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Genomic identification and quantification of microbial species adhering to toothbrush bristles after disinfection: A cross-over study

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ABSTRACT

Purpose: The aim of this clinical investigation was to identify and quantify the microbial species adhering to toothbrush bristles after controlled brushing and storage in different antimicrobial agents.

Methods: Sixteen healthy participants were enrolled in this study and randomly submitted to 4 interventions in a cross-over design: brushing and toothbrush storage in (I) Periogard/(II) Periobio (Chlorhexidine gluconate 0.12%), (III) Cepacol (cetylpyridinium chloride 0.05%) and (IV) distilled water (positive control). Thirty-eight bacterial species including putative pathogens and 5 *Candida* spp. were assessed by Checkerboard DNA-DNA hybridization.

Results: The results of the study have shown a striking reduction of the total microbial counts, including bacteria and *Candida* spp., on the toothbrush bristles after storage in cetylpyridinium chloride 0.05% ($p < 0.0001$). Chlorhexidine gluconate 0.12% showed no differences on the total bacterial count when compared to distilled water ($p > 0.05$). Cetylpyridinium chloride solution also presented the lowest genome counts and frequency of detection for individual target species; distilled water showed the highest individual genome counts ($p < 0.05$). Potential pathogenic species were recorded in moderate to high levels for chlorhexidine gluconate and distilled water.

Conclusion: Cetylpyridinium chloride 0.05% was the most effective storage solution in the reduction of total and individual microbial counts, including pathogenic species.

1. Introduction

The human oral cavity comprehends a complex and heterogenic ecosystem including soft and hard tissues and fluids, which are colonized by several species of microorganisms.[1,2] Besides high diversity, the oral ecosystem is characterized by constant transitions over the time, which are influenced by diverse factors, including external disturbances (such as presence of food debris as result of mastication process, mechanical oral hygiene, antimicrobial solutions, antibiotics, food), intra-biogeographical differences (such as hard or soft tissues), interaction with host and secretions.[3,4] Interaction between these factors may favour microorganisms' colonization and proliferation on the oral surfaces predicting the transitions from health to disease in the oral cavity.[3] Saliva

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also may be considered an important source of nutrients and it is primordial for oral biofilm formation and maturation. Many salivary components (such as proteins) can promote microbial adhesion on the surfaces and may cause aggregation of oral microorganisms resulting in their increasing in the oral cavity.[3]

Over 700 microbial species, including fungi, viruses and unclassified microorganisms could be found colonizing the different surfaces in the oral cavity.[5,6] Most of them are commensal species and are beneficial in promoting the oral health.[5] However, some of these species, under specific conditions, are able to overcome protective host responses and have been implicated in oral diseases, and are referred as pathogenic species. The disruption of the oral microbiota balance resulting in the overload of pathogenic species may cause caries, periodontitis and stomatitis, which are among the most common oral microbial infections in humans. *Streptococcus* spp. and *Actinomyces* spp. are pioneer colonizers, abundant and commonly related to supragingival plaque at the early stage of biofilm formation.[7] They can acidify the oral biofilm increasing their cariogenic potential.[1,8] Following the biofilm maturation, anaerobic and proteolytic bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* and *Fusobacterium* spp. are frequently found harbouring the subgingival biofilm.[9] Bacterial proteases and metabolic products for their lysis, have the potential to induce host responses such as inflammation and immunoreactions leading to periodontitis/peri-implantitis.[10,11] *Candida* spp. are the most incident fungi in the oral cavity and they are strongly associated with denture stomatitis.[12,13] Furthermore, they have been detected as an opportunistic species in periodontal and peri-implantar lesions.[14]

The mechanical removal of formed oral biofilm (including toothbrush, dental floss and tongue scrapers) is considered the most effective method of oral hygiene of the soft and hard tissues significantly reducing the microbial counts in the oral cavity.[15,16] In addition, complimentary hygiene methods by means of mouth rinses and topic fluoride contribute removing the remaining debris and preventing/minimizing the microbial adhesion on the oral surfaces.[17,18] However, a major concern to be overcome in dentistry is the toothbrush contamination by microorganisms after brushing with consequent cross-infection of the oral tissues. Toothbrush bristles are colonized by several species of microorganisms after using, and the viability of these species has been reported ranging from one day to one week.[19,20] Food debris on the bristles acting as nutrient and their exposure to contaminated aerosols (*i.e.* bathroom storage) may precipitate and facilitate the growth and proliferation of various species.[20,21]

Several investigations reported toothbrushes contaminated by non-pathogenic and/or pathogenic species having a relevant impact on the development and proliferation of oral diseases.[22–24] In addition, this condition may contribute aggravating systemic diseases, including respiratory and cardiovascular problems and septicaemia.[25] Currently, it is a consensus that toothbrush disinfection is a primordial step in the oral hygiene. Many studies have assessed various protocols of toothbrushes disinfection, including immersion/spray in different disinfectant solutions or mouth rinses, and bristles impregnated with antimicrobial agents.[26–28] Most of these protocols have been shown effective in reducing the microbial colonization on the bristles. However, most of these investigations used culture-dependent methods for microbial evaluation, in which only viable cells could be detected. Using culture-independent methods (*i.e.* genetic material), we can detect and identify both viable and non-viable microorganisms. Thus, the final result of detection may be different according to the method employed. Considering that microbial cell death may occurs due to absence of significant amount of nutrients and/or microbial competition during toothbrush storage, and the products of cells lysis and degradation may interfere in the inflammatory process of oral tissues, we proposed in this cross-over randomized controlled clinical investigation identifying and quantifying, using the Checkerboard DNA-DNA hybridization method, the microbial species adhering to the toothbrush bristles after brushing and storage in different antimicrobial agents. Thirty-eight bacterial species including putative pathogens and 5 *Candida* spp. were investigated. Two hypotheses were tested: antimicrobial solutions (1) are capable to significantly reduce the total microbial counts on bristles; (2) are effective in reducing the pathogenic species.

2. Materials and methods

2.1. Participants

Participants were selected among graduate students from School of Dentistry of Ribeirão Preto (University of São Paulo, Brazil). Sixteen healthy participants (8 men and 8 women; mean age (±SD) 20.33 ± 1.67 years) were enrolled with at least 28 teeth, no clinical signs of disease in the oral mucosa and healthy gingiva. We did not accept participants who were: pregnant or lactating; had periodontal treatment or antibiotics in the previous 3 months; smoked tobacco; had a systemic disease that might disturb the periodontium, or who required pre-medication for dental treatment. Moreover, an oral exam was carried out to ensure that all participants have not signs or symptoms of pathologic changes. The parameter microbial cell count was the primary variable for the calculation of the minimum sample size in this investigation, and this parameter was calculated using the software PASS 2005 (NCSS, Kaysville, USA). Reference standard deviations were extracted from similar studies in the literature. The study was approved by the local ethics committee and all the experiments were undertaken with the understanding and written consent of each subject and according to the ethical principles (CAAE 2010.1.1125.58.5).

2.2. Toothbrushes and dentifrice selection

Sixty-four conventional toothbrushes (Oral-B Indicator®, Procter & Gamble Company, São Paulo-SP, Brazil) and a dentifrice containing sodium monofluoro-phosphate, 1500 ppm Fluoride, calcium carbonate, sodium lauryl sulfate, and sodium silicate (Sorriso™ Kolynos do Brazil Ltd., São Bernardo do Campo, SP, Brazil) were used during brushing. All the toothbrush heads had a similar regular size, shape and number of bristles.