

## Microbial contamination of toothbrushes among smokers and non-smokers

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### Original Article

#### Abstract

**BACKGROUND AND AIM:** The most common oral hygiene aid used for the maintenance of oral health is the toothbrush and its hygiene is frequently neglected. Cigarette smoking is associated with many oral and periodontal diseases. Its effect can never be neglected on the microbiota of the oral cavity. Therefore, this study was planned to evaluate the microbial contamination of toothbrushes and its relation with smoking. This study aimed to assess the microbial contamination of capped and uncapped toothbrushes of adult smoker and non-smoker men.

**METHODS:** This randomized study included 50 different used toothbrushes of healthy men, non-smoker and smoker, who may use capped and uncapped toothbrushes. This study was conducted at Karachi Medical and Dental College, Karachi, Pakistan, from September to October 2019. All toothbrushes were evaluated for microbial analysis. Samples were allocated through simple random sampling. Every toothbrush head was put in a sterile container with 10 ml brain heart agar (Oxoid) and incubated in 37 °C for 24 hours; after which, they were placed in different bases (blood agar, McConkey). Results were analyzed using SPSS software.

**RESULTS:** Staphylococcus aureus was the most offender and major organism causing contamination in all 4 groups of smokers and non-smokers with capped toothbrush and smokers and non-smokers with uncapped toothbrush. A correlation test was done using Pearson's correlation, showing a positive correlation between uncapped non-smokers and capped smokers ( $P = 0.050$ ).

**CONCLUSION:** The microbial contamination of capped and uncapped toothbrushes of adult smoker and non-smoker men was quite high and hence, it increases the risk of dental diseases.

**KEYWORDS:** Microbiota; Smokers; Non-smokers

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The toothbrush plays a crucial role in the maintenance of oral hygiene and is the most common aid used day to day to wash the teeth and mouth in both developed and developing countries. The primary and best tool for removing the oral biofilm and therefore, the soft debris out of the mouth, especially from the tooth and tongue surface, is the toothbrush.<sup>1</sup>

Toothbrushes may affect the transmission of disease and increase the danger of

infection, since they will be required as a reservoir for the microorganism in healthy and infected individuals.<sup>2</sup> The mouth includes a teeming population of various sorts of microorganism which are relocated to a toothbrush during use.<sup>3</sup> Toothbrush plays a crucial role in personal oral hygiene and effective plaque removal.<sup>4,5</sup>

Back in the early twenties of the 20<sup>th</sup> century, Cobb reported that the toothbrush could cause persistent infections within the

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mouth.<sup>6</sup> Variety of features with long microbial survival on toothbrushes ranging from two days to at least one week.<sup>7</sup> Many factors contribute to this which include the improper handling and keeping, use of toothbrush without decontamination, and use of old toothbrush that all of them result in its cleaning and may end in the repeated entry of latent pathogens cross infections within the oral cavity,<sup>8</sup> especially in children and elderly people.<sup>9</sup> Toothbrushes are the most ordinarily-used oral hygiene aid to promote oral health and stop dental diseases. Unfortunately, proper care of the toothbrushes is usually ignored and typically they are kept in bathrooms which are an honest place to harbor many microorganisms.<sup>10</sup> Some studies reported that prolonged use of toothbrushes contaminated them with various microorganisms like staphylococcus,<sup>11,12</sup> streptococcus, and lactobacilli.<sup>13</sup> The temperature conditions of the encompassing areas of toothbrushes are additionally vital in terms of bacterial survival. A study conducted by Dhifaf in 2011 revealed that toothbrushes that were kept in air conditions had fewer bacteria than those which were kept at normal temperature. The bacterial growth was 70% higher in the wet and guarded environment.<sup>14</sup> The wet environment within the bathroom, where toothbrushes are held, might enable bacterial growth and therefore, the cross-contamination, especially that one which occurs through the aerosols formed throughout the water passing in toilets, with enteric types and pseudomonas from the toilets and hygienic drainage.<sup>15</sup>

Pakistan is our dear homeland. The dental and general health of individuals is of utmost importance for the populace. But

unfortunately, after a thorough search of knowledge, no study has been published to statistically prove the microbial contamination of toothbrushes among smokers and non-smokers. Therefore, this study aimed to isolate, characterize, and recognize the bacterial contaminants on used manual toothbrushes acquired from different smokers and non-smokers and we hope that results of this study educate the general public on the need for sound oral hygiene over adequate care of toothbrushes.

## Methods

This randomized study was done from September to October 2019 at Karachi Medical and Dental College, Karachi, Pakistan. Permission to conduct the study was obtained from the Institutional Review Board of Karachi Medical and Dental College. The data were collected by simple random sampling technique (a sample chosen randomly is meant to be an unbiased representation of the total population) from different smokers and non-smokers and from different socioeconomic status (SES) groups in Karachi. Verbal informed consent was taken and the secrecy of each participant was kept confidential. People were healthy and did not have a systemic disease or medication. The sample size was calculated through Raosoft software with a 95% confidence interval (CI), 5% margin of error, and response distribution of 50%. A total of 50 toothbrushes of smoker and non-smoker men (25 in each group) were randomly collected and secured in a sterilization pouch to avoid further contamination; the sample size was divided into 25 capped and uncapped smokers versus 25 capped and uncapped non-smokers (Table 1).

**Table 1.** Statistics of toothbrush contamination in smokers and non-smokers

		Capped toothbrush contamination in smokers	Uncapped toothbrush contamination in smokers	Capped toothbrush contamination in non-smokers	Uncapped toothbrush contamination in non-smokers
Number	Valid	7	18	12	13
	Missing	14	3	9	8

The inclusion criteria were male gender, age between 18 to 70 years, having cigarette smoking habits of a minimum of six months, and consuming at least two cigarettes per day. Their toothbrushes should be 2 months old.

The exclusion criteria were using other types of tobacco combination addictions and not giving consent for the study.

All smokers and non-smokers' brush heads were detached by using sterilized gloves and forceps. Every toothbrush head was put in a sterile container with 10 ml brain heart agar (Oxoid) and incubated in 37 °C for 24 hours; after which, they were placed in different bases (blood agar, McConkey). The bases for aerobic cultivation were incubated in a thermostat for 24 hours; then the developed colonies in the blood agar and McConkey were compared for differentiation of the species.

The bacterial segregations were characterized and identified based on their colonial, molecular, and biochemical features. Gram staining, coagulase, catalase, motility, Voges Proskauer, indole production, citrate utilization, oxidase, methyl red, and sugar fermentation tests were done according to the scheme of Cheesbroug.

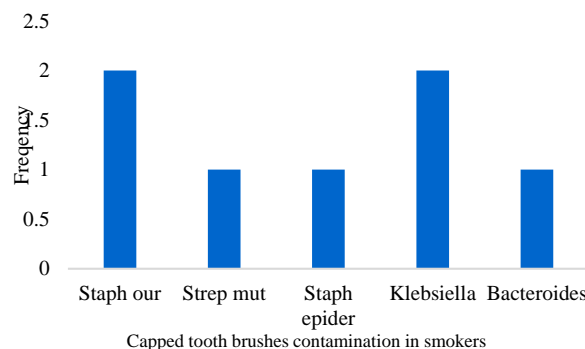
Data were entered and analyzed using SPSS software (version 17, SPSS Inc., Chicago, IL, USA) and a P-value less than or equal to 0.050 was considered statistically significant.

### Results

Capped toothbrush contamination in smokers was more with staphylococcus aureus and Klebsiella, i.e., 28.6% (n = 2), respectively (Table 2 and Figure 1).

**Table 2.** Capped toothbrush contamination in smokers

Organism type	n (%)
Staphylococcus aureus	2 (28.6)
Streptococcus mutans	1 (14.3)
Staphylococcus epidermidis	1 (14.3)
Klebsiella	2 (28.6)
Bacteroides	1 (14.3)



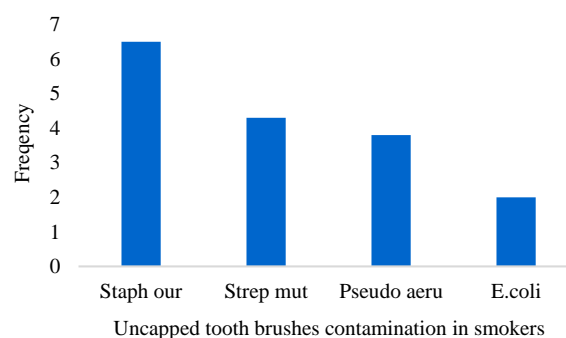
**Figure 1.** Capped toothbrush contamination in smokers

Uncapped toothbrush contamination in a smoker revealed staphylococcus aureus, streptococcus mutans, and pseudomonas aeruginosa as 38.9% (n = 7), 27.8% (n = 5), and 22.2% (n = 4), respectively (Table 3 and Figure 2).

**Table 3.** Uncapped toothbrush contamination in smokers

Microorganism type	n (%)
Staphylococcus aureus	7 (38.9)
Streptococcus mutans	5 (27.8)
Pseudomonas aeruginosa	4 (22.2)
E. coli	2 (11.1)

E. coli: Escherichia coli

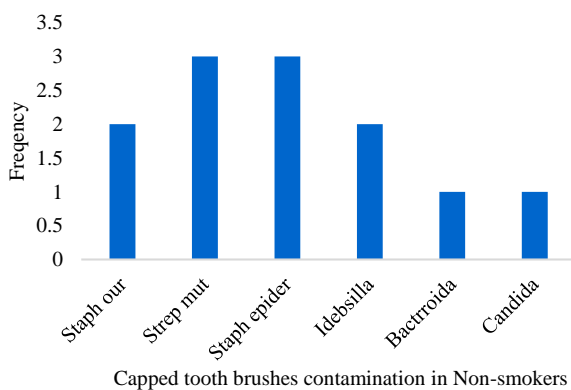


**Figure 2.** Uncapped toothbrush contamination in smokers

Staphylococcus mutans and streptococcus epidermidis constituted the highest percentage of organisms in capped toothbrush contamination in non-smokers (Table 4 and Figure 3). Staphylococcus aureus and streptococcus epidermidis constituted the highest number in uncapped toothbrush contamination in non-smokers (Table 5 and Figure 4).

**Table 4.** Capped toothbrush contamination in non-smokers

		n (%)	Valid percent	Cumulative percent
Valid	Staphylococcus aureus	2 (9.5)	16.7	16.7
	Streptococcus mutans	3 (14.3)	25.0	41.7
	Staphylococcus epidermidis	3 (14.3)	25.0	66.7
	Pseudomonas aeruginosa	2 (9.5)	16.7	83.3
	Bacteroides	1 (4.8)	8.3	91.7
	Candida	1 (4.8)	8.3	100
Total		12 (57.1)	100	
Missing	System	9 (42.9)		
Total		21 (100)		



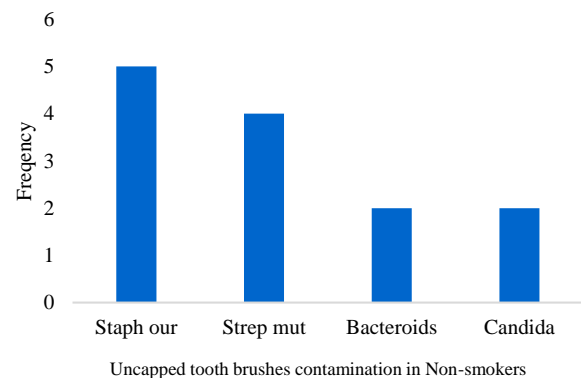
**Figure 3.** Capped toothbrush contamination in non-smokers

Pearson’s correlation test showed a correlation of P-value between smoking and toothbrush. In smokers, a capped toothbrush was statistically significant, because the P-value was 0.050, but an uncapped toothbrush was statistically insignificant, because the P-value was 0.961 (Table 6).

In non-smokers, a capped toothbrush was statistically insignificant, because the P-value was 0.961, but an uncapped toothbrush was statistically significant, because the P-value was 0.050 (Table 6).

It shows that microbial contamination of toothbrushes was significant even if the

patient did not smoke but used an uncapped toothbrush. Similarly, smoking had a significant effect on microbial contamination even if the toothbrush was capped.



**Figure 4.** Uncapped toothbrush contamination in non-smokers

Staphylococcus aureus was the most culprit and major organism causing contamination in all 4 groups.

**Table 6.** Pearson’s correlations showing P-value among smokers and non-smokers’ toothbrushes

	P (Capped toothbrushes)	P (Uncapped toothbrushes)
Smokers	0.050	0.961
Non-smokers	0.961	0.050

**Table 5.** Uncapped toothbrush contamination in non-smokers

		n (%)	Valid percent (%)	Cumulative percent (%)
Valid	Staphylococcus aureus	5 (23.8)	38.5	38.5
	Streptococcus mutans	4 (19.0)	30.8	69.2
	Bacteroides	2 (9.5)	15.4	84.6
	Candida	2 (9.5)	15.4	100
	Total	13 (61.9)	100	
Missing	System	8 (38.1)		
	Staphylococcus aureus	5 (23.8)	38.5	38.5
Missing	Streptococcus mutans	4 (19.0)	30.8	69.2
Total		21 (100)		

## Discussion

The oral cavity may be a place with the higher concentration of various microbial populations (more than 700 bacterial species, from which 400 are found within the periodontal pockets); it is especially colonized by *Staphylococcus* sp., *Streptococcus* sp., *Neisseria* sp., *Bacteroides* sp., *Actinomyces* sp., *Treponema* sp., and *Mycoplasma* sp.<sup>16</sup> Toothbrush contamination in healthy individuals occurs early after the primary use, and it gets developed with its more usage.

During the present study, the microorganisms isolated were not only oral pathogens but also general pathogens. The source of general pathogens might be due to inopportune packing of toothbrushes and/or attached bathrooms with combined toilets revealing them to the outside environment.

The incidence of *Staphylococcus* species on the analyzed toothbrushes shows that they are a great mediator for bacterial transfer; therefore, more attention should be paid, because it not only causes a variety of oral diseases, but also infection.

*Staphylococcus aureus* was most often isolated from the used toothbrushes in smokers and non-smokers (capped and uncapped).

In smokers (capped), *Staphylococcus* and *Klebsiella* had the highest percentage, while *epidermidis* and *Bacteroides* had the lowest percentage on the used toothbrushes examined.

In smokers' toothbrushes (uncapped), *Staphylococcus aureus* had the highest percentage of 38.9% (n = 7), while *Escherichia coli* (*E. coli*) (11.1%) had a rock bottom percentage occurrence of two on the used toothbrushes examined. In our study, uncapped toothbrushes showed more contamination, whereas a recent study by Mansoori et al.<sup>17</sup> reported that usage of toothbrush cover would increase the bacterial growth on a toothbrush. The presence of *E. coli* on the toothbrushes examined revealed fecal contamination. The used brushes are stowed in germ-infested environments like washbasin in the bathroom.<sup>18</sup>

In non-smokers' toothbrushes (capped),

*Streptococcus mutans* and *Staphylococcus epidermidis* had the highest percentage, while *Bacteroides* and *Candida* had rock bottom percentage in the used toothbrushes examined.<sup>19</sup>

Raiyani et al.<sup>20</sup> found that *Staphylococci* were one of the most found microorganisms on many toothbrushes; however, this finding was almost like the present study. On the contrary, in the studies of Saini and Kulkarni,<sup>21</sup> Thamke et al.,<sup>22</sup> Talaat et al.,<sup>23</sup> and Karibasappa et al.,<sup>24</sup> *Streptococcus mutans* were found the prime common bacteria on the used toothbrushes. In our study, uncapped toothbrushes in non-smokers and capped toothbrushes in smokers were exposed to the same contamination.

Various microorganisms isolated during this study cause different diseases. *Streptococcus mutans* cause beginning of cavity in people, *Candida* causes candidiasis, *Pseudomonas* causes otitis, eye infections, urinary tract infections, and burn infections, *Klebsiella* causes pyogenic infections, pneumonia, diarrhea, and tract infections, *Streptococcus pyogenes* causes tract infections, infectious disease, glomerulonephritis (GN), and *Staphylococcus aureus* causes boils, carbuncle, pustules, abscess, osteomyelitis, endocarditis, and septicemia.<sup>17</sup>

Petersen et al. divulged that regular tooth brushing seemed less common among older people than the population at large.<sup>18</sup> Toothbrushes are found to be more contaminated in men [n = 47, (51.6%)] and those who brush twice each day [n = 58, (63.7%)]. The study signifies that the same toothbrush for quite three months is found to be more contaminated (47.3%); results are quite almost like other study led in India by D'Silva et al.<sup>25</sup>

The limitations of the study were small sample size and involvement of male gender only. It is recommended that further studies with large sample sizes must be administered for both genders, so that the results are going to be more representative of the population. Smoking should be banned for all ages; further studies are needed with a large

sample size including female gender as well. The bath area and flush area should be separated. Toothbrushes should be kept capped. Toothbrush disinfection should be suggested for youngsters, elderly people, high-risk patients, and weak populations during a hospital stay.

Awareness programs and social media should increase to make people aware regarding maintenance of toothbrush hygiene and its use.

Ideally, toothbrushes should not be kept within the lavatory with or without toilets due to a higher amount of contamination, or toothbrushes should be kept within the anti-microbial solutions to avoid contamination. Toothbrushes used for 3 months have important contamination compared to a 1-month-used toothbrush. Thus, the toothbrush should be changed after every 3-4 weeks. The toothbrush should be

stored in a straight position and kept separately to avoid nosocomial infection. Every dentist educates the patients on the importance of toothbrush disinfection.

Capping of toothbrushes should be mandatory to avoid contamination. Further, similar studies are required concerning the spread of viruses through toothbrushes and their disinfection.

### Conclusion

The microbial contamination of capped and uncapped toothbrushes of adult smoker and non-smoker men was quite high and hence, it increases the risk of dental diseases.

### Conflict of Interests

Authors have no conflict of interest.

### Acknowledgments

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