



# Microbial contamination of toothbrushes and their decontamination

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## Abstract

**Purpose:** The objective was to determine the level of contamination of toothbrushes by mutans streptococci using microbiological identification, to assess the bacterial contamination using scanning electron microscopy (SEM) and to evaluate the efficacy of two toothbrush disinfectants.

**Methods:** Nineteen children used their toothbrushes once a day, for five consecutive days. The toothbrushes were then immersed into disinfectant solutions for 20 h: Group I - 0.12% chlorhexidine gluconate; Group II - 1% sodium hypochlorite; Group III - sterile tap water. They were then placed into test tubes containing CaSa B, for 3 to 4 days at 37°C. The number of MS cfu was counted and the toothbrushes were submitted to SEM analysis.

**Results:** There was no bacterial growth in Groups I and II; Group III showed MS growth (range, 21 to 120 cfu). Scanning electron microscopy showed biofilm formation on toothbrush bristles.

**Conclusion:** Immersion in 0.12% chlorhexidine gluconate and 1% sodium hypochlorite are efficient methods for toothbrush disinfection. (*Pediatr Dent* 22:381-384, 2000)

The oral cavity is free of microorganisms at birth, because the fetus develops in sterile conditions.<sup>1-3</sup> There is a great variety of microbes in the oral cavity during the first day of life, such as *Streptococcus*, *Staphylococcus*, *Neisseria*, *Candida*, *Lactobacillus*, *Veillonella* and coliforms<sup>1</sup>. However, mutans streptococci (MS), which is the primary etiological agent of dental caries in humans, is present only after dental eruption, because it establishes on hard surfaces.<sup>4,5</sup>

As early as 1920, Cobb<sup>6</sup> reported the toothbrush to be a cause of repeated infections of the mouth. Svanberg<sup>7</sup> found that toothbrushes can be heavily infected by MS after 24 h. According to Glass,<sup>8</sup> microorganisms not only adhere to and reproduce on used toothbrushes but also have the ability to transmit organisms responsible for both local and systemic diseases. He also reported that herpes simplex type I survived for 48 h on toothbrushes that had been artificially air-dried and for 7 days or more on moist toothbrushes.

Caudry et al.<sup>9</sup> reported that in spite of the millions of toothbrushes sold each year in North America, there is little public awareness that their bristles may become contaminated by microorganisms with use. The author also believes that

contaminated bristles may play an important role in the transmission and inoculation of the contaminating microorganisms through abrasions of the gingiva, as well as through existing lesions. Glass and Lare<sup>10</sup> suggested that toothbrushes could be an important means of transmission of pathogenic microorganisms to patients submitted to organ transplantation or with immunological depression, via gingival lesions.

According to the literature, the main source of MS is the mother,<sup>11,12</sup> or other family members.<sup>13</sup> Transmission can occur through direct (saliva) or indirect contacts.<sup>12</sup> Indirect contact can occur through fomites, such as spoons,<sup>14</sup> cups, toys or contaminated toothbrushes.<sup>7,12</sup> Day-care centers present a possible risk for infection among children. Outbreaks of group A streptococcal infections have been reported. The close contact between children involving biting and sucking on common toys facilitates the transmission of potential pathogenic microorganisms<sup>7,15-17</sup>. The toothbrushes are colonized by oral cavity microbiota, which can act as reservoirs to reintroduce microorganisms, such as MS, or to contaminate an unaffected surface.<sup>7</sup> Under usual conditions of storage, toothbrushes can be a source, or a vector for transmission or re-infection of diseases such as herpes<sup>8</sup> or periodontopathogenic microorganisms,<sup>18</sup> and coliforms from the bathroom environment.<sup>19</sup>

Various studies have reported toothbrush contamination<sup>7,10,15-20</sup> and recommended methods of disinfection.<sup>9,21</sup> However, in most cases different methodologies were used which do not permit comparisons.

The objective of the present study was to evaluate the contamination level of toothbrushes by MS, the biofilm formation on the bristles by scanning electron microscopy and the efficacy of 0.12% chlorhexidine gluconate and 1% sodium hypochlorite as disinfectants.

## Methods

Saliva samples from 64 children were collected from a day-care center (Lar Santana, at Ribeirão Preto, State of São Paulo, Brazil). The children were of both sexes and between 5 to 12 years old. This study was approved by the Ethical Committee of the Ribeirão Preto School of Dentistry (Process # 98.1.643.58.0) and written consent was obtained from the parents or guardians of the children.

The saliva samples were obtained as described by Kohler & Bratthall (1979)<sup>22</sup> and were plated on SB20 medium according to Davey & Rogers (1984),<sup>23</sup> modified by Azevedo (1988),<sup>13</sup> and incubated at 37° C for 72 h in microaerophilic conditions under the candle jar system. The number of colony forming units (cfu) of MS was counted with a stereoscopic microscope. The children were divided into three groups according to the number of cfu/mL of MS in the samples:<sup>22</sup> low caries risk (0-20 cfu/mL), medium risk (21-100 cfu/mL), and high caries risk (more than 100 cfu/mL).

After MS screening, thirty children considered as medium and high dental caries risk were selected to participate in this study. Their old toothbrushes were recovered and used as positive control. Each child received a tube of toothpaste (Kolynos Super Branco, Kolynos do Brazil Ltda., São Bernardo do Campo, São Paulo, Brazil), and a sterile Johnson's Jr. toothbrush (Johnson & Johnson, São José dos Campos, São Paulo, Brazil). After dental care instructions, the children were submitted to a supervised toothbrushing once a day for 5 consecutive days.

The toothbrushes were maintained separately during the toothbrushing intervals. After 5 days, 19 of the 30 toothbrushes were evaluated. Eleven toothbrushes were eliminated because the children had missed a supervised toothbrushing. During transport, a support was used to avoid contact between the toothbrushes. The toothbrushes were divided into three groups: Group I (N = 7) - immersed individually in test tubes containing 5mL 0.12% chlorhexidine gluconate (Farmácia de Manipulação Doce Erva, Ribeirão Preto, SP, Brazil), for 20 h; Group II (N = 6) - immersed individually in 1% sodium hypochlorite (Farmácia de Manipulação Doce Erva, Ribeirão Preto, SP, Brazil), for 20 h; Group III (N = 6) - immersed individually in sterile tap water, for 20 h, as control. Unused toothbrushes (N = 6) were used as a negative control.

### Microbiological procedures

After 20 h of disinfection, the toothbrushes, including the old toothbrushes recovered, were introduced vertically to avoid contact of the bristles with the test tube wall into separate 25x150 mm test tubes, containing 10.0 mL CaSa B (Bacitracin Sucrose Broth - selective enrichment broth prepared by the modification of Jensen & Bratthall,<sup>24</sup> medium specific for MS without trypan blue according to Cesco et al.<sup>25</sup>) for 3 to 4 days at 37° C. The toothbrushes were withdrawn and rinsed in sterile tap water with gentle shaking, to remove planktonic microbiota, leaving sessile bacteria adhered as "spike" or "mushroom-like" colony/biofilm. The remaining water was then discarded with gentle shaking, and the toothbrush bristles were analyzed carefully from all sides and angles, and each sessile biofilm/colonies, based on colony morphology, were counted under aseptic conditions with a stereoscopic microscope and reflected light.

MS recovery or confirmation was assessed by transferring some colonies from the bristles in the tube containing 2.0mL sterile saline, vortexed for 2 min and seeded on SB20 agar.

### Scanning electron microscopy (SEM)

Two toothbrushes from each group were submitted to SEM analysis (JEOL JMS 25-SII) according to Adriaens et al.<sup>26</sup> to study the bacterial contamination and the biofilm formation on the toothbrush bristles. A tuft chosen at random from the

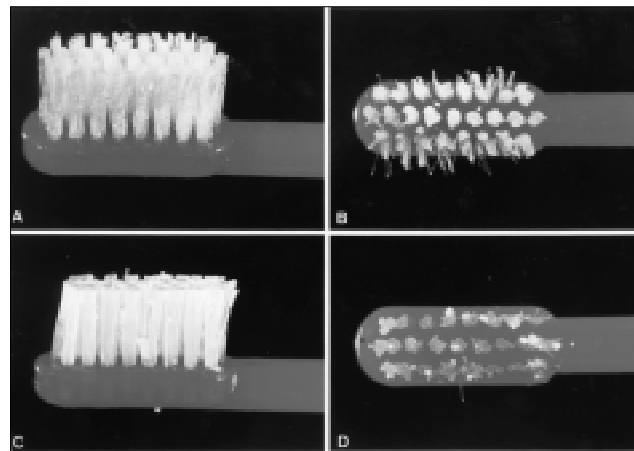


Fig 1. Colonies of mutans streptococci, adhered on toothbrush bristles (A, B, C, D), immersed in sterile tap water (Group III).

toothbrushes was joined and cut off so that the bristles of the tuft remained together. This tuft was then mounted on a preparation-carrier for SEM, coated with gold, under vacuum (Denton Vaccum Desk II), for 40 s, and examined with a scanning electron microscope at 15 kV.

### Results

Unused toothbrushes cultured as control resulted in negative culture. However, transferring some colonies from Group III toothbrush bristles seeded on SB20 agar resulted in MS, almost in pure culture, according to Azevedo.<sup>13</sup> This indicated that the colony/biofilm on bristles is formed by MS.

### Microbiological results

No bacterial growth was observed on Groups I and II toothbrushes. All Group III toothbrushes showed MS growth (range, 21 to 120 cfu). In one toothbrush, MS growth was so abundant it was not countable (case 46). MS was recovered from all six Group III toothbrushes.

Figure 1 shows the MS adhered to Group III toothbrush bristles.

### Scanning electron microscopy

Scanning electron microscopy showed MS growth on toothbrush bristles treated with sterile tap water (Fig 2A). No growth was seen on toothbrush bristles disinfected with 1% sodium hypochlorite (Fig 2B) or 0.12% chlorhexidine gluconate (Fig 2C).

### Discussion

The literature has shown that toothbrushes can be a reservoir for the direct transmission of microorganisms,<sup>12</sup> as well as a source for inoculation or reintroduction of microorganisms from infected to non-infected tissues.<sup>7</sup>

Mutans streptococci, the primary agent of the dental caries, can also be transmitted by toothbrushes, intra- or inter-individual, increasing the incidence of dental caries, especially in children.<sup>17</sup> This means of transmission is of great importance in a country such as Brazil, where the frequency of collective toothbrush use is very common, especially in low-income families.<sup>18,27</sup>

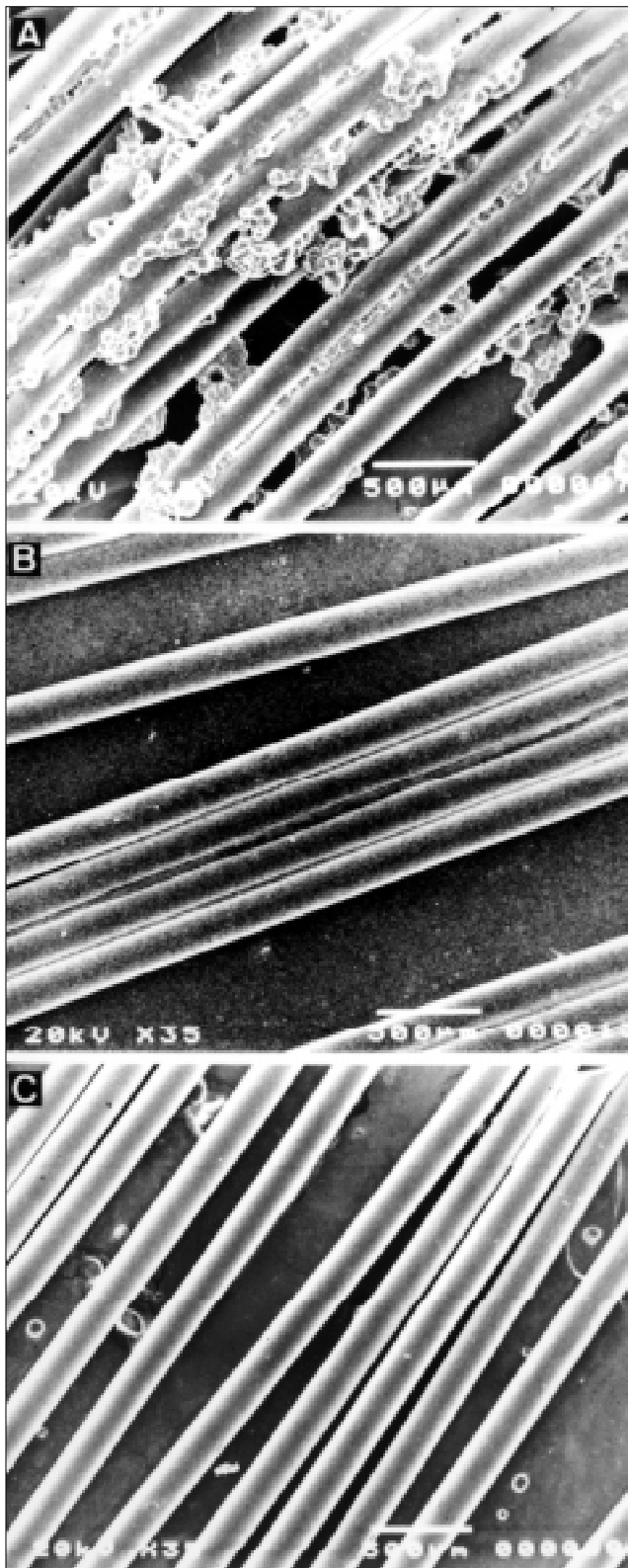


Fig 2. Scanning electron microscopy of toothbrush bristle surfaces (magnification, 35X). A: MS on the toothbrush bristles immersed in sterile tap water (Group III). B: Toothbrush bristles immersed in 1% sodium hypochlorite (Group II), no bacterial growth. C: Toothbrush bristles immersed in 0.12% chlorhexidine gluconate (Group I), no bacterial growth.

In the present investigation, mutans streptococci were found in 100% of the toothbrushes maintained in sterile tap water for 20 h as control (Group III). The biofilm mushroom-like colonies formed on the bristles of these toothbrushes seeded on SB20 agar were identified as MS. This finding, associated with the bacterial growth shown by scanning electron microscopy (Fig 2A), demonstrate that mutans streptococci survive on toothbrush bristles. Even though Taji & Rogers<sup>20</sup> reported no mutans streptococci from adult toothbrushes in a pilot study, our findings are in agreement with those of Svanberg<sup>7</sup> who reported a massive presence of mutans streptococci on the toothbrushes stored for 24 h.

Time necessary for colonization is contradictory varying from 1 to 30 days.<sup>10,20</sup> According to Cesco et al.,<sup>25</sup> colonization of toothbrushes by mutans streptococci occurs in a short time period, since after a single toothbrushing, they found the development of the microorganism in 24% of the cases. Svanberg<sup>7</sup> reported the presence of mutans streptococci on toothbrushes after 3 days. In this study, colonization by mutans streptococci was observed on bristles (Fig 1) after 5 consecutive days of toothbrush use. Biofilm on the old toothbrush bristles was also observed despite the time of use and storage conditions.

Storage conditions of toothbrushes are an important factor for bacterial survival. Dayoub et al.<sup>16</sup> and Meier et al.<sup>21</sup> reported that the number of microorganisms in the toothbrushes kept in aerated conditions was lower than in toothbrushes stored in plastic bags. Several authors have reported that bacterial contamination can be reduced by washing toothbrushes after use, and drying in aerated conditions.<sup>15,17,25,28</sup> Caudry et al.<sup>9</sup> reported that a wet environment increases bacterial growth and cross contamination. Therefore, as time increases between one toothbrushing and another, more microorganism development can occur in the toothbrushes stored in a wet/moisture environment.<sup>17</sup>

Several researchers have suggested the need for toothbrush disinfection to reduce the number of microorganisms on the bristles, using such methods as UV-radiation, microwave oven, boiling water<sup>29</sup> and chemical agents such as Listerine, Plax,<sup>9</sup> and Cepacol.<sup>9,21</sup> Caudry et al.<sup>9</sup> suggested that immersion in Listerine, for 20 min is an efficient method of disinfection. Meier et al.<sup>21</sup> used cetylpyridinium chloride spray 3 times over the bristles and observed a 100% reduction of *S. epidermidis* and 94% for *Candida albicans*. The present investigation showed that immersion of toothbrushes for 20 h in 0.12% chlorhexidine gluconate or 1% sodium hypochlorite was efficient for disinfection (100% inhibition) (Figs 2B and 2C).

The American Dental Association recommends a routine change of toothbrushes every 3 months.<sup>21</sup> As reported by Denny,<sup>28</sup> Glass<sup>29</sup> specifically recommended that healthy patients replace their toothbrush every two weeks. Patients who are sick should change their toothbrushes at the beginning of an illness, when they first feel better, and when they are completely well. Chemotherapy or immune-suppressed patients should change their toothbrushes every three days, and persons submitted to major surgery should change their toothbrushes every day. Many patients, however, reported psychological, economic, and environmental barriers to changing their toothbrushes so frequently. Establishing an easy and effective method for disinfecting a toothbrush would be an important

and economical way to prevent the continuation of reinfection of oral diseases.

The results of this study show the need for toothbrush disinfection. The use of 0.12% chlorhexidine gluconate or 1% sodium hypochlorite proved efficient in this study as a safe, economical and easy method to avoid contamination by mutans streptococci. However, other studies are necessary to decrease immersion time, to find other methods of application and to test other disinfecting agents.

## Conclusions

- 1) Group III toothbrushes, immersed in sterile tap water, showed high mutans streptococci development (range, 21 to 120 cfu).
- 2) Immersion in 0.12% chlorhexidine gluconate (Group I) and 1% sodium hypochlorite (Group II), were efficient disinfection methods.
- 3) Scanning electron microscopy showed biofilm formation on Group III toothbrush bristles.

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