EFFICIENCY OF CARES OVER TOOTHBRUSH STORAGE TO PREVENT CONTAMINATION

ABSTRACT

AIM: This study evaluated the efficiency of a toothbrush holder to prevent contamination of toothbrushes used by preschool children. **MATERIAL AND METHODS:** For that, the sample was composed by children 6 years old, enrolled in an educational and recreational center in Araraquara/SP, and divided into 3 groups: G1: same continuing routine storage toothbrushes, G2: children received only a new toothbrush holder for storage; G3: they received new toothbrushes holder for storage and solution of chlorhexidine digluconate to 0.12% to dabble in the toothbrush after use. After brushing their teeth, toothbrushes were collected for microbiological analysis. The data were analyzed using the distribution of frequencies. **RESULTS:** It was observed that, in general, higher prevalence of the microorganism in the toothbrushes was Streptococcus viridans (58.97%), followed by Estafilococcus (35.90%), the bacillus of air (28.21%) and Neisseria mucosa (5.13%). Evaluating frequency, it was noted that the contamination presented by Streptococcus is higher in G1 when compared to G2 and G3, while for Estafilococcus, the presence was more significant in G3. **CONCLUSION:** Thus, it was concluded that the use of new toothbrushes holder able to avoid direct contact between brushes and allow drying without smothering could be an excellent alternative to educational institutions that require the storage group.

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KEYWORDS

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INTRODUCTION

The use of instruments to promote oral hygiene, like toothbrushes and dental floss is essential to eliminate bacterial plaque, main etiological factor in the formation of caries and periodontal disease.[1] It is known that ideal characteristics recommended for toothbrushes are: small head with soft bristles, rounded edges and preferably transparent; this last recommendation has the aim to avoid proliferation of microorganisms which can adapt themselves to low-light environments.

This instrument is the more used and efficient to prevent oral diseases, its storage should be performed judiciously, because when it is not packed properly, it may serve as a vehicle of transmission faeco-oral.[2,3]

According to Warren et al.[4] (2001), contaminated toothbrushes not only may represent a bacterial, virus and other microorganism deposit, but also may become transmission focus of them, causing inflammatory oral diseases, as well as systemic problem. The retention and survival of cariogenic microorganisms in toothbrushes represents a possible cause of oral re-contamination.[5]

Besides, population awareness about cares over toothbrush storage is extremely important, once the public health depends on the collaboration of information transmission agents, which should be adequate and appropriate.

Then, the school environment represents a communication mean among their integrant and the community, beyond its importance in the individual formation, the school, by its meaning in children's life, has highlighted forming role and must accept explicitly the responsibility by health education, because the formation of concepts, procedures and attitudes related to it are strongly associated to values that the professor and all the school community will transmit necessarily in every-day life.[6]

The literature presents few reports about maintaining cleanliness of toothbrushes. Some authors have studied different recipients to storage toothbrushes; as well others [7-9] have tested the efficiency of several mouthwashes in order to try increase the control over infection. However, there is the necessity of more studies and disclosure of these findings to the population.

In this way, the study aimed verifying the microbiological contamination of toothbrushes used by children in an education center, according to the mode of storage.

MATERIAL AND METHODS

The performing of this study was linked to the approval of the Committee on Ethics in Research of the University Center of Araraquara – UNIARA under the protocol number 746/08; the participation of the school children was linked to the correct fulfillment and signature of the Pre-

informed statement of consent by parents or responsible.

The sample in this study was composed by 39 preschool children of 6 years old, regularly enrolled in pre-school of Elementary School in an educational and recreational center in Araraquara/ SP.

To perform the research, a toothbrush holder was elaborated from an ice-cream container with volume of two liters, in which were confectioned orifices to dovetail the toothbrushes, in order to make them stayed with their heads exposed to the external environment and the handles inside the container, with no direct contact among them.

It is important highlight that the toothbrushes used by the school children were collected and new ones were distributed in the beginning of the research, with identification by the children's name to be stored according with the study methodology.

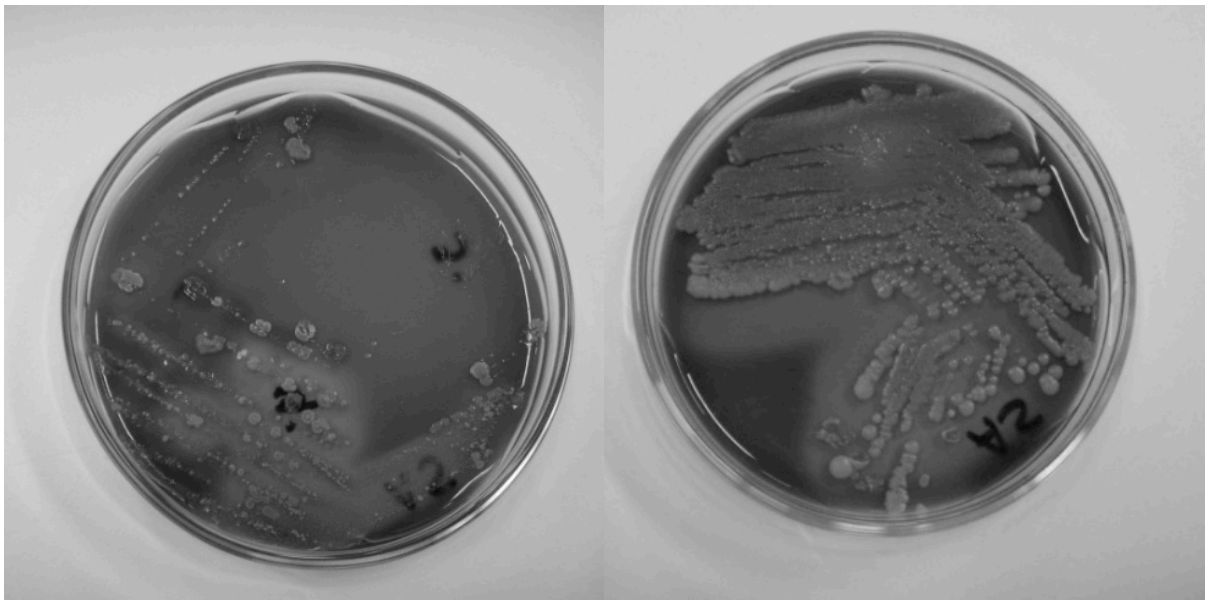
In this way, students were divided into (3) groups: the control (G1), which have continued performing the storage of all the toothbrushes in a fabric package with cocoons where they were introduced and convolved to keep it in the classroom cabinet. The group 2 (G2) have received only the toothbrush holder to storage and the teacher was oriented to remove the water excess after each tooth brushing with a movement of the toothbrush in the air. The group 3 (G3) has received the toothbrush holder to storage them and the solution of chlorhexidine gluconate 012% to be sprayed on each toothbrush after the use. All the recipients to storage were maintained in ventilated cabinet. This procedure was carried out during 30 days for each group with weekly accompaniment to verify whether the recommendations were followed. After this period, the toothbrushes were collected for microbiological analysis in order to observe the prevalence of contamination by microorganisms in the different groups evaluated. Then the children have received new toothbrushes.

Microbiological evaluation:

To evaluate the microbiological contamination, the identification of microorganisms found in the toothbrushes was performed. Hence, the toothbrushes were collected with the use of sterile procedure gloves and deposited in plastic bags properly identified containing 3 mL BHI (Brain Heart Infusion) with bristles down and taken to the microbiology laboratory, where they were vigorously agitated in a vortex agitator during one minute to release the microorganisms adhered to the bristles.

Next, the samples were seeded with a disposable bacteriological loop in Petri dishes of 80x15mm, containing Agar Blood-Agar Tryptone Soy (DIFCO) plus 5% of sterile defibrinated sheep blood, agar Mac Conkey and spread in streaks in order to obtain the isolation of bacterial colonies. Then, the plaques were incubated in microaerophilic incubator at 35-37°C by 24 hours. After this period, the colonies formed in agar were analyzed by Gram coloring according to their morpho-tinctorial characteristics and to cellular arrangement for posterior identification by biochemical-physiological tests (Figure 1).

Figure 1 – Culture of Staphylococcus coagulase negative and Streptococcus group.



Statistical Analysis:

The analysis of data were performed in descriptive way by distribution of frequency of the microorganisms found in the samples collected from each group and the results presented in graphs.

The association of presence or absence of each microorganism according with the group was studied through Fisher's exact test. The level of significance adopted was 5%.

RESULTS

It was evaluated 39 toothbrushes, which nineteen belonged to the group 1, eight to the group 2 and twelve to the group 3.

In general way, independently on the groups, it was observed that Streptococcus group viridans (mutans) was present in higher percent in toothbrushes (58.97%), followed by coagulase-negative Staphylococci (35.90%), genus Bacillus sp (28.21%) and Neisseria mucosa (5.13%).

When data were analyzed separately for each group studied, the results were presented in the Figure 2.

In relation to the contamination by Streptococcus group viridans (Figure 2), it was observed that for the group 1, higher frequency in toothbrushes (14 – 73.7%) presenting this microorganism. For the group 2, the results found were 5 (62.5%) toothbrushes, while in the group 3, it was verified in 4 (33.3%) of them. It is important highlight that there were statistically significant difference among the groups ($p<0.05$), showing that in the control group (traditional toothbrush holder), the contamination was significantly higher ($p<0.05$) when compared to the groups 2 and 3 (experimental toothbrush holder and toothbrush holder plus chlorhexidine) which presented similarity between them.

About the presence of Neisseria mucosa, it was possible verify that (Figure 3), for G1, there

was only 1 toothbrush presenting this microorganism. For G2, there was no contamination in any toothbrush, and in G3 was verified only 1 contaminated with non-significant statistically difference among the groups ($p > 0.05$).

Regarding the presence of the genus Bacillus sp, it was noticed by the Figure 4 that G1 presented slightly larger amount of these microorganisms, with 7 (36.8%) toothbrushes contaminated, when compared with other groups that obtained 2 contaminations in the toothbrushes, what represent 33.3% and 20% respectively, for the groups 2 and 3. It is important remember that this microorganism is contaminant of air microbiota and due to the humidity and food remaining in the toothbrushes, it may develop itself easily; however, there is no pathogenic profile.

Figure 2 – Frequency of Streptococcus group viridans found in toothbrush from different groups.

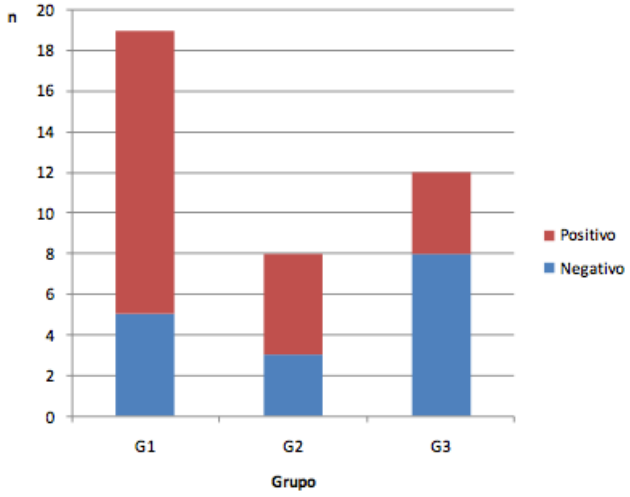


Figure 3 – Frequency of Neisseria mucosa found in toothbrushes from different groups.

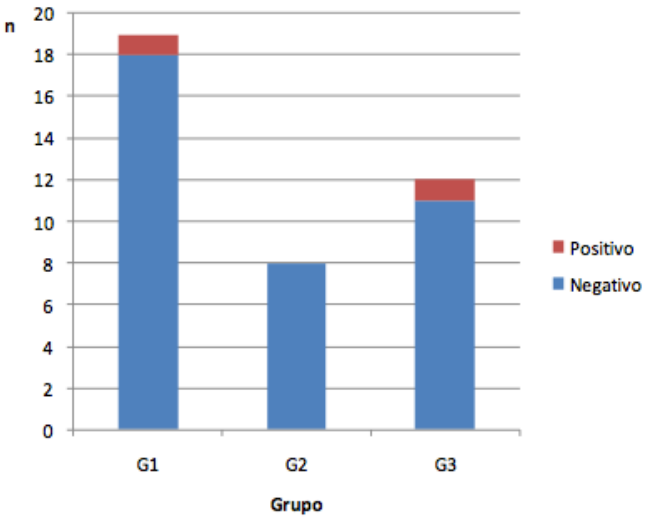


Figure 4 – Frequency of Bacillus sp found in toothbrushes from different groups.

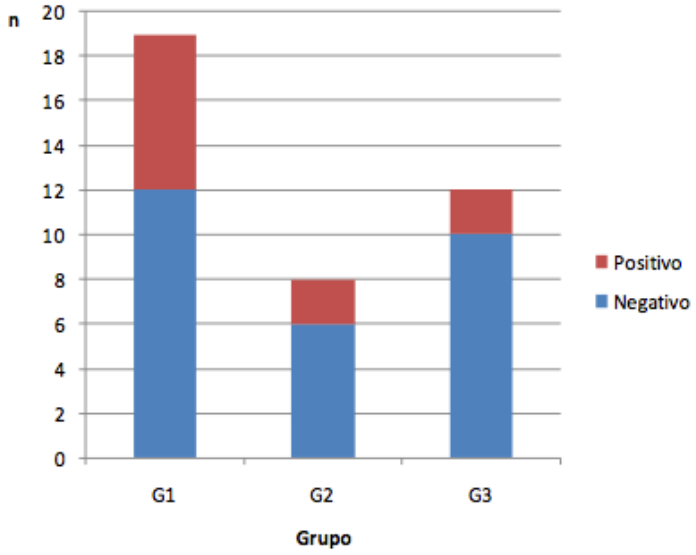
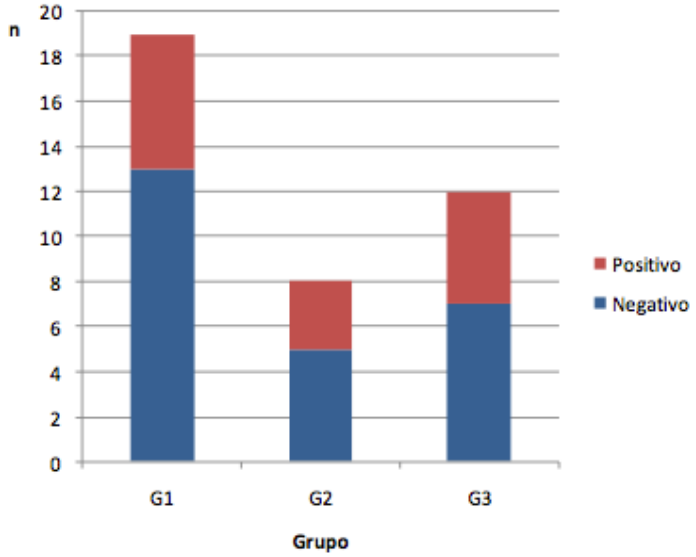


Figure 5 – Frequency of Staphylococcus coagulase negative found in toothbrush from different groups.



About this group of microorganism, it is noticed by the Figure 5 that, for the group 1, it is present in 6 (31.6%) toothbrushes of 19, while for the group 2, it was observed similar percent (3 toothbrushes of 8, what configure 37.5%). For the group 3, the presence of this microorganism was more expressive, where 5 (41.66%) of 12 toothbrushes were contaminated after storage. This microorganism is able to form a biofilm in plastic surfaces, and also in metal; its presence as expressive as Streptococcus group viridans (mutans) occurs because this capacity of fixation and adherence.

DISCUSSION

The current Dentistry advocates that, to prevent diseases as caries and periodontal, the appropriate hygiene represents an important

role in the combat of these pathologies, which may be considered public health problems, due their prevalence and severity. According to Barros et al.[10] (2001), the removal and the mechanical disruption of the bacterial plaque are the key of prevention for an effective reduction of these diseases and, for this, the toothbrushes contribute directly in these mechanism.

However, the toothbrush, the more used instrument to perform the oral hygiene, may also be responsible by the introduction of new microorganisms in the oral cavity, causing systemic or localized diseases, what increases the risk of caries and infectious diseases, mainly after hygienic procedures.[11] This fact may occur whether the toothbrush will not be stored in an appropriate way.[12] According to Vilhena et al.[6] (2008), the appropriate use of toothbrush by daily tooth brushing is very important, as well as the way of storage.

The packaging site should allow a clean toothbrush with fast dry, besides avoid the contact with other toothbrushes.[12] It allows that proliferation of pathogenic microorganisms decrease considerably, once the storage environment does not correspond to that in which bacteria, fungi and viruses survive.

It is known that, among Brazilian people as in other cultures, there is the use of storage the toothbrushes belonged to several family members in the same recipient, causing direct contact among them; many times, they are stored on the sink or in the cabinet inside the bathroom.[13,14] Eventually, beyond the cross transmission that may occur among family members, it may also occur with individuals who live in collective environment, like daycare centers and preschools. In this sense, it is important the control of occurrence of the salivary contact among individuals in environments such these institutions which shelter children in early age, because the inadvertent risk of change/share among toothbrushes. Most of toothbrushes used in a school environment is collective, and the contact among bristles is allowed, what facilitate the propagation of infectious and parasitic diseases among students by lack of care.12 Then, the disinfection of toothbrush may be an important procedure to help the prevention of several pathologies.[15]

In this study, it was observed that the toothbrushes were stored in a fabric package, in which they were disposed in cocoons and convolved and stored in a classroom cabinet. In the research performed by Coutinho et al.[16] (2007), the use of recipients in fabric was verified in 8.9% institutions, and in plastic in 28.9%. Besides, the identification by children's name in the toothbrushes handle with pen was inefficient, because with time the names became unreadable, increasing the risk of cross contamination among pre-school

students. Similar results were also verified by Coutinho et al.[16] (2007).

The toothbrushes holder proposed here, with separation of toothbrushes by the distance among orifices in the recipient demonstrated higher efficiency in the prevention of contamination, although this was not the only factor to be considered. The presence of pathogens in this kind of instrument is common due to the factors mentioned before (contact and humidity) and, for this reason, the presence of microorganisms was higher in the group 1 in relation with the groups 2 and 3, notwithstanding non-significant.

Most of microorganisms transferred from toothbrush to the oral cavity or to other toothbrushes (when they are stored together) is composed by a native microbiota1 and may act in the re-introduction or being intraoral translocation vector of bacteria, like Streptococcus group mutans, which is the main etiological agent of caries.[13] Dissemination of Streptococcus group mutans may occur by direct contact (via saliva) or indirect; it happens via fomite by spoons, cups, toys, and contaminated toothbrushes.

Besides microorganisms studied in this work, others also may be found in toothbrushes, such Candida albicans, Ancylostomatidae, Taenia sp, Entamoeba coli and fecal coliform.[3] In this study, none of these microorganisms was found in toothbrushes stored in different groups.

Microbial contamination of toothbrush bristles suffer influence from several factors, highlighting the kind of dentifrice, which may contain antimicrobial agents like Fluor or triclosan, which provide reduction of this contamination.[4,7]

Besides, there are several proposes to eliminate[3,5,6,8,15,17] these microorganisms from toothbrushes, for example, the use of microwave oven, which have shown positive results in eliminate microorganisms from toothbrush in short time of exposure,1 as well the use of solutions for sanitization, like Sodium hypochlorite,[18] mouthwash cetylpyridinium chloride-based, essential oils and chlorhexidine gluconate,[15] among others. The ways to perform toothbrush antisepsis is a subject very discussed in the literature, because toothbrush contamination is almost unavoidable with its routine use.[12] The use of anti-septic solution has been considered the more appropriate way of prevent contamination. Studies performed by Mialhe et al.[19] (2007) and Zião et al.[20] (2011) with Dentistry students showed that even this part of population is conscious of the way of drying and storage of toothbrushes. Mialhe et al.[19] (2007) observed that only 16% of the respondents asserted spraying or immerse the toothbrush in any antimicrobial solution after

tooth brushing, and Zião et al.[20] (2011) noticed that 20% of students did not use any anti-septic to disinfect the toothbrush bristles. According to Alves et al.[21] (1998), S. mutans present itself highly sensitive to the chlorhexidine. Oliveira et al.[22] (2009) also tested the proliferation of S. mutans in toothbrushes exposed to Eugenia uniflora L. essential oil and to the chlorhexidine, and they observed that, although the first one have shown efficiency, the chlorhexidine achieved much higher decontamination. On the other hand, in the study performed by Turner et al.[23] (2009), the authors reported that bacterial growing was similar among toothbrushes of groups that use or not the chlorhexidine as solution of sanitization.

In this study, the microorganisms found with higher frequency after performing the experimental storage of toothbrushes were Streptococcus viridans and the genus Bacillus sp. Corroborating these findings, Sato et al.[8] (2004), encountered high percentage of these microorganisms in toothbrushes without use of antiseptic solution (Streptococcus in 80% and aerobic Gram-negative bacilli in 46.7%).

However, the authors observed significant reduction in contamination of toothbrushes when used anti-microbial sprays in basic formulation and containing cetylpyridinium chloride. Neisseria mucosa was observed only in some collections of this study, and it may have occurred by contamination during the procedure of collection of toothbrushes and take them to the laboratory of microbiological analysis. About coagulase-negative Staphylococci, it may have shown itself resistant to the chlorhexidine, once it was more frequent in the group 3, what shows the possible formation of bacterial biofilm, protecting it from the action of chlorhexidine.

Studies like Sato et al.[8] (2004) performed shows that chlorhexidine presents great affinity by bacteria, probably due to the adsorption of cationic molecule (positive) of chlorhexidine to the cellular wall anionic (negative) of microorganism. This interaction promotes the increase of membrane permeability, opening real craters that provoke the entrance of chlorhexidine in the cytoplasm and which causes extravasation of cellular constituents with low molecular weight, beyond the precipitation of cell contents, culminating in the death of microorganism.

In the study by Moreira and Cavalcanti[24] (2008), the authors noticed that 95.7% of toothbrushes which were submitted after their use to the washing with water, aseptically by mouthwash, protection of bristles by protective cover and exposition to the natural environment, they were free of contamination, proving that good hygiene habits and adequate storage contribute for

they do not become vehicles of pathogens that compromise the health by the mouth.

Finally, it is considered that the use of aseptic techniques to storage toothbrushes is important in the maintenance of them, related to the contamination and the proliferation of microorganisms, pathogenic or not.

Besides, the use of toothbrushes holder may be an interesting alternative for teaching institutions that need collective storage, once it was observed decrease of bacterial load in the groups studied.

In this context, it is suggested that both for health professionals and educators should play better the role of transmit this information to the society, favoring the creation of healthy habits in oral hygiene among any age citizens.

CONCLUSION

It is concluded that:

1. The kinds of microorganisms found in toothbrushes were: Streptococcus viridans, Estafilococcus coagulase negative, Bacillus air and Neisseria mucosa;

2. The contamination in the traditional toothbrush holder was significantly higher when compared with the experimental one and with the toothbrush holder with sanitization by chlorhexidine.

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