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ORIGINAL ARTICLE

Children's toothbrush contamination in day-care centers: how to solve this problem?

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Abstract
Objective To evaluate the contamination level of toothbrushes by mutans streptococci (MS) and the efficacy of antimicrobial solutions: cetylpyridinium chloride 0.05 % (CPC; Cepacol™) and chlorhexidine 0.12 % (CHX; Periogard™), to disinfect toothbrushes of preschool-aged children in day-care centers.
Material and methods Fifty-two children were randomly divided into three groups, and a three-stage changeover system was used with a 1-week interval between each stage. Solutions were used by a different group of children in each stage. Children were submitted to a 1 minute brushing without dentifrice, performed by a professional calibrated, followed by random spraying over the bristles of brushes. Process and microbiological analysis were realized, and four brushes of each group were analyzed by scanning electron microscopy (SEM).
Results Friedman's test at 5 % significance level revealed difference between the antimicrobial solutions ($p<0.01$). MS were detected in 100 % cases of toothbrushes sprayed with sterile tap water (control) and in 66.7 % after spraying with CPC, but it was not detected formation of colonies/biofilms after spraying with CHX. The data were confirmed by SEM.
Conclusions The toothbrushes were contaminated with MS after a single brushing.
Clinical relevance Although CPC has shown good results in comparison with the control, CHX showed greater efficacy in disinfection bristles of toothbrushes.

Keywords Toothbrushes · Disinfection · Day-care centers · *Streptococcus mutans*

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Introduction

In an attempt to improve the quality of life [1] in industrialized society, the employment of mothers outside the home requires nonmaternal care of various kinds [2], such as day care, where young children spend a considerable amount of time [1]. The oral health education in child care centers should address concepts of transmission of oral bacteria and preventive measures, since children would benefit from the promotion of oral health [1]. The routine use of contaminated toothbrushes can contribute to disseminate microorganisms within the oral cavity [3] of a same person or between different individuals because the microorganisms can remain viable on toothbrush bristles for periods ranging from 24 h to 7 days [4]. Eventually, there may also be direct contact between toothbrushes of different family members, in bathroom drawers or cabinets [5]. Furthermore, the control of the occurrence of salivary contact among children staying in environments, such as kindergartens, preschools, and other institutions that take care of young children [6], is difficult to be realized, and toothbrushes can be carelessly exchanged or shared. Practical measures, effective and low cost, are needed to control the contamination of toothbrushes, as the proper way to wash, disinfect, and store toothbrushes.

Toothbrushes can be contaminated by different types of bacteria [3, 7–9], viruses [4], and fungi [10, 11] after being

used for mechanical oral hygiene and stored under usual conditions, thus becoming sources of inoculation and/or re-inoculation of potentially pathogenic microorganisms such as *Streptococcus mutans* [12]. Moreover, contamination can occur through variation in toothbrush design [12]. For this reason, some researchers have been interested in evaluating microbial contamination of toothbrushes by emphasizing the importance of disinfection for general and oral health [3, 7, 8, 11–14].

There is an increasing concern about oral health of young children and great interest regarding the early mutans streptococci (MS) colonization of oral cavity because of the risk of caries [15]. It is known that transmission of cariogenic microorganisms occurs not only by direct contact (e.g. saliva) [16], but also by indirect contact [5] by means of objects such as toothbrushes contaminated [17]. Nevertheless, the literature on contaminated toothbrushes of preschool-aged children is scanty [14]. Further studies are needed in order to avoid cross infection, re-inoculation of microorganisms, and reduce contamination of non-contaminated surfaces.

The objective of the present study was to assess in vivo the MS contamination of toothbrushes used by preschool-aged children as well as the efficacy of antimicrobial spray solutions cetylpyridinium chloride 0.05 % (CPC; Cepacol™) and chlorhexidine 0.12 % (CHX; Periogard™) for toothbrush disinfection by microbial culture and scanning electron microscopy (SEM), by randomized clinical trial.

Material and methods

Randomized clinical trial

This study was independently reviewed and approved by the Ethics Committee in Research of the School of Dentistry of Ribeirão Preto, University of São Paulo (Process #2002.1.999.58.3). Written informed consent of parents and verbal willingness of the children were granted for those included.

Initially, 53 children were preselected, but only 52 children of both sexes (29 boys and 23 girls) 24 to 48 months old (mean age=39 months) were recruited from the day-care center Institution Betânia House, Ribeirão Preto, São Paulo, Brazil, for study according to the inclusion/exclusion criteria. The participants should have complete primary dentition, not under dental treatment, and not under therapy with antibiotics or antiseptic mouth rinses for at least 3 months. Furthermore, they should have the presence of MS in saliva, as detected in SB-20 M culture medium, which was prepared according to Davey and Rogers [18] and modified by replacement of sucrose with sugar-cane [8, 19]. The baseline salivary levels of MS in all 52 children before tooth brushing procedures ranged from 20 to 3×10^6 cfu/ml. Exclusion criteria used were: children with special needs (poor health), children who were in dental treatment and under therapy with antibiotics or antiseptic mouth rinses for at least 3 months, and absence of MS in saliva. It was observed that 52 of the 53 children had MS in saliva, ranging from 20 to 3,000,000 cfu. Among the 52 children, a total of 100 % showed *S. mutans* at levels varying from 20 to 2,720,000 cfu/ml, whereas only 11.5 % were *Streptococcus sobrinus* at levels ranging from 1,800 to 280,000 cfu/ml of saliva.

The following solutions were evaluated: Sterile tap water (control group), CPC 0.05 % (Cepacol™; Aventis Pharma Ltda — Suzano, São Paulo, Brazil), and CHX 0.12 % (Periogard™; Colgate-Palmolive Company, São Paulo, Brazil).

The solutions CPC, CHX, and sterile tap water were individually placed in plastic trigger-spray bottles (Elyplast, São José dos Campos, São Paulo, Brazil). Aluminum foils codified were used to cover bottles and perform a blind evaluation of the solutions.

The fifty-two children were divided by lot (randomization), using the table of random numbers into three groups that followed the protocol "cross-over" random, thus forming two groups of 17 children each and one group of 18 children. A three-stage cross-over system was used with a 1-week interval between each stage. All three solutions were used in all stages, however each solution was used by a different group, in the form of rotation, to minimize the impact of variables that could interfered in the results. Knowing that the type of dentifrice can influence the microbial contamination of the bristles of toothbrushes [20, 21], at each stage, children's teeth were brushed by a dentist without dentifrice using new toothbrushes taken from their original packages (Colgate Baby-Barney, Colgate-Palmolive Company, São Bernardo do Campo, São Paulo, Brazil).

After each tooth brushing, the bristles were carefully rinsed, and excess water was removed. The toothbrushes were held in a vertical position, and the solutions were sprayed six times onto the bristles at a distance of 5 cm (approximately 0.6 ml of solution per toothbrush) in all sides of the toothbrush head. Excess solution was removed from the bristles hitting carefully the handle of toothbrush against the sink.

The toothbrushes were maintained in a closed custom container to avoid contact between them, but allowing air circulation for drying, and kept at room temperature for 4 h to simulate the interval between brushings [8, 21].

Five unused toothbrushes (additional control) were taken from their original packages and submitted to microbiological processing to investigate whether the new toothbrushes were contaminated during manufacture and packaging processes.

All examiners were blinded to the group being examined by culture or by SEM.

Microbiological procedures

After 4 h of interval between brushings, the toothbrushes of each group were separated and vertically placed into 25× 150 mm test tubes containing 10.0 ml CaSa B (Bacitracin Sucrose Broth — selective enrichment broth prepared by the modification of Jensen and Brattall [22], medium specific for MS without trypan blue) for 3 to 4 days at 37 °C. It was care taken to avoid contact of the bristles with the test tube walls. The toothbrushes were withdrawn and rinsed in the broth with gentle shaking to remove planktonic microbiota, leaving sessile bacteria adhered as "spike" or "mushroom-like" colony/biofilms. The toothbrush bristles were carefully examined from all directions, and MS colonies were counted using a stereomicroscope (Nikon, Tokyo, Japan) with reflected light.

The number of MS colonies/biofilms on the bristle surfaces was expressed according to a ranking scale: Score 0=no colonies/biofilms were detected, indicating the absence of microorganisms on bristle's surface; score 1=1 to 50 colonies/biofilms; score 2=51 to 100 colonies/biofilms; and score 3=over 100 colonies/biofilms (intense bacterial growth with confluent colonies and not allowing an accurate counting of the number of colonies/biofilms).

Four to five colonies were collected from the bristles of 3 to 4 toothbrushes in each group and transferred to tubes containing 2.0 ml of phosphate-buffered solution and glass beads. The colonies were vortexed for 2 min, and the resulting suspension was seeded on SB-20 M agar (tryptone soy yeast agar plus 20 % sucrose and 0.2 U/ml Bacitracin, Sigma, Saint Louis, MO, USA) and incubated in microaerophilia at 37 °C for 72 h. The growth of colonies/biofilms was verified after the incubation period.

Tests were conducted to biochemical identification by fermentation of mannitol, sorbitol, raffinose, and melibiose [23] and hydrogen peroxide production [24].

Microbiological results were analyzed using Friedman's nonparametric test at 5 % significance level and 8.1 GMC statistical software package (Dr. Campos, Faculty of Dentistry of Ribeirão Preto, University of São Paulo, São Paulo, Brazil).

Scanning electron microscopy (SEM)

After microbiological processing, four representative toothbrushes of each group were fixed in 4 % glutaraldehyde in cacodylate buffer, pH 7.4, at 37 °C. Two bristle tufts of each toothbrush were removed, post-fixed with 1 % osmium tetroxide, dehydrated in ascending ethanol grades, and critical-point dried with liquid carbon dioxide. Subsequently, eight bristles of these tufts were separated, mounted on stubs, sputter-coated with gold, and examined on scanning electronic microscope Zeiss (DSM 940A, Jena, Germany) at 15 kV.

Table 1 Number of colonies/biofilms of *Streptococcus mutans* in the bristles of toothbrushes for children after brushing and using different solutions

Case	Sterile tap water	CPC	CHX
1	Uncountable	26	0*
2	Uncountable	31	0*
3	Uncountable	53	0*
4	Uncountable	29	0*
5	Uncountable	+100	0*
6	Uncountable	0*	0*
7	Uncountable	0*	0*
8	Uncountable	5	0*
9	Uncountable	0*	0
10	Uncountable	83	0*
11	Uncountable	0*	0*
12	Uncountable	0*	0*
13	Uncountable	0*	0*
14	63	17	0*
15	Uncountable	38	0*
16	Uncountable	5	0*
17	Uncountable	0	0*
18	Uncountable	11	0*
19	Uncountable	0*	0*
20	Uncountable	0*	0*
21	Uncountable	28	0*
22	Uncountable	42	0*
23	Uncountable	100	0*
24	Uncountable	100	0*
25	Uncountable	0*	0*
26	Uncountable	73	0*
27	Uncountable	25	0*
28	72	0*	0*
29	Uncountable	13	0*
30	18	16	0*
31	Uncountable	8	0*
32	2	4	0*
33	Uncountable	21	0*
34	Uncountable	100	0*
35	Uncountable	47	0*
36	Uncountable	75	0*
37	Uncountable	0*	0*
38	Uncountable	0*	0*
39	Uncountable	0*	0*
40	Uncountable	19	0*
41	Uncountable	33	0*
42	Uncountable	12	0*
43	Uncountable	63	0*
44	Uncountable	0*	0*
45	Uncountable	4	0*
Total number of positive cases for MS	45 (100 %)	30 (66.7 %)	0 (0 %)

0*: absence of microbial growth, 0: turbidity of the culture medium

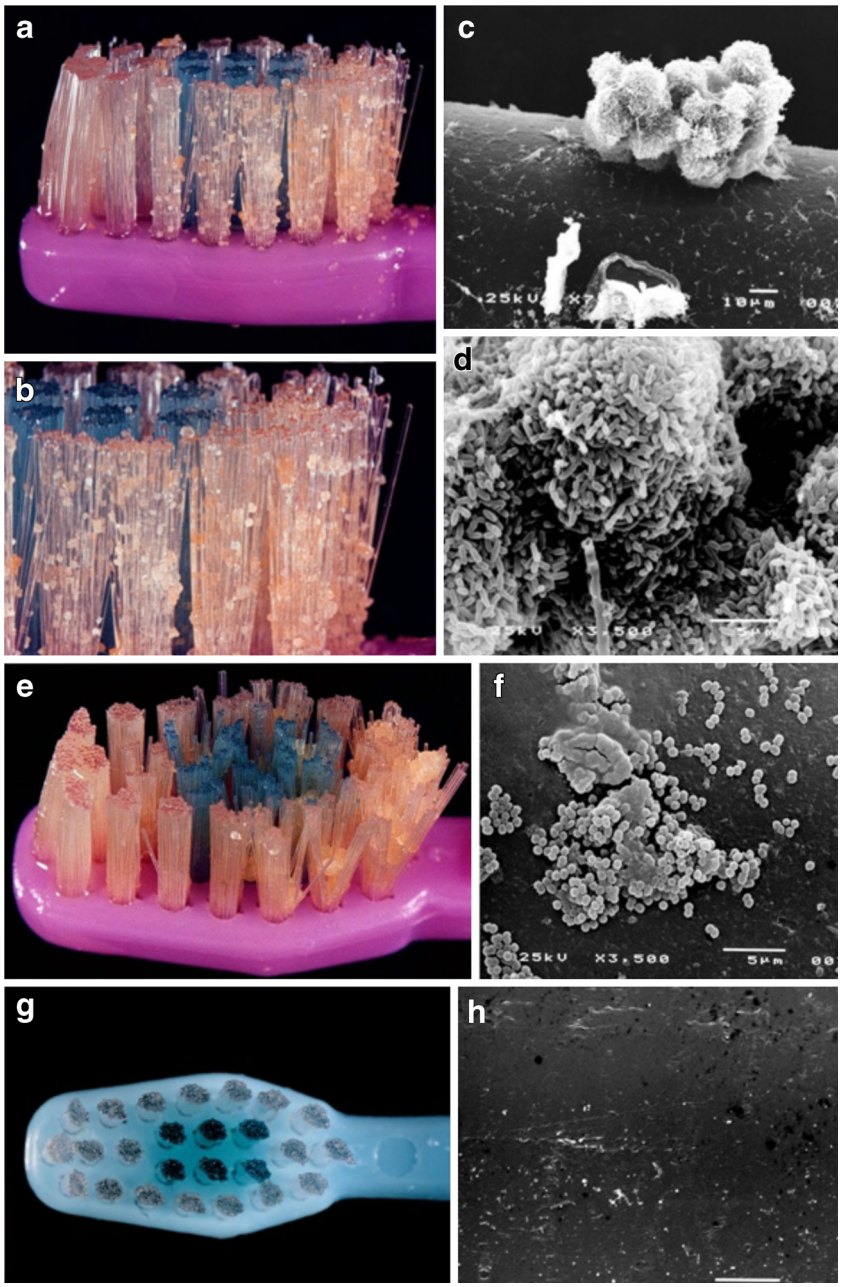

Fig. 1 Sterile tap water: **a** and **b** Many mutans streptococci colonies/biofilms on the toothbrush bristles, after microbial culture. **c** and **d** SEM micrograph showing the formation of mutans streptococci colonies/biofilms (×750 and 3,500 magnification). Cepacol™. **e** Few mutans streptococci colonies/biofilms on the toothbrush bristles, after microbial culture. **f** SEM micrograph showing the formation of mutans streptococci colonies/biofilms after microbial culture (×3,500 magnification). Periogard™. **g** No mutans streptococci colonies on toothbrushes after chlorhexidine 0.12 % treatment. **h** SEM micrograph representative of toothbrush bristles sprayed with chlorhexidine 0.12 %, showing the absence of microorganisms (×3,500 magnification)

Results

Randomized clinical trial

From 52 children initially enrolled in this study, 45 (87 %) participated in all three stages of the randomized clinical trial. Table 1 shows the number of cases according to the number of MS colonies on the toothbrush bristles after tooth brushing and spraying with the tested solutions.

MS were detected on the bristles of all toothbrushes (100 %) in the control group (sprayed with sterile tap water) with a strong predominance of score 3. The number of colonies/biofilms ranged from 2 to uncountable (Fig. 1a and b).

After using CPC, it was observed the presence of MS in 30 toothbrushes (66.7 %), with the number of colonies/biofilms ranging from 4 to uncountable (Fig. 1e). No colonies were observed in 15 toothbrushes (33.3 %).

On the other hand, after CHX use, no colonies were observed in all toothbrushes (100 %) (Table 1).

There were significant differences in numbers of MS colonies detected after treatment with the antimicrobial solutions (x^2=74.10; p<0.01) by Friedman's test. Both CPC and CHX

reduce colony formation on toothbrushes, with the latter solution giving the better results.

Seeding the colonies in SB-20 M medium confirmed that those microorganisms growing on the bristle's surface were MS. No microbial growth was observed on the unused toothbrushes after incubation at 37 °C for 20 days.

Scanning electronic microscopy

In all groups, when microbiological culture was positive by stereomicroscopy, bacteria were observed on toothbrush using SEM analysis (Fig. 1c, d, f). Bacteria were not observed using a stereomicroscope. No microorganisms or only sparse one were observed on SEM examination (Fig. 1h).

Discussion

Despite the increasing concern about prevention of dental caries in young children, the evaluation of toothbrush contamination by children less than 4 years old has received little attention [14]. In the present study, there was 100 % of contamination by MS on toothbrush bristles after a single 1-minute brushing followed by spraying with sterile tap water (control). These findings are in agreement with those of Nelson-Filho et al. [8], Nelson-Filho et al. [21], and Sato et al. [13] who observed high levels of *S. mutans* contamination in toothbrushes used by 5–12-year-old children and adults.

The present study indicated the need to disinfect toothbrushes following use because drying for 4 h did not eliminate viable bacteria. According to Saravia et al. [25], *S. mutans* can remain viable on dried bristles for up to 8 h.

In addition to cariogenic microorganisms, toothbrushes can be contaminated by other bacteria [7, 9], viruses [4], yeasts, and fungi [10, 11], which may promote dissemination of these pathogens [26]. This is important in immune-suppressed, cardiac, and transplant patients, since tooth brushing usually causes a transient bacteremia [27] that may trigger bacterial endocarditic in the case of cardiac abnormalities [28].

Poor toothbrush care results from lack of knowledge, including by dental practitioners, that toothbrushes can be contaminated and require disinfection. Generally, toothbrushes are rinsed with water before storing.

Among the antimicrobial solutions being used for toothbrush disinfection, one can cite the cetylpyridinium chloride, commercially known as Cepacol™, which has been evaluated elsewhere with satisfactory results [13, 29]. This agent is a quaternary ammonium compound whose antibacterial activity is the result of inactivation of energy-producing enzymes, denaturation of essential proteins, and disruption of cell membrane. Also, cetylpyridinium chloride acts upon Gram-positive microorganisms and some Gram-negative ones in addition to fungi [30]. In the present study, CPC promoted complete suppression of MS in only 33.3 % of the cases. This low efficacy, compared to the results obtained by Sato et al. [13] and Caudy et al. [29], was probably due to the randomized clinical trial design as the study was carried out in vivo using no dentifrice, and the subjects had high levels of MS in their saliva.

On the other hand, analysis of the results regarding CHX showed inhibition of colony/biofilm formation by MS in all toothbrushes, a finding also reported by Nelson-Filho et al. [8] and Nelson-Filho et al. [14].

These finding suggest that MS can be transferred between individuals after toothbrush contamination, and that antimicrobial solutions can inhibit toothbrush contamination. Further studies are needed to know the effect of disinfecting procedures on oral health.

Conclusion

In summary, after a single use, all toothbrushes used by preschool-aged children in day-care center were contaminated by MS. Although the CPC showed better results compared to controls, the CHX was found to be more efficacious in disinfecting the toothbrushes. The disinfection of toothbrushes must be made daily after the brushing of teeth with dentifrice, with a spray containing CHX, as a control routine and complementary to infection by MS.

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Conflict of interest The authors declare that they have no conflict of interest.

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