IDENTIFICATION AND ANTIBIOGRAM OF BACTERIA FROM USED TOOTHBRUSHES BY STUDENTS OF FEDERAL UNIVERSITY WUKARI, NORTH EAST, NIGERIA

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Abstract: Toothbrushes play a significant role in disease transmission and increase the risk of infection since they can serve as a reservoir for microorganisms. This study was carried out to investigate the presence of bacteria on regularly used toothbrush. One hundred and twenty (120) in-use toothbrushes were used for this study. Sixty (60) pieces each from males and female; with half the number obtained from both the males and females living within the school hostel and males and females living off school campus were examined using standard bacteriological techniques. The results showed a total of six (6) bacteria isolates; *Staphylococcus aureus* 52%, *Streptococcus species* 22%, *Staphylococcus epidemidis* 11%, *Pseudomonas aeruginosa* 9%, *Escherichiacoli* 4%, *and Klebsiella oxytoca* 2% which clearly showed *Staphylococcus aureus* as the most commonly isolated bacteria from toothbrushes in the localities. The antibiogram of the isolates show that all isolates were susceptible to ciprofloxacin and resistant to augmentin. Other antibiotics (gentamycin, streptomycin, chloramphenicol and ampiclox) has various degree of susceptibility and resistant. Also, the presence of these bacteria in the various toothbrushes samples investigated could have been as a result of exposure to dirty environment, contaminated water or all left over materials from food consumed. In conclusion, the presence and multiplication of the above bacterial in toothbrushes may lead to infection and decaying of teeth and hence lead to smelling mouth and breath. Therefore, whenever there is decaying of teeth or mouth infection, especially one which delays in healing, routine culture should be carried out to determine bacterial associated with such decay and its susceptibility to various antibiotics should also be carried out to determine the choice of antibiotic for treatment. Hence good hygiene and proper care of toothbrushes plus in-cooperation of antimicrobial drugs during mouth wash is advised.

Keywords: Bacteria, identification, students toothbrushes, University, Wukari

Introduction

A toothbrush is a dental instrument used for cleaning teeth, ideally in conjunction with toothpaste or mouthwash. The toothbrush consists of a plastic handle and nylon bristles attached to the head of the brush. A toothbrush plays a pertinent role in oral hygiene and it is commonly found in homes and other places of human residence. It could also play a significant role in disease transmission since it can serve as reservoir for microorganisms in healthy and medically ill adults. Many bacteria are found on toothbrushes after brushing. These microorganisms can remain viable for a day to a week after brushing (Efstratius *et al.,* 2007; Downes *et al.,* 2008). Toothbrushes are most commonly located near the bathroom sink, which is very conducive for microbial growth. A new toothbrush is usually not a favourable habitat for bacteria, but in some cases, it is already slightly contaminated because of the absence of regulations that ensure its sterility when packaged for sale (Glass, 2012). The mouth is a hospitable niche for all kinds of microbes and thus, the toothbrush will always be contaminated through brushing (Quirynen *et al.*, 2011).

Oral diseases can be greatly controlled by reducing the microbial load in the oral cavity and this can be achieved by maintaining proper oral hygiene (Karibasappa *et al*., 2011). The human oral cavity is colonized by a larger variety of bacteria flora than any other anatomic area. More than seven hundred (700) species of bacteria have already been identified, four hundred (400) of which were found in the periodontal pocket adjacent to teeth. Organisms not normally associated with oral flora also have been isolated from toothbrushes including enterobacteria, Pseudomonads (Sammons *et al.,* 2004). The infectious microorganisms remaining on the brushcan re-infect our mouth and teeth again, with some of them even spreading to the rest of our body and causing serious health problems, including heart disease, stroke, arthritis etc. (Warren *et al*., 2001).

A new toothbrush is usually not a favourable habitat for bacteria and fungi, but in some cases, toothbrushes are already slightly infected because no regulation that stating that toothbrushes must be sold in a sterile packages exist (Efstratius *et al* 2007). Tooth brushing plays an important everyday role for personal oral hygiene and effective plaque removal. Appropriate toothbrush care and maintenance are also important considerations for sound oral hygiene. The oral cavity is home to hundreds of different types of microorganisms (Mehta *et al*., 2007). Therefore, it is not surprising that some of these microorganisms are transferred to a toothbrush during use. It may also be possible for microorganisms that are present in the environment where the toothbrush is stored to establish them on the brush, since they are not required to be sold in a sterile package (Dabas *et al*., 2008). The toothbrush is not naturally favourable towards the growth of microbes, but can sustain bacterial life once they are transferred onto the toothbrush. Different modes of transfer are responsible for the bacteria on the toothbrush such as contact with the mouth, cross contamination, and the bacteria in the toilet community (Alm *et al*., 2007). The organisms that can survive for a certain amount of time on the toothbrush are diverse, ranging from fungus to bacteria to yeast. The environment of the toothbrush is affected by many conditions whether it is the architecture of the toothbrush itself regarding bristles or by adjusting the pH level. These conditions alter the population of bacteria on the toothbrush. While the toothbrush is not the ideal niche for a microbe, the toothbrush is capable of supporting microbial life (Ismail *et al*., 2007). Toothbrushes are necessary for daily oral hygiene, but residues remaining on their bristles may precipitate the growth of several microorganisms. Oral biofilms develop over time into exceedingly complex communities.

Hundreds of species of bacteria has been identified in such biofilms (Johansson *et al*., 2009). The oral cavity, the skin, and the upper respiratory tract are the primary portals for *Streptococcus viridans, Staphylococcus species*and

Haemophilus aphrophilus, Aggregatibacter (formerly Actinobacillus) *Actinomycetem comitans, Cardiobacterium hominis, Eikenella corrodens,* and *Kingella kingae* (HACEK) organisms with streptococcal and staphylococcal organisms responsible for more than 80% of cases of bacterial endocarditis (Imarenezor *et al*., 2016). The overall survival rate for patients with native valve endocarditis caused by *Streptococcus viridans,* HACEK organisms, or enterococci ranges from 85-95%. For *Staphylococcus aureus* native valve endocarditis, the mortality rate is 55-70% in persons who do not abuse intravenous drugs and is 85-90% in those who do. Prosthetic valve endocarditis beginning within 2 months of valve replacement results in mortality rates of 40-50% (Burt *et al*., 2006). The administration of antibiotic prophylaxis to at-risk patients who are undergoing dental manipulations is a reasonably well accepted clinical practice (Cook *et al*., 2008). Numerous studies have demonstrated that antibiotics can reduce the prevalence and the magnitude of bacteremia (Baltch *et al*., 2012).

Today, mutansstreptococci are considered to be the main aetiological microorganisms in caries disease, withlactobacilli and other microorganisms participating in the disease progression. The mouth is home to millions of microorganisms (germs). In removing plaque and other soft debris from the teeth, toothbrushes get contaminated with bacteria, blood, saliva, oral debris, and toothpaste. Because of this contamination, a common recommendation is to rinse one's toothbrush thoroughly with tap water following brushing. Limited research has suggested that even after being rinsed visibly clean, toothbrushes can remain contaminated with potentially pathogenic organisms. In response to this, various means of cleaning, disinfecting or sterilizing toothbrushes between uses have been developed. This research is therefore aim at isolation and identifying the various contaminating bacterial isolates on regularly used toothbrush.

Materials and Methods

Study area

This study was carried out in the Department of Microbiology, Federal University Wukari, Taraba State, Nigeria. Wukari metropolis is a large town which is the Headquarter of Wukari Local Government Area of Taraba State. Geographically, Wukari lies between latitude 7°55'42" North and longitude 9°47'59" East. It has an area of 4,308 km^2 and a population of about thirty thousand (30000). Wukari is home to Federal University Wukari and Kwararafa University. The major languages spoken are Jukun, Kutep, Tiv, Hausa and Fulani. The population is dependent on factors such as migration and economy. The inhabitants of Wukari are mostly farmers while a few indulge in commerce and civil service (Imarenezoe *et al*., 2016).

Collection of samples

One hundred and twenty (120) samples of already in–use toothbrushes were randomly collected aseptically from the students. The students only agreed to release their toothbrushes upon receiving a new replacement. The samples were labeled accordingly and taken to the laboratory for investigation

Sample preparation

The head region of the toothbrushes was cut off with a sterile scissor according to Sammons *et al.* (2004) standard and soaked in a 10 ml peptone water solution for 60 min. After which they were vortexed slowly for a minute to dislodge adherent bacteria from the samples.

Culture procedure

The bacterial suspension was one fold diluted for 10 and (0.1 ml) of broth was plated out by use of a sterile pipette into MacConkey agar (MCA), Nutrient agar (NA), and Trypton soy agar (TSA) where NA served as the non-selective medium, MCA served as a selective media for the isolation of enterobacteria and TSA for staphylococci and other Gram-positive bacteria.

Results and Discussion

The occurrence and percentage of the bacterial isolates from used toothbrushes is shown on Table 1 while Table 2 indicated numbers of contaminated toothbrushes according to sex and the bacteria isolated. Tables 3 and 4 give the overall cultural and biochemical characteristics of bacteria isolates from the various toothbrushes and antimicrobial susceptibility test of bacterial isolates against selected antibiotics, respectively.

Table 1: Occurrence and percentage of the bacterial isolates from used toothbrushes

Bacterial isolates	Number of Isolates	% of Isolates
Staphylococcus aureus	63	(52.5%)
Streptococcus species	26	(21.6%)
Staphylococcus epidermidis	13	(10.83%)
Pseudomonas aeroginosa	11	(9.17%)
Escherichia coli	5	(4.17%)
Klebsiella oxytoca	2	(1.67%)

Table 2: Number of contaminated toothbrushes according to sex

Bacterial isolates	Males	Females	Total
Staphylococcus aureus	31(50)	32(50)	**32**
Streptococcus species	11(45.5)	15(55.5)	**11**
Staphylococcus epidermidis	9(62.5)	4(37.5)	**8**
Pseudomonas aeroginosa	6(50)	5(50)	**4**
Escherichia coli	1(25)	4(75)	**4**
Klebsiellia oxytoca	2(100)	0(0)	**1**
Total specimen	**30**	**30**	**60**

Most of the investigated toothbrushes were heavily dirty with chopped bristles irreversibly bending away from their normal positions and with the smell of toothpaste. Of the one hundred and twenty (120) samples investigated for bacterial contamination, the result showed that bacteria were isolated from all the used toothbrushes and these bacterial isolates were identified as *Streptococcus* species, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella oxytoca*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, this aligned with the work of Imarenezor *et al.* (2016). The susceptibility of the isolated bacteria to a selection of six (6) antibiotics is shown in varying degree of susceptibility which showed that ciprofloxacin was the most effective as it inhibited all the organisms with a 0% resistivity followed by gentamycin, chloramphenicol and ampiclox with 50% sensitivity each.

Table 3: Overall cultural and biochemical characteristics of bacteria isolates from the various toothbrushes

S/N	Morphology	Gram stain	coa	cat	Citr	Oxi	Suc	Glu	lac	Gas	H_2S	Indole	Organism
1	Pink, round, flat, dry	-ve rod	_	+	_	_	+	+	+	+	_	+	*E. coli*
2	yellow, round, moist	+ve cocci	+	+	+	_	+	_	+	_	_	_	*S. aureus*
3	Greenish, round, flat, dry	-ve rod	_	+	+	+	+	_	+	_	_	+	*P. aeruginosa*
4	whitish, round, moist	+ve cocci	_	+		_	+	+	+	+	+	_	*S. epidemidis*
5	Creamy, round, flat	-ve rod	_	+	+	_	+	+	+	+	_	+	*K. oxytoca*
6	Yellow, round, moist	+ve cocci	_	_	_	_	+	+	+	_	_	_	*S. species*

cit = citrate, oxi = oxidase, coa = coagulase, cat = catalase, glu = glucose, lac = lactose, suc = sucrose, and H_2S = hydrogen sulphide, + = positive and - = negative

The least effective was septrin with a mere 20% sensitivity. Interestingly, chloramphenicol inhibited all the enterobacteria (*Klebsiella* species, *Escherichia coli* and *Pseudomonas aeruginosa*) isolated. Six strains of bacteria were isolated from the one hundred and twenty (120) toothbrushes investigated (Table 2). This is probably because the toothbrushes were poorly stored after use in closed containers or kept in moist toilet places devoid of solar radiation (disinfection) and ventilation. This is in agreement with Sammone *et al.* (2004) and Baltch *et al.* (2012). The leading cause of presence of these bacterial types on toothbrushes could be due to the moist environment of bathrooms and toilets especially when these environments are stabilized and the brush is not aired (Caudry *et al.,* 2015). The occurrence of the bacterial isolates, on the used toothbrushes is presented in Table 2. *Staphylococcus aureus* was most frequently isolated (52%) followed by *Streptococcus* species (22%), *Staphylococcus epidermidis* (11%), *Pseudomonas aeruginosa* (9%), *Escherichia coli* (4%) while *Klebsiella oxytoca* had the least occurrence 2%. These result in agreement with previous researched work by Warren *et al.* (2001); Dabas *et al*. (2008) and Baltch *et al.* (2012). The overwhelming availability of *Staphylococcus aureus* could be linked to poor storage and handling by the individuals, this is in agreement also with Imarenezor *et al.* (2016).

Table 4: Antimicrobial susceptibility test of bacterial isolates against selected antibiotics

S/N	Isolate	CIP	AU	CN	SXT	CH	APX
1	*E. coli*	S	R	S	S	R	R
2	*S. aureus*	S	R	R	S	S	S
3	*P. aeruginosa*	S	R	R	R	R	R
4	*S. epidermidis*	S	R	S	S	S	S
5	*K. species*	S	R	S	S	R	R
6	*S. species*	S	R	R	S	S	S

S = Sensitive, R = Resistant, CIP = ciprofloxacin, AU = augmentin, CN = gentamycin, SXT = septrin, CH = chloramphenicol and APX=ampicilin/cloxacillin

Conclusion

In conclusion, all the used toothbrushes examined in this study were contaminated with bacteria, which are known to cause serious health problems in humans. Since toothbrushes serve as a reservoir for microorganisms and play a major role in disease transmission and can also increase the risk of infections to users, their care should be given adequate attention. They must be adequately rinsed with good water and allowed to dry in air before storing in hygienic dry containers. In addition, disinfection of toothbrushes before use should be encouraged and sharing of toothbrushes should be discouraged.

Conflict of Interest

Authors have declared that there is no conflict of interest in this study.

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