



Evaluation of the Antimicrobial Effects of Various Methods to Disinfect Toothbrushes Contaminated with *Streptococcus mutans*

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ABSTRACT

Toothbrushes sterilization has strongly taken into consideration in order of prohibiting of transmission and re-infection. The differences of materials, procedures and duration, made the problem more complicated. Our objective of the present research was assessment of antibacterial effects of different procedures which are used for disinfection of those toothbrushes which are infected to *Streptococcus mutans*. 144 fresh toothbrushes were immersed in a 0.5 McFarland culture of *S. mutans* for 15 minutes. The toothbrushes were then classified into 8 groups (6 in each group) according to their disinfecting procedure including White vinegar 50%, Sodium hypochlorite 1%, Ethyl alcohol and Povidone Iodine 10%. The disinfecting procedures which used were Microwave and Dishwasher. Bacterial cultivation was done and microbial colonies had been counted before toothbrushes were exposed to the above disinfectants. After exposure of toothbrushes to above disinfectants for 1, 5 and 10 minutes, the colonies were recounted. SPSS ver.19 (Kruskal-Wallis Test) was used for statistical analysis. Bacterial cultures of samples at Chlorhexidine group (negative control) were negative and White vinegar did not make a considerable sense. On the other hand, Sodium hypochlorite 1%, Ethyl alcohol, Povidone Iodine 10% and dishwasher decreased the numbers of *S. mutans* considerably ($p < 0.05$). Microwave by increasing the time of exposition, decreased the numbers of microbes but it was not statistically significant. Povidone Iodine 10%, Sodium hypochlorite 1% and Ethyl alcohol, could be effective in disinfection of toothbrushes against *S. mutans* if be used for 10 minutes.

Key words: Disinfection, *Streptococcus mutans*, toothbrush.

INTRODUCTION

Using toothbrush or dental floss to remove microbial plaques and prevent tooth decay and periodontal diseases is essential. Some studies has shown that oral microbes form colonies on toothbrush bristles and act as a source for re-entering microorganisms particularly *S. mutans* to the mouth or contaminate regions free from infection. Under normal conditions of storage, a toothbrush can be a source for transmitting infections or re-infection with certain viruses such as HSV.¹ Within two days after the first use of a toothbrush, bristles get strongly infected with viruses, bacteria and fungi. Even new toothbrushes are infected as they get out of the package. Several studies have shown that up to 10^8 microbes from toothbrushes bristles are obtained even in healthy individuals. In addition, microbes having a significant role in the etiology of dental decays and periodontal diseases, can survive on toothbrush bristles up to 6 hours in dry weather. A wide range of oral diseases can be controlled by toothbrush infection reduction. To achieve this goal, a new toothbrush can be used each time, or at least changed to a new one regularly. As this approach is not cost effective and has economic and environmental impacts and on the other hand sterilizing toothbrush after using each time is not practical, disinfection methods which cause reduction in the contamination of

toothbrushes are proposed.² Researchers have proposed the following methods and solutions for disinfection of toothbrushes and decreasing the concentration of microorganisms in the fibers of the brush after each time of use:

1. Use of UV
2. Putting the brush in the micro wave
3. Use of boiling water
4. Putting the toothbrush in mouthwash chemicals such as Listerine, Chlorhexidine gluconate, Sodium hypochlorite 1% and using Cetylpyridinium chloride sprayed on brush fibers.³⁻⁶

As different times, methods and materials have been proposed to disinfect and many of them are not available for all, we used methods and materials which are at reach in all homes in this study and different times has been investigated to determine the minimum time for effective disinfection of toothbrushes from *S. mutans*. Accordingly, in this study the antimicrobial effects of microwave, white vinegar 50 %, sodium hypochlorite 1 %, white alcohol, Povidone Iodine 10% and dishwasher on toothbrushes contaminated with *S. mutans* has been investigated.

MATERIALS AND METHODS

In this experimental study, lyophilized *S. mutans* (ATCC 35668) and cooked meat broth (Quelab / UK) was used. To carry out this plan, 144 first-hand toothbrushes (Oral-B, Ireland) of plus kind were used and were sterilized in the temperature of 121°C and the pressure of 15 lb/in² for 15 minutes. All the toothbrushes were kept in the cooked meat broth for 5 hours previously contaminated with *S. mutans* (1.5×10⁸ CFU / ml). After that all the brushes were placed on filter papers inside sterile plates to get dried in air. Then, all toothbrushes were cultured on mitis salivarius agar medium (Quelab / Canada) and plates were incubated for 48 hours at 37 ° C. After this period, the number of colonies was counted on each plate.¹ The brushes then were divided into 3 groups of 48 and each group was divided into 8 sub- groups with following respect:¹⁰

Group A: Sterile tap water

Group B: Chlorhexidine gluconate 0.2 % (pharmaceutical company Behsa , Arak - Iran)

Group C: White vinegar (Mahram - Iran)

Group D: White alcohol 70% (Taghtirkhorasan –Iran)

Group E: Sodium hypochlorite 1 % (Active - Iran)

Group F: Povidone Iodine 10 % (Nazheh - Iran)

Group G: Dishwasher (Bosch-German) withdetergent (finish-German) at 45°C

Group H: Microwave (Delonghi-German)

Brushes of each group were placed in the mentioned chemicals and conditions respectively for 1, 5 and 10 minutes. Finally, all the toothbrushes were cultured again and colonies were counted. Analysis was done by SPSS software (ver19).

RESULTS

Overall, the total number of *S. mutans* before intervening of disinfectants was about 10⁵±1000.

In the presence of chlorhexidine 0.2% (negative control) no sign of bacterial growth was seen in all of the samples in each of the one, five and ten-minute intervals. In other words, no infections to microbes in any of the times in brush samples were reported.

Investigation of infection with *S. mutans* on toothbrushes placed in tap water (positive control) showed that in all samples existed about 10⁵ microbes after 1, 5 and 10 minutes.

Increasing the time of disinfection in 50% white vinegar caused no reduction in the number of bacteria ($p = 0.168$), whereas sodium hypochlorite 1% ($p = 0.038$), white alcohol ($p = 0.001$), povidone iodine 10% ($p = 0.001$), dishwasher ($p = 0.01$) and micro wave ($p=0.028$) showed a significant difference in reduction of the number of microbes (table 1). In other words, by increasing the time of disinfection the number of microbes decreased significantly.

Within one minute disinfection time, the number of bacteria in the disinfectant of white vinegar was averagely 158333.3 and the highest and it was meaningfully different from other disinfectants. The minimum number of bacteria in the disinfectant was in sodium hypochlorite 1 % with the average 7×10^3 . Number of bacteria in other disinfectants was similar.

In five minutes of disinfection, the number of microbes on six different methods showed significant differences with each other. Number of bacteria in the disinfectant of white vinegar 50 % with a mean of 44166.6 and microwaves with an average of 29166.6 was the maximum level and the minimum number of bacteria belonged to the disinfectant sodium hypochlorite 1%, povidone iodine 10 % and white alcohol. Dishwasher was also second in terms of the number of microbes and disinfecting within a 10- minute period and resulted in significant difference in the number of bacteria in 6 kinds of disinfectant. Number of bacteria in the disinfectant of white vinegar 50 % with a mean of 31666.6 and microwaves with an average of 7833 was the maximum level and the minimum number of bacteria belonged to the disinfectant sodium hypochlorite 1%, povidone iodine 10% and white alcohol. Dishwasher was also second in terms of the number of microbes.

DISCUSSION

The study was conducted in laboratory conditions. All the toothbrushes in tap water (positive control) and at different times were 100 % or nearly 100 % infected with *S. mutans*. In other words, tap water was not effective in disinfection of toothbrushes and these results were similar to reported results of Nelson,^{7, 8} Nascimento,⁹ Nanjun Swamy,¹⁰ Bhat,¹ and Sato.¹¹

In present study chlorhexidine was used as negative control because in most studies including Nanjunda Swamy,¹⁰ Konidala,²⁰ Nelson,^{7,8} Bhat,¹ Sato,¹¹ AlTalib,¹² and Komiyama,¹⁷ chlorhexidine has been proved as a strong disinfectant especially against *S. mutans*.

Our results revealed that 1% sodium hypochlorite has an acceptable disinfecting effect in some time intervals which was similar to what AlTalib,¹² Nelson,⁷ and Bhat,¹ reported. In our study, the role of microwave was significant in disinfecting brushes as Gujjari,¹³ Nelson,⁸ and Spolidorio,¹⁴ had proved.

We found that 1 min microwave irradiation is not sufficient to eliminate *S. mutans* and this was inconsistent with Spolidorio results.¹⁴ Based our results, disinfecting degree of microwaves was time dependent and 10-minute irradiation was suitable to disinfect brushes, although it is not cost effective because the heat resulted from microwave irradiation damages the plastic handles of brushes.

The role of the dishwasher to disinfect contaminated toothbrushes was studied in the present investigation and 10-minute time with lower temperature degrees was determined to be suitable. Belanger et al,¹⁶ also compared regular cleaning of brush in the dishwasher with other methods of disinfection and showed that its disinfecting impact was suitable which is in agreement with our studies but Zurawski,¹⁵ suggested a 2-minute wash with warmer water for a complete disinfection.

In our study, no change had been resulted after intervening white vinegar while Komiyama et al,¹⁷ who studied on disinfecting impacts of different chemicals including white vinegar 50%, introduced it as an effective disinfectant against brush micro-organisms except *Candida albicans* in 10 minutes. This difference could be related to the kind of white vinegar used and consequently the existing amount of acetic acid in it.

In the present study, povidone iodine 10% in 10 minutes had a similar effect compared with chlorhexidine which is introduced as golden standard among other disinfectants.^{4, 18} These findings are similar to results by Simratvir et al,¹⁹ in investigating the effect of povidone iodine 10% on the extent of reduction of salivary *S. mutans* in children but no study has been found on mere effect of povidone iodine 10% on contaminated brushes with *S. mutans*. Therefore, povidone iodine could be recommended as a suitable disinfectant for brushes.

Soaking the brush in alcohol was one of the first recommended methods to disinfect toothbrushes and was introduced by Cobb.¹² In the present investigation, this method was re-tested and the results showed that plunging contaminated toothbrushes in white alcohol is an acceptable way for disinfection.

Table1. Comparison of the amount of bacteria in each of the disinfectants in three different times

	Disinfection time(min)			p value
	1	5	10	
White vinegar 50%	158333.3±1543	44166.6±405	31666.6±278	0.168
Sodium hypochlorite 1 %	7000 ± 42	6500 ± 61	1250 ± 14	0.038
White alcohol	36583.3 ± 336	3667 ± 21	700 ± 7	0.001
Povidone Iodine 10 %	17920 ± 161	8500 ± 64	16 ± 4	0.001
Dishwasher	34267 ± 340	13500 ± 10	1500 ± 18	0.01
Microwave	61667 ± 577	29166.6 ± 253	7833 ± 66	0.028

CONCLUSION

Results of the present investigation showed that povidone iodine 10% is placed in the first rank after chlorhexidine in terms of overall effectiveness among the disinfectants tested and after that sodium hypochlorite 1% and white alcohol could be sufficient disinfectants for *S. mutans* contaminated brushes. Also according to the present study, dishwasher is an acceptable method for disinfecting brush and the effect is time-dependent. On the other hand, microwave will reduce the contamination amount of toothbrushes. White vinegar 50% on the other hand, has no effect on reducing the amount of infection. Therefore it is suggested that families use povidone iodine 10% and sodium hypochlorite 1% to disinfect used toothbrushes.

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