EVALUATION OF MICROBIAL CONTAMINATION AND EFFICACY OF ANTIMICROBIAL AGENTS IN DISINFECTION OF HANDICAPPED PATIENTS' TOOTHBRUSHES

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Palavras-chave: Desinfecção. Estreptococos do grupo mutans. *Streptococcus mutans*. Escova de dentes. Pacientes especiais.

RESUMO

Objetivo: O presente estudo teve como objetivo avaliar a contaminação de escovas de dente utilizadas por pacientes especiais, por meio de cultura microbiana e formação de biofilme cariogênico, explorando dois métodos de desinfecção. **Métodos**: O estudo foi dividido em três estágios, com o mesmo intervalo de tempo entre cada estágio. No primeiro estágio, os pacientes escovaram os dentes e enxaguaram com água, em seguida, suas escovas foram borrifadas com água destilada. No segundo e terceiro estágios, as etapas foram semelhantes às do estágio I, exceto que as escovas de dente foram borrifadas com soluções de clorexidina 0,12% e cloreto de cetilpiridínio 0,05%, respectivamente. Ao final de cada etapa, as cerdas das escovas de dente foram cultivadas em meio de Caldo Sacarose Bacitracina (CaSaB). Os dados foram analisados por meio do teste não paramétrico de Friedman (nível de significância de 5%). **Resultados**: No estágio I, os estreptococos do grupo *mutans* (EM) estavam presentes em 30 escovas de dente (76,9%), e o número de colônias / biofilmes variou de 0 a +100. No estágio II, nenhuma colonização por MS foi observada. No estágio III, apenas 10,2% das escovas de dente estavam contaminadas com MS, e o número de colônias / biofilmes variou de 1 a 31. **Conclusão**: As cerdas das escovas de dente utilizadas por pacientes especiais contaminaram-se com EM após uma única escovação. A solução de clorexidina 0,12% eliminou todos os microrganismos das cerdas das escovas de dente utilizadas pelos pacientes. Ambas as soluções em spray (gluconato de clorexidina 0,12% e cloreto de cetilpiridínio 0,05%) podem ser utilizadas com eficácia para desinfecção das escovas de dente para reduzir a contaminação.

Keywords: Disinfection. Mutans group streptococci. *Streptococcus mutans. Toothbrushes. Handicapped patients.*

ABSTRACT

Objective: This study aimed to evaluate the contamination of toothbrushes used by patients with disabilities, by microbial culture and cariogenic biofilm formation, and to explore two methods of disinfection. **Methods**: Experimental procedures were divided into three stages, with the same interval between each stage. In the first stage, the patients brushed their teeth, rinsed them with water, and their toothbrushes were sprayed with sterilized tap water. In the second and third stages, the steps were similar to those of Stage I, except the toothbrushes were sprayed with 0.12% chlorhexidine and 0.05% cetylpyridinium chloride solutions, respectively. At the end of each stage, the toothbrush bristles were cultured in bacitracin sucrose broth (CaSaB) medium. Data were analyzed through Friedman's nonparametric test (5% significance level). **Results**: In Stage I, mutans group streptococci (MS) were present in 30 toothbrushes (76.9%), and the number of colonies/biofilms ranged from 0 to +100. In Stage II, no MS colonization was observed. In Stage III, only 10.2% of the toothbrushes were contaminated with MS, and the number of colonies/biofilms ranged from 1 to 31. **Conclusion**: Bristles of toothbrushes used by patients with disabilities became contaminated with MS after a single brushing. The 0.12% chlorhexidine solution eliminated all microorganisms from the bristles of the toothbrushes used by the patients. Both 0.12% gluconate chlorhexidine and 0.05% cetylpyridinium chloride spray solutions can effectively be used for toothbrush disinfection to reduce contamination.

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INTRODUCTION

According to the Clinical Guidelines and Integrated Pathways of Care for Oral Health of Persons with Learning Disabilities (2012),[1] a good oral care routine is important for everyone, especially for patients with disabilities. Unfortunately, there is evidence that patients with disabilities present poor general and oral health, they also have unmet health needs and less acceptance of screening services.[2,3] Currently, people with special needs have more dental issues, including dental caries, periodontal diseases, and missing teeth; they also experience more difficulty obtaining dental care compared with other segments of the population.[4-9] Additionally, children with disabilities appear to have higher incidence of caries and higher levels of unmet dental needs and poor oral hygiene compared with healthy controls.[6-9]

The general state of oral health is related to poor oral hygiene routine and contributes to the occurrence of systemic diseases. This fact should raise greater concern when it comes to people with special needs, who usually have systemic alterations, including immune deficiency. Toothbrushes are the primary method for removing dental biofilm. However, when bacteria survive on toothbrushes, they can reinoculate the oral cavity of the original host. The multiplication and increase in the number of these microorganisms may represent a significant risk of dissemination.[10-13] Several studies have shown that toothbrushes can be contaminated after use[14-17] by different types of bacteria,[18] viruses,[19] and fungi,[20,21] that are present in the oral cavity; *Streptococcus mutans* remain alive on toothbrushes for 44 hours. For this reason, disinfection methods for toothbrushes should be indicated, especially for patients with disabilities.[22] There are no studies that specifically examine toothbrush contamination and its effect on oral health in vulnerable populations.[23]

This study aimed to evaluate toothbrush contamination by mutans group streptococci (MS) after use in patients with disabilities. The efficacy of the use of 0.12% chlorhexidine and 0.05% cetylpyridinium chloride solutions in toothbrush disinfection was also examined.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of the Faculty of Dentistry of the University of São Paulo, Brazil (Protocol number 2002.1.471.58.9), and written informed consent was obtained from all parents or guardians of the participants.

A total of 39 individuals with disabilities, aged 6 to 20, of both genders, participated in this study. The experimental procedures were divided into 3 stages, with a 3-day interval between Stages I and II, and II and III.

In Stage I, patients underwent a 1-minute brushing performed with dentifrice by a single dentist (Sorriso – Kolynos do Brasil Ltd, São Bernardo do Campo, São Paulo, Brazil) and using new toothbrushes taken directly from their original packaging. Then the bristles were rinsed and excess water was removed. The toothbrushes were held upright and the bristles were sprayed with sterilized tap water. In Stages II and III, a new brushing was performed with the same dentifrice and 0.12% chlorhexidine (PerioGard, Colgate-Palmolive Company, NY, NY, USA) and 0.05% cetylpyridinium chloride solutions (Reach oral antiseptic, Johnson & Johnson, New Brunswick, NJ, USA) were sprayed 6 times on the bristles at a distance of 5 cm (approximately 0.6 mL solution per toothbrush) in different areas: (1) right side, (2) left side, (3) top, (4) bottom, (5) front, and (6) the back of the toothbrush head. The excess antimicrobial solution was removed from the bristles by tapping the toothbrush gently against the sink. Afterward, the toothbrushes were kept in a closed container to avoid contact between them. They were also kept at room temperature for 4 hours to simulate the interval between uses.

After this period, the toothbrushes of each group were placed individually and vertically in 25 x 150 mm test tubes containing 10.0 mL bacitracin sucrose broth (CaSaB)-selective enrichment broth prepared with the modification of Jensen and Brattall[14] (specific medium for *S. mutans* without trypan blue) for 3 to 4 days at 37°C. The toothbrushes were placed with care in order to avoid contact between the bristles and the walls of the test tube. They were removed and rinsed in the broth with gentle agitation to remove the planktonic microbiota, leaving the sessile bacteria adhered as spike or mushroom-like colonies/biofilms. The toothbrush bristles were carefully analyzed on all sides, and *S. mutans* sessile colonies/biofilms based on colony morphology were counted under aseptic conditions with a stereomicroscope (Nikon, Tokyo, Japan) with reflected light.

After incubation, the toothbrushes with no colonies/biofilms found in the bristles (score 0) were immersed in the culture medium for 20 days to evaluate the turbidity of the medium; this would indicate growth of microorganisms other than *S. mutans*. If there was no turbidity of the medium after this period, the specimens were classified as 0 *, meaning that they were considered free of microorganisms. The confirmation that the adhered microorganisms were *S. mutans* was obtained through a sequence of steps: (1) Four to five colonies/biofilms representative of bacterial development were collected from 3 to 4 toothbrushes in each group and transferred to tubes containing 2.0 mL of phosphate buffer and glass beads; (2) Colonies were shaken for 2 minutes; (3) The resulting suspension was seeded on SB20 agar (tryptone soy yeast agar + 20% sucrose and 0.2 U/

mL bacitracin; Sigma, Saint Louis, MO, USA) and incubated in microaerophilic conditions at 37°C for 72 hours; (4) Growth of colonies biofilms was verified after the incubation period; (5) The following tests were performed for biochemical identification: fermentation of mannitol, sorbitol, raffinose and melibiose; hydrolysis of arginine and esculin; H2O2 production; and sensitivity to 2.0 IU of bacitracin.[24]

The microbiological results were statistically analyzed by Friedman's nonparametric test at a significance level of 5%, using GMC statistical software, version 8.1 (Geraldo Maia Campos - School of Dentistry of Ribeirão Preto, University of São Paulo, São Paulo, Brazil).

RESULTS

Thirty-nine patients with disabilities participated in the randomized clinical trial. All participants completed the three stages.

S. mutans colonies/biofilms were detected in 30 of 39 toothbrushes (76.9%) in Stage I (sprayed with sterilized tap water), with colonies/biofilms ranging from 2 to +100. The 9 toothbrushes (23.1%) that did not show colonization of the *S. mutans* showed turbidity of the medium, which were considered to be positive cultures.

In Stage II, in which the 0.12% chlorhexidine solution was used for disinfection, no *S. mutans* colonies/biofilms were observed in all cases, showing 100% efficacy. However, after the turbidity of the medium, other microorganisms were evidenced in 8 toothbrushes (20.5%). Absence of microorganisms (classification 0 *) was evidenced in 31 toothbrushes (79.5%).

In Stage III (disinfection with 0.05% cetylpyridinium chloride solution), only 4 toothbrushes (10.3%) were contaminated with *S. mutans*, with the number of colonies/biofilms ranging from 1 to 31. A total of 35 toothbrushes (89.7%) were not contaminated with *S. mutans*. However, the presence of other microorganisms evidenced by the turbidity of the medium was observed in 17 toothbrushes (43.6%). No microorganisms (classification 0 *) were observed in 18 toothbrushes (46.2%). All solutions differed statistically from each other (p <0.01) (Figures 1 and 2).

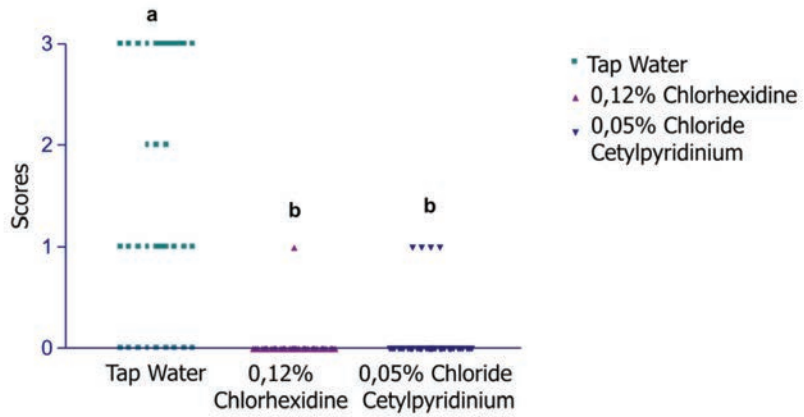

***Figure 1**: Stage I (sterilized tap water) 0 (0%) microorganism-free toothbrushes (0 *); Stage II (chlorhexidine gluconate 0.12%) 31 (79.5%) microorganism-free toothbrushes (0 *); Stage III (0.05% cetylpyridinium chloride) 18 (46.2%) microorganism-free toothbrushes (0 *). In this figure, different letters indicate statistically significant differences.*

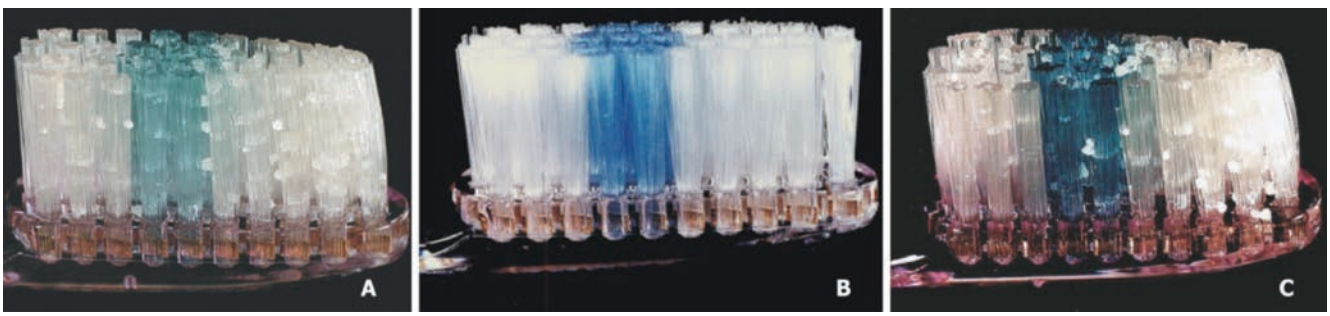

***Figure 2**: (A) Stage I (sterilized tap water) Intense development of Streptococcus mutans colonies/biofilms on toothbrush (bacterial biofilm); (B) Stage II (0.12% chlorhexidine) No development of S. mutans colonies/biofilms on toothbrush; (C) Stage III (0.05% cetylpyridinium chloride) Presence of small number of S. mutans colonies/biofilms on toothbrush (bacterial biofilm).*

DISCUSSION

The present study indicates that among the solutions tested, the best solution for the disinfection of toothbrushes for individuals with disabilities is 0.12% chlorhexidine. Our finding is in line with those of Nelson-Filho et al.[22] who evaluated the disinfection of toothbrushes in the general population. In the study by Nelson-Filho et al.[22], *S. mutans* contamination was detected in toothbrushes used by patients, after a single use. However, given the motor difficulty and immunodeficiency that patients with disabilities present, the present study becomes relevant. Additionally, patients with disabilities have a higher risk of caries and greater difficulty in finding quality treatment.[6,7,8,9,25]

The present study found that *S. mutans* contamination in toothbrush bristles used by patients with disabilities was almost completely eliminated (79.5%) through the use of chlorhexidine spray. This finding is consistent with those of other studies[4,21,22,26,27] that have found 0.12% chlorhexidine solution to be highly effective in disinfecting toothbrushes in children and adults.

The use of cetylpyridinium chloride solution as a disinfection method showed good results in the present study. Several other studies[14,20,23] have also demonstrated high efficacy in disinfecting toothbrushes with the solution spraying method.

An important aspect to consider is that toothbrushes can be contaminated by other pathogens responsible for different local and systemic diseases as well as by cariogenic microorganisms. According to Glass[15], the microorganisms found in the bristles of toothbrushes can not only cause oral diseases, but also gastrointestinal respiratory infections. In patients with disabilities, who often have several mental deficiencies and physical issues, this information is even more relevant in relation to the risk of bacteremia.

Considering the contamination of toothbrushes by a wide range of microorganisms and the effectiveness of antimicrobial sprays in preventing microbial growth and accumulation, the need to disinfect toothbrushes after each use should be widely diffused and strongly emphasized.

CONCLUSION

Bristles of toothbrushes used by patients with disabilities became contaminated with MS after a single brushing. The 0.12% chlorhexidine solution eliminated all microorganisms from the bristles of the toothbrushes, and was the most effective among the evaluated solutions (sterilized tap water, 0.12% chlorhexidine, and cetylpyridinium chloride solution). Both 0.12% gluconate chlorhexidine and 0.05% cetylpyridinium chloride spray solutions can effectively be used for toothbrush disinfection to reduce contamination.

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