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RESEARCH ARTICLE **OJBR0501001-530**

PREVALENCE AND SUSCEPTIBILITY OF BACTERIA FROM USED TOOTHBRUSHES OF STUDENTS RESIDING IN HALL I AND II UNIVERSITY OF BENIN.

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ABSTRACT

Used toothbrushes are reservoirs for varieties of bacteria that are implicated in human disease transmission. Personal oral hygiene and removal of plaque are important roles played by the use of toothbrushes in an act endorsed for oral hygiene resolution and ubiquitously practiced in developing and developed Nations. The study aims to evaluate the prevalence and susceptibility of bacteria present in used toothbrushes of students residing in Halls I and II, University of Benin. New toothbrushes ninety (90) were bought and forty-five (45) each were distributed to students in each hall of residence, these toothbrushes were used for one month and collected for bacteria analysis. Standard bacteriological procedures were observed for the analysis. Data were analyzed using the SPSS 22.0 computer software package. Independent t-test was used to find the differences between the two variables. The value of $p < 0.05$ was taken to be statistically significant. Heavy bacteria contamination was associated with used toothbrushes while no bacteria contamination in the unused ones, which serves as a control. *Klebsiella* species 16 (35.53 %), and 19 (42.2 %) were more prevalent than coagulase-negative *staphylococci* species 2 (4.4 %) and 2(4.4 %) respectively from both halls. All the toothbrushes analyzed in this study had bacteria contaminates that are known to harm human health, contributing significantly to the spread of diseases, and increasing infection risks. Establishing a high aseptic protocol, storage, and management be encouraged in tertiary institution halls of residence as the incidence of these oral bacteria and individual health risks will be minimized.

Keywords: *Bacteria, contamination, Toothbrushes, Oral health, Prevalence*.

INTRODUCTION

Oral health and disease prevention require routine oral hygiene where toothbrushes play a vital role (Jagadeeshwar *et al*., 2015). Unhealthy sanitary conditions like wardrobes, drawers, bathrooms, kitchens, and toilets are the most common places where toothbrushes are kept. Different populations of microorganisms are associated with the oral cavity (Mehta *et al*., 2007), which in the course of usage are transferred to toothbrushes. Personal oral hygiene and removal of plaque are important roles played by the use of toothbrushes an act endorsed for oral hygiene resolution and ubiquitously practiced in developing and developed Nations. An unused toothbrush is not a friendly land for the proliferation of bacteria; however, it can be partially contaminated right from the production floor (Efstratiou *et al*., 2007; Downes *et al*., 2008). Microtrauma, storage environments, aerosols, and hands are common places where toothbrushes can easily be contaminated (Tagi and Roger1998; Frazella and Munro, 2012). The re-introduction of potential microbes to the oral cavity is a result of the storage condition observed for toothbrushes especially from the kitchen and bathroom environment (Wetzel *et al*., 2005).

The accumulation, survival, and attachment of bacteria on toothbrushes could be transmitted through the individual storage conditions, as a reservoir of microbes causing disease (Goldschmidt *et al*., 20004; Caudy *et al*., 1995). Reports abound on the contamination of toothbrushes by bacteria, with lower or higher contamination associated with large illegal obstructions placed between the toothbrush and the handle (Mehta *et al*., 2008). Bacteria retention, growth, and transport are quite associated with toothbrushes and re-infection which is a risk factor for periodontal disease (Goldschmidt *et al*., 2004). Community and hospital settings are common places where toothbrushes are found due to the essential role they play in individual oral health (Tagi and Rogar, 1998). When in regular use they are reported to be heavily contaminated by microbes (Malmberg *et al*., 1994; Osho *et al*., 2013) and could express a significant role in disease transmission and increase the risk of infection since they serve as a reservoir for microorganisms in healthy, medically-ill and oral diseased adults (Efstratiou *et al*., 2007; Glass 1992). Systemic and localized diseases associated with contaminated toothbrushes have been suggested to play a role in both. Bacteremia, heart diseases, arthritis, and stroke have also been reported to be associated with toothbrushes (Warren *et al*., 2001; Sammons *et al*., 2004). This study aims to isolate and identify the possible bacterial contaminants associated with used toothbrushes obtained from students' residences in hall I and II hostels in the University of Benin, Nigeria.

Heavily contaminated toothbrushes and inappropriate storage can cause so many health problems as some toothbrushes stored in an improper storage facility will increase or serve as a reservoir for bacteria growth, retention transportation, and re-infection which is a risk factor for periodontal diseases.

MATERIALS AND METHODS

Sample Collection

Ninety (90) new toothbrushes were bought, forty-five (45) were distributed in each hall of residents (I and II) and each student received a brand-new toothbrush of Doctor White or Evergreen products. Students were instructed to use the toothbrush twice daily (morning and evening) for routine oral hygiene in a month. After the end of one month, the toothbrushes were collected from each recruited participant, and collected toothbrushes were rinsed in running tap

water and placed in the zip lock pouch, which was then transported to the laboratory for analysis on the same day. At the time of sample collection, questionnaires were administered to each participant on how and where the brush was preserved during the last thirty days.

Isolation of Bacteria

Tryptone soya broth was prepared according to manufacturer instructions and 15 ml was aseptically dispensed in McCartney bottles and sterilized at 120 ^{0} C for 15 minutes. Each of the used toothbrushes bearing the head was decapitated and aseptically transferred into the sterile 15 ml tryptone soya broth. The contents were allowed to stand for thirty minutes and vortexed for sixty seconds before usage.

Nutrient agar was prepared and sterilized at 121^{0}C for 15 minutes, when the molten agar cooled to 40^0 C, 0.05mg/ml of Ketoconazole was added to inhibit fungal growth. 1ml appropriate dilution of 10^5 was spread onto sterile solidified nutrient agar contained in Petri dishes. The Petri dishes were then incubated aerobically at 37^0 C for 24 hours. New (unused) toothbrushes were subjected to the same procedure serving as controls. Colonies from the plates were purified and stored on nutrient agar slants for identification.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was carried out using the Kirby-Bauer-CLSI modified Disc Agar Diffusion technique (DAD) (Cheesebrough, 2006). One milliliter (1.0 ml) of a standardized overnight culture of each isolate (10^6/ml) was used to flood the surface of Mueller Hinton Agar (MHA) plates and the excess drained off and dried while the Petri dish lid was in place. The standard antibiotic discs were then aseptically placed at reasonable equidistance on the inoculated MHA plates and allowed to stand for 1 h. The plates (prepared in duplicates for each isolate) were incubated at 37°C for 18 h (Ehinmidu, 2003). The diameter of the zones of inhibition produced by each antibiotic disc was measured and recorded. An agar plate containing just agar was used as a positive control while a plate inoculated with antibiotics was used as a negative control. Zone diameter was recorded and interpreted as susceptible, intermediate, and resistant according to Clinical Laboratory Standard Institute (CLSI, 2020)

DATA ANALYSIS

Data were analyzed using the statistical package for Social Sciences (SPSS) 22.0 computer software package. An Independent t-test was used to find the differences between the two variables. The value of $p< 0.05$ was taken to be statistically significant.

RESULTS

The variable below showed that 55. 6 % and 57.8 % of the participants kept their toothbrushes in the toilet, whereas 44.4 % and 48.9 % stored them in other locations including their bedroom and closet respectively. Only 20% of individuals kept their toothbrushes in the closet, compared to 51.1 % used toothbrush caps for safeguarding. Although 15.6 % of individuals clean their teeth thrice daily. Only 40.0 % of participant observed a distance of 0 -50 meters between the toilet and the top of the sink where they keep their toothbrushes, while 6.7 % had a distance between 181 to 240.

Table 1: Storage area of toothbrushes among residence of Hall I and II hostel, University of Benin

Variable	Hall I (n = 45)						Hall II (n = 45)					
	Yes	**No**	**Prevalence (%)**	**t**	**Df**	**p-value**	**Yes**	**No**	**Prevalence (%)**	**t**	**df**	**p-value**
Toothbrush Storage (90)				0.514	88	0.322				0.514	88	0.322
Toilet	25	20	55.6				26	19	57.8			
Others (Bedroom, Closet)	20	25	44.4				22	23	48.9			
Toothbrush Handing (90)				0.228	88	0.207				0.228	88	0.207
Top of the sink	13	32	28.9				14	31	31.1			
Toothbrush Cap	23	22	51.1				28	17	62.2			
Closed Cabinet	9	36	20.0				8	37	17.8			
Frequency of use/day (90)				0.650	88	1.622				0.650	88	1.622
Once / Day	15	30	33.3				17	28	37.8			
Twice/Day	23	22	51.1				28	17	62.2			
Thrice/Day	7	38	15.6				9	36	20.0			
More than three times /Day	00	00	00				00	00	00			
Distance (cm) Toilet to Toothbrush (90)				0.214	88	0.671				0.214	88	0.671
0 – 59	18	27	40.0				17	28	37.8			
60 – 120	11	34	24.4.				11	34	24.4			
121 – 180	13	32	28.9				12	33	26.7			
181 - 240	3	42	6.7				3	42	6.7			

following bacteria; *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Pseudomonas* sp., *Escherichia* sp., *Klebsiella* sp., coagulase-negative *Staphylococci* sp. and *Staphylococcus* sp. was isolated from used toothbrushes obtained in Hall I and Hall II. *Klebsiella* sp. (35.5 %) and 42.2 % were frequently isolated while *Enterobacter* sp. and *Escherichia coli* (17.8%) had the same number of positive samples and percentages respectively. *Pseudomonas* sp. and *Citrobacter* sp. 3(6.7%) had the same frequency of occurrence. *Staphylococcus* sp. and *Klebsiella* sp. (2.2%) had the same occurrence, whereas coagulase-negative *Staphylococci* sp. had a 4.4 % prevalence.

The following bacteria which include *Klebsiella* sp. *Enterobacter* sp., *Citrobacter* sp., *Pseudomonas* sp., *Escherichia coli, Klebsiella* sp., coagulase-negative *Staphylococci* sp., *Staphylococcus aureus*, were isolated from used toothbrushes obtained in Hall I and Hall II. *Klebsiella* sp. (35.5 %) and (42.2 %) were frequently isolated while *Enterobacter* sp. and *Escherichia* sp. (17.8 %) had the same number of positive samples from both halls and percentages respectively. *Pseudomonas* sp. (6.7%) respectively and *Citrobacter* sp. had (6.7 %) and (4.5 %) for Hall I and II with different frequency of occurrence. *Staphylococcus* sp. recorded (11.1 %) for Hall I and (8.9 %) for Hall II whereas coagulase-negative *Staphylococci aureus* had a 4.4 % prevalence from both halls of residence.

Table 2. Bacterial Isolates occurrence on the used toothbrushes

	Hall I		**Hall II**	
Isolates	**Frequency of occurrence (n=45)**	**Prevalence (%)**	**Frequency of occurrence (n=45)**	**Prevalence (%)**
klebsiella sp.	16	35.5	19	42.2
Enterobacter sp.	8	17.8	8	17.8
Citrobacter sp.	3	6.7	2	4.5
Pseudomonas sp.	3	6.7	3	6.7
Escherichia sp.	8	17.8	7	15.6
CN *Staphylococci* sp.	2	4.4	2	4.4
Staphylococcus sp.	5	11.1	4	8.9

Key: CN - Coagulase Negative

All isolates were susceptible to PEF, CN and CTX and resistant to APX, Z, AM and AU. *Enterobacter* species, *Citrobacter* species, *Pseudomonas* species, *Escherichia coli*, and *Klebsiella* species *were all susceptible* to Ofloxacin.

Table 3: Sensitivity Test (mm) on Bacterial Isolates from used tooth brushes

Bacterial Isolates	**PEF 30µg**	**CN 30µg**	**APX 30µg**	**Z 30µg**	**AM 30µg**	**R 25 µg**	**CTX 30µg**	**S 30µg**	**SXT 30µg**	**E 10µg**	**AU 10µg**	**SP 10µg**	**CH 30µg**	**OFX 10µg**
K. sp.	S (19.5)	S (17.5)	R	R	R	R	S (23.0)	R	R	R	R	R	R	R
Enterobacter Sp.	S (25.5)	S (21.5)	R	R	R	R	S (25.5)	R	R	R	R	S (25.5)	R	S (34.5)
Citrobacter sp.	S (25.5)	S (21.5)	R	R	R	R	S (25.5)	R	R	R	R	S (25.5)	R	S (34.5)
Pseudomonas sp.	S (24.5)	R	R	R	R	R	S (30.0)	R	R	R	R	S (17.5)	R	S (24.5)
E. coli	S (30.0)	S (22.0)	R	R	R	R	S (30.0)	S (22.5)	S (26.0)	R	R	S (25.5)	S (23.5)	S (30.0)
Klebsiella sp.	S (27.5)	S (19.5)	R	R	R	R	S (27.5)	R	S (24.0)	R	R	S (23.5)	S (22.0)	S (22.0)
Coagulase-negative *Staphylococcus* sp.	S (32.5)	S (30.0)	R	R	R	S (32.5)	S 25.0()	S (20.0)	R	R	R	R	R	R
Staphylococcus sp.	S (30.0)	S (22.0)	R	R	R	S (30.5)	S (30.0)	S (29.0)	S (30.5)	S (23.0)	R	R	R	R

Key: R: Resistance; PEF: pefloxacin; CN: Gentamicin; APX: Ampicillin/Clavulanic; S: Streptomycin; Z: Zeocin; AM: Ampicillin; R:Rifampin; CTX: Cefotaxime; SXT: Sceptrin; E: Erythromycin; AU: Augmentin; SP: Spiramycin; CH: Chloramphenicol; OFX: Ofloxacin

Table 4: Clinical Laboratory Standard of antibiotics for the determination of susceptibility of bacteria isolates

Micro organism	Susceptible range	Resistant
Staphylococcus sp.	Ofloxacin ≥16	≤ **13**
Coagulase-negative *staphylococcus* sp.	Gentamicin ≥ 15	≤ **1**2
Pseudomonas sp.	Erythromycin ≥23	≤ 12
	Pefloxacin ≥	≤ 23
Citrobacter sp.	Cefotaxime ≥23	≤ 14
Enterobacter sp.	Sceptrin ≥ 16	≤ 10
Klebsiella sp.	Ampicilin/clavulanic acid	≤ 13
E. sp.	Streptomycin ≥ 15	≤ 11
	Ampicillin ≥ 17	≤ 10
	Rifampin ≥ 20	≤ 16
	Chloramphenicol ≥ 18	≤ 12

Source: CLSI, 2020

DISCUSSION

The findings indicate that toothbrushes taken from the University of Benin's Hall I and Hall II hostels were heavily contaminated with various microorganisms, in contrast to the unused toothbrushes used as control, which exhibited no bacterial growth. The storage environment, oral cavity, and storage container may have all contributed to the contamination of toothbrushes. The result from Table 1 showed that 25(55.6 %) participants stored their toothbrushes in the bathroom, while 20(44.4 %) participants kept their toothbrushes in their bedrooms or other suitable locations. Contamination starts with handling and closeness to toilets. Another possibility is that members of the household frequently keep their toothbrushes in small containers next to one another, which might lead to cross-contamination. Location of storage and lack of maintenance of the bathroom might have contributed to the contamination. Frazzelle and Munro (2012) reported that contamination of toothbrushes could be caused by a short distance to the restroom and a lack of sufficient maintenance of the restroom. It was found that 23 (51.1 %) participants cover their toothbrushes with caps, oblivious to the fact that doing so moistens the air and encourages the growth of microorganisms, therefore, increasing their microbial load, this is in line with Frazzelle and Munro (2012) who reported that toothbrush caps and the moist in bathroom environment are critical elements that boost the proliferation of microorganisms in comparison to capped toothbrushes. The frequency of use and the environment of storage can have a significant impact on the growth of microorganisms, which ultimately leads to a high rate of contamination. According to the literature, toothbrushes should be replaced after three months of usage. Several microorganisms can develop mature biofilm on the synthetic bristles of toothbrushes and invade oral structure (Dayoub *et al*., 1997; Eaton and Carlile, 2008; Frazella and Munro, 2012). In this study, even though the toothbrushes were only used for a month, substantial bacteria contaminants were present. So, a key component of oral hygiene should be the disinfection of toothbrushes. In all the variables, hall I is not different from hall II. The P-value of 0.322 is greater than the 0.05 level of significance. t value of 0.514 is less than the t critical 2.009. Therefore, there is no significant difference between halls I and II for bacteria contamination of the toothbrushes.

Klebsiella sp., *Enterobacter* sp., *Citrobacter* sp., *Escherichia* sp., *Klebsiella* sp., *Pseudomonas* sp., and coagulase-negative *Staphylococcus* sp. are among the bacterial isolated from used toothbrushes. This might be a result of the

level of cleanliness observed in the bathroom, as many of these organisms are associated with a dirty environment. Sammons *et al*., (2004*)* identified *Staphylococci* presumptive coliform and *Pseudomonas* sp. from the toothbrush they studied. This is reinforced by the fact that *Staphylococcus* sp. is a natural flora of the epidermis. Osho *et al.* (2013) isolated, *Enterobacter, Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli,* and *Pseudomonas aeruginosa.* Additionally, *Staphylococcus epidermidis* and Streptococci were isolated from toothbrushes after use by Malmberg *et al.,* (1999), while Glass (1992) found potentially pathogenic bacteria in toothbrushes from both healthy and diseased patients, including *Pseudomonas species, Staphylococcus* species. and *Escherichia coli.* While Contreras *et al.,* (2010*)* revealed that the most frequent microbes detected in toothbrushes used by parents and children for one month were *Pseudomonadaceae* and *Enterobacteriaceae*. Bello *et al*., (2013) found *Escherichia*, *Pseudomonas*, and *Staphylococcus,* in used toothbrushes. According to Kozai *et al.,* (1989*)*, using a toothbrush increases the chance of contracting harmful microorganisms including *Streptococcus mutants* and other bacteria that can be transferred increasing the risk of dental caries and infectious diseases, he also, isolated *Streptococcus mutants* from used toothbrushes. *Escherichia* species, *Enterobacter* species, and *Klebsiella* species had the highest percentage incidence of bacteria contaminates (17.8%) (15.6 %), (17.8%) (17.8 %) and (35.5 %) (42.2 %) in Hall I and II respectively. However, Osho *et al*., (2013) recovered *Escherichia coli* (10%), *Enterobacter* (10%), *Staphylococcus aureus* (20%), *Staphylococcus saprophyticus* (20%), and *Pseudomonas aeruginosa* (40%), and Sammons *et al.,* (2004) isolated *Staphylococci* (48%) and *Pseudomonas* (16%) from used toothbrushes.

Table 3, shows that all isolates were susceptible to pefloxacin and ciprofloxacin. Pefloxacin and cefotaxime are both members of the fluoroquinolone antibiotic class, which inhibits the enzymes DNA gyrase and topoisomerase, which are necessary for DNA replication. Although they are both broad-spectrum antibiotics mostly used to treat infections and sexually transmitted diseases. *Pseudomonas* sp. was susceptible to Gentamicin; however, some were resistant to it. Gentamicin is an antibiotic in the aminoglycoside class that operates by preventing bacterial protein synthesis. It is used to treat severe bacterial infections such as meningitis, infections of the blood, abdomen, lungs, skin, and bones, as well as infections of the urinary tract if *Pseudomonas aeruginosa* is involved (Chaves and Tadi, 2022).

CONCLUSION

All the toothbrushes analyzed in this study had bacteria contaminates that are known to harm human health. Toothbrushes are reservoirs for germs, contributing significantly to the spread of diseases, and increasing infection risks, toothbrushes should be properly cared for like, washing with clean water after use and allowed to air dry before storage.

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