

**ISOLATION AND CHARACTERIZATION OF MICROBES ON USED  
TOOTHBRUSH OF SELECTED STUDENTS OF OGUN STATE  
COLLEGE OF HEALTH TECHNOLOGY ILESE-IJEBU**

**BY**

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## ABSTRACT

*This study is based on isolation and characterization of microbes on used toothbrush of selected students of Ogun State College of Health Technology Ilese-Ijebu. Convenience and purposive sampling technique was used to select 51 students from Ogun State College of Health. 28g of the product was weighed, and added to mix 1 litre of deionized water and allowed to soak for minutes, stirred or swirled to mix them up, allow to cool at 47<sup>0</sup>C so as to have homogeneous mixture and then poured on plate after cooling. Microorganisms were isolated and identified, by characterizing and identifying on the basis of their colonial, molecular, biochemical characteristics, and gram staining, coagulase, catalase, and oxidase test were performed. This study showed that all the used toothbrush were contaminated with pathogenic bacteria which are known to cause serious health problem in human, such as heart disease, bacteremia, and stroke, since toothbrush serve as a reservoirs for microorganism and play a major role in disease transmission and increase the risk of infection, their care should be given adequate attention. Based on the outcome of this study, it was discovered that staphylococcus aureus occur mostly and have the highest percentage among the bacteria isolation. Its was recommended that toothbrushes should be soaked in salt and warm water solution for 15-20 minutes for removal of bacteria present on toothbrush and should be kept in a ventilated area and not closed to the toilet and should be covered after dried with a perforated cover for easy flow of air.*

## INTRODUCTION

The most common way to maintain the complex of proper oral hygiene is the tooth brushing; its main goal is to remove the dental plaque which is responsible for a number of oral diseases: tooth decay, periodontitis, as well as halitosis. The first and most efficient tool for removing the oral biofilm and the soft debris out of the mouth, especially from the tooth and tongue surfaces, is the toothbrush (Beneduce, Baxter, Bowman, Haines, and Andreana, 2010).

The oral cavity contains a teeming population of different types of microorganisms some of which are transferred to a toothbrush during use. Tooth brushing plays an important everyday role for personal oral hygiene and effective plaque removal. It is the most commonly recommended and performed oral hygiene behaviour and is done ubiquitously in both developed and developing world. The toothbrush is used on a daily basis to clean the oral cavity (Frazelle and Munro, 2012).

Oral hygiene is the practice of keeping the mouth and teeth clean to prevent dental problems like, dental caries, gingivitis, periodontitis and bad breath. Tooth brushing, tongue cleaning, flossing, mouth rinsing with disinfectant mouth washes are some of the methods for maintaining oral hygiene. Tooth brushing is the most effective and commonly used method among them. Along with the brushing methods, disinfection of toothbrush is also equally important for maintenance of health of oral tissues (Susheela and Radha, 2016).

Unfortunately, proper care of toothbrush is often neglected as toothbrushes are usually kept in bathrooms that harbor millions of microorganisms. This is mainly due to a lack of awareness regarding proper toothbrush maintenance. Therefore, the survival of microorganism on a toothbrush after brushing presents a possible mode of re-contamination upon a second usage.

Back in early 20's of the 20th century, it was reported that the toothbrush can cause recurrent infections in the mouth. A number of factors, including the long microbial surviving in the toothbrushes – from 2 days to one week , the inadequate keeping, the toothbrush use without decontamination – which leads to autoinoculation and the untimely changing of the toothbrush with new ones, may result in repeated entry of potential pathogens and crossed infection in the oral cavity, especially in children, elderly people, those with concomitant somatic disease, patients with high risk i.e. immunocompromised ones, those with transplanted organs or oncologic patients (Sammons, Kaur and Neal, 2014).

Although researches show that different microorganisms can grow on the toothbrushes after its usage (Beneduce et al, 2010), different microorganisms have been established while studies have recorded very few microbes, this makes the subject a controversial one and as a result catching the attention of researcher worldwide.

The pathogenic contamination in the vulnerable population, like critically ill patients, immunosuppressed patients, elderly persons, pregnant women and children may raise the risk of infection and its transfer. It is obvious that there is insufficient information about microbes found on toothpaste residue on used toothbrush while it is also evident that enough researcher has not been done on the area of study which prompts the need for a study of this nature.

## **Research Questions**

1. Are there microbes present on used toothbrush?
2. What are the different microbes present on used toothbrush?
3. What are the microbes often found on used toothbrush?
4. Are the microbes found dangerous to the oral health?

## **Broad Objective**

This study is based on isolation and characterization of microbes on used toothbrush of selected students of Ogun State College of Health Technology, Ilese-Ijebu.

## **Specific Objectives**

1. To investigate the presence of microbes on used toothbrush,
2. To ascertain different microbes found on used toothbrush,
3. To examine most frequent microbe on used toothbrush.
4. To examine the effect of microbes found on oral health.

## **Operational Definition of Terms**

**Isolation:** the separation of a strain from a natural, mixed population of living **microbes**, as present in the environment

**Characterization:** Examination of Biochemical, physiological & molecular characteristics of microbes.

**Microbes:** Minute organism typically visible under a microscope and often causes danger to the oral health. Microbes include bacteria, fungi, and protozoan parasites

**Toothpaste Residue:** The leftover of toothpaste after routine brushing of the teeth with toothpaste and toothbrush.

**Toothbrush:** An oral hygiene instrument used to clean the teeth, gums, and tongue. It consists of a head of tightly clustered bristles--atop of which toothpaste can be applied--mounted on a handle which facilitates the cleaning of hard-to-reach areas of the mouth.

## METHODOLOGY

This study is an experimental study which was carried out at Ogun State College of Health Technology, Ilese-Ijebu.

Toothbrushes are manufactured in a variety of styles. Toothbrush bristles range from soft to hard with different cluster patterns and plastic shapes while toothbrush handles included different plastic shapes and decorative moldings. Different toothbrush design elements will be examined by some of the studies. It will be examined if bacteria is trapped inside the bristles of the toothbrush, bacterial survival may be dependent upon the bacteria (aerobic versus anaerobic) and toothbrush design. In addition, the researchers will look at the possibility of solid handles having retention of bacteria and that as the surface area.

### Materials

Nutrient agar	MacConkey agar
Blood agar	Normal saline water
Iodine solution	Safranin
Methanol	Cotton wool
Foil paper/ aluminum	Sterile polythene bag

### Preparation of Nutrient Agar

28g of the product will be weighed, and added to mix 1 litre of demonized water and allowed to soak for minutes, stirred or swirled to mix them put in cool to 47<sup>0</sup>C so as to have homogeneous mixture and will then be poured on plate after cooling.

MacConkey preparation of 46.53gm of medium will be suspended in 1000ml of distilled water, heated to boiling point. The agar will be completely put in autoclave for 121C<sup>0</sup> for 15mins.

## Characterization and Identification

The microorganisms' isolates will be characterized and identified on the basis of their colonial molecular biochemical characteristics.

Gram staining, coagulase, catalase, and oxidase will be performed according to Chees brought.

## RESULTS

### Age Group of Subjects

The age group of subjects is shown in the table below:

Age Group	Frequency	Percentage (%)	Mean	S.Dev.
15-24	45	88.24		
25-34	2	3.92		
35-42	4	7.84		
<b>Total</b>	<b>51</b>	<b>100</b>	<b>1.196</b>	<b>.5664</b>

From the table above, subjects aged 15-24 were 88.24%, other age groups were 25-34 (3.92%) and 35-42 (7.84%). The result revealed that majority of subjects were between the age of 15 and 24.

### Gender of Subjects

The gender of subjects is shown in the table below:

Gender	Frequency	Percentage (%)	Mean	S.Dev.
Male	12	23.53	<b>1.765</b>	<b>.4284</b>

Female	39	76.47
<b>Total</b>	<b>51</b>	<b>100</b>

The table above shows that, 12% of subjects were male while 39% were female. It can be inferred that majority of subjects used for the study were female.

**Table 3: Percentage Growth of Organism on Used Toothbrush**

Percentage Growth of Organism on Used Toothbrush is shown in the table below:

Gender	Frequency	Percentage (%)	Mean	S.Dev.
Growth (G”	47	92.2		
No Growth “NG”	4	7.8	<b>1.078</b>	<b>.272</b>
<b>Total</b>	<b>51</b>	<b>100</b>		

From table 3, the percentage of growth of microorganisms on used toothbrush was **92.2%**, also the mean value of **1.078 (SD=.272)** shows that majority of used toothbrushes harbours microorganism.

**Table 4: Microorganisms isolated from the used manual toothbrush bristle.**

Microorganisms isolated from the used manual toothbrush bristle are shown in the table below:

Isolate	Identity
1	<i>Klebsiella SPP</i>
2	<i>Escherichia coli</i>
3	<i>Pseudomonas auruginosa</i>

4	<i>Staphylococcus aureus</i>
5	<i>Proteus</i>

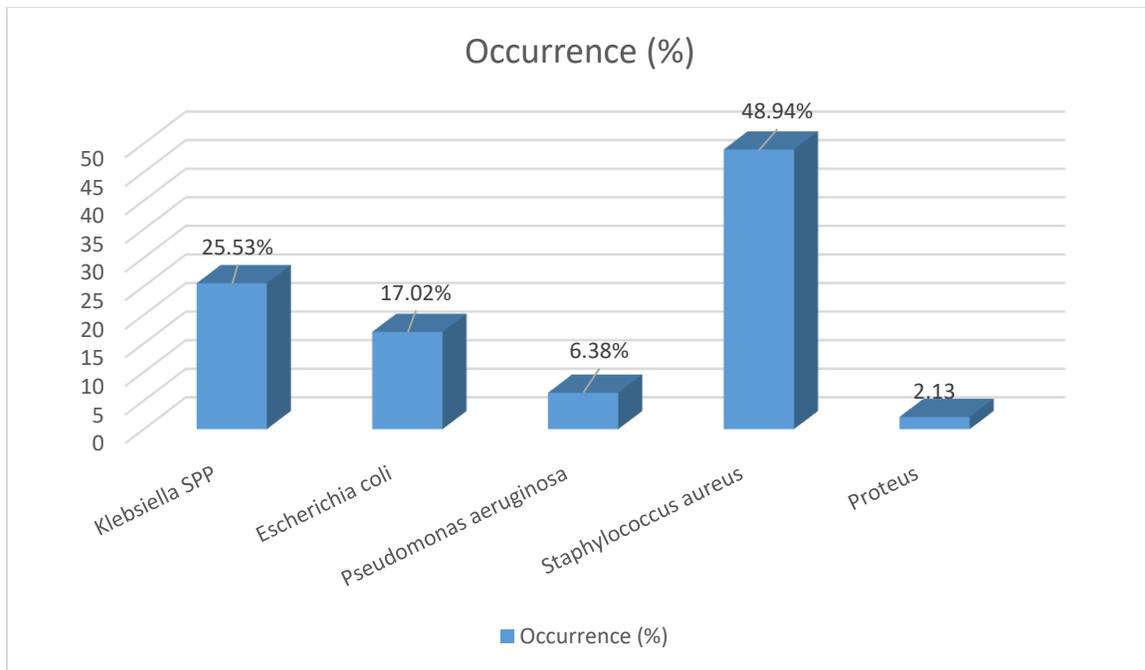
The table revealed microorganisms isolated from used toothbrush. The microorganism isolated from samples include: *Klebsiella SPP*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus*.

**Table 5: Occurrence of bacteria Isolates on used manual toothbrush bristle**

The occurrence of the microorganisms isolate on the used toothbrush is presented in the table below.

<b>Organisms Isolated</b>	<b>No Isolated</b>	<b>Occurrence (%)</b>
<i>Klebsiella SPP</i>	12	25.53
<i>Escherichia coli</i>	8	17.02
<i>Pseudomonas aeruginosa</i>	3	6.38
<i>Staphylococcus aureus</i>	23	48.94
<i>Proteus</i>	1	2.13
<b>Total</b>	<b>47</b>	<b>100</b>

Table 5 revealed that the occurrence of *Klebsiella SPP* was 25.53%, *Escherichia coli* was 17.02%, *Pseudomonas aeruginosa* was 6.38%, *Staphylococcus aureus* was 48.94% and *Proteus* was 2.13%.



**Figure 1:** Showing occurrence of microorganisms isolated from used toothbrush

### Discussion

The oral cavity contain a teaming population of different types of microorganisms some of which are transferred to toothbrush during use and some are acquired from the environment where the toothbrush is kept for instance those kept in bathroom or toilet acquired more microorganisms due to the flushing of the toilet when the lid of the closet is not covered as well some carriers, vectors, serve as infection transmission agent.

A total of fifty one (51) used toothbrush bristle obtained from 51 students of Ogun State College of Health were examined, bacteriologically the result shows majority (92.2%) of the used toothbrushes were contaminated with bacteria, the contamination of the used toothbrush by microorganisms come from the oral cavity, storage containers, storage environment, the water used for rinsing and also from users. Microorganisms isolated were identified from the used

toothbrush. The bacterial were *Klebsiella SPP*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus*.

Result from the study conforms with the study of Bello et al (2013) reported staphylococcus Escherichia, and pseudomonas in used toothbrush. This also supports the result in the study of Contreras et al (2015) who reported that the most frequent microorganisms found in toothbrush used by parents and children for one month were *enterobacteriaceae* and *pseudomonadacea*.

Kozai et al (2014) also reported that pathogenic microorganisms can be transferred readily when a toothbrush is used increasing the risk of dental caries and infectious diseases. *Staphylococcus aureus* was most frequently isolated from the used toothbrushes with a percentage occurrence of 48.94% while *Proteus* had the lowest percentage occurrence of 2.13% on used toothbrush examined. Others are *Klebsiella spp.* (25.53%), *Escherichia coli* (17.02%), *Pseudomonas aeruginosa* (6.38%)

Sammons et al (2014) however isolated pseudomonas 15% from used toothbrushes while Osho et al (2013) isolated Escherichia coli, staphylococcus aureus, and pseudomonas aeruginosa from used toothbrush.

*Staphylococcus aureus* was detected in 23 of the used toothbrushes examined, *Klebsiella spp.* from 12 toothbrushes, *Escherichia coli* from 8 toothbrushes, *Pseudomonas aeruginosa* from 3 toothbrushes and *Proteus* from 1 toothbrush.

Staphylococcus are common skin in habitants. Their presence on the used toothbrushes in high number may come from handling and rinsing of toothbrushes after use, they are known to produce potent toxins which are injurious to health. They are also capable of producing many oral infectious diseases. Pseudomonas aeruginosa are opportunistic pathogens responsible for many nosocomial infections. They are also ubiquitous in nature including water. They are presence in

use toothbrushes may be attributable to the storage environment such as a bathroom, toilet, and washing sinks, rinsing water in the air. The bathroom provides a humid environment that encourages the growth of microorganisms.

*Escherichia coli* are coliforms and member of the family Enterobacteriaceae. They are also pathogenic to human insignificant numbers. The presence of *Escherichia coli* on the toothbrushes examined was indicative of fecal contaminations. The used toothbrushes must have been stored in unhygienic environment such as toilet, bathroom, sinks. These bacteria may also have entered the toothbrush through the rinsing water.

Different brands of toothbrushes are marketed to the public every year with little information on their contaminations by bacteria with use. The use of uncontaminated toothbrushes will assist in the maintenance of sound oral hygiene and reduce the health risk posed by the contaminating bacteria to humans.

### **Conclusion**

Majority of the used toothbrush bristle used in this study were contaminated with pathogenic bacteria which are known to cause serious health problem in human, since toothbrush serve as a reservoirs for microorganism and play a major role in disease transmission and increase the risk of infection, their care should be given adequate attention. They must be adequately rinsed with sterile water and allowed to dry in air before storage in hygienic dry container. In addition, sharing of toothbrush should be discourage.

### **Recommendations**

- ❖ Toothbrushes should not be kept in toilet or close to toilet to avoid contamination.

- ❖ Tooth brush should be soaked in salt and warm water solution for 15-20 minutes in removing of bacteria present on toothbrush then it should be kept in a ventilated area i.e (presence of air),
- ❖ Toothbrushes should be covered after use with a perforated cover for easy flow of air.
- ❖ Toothbrushes should be replaced every 3 to 4 months
- ❖ It is advised that people should not share toothbrushes as this may compromise oral health.

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