

Evaluation of Bacterial Contamination of Used Toothbrushes in Smokers' Washroom and Non-Washroom Environments

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ABSTRACT: *Background:* The toothbrush, a vital tool for oral hygiene, is often overlooked regarding its cleanliness. Smoking, a known risk factor for oral diseases, alters oral microbiota. This study investigates microbial contamination of toothbrushes in different storage conditions and its association with smoking. By analyzing contamination in toothbrushes used by adult male smokers and non-smokers, we aim to highlight this neglected aspect of oral hygiene. *Methods:* Sixty used toothbrushes from healthy smokers and non-smokers were analyzed. Participants stored toothbrushes either inside (washroom) or outside (non-washroom) the bathroom. The study was conducted at Rajshahi Medical College, Bangladesh (2019–2022). Samples were randomly selected, placed in sterile containers with brain heart agar, and incubated at 37°C for 24 hours. Further analysis was conducted using blood agar and McConkey's agar. Data was analyzed using SPSS. *Results:* Staphylococcus aureus was the primary contaminant in all groups. A significant correlation was found between non-smokers storing toothbrushes outside the washroom and smokers keeping them inside, as indicated by Pearson's correlation test ($P = 0.050$). *Conclusion:* Toothbrushes of both smokers and non-smokers showed significant microbial contamination, irrespective of storage conditions, increasing the risk of dental diseases. Proper toothbrush hygiene is essential to minimize contamination.

Keywords: Microbiota, Non-washroom, Non-smokers, Smokers, Washroom.

Article at a glance:

Study Purpose: To assess bacterial contamination on toothbrushes in washroom and non-washroom environments among smokers and non-smokers.

Key findings: Staphylococcus aureus was the main contaminant, with significant microbial contamination in both groups, increasing dental disease risk.

Newer findings: Non-smokers' toothbrushes outside washrooms and smokers' inside showed significant contamination, with E. coli and Pseudomonas more common in washroom-stored brushes.

Abbreviations: WR: Washroom, NWR: Non-washroom.

INTRODUCTION

The toothbrush is the most widely utilized tool for regular mouthwash use in both developed and developing nations, and it is essential for maintaining good oral hygiene. The toothbrush is the primary and most effective instrument for eliminating oral biofilm and soft debris from the mouth, particularly from the surface of the teeth and tongue.¹ Since toothbrushes act as a reservoir for the bacterium

in both healthy and affected individuals, they may have an impact on the spread of disease and raise the risk of infection². A diverse population of microorganisms coexists in the mouth and is transferred to a toothbrush when brushing.³ A toothbrush is an essential tool for maintaining good oral hygiene and efficiently removing plaque from teeth.^{4, 5} A range of characteristics on toothbrushes that have a long microbial survival period of two days

to at least one week.^{6,7} This is caused by a variety of factors, such as improper handling and storage, using a toothbrush without first decontaminating it, and using an old toothbrush increase the risk of latent pathogen cross-infections in the oral cavity, particularly in young children and the elderly.⁸ A toothbrush is the most common oral hygiene tool for maintaining good oral health and preventing dental diseases.⁹ Sadly, toothbrush maintenance is frequently neglected, and most of the time, they are stored in the washroom (Bathroom), which is known to be a breeding ground for bacteria, especially enteric bacteria.^{10,11} According to specific research, using toothbrushes for extended periods can infect them with bacteria, lactobacilli, and streptococcus, among other microorganisms.¹² For bacteria to survive, the surrounding areas of toothbrushes must have appropriate temperature conditions.¹³ Toothbrushes stored in air conditions contained fewer bacteria than those at room temperature. The moist and protected environment with limited airflow had 70% more bacterial growth.¹⁴ The wet and moist atmosphere in bathrooms where toothbrushes are kept may promote bacterial growth and, consequently, cross-contamination, particularly via aerosols created in the water passing through toilets and hygienic drainage systems with enteric types and *Pseudomonas*.¹⁵ Despite a comprehensive literature search, a study that provides scientific and statistical evidence of used toothbrush microbial contamination among smokers and non-smokers in toothbrush-stored environments

has yet to be released. To better inform the public about the importance of good oral hygiene over proper toothbrush care, this study set out to isolate, characterize, and identify the bacterial contaminants on used manual toothbrushes obtained from various smokers and non-smokers stored in washrooms and other environments.

METHODS

This study was conducted at the Dental Unit, Rajshahi Medical College, Rajshahi, Bangladesh, from 2019 to 2022. Data was gathered using simple random samplings from smokers and non-smokers, washroom and non-washroom environments, and individuals from various socioeconomic status (SES) groups in Rajshahi. Verbally informed consent was obtained from all participants who were healthy and free from systemic diseases or medication. The sample size was determined using software, with a 95% confidence interval (CI), a 5% margin of error, and a response distribution of 50%. 60 toothbrushes from both smoker and non-smoker men were randomly collected and stored in sterilization pouches to prevent contamination. The sample size was then divided into 15 washroom environments (WR) and 15 non-washroom environments (NWR) in 30 smokers and, 15 washroom environments (WR) and 15 non-washroom environments (NWR) in 30 non-smokers (Table 1).

Table 1: Statistics of Toothbrush Contamination in Smokers and Non-Smokers

Category	Smokers	Non-Smokers	Total
Washroom (WR) Toothbrush Contamination	15	15	30
Non-Washroom (NWR) Toothbrush Contamination	15	15	30
Overall Total	30	30	60

The male participants in this study had to meet specific inclusion criteria, including being between 18 and 70 years old, having smoked cigarettes for at least six months, and smoking a minimum of two cigarettes per day. Additionally, their toothbrushes had to be at least two months old. On the other hand, individuals with other tobacco addictions and those who did not consent to participate were excluded from the study. All participants' toothbrush heads, regardless of their smoking habits, were removed using sterilized gloves and forceps. These brush heads were placed in a sterile container with brain heart agar and incubated

at 37 °C for 24 hours. After incubation, the brush heads were transferred to different bases for further cultivation and comparison of bacterial colonies. The bacterial species were identified based on various characteristics, including colonial and biochemical features. A series of tests were conducted to differentiate the species, following the Chesebrough scheme. The data collected were then analyzed using SPSS software, with a P-value of 0.050 or less considered statistically significant.

RESULTS

Streptococcus mutants (85.5%, n=51) were the all groups, followed by Escherichia coli and culprit and major organisms causing contamination in Pseudomonas, comprising 66.6% (n=40). (Table 2)

Table 2: Bacterial Contamination in Used Toothbrush

Organism Type	WR n (%)	NWR n (%)	Total n (%)
Staphylococcus Aureus	17(56.6)	17(56.6)	34(56.6)
Streptococcus Mutans	25(83.0)	26(86.6)	51(85.5)
Lactobacilli	11(73.3)	12(40.0)	23(38.3)
Escherichia Coli	26(86.6)	14(46.6)	40(66.6)
Klebsiella	12(40.0)	13(43.3)	25(41.6)
Pseudomonas	24 (80.0)	16(53.3)	40 (66.6)

A toothbrush kept in a washroom environment (WR) was more contaminated with Escherichia coli (86.6%, n=26) and Pseudomonas (80.0%, n=24), respectively. (Table 3). In non-washroom environments (NWR), toothbrushes in smokers and non-smokers were more contaminated with streptococcus mutans (86.6%, n=13) (Table 4).

Table 3: WR Toothbrush Contamination in Smokers and Non-Smokers

Organism Type	Smokers n (%)	Non-Smokers n (%)	Total n (%)
Staphylococcus Aureus	9 (60.0)	8 (53.3)	17 (56.6)
Streptococcus Mutans	13 (86.7)	12 (80.0)	25 (83.0)
Lactobacilli	5 (33.3)	6 (40.0)	11 (73.3)
Escherichia Coli	14 (93.3)	12 (80.0)	26 (86.6)
Klebsiella	7 (46.6)	5 (33.3)	12 (40.0)
Pseudomonas	13 (86.7)	11 (73.3)	24 (80.0)

Table 4: NWR Toothbrush Contamination in Smokers and Non-Smokers

Organism Type	Smokers n (%)	Non-Smokers n (%)	Total n (%)
Staphylococcus Aureus	8(53.3)	9(60.0)	17(56.6)
Streptococcus Mutans	13(86.7)	13(86.7)	26(86.6)
Lactobacilli	7(46.6)	5 (33.3)	12(40.0)
Escherichia Coli	8 (53.3)	6 (40.0)	14(46.6)
Klebsiella	7(46.6)	6 (40.0)	13(43.3)
Pseudomonas	7(46.6)	9(60.0)	16(53.3)

Staphylococcus mutans and Escherichia coli constituted the highest percentage of organisms in WR toothbrush contamination in smokers (Table 5). Streptococcus mutans and Pseudomonas constituted the highest number of NWR toothbrush contamination in non-smokers (Table 6).

Table 5: Smokers' Toothbrush Contamination in WR And NWR Environment

Organism Type	WR n (%)	NWR n (%)	Total n (%)
Staphylococcus Aureus	9 (60.0)	8 (53.3)	17 (56.6)
Streptococcus Mutans	13 (86.7)	13 (86.7)	26 (86.6)
Lactobacilli	5(33.3)	7 (46.6)	12 (40.0)
Escherichia Coli	14 (93.3)	8 (53.3)	22(73.3)
Klebsiella	7 (46.6)	7 (46.6)	14 (46.6)
Pseudomonas	13 (86.7)	7 (46.6)	20(66.6)

Table 6: Non-Smokers' Toothbrush Contamination in WR and NWR Environment

Organism Type	WR	NWR	Total
	n (%)	n (%)	n (%)
Staphylococcus Aureus	8 (53.3)	9 (60.0)	17 (56.6)
Streptococcus Mutans	12 (80.0)	13 (86.7)	25(83.3)
Lactobacilli	6 (40.0)	5 (33.3)	11(36.6)
Escherichia Coli	12 (80.0)	6 (40.0)	18(60.0)
Klebsiella	5 (33.3)	6 (40.0)	11 (36.6)
Pseudomonas	11 (73.3)	9 (60.0)	20 (66.6)

Pearson's correlation test showed a correlation of P-value between smoking and toothbrushes. In smokers, a WR toothbrush was statistically significant because the P-value was 0.050, but an NWR toothbrush was statistically insignificant because the P-value was 0.961 (Table 7). In non-smokers, a WR toothbrush was statistically insignificant because the P-value was 0.961, but an

NWR toothbrush was statistically significant because the P-value was 0.050 (Table 7). It shows that microbial contamination of toothbrushes was significant even if the patient did not smoke but used a WR toothbrush. Similarly, smoking significantly affected microbial contamination even if the toothbrush was kept in an NWR environment.

Table 7: Pearson's Correlations Show P-Value Among Smokers and Non-Smokers' Toothbrushes

Category	p-value	
	WR Toothbrushes	NWR Toothbrushes
Smokers	0.050	0.961
Non-Smokers	0.961	0.050

DISCUSSION

Approximately 700 bacterial species (400 of which are found in periodontal pockets) are concentrated in the oral cavity.¹⁶ *Staphylococcus* sp. is the most common colonizer of this area. Toothbrush contamination develops with repeated use and happens soon after the first use in healthy individuals. In the current investigation, the microorganisms isolated included both oral and general pathogens. The source of general pathogens may be toothbrushes packed too soon or attached bathrooms with combined toilets that expose them to the outside world. Since *staphylococcus* species are a significant mediator for bacterial transfer, as evidenced by their incidence on the examined toothbrushes, greater attention should be given to this issue as it leads to infection and a variety of oral diseases. Most frequently, toothbrushes used by smokers and non-smokers (washroom and non-washroom) contained *Staphylococcus aureus*. On the used toothbrushes analyzed, *staphylococcus* and *Klebsiella* were most common in smokers (WR), whereas *Bacteroides* and *epidermidis* were less common. In washroom toothbrushes, *streptococcus mutans* had the highest percentage of 85.5% (n = 51), while *Lactobacilli*

(138.3%) had a rock-bottom percentage occurrence on the used toothbrushes examined. In our study, Washroom toothbrushes showed more contamination, whereas a recent study by Mansoori *et al.* reported that washroom-stored toothbrushes would increase bacterial growth on a toothbrush.¹⁷ 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.¹⁸ in non-smokers' toothbrushes (Washroom), in the used toothbrushes examined, the highest percentages were found in *Streptococcus mutans* and *Staphylococcus epidermidis*, while the lowest percentages were found in *Bacteroides* and *Candida*.¹⁹ *Staphylococci* were among the most common microorganisms on many toothbrushes in a study conducted in; however, this finding was like the current study.²⁰ Conversely, in the research conducted by Saini and Kulkarni, Thamke *et al.*, Talaat in collaboration.^{21, 22} Additionally, Karibasappa *et al.* The predominant common bacteria on the used toothbrushes were discovered to be *streptococcus mutans*.²³ The same contamination was found in our study's uncapped toothbrushes used by non-smokers and capped toothbrushes used by smokers.

Various diseases are caused by different microorganisms that were isolated during this study. People get cavities because of *Streptococcus mutans*; they also get candidiasis from *Candida*; burns, otitis, eye infections, urinary tract infections, and pneumonia from *Klebsiella*; glomerulonephritis (GN), infectious disease, and tract infections from *Streptococcus pyogenes*; and boils, carbuncles, pustules, abscesses, osteomyelitis, endocarditis, and septicemia from *Staphylococcus mutans*. Compared to the general population, regular tooth brushing seemed to be less common among older people.¹⁸ Men [$n = 47$, (51.6%)] and those who brush twice a day [$n = 58$, (63.7%)] are found to have more contaminated toothbrushes. According to the study, using the same toothbrush for three months or longer is associated with higher contamination levels (47.3%); the findings are like those of another study conducted in India by D'Silva *et al.*²⁴⁻³⁹ The study's limitations included its small sample size and exclusive focus on men. To ensure that the findings are more representative of the population, it is advised that more research be conducted on both genders using sizable sample sizes. All ages should be prohibited from smoking; more research with a sizable sample size that includes the female gender is required. It is best to keep the bath area and the flush area apart. It is best to keep toothbrushes capped. During a hospital stay, toothbrush disinfection should be advised for young patients, the elderly, high-risk patients, and weak populations. Social media and awareness campaigns should be expanded to raise public awareness of the importance of maintaining toothbrush hygiene. Because there is a higher risk of contamination in restrooms with or without toilets, toothbrushes should ideally be stored in anti-microbial solutions to prevent contamination. Compared to a toothbrush used for just one month, those used for three months have significant contamination. As a result, the toothbrush needs to be replaced every three to four weeks. The toothbrush needs to be stored separately and upright to prevent nosocomial infections. Every dentist informs their patients of the value of cleaning their toothbrushes. Toothbrush caps ought to be required to prevent contamination. Similar research is needed to determine how viruses spread via toothbrushes and how to disinfect them.

CONCLUSION

The risk of dental diseases is increased by the high level of microbial contamination found in adult

smokers and non-smokers with capped and uncapped toothbrushes.

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Authors' Contributions

S A: Conceptualized the study, designed the methodology, analysis, and interpretation of results, writing manuscript and supervised the research process. S A and A F M S R contributed to data collection and statistical analysis. K M F A: Provided expertise and guidance in microbiological analysis and laboratory procedures. S M S I: Supervised the research process and manuscript preparation. All authors reviewed and approved the final manuscript for submission.

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Conflict of Interest: Authors declared no conflict of interest.

Ethical Approval

Ethical approval of the study was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi and informed consent was taken from all participants. Methodology of the study was carried out following the relevant ethical guidelines and regulations.

Consent for Publication: Taken.

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