Effect of Triclosan Dentifrice on Toothbrush Contamination

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Abstract

Purpose: The objective of this study was to evaluate by culture and scanning electron microscopy (SEM) the contamination of toothbrushes of 30 children (5-7 years old) by mutans streptococci (MS) when dentifrices with or without triclosan are used.
Methods: The clinical procedures were divided into 3 phases at 1-week intervals. In phase 1 (group I), the children brushed their teeth without dentifrice for 4 minutes; phase 2 (group II) brushed with fluoridated dentifrice (Tandy); phase 3 (group III) brushed with dentifrice containing triclosan (Colgate Total). The toothbrushes were then submitted to microbiological processing for the counting of colony-forming units (CFUs) of MS adhered to the bristles. Four toothbrushes from each group were analyzed by SEM.
Results: MS were present on 93% of group I toothbrush bristles and on 77% of group II toothbrush bristles. Only 40% of group III toothbrushes were contaminated with MS. When there was a positive microbiological culture, the formation of cariogenic bacterial biofilm adhered to the bristles of all groups was identified by SEM.
Conclusions: Toothbrush bristles were contaminated by MS after only one use. A dentifrice containing triclosan significantly reduced bacterial contamination of these toothbrushes. (*Pediatr Dent*. 2004;26:11-16)

KEYWORDS: TOOTHBRUSH CONTAMINATION, DENTIFRICE, MUTANS STREPTOCOCCI, TRICLOSAN

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Different studies have shown that toothbrushes are contaminated by different bacteria, viruses, and fungi after use.[1-8] Since modern dentistry emphasizes prevention and infection control, toothbrushes should be correctly stored, disinfected, and changed at regular intervals. However, the literature presents few articles on the disinfection of toothbrushes.[1,7,9-10]

Since adult individuals are sometimes incapable of efficiently controlling dental biofilm formation, antimicrobial agents such as triclosan are associated with fluoridated dentifrices[11] to increase clinical performance. Triclosan is a synthetic antimicrobial agent, originated from phenol, used as an adjunct in cosmetic products such as soaps and dermatological formulas and recently used in dentistry. It is compatible with two of the most used salts in dentistry, sodium fluoride and sodium monofluorophosphate.[12] When the antimicrobial activity of dentifrices with or without triclosan are compared, it has been suggested that those with triclosan are more effective.[13,14]

Clinical studies utilizing real-life conditions have been conducted to document a variety of benefits, including decreased plaque and gingivitis following use of a dentifrice with triclosan/copolymer.[15] However, according to Sreenivasan and Gaffar[16] in other clinical tests with triclosan, no alterations of the microbial flora of dental plaque were reported. Examination of clinical isolates of bacteria from patients using this antiplaque biocide show no emergence of resistant microflora.

The lack of studies led us to evaluate microbiologically and by scanning electron microscopy (SEM) the effect of dentifrices with or without triclosan on contamination levels of children's toothbrushes by mutans streptococci (MS).

Methods

Forty children of both sexes (5-7 years old) were selected from the Albert Einstein School (Ribeirão Preto, State of São Paulo, Brazil). They were neither under dental treatment nor using antibiotics or antiseptic mouthwashes for

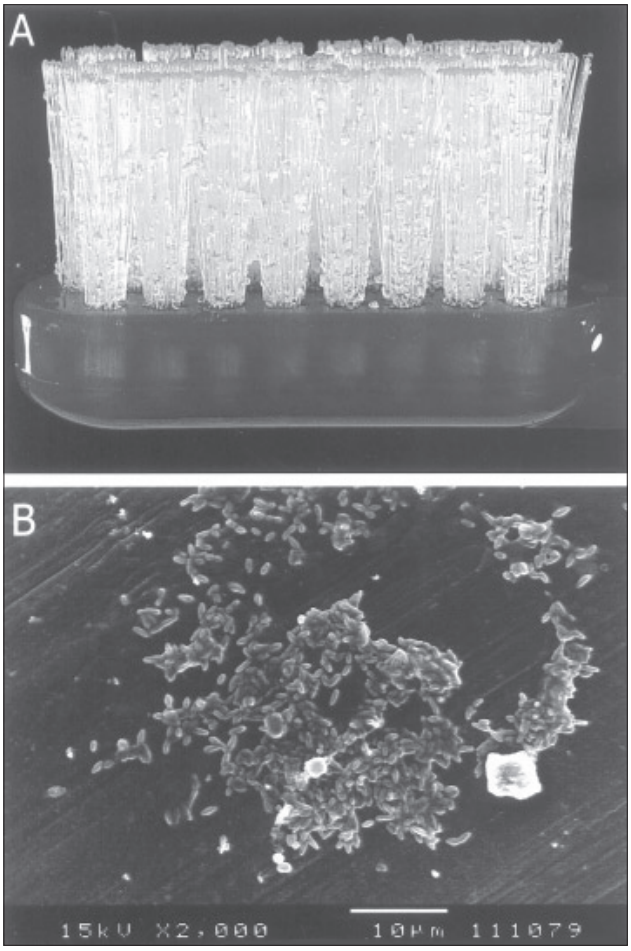

Figure 1A. Group I (tooth-brushing without dentifrice): Intense presence of mutans streptococci colonies adhered on toothbrush bristles.

Figure 1B. Scanning electron microscopy of toothbrush bristle surfaces showing the cariogenic bacterial biofilm formation (magnification ×2000).

at least 3 months. This study was approved by the Ethics Committee of the Ribeirão Preto School of Dentistry, University of São Paulo, and written informed consent was obtained from the parents or guardians of the children.

The study was divided into 3 phases, with a 1-week interval between each phase. In the first phase, each child received a Johnson's Jr. toothbrush (Johnson & Johnson, São Bernardo do Campo, São Paulo, Brazil) and was submitted to supervised tooth-brushing during 4 minutes, using only tap water (group I–control), without dentifrice.

In the second phase (group II–experimental), the same children received another Johnson's Jr. toothbrush and were submitted to supervised tooth-brushing during 4 minutes, with a standardized quantity (covering one third of the bristle) of fluoridated dentifrice (Tandy, Kolynos do Brazil Ltda, São Paulo, Brazil).

In the third phase, the same children (group III–experimental) received another Johnson's Jr. toothbrush and performed supervised tooth-brushing during 4 minutes, with a standardized quantity (covering one third of the bristle) of dentifrice containing triclosan (Colgate Total, Colgate–Palmolive, Osasco, Brazil).

After tooth-brushing, the toothbrushes were carefully rinsed with tap water and excess liquid was removed with gentle shaking by the same professional. They were then collected, numbered sequentially, transported in a special container to avoid contact, and submitted to microbiological processing. The closed container with the toothbrushes was kept at room temperature for 4 hours, simulating the period between brushing.[8,17]

An additional 3 unused toothbrushes (additional control) were removed from their original packages and submitted to microbiological processing before use.

All examiners were blinded as to the group being examined microbiologically or by SEM.

Microbiological procedures

After 4 hours, the toothbrushes of each group were individually placed vertically to avoid contact of the bristles with the test tube wall into separate 25×150 mm test tubes, containing 10 mL CaSa B (bacitracin sucrose broth–selective enrichment broth prepared by the modification of Jensen and Bratthall,[18] medium specific for MS without trypan blue, according to Cesco et al[17]) for 3 to 4 days at 37°C. The toothbrushes were withdrawn and rinsed in the broth with gentle shaking to remove planktonic microbiota, leaving sessile bacteria adhered as "spike" or "mushroom-like" colony/biofilm. The toothbrush bristles were analyzed carefully from all sides and angles, and sessile biofilm/colonies of MS, based on colony morphology, were counted under aseptic conditions with a stereoscopic microscope (Nikon) under reflected light.

The number of colonies was expressed as:

1. 0s=no biofilm or turbid culture medium after 20 days, indicating absence of microorganisms on toothbrush bristles.
2. 0=lack of cariogenic biofilm adhered to the bristles but with turbid medium, indicating presence of other microorganisms.
3. 1 to 100=number of CFUs were countable.
4. +100=colonies were not confluent and had values greater than 100.
5. Uncountable=bacterial development was intense, with confluent colonies, not allowing exact counting of CFUs.

Confirmation that the microorganisms were MS was done by:

1. transferring 4 to 5 colonies/biofilms from the bristles of 3 to 4 toothbrushes, representative of the bacterial development of the group, in the tubes containing 2.0 mL of phosphate buffered solution and glass beads.
2. vortexing the colonies for 2 minutes.
3. seeding of resulting suspension on bacitracin sucrose agar plates (SB_{20}).

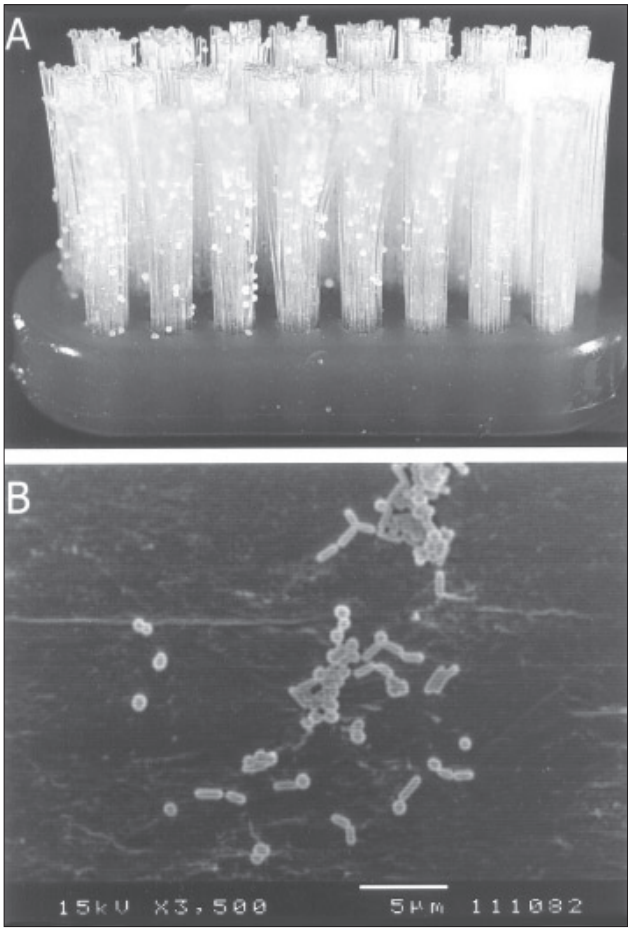

Figure 2A. Group II (tooth-brushing with fluoridated dentifrice): MS colonies adhered on toothbrush bristles.

Figure 2B. Scanning electron microscopy of toothbrush bristle surfaces showing the cariogenic bacterial biofilm formation (magnification ×3500).

This is selective for MS and was prepared according to Davey & Rogers.[19] modified by the substitution of sucrose with cane sugar, according to Torres et al.[20] The colonies that developed were submitted to tests of:

1. fermentation of mannitol, sorbitol, raffinose, and mellibiose.
2. hydrolysis of arginine and sculin.
3. production of H_2O_2.
4. sensitivity to 2.0 IU bacitracine.[21]

Scanning electron microscopy (SEM)

After being submitted to microbiological processing, 2 tufts of bristles from 4 representative toothbrushes of each group were removed. Subsequently, 4 to 5 bristles of these tufts were separated and mounted on stubs and submitted to processing for SEM analysis (JSM 5410 microscope) according to Adriaens et al,[22] to evaluate the cariogenic bacterial biofilm formation on the toothbrush bristles. The bristles were cut and dried (to a critical point) mounted on a preparation-carrier for SEM, coated with gold under vacuum (Denton Vacuum Desk II) for 60 seconds and examined with a scanning electron microscope at 15 kV.

Statistical analysis

The microbiologically obtained results were submitted to statistical analysis for paired data (Cochran and sign test) using the 8.1 GMC software (http://www.forp.usp.br/restauradora/gmc/gmc.html).

Results

Microbiological results

From the initial 40 patients, only 30 completed the 3 phases of this study. The number of CFUs of MS observed in the bristles of groups I, II, and III are presented in Table 1. MS were present on 28 of 30 (93%) group I toothbrushes, and the CFUs ranged from 11 to more than 100, being uncountable in 12 toothbrushes (Figure 1A). Only 2 toothbrushes (7%) were not colonized by MS, however, they did have a positive culture (turbid).

A total of 23 toothbrushes (77%) from group II were contaminated with MS, with the number of CFUs ranging from 5 to more than 100 and uncountable in 11 toothbrushes (Figure 2A). There was no MS contamination in 23% of these toothbrushes; however, microorganisms were absent in only 1 case (3%).

The bristles of only 12 toothbrushes (40%) from group III were contaminated by MS, with CFUs ranging from 2 to more than 100 and uncountable in only 2 (Figure 3A). Eighteen toothbrushes (60%) were not colonized by MS; however, the culture was positive for other microorganisms for 47% of the toothbrushes. There were no microorganisms in 4 toothbrushes (13%).

Contamination was greater in group I (93%), with the least contamination seen in group III (40%). Statistical analysis showed that these differences were statistically significant between groups (I≠II, II≠III, I≠III; chi-square=23.64; $P<.01$).

There was no bacterial contamination of the 3 unused toothbrushes after incubation at 37°C for 20 days.

Scanning electron microscopy

When there was a positive microbiological culture, there was also cariogenic bacterial biofilm adhered to the toothbrush bristles of all groups (Figures 1B, 2B, and 3B).

Discussion

Although MS are considered the primary etiologic agents for dental caries lesions, not many studies have been published on their presence on toothbrush bristles[1,6,7,17,23].

In this study, the authors detected MS in 93% of the toothbrushes from group I (toothbrush without dentifrice). Although Taji & Rogers[5] did not show the development of MS in a pilot study with adult toothbrushes, the findings of the present study are in agreement with those of Svanberg,[23] who reported a massive presence of MS on toothbrushes ($1.5\text{-}6\times10^8$ CFUs). Nelson-Filho et al[7] also observed the development of MS in 100% of child toothbrushes kept in sterile water after brushing.

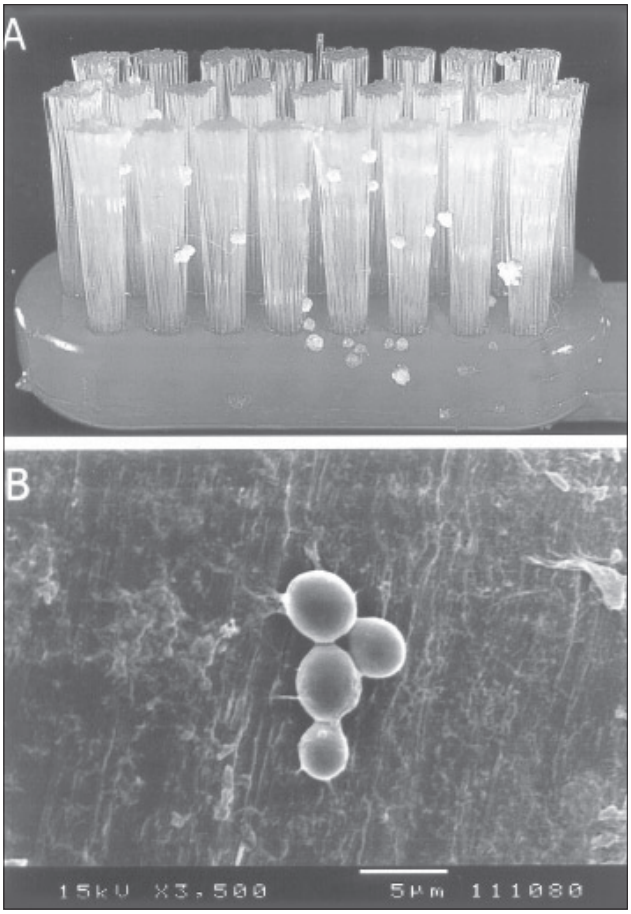

Figure 3A. Group III (toothbrush with dentifrice containing triclosan): A small number of MS colonies adhered on toothbrush bristles.

Figure 3B. Scanning electron microscopy of toothbrush bristle surfaces showing the cariogenic bacterial biofilm formation (magnification ×3500).

In a study at Hiroshima University, Kozai et al,[1] reported that MS were present on toothbrushes at high levels (2.55×10^4 CFUs) even 6 hours after use and exposed to air. The present study showed MS on the bristles of group I at 4 hours after exposure at room temperature.

In 1999, Motzfeld et al,[6] detected MS in only 3% of the analyzed toothbrushes. This difference, compared to the authors' results, is probably because the population studied was from the Faculty of Dentistry of Chile, which is probably more informed about the importance of dental hygiene.

Toothbrush bristles in this study were contaminated by MS after only a single use by children. This is important because the use of community toothbrushes is high in the low socioeconomic level population in Brazil. According to Fratto et al,[9] contact between toothbrushes can occur in a cup or bathroom cupboard. Also, the control of salivary contact is very difficult between individuals in daycare centers, preschools, and other institutions with small children.[3]

Table 1. Number of Colony-forming Units (CFUs) of Mutans Streptococci (MS) on the Bristles of Toothbrushes Used Once by Children*

Case	Group I†	Group II‡	Group III§
1	0	0	0
2	0	0	0
3	+100	0s	0
4	+100	0	0s
5	Unc	5	0
6	Unc	+100	+100
7	+100	Unc	0s
8	26	14	18
9	Unc	Unc	0s
10	11	Unc	+100
11	Unc	19	2
12	14	0	8
13	Unc	+100	72
14	Unc	Unc	Unc
15	Unc	17	0
16	Unc	Unc	0
17	Unc	Unc	+100
18	+100	+100	0
19	+100	22	2
20	Unc	+100	+100
21	22	8	0
22	31	0	0
23	21	Unc	0
24	14	0	0s
25	+100	11	0
26	Unc	Unc	+100
27	Unc	Unc	Unc
28	+100	Unc	0
29	+100	52	0
30	63	Unc	0
Total number of positive cases for MS	28 (93%)	23 (77%)	12 (40%)

*The number of colonies was expressed as: 0s=no biofilm or turbid culture medium, indicating absent microorganisms; 0=lack of cariogenic biofilm adhered to the bristles but with turbid medium indicating presence of other microorganisms; 1 to 100=number of CFUs were countable; +100=colonies were not confluent and had values greater than 100; and Unc=uncountable; bacterial development so intense, with confluent colonies, not allowing exact CFU counting. Groups I≠II, II≠III, I≠III ($P<.01$; Cochran and sign tests).

†Group I (control): brushing without dentifrice.
‡Group II: brushing with fluoridated dentifrice.
§Group III: brushing with dentifrice containing triclosan.

Macro- and microscopic analysis of toothbrushes with positive microbiological culture showed that the presence of cariogenic bacterial biofilm adhered to the bristles, in agreement with Cesco et al[17] and Nelson-Filho et al.[7]

In the toothbrushes from group II (toothbrush with fluoridated dentifrice), the development of MS was observed in 77% of the cases, which was significantly different when compared to group I ($P<.01$). The presence of fluoride in the dentifrice can act as an antibacterial agent, since fluoride can accumulate in the interior of bacteria and cause metabolic changes inhibiting glycolysis, which is responsible for the acidogenic potential of MS which inhibits the adhesion of this microorganism to hydroxyapatite.[24] The Tandy dentifrice contains both fluoride and lauryl sodium sulfate, which also has antibacterial properties.[25] These properties can be responsible for the difference between toothbrush bristle contamination of groups I and II.

Triclosan has been added to dentifrice to reduce dental biofilm formation, caries lesion development, and gingivitis. In a 30-day study, Hawley et al,[26] observed no side effects when using dentifrice with triclosan and reported that it does not affect the anti-caries lesion effect of fluoride. Fine et al,[27] reported that the use of triclosan does not cause a shift in the cariogenic or opportunistic periodontopathogenic microbiota, nor the development of resistant strains even after 6 months use, indicating that triclosan is a safe product. To increase retention by the buccal cavity surface, a copolymer[28] was added to its formula. Zinc citrate was also added to increase the antibacterial potential of triclosan.[13] Colgate Total dentifrice has been reported[25] to be effective, and according to Bradshaw et al,[29] *S mutans* is highly sensitive to triclosan.

The present study showed that only 40% of the toothbrushes from group III (toothbrush using dentifrice with triclosan) were contaminated with MS, indicating that the use of fluoridated dentifrice with triclosan significantly reduced toothbrush bristle contamination by MS. Statistical analysis showed greater efficacy of group III than groups I and II ($P<.01$).

In 1999, Motzfeld et al,[6] reported that toothbrush contamination can be influenced by many factors, such as the type of dentifrice with chemical products that inhibit certain microorganisms or present anti-enzymatic properties. According to Gilbert,[30] after the use of a dentifrice containing triclosan, this antibacterial agent can continue to adhere to the toothbrush when incorporated by residual dentifrice not removed with toothbrush rinsing or directly adhered to the bristles.

However, dentifrice effect on bristle contamination has been examined in very few studies. Verran et al,[4] reported its effect on enterococci, streptococci, and total staphylococci, and Warren et al,[8] evaluated the effect of Colgate Total on periodonto-pathogenic microorganisms (*Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis,* and *Prevotella).*

The comparison of the present study with the literature is not possible because there are no studies evaluating the effect of dentifrice on bristle bacterial contamination by cariogenic microorganisms in children's toothbrushes. This study provides somewhat interesting pilot data to indicate that the use of antibacterial or fluoride-containing dentifrices may influence the potential contamination of toothbrushes in between use. Thus, additional studies should be performed to evaluate different dentifrice brands and compositions containing antibacterial agents to reduce toothbrush contamination as well as various species of bacteria, viruses, and fungi.

Conclusions

1. Toothbrush bristles were contaminated by MS after only a single use.
2. The dentifrice containing triclosan significantly reduced bacterial contamination of the toothbrushes and can be indicated to reduce bacterial contamination of children's toothbrush bristles by MS.

Acknowledgments

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References

1. Kozai K, Iwai T, Miura K. Residual contamination of toothbrushes by microorganisms. *J Dent Child*. 1989;56:201-204.
2. Glass R.T, Jensen HG. The effectiveness of a U-V toothbrush sanitizing device in reducing the number of bacteria, yeasts, and viruses on toothbrushes. *J Okla Dent Assoc.* 1994;84:24-28.
3. Malmberg E, Birkhed D, Norvenious G, Norén JG, Dahén G. Microorganism on toothbrushes at daycare centers. *Acta Odontol Scand*. 1994;52:93–98.
4. Verran J, Leahy-Gilmartin A, Watson GK, Hammond K, Huntington E, Raven SJ. Microbial contamination of toothbrushes during an in-home trial. *J Dent Res*. 1997;76:437.
5. Taji SS, Rogers AH. The microbial contamination of toothbrushes. A pilot study. *Aust Dent J*. 1998;43:128-130.
6. Motzfeld R, Huerta J, Apip A, Araya E. Tipo y grado de contaminación por bacterias bucales y leveduras de cepillos dentales con uso habitual. *Rev Fac Odontol Univ Chile.* 1999;17:9-14.
7. Nelson-Filho P, Macari S, Faria G, Assed S, Ito IY. Microbial contamination of toothbrushes and their decontamination. *Pediatr Dent*. 2000;22:381-384.
8. Warren DP, Goldschimidt MC, Thompson MB, Adler-Storthz K, Keene WJ. The effects of toothpastes on the residual microbial contamination of toothbrushes. *J Am Dent Assoc*. 2001;132:1241-1245.

9. Fratto G, Nazzicone M, Ortolani E. Disinfezione degli spazzolini dentali. Ricerca sperimentale. *Prev Assist Dent.* 1990;16:7-10.
10. Caudry SD, Klitorinos A, Chan ECS. Contaminated toothbrushes and their disinfection. *J Can Dent Assoc.* 1995;61:511-516.
11. Johnson NW. Hygiene and health: The value of antiplaque agents in promoting oral health. *Int Dent J*. 1993;43:375-386.
12. Kjcerheim V. *Experiments with triclosan* [thesis]. Oslo, Norway: University of Oslo; 1995.
13. Saxton CA. The effect of a dentifrice containing zinc citrate and 2,4,4'trichloro-2'-hydroxydiphenyl ether. *J Periodontol.* 1986;57:555-561.
14. Renvert S, Birkhed D. Comparison between 3 triclosan dentifrices on plaque, gingivitis, and salivary microflora. *J Clin Periodontol.* 1995;22:63-70.
15. Volpe AR, Petrone ME, De Vizio W, Davies RM, Proskin HM. A review of plaque, gingivitis, calculus, and caries lesion clinical efficacy studies with a fluoride dentifrice containing triclosan and PVM/MA copolymer. *J Clin Dent.* 1996;7:S1-S14.
16. Sreenivasan P, Gaffar A. Antiplaque biocides and bacterial resistance: A review. *J Clin Periodontol*. 2002;29:965-974.
17. Cesco RT, Bignelli P, Santos CP, Ito IY. Toothbrushes: Evaluation of contamination level by streptococci of mutans group. 5th World Congress on Preventive Dentistry, 27-30 April 1995. São Paulo, Brazil: Anais do 5th World Congress on Preventive Dentistry; 1995:103.
18. Jensen B, Bratthall D. A new method for the estimation of mutans streptococci in human saliva. *J Dent Res.* 1989;68:468-471.
19. Davey AL, Rogers AH. Multiple types of bacterium *Streptococcus mutans* in the human mouth and their intrafamily transmission. *Arch Oral Biol.* 1984;29:453-460.
20. Torres AS, Pizzolitto, AC, Ellias am, Ito IY. Estreptococos do grupo mutans: Avaliaçáo do agar SB20 e MSB na determinaçáo de ufc na saliva e na placa dental de adolescentes. *Rev Bras Odontol.* 1993;50:18-21.
21. Shklair IL, Keene HJ. A biochemical scheme for the separation of 5 varieties of *Streptococcus mutans*. *Arch Oral Biol.* 1974;19:79-81.
22. Adriaens PA, Seynhaeve TM, Be Boever JA. A morphologic and SEM investigation of 58 toothbrushes. *Clin Prev Dent*. 1985;7:8-16.
23. Svanberg M. Contamination of toothpaste and toothbrushes by *Streptococcus mutans*. *Scand J Dent Res*. 1978;86:412-414.
24. Meurman JM. Ultrastructure, growth, and adherence of *Streptococcus mutans* after treatment with chlorhexidine and fluoride. *Caries Res*. 1988;22:228-287.
25. Renvert S, Birkhed D. Comparison between 3 triclosan dentifrices on plaque, gingivitis, and salivary microflora. *J Clin Periodontol*. 1995;22:63-70.
26. Hawley GM, Hamilton FA, Worthington HV, Davies RM, Holloway PJ, Davies TGH, Blinkhorm AS. A 30-month study investigating the effect of adding triclosan/copolymer to a fluoride dentifrice. *Caries Res.* 1995;29:163-167.
27. Fine HD, Furgang D, Bontá Y, Devizio W, Volpe AR, Reynolds H, Zambon JJ, Dunford RG. Efficacy of a triclosan/NaF dentifrice in the control of plaque and gingivitis and concurrent oral microflora monitoring. *Am J Dent.* 1998;11:259-270.
28. Nabi N, Mukerjee C, Schmid R, Gaffar A. In vitro and in vivo studies on triclosan/PVM/M A copolymer/NaF combination as an antiplaque agent. *Am J Dent*. 1989;2:197-206.
29. Bradshaw DJ, Marsh PD, Watson GK, Cummins D. The effects of triclosan and zinc citrate, alone and in combination, on a community of oral bacteria grown *in vitro*. *J Dent Res*. 1993;72:25-30.
30. Gilbert RJ. The oral clearance of zinc and triclosan after delivery from a dentifrice. *J Pharm Pharmacol*. 1987;39:480-483.