



Evaluation of antimicrobial efficacy of Chlorhexidine, Listerine, and herbal mouthwashes in decontamination of toothbrushes-An invitro study

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ABSTRACT

Toothbrushing and toothbrushes play an important role in oral hygiene. Disinfection, storage and changing of toothbrushes at regular intervals are essential. The aim of the present study is to evaluate the invitro efficacy of Chlorhexidine, Listerine® and herbal mouthwash (Himalaya Oro-T™) against microorganisms as toothbrush disinfectants. A total of 20 participants were recruited. Brand new identical toothbrushes were given to each participant and they were asked to brush their teeth twice daily. They were instructed to use tap water to rinse the used toothbrushes. The toothbrushes were collected after a week. The bristles of each toothbrush were cut and kept in separate vials containing transport media. Then, they were sent for microbiological evaluation. The number of colony-forming units were measured. The vials containing bristles were divided into four groups, each group containing 5 vials: Group I: Chlorhexidine mouthwash; Group II: Listerine mouthwash; Group III: Herbal mouthwash; Group IV: Distilled water. The bristles were immersed in test-tube containing respective mouthwashes for one hour. And, colony-forming units were measured. In all three test groups, there was a significant difference in the reduction of microbes after sanitization with mouthwashes. The highest reduction was seen in group I and the least reduction was group IV. Disinfection of toothbrush may prevent the occurrence of oral infections. Chlorhexidine mouthwash was found to be more effective than other mouthwashes as a disinfecting agent in reducing the microbial load.

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INTRODUCTION

Toothbrushing helps to maintain oral hygiene which is essential for good oral health. In spite of the widespread use of toothbrushes, the awareness of proper storage of toothbrushes is lacking. The microorganisms may contaminate the toothbrushes to a great extent. Aerosols from toilet flushing may cross-contaminate and facilitate the bacterial growth in the used toothbrushes which are kept in the bathroom. The possible cause for recontamination of the oral cavity may be due to the retention and survival of microorganisms on the toothbrush after brushing. In 1920, Cobb, in his study, reported that contaminated toothbrush might cause

mouth infection to recur and it may extend further to the throat (Cobb, 1920). Malmberg *et al.* reported that toothbrushes used by children had growth of molds and enteric yeasts. The coliforms which originate from the toilet have also been found in toothbrushes (Saleh, 2011). Kauffman gave a few techniques of sanitisation like drying toothbrushes under sunlight, immersion in formaldehyde and use of table salt on the toothbrush surface in order to absorb moisture from it. The other procedures include spraying antimicrobial solutions, washing the toothbrushes in a dishwasher, use of disinfecting solution. Microwave and ultraviolet light are the other methods (Kauffman, 1930).

Among the chemical agents, Chlorhexidine solution (CHX) has been proven to be an effective toothbrush disinfectant. It has also been reported that it can kill various microbial species (Bhat *et al.*, 2003). It has been shown that Listerine presented an efficient reduction of microorganisms. Neem and turmeric are some of the herbal products which were investigated for their effectiveness as antimicrobial solutions (Muller *et al.*, 1989). Though various sanitization techniques have been tried, procedures for sustaining the cleanliness of toothbrushes are not yet described properly (Sato *et al.*, 2005). Therefore, this study was undertaken to find the effectiveness of Chlorhexidine, Listerine, and herbal mouthwashes for toothbrush disinfection.

MATERIALS AND METHODS

The patients visiting the department of periodontics, SRM Kattankulathur Dental College and Hospital were recruited for the study. This study was approved by and ethical clearance was obtained from the Institutional Ethical Committee (IEC/2020/564). The participants were informed about the procedure and were asked to sign the informed consent. 20 systemically healthy gingivitis patients aged between 22-28 years having minimum of 20 teeth were included in the study. Patients with periodontitis, patients using any form of partial dentures, patients who cannot follow brushing technique, patients who are edentulous, patients undergoing orthodontic treatment, patients with systemic conditions predisposing to periodontal disease were excluded from the study.

New identical multi-tufted nylon toothbrushes with soft bristles were given to each participant, before the start of the study. The participants were instructed to brush twice daily with the toothbrush that has been given and to use running tap water to rinse the used toothbrushes. They were collected from all the study participants after one week. The

bristles of each toothbrush were cut and kept in separate vials containing 1ml of transport media (thioglycolate medium). Then, they were sent for microbiological evaluation (Maratha Mandal's Central Research Laboratory, Belgaum). Along with that, two unused packed toothbrushes were also cultured to assess bacterial growth, if any.

Microbial analysis

Pre-sanitization

The bristles were transferred from each vial to the test tube containing distilled water. Then, the microbial analysis was done using distilled water on blood agar by spread plate technique. The incubation period was 24-48 hours at 37°C to facilitate bacterial growth. Then, the colonies were counted using a digital colony counter and measured as colony forming units (CFU).

Post-sanitization

After measuring the colonies, the vials containing bristles were divided into four groups, each group containing five vials; then, the bristles were immersed in the respective mouthwashes for one hour.

Group I: CHX(Chlorhexidine) mouthwash

Group II: Listerine[®] mouthwash

Group III: Herbal mouthwash (Himalaya Oro T[™] oral rinse)

Group IV: Distilled water (control)

Then, they were vortexed for 2 mins. The microbial analysis was done similarly and colony-forming units were counted.

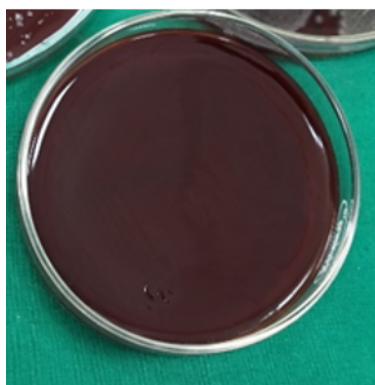
RESULTS AND DISCUSSION

Microorganisms present in the mouth contaminate the toothbrush during oral use. After the use of a toothbrush, it is rinsed with plain water and stored in a place near or in the bathroom, where the moist conditions will enhance bacterial growth (Balappanavar, 2009).

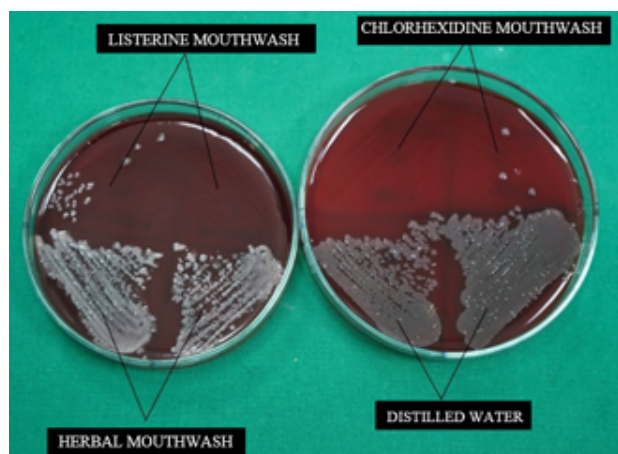
The microorganisms can remain on the toothbrushes for a certain period and can colonize it. After 24 hours of use of a toothbrush, it gets colonized to a great extent which was observed by Svanberg (1978). *Streptococci* harboured on children's toothbrushes may cause pharyngitis or tonsillitis, which has been shown in some studies (Fischer, 1999). Furthermore, improperly rinsed toothbrushes may lead to bacteremia (Muller *et al.*, 1989). Therefore, the decontamination of toothbrushes is essential to maintain good oral health.

Table 1: The comparison of colony-forming units before and after sanitization of toothbrushes with mouthwashes. PS-Pre-Sanitization & POS-Post Sanitization

S.no	Chlorhexidine		Listerine		Herbal		Distilled Water	
	PS	POS	PS	POS	PS	POS	PS	POS
1	>500	No growth	>500	18	>350	>100	>500	>450
2	>300	No growth	>300	No growth	>400	>120	>300	>300
3	>350	No growth	>400	22	>500	>150	>600	>600
4	>300	No growth	>600	No growth	>600	>130	>400	>300
5	>600	No growth	>600	No growth	>400	>100	>350	>300

**Figure 1: Microbial analysis in fresh toothbrushes showed no colony formation**

Hexidine mouthwash, Hydrogen peroxide, Dettol and Cetylpyridinium chloride are some of the chemical disinfecting solutions that have been tried. Neem and turmeric are the herbal products which were explored for their efficacy as antimicrobial solutions. Some studies suggest that herbal products and chemical disinfection are effective in decontamination of toothbrushes (Muller *et al.*, 1989).

**Figure 2: Colonies formed after sanitization of toothbrushes**

The present study was undertaken to evaluate the efficacy of three types of mouthwash, namely Chlorhexidine, Listerine and herbal mouthwashes

as a disinfecting solution. For this purpose, 20 systemically healthy patients were asked to brush twice daily and wash it under running tap water. Then, the brushes were collected in 7 days which was in accordance with the studies done by Sogi *et al.* (2002); Bhat *et al.* (2003).

On microbial analysis, fresh toothbrushes showed no colonies, which is represented in Figure 1. The colony-forming units of pre-sanitization procedure were in the range of 300-500 CFUs in all four groups. Before intervention (pre-sanitization), there was no significant difference in the CFUs among the groups.

In all the three test groups, there was a significant difference in the reduction of microbes after sanitization with respective mouthwashes. Table 1 shows the comparison of colony-forming units before and after sanitization. Figure 2 shows colonies formed after sanitization of toothbrushes. The highest reduction in colony-forming units among test groups was seen in group I (Chlorhexidine mouthwash) followed by group II (Listerine), then group III (herbal mouthwash) and least reduction was the group IV (distilled water).

In the present study, the stored toothbrushes showed different species of bacteria after oral use. Previous studies which found similar outcomes have indicated that toothbrushes will have a risk of colonization by bacteria after daily use (Fischer, 1999; Sconyers *et al.*, 1973; Schlein *et al.*, 1991).

CHX solution results in the present study are in accordance with the results of Balappanavar (2009); Bhat *et al.* (2012) in which bacterial count reduction was seen with CHX solution. CHX has a broad-spectrum antimicrobial effect and it is has been established as the gold standard. In contaminated toothbrushes, this solution has shown to inhibit bacterial count effectively, is cost effective and easily available. Therefore, CHX, as a disinfecting solution is recommended.

Results in Listerine group reveals a more effective reduction in the total viable bacterial count when

compared to herbal mouthwash. A disinfecting solution like herbal mouthwash which was used in the present study also showed a reduction in the number of colony-forming units. This shows that it has an antimicrobial property. Storage conditions of toothbrushes play the main role in bacterial survival (Dayoub *et al.*, 1977).

Significant differences were found before and after the sanitization of toothbrushes. This implies that a proper and definite decontamination procedure is required for the toothbrushes after oral use. The limitations of the study include small sample size, no direct observation of the study participants and no identification of specific microorganism. Further research is needed to understand the disinfection of toothbrushes to come up with an effective protocol.

CONCLUSION

Toothbrushes kept inside the bathroom can be a source of contamination with microbes; its disinfection may prevent the occurrence of oral infections. It can be concluded that chlorhexidine mouthwash is more effective than other mouthwashes as a disinfecting agent in reducing the microbial counts. Further investigations are required to analyse different herbal products as disinfecting solution at different concentrations.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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