



MICROBIAL CONTAMINATION; A SURVEY OF MICROBIAL CONTAMINATION OF TOOTHBRUSHES AMONG GENERAL POPULATION OF KARACHI

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INTRODUCTION

Toothbrushes play an essential role in oral hygiene and is commonly practiced on daily basis to clean the oral cavity in both developed and developing world. Toothbrushes are also significantly affect the transmission of disease and increase the risk of infection since they can serve as a reservoir for microorganisms in healthy and infected persons.¹ Various type of microorganism resides in oral cavity, some of which are transferred during tooth brushing.²

Contamination is the retention and survival of infectious organisms that occur on animate or inanimate objects. In healthy adults, contamination of toothbrushes occurs early after

ABSTRACT... Introduction: Toothbrushes play an essential role in oral hygiene and is commonly practiced on daily basis to clean the oral cavity. It can serve as a reservoir for microorganisms in healthy and infected person. **Objectives:** To isolate the bacterial contaminants on used toothbrushes and to compare the type of bacterial growth in capped and uncapped toothbrushes among general population of Karachi. **Study Design:** Cross-sectional study. **Setting:** Study was conducted among general population of Karachi. Hamdard College of Medicine and Dentistry, Hamdard University, Karachi. **Period:** February to September 2014. **Methodology:** Using convenient sampling technique, data was collected from subjects of different socioeconomic status. Toothbrushes for culture was collected under standard precautions to observe aseptic conditions. Descriptive analysis was done for all independent variables using SPSS version 20. The frequency of various bacterial contaminants on used toothbrushes were determined and chi square test was used to observe the association of bacterial growth among capped and uncapped toothbrushes. P-value <0.05 was considered statistically significant. **Results:** Out of the total 106, 51 (48.1%) were female and 55 (51.9%) male participants. Substantial number of toothbrushes 91 (85.8%) were found to have bacterial contamination. Majority of the participants (82.1%) were between 19 – 59 years of age, intermediate or above (61.3%), monthly income rupees ≤ 20000 (46%), using same brush >3 months (50.0%), brushing teeth twice a day (62.3%) and keeping toothbrushes uncapped were (66.0%). Statistically significant (P<0.05) difference was found between capped and uncapped toothbrushes and presence of Klebsiella and Pseudomonas. **Conclusion:** The study concluded that used toothbrushes are significantly contaminated with bacteria which may cause serious health problems among individuals and families.

Key words: Toothbrush, Bacteria, Contamination, Streptococcus, Lactobacillus.

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initial use and increases with repeated use.³ Oral cavity environment, hands, aerosols and storage conditions can contaminate toothbrushes.⁴ Micro trauma that may cause by brushing of teeth results in colonization of bacteria in oral cavity.⁵ Disease transmitted in individuals due to attachment, accumulation and survival of bacteria present on toothbrushes.⁶

Toothbrushes may act as medium of microbial transport, retention and growth. Several articles have reported that level of contamination of toothbrush by bacteria and fungus, being associated with numerous interferences placed between the brush and the handle.^{7,8} Contaminated brushes may cause a possible

constant re-infection which is a risk factor for periodontal disease.⁹ It is suggested that contaminated toothbrushes can possibly be a source of both localized and systemic diseases such as endocarditis, bacteremia, arthritis and stroke have been reported.^{10,11}

Being a household object of common use, toothbrush usually is not given consideration that is its due. Any object being used on the body or in the body carries its good effects and bad effects. When the good effects are overwhelming, the bad effects are generally ignored, but they are still there. Research conducted in different countries on toothbrushes have proved that those brushes that were disinfected before or after use showed minimal or no growth.¹² Several studies^{13,14,15} found that toothbrushes can be contaminated by different types of bacteria, viruses and fungi after being used for mechanical oral hygiene and stored under usual conditions, thus becoming sources of inoculation and/or re-inoculation of potentially pathogenic microorganisms.^{16,17} A study reported that prolonged use of the toothbrush facilitates contamination by various microorganisms such as *Streptococcus*, *Staphylococcus* and *Lactobacilli*.¹⁸

Tooth brushes are the most commonly used oral hygiene aid to promote oral health and prevent dental diseases. Unfortunately, proper care of toothbrush is often neglected and is kept in bathrooms which are a good place to harbor millions of micro-organisms.¹⁹

In Pakistan there is no searchable data present regarding the health effects of contaminated toothbrushes. The objective of this study was to isolate the bacterial contaminants on used manual toothbrushes and to compare the type of bacterial growth in capped and uncapped toothbrushes obtained from general population of Karachi.

METHODOLOGY

This cross-sectional study was done during February to September 2014 at Hamdard College of Medicine and Dentistry, Hamdard University, Karachi. The protocol was approved by the Ethical

Review Committee of the institute. The data was collected using convenient sampling technique from families residing in Karachi, representing different socioeconomic status. Verbal consent was taken and anonymity of each participants were assured. A total of 106 samples of culture from tooth brushes were taken on gel swab to prevent dryness and loss of microorganisms present on tooth brushes.

A pre-designed questionnaire was also given to each participant to collect demographic information, type and habit of brushing, history of any disease of oral cavity (mouth, teeth, gums) and history of any addiction were also inquired from the participants.

The sample for culture was collected under standard precautions and technique to observe aseptic conditions. Transwab® Amies Plain Transport (Self-contained transport swab with semi-solid medium for both aerobes and anaerobes) was used. After wearing sterile gloves and mask to prevent any contamination or cross infection, the container seal was then opened and transport tube containing Rayon Bud (it is non-toxic and allows best specimen uptake and release, particularly for microorganisms) at the end from the swab was taken. The tube was then rubbed thoroughly on the toothbrushes for 15-20 seconds and was placed in the container containing 1.2 ml of liquid medium, Amies (inorganic buffer ensures maintenance of microorganisms without overgrowth and also prevents drying/killing of microbes) and closed tightly. The specimen was then taken to the laboratory within 3 hours for culture and sensitivity.

Tooth brush users without any age and sex consideration are included in the study. Participants having any dental procedure/surgery in recent past were excluded.

The data was entered and cleaned into SPSS version 20 for statistical analysis. Descriptive analysis was done for all independent variables. Cross-tabulation of different variables was done with microbial contamination. The frequency of various bacterial contaminants on used toothbrushes were determined and chi square

test was used to observe the association of bacterial growth among capped and uncapped toothbrushes. P-value <0.05 was considered statistically significant.

RESULTS

Out of the total 106, 51 (48.1%) were female and 55 (51.9%) male participants. Substantial number of toothbrushes 91 (85.8%) were found to have bacterial contamination. Age equal to or less than 18 years were 14 (13.2%), participants 87 (82.1%) between 19 to 69 years, and 5 (4.7%) participants were equal to or above 60 years of age. Education level of 41 (38.7%) participants was upto matric and 65 (61.3%) were intermediate or above. The monthly income (rupees) of most of the participants 46 (43.4%) was equal to or less than twenty thousand, participants having monthly income between twenty one thousand to forty thousand were 22 (20.8%) and 38 (35.8%) participants have monthly income more than forty thousand. Participants using toothbrush for less than or equal to one month were 16 (15.1%), toothbrush used for 2 to 3 months by 37 (34.9%) and 53 (50.0%) participants were a single using toothbrush for more than 3 months. Most of the participants, 66 (62.3%) were brushing teeth twice a day whereas 40 (37.7%) were brushing teeth once in a day. Majority 70 (66.0%) were kept there tooth brushes uncapped and 36 (34.0%) of participants keep their toothbrushes capped. Tooth brush washed by tap water by 93 (87.7%) of the participants while 13 (12.3%) were washing there toothbrush by bore water. Participants who were addicted were 16 (15.1%) whereas 90 (84.9%) were not using any addictive substance. Only 6 (5.7%) participants were used antibiotics while 100 (94.3%) were not used any antibiotics during last two weeks. No statistical significant difference was observed between different practices of brushing teeth and bacterial growth. (Table-I)

Toothbrushes are found to be contaminated with Staphylococcus, 5 (13.9%) in capped whereas 17 (24.3%) uncapped, Klebsiella 22 (61.1%) in capped and 23 (32.9%) in uncapped, Pseudomonas 17 (47.2%) in capped and 18 (25.7%) in uncapped, Proteus 3 (8.3%) in capped and 2 (2.9%) in

uncapped, Enterococcus 1 (2.8%) in capped and 6 (8.6%) in uncapped, Micrococcus was present only in 4 (5.7%) uncapped, E.coli 2 (5.6%) in capped and 2 (2.9%) in uncapped, Bacillus 5 (13.9%) in capped and 17 (24.3%) in uncapped and Streptococcus was only found in 1 (1.4%) uncapped toothbrush. Statistically significant (P < 0.05) relationship has been observed between presence of Klebsiella and Pseudomonas and uncapped toothbrushes. (Table-II).

DISCUSSION

All used toothbrushes obtained from participants were examined bacteriologically. The result showed that 91 (85%) toothbrushes were contaminated with bacteria. A study done in Nigeria on forty toothbrushes reported that all used toothbrushes are contaminated with bacteria²⁰. The source of contamination of the used toothbrushes may come from the oral cavity, storage container, surroundings and water used for rinsing of brush.

Toothbrushes of participants between 19 to 59 years of age showed high frequency of bacteria 75 (82.4%). Study done by Petersen et al. revealed that regular tooth brushing appears less common among older people than the population at large.²¹ Toothbrush are found to be more contaminated of males 47 (51.6%) and who are brushing twice a day 58 (63.7%). Kudirkaite et al. reported in their study that females brush their teeth more regularly than males.²² According to Nanys et al. females have a better condition of teeth as they pay more attention to their oral health. It has been found that compared to males, females tend to carry oral hygiene measures with themselves more often and more frequently tend to brush their teeth three or more times a day.²³ The study demonstrated that using same toothbrush for more than three months (47.3%) is found to be more contaminated, results are similar to another study conducted in India by D'Silva et al.²⁴ In our study uncapped toothbrushes showed more contamination whereas a recent study by Daloğlu et al. reported that usage of toothbrush cover will increase the bacterial growth on a toothbrush.²⁵

Variable*-	Bacterial Contamination		Total n = 106
	No Growth n = 15	Growth* n = 91	
Age in years			
≤ 18	2 (13.3%)	12 (13.2%)	14 (13.2%)
19 - 59	12 (8.0%)	75 (82.4%)	87 (82.1%)
≥ 60	1 (6.7%)	4 (4.4%)	5 (4.7%)
Gender			
Female	7 (46.7%)	44 (48.4%)	51 (48.1%)
Male	8 (53.3%)	47 (51.6%)	55 (51.9%)
Education level			
Upto Matric	5 (33.3%)	36 (39.6%)	41 (38.7%)
Intermediate or above	10 (66.7%)	55 (60.4%)	65 (61.3%)
Monthly income (rupees)			
≤ 20000	5 (33.3%)	41 (45.1%)	46 (43.4%)
21000 – 40000	4 (26.7%)	18 (19.8%)	22 (20.8%)
> 40000	6 (40.0%)	32 (35.2%)	38 (35.8%)
Brush usage (months)			
≤ 1	3 (20.0%)	13 (14.3%)	16 (15.1%)
2 -3	2 (13.3%)	35 (38.5%)	37 (34.9%)
> 3	10 (66.7%)	43 (47.3%)	53 (50.0%)
Brushing per day			
Once	7 (46.7%)	33 (36.3%)	40 (37.7%)
Twice	8 (53.3%)	58 (63.7%)	66 (62.3%)
Brush keeping			
Capped	3 (20.0%)	33 (36.3%)	36 (34.0%)
Uncapped	12 (80%)	58 (63.7%)	70 (66.0%)
Water source			
Bore water	3 (20.0%)	10 (11.0%)	13 (12.3%)
Tap water	12 (80%)	81 (89.0%)	93 (87.7%)
Addiction			
Yes	2 (13.3%)	14 (15.4%)	16 (15.1%)
No	13 (86.7%)	77 (84.6%)	90 (84.9%)
Antibiotics used (last 2 wks.)			
Yes	1 (6.7%)	5 (5.5%)	6 (5.7%)
No	14 (93.3%)	86 (94.5%)	100 (94.3%)

Table-I. Relationship of different variable with bacterial growth.

Microorganism	Capped Brushes n = 36	Uncapped Brushes n = 70	Total n = 106	p-value
Staphylococcus	5 (13.9%)	17 (24.3%)	22 (20.8%)	NS
Klebsiella	22 (61.1%)	23 (32.9%)	45 (42.5%)	0.005
Pseudomonas	17 (47.2%)	18 (25.7%)	35 (33.0%)	0.023
Proteus	3 (8.3%)	2 (2.9%)	5 (4.7%)	NS
Enterococcus	1 (2.8%)	6 (8.6%)	7 (6.6%)	NS
Micrococcus	0	4 (5.7%)	4 (3.8%)	NS
E.coli	2 (5.6%)	2 (2.9%)	4 (3.8%)	NS
Bacillus	5 (13.9%)	17 (24.3%)	22 (20.8%)	NS
Streptococcus	0	1 (1.4%)	1 (0.9%)	NS

Table-II. Comparison of microbial growth among capped & uncapped brushes.
p-value < 0.05 NS= Not significant

* = Moderate & heavy growth are combined for statistical significance

Nine bacterial isolates were identified from used toothbrushes, among all *Klebsiella* (42.5%) and *Pseudomonas* (33.0%) are most frequently isolated. Other studies also reported higher frequency of *Pseudomonas*^{20,26,27} and *Klebsiella*.^{26,27} *Staphylococcus* and *Bacillus* (20.8%) followed by *Enterococcus* (6.6%), *Micrococcus* and *Escherichia. Coli* (3.8%) while *Streptococcus* had the least occurrence (0.9%). Result from other study showed that *Staphylococcus aureus* was isolated from 60% of the samples while *Staphylococcus epidermidis*, *Streptococcus mutans*, *Escherichia coli* and *Enterobacter aerogenes* was isolated from 45%, 25%, 20% and 10% of the samples respectively.²⁰ Statistically significant difference was observed between presence of *Pseudomonas* and *Klebsiella* with uncapped toothbrush. More studies should be done to identify the type of bacteria and health risk by the contaminated toothbrushes among different communities and create awareness regarding the use of toothbrush and maintenance of oral hygiene.

CONCLUSION

The study concluded that used toothbrushes are significantly contaminated with bacteria which may cause serious health problems among individuals and families. As toothbrush act as a reservoir for microorganism and responsible of transmission and increase risk of infections, proper guidelines regarding keeping and maintaining the hygiene of the toothbrushes, as well as their replacement in an optimal time intervals should be encouraged.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Naveed Mansoori	Study design, Writing of manuscript and editing.	
2	Imran Bakar	Interpretation of data, editing and proof reading.	
3	Naveen Shahid	Manuscript editing and proof reading.	
4	Syed M. Mubeen	Critical revision and final approval.	