The Efficacy of Chlorhexidine Spray vs Mouthwash in the Microbial Contamination of Child Toothbrushes

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ABSTRACT

Purposes: The purposes of this study were to, determine the level of contamination by mutans streptococci on the toothbrushes of children using microbial identification and evaluate the efficacy of 0.12% chlorhexidine spray vs chlorhexidine mouthwash as toothbrush disinfectants.

Methods: Seventy-one 7-year-old children were randomly selected for this study. The children were divided into 3 groups, according to the number of cfu/ml of mutans streptococci in the samples: (1) low caries risk (0-21 cfu/ml); (2) medium caries risk (21-100 cfu/ml); and (3) high caries risk (>100 cfu/ml). According to this evaluation, 24 children from the high dental caries risk group were selected to participate in this study. After oral hygiene instruction, the children participated in a supervised daily tooth-brushing for 5 consecutive days. At the end of these days, all toothbrushes were collected. The toothbrushes were divided into 3 groups randomly: (1) group 1 (chlorhexidine [CHX] mouthwash)—toothbrushes were immersed individually in test tubes containing 10 ml of 0.12% CHX gluconate and 0.15% benzidamin; (2) group 2 (CHX spray)—toothbrushes were sprayed with the solutions, including 0.12% chlorhexidine gluconate and 0.15% benzidamin, onto the bristles twice; (3) group 3 (sterile saline)—toothbrushes were immersed individually in test tubes containing 10 ml sterile saline as a control. After the microbiologic procedures, the number of mutans streptococci colonies were counted and statistically evaluated. The toothbrush bristles were carefully inspected, and the biofilm formation was evaluated under aseptic conditions with a stereoscopic microscope.

Results: There was no statistically significant difference between the CHX mouthwash and spray groups ($P>.05$), but a statistically significant difference was observed between the control group and the other test groups ($P<.05$).

Conclusion: All toothbrushes immersed in sterile saline showed high mutans streptococci counts and biofilm formation. There was no statistically significant difference between the chlorhexidine mouthwash and spray groups, and both approaches had the ability to disinfect the toothbrushes if maintained for 2 hours. (J Dent Child 2007;74:177-81)

KEYWORDS: CHLORHEXIDINE, MOUTHWASH, DISINFECTION, TOOTHBRUSHING

Toothbrushes are mainly used to remove dental plaque bacteria. When bacteria survive on toothbrushes, they may reinoculate the oral cavity of the original host. The multiplication and increased in number of these micro-organisms can pose a significant risk of dissemination.[1-4,6]

As early as 1920, Cobb[5] reported the toothbrush to be a cause of repeated infections of the mouth. Svanberg[6] found that toothbrushes can be heavily infected by mutans

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streptococci (MS) after 24 hours. According to Glass,[7] micro-organisms not only adhere to and reproduce on used toothbrushes, but also have the ability to transmit organisms responsible for both local and systemic diseases.

Under usual storage conditions, toothbrushes can be a source for transmission or reinfection of diseases, such as: herpes[7], periodontopathogenic micro-organisms[8], or coliforms from the bathroom environment.[9] Toothbrushes kept in a moist environment like that of a bathroom retained up to 50% of herpes simplex virus type 1 for a week.[10] Other studies have reported that MS was found on toothbrushes after 6 hours of drying time, thus increasing the risk of dental caries.[6,11] MS cells exist in moist dental plaque that adheres to and can remain on toothbrushes.[6]

In response to these reports, several studies have focused on toothbrush disinfection methods. It is not feasible to sterilize toothbrushes between uses, but a decontamination procedure that reduces the infectious burden might be acceptable. In most cases, different methodologies were used that do not permit comparisons. Caundry et al[12] found that soaking toothbrushes for 20 minutes in a mouthrinse containing essential oils killed 100% of the bacteria present. The use of a UV toothbrush-sanitizing device has also been shown to be effective.[13] Meier *et al*,[14] tested the use of cetylpyridinium chloride spray with toothbrushes and found it to be bactericidal. Warren *et al*[15] and Nelson-Filho *et al*[16] found that a triclosan-containing toothpaste was effective on the residual anaerobic microbial contamination of toothbrushes. Quirynen et al[17] also found that toothpaste detergents decreased the survival rate of pathogenic species on a toothbrush and could limit the risk for bacterial translocation. More recently, a proprietary toothbrush spray disinfectant (Brushtox) was produced against specific bacteria and fungi and was found to be effective on toothbrushes.[18]

The efficacy of chlorhexidine (CHX) gluconate as a bacteriostatic and bactericidal agent in dental plaque control has also been shown.[19-22] Several comparative studies with other chemical agents found CHX to be the most effective agent.[23-25] The superior antiplaque activity of CHX apparently is due to its capacity of persistence or substantivity.[26-28]

The spray form has proven to be a helpful therapeutic aid, since it allows the patient to confine the administration of the chemical agent to the region where mechanical oral hygiene has to be avoided. The spray also represents a reliable method for the control of dental plaque accumulation, with its efficacy being similar to that of CHX mouthwash.[29-31]

Although there are sufficient reports on dental plaque control of CHX, only 1 study[32] could be obtained on the effectiveness of CHX mouthwash as a toothbrush disinfection.

The study's purposes were to determine the MS contamination level of toothbrushes using microbial identification; and evaluate the efficiacy of 0.12% chlorhexidine spray vs chlorhexidine mouthwash as toothbrush disinfectants.

METHODS

Seventy-one 7-year-old children were randomly selected for this study from an elementary school (The Foundation of Gazi University, Private Elementary School, Ankara, Turkey). Written, informed consent was obtained from each child's parents. The participants chewed a piece of paraffin wax, about 0.9 g, for 1 minute.[33] With a sterile Pasteur pipette, 0.5 ml of paraffin-stimulated saliva from each child was transferred to sterile test tubes containing 4.5 ml sterile saline. The tubes were placed in ice immediately . These collected samples were transported to the laboratory within 1 hour. The test tubes were vortexed for 30 seconds. The suspensions were diluted serially from 10^{-1} to 10^{-2}, and 10 µl of each diluted saliva was spread on the surface of a plate containing SB-20 agar medium.[34] Plates were incubated at 37°C for 72 hours in microaerophilic conditions (air+5% CO_2). After this process, the MS colonies were counted by means of colony-forming units (cfu/ml).

The children were divided into 3 groups, according to the number of cfu/ml of MS in the samples: (1) low caries risk (0-21 cfu/ml); (2) medium caries risk (21-100 cfu/ml); and (3) high caries risk (>100 cfu/ml).

According to this evaluation, 24 children from the high dental caries risk group were selected to participate in this study. Each of the children received a toothpaste (Signal, Lever, Kocaeli, Turkey) containing 1,000 ppm sodium fluoride and a new toothbrush (Oral-B Stages 3, Oral-B, Boston, Mass).

After oral hygiene instruction, the trained children participated in a supervised daily tooth-brushing for 5 consecutive days. After each tooth-brushing, the toothbrushes were rinsed under running tap water for 10 seconds and excess liquid was removed with shaking by the same superviser. The toothbrushes were stored separately to avoid contact with each other at room temperature in a dry environment between brushings.[17] At the end of 5 consecutive days, all toothbrushes were collected for examination. Before being tested, toothbrushes were kept at room temperature for 4 hours, enabling the toothbrushes to dry.[16]

The toothbrushes were randomly divided into 3 treatment groups:

1. Group 1 (CHX mouthwash; N=8) toothbrushes were immersed individually for 2 hours in test tubes containing 10 ml of 0.12% CHX gluconate and 0.15% benzidamin.
2. Group 2 (CHX spray; N=8) toothbrushes were sprayed in a 5-cm distance for 2 hours, with solutions containing 0.12% chlorhexidine gluconate and 0.15% benzidamin, onto the bristles twice.
3. Group 3 (sterile saline; N=8) toothbrushes were immersed individually for 2 hours in test tubes containing 10 ml steril saline as control.

Unused toothbrushes (N=8) were used as a sham control.

MICROBIOLOGICAL PROCEDURES

After disinfection for 2 hours, the toothbrushes were placed vertically into the 25x150 mm sterile test tubes containing 10 ml SB-20 broth medium (selective enrichment broth prepared by the modification of de Stoppelaar et al (35) for 4 days at 37°C in air and 5% CO_2 . After 4 days the toothbrushes were withdrawn and rinsed in sterile distilled water with gentle shaking to remove nonadhered bacteria. The toothbrush bristles were inspected carefully, and biofilm formation was evaluated under aseptic conditions with a stereoscopic microscope.

To recover the MS colonies, 2 bristles were detached from the toothbrushes on which the biofilm formed, placed into the test tubes containing 500 µl sterile saline, and stirred with a vortex mixer for 5 minutes.

After stirring, 20 µl of the suspension were spread onto the SB-20 agar plates and incubated at 37°C in microaerophilic conditions for 4 days. After this period, the number of MS colonies were counted and evaluated.

Statistical analyses were performed using Kruskal-Wallis and Mann-Whitney U tests. Results were given for a probability level of P=.05.

RESULTS

According to the treatment groups, the number of MS colonies and the presence of the biofilm were expressed at the end of the incubation period as:

1. In group 1 (CHX mouthwash), biofilm formation on the toothbrushes and MS growth on agar plates were not observed.
2. In group 2 (CHX spray), biofilm formation was not observed in all the toothbrushes, but MS growth was observed on the 3 agar plates (range=10-15 cfu/ml).
3. In group 3 (sterile saline), biofilm formation on all toothbrushes and MS growth on all agar plates (range=54-110 cfu/ml) were observed.

In the negative control group (N=8), there was no biofilm formation or MS growth.

As a result, there was no statistically significant difference between the CHX mouthwash and CHX spray groups (P>.05), but a statistically significant difference was observed between the control group and the other test groups (P<.05).

DISCUSSION

Clinical interest in toothbrush contamination has increased in recent years. In households, daily procedures for preventing contamination consist mainly of rinsing and drying the toothbrushes. Kozai et al[11] revealed that many micro-organisms remained on the toothbrush bristles after usage and cleaning with this general method. Air drying of toothbrushes may be an incomplete method for disposing of micro-organisms. The more economically acceptable alternative is to decontaminate the brushes. This study's results showed that CHX mouthwash or spray can produce such desired effects, since it is highly effective against MS. According to this study's results, it may be thought that toothbrushes can be a reservoir for direct transmission of micro-organisms as well as a source for inoculation or reintroduction of micro-organisms from infected to noninfected tissues.

Although MS is considered the primary etiologic agent for dental caries lesions, not many studies have been published on the contamination of toothbrush bristles.[6,11,16,32] Svanberg[6] reported a massive presence of MS on toothbrushes. Nelson-Filho *et al*[16] observed the development of MS in 100% of children's toothbrushes kept in sterile water after brushing. They also showed MS on the bristles after 4 hours exposure at room temperature. Kozai *et al*[11] reported that high levels of MS was present on toothbrushes in 6 hours after use and air exposure. In another study,[32] MS was found in 100% of the toothbrushes maintained in sterile tap water for 20 hours as a control. In the present study, MS was found in 100% of the toothbrushes maintained in sterile saline for 2 hours as a control. The presence of cariogenic bacteria adhering to bristles was shown microbiologically by the toothbrush cultures, and the results were confirmed with these cited studies.

The time necessary for MS colonization varies from 1 to 30 days.[36,37] According to Cesco *et al*,[38] colonization of toothbrushes by MS occurs in a short time period within the bristles of the toothbrushes. Svanberg[6] reported the presence of MS on toothbrushes after 3 days. In this study, the colonization of MS was observed on toothbrushes after 5 consecutive days of toothbrush use. This result is in agreement with Nelson-Filho et al's study.[32] This may be because MS chiefly exists in dental plaque, is adhesive, is moist, and is difficult to remove from toothbrushes and is difficult to dry.

The need for toothbrush disinfection to reduce the number of micro-organisms on the bristles has been suggested using such methods as UV-radiation, the microwave oven, boiling water[10], and chemical agents like Listerine, Plax,[12] and Cepacol.[12,14] Microwave disinfection was hampered by the arching of the metal cleats used to anchor the toothbrush bristles. Disinfection could be achieved using the microwave, however the resultant distorted and convoluted toothbrush was not functional. Ultraviolet light disinfection may be promising in killing micro-organisms, but needs further investigation. Chemical disinfectants had difficulty penetrating the aggregates of micro-organisms and in penetrating the toothbrush bristle depth and defects.[39]

Establishing an easy and effective method for disinfecting a toothbrush would be an important and economical way to prevent the continuation of reinfection of oral diseases. This study's results indicate that toothbrushes can be contamined with MS for up to 5 days following use. The use of 0.12% chlorhexidine gluconate in spray or mouthwash form was effective to reduce MS contamination. This result agrees with Nelson-Filho and other studies showing that immersion of toothbrushes for 20 hours in 0.12% CHX mouthwash or 1% sodium hypochlorite was efficient for disinfection. But there was a difference between the studies, such as the immersion time. Although the immersion time was 2 hours in this study, high MS growth could be obtained in

the sterile saline group. Also, other studies need to be done, such as decreasing immersion time, finding other methods of application, and other disinfecting agents.

In a previous study,[30] CHX spray did not show any difference regarding CHX mouthwash in the control of dental plaque, confirming previous findings in a study involving healthy volunteer subjects. A further study[40] comparing CHX delivered by mouthwash and spray confirmed the spray to be as effective as the mouthwash in controlling plaque regrowth.

In this clinical trial, 2 different means of delivering CHX were compared. The main purpose of this study was to verify the efficacy of a spray and mouthwash solution of CHX in the control of sterile saline. Similar results were found between CHX mouthwash and spray forms as disinfectants. Although no significant difference was found between CHX mouthwash and CHX spray in terms of disinfection, the solution form seemed to be cost effective for daily uses. Conversely, there may be some resistant bacterial strains to this chemical agent. As this solution ought to be frequently changed for precaution, this is not cost effective. Although CHX spray costs more than CHX mouthwash, the former is easier to use and provides longer preventive benefits. Either form of CHX, however, is a prerequisite for preventing MS contaminations oon child toothbrushes and can also reduce the number of planktonic mutans streptococci on used toothbrush bristles that had been rendered undetectable before.

CONCLUSIONS

Based on this study's results, the following conclusions can be made:

1. All toothbrushes immersed in sterile saline showed high mutans streptococci development and had biofilm formation.
2. There was no statistically significant difference between the chlorhexidine mouthwash and spray groups, and both had the ability to disinfect toothbrushes for 2 hours.

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