

1 Priyal Matreja<sup>1</sup>, Rajshree Bhandari<sup>2</sup> and Meena Anand<sup>3</sup>

2 <sup>1</sup> Manipal College Of Dental Sciences,Manipal

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## 5 **Abstract**

6 Toothbrushes get easily contaminated with different microorganisms originating not only from  
7 the oral cavity but also from the surroundings in which they are stored. Contaminated  
8 toothbrushes might serve as a possible cause in infection or reinfection especially in patients  
9 undergoing periodontal treatment. The purpose of the present study was to evaluate and  
10 compare the efficacy of five different disinfectant solutions like hydrogen peroxide (3

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12 **Index terms**— toothbrush, chlorhexidine, cetylpyridium chloride, essential oil, hydrogen peroxide.

## 13 **1 Introduction**

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

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

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

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

31 There is a need of disinfection of toothbrush, which can be done by methods which acts rapidly, costeffective,  
32 non-toxic and which can be easily implemented. Various methods for toothbrush disinfection have been listed in  
33 literature like immersion in antimicrobial solution, use of anti-bacterial tufted toothbrushes, UV sterilization etc  
34 8 . Based on this, the present study was done to compare the efficiency of different antimicrobial solutions for  
35 disinfection of toothbrush.

## 36 **2 II.**

## 37 **3 Materials and Methods**

38 The present study was done in Manipal College of Dental Sciences, Manipal. Permission from ethical committees  
39 of Manipal College of Dental Sciences and Kasturba Medical College, Manipal were taken. A total of sixty  
40 (volunteers) dental graduates aged in the range of 22-27 years were selected for the study. They were explained  
41 verbally about the study and they were language they could easily understand (Kannada or English). Written  
42 consent and contact information was collected from the volunteers. It was ensured that the selected volunteers  
43 are not taking any antimicrobial substances or antibiotics. Following this a routine dental checkup and oral  
44 prophylaxis was performed on the selected volunteers and plaque & gingival scores were brought down to zero.

## 6 DISCUSSION

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

48 For purpose of standardization, same brand of toothbrush (Colgate, medium hard) and toothpaste (Colgate)  
49 were provided to all the volunteers. Toothbrushes were labeled as T1-T10 (Tap watercontrol), T11 -T20 (3%  
50 Hydrogen peroxide), T21 -T30 (0.9 % Saline), T31-T40 (0.2% Chlorhexidinegluconate), T41 -T50 (essential oils),  
51 T51-T60 (Cetylpyridinium chloride).The first group of ten volunteers were asked to brush using the standard  
52 modified bass technique for 3 min, twice daily for three days using the toothbrush and toothpaste provided to  
53 them. Following which they were instructed to rinse their brushes under tap water for 20 seconds, shake and leave  
54 the toothbrush to air dry in bathroom. In the same manner remaining five groups with ten subjects were asked to  
55 rinse their brushes under tap water for 20 seconds, shake well and keep the toothbrush in 3% hydrogen peroxide,  
56 0.9% saline, 0.2% Chlorhexidinegluconate, essential oils and cetylpyridinium chloride containing mouthwashes  
57 respectively for 10 minutes. The tooth brush head was completely immersed in the disinfectant. A small sterile  
58 white bottle was provided to volunteers to put the disinfectant and to immerse brush head in disinfectant.  
59 Volunteers belonging to 3% hydrogen peroxide, 0.9% saline, 0.2% Chlorhexidinegluconate , essential oils and  
60 cetylpyridinium chloride mouthwashes groups were asked to dip the brush head in 1:1 dilution of the solution  
61 respectively. After ten minutes of immersion in disinfectant solution, volunteers were instructed to take out the  
62 toothbrush head from it and shake it once to remove the excess disinfectant solution. After this, volunteers were  
63 asked to keep their toothbrushes erect with its head facing upwards and left it for drying. Volunteers were given  
64 reminders for all the three days to follow the post-brushing instructions with the help of text messages in morning  
65 and at night.

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

68 For the microbiological analysis, back and handle of each toothbrush was disinfected with cotton soaked in  
69 70% isopropyl alcohol (spirit), following which each toothbrush head (pre labeled as T1 to T60) was immersed in  
70 separate 10 ml of thioglycolate broth solution bottles and shaken for 2 minutes to transfer the microbial content  
71 present on bristle surface to the broth solution.

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

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

## 81 4 III.

## 82 5 Results

83 Sixty volunteers between the age group of 22 to 27 years (mean age=?) participated in this study. The  
84 toothbrushes were labeled and subjected to microbial analysis after twice daily use for three days to determine  
85 the total number of CFUs. The mean log CFU and standard deviation after treatment with six different solutions  
86 used to disinfect toothbrushes is presented in Table 1. The mean difference in the log CFUs among the six groups  
87 was analyzed using ANOVA and they were significantly different ( $P < 0.001$ ). 3 % Hydrogen peroxide ( $4.24 \pm$   
88 1.0) produced the lowest number of CFUs among all the six groups followed by mouthwashes containing 0.2 %  
89 Chlorhexidinegluconate ( $4.47 \pm 1.7$ ) and essential oils( $4.75 \pm 1.2$ ) respectively.

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

## 95 6 Discussion

96 Overview of the literature suggests that contamination of toothbrush and its role in transmission of oral and  
97 systemic disease 7,10,11 . Several studies (a-log transformed, b-one way ANOVA, \*-p value  $< 0.05$  ) conducted  
98 in past used different disinfection techniques like UV radiation 8,9 , microwave irradiation 12 , boiling water,  
99 chemical agents 13 like hydrogen peroxide, cetylpyridinium chloride, chlorhexidine , etc., had shown reduction  
100 in microbial count on toothbrush bristles suggesting need for toothbrush disinfection. The present study was  
101 undertaken to analyze the disinfection property of five different antimicrobial solutions (saline 0.9%,hydrogen  
102 peroxide 3%, 0.2% Chlorhexidinegluconate , essential oils and Cetylpyridinium chloride containing mouthwashes)  
103 and tap water as control. Sixty volunteers with average age ranging from 22 to 27 years were randomly assigned  
104 one of the six groups with ten subjects in each.

105 Volunteers were asked to brush twice daily for three days and follow the post brushing disinfection instructions  
106 given to them. At the end of three days used toothbrushes were collected and sent for microbiological analysis.  
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108 Hydrogen peroxide showed maximum reduction in microbial count ( $MD = -2.02$ ,  $p < 0.001$ ) .This result agrees  
109 with the finding of a study done Sogi et al 14 .The antimicrobial activity of hydrogen peroxide is based on release  
110 of nascent oxygen and its effervescence removes the debris from otherwise in accessible regions 15 .

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

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

## 127 **7 Conclusion**

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

## 131 **8 P value**



Figure 1: Figure 1 :

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Disinfectant groups	Number of case s	Mean ± SD (Log CFU) g	Range (Minim um to Maxim um)	(Log um) (P value)
Water	10	6.27 ± 0.8	4.9	7.6 0.001*
3% H <sub>2</sub> O <sub>2</sub>	10	4.24 ± 1.0	3.0	4.2
.9% Saline	10	6.13 ± 0.7	4.8	6.9
.2% Chlorhexidinegluconate mouthwash	10	4.47 ± 1.7	3.0	6.9
Essential mouthwash oils	10	4.75 ± 1.2	3.0	7.0
Cetylpyridinium chloride mouthwash	10	5.48 ± 1.0	3.0	6.8
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Figure 2: Table 1 :

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132 [Duarte et al.] , C A Duarte , P C Marcondes , A T Rayel .  
133 [Da Microbiotabucalemhumanos] , Transmissibilidade Da Microbiotabucalemhumanos .  
134 [Edson Yukio Komiyama] , Edson Yukio Komiyama .  
135 [Back-Brito] , Graziellauernberg Back-Brito .  
136 [Navneet and Kaur ()] 'A study of toothbrush contamination at different time intervals and comparative effectiveness of various disinfecting solutions in reducing toothbrush contamination' G Navneet , S Kaur . *J Indian Soc.Pedo.Prev Dent* 1996. 14 p. .  
137  
138  
139 [Meier et al. ()] 'An in vitro investigation of the efficacy of CPC for use in toothbrush decontamination' S Meier , C Collier , M G Scaletta , J Stephens , R Kimbrough , J D Kettering . *J Dent Hyg* 1996. 70 p. .  
140  
141 [Antimicrobial mouthrinses: overview and update. Mandel, I.D *Journal of the American Dental Association* ()]  
142 'Antimicrobial mouthrinses: overview and update. Mandel, I.D'. *Journal of the American Dental Association*  
143 1939. 1994.  
144 [Mehta et al. ()] 'Bacterial Contamination and Decontamination of toothbrushes after use' A Mehta , P S  
145 Sequeira , G Bhat . *NYSJ* 2007. p. .  
146 [Mythili et al. ()] 'Comparative effectiveness of various disinfecting solutions in preventing toothbrush contami-  
147 nation'. R Mythili , Sreedhar , M Jaya . *J Indian Soc Periodontal* 1997. 21 (2) p. .  
148 [ H ()] 'Contaminated toothbrushes and pharyngitis'. H . *Arch Otolaryngol Head Neck Surg* 1999. 125 p. 479.  
149 [Caudry et al. ()] 'Contaminated toothbrushes and their disinfection' S D Caudry , A Kklitorinos , E C Chan .  
150 *J Can Dent Assoc* 1995. 61 p. .  
151 [Sogi et al. ()] 'Contamination of toothbrush at different time intervals and effectiveness of various disinfecting  
152 solutions in the contamination of toothbrush'. S Sogi , Subbareddy , S Kiran . *J Indian Soc.Pedo.Prev Dent*  
153 2002. 20 (3) p. .  
154 [Dhifaf and Saleh ()] 'Effectiveness of different cleanser solutions on the microbial contamination of toothbrushes'.  
155 Mohammad Dhifaf , Saleh . *Journal of Kerbala University* 2011. 9 (3) p. . (Scientific)  
156 [Balducci and Koga-Ito (2010)] 'Evaluation of alternative methods for the disinfection of toothbrushes'. Ivan  
157 Balducci , ; Cristianeyumi Koga-Ito . *Braz. oral res* Jan. Mar 2010. 24 (1) .  
158 [Chibebe ()] 'Evaluation of sterilization of toothbrushes in a microwave oven (in vitro study)'. J ChibebeJr ,  
159 Pallos . *Rev Biocienc* 2001. 7 p. .  
160 [Verran and Leahy-Gilmartin ()] 'Investigations into the microbial contamination of toothbrushes'. J Verran , A  
161 A Leahy-Gilmartin . *Microbios* 1996. 85 p. .  
162 [Verran and Leahy-Gilmartin ()] 'Investigations into the microbial contamination of toothbrushes'. J Verran , A  
163 A Leahy-Gilmartin . *Microbios* 1996. 85 p. .  
164 [Sato et al.] *Izabel Yoko Ito:Antimicrobial spray for toothbrush disinfection: An in vivo evaluation*, Sandra  
165 Sato , Elza Helena Viníciuspedrazzi , Guimarães Lara , Rubens Heitorpanzeri , Ferreira De Albuquerque  
166 . (Quintessence internationalVol)  
167 [Dayoub et al. (1977)] 'Microbial contamination of toothbrushes'. M B Dayoub , D Rusliko , A Gross . *J Dent*  
168 Res 1977. november/december 2005. 56 (10) p. 706.  
169 [Malmberg et al. ()] 'Microorganisms on toothbrushes at day-care centers'. E Malmberg , D Birkhed , G  
170 Norvenius , J G Noren , G Dahlén . *ActaOdontolScand* 1994. 52 p. .  
171 [Glass and Shapiro ()] 'Oral inflammatory diseases and the toothbrush'. R T Glass , S Shapiro . *J Okla Dent*  
172 *Assoc* 1992. 82 p. .  
173 [Brool] *Persistence of group A beta haemlytic streptococci in toothbrushes and*, I Brool , Goberae .  
174 [Boylanr et al. ()] 'Reduction in bacterial contamination of toothbrushes using the Violet ultraviolet light  
175 activated toothbrush sanitizer'. Liy Boylanr , Simeonoval , Sherwing , Cra Kreismannj . *Am J Dent* 2008. 21  
176 p. .  
177 [Sconyersjr ()] 'Relationship of bacteremia to toothbrushing in patients with periodontitis'. Crawfordjj Sconyersjr  
178 . *J Am Dent Assoc* 1973. 87 p. .  
179 [Kozai et al. ()] 'Residual contamination of toothbrushes by microorganisms'. K Kozai , T Iwai , K Miura . *J*  
180 *Dent Child* 1989. 56 p. .  
181 [Glass and Hg ()] 'The effectiveness of a u-v toothbrush sanitizing device in reducing the number of bacteria  
182 ,yeasts and viruses on toothbrushes'. R T Glass , Jensen Hg . *JOkla DENT Assoc* 1994. 84 p. .  
183 [Taji and Rogers ()] 'The microbial contamination of toothbrushes. A pilot study'. S S Taji , A H Rogers . *Aust*  
184 *Dent J* 1998. 43 p. .  
185 [Aznita and Fathilah ()] 'The potential use of chlorhexidine (CHX) and Hexetidine containing mouth rinse in  
186 maintaining toothbrush sterility'. W H Aznita , A R Fathilah . *J.Med.Sci* 2006. 6 (1) p. .  
187 [Cobb ()] 'Toothbrushes as a cause of repeated infections of the mouth'. C M Cobb . *Boston Med Surg J* 1920.  
188 183 p. .