Original Article

Comparative Evaluation of Various Disinfectant Agents to Disinfect Toothbrush Microbiota

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Abstract

Introduction: Toothbrush decontamination is essential to eliminate pathogenic microorganisms transmitted on toothbrushes during brushing from the oral cavity or from the other toothbrushes and storage area. Rinsing the toothbrush with plain tap water may not be sufficient in regular use. Hence, the aim of the study is to evaluate the effectiveness of different disinfectant agents in decontaminating the toothbrushes and educate the children, parents, and the community about toothbrush decontamination. **Materials and Methods:** Seventeen children were asked to brush their teeth for 1 month. After 1 month, toothbrushes were collected. The bristles from these brushes were then placed in disinfectants such as 0.2% chlorhexidine (Group I), water (Group II), hydrogen peroxide (Group III), 5% sodium hypochlorite (Group IV), and Group V as a control for 12 hrs and then cultured. **Results:** Hydrogen peroxide (Group III solution) considerably reduced the bacterial colonies. **Conclusion:** It can be concluded that the use of hydrogen peroxide is a useful for every individual at regular intervals for day-to-day use for toothbrush decontamination.

Keywords: Decontamination, oral hygiene, toothbrush, toothbrush microbiota

Introduction

Oral health is an integral part of general health. It directly and indirectly reflects the overall well-being of an individual; thus, maintaining oral hygiene becomes a crucial factor. Even though there are different oral hygiene aids, none of them gives complete protection because of various reasons. In chemico-mechanical plaque control, toothbrush plays an important role for personal oral hygiene and effective plaque removal. Furthermore, toothbrush is the most common oral hygiene aid used, but maintaining and storing the toothbrush hygienically are commonly neglected.[1]

Further, in day-to-day life, it is very commonly seen that toothbrush storage place is common for all family members/roommates without any specific protection, which is highly unexpected.

Studies have shown that toothbrushes are colonized by the oral microbiota which acts as a reservoir to reintroduce microorganisms, especially mutans streptococci (MS) or contaminate uninfected surfaces. Under usual conditions of storage, toothbrushes can be a vector for the transmission or reinfection of certain viruses such as HSV-1.[2]

There is a need for standardized guidelines to prevent toothbrush contamination, which may increase the risk of infections from potentially pathogenic microorganisms and is clinically relevant for assessing the risks and benefits of oral care.

The present study was carried out to evaluate the presence of microorganisms in the toothbrushes and compare the effect of different disinfectants on toothbrush microbiota. The disinfectants used in this study are chlorhexidine (CHX) 1.5% + cetrimide 3.0%, 30% hydrogen peroxide, tap water, 5% sodium hypochlorite to decontaminate them. Sample size calculation was done with the help of a statistician and 17 children in the age group of 5–12 years were randomly

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Access this article online

Quick Response Code:

Website: www.ijpedor.org

DOI: 10.4103/ijpr.ijpr_11_17

How to cite this article: Jathar P, Panse A, Desai AR. Comparative evaluation of various disinfectant agents to disinfect toothbrush microbiota. Int J Pedod Rehabil 2018;3:12-7.

selected for the present study, ethical clearance was taken before the commencement of study.

Materials and Methods

Patients with a history of taking antibiotics 3 months before the study and patients undergoing orthodontic treatment or with extensive intraoral prosthesis were excluded from the study. Dental hygiene instructions were explained to all the subjects after which each of them was given a toothbrush and a paste. The children were subjected to supervise brushing using Fone's technique, twice daily for 1 month. At the end of the month, toothbrushes were collected from them.

Seventeen toothbrushes were randomly divided into four groups according to disinfectant used and each group was containing four toothbrushes, i.e., Group 1: CHX 1.5% + cetrimide 3.0%, Group 2: water, Group 3: hydrogen peroxide, Group 4: sodium hypochlorite and one toothbrush was taken as a positive control in Group 5 [Table 1].

Then, the bristle from one tuft from each toothbrush of the group was immersed in respected disinfectant for 12 h in four sterile test tubes [Figures 1 and 2]. After 12 h, the bristles from test tube were taken out and rinsed with water and immersed in Robertson Cooked Meat Medium (RCMM) for 5 h in the sterile test tube [Figures 3 and 4]. After that, RCMM was transferred on sheep blood agar by streak and by spread method. Blood agar plates were incubated at 37°C for 24 h and examined for CFU/ml.

For control group, the bristle from one tuft of the toothbrush from Group 5 was immersed directly in RCMM in one sterile test tube for 5 h. Before spreading the RCMM of control group, 10^{-9} dilutions were made. To make the dilutions first, 1 ml of pure RCMM liquid was added with the help of pipette in 9 ml of sterile water in the sterile test tube, and it became dilution of 10^{-2}. After that, 1 ml solution from 10^{-2} dilution was added in 9 ml of sterile water in sterile test tube, and now, it became solution with 10^{-3} dilution and the same procedure was repeated from each newly formed dilution till the getting of solution with 10^{-9} dilution [Diagram 1]. Then, spread culture was made at 10^{-4}, 10^{-6}, and 10^{-8} dilution and additionally one undiluted spread and one streak culture were done on sheep Blood agar plates and incubated for 37°C for 24 h and examined for CFU/ml.

Table 1: Disinfectant groups and no. of toothbrushes in each group

Five groups	Number of toothbrushes in each group
Group 1: Chlorhexidine 1.5% + cetrimide 3.0%	4
Group 2: Water	4
Group 3: Hydrogen peroxide	4
Group 4: Sodium hypochlorite	4
Group 5: Control	1

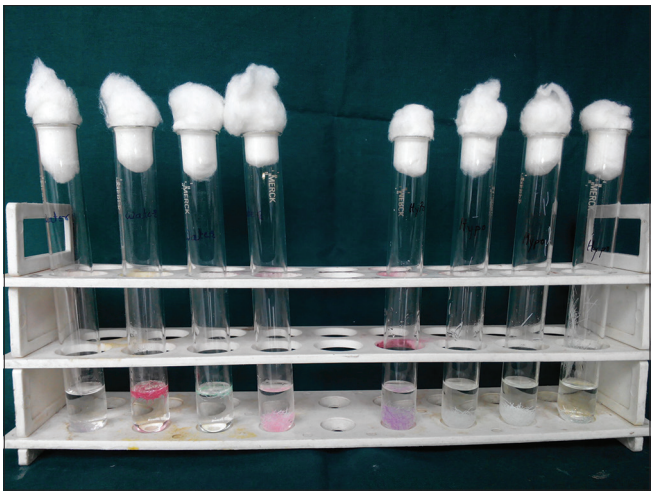

Figure 1: The bristle from one tuft from each toothbrush of the group was immersed in respected disinfectant for 12 h in four sterile test tubes (Hydrogen peroxide group & Sodium hypochlorite group).

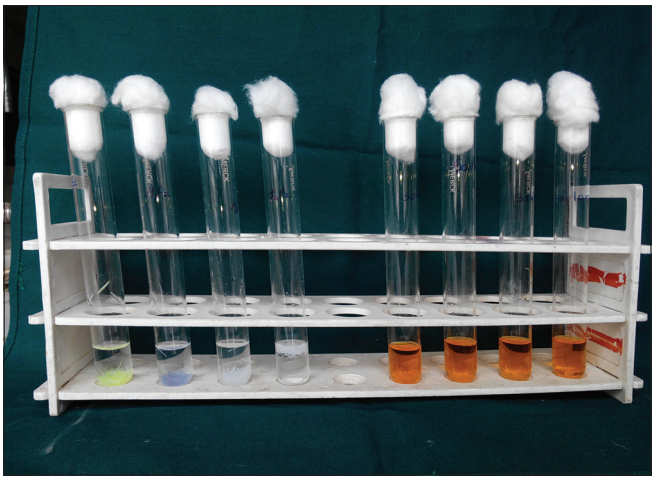

Figure 2: The bristle from one tuft from each toothbrush of the group was immersed in respected disinfectant for 12 h in four sterile test tubes (Chlorhexidine 1.5% + cetrimide 3.0% group and Water group).

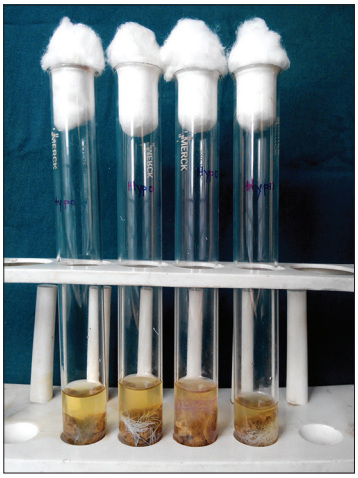

Figure 3: After 12 h, the bristles from test tube were taken out and rinsed with water and immersed in Robertson Cooked Meat Medium for 5 h in sterile test tube.

Results

Results showed the presence and cultural characteristics of bacteria in spread and streak culture [Tables 2 and 3].

In the present study, two types of culture were carried out, i.e., one is spread and one is streak method. It was done to make the colonies countable by streak method and to see cultural characteristics by spread technique.

In Group I (CHX 1.5% + cetrimide 3.0%), growth of microorganisms was present and uncountable white-colored mat pin-point colonies were seen in spread and streak technique [Figure 5].

In Group II (water), growth of microorganisms was present and uncountable white-colored mat pin-point colonies were seen in spread and streak technique additionally complete hemolysis was seen [Figure 6].

In Group III (hydrogen peroxide), growth of microorganisms was present and white-colored mat pin-point colonies were seen in spread and streak technique. However, the colonies were countable, and for streak and spread culture, it was respectively, 102 CFU/ml and 108 CFU/ml [Figure 7].

In Group IV (sodium hypochlorite), growth of microorganisms was present and uncountable white-colored mat-pin point

Table 2: Result in streak culture method

Disinfectant	Streak
Chlorhexidine 1.5% + cetrimide 3.0%	+
Water	+
Hydrogen peroxide	102
Sodium hypochlorite	+

+Indicates presence of colonies and uncountable. Expressed in cell forming unit CFU/ml

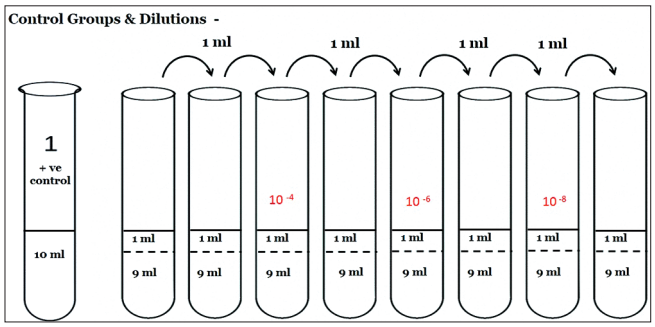

Diagram 1: Procedure of making dilutions.

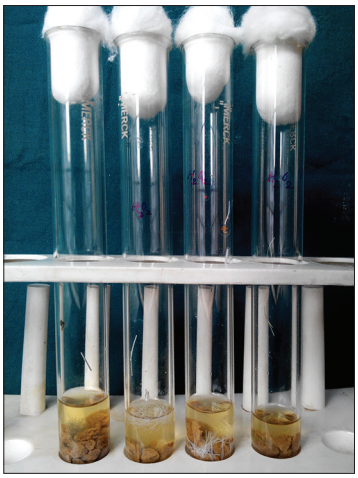

Figure 4: After 12 h, the bristles from test tube were taken out and rinsed with water and immersed in Robertson Cooked Meat Medium for 5 h in sterile test tube.

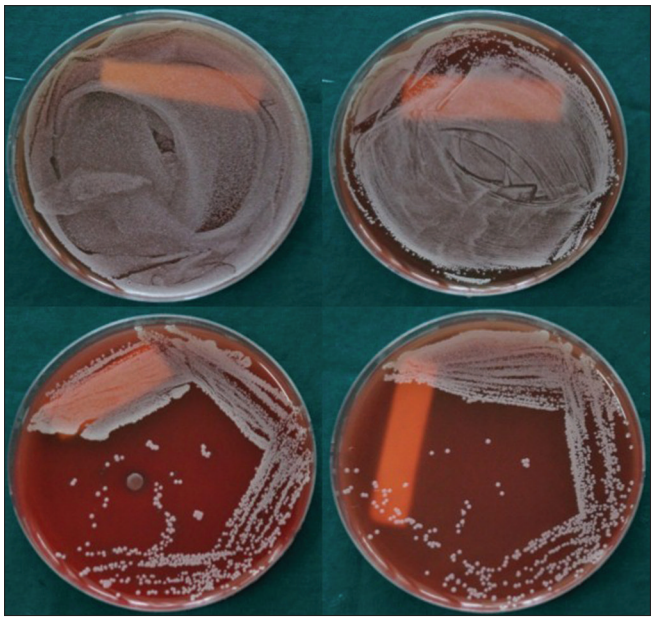

Figure 5: In Group I (chlorhexidine 1.5% + cetrimide 3.0%) uncountable white-colored mat pin-point colonies present in spread and streak technique.

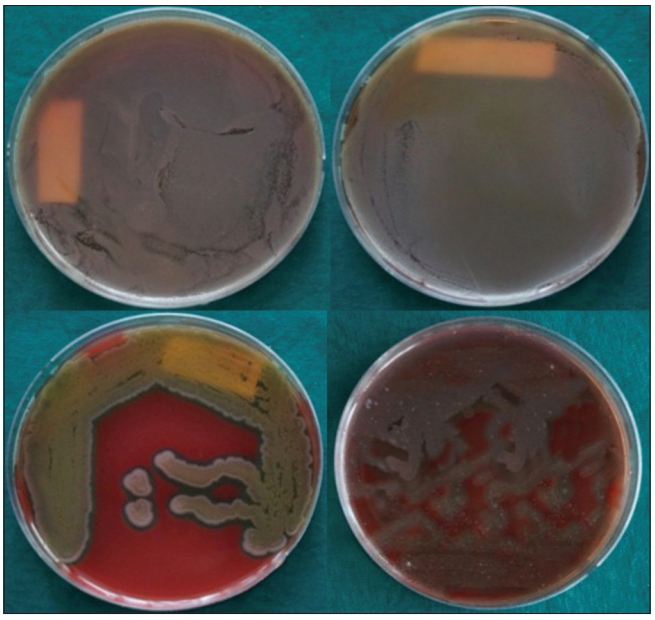

Figure 6: In Group II (water) uncountable white-colored mat pin-point colonies present in spread and streak technique with complete hemolysis.

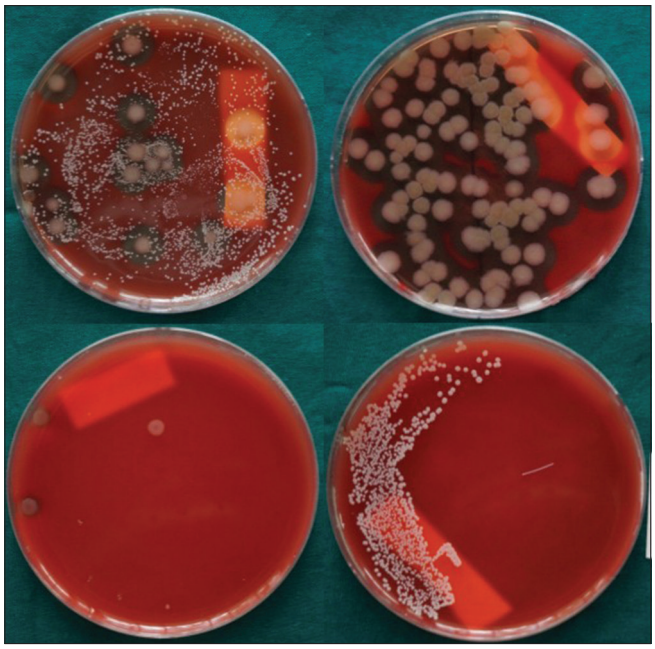

Figure 7: In Group III (hydrogen peroxide) white-colored mat pin-point countable colonies present in spread and streak technique, respectively, 102 CFU/ml and 108 CFU/ml.

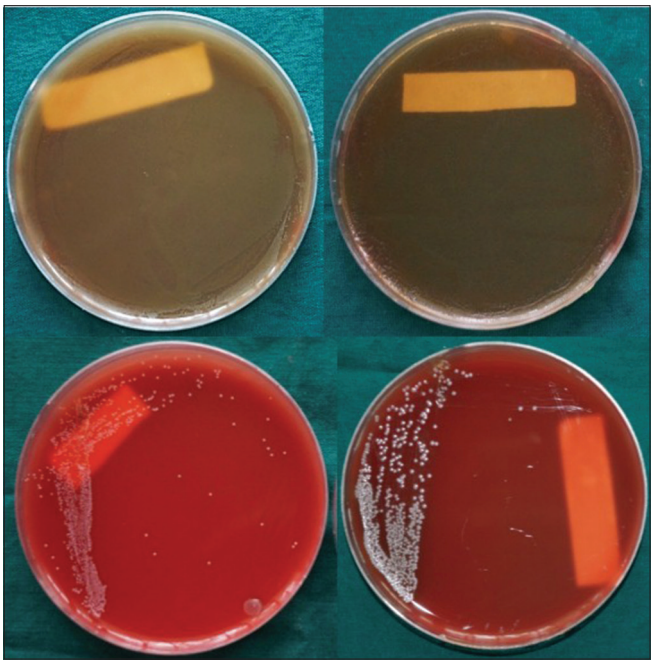

Figure 8: In Group IV (sodium hypochlorite) uncountable white-colored mat pin-point colonies present in spread and streak technique with hemolysis around some colonies in spread culture.

colonies were seen in spread and streak technique. In addition, around some colonies hemolysis was seen in spread culture [Figure 8].

In control group, [Table 4 and Figures 9, 10].

Discussion

Routine household procedures for preventing contamination mainly consist of rinsing and drying the toothbrushes. However, during tooth brushing, the toothbrush gets contaminated with different types of microorganisms which may act as a source for inoculation or reintroduction of microorganisms from infected to uninfected tissues and causes recurrent infections in the mouth.[3] It also can introduce microorganisms which are not residents of oral cavity, thereby disturbing the oral flora. Air drying of toothbrushes may be an incomplete method for disposing of microorganisms.[2] The more acceptable alternative is to decontaminate the toothbrushes with antimicrobial agents.

A great variety of microbes such as *streptococcus*, *neisseria*, and *candida* infect the oral cavity in the very first day of life itself.[4] however, *ms* (*Streptococcus mutans*) the main etiological agent of dental caries in humans infects the oral cavity only after the eruption of teeth. cobb has reported that toothbrush is a major cause of repeated infection in the mouth.[5] svanberg found that toothbrushes could be heavily infected with microorganisms especially ms within 24 h of use.[6] transmission can occur directly through saliva or indirectly through the use of fomites such as cups, spoons, or toothbrushes. it can be transmitted inter- or intra-individually which increases the incidence of dental caries, especially in children.[7]

Table 3: Result in spread culture method

Disinfectant	Spread
Chlorhexidine 1.5% + cetrimide 3.0%	+
Water	+ (CH)
Hydrogen peroxide	108
Sodium hypochlorite	+

+Indicates presence of colonies and uncountable. CH: Complete hemolysis

Table 4: No. of colonies and hemolysis in control group

Undiluted streak	Undiluted spread	10^{-4} (dilution)	10^{-6} (dilution)	10^{-8} (dilution)
+ (CH)	+ (CH)	Mat	540×10^{6}	360×10^{8}

+Indicates presence of colonies and uncountable. Expressed in cell forming unit CFU/ml. CH: Complete hemolysis

It is seen that improperly cleaned or rinsed toothbrushes act as a factor for the growth of group A hemolytic streptococci which causes pharyngitis or tonsillitis in children.[8]

Dayoub *et al*. stated that wet environment is an ideal factor for the growth of microorganisms and the use of a disinfectant is a must at regular intervals.[9]

In the present study, 10^{-2}–10^{-10} dilutions were made of control group to make the colonies countable, and at each dilution, culture was made. Finally, first countable colonies started appearing at 10^{-6} dilution and accordingly CFU/ml calculated [Table 4].

In the present study, the hemolysis was seen prominently with some of the specimens which strongly suggest microorganisms

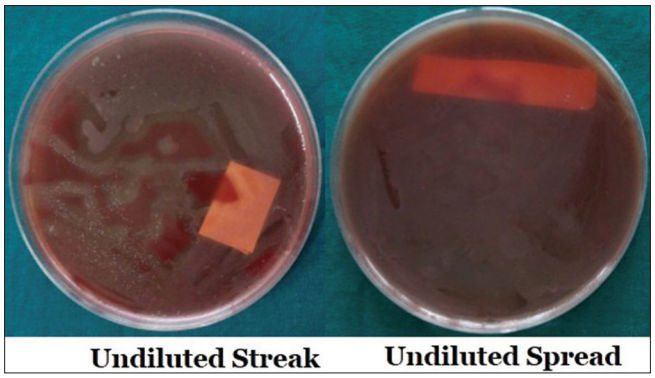

Figure 9: Growth of microorganisms was present and uncountable bacterial colonies seen on undiluted spread and streak culture.

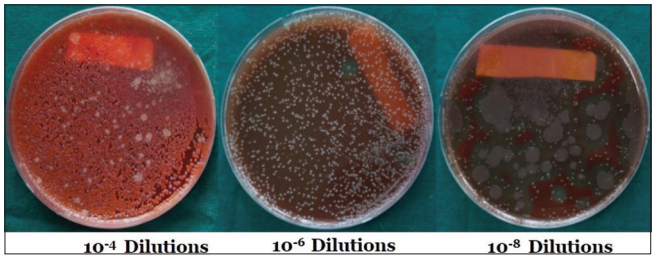

Figure 10: Appearance of bacterial colonies at various dilutions: 10^{-4} dilution, 10^{-6} dilution, 10^{-8} dilution.

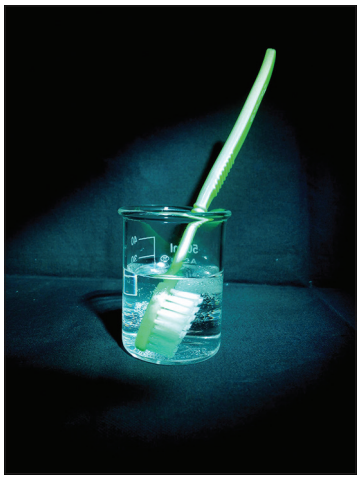

Figure 11: Toothbrush can be stored overnight in small container containing 3% hydrogen peroxide.

from *Staphylococcus* family and commonly found microorganism in abscessed lesions. Observation from Tables 3 and 4 shows that there is marked difference in number of colonies in control and toothbrush treated with hydrogen peroxide.

Within the limitations of the present study, the sample size was comparatively small and different level of concentrations of these solutions and at different time intervals were not focused in depth. There would be strong correlation between concentration of disinfectant and its antimicrobial efficacy. In addition, the culture media which were used are not specific media for specific bacteria. Hence, bacterial colony count simply showing amount of bacterial load on toothbrush and not count of specific bacteria. From these points of view, further studies are inevitable.

To maintain good oral hygiene, frequent change of toothbrushes was suggested by various authors previously. The American Dental Association in 1996 has recommended the change of toothbrushes after every 3 months.[10] Glass and Jenson[11] and Denny[12] had advised the change of toothbrushes after every 3 days for patients undergoing chemotherapy; those subjected to major surgery should change their toothbrushes every day, and those who are sick should change their toothbrushes at the beginning of illness, when they first feel better and when they are completely well.[11,12] Glass and Jensen reported that due to the longevity of viruses, it may be appropriate to replace toothbrushes every 2 weeks, and for the medically compromised community, changing toothbrushes every 3–7 days was suggested.[11]

Nelson-Filho *et al*. evaluated contamination level of toothbrushes by MS and the efficacy of antimicrobial solutions: cetylpyridinium chloride 0.05% (CPC; Cepacol™) and CHX 0.12% (Periogard™), to disinfect toothbrushes of preschool-aged children in day-care centers, and stated that MS was detected in 100% cases of toothbrushes sprayed with sterile tap water (control) and in 66.7% after spraying with CPC, but it was not detected formation of colonies/biofilms after spraying with CHX.[13]

Saleh concluded that the use of Dettol was very effective in reducing the number of contamination of toothbrushes, but it is unacceptable flavor limiting its use.[14]

Raj *et al*. evaluated the effectiveness of vinegar, lime, and salt water as potential household decontaminants for toothbrushes and concluded that commonly used household materials can be potential decontaminants for toothbrushes and showed that vinegar was the most effective decontamination agent followed by lime and salt water.[15]

The frequent change of toothbrush increases the cost of maintenance of oral hygiene which becomes a burden to the common man. Hence, instead of changing the toothbrush, decontamination of toothbrushes with the disinfectant is more economical. Thus, it is important for every individual to disinfect the toothbrush at regular intervals to maintain a good oral hygiene. In the present study, the toothbrushes were immersed for overnight period, and there was considerable bacterial count reduction seen with hydrogen peroxide and sodium hypochlorite. Among these disinfectants, the most economical is 3.0% hydrogen peroxide [Figure 11], which can be recommended as a routine for the community.

Conclusion

Within the limitations of the study, we conclude that there is a necessity to concentrate on disinfection of toothbrushes with antimicrobial solutions which benefits in preventing reinfections or cross infections. Every dentist should educate

and motivate the patients about toothbrush disinfection. Further, similar studies are needed regarding the effectiveness of different concentration of disinfectant and duration of immersion of toothbrush.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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