Evaluation of alternative methods for the disinfection of toothbrushes

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Abstract: The aim of this study was to evaluate alternative methods for the disinfection of toothbrushes considering that most of the previously proposed methods are expensive and cannot be easily implemented. Two-hundred toothbrushes with standardized dimensions and bristles were included in the study. The toothbrushes were divided into 20 experimental groups (n = 10), according to microorganism considered and chemical agent used. The toothbrushes were contaminated *in vitro* by standardized suspensions of *Streptococcus mutans*, *Streptococcus pyogenes*, *Staphylococcus aureus* or *Candida albicans*. The following disinfectants were tested: 0.12% chlorhexidine digluconate, 50% white vinegar, a triclosan-containing dentifrice solution, and a perborate-based tablet solution. The disinfection method was immersion in the disinfectant for 10 min. After the disinfection procedure, the number of remaining microbial cells was evaluated. The values of cfu/toothbrush of each group of microorganism after disinfection were compared by Kruskal-Wallis ANOVA and Dunn's test for multiple comparisons (5%). The chlorhexidine digluconate solution was the most effective disinfectant. The triclosan-based dentifrice solution promoted a significant reduction of all microorganisms' counts in relation to the control group. As to the disinfection with 50% vinegar, a significant reduction was observed for all the microorganisms, except for *C. albicans*. The sodium perborate solution was the less effective against the tested microorganisms. Solutions based on triclosan-containing dentifrice may be considered effective, nontoxic, cost-effective, and an easily applicable alternative for the disinfection of toothbrushes. The vinegar solution reduced the presence of *S. aureus*, *S. mutans* and *S. pyogenes* on toothbrushes.

Descriptors: Disinfection; Toothbrushing; Triclosan; Chlorhexidine; Acetic acid.

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Introduction

The use of toothbrushes and/or dental floss is essential to removing dental biofilm and to preventing dental caries and periodontal disease.[1] Brushing methods are widely described in the literature, but the disinfection of toothbrushes after their use is rarely discussed.

The contamination of toothbrushes was first described in the decade of 20 of the XXth century, and may be the cause of successive infections in the oral cavity after their use.[2] Glass[3] (1992) observed that injuries to oral tissues are aggravated by the use of contaminated toothbrushes when compared with sterile ones, and may even cause septicemia after brushing. Other studies concluded that toothbrushes of both healthy and diseased individuals contain a significant number of opportunistic and pathogenic microorganisms, and might induce respiratory, gastrointestinal, cardiovascular and renal problems.[3] Taiji, Rogers[4] (1998) reported that toothbrushes are usually stored in bathrooms and present a high level of contamination, considering that this environment is highly contaminated, mainly by enteric bacteria dispersed by aerosols.

According to Devine *et al.*[5] (2007), there is a need for disinfection methods that are rapidly effective, cost-effective, nontoxic and that can be easily implemented. However, few studies on the methods of disinfection of toothbrushes are found in the related literature. Immersion in chlorhexidine gluconate is suggested as an efficient method for toothbrushes contaminated by the use by children or adults.[6,7] Previous studies reported that brushing with dentifrices with specific components may reduce the bacterial contamination of toothbrushes.[7,8,9] On the other hand, toothbrushes with antibacterial tufts failed to prevent contamination by cariogenic and periodontopathogenic microorganisms.[10] The effectiveness of tetrasodium EDTA and UV sanitization devices for toothbrush disinfection has been recently reported.[5,11]

Considering that the proposed methods for toothbrush disinfection reported in the literature are mostly expensive and cannot be easily implemented, the aim of this study was to evaluate alternative chemical agents for the disinfection of toothbrushes, aiming particularly at its home and widespread use.

Material and Methods

Two-hundred toothbrushes with standardized dimensions, bristles, and color (Dentalprev®, São Paulo, SP, Brazil) were included in the study. The toothbrushes were divided into 20 experimental groups (n = 10), according to microorganism and chemical agent used.

Standardized suspensions (1×10^6 cfu/ml) of the following microorganisms were obtained spectrophotometrically: *Streptococcus mutans* ATCC 35688 (λ = 398 nm, O.D. = 0.620); *Streptococcus pyogenes* ATCC 700294 (λ = 398 nm, O.D. = 0.620), *Staphylococcus aureus* ATCC 6538 (λ = 490 nm, O.D. = 0.374) and *Candida albicans* ATCC 18804 (λ = 530 nm, O.D. = 0.284). These parameters were previously determined.[12] For the preparation of the suspensions, bacterial strains were previously plated on Tryptic Soy agar (Difco, Detroit, MI, USA) and *C. albicans* was plated on Sabouraud dextrose agar. The plates were incubated at 37°C (and 5% CO_2 for *S. mutans* and *S. pyogenes*; CO_2 Water Jacketed Incubator, Nuaire, MN, USA) for 24 h.

Each toothbrush, previously sterilized in an autoclave, was immersed into 10 ml of Tryptic soy broth (Difco, Detroit, MI, USA) for tests with bacterial strains and Sabouraud broth for tests with *C. albicans*. An aliquot of 0.2 ml of the respective standardized suspension was inoculated in each tube[12] and the tubes were incubated at the temperature and CO_2 conditions previously described for 24 h.

Groups of 10 toothbrushes contaminated by each microorganism was disinfected by the following products: 0.12% chlorexidine digluconate (Periogard®, Colgate, São Paulo, SP, Brazil); 50% white vinegar in sterile deionized water (Agrin, Castelo, Jundiaí, SP, Brazil); a triclosan-containing dentifrice solution (Colgate gel refrescância confiável®, Colgate, São Paulo, SP, Brazil); a solution, obtained according to the manufacturer's instruction, of sodium perborate-based tablets for the disinfection of toothbrushes (Aquafresh®, GlaxoSmithKline, Tokyo, Japan). The dentifrice solution was obtained by diluting 5 g of the dentifrice in 20 ml of sterile deionized water, as proposed in a previous study.[13]

The method used for the disinfection of the toothbrushes was immersion in the chemical agent for 10 min.[12] Control groups of 10 toothbrushes contaminated with the tested microorganisms were immersed into sterile deionized water instead of the disinfectant solution.

After the immersion period, the toothbrushes were transferred to tubes containing sterile distilled water for 2 seconds to eliminate the excess of the disinfectant. They were then transferred to tubes containing 10 ml of sterile physiologic solution (0.85% NaCl) and glass beads and submitted to agitation (Phoenix AP56, São Paulo, SP, Brazil) for microbial cell detachment. From the initial suspension, 10^{-1}, 10^{-2} and 10^{-3} dilutions were obtained in sterile physiologic solution, and then 0.1 ml aliquots were plated in duplicate in Sabouraud dextrose agar (Difco, Detroit, MI, USA) or Tryptic soy agar (Difco, Detroit, MI, USA). The plates were incubated at 37°C (5% CO_2 for *S. mutans* and *S. pyogenes*) for 48 h. After this period of incubation, the number of colonies in each plate was counted and the number of colony-forming units per toothbrush was obtained (cfu/toothbrush).

The cfu/toothbrush values for each group of microorganism after disinfection with the disinfectants tested in the study were compared by Kruskal-Wallis ANOVA and Dunn's test for multiple comparisons (5%).

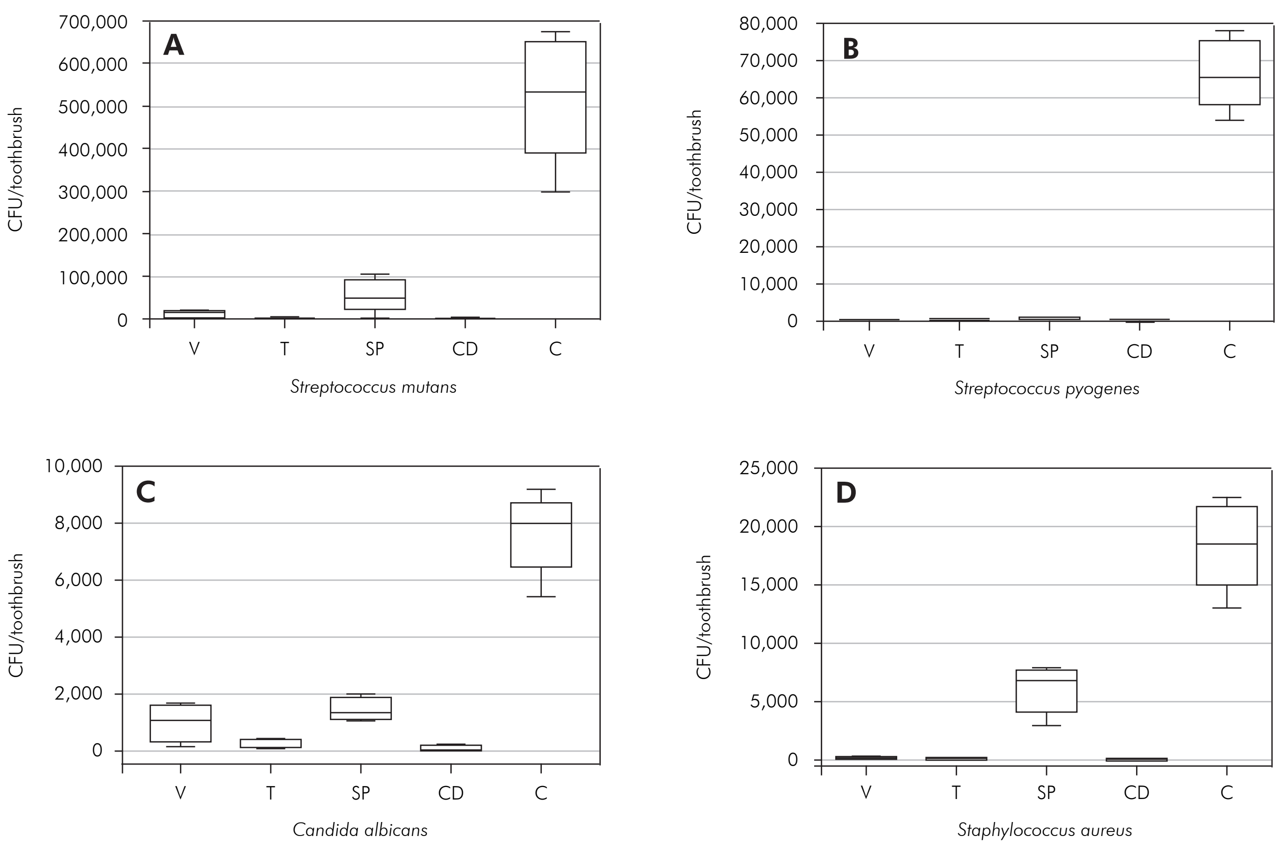
Results

The data obtained, expressed in cfu/toothbrush, for the control groups and experimental groups are shown in Graph 1.

Analysis of the data indicated statistically significant differences among the distributions of the values obtained for the different groups of disinfectants (p = 0.0001) for all the tested microorganisms (Table 1). The chlorhexidine digluconate solution was the most effective for the disinfection of toothbrushes.

The dentifrice solution promoted a significant reduction of all the microorganisms' counts in relation to the control group.

The final counts of *C. albicans* after disinfection with the vinegar solution did not show statistically significant difference in relation to the control group. For the other microorganisms tested, a



Graph 1 - Box-plot of the data obtained before (control group - C) and after (V - vinegar; T - toothpaste; SP - sodium perborate; CD - chlorhexidine digluconate) disinfection of the toothbrushes contaminated *in vitro* by *Streptococcus mutans*, *Streptococcus pyogenes*, *Candida albicans* and *Staphylococcus aureus*.

Table 1 - Median values (cfu/toothbrush) of the four microbial species' counts according to disinfectant used. Homogeneity among groups is indicated by the same letters in HG columns; the comparison among the disinfectant groups for each microbial species was evaluated by the application of the Kruskal-Wallis test followed by the Dunn test (multiple comparison procedure).

Disinfectant	*Candida albicans*				*Staphylococcus aureus*				*Streptococcus mutans*				*Streptococcus pyogenes*				
	median	HG			median	HG			median	HG			median	HG			
Control	8,000	A			18,500	A			532,500	A			65,250.0	A			
Sodium perborate (tablets)	1,325	A			6,750	A	B		49,250	A	B		860	A	B		
Vinegar (50%)	1,075	A	B		187.5		B	C	15,500		B	C	682.5		B	C	
Toothpaste (solution)	95		B	C	175.5		B	C	785		B	C	337.5			C	D
Chlorhexidine digluconate (0.12%)	0			C	0			C	0			C	0				D

*Median values in the same column with the same letter are not significantly different by the Dunn test (5%).
**for *Candida albicans*, T = 43.9661 (p = 0.0001); for *Staphylococcus aureus*, T = 45.0836 (p = 0.0001); for *Streptococcus mutans*, T = 46.6599 (p = 0.0001); for *Streptococcus pyogenes*, T = 47.4507 (p = 0.0001).

significant reduction was observed in relation to the control group after disinfection with this solution.

The sodium perborate solution was the less effective, and the counts of microorganisms after disinfection with this product were not statistically different in relation to the control group.

Discussion

Although the related literature has demonstrated the contamination of toothbrushes, as well as its potential for the transmission of oral and systemic diseases, little attention has been given to the disinfection of toothbrushes. Even the use of a single toothbrush per family is still observed, mainly among low-income populations. Taiji, Rogers[4] (1998) identified *Candida*, *Corynebacterium*, *Pseudomonas* and coliforms in used toothbrushes. Other studies concluded that these microorganisms may survive for more than 6 hours after utilization of the toothbrush. These authors correlated these results with the possibility of cross-infection, which is of great importance, particularly among children and immunocompromised patients, and reinforced the role of the daily disinfection of toothbrushes.[11,14]

The selection of microorganisms used in the present study was based on their pathogenic potential. *C. albicans* is the main cause of oral candidosis, and the prevalence of this fungal infection has increased significantly over the past decades.[15] In patients with AIDS, bone marrow transplantation and aggressive anti-neoplasic therapy, this infection is cited as an important cause of mortality and morbidity.[16] *S. mutans* is the main species related to dental caries, and the presence of viable forms of this microorganisms in toothbrushes has been reported.[17,18] *S. aureus* is correlated with several human infections as pneumonia, sepsis, osteomielitis, and abscesses.[19] This species can cause potentially severe infections in immunocompromised patients.[20] *S. pyogenes* is a major causative agent of pharyngitis and tonsillitis.[21] A previous study reported the persistence of group A beta-hemolytic streptococci in toothbrushes and suggested that it could contribute to the persistence of these microorganisms in the oral pharynx and may account for the failure of penicillin therapy in some cases of pharyngotonsillitis.[22]

According to Devine *et al.*[5] (2007), there is a need for disinfection methods that are rapidly effective, cost-effective, nontoxic and that can be easily implemented. However, most of the proposed methods, such as chlorhexidine gluconate,[6,7] tetrasodium EDTA and UV sanitization[5,11], fail mainly in terms of cost-effectiveness and ease of implementation.

The results of the present study regarding the high effectiveness of chlorhexidine digluconate are in accordance with previous results, and the total absence of viable microorganisms was observed after immersion for 10 minutes. Chlorhexidine digluconate is usually indicated as the first-choice antiseptic in Dentistry due to its high antimicrobial activity and effectiveness for pre-surgical antisepsis.[3,23] Nelson-Filho *et al.*[7] (2000) studied the disinfection of children's toothbrushes using 0.12% chlorhexidine digluconate for 20 hours and reported total destruction of microorganisms. In spite of the high efficacy observed with this substance, it is considered expensive, thus limiting its widespread use by the population.

Previous studies reported that brushing with dentifrices with specific components may reduce the bacterial contamination of toothbrushes.[6,7,8,9] Komiyama *et al.*[13] (2004) observed that triclosan-containing dentifrices were the most effective against *Streptococcus mutans*. Triclosan is a phenolic substance with antimicrobial properties and also anti-inflammatory activity.[4,6,7] Based on these studies, an experimental group including the solution obtained from the soluble portion of a triclosan-containing dentifrice was included in the present study. *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pyogenes* and *Candida albicans* counts were significantly reduced in relation to the control group after the disinfection in this solution for 10 min, suggesting that it may have potential home-use application.

Considering its low toxicity and low cost, and aiming at proposing a viable home-use application, acetic acid, one of the components of vinegar, was tested. This solution has been suggested as an interesting alternative as a disinfectant in other areas. The use of an acetic acid-based solution for the disinfection of semi-critical articles, for the treatment of oral inflammatory processes (as a mouthwash) and as an anti-septic for sores has been reported in the literature.[24] Few studies have reported the use of acetic acid-based solutions (vinegar) in Dentistry. Silva *et al.*[12] (2008) observed a good antimicrobial effectiveness against *C. albicans* and *S. aureus* in the disinfection of acrylic resin. The reduction in the number of *S. pyogenes* experimentally inoculated in toothbrushes was observed after disinfection with vinegar.[2] Azuma *et al.*[25] (2006) analyzed different concentrations of several trademarks of vinegar and observed that low concentrations (3-6%) were effective *in vitro* against *C. albicans*. On the other hand, in the present study, the vinegar-based solution did not reduce the *C. albicans* counts significantly. These results might be correlated with the different experimental model adopted in the present study when compared to that of previous reports. The formation of biofilm by *C. albicans* on toothbrushes' bristles might be different when compared to that occurring on other materials. Meanwhile, a significant reduction of *S. aureus*, *S. mutans* and *S. pyogenes* was observed after disinfection with this substance, and this result can be considered promising and suggests a possible application.

Sodium perborate-based tablets are indicated for the cleansing of prostheses and orthodontic appliances associated with mechanical action.[6] Some authors have observed the antimicrobial activity of these products on prostheses.[12,26] Harrison *et al.*[26] (2004) and McCabe *et al.*[27] (1995) observed that sodium perborate-based tablets contributed significantly to the treatment of prosthetic stomatitis. There are no studies in the literature on the effectiveness of sodium perborate-based tablets for the disinfection of toothbrushes. In the present study, they did not present good antimicrobial effectiveness. However, further studies on this product are recommended, perhaps testing different periods of evaluation and procedures.

Conclusion

Based on the results, it could be concluded that the solutions based on the triclosan-containing dentifrice tested may be considered effective, nontoxic, cost-effective, and an easily applicable alternative for the disinfection of toothbrushes. The vinegar solution tested reduced the presence of *S. aureus*, *S. mutans* and *S. pyogenes* on toothbrushes.

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