36.6%) and Lactobacilus spp (36.6%) were the most predominant organism followed by *Bacillus cereus* (14.6%), Coagulase negative Staphyloccous (7.3) while *Pseudomonas aeroginosa* and Klebsiella spp were both (2.4%). No significant relationship existed between gender, age and toothbrush contamination. Tooth brush keeping place, length of usage and toothbrush casing had no significant statistical correlation with the toothbrush contamination.

***Conclusion:*** From our study, bacteria contamination of toothbrushes and toothbrush keeping places is very high. Toothbrushes and toothbrush keeping places should be properly maintained.

***Keywords:*** Apparently Healthy Individuals, Egor LGA, Keeping Place, Microbial Contamination, Toothbrush.

## 1. INTRODUCTION

A toothbrush is an oral hygiene instrument used to clean the teeth, gums and tongue so as to improve oral health and prevent oral diseases in an individual [1, 2]. Toothbrushes are usually made of bristles enhanced with round active ends that are stiff enough to remove accumulations of microorganisms, mono or poly-microbial aggregates, dental cavities and dental decay from the mouth without causing bruises to the gum [1]. Toothbrushes may become contaminated with microorganisms as they are often rinsed with plain water after use and kept in unhygienic conditions such as the bathrooms and toilets [3]. The unhygienic wet condition in the bathroom and toilet promote bacteria growth and crossed contamination through the aerosols produced when the toilets is flushed, thereby causing enteric pathogens and pseudomonas from the toilet and drainage to contaminate the toothbrush kept in the toilet and bathroom [4]. Toothbrush plays a significant role in disease transmission and increases the risk of infection to the user. This is because they can serve as reservoir for microorganisms in healthy, oral diseased and medically ill individuals. The microorganisms contaminating toothbrush may arise from: the environment where these toothbrushes are kept, from the hands that touch them, from the mouth in which it is used and their storage containers [5]. Thus, the bacteria which attach to these toothbrushes accumulate and survive on these toothbrushes thereby becoming vehicles for disease transmission to the individuals that use them. The mouth presents one of the most concentrated and varied microbial population particularly colonized by *Staphylococcus aureus*, Streptococcus spp, Bacteriodes spp, mycoplasma spp including fungi and viruses [6, 7]. These microorganisms tend to colonize the mouth as well as the environment where these toothbrushes are kept [8]. In healthy individuals, contamination of toothbrushes occurs early after initial use and increases with repeated use [9]. More so, retention and survival of these microorganisms on toothbrushes after brushing can lead to re-contamination

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and re-infection of the mouth of the user [5]. These microorganisms can therefore produce tooth decay, gum disease, stomatitis or infectious endocarditis in an individual which can affect both the mouth and general health of individuals. The possibility of these toothbrushes being associated with the transmission of severe health problems such as heart disease (infective endocarditis), arthritis, bacteraemia and stroke has been known [10].On the average, the lifespan of toothbrush is approximately three months. The American Dental Association recommends regular change of the toothbrush as the cleaning effectiveness decreases [1]. While these measures are aimed at reducing the microbial load in the mouth, mouth disease as well as other systemic disease; the microbial contamination and conditions where these toothbrushes are kept were not given attention while recommending the frequency for the change of toothbrush [11]. In Nigeria today, there is inadequate awareness among the general public on proper storage, replacement and disinfection of toothbrushes, as such apparently healthy individuals may be living with various mouth related diseases without recognizing them. A study on toothbrush contamination will help to sensitize the general public about the importance of toothbrush care especially in areas of proper storage and maintenance of toothbrushes. This study is therefore designed to evaluate the microbial contamination of toothbrushes and toothbrush keeping place in our locality.

## 2. MATERIALS AND METHODS

### 2.1 Materials

*2.1.1 Study Area*

This study was carried out among apparently healthy individuals residing in Egor local government area, Benin City, Nigeria. Benin City is the capital and largest City in Edo State, Southern Nigeria.

*2.1.2 Study Population*

The study population comprised of sixty (60) randomly selected, apparently healthy individuals residing in Egor LGA, Benin City, Nigeria. The participants were requested not to use any antimicrobial mouth rinses during the study period. All consenting participants were given new toothbrushes and told to use the toothbrushes as they normally do and keep where they normally keep. The toothbrushes were collected from them after a period of 4months and were used for this study. A structured questionnaire was administered to each participant for demographic information and other relevant information including where the participants preserved their toothbrush after brushing. Based on this information, the participants were assigned to one of the three categories:
Group 1: Participants who preserved their toothbrush outside the bathroom.
Group 2: Participants who preserved their toothbrush within the bathroom without attached toilets.
Group 3: Participants who preserved their toothbrush within the bathroom with attached toilets.

*2.1.3 Ethical Approval*

Approval for this research was sought and obtained from the Ethics and research committee Ministry of Health, Edo State, Nigeria.

*2.1.4 Sample Collection*

On the intended day for sample collection, each of these participants was given a sterile transparent zip lock plastic pouch and instructed to rinse the toothbrush in tap water after brushing and place their toothbrush in the zip lock pouch before the samples were collected.

### 2.2 Methods

*2.2.1 Sample Processing*

*2.2.1.1 Isolation of Organisms*

The samples were labeled according to the number on the questionnaire. The head of each toothbrush was cut off using a sterile scissors and placed in a sterile plain container into which nutrient broth was added aseptically and incubated at room temperature for 24 hours. After which serial dilution was carried out in a vacuum tube; 9ml was added to 3 graduated tubes. To the first tube, 100ml of the broth was added to 9.9ml of sterile water. Then 1ml was removed from tube 1 into tube 2 until it reached tube 4. The Petri dishes were labeled as 1 x $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$. Then 50ml of tube 2 ($10^{-3}$) was added up to the Petri dish labeled 1 x $10^{-3}$, the same was done for tube 3 and tube 4. After which nutrient Agar was poured and mixed with the diluents present in the Petri dish and allowed to solidify and incubated at 37°C for 24hours. After 24hours of incubation, it was checked for growth, the growth of the organisms were sub-cultured into chocolate and Mac Conkey agar plates and incubated for 24hours.

2.2.1.2 Bacteria Identification

Pure bacteria colonies isolated from the chocolate and Mac Conkey agar were preliminarily examined using colonial morphology, haemolytic reactions on chocolate agar plates and Gram stained. Identification of bacteria was done by employing an array of routine biochemical tests such as catalase and coagulase for Gram positive

bacteria, indole production, motility test, urease test, citrate utilization tests and oxidase test for Gram-negative bacteria.

**2.3 Statistical Analysis**

Categorical variables obtained from the laboratory investigations were tabulated, encoded and statistically analyzed using Statistical Package for Social Sciences (SPSS version 16) program. Test of significance was carried out using Chi square and the levels of significance were accepted at $p<0.05$.

**3.0 RESULTS**

Different species of microorganisms were recovered from 41 (68.3%) out of the 60 (100%) toothbrushes examined. No statistical significant relationship existed between gender, age and toothbrush contamination (p=0.605, p=0.698). Tooth brush keeping place, length of usage and toothbrush casing as a risk factor for toothbrush contamination had no significant statistical correlation with the toothbrush contamination (p=0.737, p=0.290, p=0.566).

Table 1: Microorganisms Isolated From Toothbrushes of Apparently Healthy Individuals in Egor LGA, Edo State, Nigeria

| Organism isolated | No. of Org Isolated | % positive | P value |
|---|---|---|---|
| *E. coli* | 15 | 36.6 | 0.005 |
| Lactobacillus spp | 15 | 36.6 | |
| Coagulase Negative Staph. | 03 | 07.3 | |
| Klebsiella spp | 01 | 02.4 | |
| *Pseudomonas aeruginosa* | 01 | 02.4 | |
| *Bacillus cereus* | 06 | 14.6 | |
| Total | 41 | 100 | |

P<0.05

Table 2: Microorganisms Isolated From Toothbrushes of Apparently Healthy Individuals In Egor LGA In Relation To Gender

| **Organism** | **Gender** | | **P value** |
|---|---|---|---|
| | **Male (%)** | **Female (%)** | |
| *Bacillus cereus* | 03 (21.4) | 03 (11.1) | |
| *Lactobacillus spp* | 02 (14.3) | 13 (48.2) | |
| *Escherichia coli* | 09 (64.3) | 06 (22.2) | 0.605 |
| *CoagulaseNegative Staph* | 00 (0) | 03 (11.1) | |
| *Klebsiella spp.* | 00 (0) | 01 (3.7) | |
| *Pseudomonas aeruginosa* | 00 (0) | 01 (3.7) | |
| Total | 14 (100) | 27 (100) | |

Table 3: Microorganisms Isolated From Apparently Healthy Individuals In Egor LGA In Relation To Age

| **Organism** | **Age (Years)** | | | | **Pvalue** |
|---|---|---|---|---|---|
| | **16-20 (%)** | **21-25 (%)** | **26 – 30 (%)** | **>31 (%)** | |
| *Bacillus cereus* | 01 (17) | 04 (66) | 01 (17.0) | 00 (0.0) | |
| *Lactobacillus spp* | 01 (7) | 13 (86) | 01 (7.0) | 00 (0.0) | |
| *Escherichia coli* | 00 (0.0) | 13 (87) | 02 (13.0) | 00 (0.0) | 0.698 |
| *Coagulase Negative Staph* | 00 (0.0) | 03 (100) | 00 (0.0) | 00 (0.0) | |
| *Klebsiella spp.* | 00 (0.0) | 01 (100) | 00 (0.0) | 00 (0.0) | |
| *Pseudomonas aeruginosa* | 00 (0.0) | 00 (0.0) | 00 (0.0) | 01 (100) | |

Table 4: Toothbrush Keeping Place as a Risk Factor for Tooth Brush Contamination Among Apparently Healthy Individuals In Egor LGA

| Organism Isolated | Tooth Brush Keeping Place | | | | | P Value |
|---|---|---|---|---|---|---|
| | Bedroom (%) | Kitchen (%) | Bathroomwith Toilet (%) | Bathroom without toilet (%) | Others (%) | |
| *Bacillus cereus* | 04 (67.0) | 00 (0.0) | 02 (33.0) | 00 (0.0) | 00 (0.0) | 0.737 |
| *Lactobacillus spp* | 14 (93.0) | 00 (0.0) | 00 (0.0) | 00 (0.0) | 01 (6.7) | |
| *E. coli* | 07 (47.0) | 30 (20.0) | 02 (13.0) | 00 (0.0) | 03 (20.0) | |
| Coagulase Negative Staph | 03 (100) | 00 (0.0) | 00 (0.0) | 00 (0.0) | 00 (0.0) | |
| Klebsiella spp. | 00 (0.0) | 00 (0.0) | 01 (100) | 00 (0.0) | 00 (0.0) | |
| *Pseudomonas aeruginosa* | 01 (100.0) | 00 (0.0) | 00 (0.0) | 00 (0.0) | 00 (0.0) | |

Table 5: Duration of Toothbrush Usage as a Risk Factor For Toothbrush Contamination Among Apparently Healthy Individuals In Egor LGA

| **Organism Isolated** | **Tooth brush Usage in Months** | | | | | | **P value** |
|---|---|---|---|---|---|---|---|
| | **1 – 2Months (%)** | | **2 – 3Months (%)** | | **4 Months (%)** | | |
| *Bacillus cereus* | 02 | (33.3) | 04 | (66.7) | 00 | (0.0) | 0.290 |
| Lactobacillus spp | 04 | (27.0) | 04 | (27.0) | 07 | (46.0) | |
| *E. coli* | 04 | (27.0) | 07 | (46.0) | 04 | (27.0) | |
| Coagulase Negative Staph | 01 | (33.3) | 02 | (66.7) | 00 | (0.0) | |
| Klebsiella spp. | 00 | (0.0) | 01 | (100) | 00 | (0.0) | |
| *Pseudomonas aeruginosa* | 00 | (0.0) | 00 | (0.0) | 01 | (100) | |

Table 6: Toothbrush Casing as a Risk Factor for Toothbrush Contamination Among Apparently Healthy Individuals In Egor LGA

| **Organism Isolated** | **Toothbrush Casing** | | | | **P Value** |
|---|---|---|---|---|---|
| | **Yes (%)** | | **No (%)** | | |
| *Bacillus cereus* | 02 | (33.3) | 04 | (66.7) | 0.566 |
| Lactobacillus spp | 06 | (40.0) | 09 | (60.0) | |
| *E. coli* | 04 | (27.0) | 11 | (73.0) | |
| Coagulase Negative Staph | 00 | (0.0) | 03 | (100) | |
| Klebsiella spp. | 01 | (100) | 00 | (0.0) | |
| *Pseudomonas aeruginosa* | 00 | (0.0) | 01 | (100) | |

## 4.0 DISCUSSION

Toothbrushes may become contaminated with microorganisms when kept in unhygienic conditions such as the bathroom and toilets [12, 13]. An overall predominance of 68.3% microorganisms was isolated from toothbrushes of apparently healthy individuals in this study. *E. coli* and Lactobacillius 15(36.6%) respectively were the most predominant organisms isolated; followed by *Bacillus cereus* 6(14.6%), Coagulase negative staphylococcus 3 (7.3%), while *Pseudomonas aeruginosa* and Klebsiella spp were the least 1 (2.4%) respectively. The organisms isolated in this study is in agreement with that established from previous study were it was observed that although the toothbrush is not the ideal environment for microorganisms growth, it is capable to obtain the life of microbes [12]. Out of the 14 male toothbrushes that had bacteria contamination in this study, *E. coli* had the highest predominance, occurring in 9 toothbrushes (64.3%), followed by *Bacillus cereus* 3(21.4%) while Lactobacillus spp 2 (14.3%) recorded the least. Of the 27 female toothbrushes that had bacteria contamination, Lactobacillus spp had highest predominance 13 (48.2%), followed by *E. coli* 6 (22.2%), *Bacillus cereus* and Coagulase negative staphylococci were found in 3(11.1%) respectively, while Klebsiella spp and *Pseudomonas aeruginosa* both recorded least 1 (3.7%) respectively. This was not statistically significant (p=0.605). Age range 21-25years had the highest predominance of coagulase negative Staphylococci 3 (100%) and Klebsiella spp 1(100%), *E. coli* 13 (87.0%), Lactobacillus 13 (86.0%) with *Bacillus cereus* recording the least 4 (66.0%). *Pseudomonas aeruginosa* was observed in 1 (100%) individual in the age range >31 years. Age range 26-30years had highest predominance of *E. Coli* 2(13.0%), *Bacillus cereus* 1 (17.0%) while Lactobacillus was least 1(17.0%). This study revealed that toothbrushes kept in the bedroom had the highest occurrence of coagulase negative Staphylococci 3 (100%),

*Pseudomonas aeruginosa* 1 (100%), Lactobacillus 14 (93.0%), then *Bacillus cereus* 4 (67.0%), with the least being *E. coli* 7 (47.0%). Those kept in the Kitchen recorded only *E. coli* 3 (20.0%). Toothbrush kept in the bathroom with toilet had Klebsiella spp as highest 1 (100%), *Bacillus cereus* 2 (33.0%) while *E.coli* recorded the least 2 (13.0%). Toothbrushes kept in bathroom without toilet had no organism isolated. *E. coli* recording 2 (20.0) was predominantly isolated from toothbrushes kept in other places with Lactobacillus recording least. This agrees with the study that Identified: short distance from the toilet, humidity in the bathroom, interaction with other items and moisture as the environmental conditions that affects toothbrush contamination and influence the development of microbes on the toothbrush [14]. Tooth brushes exposed to the bathroom environment are known to be heavily infected with Enterobacteriaceae and Pseudomonas species [3]. The duration of toothbrush usage as a risk factor for microbial contamination of toothbrushes recorded the highest predominance in toothbrushes used between 2-3months 18 (43.9%), followed by toothbrushes used for 4months 12 (29.3%) with toothbrushes used for 1month recording the least microorganisms 11 (26.8%). This study agrees with researches which states that microbial colonization reaches higher level with every further use of the toothbrush. Also, the presence of *E.coli*, Proteus spp, Klebsiella spp and *Pseudomonas aerudinosa* as microorganisms contaminating toothbrushes is related to storage places of those toothbrushes [15, 16, 17, 18]. Toothbrush casing was accessed as a risk factor for developing isolated organisms in the toothbrushes studied. This is because toothbrushes that had no casing recorded the highest presence of contaminating bacteria 28 (68.3%) while toothbrushes that had casing recorded the least 13 (31.7%). This agrees with studies that shows there is substantial association between the existence of Klebsiella and Pseudomonas with uncapped toothbrushes, while Enterococcus, Micrococcus, *E. coli*, Bacillus, Streptococcus were also isolated in capped and uncapped toothbrushes [14].

## 5.0 CONCLUSION

Result from this study has shown that bacteria contamination of toothbrushes and toothbrush keeping places is very high with a prevalence of 58.33%. The bacteria isolated from the toothbrushes were *E. coli*, Lactobacillus spp., Coagulase negative staphylococcus, Klebsiella spp, *Pseudomonas aeruginosa* and *Bacillus cereus* with *E. coli* and Lactobacillus spp being the most predominant.

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**Conflicts of Interest**

The authors declare that they have no conflicting interest.

**Authors Contributions**

Laboratory investigations and statistical analysis were performed by Igbineweka Obehi Mya, assisted by Mr Idemudia Nosakhare Lawrence. Moses-Otutu Ifueko Mercy designed the study and drafted the manuscripts. All the authors have read and approved the manuscript.

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