Australian Dental Journal 1998;43:(2):128-30

ADRF Trebitsch Scholarship

The microbial contamination of toothbrushes . A pilot study

S. S. Taji*
A. H. Rogers*

Abstract

Ten individuals were each supplied with a new toothbrush of the same type and brand, together with identical tubes of fluoridated toothpaste. After a three-week period, during which subjects were asked to follow their usual oral hygiene practices, the toothbrushes were collected and assayed for microbial contamination using a range of selective growth media. The total microbial load per toothbrush was found to be 10^4 to 10^6 colony forming units. Staphylococci were found on all toothbrushes and streptococci on all but one. These two genera were also quantitatively dominant. Candida, corynebacteria, pseudomonads and coliforms were identified in 70, 60, 50 and 30 per cent of toothbrushes, respectively. However, mutans streptococci, lactobacilli and black-pigmented Gram-negative anaerobic rods were not detected on any of the toothbrushes. For each individual, information on variables such as toothbrush rinsing practices and post-brushing storage methods and environment was collected. No obvious relationship between such variables and microbial load was apparent but it is suggested that more extensive studies are needed, taking into account additional parameters such as age and degree of toothbrush wear and the use of pre-brushing mouthwashes.

Key words: Toothbrush, contamination, micro-organisms.

(Received for publication May 1997. Accepted June 1997.)

Introduction

There is evidence that toothbrushes in regular use can become heavily contaminated with micro-organisms.[1-3] Depending upon storage conditions, the toothbrush can, therefore, serve as a reservoir for the reintroduction of potential pathogens, such as mutans streptococci.[4] Micro-organisms from storage environments can also be introduced; these include enteric bacteria dispersed via aerosols from toilet flushing or from contaminated fingers and skin commensals and pseudomonads emanating from the bathroom and other wet areas.[5]

Glass and co-workers suggested that contaminated toothbrushes may play a role in both systemic and localized diseases.[6] In this context, Sconyers *et al.* found bacteraemia in five of 30 periodontal patients four minutes after tooth brushing, but failed to detect circulating bacteria in any of the 50 clinically-healthy subjects examined.[7] Silver *et al.* detected bacteraemia in three of 36 clinically-healthy patients but they did not take pre-brushing control blood samples.[8]

Studies on the microbiota of toothbrushes have not only been sporadic but varying methodologies have been employed. One critical factor is the manner in which micro-organisms are removed from sample toothbrushes. Such a method should be maximally efficient while causing minimal damage to the micro-organisms. Various methods such as sonication,[1] shaking with glass beads[2] and vortex mixing[4] have been used. A recent study indicated that a combination of vortex mixing and sonication was the most effective method.[3] This has been adopted in the present pilot study, the aim of which was to investigate the microbial contamination of toothbrushes.

Materials and methods

Ten adults were each given a new toothbrush of the same brand and type, together with identical tubes of fluoridated toothpaste.† They were requested to follow their normal oral hygiene practices for a three-week period at the end of which each toothbrush was collected in a sterile paper bag and processed within 18 hours of its last use. Each toothbrush was then decapitated and the head transferred to a tube containing 10 mL of sterile phosphate-buffered saline (PBS). The contents were then subjected to vigorous vortex mixing for 60

*Department of Dentistry, The University of Adelaide.

†Colgate professional medium size soft toothbrush.
Colgate Total toothpaste.
Colgate Oral Care, Sydney, NSW.

Table 1. Numbers of micro-organisms isolated on various media from used toothbrushes – counts are expressed as $\log_{10}$ colony forming units per toothbrush

Subject (brush)	Medium								
	CB	AN	MS	TS	MA	RO	MC	CF	SD
1	6.23	5.81	5.74	5.53	4.84	0	5.89	5.79	5.04
2	4.53	4.19	3.34	3.29	0	0	3.38	0	0
3	3.46	3.26	3.20	2.92	3.30	0	0	0	2.60
4	6.68	6.69	6.73	6.14	6.15	0	6.48	5.29	5.88
5	5.26	5.30	5.16	5.06	3.04	0	5.03	4.18	3.62
6	3.99	4.00	3.67	3.37	3.85	0	3.08	0	3.18
7	5.04	4.95	3.85	3.25	3.00	0	0	0	0
8	6.14	5.46	4.57	5.70	4.09	0	5.98	5.96	3.52
9	4.20	3.28	3.56	3.69	3.04	0	3.56	0	3.18
10	6.80	6.90	6.44	6.30	2.00	0	6.62	5.36	6.12

CB=Columbia blood agar; AN=anaerobic blood agar; MS=mitis-salivarius agar; TS=TSY20 medium; MA=mannitol salt agar; RO=Rogosa agar; MC=MacConkey agar; CF=CFC agar; SD=Sabouraud dextrose agar.

seconds, ultrasonication for 30 seconds, followed by further vortex mixing for 15 seconds. Ten-fold dilutions in PBS were then prepared and 0.1 mL of appropriate dilutions spread onto plates of the following media: Columbia blood agar (CB) for a total aerobic count; anaerobic blood agar (AN) for Gram-negative anaerobes; mitis-salivarius agar (MS) for total streptococci; TSY20 medium (TS) for mutans streptococci; mannitol salt agar (MA) for staphylococci; rogosa agar (RO) for lactobacilli; MacConkey agar (MC) for enterobacteria; CFC agar (CF) for pseudomonads; and Sabouraud dextrose agar (SD) for yeasts and moulds. The MS, TS and AN plates were incubated anaerobically at 37°C in an atmosphere of $CO_2/H_2/N_2$ (5:5:90) for a minimum of 72 hours while the remaining plates were incubated aerobically for 48-72 hours. Total counts and counts of individual colony types were done. Representative colonies from appropriate plates were Gram-stained. They were also tested for the presence of oxidase and catalase and fermentation tests were carried out as required for preliminary microbial identification to genus level.[9]

In order to identify some of the variables which might influence microbial counts, the following information was recorded for each individual: method used for toothbrush rinsing; post-rinsing storage method and environment (for example, bathroom within which there is a toilet); frequency of tooth brushing; and use of pre-brushing mouthwash. At the beginning of the study each individual was given a mirror and probe dental examination; open carious lesions, evidence of periodontal disease and mucosal abnormalities were noted.

Results

The numbers of micro-organisms isolated on the various media are shown in Table 1. No brush was bacteria-free and the total numbers recovered varied from 10^4 to 10^6 colony forming units. Growth occurred on all AN, MS and TS plates, and growth occurred on MA, MC, SD, and CF for 90, 80, 80 and 50 per cent of brushes, respectively. No growth was detected on any of the RO plates. In almost every case, the AN (anaerobic) counts were essentially the same as for those on CB. Indeed, all of the isolates growing on AN were subsequently found to grow aerobically, indicating that they were facultative anaerobes. No black-pigmented colonies were detected on any of the AN plates even after 7-10 days incubation.

Identification to genus level of the various colony types on selective media revealed that all but the MA, CF and SD – selective media for staphylococcus, pseudomonas and yeasts, respectively – were not, in fact, selective. For example, coliforms were detected on MacConkey agar only from brushes 4, 5 and 9 (Table 2) despite the fact that high counts on this

Table 2. Contribution of various micro-organisms to the total microbiota found on used toothbrushes

Subject (brush)	Percentage contribution							
	Staphylococcus	Streptococcus	Aerococcus	Corynebacterium	Pseudomonas	Coliforms	Yeasts	Unidentified
1	22	37	2	13	8	0	0	18
2	32	55	3	0	0	0	0	10
3	46	0	30	0	0	0	14	10
4	17	50	0	1	5	2	15	10
5	20	45	10	0	6	1	3	15
6	30	15	19	0	0	0	16	20
7	35	16	14	0	0	0	0	35
8	26	25	15	5	6	0	3	20
9	36	15	17	9	0	1	10	12
10	10	20	6	0	31	0	18	15

medium were recorded for brushes 1, 8 and 10. Many of the colony types growing on mitis-salivarius agar were not streptococci, but staphylococci/aerococci. The TSY20 medium, designed to favour the growth of mutans streptococci, showed no such organism on any of the brushes. Indeed, colonies growing on this medium proved to be streptococci or staphylococci.

Nevertheless, quantitation of individual genera was attempted and the results can be seen in Table 2. Staphylococci were found on all toothbrushes and were often numerically dominant. Streptococci and aerococci were found on all but one of the toothbrushes, the former often in high numbers. Yeasts (all identified as candida), corynebacteria, pseudomonads and coliforms were identified in 70, 60, 50 and 30 per cent of toothbrushes, respectively, but their contributions to the microbial populations were comparatively low. A significant number of colony isolates could not, with any degree of certainty, be identified at genus level.

Tests were also carried out on three unused toothbrushes. A few colonies of staphylococci were recovered from one of them but the other two were apparently bacteria-free.

Discussion

These findings that most toothbrushes were extensively contaminated with a variety of microorganisms are comparable to those recorded by other investigators.[1–3] It is of interest that the user of the toothbrush showing the lowest level of contamination routinely used a pre-brushing mouthwash which may have exerted an anti-microbial effect. The non-selective nature of some of the supposedly selective media also confirms the findings of Verran and Leahy-Gilmartin[3] who urged that care be taken in the types of selective media used and in the subsequent interpretation of results. One of the undoubted problems is that non-fastidious organisms, such as staphylococci, which are common skin inhabitants, grow well on a range of selective media. The ubiquity of this group of organisms on the tested toothbrushes may well be related to the fact that most of the subjects used their fingers during post-brushing rinsing of their toothbrushes. Corynebacteria could have originated from either the skin or the mouth. Streptococci almost certainly originated from plaque trapped in toothbrush bristles and candida would also have had oral origins. The origin of pseudomonads, aerococci and coliforms would be environmental; pseudomonads from tap water.[5]

No toothbrush was found to harbour mutans streptococci, lactobacilli or black-pigmented Gram-negative anaerobic rods, all of which are potential oral pathogens. Although the number of toothbrushes tested in the present pilot study was small, it would appear that the toothbrush is not a source or oral pathogens.

Eight toothbrushes were stored in bathrooms, five of which were combined toilet/bathroom facilities. Eight of the 10 toothbrushes were stored open to the environment and six of the 10 were rinsed using a finger to manipulate the bristles. No clear correlation could be found between these rinsing techniques and storage methods/environments, and the microbiological data, bearing in mind that only 10 toothbrushes were examined in this pilot study and that statistical analyses were not carried out. There was also no apparent correlation between oral health status and level of toothbrush contamination. The only factor common to the toothbrushes was the fact that they were all new and of the same brand and type. This is another variable which should be further investigated since the relationship between age and wear of toothbrushes and microbial contamination is not known.[3] Furthermore, tests for levels of microbial contamination on brushes left in storage in the bathroom but not actually used, were not conducted. Such tests might have yielded useful data. A more extensive study, taking into account the variables noted above, might pinpoint those factors most likely to influence toothbrush contamination.

Acknowledgement

The principal author (S.S.T.) gratefully acknowledges the award of the Trebitsch Scholarship from the Australian Dental Research Foundation Inc., enabling this study to be undertaken.

References

1. Kozai K, Iwai T, Miura K. Residual contamination of toothbrushes by microorganisms. JDent Child 1989;56:201-4.
2. Malmberg E, Birkhed D, Norvenius G, Noren JG, Dahlen CT. Microorganisms on toothbrushes at day-care centres. Acta Odontol Scand 1994;52:93-8.
3. Verran J, Leahy-Gilmartin AA. Investigations into the microbial contamination of tooth brushes. Microbios 1996;85:231-8.
4. Svanberg M. Contamination of toothpaste and toothbrush by *Streptococcus mutans*. Scand J Dent Res 1978;86:412-4.
5. Scott E, Bloomfield SF, Barlow CG. An investigation of microbial contamination in the home. J Hyg 1982;89:279-93.
6. Glass RT, Lare MM. Toothbrush contamination: a potential health risk? Quintessence Int 1986;17:39-42.
7. Sconyers JR, Crawford JJ, Moriaty JD. Relationship of bacteremia to toothbrushing in patients with periodontitis. J Am Dent Assoc 1973;87:616-22.
8. Silver JG, Martin AW, McBride BC. Experimental transient bacteremias in human subjects with clinically healthy gingivae. J Clin Periodontol 1979;6:33-6.
9. Barrow GI, Feltham RKA, eds. Cowan and Steel's Manual for the identification of medical bacteria. 3rd end. Cambridge: Cambridge University Press, 1993:50-164.

Address for correspondence/reprints:
Associate Professor A. H. Rogers,
The Department of Dentistry,
The University of Adelaide,
Adelaide, South Australia 5005.