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ORIGINAL RESEARCH

Efficacy of Microwaves and Chlorhexidine for Disinfection of Pacifiers and Toothbrushes: An *in vitro* Study

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ABSTRACT

Purpose: The purpose of this study was to evaluate, *in vitro*, the contamination of toothbrushes and pacifiers by *Streptococcus mutans*, and the efficacy of microwave and chlorhexidine for their disinfection.

Materials and methods: Sixty pacifiers and 60 toothbrushes were contaminated with *S. mutans* and then divided into groups according to the disinfection protocol: Group 1—chlorhexidine solution; group 2—microwave sterilization; and group 3—sterile tap water. The devices were evaluated microbiologically after disinfection for the survival of *S. mutans* colonies and were examined. The results were analyzed statistically by ANOVA and Turkey test.

Results: The results of both types of evaluation showed a large number of *S. mutans* colonies after spraying with sterile tap water, and chlorhexidine spraying and microwaving were effective in eliminating colonies. Groups 1 and 2 were statistically similar to each other ($p > 0.05$) and differed significantly from group 3 ($p < 0.05$).

Conclusion: The 0.12% chlorhexidine solution spray and 7 minutes of microwave irradiation were almost equally effective for disinfection of pacifiers and toothbrushes.

Keywords: Chlorhexidine solution, Pacifier, Microwave, Toothbrush.

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INTRODUCTION

Toothbrushes are free from microorganisms after the manufacturing process.[1,2] They can, however, be contaminated after only one toothbrushing during 30 seconds to 4 minutes,[3,4] by different types of bacteria,[2,5] viruses[6,7] and fungi[8,9] present in the oral cavity or the environment.[10] As public health policies emphasize the concepts of prevention and biosecurity, it is important to disseminate the idea that the toothbrushes should be stored, disinfected and replaced properly. There are, however, few studies that have evaluated toothbrush contamination and disinfection methods.[2,9,11-13]

Similarly, some authors[14,15] have demonstrated that because the silicone or latex pacifiers are in permanent contact with saliva and the oral microflora, they constitute a site for the growth of microorganisms. The literature also emphasizes that the use of pacifiers is associated with the occurrence of otitis media,[16] dental caries[17] and intestinal parasites as protozoan cysts and helminths eggs or larva.[15] Indeed, these pacifiers can be considered a vehicle of contamination and microbial transmission in children and disabled patients.

While studies related to the contamination of pacifiers can be found in the literature, there are no studies referring to the use of disinfection methods to eliminate contamination by oral microorganisms. From a social point of view, these methods of disinfection should be effective, simple and inexpensive.

Some studies have suggested the importance of the disinfection of toothbrushes to reduce the number of microorganisms present on the bristles using UV radiation,[13] electrolyzed water[18] and chemical agents, such as listerine, plax, cepacol[10] and chlorhexidine.[12,19] Although there are a few antimicrobial agents that could be used for disinfection, chlorhexidine is still considered the 'gold standard'.

Recently, several studies have evaluated microwave efficacy for the disinfection of dental devices and materials, such as prosthesis,[20] acrylic resins[21] and composites.[22] Microwave irradiation is claimed to be a simple, effective and inexpensive disinfection method.

The purpose of the present study was to evaluate, *in vitro*, the contamination of toothbrushes and pacifiers by *Streptococcus mutans* and the efficacy of microwave and chlorhexidine spray on their disinfection.

MATERIALS AND METHODS

Sixty silicone pacifiers (Kuka Baby) and 60 toothbrushes (Johnson Jr, Johnson and Johnson) were used from their original packages and soaked in a suspension of *S. mutans* (MTCC 890) at 39 × 10^6 CFU/ml for 5 minutes.

After exposure to *S. mutans*, the pacifiers and toothbrushes were randomly divided to three groups of 20 specimens each, according to the following protocols described: Group 1—spraying four times with 0.12% chlorhexidine solution (Periogard, Colgate/Palmolive); group 2—disinfection in a microwave oven adjusted to potency level 7 (corresponding to 70% of full power) for 7 minutes and group 3—spraying four times with sterile tap water.

The microwave parameters used for disinfection of toothbrushes were established based on the results of a pilot study in which different potencies and periods of exposure to microwaves were evaluated. In the pilot study, 9 pacifiers were contaminated by a known *S. mutans* concentration before exposure to microwave cycles of 7, 8 and 9 minutes. Two microwave potencies were compared (7 and 10). The best results for preventing the growth of microorganisms were obtained with 7 minutes of exposure to microwaves at potency level 7.[31]

The toothbrushes and pacifiers were transferred from the *S. mutans* solution to empty sterile glass containers (10 specimens per container) and exposed to microwave radiation for 7 minutes at potency 7 in a microwave oven. The containers were kept or arranged at a distance to avoid contact among them, and the toothbrush bristles and silicone pacifiers were placed in a vertical position (Fig. 1).

After treatment, the toothbrushes and pacifiers were transferred to flasks containing BHI medium and incubated overnight at 37ºC at 120 rpm. This was followed by plating the medium on brain heart infusion (BHI) agar plates for CFU measurement.

Microbiological Analysis

Streptococcus mutans (MTCC 890) was obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The strain was maintained on BHI medium agar slants (composition per liter: BHI 10 gm, glucose 20 gm, tryptone 20 gm, yeast extract 20 gm, agar 15 gm, pH 7.2 ± 0.2). The culture was stored at 40ºC and subcutured before use.

After 4 hours, the toothbrushes of each group were individually and vertically placed into 25 × 150 mm test tubes containing 50 ml, MRS medium for 24 hours at 37ºC.[23] Each pacifier was individually placed in Borel tubes containing 50 ml MRS medium and incubated in the same way as the toothbrushes. Evaluations of the surfaces for the presence of bacteria were made after incubation. At each time, the toothbrushes and pacifiers were withdrawn, rinsed in the broth, and gently shaken to remove planktonic microbiota, leaving sessile bacteria adhered as 'spike' or 'mushroom-like' colonies. The toothbrush bristles and silicone pacifier surfaces were carefully analyzed on all sides, and sessile *S. mutans* colonies, based on colony morphology, were counted under aseptic conditions with a stereomicroscope (Nikon, Tokyo, Japan) with reflected light by a blinded examiner. The microbiological results were analyzed statistically with ANOVA and Turkey test.

RESULTS

In groups 1, treated with chlorhexidine, the initial CFU of the culture was 39 × 10^6 and 32 × 10^6 for toothbrush and pacifiers respectively. After treating, it got reduced to 1 and 1 for toothbrush and pacifiers respectively (see Fig. 1).

In the groups 2 treated with microwave, the initial CFU of the culture for toothbrush and pacifiers was 39 × 10^6 and 32 × 10^6 respectively. After treatment it got reduced to 6 and 0 respectively (see Fig. 1).

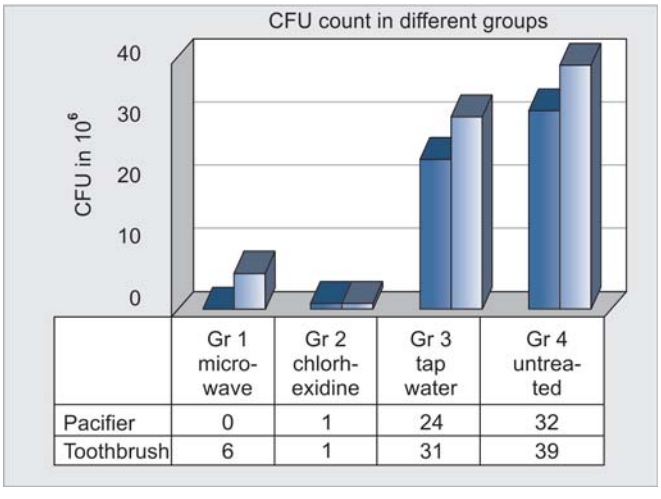
In groups 3 that was treated with tap water, the initial CFU of the culture was 39 × 10^6 and 32 × 10^6 for toothbrush and pacifiers respectively. After treating it got reduced to 31 and 24 for toothbrush and pacifiers respectively. When 0.12% chlorhexidine spray and microwave were used (groups 1 and 2), colonies were absent in 100% of the cases.

Groups 1 and 2 were statistically similar to each other ($p > 0.05$) and differed significantly from group 3 ($p < 0.05$) (Fig. 2).

DISCUSSION

In the present *in vitro* study, there was *S. mutans* contamination on 100% of group 3 toothbrushes, which were sprayed with sterile water. This result shows that the methodology is correct and can be used as a control in *in vitro* tests to evaluate the efficacy of different disinfection protocols (physical and chemical) before the *in vivo* evaluations. The present study's findings are similar to those of *in vivo* studies by Motzfeld et al,[24] Nelson Filho et al,[12,25] and Quirynen et al,[11] which described an intensive contamination by *S. mutans* on adult's and children's toothbrushes after use.

Fig. 1: The CFU count and disinfection of pacifiers and toothbrushes with chlorhexidine



	Gr 1 micro-wave	Gr 2 chlorh-exidine	Gr 3 tap water	Gr 4 untrea-ted
Pacifier	0	1	24	32
Toothbrush	6	1	31	39

Fig. 2: The CFU of the culture for toothbrush and pacifiers

Several studies have suggested the importance of disinfection in reducing the number of microorganisms on toothbrush bristles. In the present study, the 0.12% chlorhexidine spray showed 100% efficacy for toothbrush disinfection, eliminating all colonies in this group. These results agree with Nelson-Filho et al[2,12] and Saravia et al,[2] who observed absence of *S. mutans* growth in toothbrushes after the use of a 0.12% chlorhexidine solution. We agree with Moshrefi[26] in that chlorhexidine is the 'gold standard' antimicrobial, compared to other agents used for dental biofilm control.

Although, the microwave efficacy has been evaluated only in prothèses,[20] acrylic resins[21] and composites,[22] in the present study this method was also effective in eliminating microorganisms from toothbrushes. It is important to highlight that in the present study efficacy was evaluated at a single power parameter (potency level, 7-70% from 1,100 watts), and the results could not be generalized to other microwaves with different parameters. Dental practices must take this into account when they will choose power levels and microwave models.

Pacifier nipples, made of silicone or latex, are in permanent contact with saliva and, therefore, oral microflora. For this reason, their surface is a preferential site for the growth of biofilms.[27,28] As biofilm micro-organisms are components of the oral microflora, it may be assumed that they also attach to pacifier nipple material.[15] A few authors have searched for specific microbial species on pacifiers. Pedroso and Siqueira[29] demonstrated that pacifiers were an important source of infection by intestinal parasites. Mattos-Graner et al[28] found a relationship between the use of pacifier and yeast proliferation in the oral cavity. According to the American Academy of Pediatrics, gastrointestinal infections and oral colonization with *Candida albicans* are more common among pacifier users. Comina et al[15] observed 80% of biofilm colonization on pacifier nipples and stated that the pacifiers can be seen as potential reservoirs of pathogens.

In the present study, 75% of group 3 pacifiers were contaminated with *S. mutans*, which is the primary etiologic agent of dental caries. According to Ollila et al[30] prolonged pacifier sucking is a possible risk factor for dental caries in children. Moreover, microorganisms from the environment are able to adhere to pacifiers, as children often drop their pacifiers on the floor and do not take good care of them. This contact with a wide range of microbial species might boost biofilm formation on the surface of pacifier nipples. Therefore, pacifiers can be contaminated by *S. mutans* after use and should be disinfected.

We agree with Comina et al[15] in that a correct disinfection of pacifiers is important to limit contamination. Hospital nurseries give strict instructions for the disinfection and sterilization of feeding bottles, but they do not give similar instructions for pacifiers. Strict rules of hygiene and an efficient antibiofilm cleaning protocol should be established to answer the worries of parents concerning the safety of pacifiers. Regarding this issue, we observed that simply cleaning pacifiers with water (group 3) was not able to control the microbial contamination.

On the other hand, the use of chlorhexidine solution or microwaves as disinfections methods resulted in 100% elimination of *S. mutans*. It was not possible to compare our results to the literature, however, because there are few papers addressing microbial contamination on pacifiers. Similarly, there is no paper related to pacifier disinfection with 0.12% chlorhexidine or microwaving. Further, *in vitro* studies and clinical trials are needed at other research levels to evaluate disinfection methods with different types of microorganisms.

CONCLUSION

1. When 0.12% chlorhexidine spray and microwave were used, colonies were absent in 100% of the cases.
2. Chlorhexidine solution spray (0.12%) and 7 minutes of microwave irradiation were effective for disinfection of pacifiers and toothbrushes.

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