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Is your Tooth Cleaner, Clean...???

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Keywords: toothbrush, chlorhexidine, cetylpyridium chloride, essential oil, hydrogen peroxide.

I. Introduction

Oral hygiene is the practice of keeping the mouth and teeth clean to prevent dental problems like, dental caries, gingivitis, periodontitis and bad breath[1]. Tooth brushing, tongue cleaning, flossing, mouth rinsing with disinfectant mouth washes are some of the methods for maintaining oral hygiene. Tooth brushing is the most effective and commonly used method among them. Along with the brushing methods, disinfection of toothbrush is also equally important for maintenance of health of oral tissues[1].

Toothbrushes often become contaminated with microorganisms which originate not only from oral cavity but also from environment in which they are stored[2,3,4]. Wet environment of bathroom, dispersed aerosols from toilet flushing and contaminated finger contact contribute to toothbrush contamination.

Several families generally store their tooth-brushes in a common container which can lead to cross- infection.There is a possibility of re-infection when the individual uses the contaminated toothbrush. In 1920, Cobb was the first investigator to report the recurrence of infection in mouth in patient using contaminated toothbrush. When patient was advised to soak the toothbrush in alcohol before and after using it patient recovered from disease[5].

Glass and Shapiro[6] observed that changing the toothbrush at short intervals, helped patient achieve elimination of inflammatory disease symptoms, suggestive that toothbrush acted as a reservoir for microorganisms capable of producing diseases. Few studies have also reported chances of bacteremia and other systemic problems due to the use of contaminated toothbrush[7].

There is a need of disinfection of toothbrush, which can be done by methods which acts rapidly, cost-effective, non-toxic and which can be easily implemented. Various methods for toothbrush disin-fection have been listed in literature like immersion in antimicrobial solution, use of anti-bacterial tufted toothbrushes, UV sterilization etc[8]. Based on this, the present study was done to compare the efficiency of different antimicrobial solutions for disinfection of toothbrush.

II. Materials and Methods

The present study was done in Manipal College of Dental Sciences, Manipal. Permission from ethical committees of Manipal College of Dental Sciences and Kasturba Medical College, Manipal were taken. A total of sixty (volunteers) dental graduates aged in the range of 22- 27 years were selected for the study. They were explained verbally about the study and they were provided with subject information sheet to them for delivering complete information regarding the study in a

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language they could easily understand (Kannada or English). Written consent and contact information was collected from the volunteers. It was ensured that the selected volunteers are not taking any antimicrobial substances or antibiotics. Following this a routine dental checkup and oral prophylaxis was performed on the selected volunteers and plaque & gingival scores were brought down to zero.

Five antimicrobial mouth rinses containing different active compounds namely 0.9% saline, 3% hydrogen peroxide, mouthwash 0.2% Chlorhex-idinegl-uconate, essential oils and Cetylpyridinium chloride along with tap water as control were selected to conduct the study.

For purpose of standardization, same brand of toothbrush (Colgate, medium hard) and toothpaste (Colgate) were provided to all the volunteers. Toothbrushes were labeled as T1–T10 (Tap water-control), T11 – T20 (3% Hydrogen peroxide), T21 –T30 (0.9 % Saline), T31- T40 (0.2% Chlorhexidinegluconate), T41 –T50 (essential oils), T51- T60 (Cetylpyridinium chloride).The first group of ten volunteers were asked to brush using the standard modified bass technique for 3 min, twice daily for three days using the toothbrush and toothpaste provided to them. Following which they were instructed to rinse their brushes under tap water for 20 seconds, shake and leave the toothbrush to air dry in bathroom. In the same manner remaining five groups with ten subjects were asked to rinse their brushes under tap water for 20 seconds, shake well and keep the toothbrush in 3% hydrogen peroxide, 0.9% saline, 0.2% Chlorhexidinegluconate, essential oils and cetylpyridinium chloride containing mouthwashes respectively for 10 minutes. The tooth brush head was completely immersed in the disinfectant. A small sterile white bottle was provided to volunteers to put the disinfectant and to immerse brush head in disinfectant. Volunteers belonging to 3% hydrogen peroxide, 0.9% saline, 0.2% Chlorhexidinegluconate , essential oils and cetylpyridinium chloride mouthwashes groups were asked to dip the brush head in 1:1 dilution of the solution respectively. After ten minutes of immersion in disinfectant solution, volunteers were instructed to take out the toothbrush head from it and shake it once to remove the excess disinfectant solution. After this, volunteers were asked to keep their toothbrushes erect with its head facing upwards and left it for drying. Volunteers were given reminders for all the three days to follow the post-brushing instructions with the help of text messages in morning and at night.

Volunteers were asked to return the toothbrush after three days. Toothbrushes were collected from the volunteers, placed in a sterile box and transported within an hour to the laboratory for microbiological analysis.

For the microbiological analysis, back and handle of each toothbrush was disinfected with cotton soaked in 70% isopropyl alcohol (spirit), following which each toothbrush head (pre labeled as T1 to T60) was immersed in separate 10 ml of thioglycolate broth solution bottles and shaken for 2 minutes to transfer the microbial content present on bristle surface to the broth solution.

Each broth solution was subjected to vortexing for 3 minutes, following which 1:10 and 1:100 dilutions were made for each broth solution in small vials using preset standard pipettes. Dilutions were labeled as T1-1:10 and T1-1:100 and same for remaining broth solutions till T60 -1:10 to T60-1: 100. After this, freeze dried blood agar plates were taken and labeled for example as follows, T1 undiluted, T1-1:10 dilution and T1-1:100 dilutions corresponding to each broth solution and its respective dilutions. Same was done for the remaining 59 broth solutions and their dilutions till T60 undiluted, T60-1:10 dilution and T60-1:100 dilutions.

With the help of sterilized end of inoculation loop, sub culturing (spreading) of individual pre labeled blood agar plates was done using 10μl of its corresponding solution. The inoculated plates were then incubated at 37°C for the next 48 hrs. At the end of 48 hrs, blood agar plates were recovered from incubator for microbial counting.

III. Results

Sixty volunteers between the age group of 22 to 27 years (mean age=?) participated in this study. The toothbrushes were labeled and subjected to microbial analysis after twice daily use for three days to determine the total number of CFUs. The mean log CFU and standard deviation after treatment with six different solutions used to disinfect toothbrushes is presented in Table 1. The mean difference in the log CFUs among the six groups was analyzed using ANOVA and they were significantly different ($P< 0.001$). 3 % Hydrogen peroxide (4.24 ± 1.0) produced the lowest number of CFUs among all the six groups followed by mouthwashes containing 0.2 % Chlorhexidinegluconate (4.47 ± 1.7) and essential oils(4.75 ± 1.2) respectively.

Dunnett post hoc analysis was performed among the six respective groups with the group using water as the control is presented in Table 2. 3 % Hydrogen peroxide (MD= -2.02, $p<0.001$), 0.2% chlorhexdinegluconate mouthwash (MD= -1.79, $P<0.001$) and essential oils mouthwash (MD=-1.51, $P<0.008$) gave a significantly lower CFUs score when compared with water as the disinfectant. Saline and cetylpyridinium chloride containing mouthwash failed to produce a significant difference in the number of CFUs when compared with that of water.

IV. Discussion

Overview of the literature suggests that contamination of toothbrush and its role in transmission of oral and systemic disease[7,10,11]. Several studies

Table 1 : Mean and standard deviation of the microbial counts in log CFU for various solutions used for disinfecting tooth brush after brushing

Disinfectant groups	Number of cases	Mean ± SD (Log CFU)[a]	Range (Log CFU)		P value[b]
			Minimum	Maximum	
Water	10	6.27 ± 0.8	4.9	7.6	0.001*
3% H_2O_2	10	4.24 ± 1.0	3.0	4.2	
.9% Saline	10	6.13 ± 0.7	4.8	6.9	
.2% Chlorhexdinegluconate mouthwash	10	4.47 ± 1.7	3.0	6.9	
Essential oils mouthwash	10	4.75 ± 1.2	3.0	7.0	
Cetylpyridinium chloride mouthwash	10	5.48 ± 1.0	3.0	6.8	

(a-log transformed, b- one way ANOVA, *- p value <0.05)

conducted in past used different disinfection techniques like UV radiation[8,9], microwave irradiation[12], boiling water, chemical agents[13] like hydrogen peroxide, cetylpyridinium chloride, chlorhexidine , etc., had shown reduction in microbial count on toothbrush bristles suggesting need for toothbrush disinfection. The present study was undertaken to analyze the disinfection property of five different antimicrobial solutions (saline 0.9%,hydrogen peroxide 3%, 0.2% Chlorhexidinegluconate , essential oils and Cetylpyridinium chloride containing mouthwashes) and tap water as control. Sixty volunteers with average age ranging from 22 to 27 years were randomly assigned one of the six groups with ten subjects in each. Volunteers were asked to brush twice daily for three days and follow the post brushing disinfection instructions given to them. At the end of three days used toothbrushes were collected and sent for microbiological analysis.

Hydrogen peroxide showed maximum reduction in microbial count (MD= -2.02, p<0.001) .This result agrees with the finding of a study done Sogi et al [14].The antimicrobial activity of hydrogen peroxide is based on release of nascent oxygen and its effervescence removes the debris from otherwise in accessible regions[15].

Chlorhexidinegluconate that was used as disinfectant showed next least microbial count (MD= -1.79, P<0.001). Chlorhexidine destroys the integrity of cell membrane, penetrates the cell and precipitates the cytoplasmic proteins leading to bacterial cell destruction. It acts mainly against gram positive organisms, some gram negative bacteria and fungi[16]. Essential oils containing mouthwash (MD=-1.51, P<0.008) showed comparable results with that of chlorhexidinegluconate. Essential oils cause bacterial cell wall destruction, their enzymatic inhibition and extraction of bacterial polysaccharide[17]. Volunteers in group six which used cetylpyridinium chloride containing mouthwash as disinfectant showed reduction in microbial count but much less in comparison with other three groups. A similar result was obtained in previous study conducted by Meier S et al 1996 using cetylpyridinium chloride spray as disinfectant for toothbrushes[18]. It was observed that the brushes of volunteers in group 3 using 0.9% saline and group 1 using tap water showed maximum microbial count on toothbrush bristles suggesting these two as least effective method for toothbrush disinfection. Similar results were also obtained in previous studies[19,20].

The present study used a wide range of disinfectant solutions that are commercially available and compared it with the routine use of tap water for cleaning toothbrush. Results suggest the use of disinfectant to be beneficial in reducing the microbial count. The study design incorporated the use of toothbrush disinfectant twice daily for three days to correlate it with the lifestyle pattern of individuals to obtain effective results. Further qualitative in vivo studies using disinfectant methods that are economical, non-toxic and easy to use can be done.

Table 2 : Post hoc analysis of various disinfecting agents with water as the control

Post hoc comparisons °		Mean Difference	P value	Effectiveness	Confidence interval	
					lower	upper
3% H_2O_2	Control	-2.02	0.001*	H2O2>Water*	-3.22	-0.82
.9% Saline	Control	-0.13	0.99	Saline = Water	-1.33	1.06
.2% Chlorhexid-ineGluconate mouthwash	Control	-1.79	0.001*	Hexidine> water*	-2.99	-0.59
Essential oils mouthwash	Control	-1.51	0.008*	Listerine > Water*	-2.71	-0.32
Cetylpyridinium chloride mouthwash	Control	-0.78	0.30	Colgate Plax = water	-1.98	0.40

* . The mean difference is significant at the 0.05 level, c-Duuunnett test Dunnett test

Figure 1 : Picture showing microbial colonies on red blood agar plates of samples collected from group 1(Tap water),group (.2 % Chlorhexidine gluconate) and group (3% Hydrogen peroxide) respectively

V. Conclusion

The present study shows that use of 3% hydrogen peroxide to disinfect toothbrush is one the most effective methods to decontaminate it followed by using mouthwashes containing 0.2% chlorhex-idinegluconate and essential oils as disinfectants.

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