

Assessment of Microbial Contamination of a Toothbrush Head with and without a Protective Cover: An *Ex Vivo* Study

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ABSTRACT

Introduction: The oral cavity is colonized by various groups of microorganisms. Toothbrushing is a common method of maintaining oral hygiene and, upon regular use, can become heavily contaminated with microorganisms. To avoid contamination of the toothbrush with microorganisms from the external environment, toothbrushes can be protected by a protective cap, the significance of which is unknown.

Objectives: To assess the microbial contamination of the toothbrush with and without a protective cap and to find the significance of the same against microbial contamination.

Materials and methods: An *ex vivo* study was conducted in the Faculty of Dental Sciences, Sri Ramachandra University. A total of 40 toothbrushes were distributed among dental students aged 18–25 years; 20 were protected by a cap, 20 were unprotected, and instructions were given to recap the toothbrush after brushing. After 1 month of regular usage, toothbrushes were collected, and organisms were identified based on Gram's reaction followed by a biochemical test.

Results: From the study conducted, it is evident that the microbial contamination of the unprotected toothbrush is higher than that of the toothbrushes that were protected with a cover.

Keywords: Manual toothbrush, Microbial count, Toothbrush disinfection.

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INTRODUCTION

Oral health is a vital part of general health. It reflects the overall well-being of an individual; thereby, maintaining oral hygiene becomes an essential factor.¹ The oral cavity is free of microorganisms at birth because the fetus develops in sterile conditions.² Gradually, the oral cavity is colonized by various groups of microorganisms.

Toothbrushing is the most common method of maintaining oral hygiene.³ There is evidence that toothbrushes in regular use can become heavily contaminated with microorganisms.⁴ Microorganisms, such as *Pseudomonas*, *Enterobacter*, and *Escherichia coli*, can also contaminate the toothbrush from storage environments.^{1,5,7} Toothbrush can also be contaminated by external environment, aerosols and hands.⁸ Thus, rather than cleaning the teeth, the toothbrush could possibly be contaminating them.⁶ In literature, there is immense information on the brushing techniques, but there is inadequate information about the maintenance of the toothbrush to avoid contamination with microorganisms.⁹

Various studies have been performed on the contamination of toothbrushes. Thamke et al.¹⁰ studied the difference between charcoal bristles and non-charcoal bristles toothbrushes and found charcoal bristles toothbrush had lesser contamination.

To avoid contamination of the toothbrush with microorganisms from the external environment, toothbrushes can be protected by a protective cap, the significance of which is unknown. Therefore, this study is conducted to assess the microbial contamination of the toothbrush with and without a protective cap and to find the significance of the same against microbial contamination.

MATERIALS AND METHODS

An *ex vivo* study was conducted in the Faculty of Dental Sciences, Sri Ramachandra University, with the help of the Department of

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Microbiology, Sri Ramachandra University. The study protocol was reviewed and approved by the Institutional Ethics Committee of Sri Ramachandra University, and permission was obtained from the concerned authorities for conducting the study. A total of 40 toothbrushes were distributed among dental students aged 18–25 years. Among the 40 toothbrushes, 20 were protected by a cap, 20 were unprotected, and instructions were given to recap the toothbrush after brushing. After 1 month of regular usage, toothbrushes were collected from the dental students in sterile covers and transported to the microbiology lab. In the lab, the toothbrushes were divided into two groups: group I (20 protected toothbrushes) and group II (20 unprotected toothbrushes).

Test tubes of size 25 × 125 were used with 5 mL of Brain heart infusion agar (BHA) broth. Mouths of the test tubes were plugged with cotton. The toothbrushes were immersed into the test tube with BHA broth and vortexed for 3 minutes at a speed of 2000 rpm.

After vortexing, 100 µL of vortexed BHA broth was diluted with 900 µL of BHA broth. Duplicates were made of the same. The solution was streaked into blood agar plates. Colony-forming units were counted after 24 hours of incubation at 37°C. The mean of the duplicates was taken as the final colony count, and the colony morphology was studied. Organisms were identified based on Gram's reaction followed by a biochemical test.

RESULT

From the study conducted, it is evident that the microbial contamination of the unprotected toothbrush is higher than that of the toothbrushes that were protected with a cover.

Table 1 shows the microorganisms found in an unprotected toothbrush that was used for a period of 1 month. In the majority, organisms such as *Pseudomonas*, *E. coli* and micrococci were predominantly seen. A few samples showed the growth of microorganisms such as *Klebsiella*. Two samples showed no growth.

Table 2 shows the microorganisms found in a toothbrush protected with a cover. In the majority, no growth was seen in a few, growth of organisms such as *Pseudomonas* and *E. coli* were seen.

Results showed that the load of microorganisms in an unprotected toothbrush was comparatively higher than in a toothbrush protected with a cover.

DISCUSSION

In the present study, the head of the toothbrush between the tufts was selected to assess the microbial contamination because it

provides a favorable environment for the microorganisms. Microbial contamination can be a positive factor for various inflammatory diseases in oral tissues.¹¹

In the present study, the microorganisms that were present in the toothbrush, used for a period of 1 month without a protective cap, were *Pseudomonas*, *E. coli* and micrococci predominantly, and *Klebsiella* in a few samples, and few samples showed no growth and with protective cap showed that majority had no growth, in a few growths of organisms such as *Pseudomonas* and *E. coli* were seen. But, in a study performed by Paulo Nelson Filho et al. it showed major growth of Mutans streptococci.² So at the end of 1 month, unprotected toothbrushes showed more growth of microorganisms than protected toothbrushes. In the study conducted by Karibasappa et al., results showed that the microorganisms present were not only oral pathogens but also general pathogens because of improper storage conditions.¹ In a study performed by SS Taji and AH Rogers, showed growth of 10⁴–10⁶ colony-forming unit growing aerobically, indicating that they were facultative anaerobes.⁴ In the study conducted by Suma Sogi et al. showed toothbrush immersed in chlorhexidine, 3% hydrogen peroxide, Dettol showed no significant difference between three experimental groups in any stage of the study period, but the significant difference only between study and control group which were toothbrushes not immersed in any solution after brushing and showed growth of microorganisms such as *Streptococcus pyogenes*, *Klebsiella*, *E. coli*, *Proteus* spp., β-hemolytic *Enterococcus faecalis* were found.⁶

The study's limitations are that environmental variations such as placing toothbrushes in the living room and bathroom could play a role in the growth of organisms. The duration of the toothbrush used was for 1 month; it could have been varied. In this study, only bacterial analysis was done, growth of fungi and viruses was not taken into account.

Table 1: Showing values obtained with unprotected toothbrush (predominant organisms include *Pseudomonas*, *E. coli* and micrococci)

S. no.	Micrococci	Streptococci	Pseudomonas	E. coli	Enterobacter	Klebsiella
1.	0	0	0	>100,000	0	>100,000
2.	160,000	0	100,000	0	0	0
3.	0	0	143,0000	950,0000	0	0
4.	0	0	101,0000	0	0	0
5.	0	0	100,000	0	0	0
6.	0	0	0	0	0	100,000
7.	0	0	100,000	0	0	0
8.	0	0	100,000	0	0	0
9.	0	0	100,000	0	0	0
10.	0	0	0	100,000	0	0
11.	0	0	0	0	100,000	0
12.	0	0	0	0	0	0
13.	0	0	0	0	0	0
14.	0	0	0	100,000	0	0
15.	1000	0	0	0	0	0
16.	100,000	0	100,000	100,000	0	0
17.	0	0	100,000	0	0	0
18.	0	0	10,000	0	0	0
19.	0	0	100,000	0	0	0
20.	10,000	0	0	0	0	0

Table 2: Showing the values obtained with protected toothbrush (most of the samples showed less or no growth)

S. no.	Micrococci	Staphylococci	Pseudomonas	E. coli	Enterobacter	Klebsiella
1.	1000	0	0	0	0	0
2.	300	0	0	0	0	0
3.	200	0	0	0	0	0
4.	0	100	0	0	0	0
5.	400	0	0	0	2900	0
6.	0	0	15,400	0	0	0
7.	300	0	0	0	0	0
8.	0	0	0	100,000	0	0
9.	100	0	0	0	0	0
10.	0	0	0	0	0	0
11.	0	0	0	100,000		0
12.	0	0	100,000	0		0
13.	0	0	0	0	0	0
14.	0	0	0	100,000	0	0
15.	0	0	0	0	0	0
16.	0	0	0	0	0	0
17.	0	0	10,000	0	0	0
18.	0	300	0	0	0	0
19.	0	0	0	100,000	0	0
20.	0	0	100,000	0	0	0

CONCLUSION

The microbial seen to be significantly increased in toothbrushes without protective caps; a few toothbrushes with protective caps showed no growth. So, at the end of this study, we would like to recommend using a toothbrush with a protective cap to maintain good oral hygiene and for the individual's general well-being.

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