

Microbiological evaluation of bristles of frequently used toothbrushes

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Introduction: Brushing teeth is probably the practice of oral hygiene most common in the world; however, inadequate use can become a risk to the population health, once they may be contaminated with various microorganisms.

Objective: The aim of this study was to evaluate the bacterial contamination on toothbrush bristles using different methodologies.

Method: We used 40 toothbrushes from healthy individuals aged 3 to 58 years. The samples were grown in test tubes containing trypticase soy broth sterile, and with the help of a tracking 0.1 µl samples were placed on plates containing sheep blood agar 5% and MacConkey agar then the samples were stored in a bacteriological incubator at 37°C for 24 hours for later analysis. It was counted the colony forming units and bacteria identification present in the brush.

Results: On the microbiological analysis, there was a growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus coagulase* negative.

Conclusion: According to the results presented in this study, we observed a high incidence of bacterial contamination in the brushes analyzed. The most frequent microorganisms were members of the Enterobacteriaceae. The usage time of toothbrushes may be related to contamination found and, therefore, not only good hygiene ensures the reduction of microbial load, but replacing the toothbrush can also ensure individuals better oral health.

Keywords: Toothbrushing. Contamination. Oral hygiene.

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How to cite this article: Ferreira CA, Savi GD, Panatto AP, Generoso JS, Barichello T. Microbiological evaluation of bristles of frequently used toothbrushes. Dental Press J Orthod. 2012 July-Aug;17(4):72-6.

Submitted: December 25, 2009 - **Revised and accepted:** October 5, 2011

» The authors report no commercial, proprietary or financial interest in the products or companies described in this article.

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INTRODUCTION

One of the biggest public health problems of most developing countries concerns the reduction or elimination of periodontal disease and caries, even with significant prevalence in the population, related indirectly to factors such as financial, housing, culture level and eating habits.¹ The toothbrush is the only instrument that the majority of the population has to control the dental biofilm². Brushing is probably the oral hygiene practice most commonly performed in the world. The first brushes were made of pig fur and bristles made of horse mane or tail mounted in bovine bone or ivory.³ This tool has as main function the reduction of dental plaque that may be responsible for oral diseases including dental caries, periodontal disease and halitosis.¹³ Toothbrushes must present minimum requirements for the plaque removal such as: the bristles should be stiff enough to remove biofilm without causing trauma to the teeth and gums (optimized by rounding the active end of the bristles) and the head should be small with soft bristles.¹⁷ Another point of concern, is the fact that toothbrushes can be a source of contamination within users,¹³ through direct contact of different brushes from family members, or contamination from the containers that are usually moistured on the sink or in bathroom cabinets.^{6,10} Toothbrushes become rapidly contaminated with oral microorganisms, including bacteria, viruses and fungi.²⁰ The oral cavity presents one of the most concentrated and varied microbial populations, notably colonized by *Staphylococcus* sp, *Streptococcus* sp., *Neisseria* sp, *Bacteroides* sp, *Actinomyces* sp, *Treponema* sp, *Mycoplasma* sp.¹⁹ These microorganisms can colonize the oral cavity, and also the environment where the toothbrushes are stored.²¹ In addition, organisms normally associated with the oral flora have been isolated from toothbrushes, including the family *Enterobacteriaceae*²⁰ and its origin is probably the bathroom.²¹ The toothbrush should be sanitized or disinfected and replaced in regular time periods. In this context, studies of microbial contamination of toothbrushes *in vivo*, proposing methods for its sanitation or disinfection,³ have emerged over the last two decades. Many consumers buy less than a toothbrush

for year despite recommendations made by dentists, where a toothbrush must be used for a maximum period of three months. The brushes can become less effective for removing plaque compared with a new toothbrush, yet consumers are usually reluctant to replace the brushes more frequently.^{3,24} This present study aimed to evaluate the microbiological contamination of toothbrush bristles in continuous use, using two different methods.

MATERIALS AND METHODS

Sample collection

The samples were randomly collected from apparently healthy individuals. The inclusion criteria were: 1) free age, 2) used the toothbrush at least three times a day; 3) the duration of use was over a month. Were excluded from the study subjects who had one or more of the following criteria: 1) bleeding gums and mouth, 2) dental post surgery 3) hospitalized subjects. The number of samples used comprised of 40 frequently used toothbrushes. The average use of toothbrushes in four participants was 3 months, in 9 participants was 5 months of use, in seven subjects was 1 month and 20 participants was 7 months of use. These brushes were collected aseptically in a sterile container and send immediately to the microbiology laboratory at the University of the Extreme South of Santa Catarina for microbiological analysis of the bristles.

Microbiological processing

For the analysis two different methodologies were used. The first method was developed by Cleri, Corrado and Seligman⁵: 20 toothbrushes were placed in test tubes containing 5 mL of tryptic soy broth (TSB). Sterile tubes were homogenized three times added 10.1µl loop and plated on sheep blood agar containing 5% and MacConkey agar. The plates were stored in a bacteriological incubator at 37 ° C for a 24 hours period for microbial growth analysis. The second method was developed by Sherertz, Raad and Balani²²: 20 toothbrushes were placed individually in a test tube containing 5 mL of tryptic soy broth (TSB), then the samples were homogenized for 15s with vortex. The brushes were removed from the culture medium and passed through 0.05 mL to 5 mL of sterile TSB broth with a micropipette, resulting in a dilution of 1:100.

This broth was homogenized again and with addition of a 0.1 mL loop was streaked on plates containing sheep blood agar and 5% MacConkey agar. The plates were stored in a bacteriological incubator at 37° C for a period of 24 hours for microbial growth analysis. After 24h growth the colony-forming units (CFU) were counted and subsequent microorganisms identification. For the microorganisms identification was performed Gram stain and biochemical tests.

RESULTS

According to the methodology of Cleri, Corrado and Seligman,⁵ in the present study the results showed that there was contamination in 14 samples totaling 70%. In the remaining 30% there was no microbial growth. The bacteria identified in the seeded culture media growth were *Escherichia coli* with 45%, followed by *Klebsiella pneumoniae* 35%, *Streptococcus pyogenes* and *Staphylococcus coagulase negative* with 10%, as shown in Table 1. Table 2 shows the frequency of colony-forming units (CFU), in which 30% of samples were considered negative and there were no growth in plates containing culture media. Moreover, 25% of the sample had an average of 220500 CFU/mL, 20% 367500 CFU/mL, 15% 514500 CFU/mL and 10% 661500 CFU/mL totaling 70% with microbial growth. In Figure 1, it is seen an exponential growth in the number of bacteria related to the time of toothbrushes use, and increased toothbrushes contamination in accordance with its longer usage. In the second method mentioned by Sherertz, Raad and Balani,²² was detected microbial growth in just five toothbrushes totaling 25% of the samples and on 15 brushes the result was an absence of growth in total percentage of 75%. Table 3 shows the microorganisms found, *Escherichia coli* in 80% brushes and *Klebsiella pneumoniae* in 20% and the average colony-forming units (CFU) was 126555 CFU/mL, and the brushes usage time was on average 5 months.

DISCUSSION

The toothbrushes contamination can play an important role in the development of various diseases. Brushes should be replaced monthly and should not be stored in closed or wet containers, these locations associated with the substrate food scraps facilitate

Table 1 - Frequency of microorganisms found using the method of Cleri, Corrado and Seligman⁵.

Bacteria	Percentage
<i>Escherichia coli</i>	45%
<i>Klebsiella pneumoniae</i>	35%
<i>Streptococcus pyogenes</i>	10%
<i>Staphylococcus coagulase negative</i>	10%

Table 2 - Frequency of CFU/mL in toothbrushes.

Class	Midpoint	Absolute frequency	Relative frequency	
0	147000	73500	6	30%
147000	294000	220500	5	25%
294000	441000	367500	4	20%
441000	588000	514500	3	15%
588000	735000	661500	2	10%

Table 3 - Frequency of microorganisms found using the method Sherertz, Raad and Balani²² (1990).

Bacteria	Percentage
<i>Escherichia coli</i>	80%
<i>Klebsiella pneumoniae</i>	20%

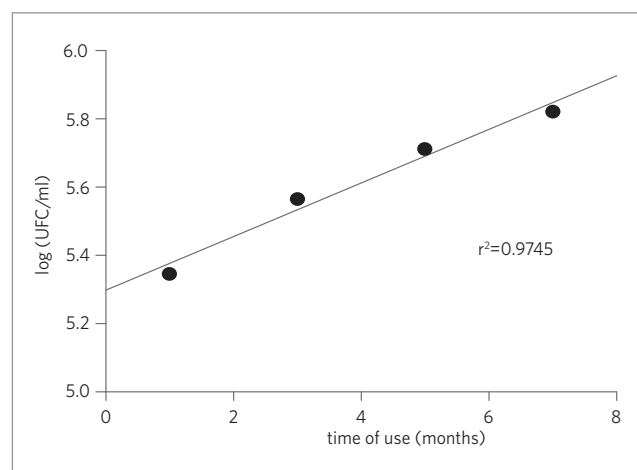


Figure 1 - Relation of bacterial growth medium (log(CFU/mL)) using the method of Cleri, Corrado and Seligman⁵, and the time of use of toothbrushes (n=20).

the microorganisms growth.^{7,11} The second method by Cleri, Corrado and Seligman,⁵ was suitable for studying microorganisms in toothbrushes. In this method, no dilution was performed to facilitate the growth and identification of contaminating microorganisms.

Taji and Rogers,²³ in their studies with toothbrushes, assessing contamination of utensils, identified the growth of *Staphylococcus* sp, *Streptococcus*,

Aerococcus sp, Pseudomonas sp, fecal coliform, among others. Toothbrushes can be a growth site for Streptococcus pyogenes and this microorganism is responsible for pharyngitis in children.² A study conducted by Moreira and Cavalcante¹⁶ reported that 100% of contaminated brushes were positive for four species of microorganisms: Candida albicans, Escherichia coli, Streptococcus mutans and Bacillus subtilis. In our results 81% and 100% of the microorganisms identified in both methodologies were members of the family Enterobacteriaceae. Toothbrushes are usually stored in the toilets and exposed to contamination, since it is a microbial environment with the presence of mainly enteric bacteria spread by aerosols from the toilet.²³ A study of Long and Santos¹⁴ indicated that none of the brushes that are kept inside the bathroom cabinet showed a growth of enterobacteria, whereas the degree of contamination with the presence of two major sorts of fecal coliforms (Enterobacter sp and Citrobacter sp) in brushes kept on the bathroom sink was 70%. The cabinet seems to be the safest place in the bathroom to prevent bristles contamination.

In previous studies we examined the toothbrushes time of use for each subject: 17.5% reported that they change their toothbrush every 30 days, 32.5% every six months and 50% of participants reported that only replace brush once a year. In this work, we found that the brush exchange is essential for good oral hygiene, since the longer is the use, the greater is the wear and microorganisms accumulation in the bristles. The presence of these organisms can be related to the lack of cleaning in the bristles or the brushes storage in wrong places with high rates of heat and humidity, which facilitates the spread and growth of these microorganisms. Among the survey members we found that 90% store the brushes in the bathrooms and among them 30% placed in lockers and 60% reported that the brushes are stored in a location such as exposed in the bathroom, over the sink, counter, glasses, among others. Regarding the use of protective cover on the brush, 90% of respondents did not use the cover protection on their brushes. The average number of persons using the bathroom was 3 per household, and 97.5% did not have habit of lowering the toilet lid to flush. Regarding the frequency of visits to the dentist,

7.5% reported visiting once a month, 15% every 6 months, 77.5% once a year and 97.5% reported that they never received instructions on how to properly store the toothbrush.

Grigoletto¹² and colleagues stated in their studies that the ideal use rate of toothbrushes is four per year, or one every three months. Study conducted in Brazil in 1997 showed that half of the population (about 85 million people) had no toothbrush and the per capita toothbrush consumption was considered low. Brazilians usually buy a brush every 17 months but the recommendation of the oral health society is to change the toothbrush every three months and every three days for patients undergoing chemotherapy.² According to Barros, Pernambuco and Tomital the brush should be kept clean without waste (food or toothpaste) and should be stored where it can dry and without direct contact with other brushes. According to the literature, there are some controversies regarding the storage of the brushes in the bathroom cabinets. Meier¹⁵ and colleagues reported that the bathroom cabinet, boxes and bristles protectors are not the most appropriate location for the storage of toothbrushes, this is justified by the fact that those places maintain a moist environment and warm around the bristles and it may promote the microbial growth. Caudry and Klitorinos³ and Coutinho⁶ et al mentioned that the bathroom cabinet can favor the occurrence of cross-contamination, because the brushes are often stored with their heads in contact with other microorganisms that can pass to each other pathogens also related to respiratory infections, intestinal and other diseases. Brushes should be washed with running water and the water excess must be removed by tapping the edge on the sink and never dry in a towel, then you should spray on the bristles with mouthwash and store it in an open and airy place.¹⁶ There are already some studies on the toothbrushes disinfection, Chaves⁴ and colleagues (2007), reported that the decontamination process of brushes must have a longer range, including the poorest populations and should be evaluated by disinfection methods that are effective and easy to perform, such as the use of sodium hypochlorite 1% and 0.05% acetic acid. Good oral hygiene habits are common when oral health values are accepted as part of the family lifestyle,

and historically, women are more involved in this process. The mothers' example is crucial for their children to acquire good habits.⁸

CONCLUSION

According to the results presented in this study, we observed a high incidence of bacterial contamination

in the analyzed brushes. The microorganisms more frequently found were members of the Enterobacteriaceae family. The toothbrushes time of use can be related to contamination found and therefore not only a good cleaning will ensure the reduction of microbial load, but the toothbrush exchange can also ensure better oral health to individuals.

REFERENCES

- Barros OB, Pernambuco R, Tomita N. Escovas dentais. Pós-Grad Rev Fac Odontol. 2001; 4:33-8.
- Bhat SS, Hegde KS, George RM. Microbial contamination of tooth brushes and their decontamination. J Indian Soc Pedod Prev Dent. 2003 Sep;21(3):108-12.
- Caudry SD, Klitorinos A, Chan EC. Contaminated toothbrushes and their disinfection. J Can Dent Assoc. 1995 Jun;61(6):511-6.
- Chaves RAC, Ribeiro DML, Zaia JE, Alves EG, Souza MGM, Martins CHG. Avaliação de soluções antibacterianas na descontaminação de escovas dentais de pré-escolares. Rev Odont UNESP. 2007;36:29-33.
- Cleri DJ, Corrado ML, Seligman SJ. Quantitative culture of intravenous catheters and other intravascular inserts. J Infect Dis. 1980 Jun;141(6):781-6.
- Coutinho PG, Bittar P, Ditterich RG, Rastelli MC, Romanelli MCMOV, Wambier D, Stadler Evaluation of the storage and preservation means of toothbrushes used by preschool. Rev Odonto Ciênc. 2007 Out-Dez;22(58):335-9.
- Dayoub MB, Rusilko D, Gross A. Microbial contamination of toothbrushes. J Dent Res. 1977 Jun;56(6):706.
- Faustino-Silva D, Ritter F, Nascimento IM, Fontaine PVN, Persici S, Rossoni E. Cuidados em saúde bucal na primeira infância: percepções e conhecimentos de pais ou responsáveis de crianças em um centro de saúde de Porto Alegre, RS. Rev Odonto Ciênc. 2008;23:375-9.
- Figueiredo MC, Faustino-Silva DD. Efetividade da dedeira de gaze comparada à escova dental convencional no controle do biofilme dentário em bebês. ConScientiae Saúde. 2008;7:357-66.
- Fratto G, Nazzicone M, Ortolani E. Disinfezione degli spazzolini dentali. Ricerca sperimentale. Prev Assist Dent. 1990;16:7-10.
- Glass RT, Lare MM. Toothbrush contamination: a potential health risk? Quintessence Int. 1986 Jan;17(1):39-42.
- Grigoletto JC, Watanabe MGC, Mestriner Junior W, Bregagnolo J. Higiene oral e uso compartilhado de escova dental. Rev Odont UNESP. 2006;35(2):175-81.
- Kozai K, Iwai T, Miura K. Residual contamination of toothbrusher by microorganisms. ASDC J Dent Child. 1989 May-Jun;56(3):201-4.
- Long SR, Santos AS, Nascimento CMO. Avaliação da contaminação de escovas dentais por enterobactérias. Rev Odontol Univ St Amaro. 2000;5:21-5.
- Meier S, Collier C, Scaletta MG, Stephens J, Kimbrough R, Kettering JD. An in vitro investigation of the efficacy of CPC for use in toothbrushes decontamination. J Dent Hyg. 1996 Jul-Aug;70(4):161-5.
- Moreira ACS, Cavalcante GM. Influência da higienização na contaminação de escovas dentais. Arq Ciênc Saúde Unipar. 2008;12: 99-103.
- Panzeri H, Lara EHG, Zaniquelli O, Schiavetto F. Avaliação de algumas características das escovas dentais do mercado nacional. Rev. ABO Nac. 1993;1(1):23-9.
- Paschoal AD, Rotta JCP. Conservação e uso das escovas. RGO- Rev Gaúcha Odont; 1992;40:276-8.
- Porto SMMS, Viana MT, Silva KMF, Diniz MFA, Castro CMMB. Neonatal malnutrition and normal microbiota of the oral cavity in rats. Rev Nutr. 2007;20(6):625-32.
- Sammons RL, Kaur D, Neal P. Bacterial survival and biofilm formation on conventional and antibacterial toothbrushes. Biofilms. 2004;1: 123-30.
- Sato S, Ito IY, Lara EH, Panzeri H, Albuquerque Junior RF, Pedrazzi V. Bacterial survival rate on toothbrushes and their decontamination with antimicrobial solutions. J Appl Oral Sci. 2004 Jun;12(2):99-103.
- Sherertz RJ, Raad II, Belani A, Koo LC, Rand KH, Pickett DL, et al. Three-year experience with sonicated vascular catheter cultures in a clinical microbiology laboratory. J Clin Microbiol. 1990 Jan;28(1):76-82.
- Taji SS, Rogers AH. The microbial contamination of toothbrushes. A pilot study. Aust Dent J. 1998 Apr;43(2):128-30.
- Tan E, Daly C. Comparison of new and 3-month-old toothbrushes in plaque removal. J Clin Periodontol. 2002 Jul;29(7):645-50.