ANTIBIOGRAM AND PLASMID PROFILE OF ISOLATED ENTEROBACTERIACEAE IN TOOTHBRUSHES

Juliana Santana de Curcio[1]; Luis Artur Mendes Bataus[2]; Warita Alves de Mello[3]; Robson Botelho de Araújo[3] & Lilian Carla Carneiro[2]

1. Scholarship graduation- State University of Goiás - UEG;
2. Teacher from Federal University of Goiás - UFG;
3. Volunteer graduation - State University of Goiás - UEG;

Corresponding Author: Lilian Carla Carneiro, Dr. Teacher from Federal University of Goiás, **BRAZIL**

ABSTRACT

The Enterobacteriaceae family is represented by Gram negative bacillus bacteria, which can contaminate toothbrushes. Objective: The research aimed to identify enterobacteria and to analyze and check its plasmid resistance profile. Material and methods: The brushes were stored in three different ways: 1/3 in a plastic brush holder, 1/3 in an open cardboard brush holder and 1/3 in an open cardboard brush door sprayed with 1% sodium hypochlorite. Results: After microbiological characterization, it was observed that the open cardboard toothbrush holder had the highest number of colonies countless. Of 45 samples, seven showed the presence of plasmid. Conclusions: This study showed that among the three forms of packaging, the plastic and cardboard brush holders sprayed with 1% sodium hypochlorite can be used to reduce toothbrush contamination.

Keywords: Bacteria; Packaging; 1% Sodium Hypochlorite, Hygienization; Contamination.

INTRODUCTION

The toothbrush is recent invention of mankind. Oral hygiene is currently understood to be a preventative measure for oral diseases and occupies an important place in society. Dentistry has emphasized the importance of oral health care, highlighting the need to use a toothbrush that allows the application of effective preventative methods, which have a collective aspect and a social impact (1). However, toothbrushes present some problems, such as reinfection by microorganisms and contaminated toothbrushes can act as reservoirs for microorganisms from the environments in which they are stored. Viable decontamination methods that can be used by the population are still not well understood or used in households. Thus, the study of these methods is important in preventing the transmission of pathogenic microorganisms, as evidenced by its importance within the concepts of prevention currently adopted in the area of oral health. Research that found the presence of pathogenic microorganisms on toothbrushes revealed the presence of the following: *Enterobacter* sp. *Citrobacter* sp., *Serratia* sp., *Candida albicans*, *Escherichia coli* and *Bacillus subtilis* (2, 1, 3).

Another factor that favors the survival of microorganisms is antimicrobial resistance, which constitutes a public health problem. Many studies have reported the emergence of bacteria resistant to antibiotic treatment. Although classically attributed to chromosomal mutations, resistance is most commonly associated with extrachromosomal elements acquired from other bacteria in the environment. These include different DNA mobile segment types, of which plasmids are one example (4).

Due to the loss of efficiency of toothbrushes through constant use, the risk of transmitting bacteria and other microorganisms that exhibit resistance due to the presence of plasmids and to know that the areas that remain dusty with food debris, damp or wet, host bacteria and facilitate their reproduction. This paper proposes an experiment with different types of toothbrush packaging in order to characterize the microorganisms contained in them, analyzing how these organisms behave in the presence of 1% sodium hypochlorite and checking for the possible presence of plasmids that may confer resistance to antibiotics. In addition, the research seeks to assess how students and educators care for oral hygiene.

MATERIALS AND METHODS

The research was conducted at the Gertrudez Lutz School in the city of Morrinhos – GO, Brazil.Toothbrushes was distributed to collect samples to perform microbiological analyses. Forty-five children aged 6-12 were selected for this study, without exclusion criteria. The collection of data to analyze the degree of public knowledge about correct oral hygiene methods and how they are to be implemented was done through a standardized questionnaire.
An experiment was conducted in which toothbrushes were stored in different ways. One third of the brushes were stored in a plastic package, 1/3 in an open cardboard container and 1/3 in open cardboard container sprayed daily with 1% sodium hypochlorite after use. After three months of use, the brushes were collected and dipped into test tubes containing peptone water. They were kept in the tubes for 24 hours for analysis of positive cultures and further identification. This was performed by Gram staining the samples from the positive peptone water. The positive samples were inoculated in MacConnkey SS (Shigella and Salmonella) culture medium. The 10^1 dilution as specified by the Ministry of Agriculture of Brazil (2003) was done in Plate Count Agar (PCA). Afterwards, incubation for 24 hours at room temperature (37°C) was used to count the colonies present using the presumptive method (CFU / mL colony-forming unit). The data were then statistically analyzed using the x^2 U test and the Mann-Whitney test.

The following biochemical tests were performed to confirm the results: lactose (5), glucose (6), H_2S (7), urease (8) and lysine (9). Ampicillin with a concentration of 10 mcg and ceftriaxone with a concentration of 30 mcg were introduced into the culture of a small colony of bacteria using the disk diffusion method (10). After the growth of positive samples, this was performed on plasmid DNA extract using the Flex-Prep Kit (Amersham ®) to investigate the existence of plasmid according to the methodology described by Sambrook et al. (11), with modifications. Plasmid DNA samples were analyzed in 1% agarose gel (w / v) stained with ethidium bromide (0.2 mg / mL) and dissolved in TEB 0.5 X.

RESULTS

Daily tooth brushing frequency was noted based on the proportion of respondents. Of the 40 respondents, 80% reported brushing their teeth three times a day, with only 2.5% reporting brushing their teeth five times or more per day (data not shown).

Respondents were asked how frequently they visited the dentist--72.5% reported going to the dentist every six months, 12.0% reported going only when they have a toothache and 10% reported going annually (data not shown).

From the Gram staining procedure, we observed that the morphology of bacteria that predominated on the slides were gram negative bacilli (100%) and gram positive cocci

(58%), gram positive and gram negative diplococci (9% and 22% respectively), gram positive (11%), gram-positive streptococci (7%), gram negative micrococus (7%), and gram-positive staphylococci (4%). Thus, this demonstrated that, in addition to the Enterobacteriaceae, the toothbrushes were contaminated by other types of microorganisms (data not shown). Table 1 shows the bacteria colonies found on the toothbrushes, according to type of packaging.

Among the three forms of brush packaging (shown in Table 1), the open cardboard brush holder had the largest number of colonies countless a total of thirteen samples. However, the brush door sprayed with sodium hypochlorite 1% was the only one with a 10^1 CFU / mL and 10^2 CFU / mL count.

Thus, a need has been demonstrated to disseminate viable methods for public use, such as decontaminating the brushing process or using a brush holder. This information is consistent with the data of this research. The data counting the SS agar colonies revealed that, among the three types of packaging, the cardboard brush holder and the open cardboard holder sprayed with 1% sodium hypochlorite had eight samples with growth in this medium. The plastic holders had the largest number of bacteria colonies in MacConkey agar--a total of 14 samples.

Enterobacteriaceae were identified in the biochemical tests performed in this study, including *E. coli* (22 samples), *Shigella* spp. (12 samples), *Citrobacter* spp., *S. typhi* (four samples), *Salmonella* spp. (one sample), and *Aeromonas* spp. (two samples), which are bacteria belonging to the Aeromonadaceae family.

We analyzed the degree of toothbrush contamination to show what methods were most effective in reducing such contamination. We will now present the statistical analysis of the Mann Whitney U test on the brush holders included in this study (data shown in Figures 1, 2 and 3).

Between the the plastic and cardboard brush holders, we observed a difference ($U = 52$, $z = -2.51$, $p < 0.02$), noting that the contamination was significantly greater in the open cardboard brush holder than in the plastic holder. The plastic brush door acted as a barrier method in preventing toothbrush contamination by microorganisms in the environment.

Figure 2 shows the statistical analysis of the brush holders made of cardboard and cardboard sprayed with 1% sodium hypochlorite. There was a significant difference between the two models ($U = 51$, $z = -2.55$, $p < 0.02$), thus showing that the 1% sodium hypochlorite acted as a decontaminant for toothbrushes.

There was no difference between the brush holder made of plastic and the one make of cardboard sprayed with hypochlorite (Figure 3). There was no significant difference in the degree of contamination ($U = 106.5$, $z = -0.25$, $p = 0.80$) between the two containers.

The X^2 test revealed significant differences in the degree of contamination between the cardboard and open plastic containers($X^2 = 7.22$ $P < 0.05$). The proportion was 86.7% for the open cardboard brush holder, compared to the plastic brush holder (33.3%). Among the plastic brush door containers (33.3%) and brush holders sprayed with sodium hypochlorite 1% (33.3%), the proportion of contamination was the same ($X^2 = 0 > P$ 0.05). There was a significant difference in the degree of contamination ($X^2 = 7.22$ $P < 0.05$).between the open cardboard containers (86.6%) and the cardboard containers sprayed with 1% sodium

hypochlorite (33.3%). The samples that tested positive for bacterial growth, underwent the DNA plasmid extraction procedure, unlike the brush holders.

Among the 45 samples of different brush holders, seven had plasmids (Figure 4). Samples from Lines 1 and 2 originated from the open cardboard brush holder (there were two other plasmids in those samples– data not shown). Line 3 was from the plastic brush holder, which had the same profile as the plasmid of the sample originating from the cardboard holders sprayed with 1% sodium hypochlorite (data not shown). These samples proved to be sensitive to treatment with ampicillin, but showed intermediate resistance to treatment with ceftriaxone.

In this study, we observed that the participants had oral hygiene habits. However a significant percentage reported visiting the dentist as a remedial measure. Bacteria was shown to be sensitive, resistant and having intermediate resistance to the use of two antibiotics, and among the 45 samples, seven had plasmid. It was possible to verify the presence of enterobacteria on the toothbrushes, including *E. coli, Shigella* spp., *Salmonella* spp., *S. typhi* and *Citrobacter* spp. Among the three types of packaging, the sprayed plastic brush door and cardboard container sprayed with 1% sodium hypochlorite proved to be effective in reducing toothbrush contamination\, These can be used as alternatives to reduce contamination by microorganisms in collective environments.

DISCUSSION

Based on the proportion of respondents, our results are agree with those of Lisboa and Abegg (12), which found that "three times a day," was the most common (53.9%) survey response regarding toothbrushing frequency preferences. Flores and Drehmer (13), found that 90.5% of respondents reported brushing three to four times a day.

In our study, respondents reported visiting the dentist every six months. However, Peres et al.; (14) reported that children with few dental caries seek more dental care than children with many caries and that the more unfavorable their socioeconomic situation, the greater the number of teeth they have with caries, as well as greater severity. The study by Perez and collaborators showed that poor populations have less access to dental care, which agrees with the research data, in which the children who participated had low purchasing power, and 12% reported going to the dentist only when in pain, thus using it as a remedial measure rather than for disease prevention.

Studies of toothbrush contamination have shown that our results agree with those of Sato et al., (3), as their survey found gram negative microorganisms in toothbrushes. According Chaves et al, (15), 1% sodium hypochlorite antimicrobial solution was more effective for toothbrush decontamination, which agrees with the data from this study. Dias et al., (16) analyzied plaque rates and found that 87.87% of the brushes stored brush holders had a regular to very good plaque index.

Toothbrush contamination levels found in our study show that *E. coli* was the more frequent genus found. Biochemistry experiments conducted by Long et al., (2) found no bacterial growth, and 36.6%, the bacterial genera found were *Enterobacter* sp., *Citrobacter* sp. and *Serratia* sp. According to Taji and Rogers (17), toothbrushes have been found in *Staphylococci, Streptococci, Pseudomonas* spp. and coliforms. Moreira and Cavalcanti (18), reported that microbiological examination found that the toothbrushes presented

contamination by microorganisms including *Candida albicans*, *E. coli*, *Streptococcus mutans* and *Bacillus subtilis*.

This agrees with the data from Passos et al., (19) that show a high tendency to contamination due to the fact that the brushes are exposed to the environment. Chaves et al., (15) claim that the 1% sodium hypochlorite acted as a decontaminant in toothbrushes. Their research found that 1% sodium hypochlorite was most effective despite its unpleasant taste This could be a solution to toothbrush contamination, as it is accessible to various social classes due to its low cost and usability in collective environments. Dias et al., (16) reported that using plastic brush holders can be a way to reduce the exposure of toothbrushes to microorganisms in the environment. This agrees with survey data showing that these two forms of packaging reduce contamination by microorganisms. This may be due to the use of a decontaminant and also to the fact the the brushes are not exposed to the environment.

Sato et al., (3), used two different toothbrush decontamination methods and said both were effective in reducing the number of microorganisms. Dias et al., (16) gives great importance to the fact that brushes that are exposed to the environment are more prone to contamination. Relating to the survey data, where the brushes used a decontaminant and the brushes were not exposed to the contamination was minor and could be employed as an simple and easy-to-access alternative in school environments to reduce toothbrush contamination.

The presence of plasmids was analyzed in samples of different brush holders that tested positive for bacterial growth. According to Rychilik (20), the plasmids confer various benefits to cells, such as providing resistance to antimicrobial agents such as R plasmids, thus enhancing the organism's ability to adapt to adverse conditions.

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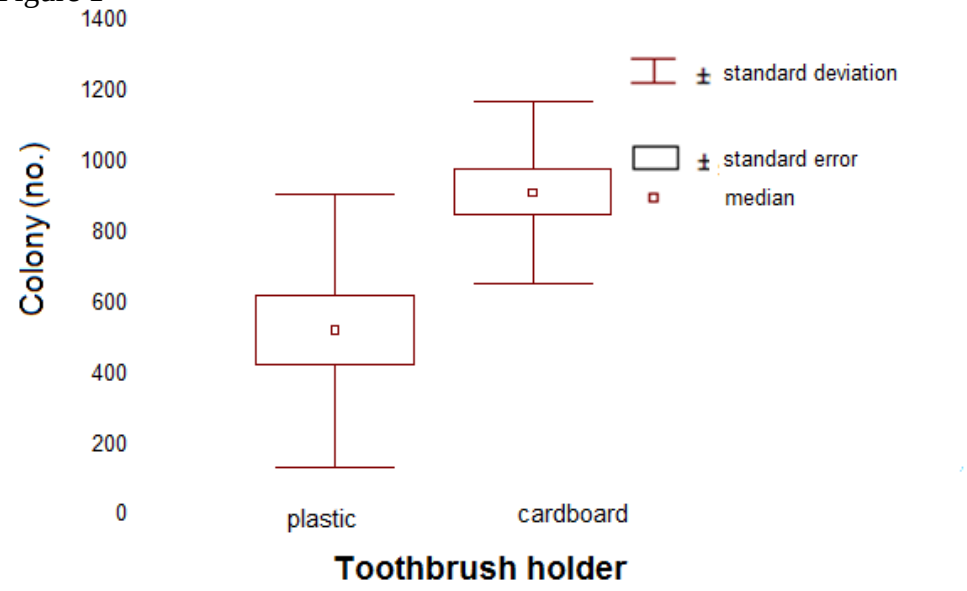
PICTURES

Table 1. Count of bacterial colonies in toothbrushes

Sample in (CFU/mL)	Plastic holder	Open cardboard holder	Open cardboard holder and 1% sodium hypochlorite spray
10^1	–	–	1
10^2	–	–	1
10^3	1	–	–
10^4	3	1	2
10^5	2	–	3
10^6	4	1	3
10^7 (countless)	5	13	5

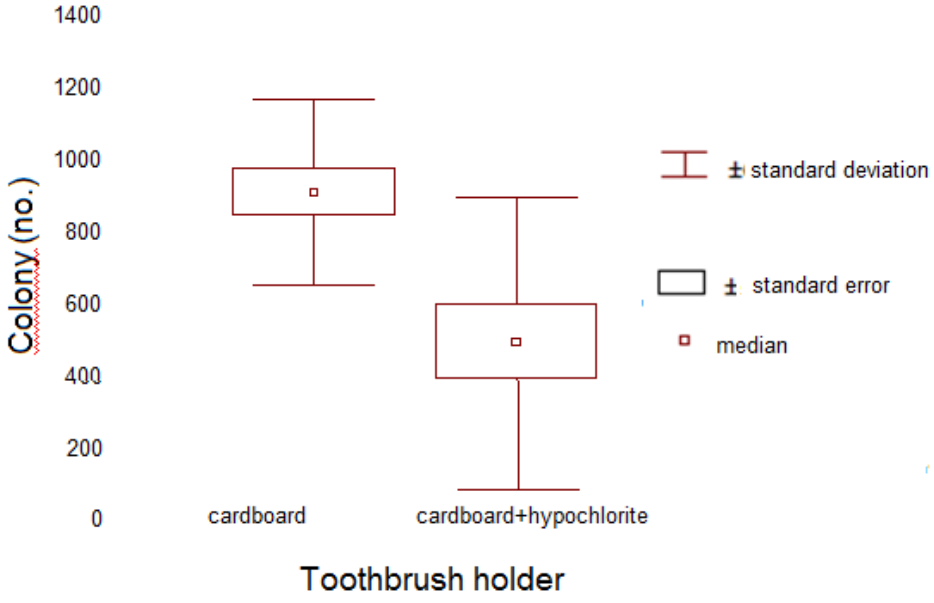
Figure 1



± standard deviation, ± standard error, median

Figure 1. Comparison of the degree of contamination of the plastic and cardboard toothbrush holders

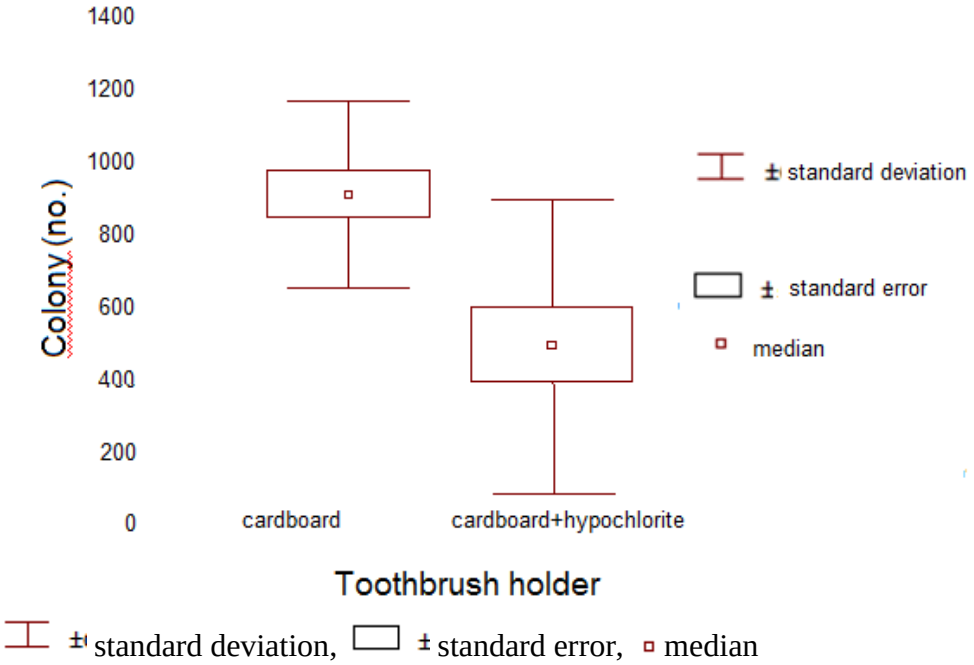
Figure 2



± standard deviation, ± standard error, median

Figure 2. Comparison of the degree of contamination of the cardboard toothbrush holder and the cardboard toothbrush holder with 1% sodium hypochlorite spray

Figure 3



± standard deviation, ± standard error, median

Figure 3. Comparison of the degree of contamination of the plastic toothbrush holder and the cardboard toothbrush holder with 1% sodium hypochlorite spray

Figure 4

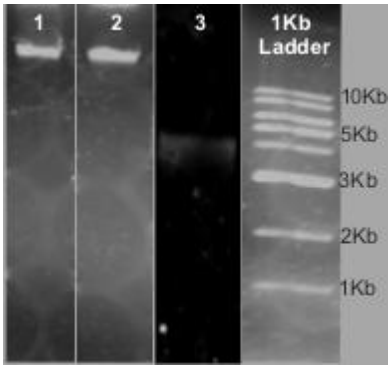


Figure 4. Profile of the plasmid found in the enterobacteria present in the brush holder studied