Efficacy of various disinfectants on microbially contaminated toothbrushes due to brushing

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Abstract

Aims: To evaluate the effectiveness of different disinfectant agents in decontaminating the toothbrushes, and to educate the children, parents and the community on the decontamination of toothbrushes. Materials and Methods: Fifty healthy male children in the age range of 8–11 years were enrolled. They were divided into five groups of 10 each and provided with toothbrushes and disinfectants. Instructions were given to the children and the toothbrushes were collected after brushing and cultured for the growth of microorganisms. The efficacy of Hexidine®, 3.0% hydrogen peroxide and Listerine® and Dettol® were evaluated. Chi-square test was used for statistical analysis. Results: Hexidine, 3.0% hydrogen peroxide and Listerine showed 100% efficacy, whereas Dettol showed 40% effectiveness in decontaminating the toothbrushes. Water as a control showed the least effectiveness in cleaning the toothbrushes. Conclusions: The study concluded that 3.0% hydrogen peroxide is the most economical and effective disinfectant when compared with the other disinfectants.

Keywords: Decontamination, disinfectants, toothbrushes

Introduction

Knowledge of toothbrush contamination is yet a void among the population and in the literature as well. Different brushing techniques have been described in the literature, but there is inadequate information about the maintenance of toothbrushes to avoid their contamination with microorganisms. Toothbrush decontamination is essential to eliminate pathogenic microorganisms transmitted to toothbrushes during brushing from the oral cavity or from the other toothbrushes and storage area. Within 2 days of first usage, toothbrushes are heavily infected with potentially disease producing bacteria, viruses and fungi. Rinsing with plain tap water may reduce this microbial load, but complete elimination is not possible. Keeping these in mind, the aim of this study was to assess the efficacy of various disinfectants to decontaminate the toothbrushes before brushing and to recommend the most economical disinfectant for the common man.

Materials and Methods

This study was carried out in the Department of Pedodontics and Preventive Dentistry, Narayana Dental College and Hospital, Nellore, Andhra Pradesh, with the help of Department of Microbiology of the same hospital. Fifty healthy male children of age 8–11 years were enrolled from Vatsalya Orphanage, Nellore. The study was explained to the children and written informed consent was taken from the orphanage authorities. Ten new packed toothbrushes were randomly taken from the package and cultured prior to the study to see whether the toothbrushes were contaminated with microorganisms.

Different time intervals were taken into consideration, i.e. after 1st, 2nd, 7th, 14th and 28th days. Each child was provided with a set of new toothbrushes and they were replaced with a new one at each time interval. Children were divided into five groups as Group I, II, III, IV and V, with each group comprising 10 members [Figure 1]. Oral hygiene devices used were five-rowed, tufted nylon toothbrushes of medium type (Morning Smile Company, Mumbai, India) and toothpaste which contained calcium carbonate as an active ingredient and an extract of Acacia Arabica (Babool®, Dabur India Ltd). The disinfectants tested for each group were: Group I – water (control), Group II – Hexidine® (ICPA Health products), Group III – 3.0% hydrogen peroxide, Group IV – Dettol® [Reckitt benckiser (India) Ltd] and Group V – Listerine® (Johnson and Johnson Ltd) [Figure 2].

Children of the control group were given instructions to brush their teeth and rinse the toothbrushes with plain tap water for 20 seconds and leave them for air drying. Then, the toothbrushes were collected and stored in the test tubes containing sterile peptone water up to the level of the head...
of the toothbrush and closed with autoclaved cotton rolls [Figure 3]. The toothbrushes were cultured by incubating for 24 hours at 37°C. Then, the incubated peptone water was subcultured in Petri dishes containing blood agar, nutrient agar and Mac Conkey’s agar media at 37°C for 24 hours for bacterial growth, and test tubes containing Sabouraud’s dextrose agar media slant were subcultured by stroking with nichrome loop and incubated at 27°C for 48–72 hours for fungal growth. The different patterns of colonies of microorganisms were identified by observing their colony morphology, gram staining and biochemical reactions. Further, the gram-positive organisms were identified by catalase, coagulase and novobiocin sensitivity tests, and the gram-negative organisms were identified by oxidase, motility tests and biochemical reactions.

Children of Group II, III, IV and V were instructed to brush their teeth and rinse their toothbrushes in running plain tap water for 20 seconds and then soak them in their respective containers with disinfectants for 20 minutes. Then, the solution was discarded and toothbrushes were kept in the containers, with the head of the toothbrushes facing outward for air drying. The toothbrushes were collected and the same method was followed as with the control group.

**Results**

Ten new unused toothbrushes were cultured directly from their respective packages and none of the toothbrushes showed microbial growth [Table 1]. Comparison of the number and percentage of contaminated toothbrushes between different disinfectant solutions was done at different time intervals [Table 2, Graph 1]. In Group I (water control), at the first interval of using for 1 day, 50% toothbrushes showed contamination (5 out of 10). This increased to 60% after 2 days and to 100% at the end of 7 days, maintaining the same level till 28 days, whereas in Group IV (Dettol), 30% of toothbrushes showed contamination (3 out of 10), which increased to 40% after 2 days and 70% after 7 days, rising to 100% at the end of 2 weeks, maintaining the level till 28 days.

In Group II (Hexidine), Group III (3.0% H\textsubscript{2}O\textsubscript{2}) and Group V (Listerine), none of the toothbrushes were contaminated after using for 1 day or thereafter, showing zero contamination. Then, the comparison was made between control and Dettol groups. After using toothbrushes for 1 day, the difference was not significant ($P = 0.47$). Similarly, the results after 2 days ($P = 0.52$), 7 days ($P = 0.46$), 14 days and 28 days were also

| Toothbrush no. | Culture report |
|----------------|---------------|
| 1              | No growth     |
| 2              | No growth     |
| 3              | No growth     |
| 4              | No growth     |
| 5              | No growth     |
| 6              | No growth     |
| 7              | No growth     |
| 8              | No growth     |
| 9              | No growth     |
| 10             | No growth     |

![Figure 1: Children brushing their teeth](image1)

![Figure 2: Disinfectants used in the study](image2)

![Figure 3: Toothbrushes stored in peptone water](image3)
not significant ($P = 1.0$). This showed no significant difference between the two groups at any time interval [Table 3, Graph 2].

The disinfectants used were compared with one another and with water for their efficacy [Graph 3]. Hexidine, 3.0% hydrogen peroxide and Listerine gave 100% results by showing no growth of microorganisms on any of the toothbrushes. Dettol showed only 40% efficacy in reducing the microbial load on toothbrushes, whereas water showed 0% reduction of the microbial load on toothbrushes.

**Discussion**

Routine household procedures for preventing contamination mainly consist of rinsing and drying the toothbrushes. But 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 in-

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**Table 2: Contaminated toothbrushes at different time intervals**

| Length of usage of toothbrush (days) | Group I (control) | Group II (Hexidine®) | Group III (3.0% H₂O₂) | Group IV (Dettol®) | Group V (Listerine®) |
|--------------------------------------|-------------------|----------------------|------------------------|-------------------|--------------------|
|                                      | No.   | %    | No.   | %    | No.   | %    | No.   | %    | No.   | %    |
| 1                                    | 5     | 50   | 0     | 0    | 0     | 0    | 3     | 30   | 0     | 0    |
| 2                                    | 6     | 60   | 0     | 0    | 0     | 0    | 4     | 40   | 0     | 0    |
| 7                                    | 10    | 100  | 0     | 0    | 0     | 0    | 7     | 70   | 0     | 0    |
| 14                                   | 10    | 100  | 0     | 0    | 0     | 0    | 10    | 100  | 0     | 0    |
| 28                                   | 10    | 100  | 0     | 0    | 0     | 0    | 10    | 100  | 0     | 0    |

**Table 3: Comparison of number of contaminated toothbrushes between control group and Dettol® group at different time intervals**

| Length of usage of toothbrushes (days) | Control group | Dettol® group | Chi-value | $P$-value | Significance |
|----------------------------------------|---------------|---------------|-----------|-----------|--------------|
| 1                                      | 5             | 3             | 0.5       | 0.47      | NS           |
| 2                                      | 6             | 4             | 0.4       | 0.52      | NS           |
| 7                                      | 10            | 7             | 0.52      | 0.46      | NS           |
| 14                                     | 10            | 10            | 0         | 1         | NS           |
| 28                                     | 10            | 10            | 0         | 1         | NS           |
fections in the mouth.[2,3] 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. The more acceptable alternative is to decontaminate the toothbrushes with antimicrobial agents.[2]

Along with the toothbrush, toothpaste is also used as an aid to maintain good oral hygiene. Studies have proved that toothpaste does not inhibit the presence of periodontal pathogens, but toothpaste detergents decrease the survival rate of pathogenic species on toothbrush and could limit the risk for bacterial translocation.[4] Considering these criteria, a pilot study was conducted to see the efficacy of toothpaste containing triclosan on decontaminating the toothbrushes, along with the main study. The results showed that the toothpaste did not eliminate all the microorganisms, but there was a reduction in the microbial load on the toothbrushes. The studies carried out by various authors suggested that the disinfectants such as cetyl pyridinium chloride,[5] sodium hypochlorite (1.0%),[3] chlorhexidine gluconate (0.2%) and hydrogen peroxide (3.0%)[6,7] were efficient in decontaminating the toothbrushes.

In this study, 50 healthy male children in the age range of 8–11 years, who fall into mixed dentition period that is the time for transitional changes in the oral microbial flora, were enrolled from an orphanage. It was also easy for supervision of tooth brushing and using disinfectants as all the children stayed in the same place. The children were asked to brush their teeth in horizontal scrub method because this method has more significant plaque removing effect than the other methods regardless of age of the child and also it is the technique most naturally adopted by the children, which was corroborated by various authors.[8]

Group I (control) toothbrushes showed 50% growth of microorganisms after 1st day of usage, which had increased to 100% by the end of 28 days, as the toothbrushes were never completely dry when stored after use and there would be formation of biofilm at the bottom of the toothbrush bristles. As the number of days increase, the number of microorganisms will also increase in the toothbrush biofilm. Just like growth media which have properties of nutrients, moisture and storing in cool environment, toothbrush may act as an enriched Petri dish on a stick which may lead to bacterial growth.[8] At the end of 28 days, the toothbrushes were contaminated with Klebsiella spp., Pseudomonas spp., Escherichia coli, Staphylococcus epidermidis and Citrobacter spp. [Figures 4–6, Table 4]. There was no fungal growth in any of the toothbrushes, which is somewhat similar to the study done by Sogi et al., where 30% growth of microorganisms was seen after 1st day of usage of toothbrush which increased to 100% by the end of 28 days. The isolated microorganisms were Staphylococcus pyogenes, Klebsiella spp., E. coli, Proteus spp. and beta-hemolytic Streptococcus faecalis.[5] Whereas another study by Grewal and Kaur reported 40% of growth of microorganisms after 1st day of usage, which reached to 100% by the end of 1 month that was maintained up to 3 months. The microorganisms isolated were Klebsiella spp., E. coli and Str. faecalis.[6]
Group II (Hexidine) toothbrushes showed 100% results. None of the toothbrushes were contaminated after using for 1 day or thereafter, which showed 0% contamination. Chlorhexidine gluconate (0.2%), the ingredient of Hexidine, acts against a wide range of gram-positive and gram-negative organisms and fungi. On the contrary, Grewal and Kaur reported that 20% of microorganisms grew in the beginning of the study; whereas Sogi et al. had shown 12.5% of growth on 14th day which had increased up to 25% on 28th day of the study.[6, 7]

Immersion in Hexidine for 20 minutes was found to be adequate to disinfect the toothbrushes. The other studies had taken the immersion time into consideration and reported that immersing in chlorhexidine (0.12%) for 2 hours[2] and 20 hours[8] and in chlorhexidine (0.2%) for 24 hours[10] was adequate to decontaminate the toothbrushes. The Group III (3.0% hydrogen peroxide) toothbrushes showed 100% results. Grewal and Kaur also reported 100% results and suggested that the toothbrush can be safely used for 3 months by soaking in 3.0% hydrogen peroxide for 30 minutes and leaving it for air drying in a clean container,[6,8] which was also supported by Sogi et al.[7]

The Group IV (Dettol) toothbrushes showed 30% contamination on using for 1 day and 100% contamination at the end of 28 days and the resistant microorganisms to Dettol were Klebsiella spp. and E. coli[Table 4, Graph 4], whereas Sogi et al. had reported only the growth of Klebsiella spp. on 14th day (i.e., 12.5%). Even though Dettol is effective against gram-positive and gram-negative microorganisms due to the presence of chloroxylenol, the efficacy in disinfecting the contaminated toothbrushes was less because of the presence of alcohol which evaporates quickly from the surface and the bacterial spores being resistant to the agent.[7]

The Group V (Listerine) toothbrushes showed 100% results. None of the toothbrushes showed the growth of microorganisms after 1 day of usage after immersion for 20 minutes and it was maintained till 28 days. Listerine contains menthol, ethanol, thymol and eucalyptol. These components have different roles to play as disinfectants. On the contrary, Grewal and Kaur had shown that 20% of toothbrushes were contaminated on the 1st day of usage, which was increased up to 40% after a period of 3 months.[8] Listerine killed all the microorganisms on the toothbrush bristles. Caudry et al. had suggested that soaking the toothbrush bristles in Listerine for 20 minutes prevents bacterial contamination.[3, 5]

In the present study Hexidine, 3.0% hydrogen peroxide and Listerine gave 100% results by showing equal efficacy in decontaminating the toothbrushes. Dettol reduced the microbial load in the toothbrushes, but did not completely eliminate the microorganisms. When compared with the other disinfectants, Dettol was the least effective.

In the present study, the toothbrushes showed contamination with Klebsiella spp., Pseudomonas spp., E. coli, Sta. epidermidis and Citrobacter spp. may cause upper respiratory and urinary tract infections, diarrhea, pyogenic infections, pneumonia, septicemia, to name a few.[11] Their origin can be environmental, from the tap water, dispersed via aerosols from toilet flushing, from contaminated fingers or from the bathroom and other humid areas.[12] 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 [13]. So, cleaning the oral cavity includes maintaining oral hygiene or oral health and also frequent changing, cleaning and disinfecting the oral hygiene devices.

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Table 4: Microorganisms isolated from contaminated toothbrushes

| Group                | Microorganisms isolated                           | Fungus          |
|----------------------|--------------------------------------------------|-----------------|
| Group I (water-control) | *Klebsiella* species, *Pseudomonas* species, *Escherichia coli*, *Staphylococcus epidermidis*, *Citrobacter* species | No growth       |
| Group II (Hexidine®)      | No growth                                        | No growth       |
| Group III (3.0% H₂O₂)       | No growth                                        | No growth       |
| Group IV (Dettol®)       | *Klebsiella* species, *Escherichia coli*        | No growth       |
| Group V (Listerine®)         | No growth                                        | No growth       |
Dental Association in 1996 has recommended the change of toothbrushes after every 3 months. Glass and Jensen and Denny 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. Glass and Jensen reported that due to the longevity of viruses, it may be appropriate to replace toothbrushes every two weeks, and for the medically-compromised community, changing toothbrushes every three to seven days was suggested.

The frequent change of toothbrush increases the cost of maintenance of oral hygiene which becomes a burden to the common man. So, instead of changing the toothbrush, decontamination of toothbrushes with the disinfectant is more economical. Thus, it is mandatory for every individual to disinfect the toothbrush at regular intervals thereby maintaining a good oral hygiene. In this study, the time intervals taken were 1, 2, 7, 14 and 28 days. The types of microorganisms were the same in toothbrushes after using them for 1 day and 2 days. Increase in the load of microorganisms was observed after using the toothbrush for 7 days. The results of our study showed that the efficacy of different media to decontaminate the toothbrushes by immersing in them for 20 minutes before brushing for every 3 days will eliminate the microorganisms present in the toothbrushes. The cost of each disinfectant for usage was estimated. Hexidine, 3.0% hydrogen peroxyde and Listerine cost around ₹6.80/-, ₹1.80/- and ₹9.40/-, when used as 20 ml once in 3 days, and the total cost per month is ₹68/-, ₹18/ and ₹94/-, respectively. Among these disinfectants, the most economical is 3.0% hydrogen peroxide, which can be recommended as a routine for the community.

Conclusions

Even though we have basic knowledge regarding the disinfection procedures for our instruments and environment, certain things are practically not implemented such as decontamination of toothbrushes. In the medical field, some of the diseases might have been unnoticed which could be transmitted through the contaminated toothbrushes. Therefore, there is a necessity to concentrate on disinfection of toothbrushes with antimicrobial solutions which really benefit in preventing reinfections or cross infections. Every dentist should be motivated to educate the patients on the importance of toothbrush disinfection. Further, similar studies are needed regarding the transmission of viruses through the toothbrushes and their disinfection.

“No one would ever use the same cloth for personal hygiene without cleaning it after each use.” The same principle should hold true for oral hygiene devices.

In our study, we tried to find the effectiveness of various disinfectants in decontaminating the toothbrushes. Even though Hexidine, 3.0% hydrogen peroxide and Listerine were 100% effective in eliminating microorganisms on the toothbrushes, 3.0% hydrogen peroxide proved to be the most economical.

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