Microorganisms on toothbrushes at day-care centers

Essan Malmberg, Dowen Birkhed, Gunnar Norvenius, Jörgen G. Norén and Gunnar Dahlén

Public Dental Service, City of Göteborg; Departments of Pedodontics, Cariology, and Oral Microbiology, Faculty of Odontology, University of Göteborg; Department of Pediatrics I, University of Göteborg; and Department of Community Medicine, Göteborg Health Care Services; Göteborg, Sweden

Malmberg E, Birkhed D, Norvenius G, Norén JG, Dahlén G. Microorganisms on toothbrushes at day-care centers. Acta Odontol Scand 1994;52:93–98. Oslo. ISSN 0001–6357.

The microflora on 44 toothbrushes at 4 day-care centers in the city of Göteborg have been investigated as a presumptive risk factor for transmission of microorganisms by children. Non-supervised toothbrushing without the use of toothpaste was performed at the day-care centers twice a day. Streptococci, predominantly *S. salivarius, S. sanguis*, and *S. mitis*, were the most frequently recorded group of microorganisms and generally constituted the greatest part of the flora (on average, 50%). Beta-hemolytic streptococci were not found in any sample. *Haemophilus* species were noted in 82% of the samples, *H. parainfluenzae* being the most frequent, and *H. influenzae* being identified in only one sample. Anaerobes constituted on average a third of the microflora. Staphylococci were identified in 86% of the samples, *S. epidermidis* dominating. Fungi including molds were found in 50% of the samples, and from one day-care center large numbers of enteric organisms were identified. Thus this study shows that unsupervised toothbrushing at day-care centers can be questioned, more from a general hygienic point of view than from the risk of transmitting serious pathogens. □ *Day-care center; microorganisms; public dental health; toothbrushes*

Jörgen G. Norén, Department of Pedodontics, Faculty of Odontology, Medicinaregatan 12, S-413 90 Göteborg, Sweden

Day-care centers have been considered places presenting a possible risk for infection among children (1–4). In particular, outbreaks of group A streptococcal infections have been recognized (5–10). The close contact between children involving biting and sucking on common toys facilitates the transmission of potential pathogenic microorganisms. In a study by Falck & Kjellander (10) it was shown that 2 weeks after the diagnosis of an outbreak of respiratory tract infections, 61% of the children were colonized with group A streptococci.

Previous studies have reported that bacterial transmission between children may take place through toothbrushes (11–13). In many Swedish day-care centers, more or less organized and supervised toothbrushing of children is carried out. Limited knowledge exists, however, as to what extent bacterial or fungal transmission occurs, and whether toothbrushes are a potential risk for spreading of infections in day-care centers.

The aim of the present investigation was therefore to study the number and composition of the microflora on toothbrushes at four day-care centers in the city of Göteborg.

Materials and methods

Four day-care centers (A–D) in Göteborg were included in the study during May 1991. At all these centers the children performed non-supervised toothbrushing without toothpaste twice a day, after breakfast and after lunch. The toothbrushes were supplied by the parents and thus were of different brands. The brushes were placed in jugs or stood apart from each other. The children in the study were between the ages of 4 and 6 years.

Two weeks before the collection of toothbrushes both the parents and the employees at the day-care centers were informed about the study and how it was to be carried out.

The toothbrushes were not individually marked, and thus, only the day-care center could be identified. The toothbrushes were collected 2 h after use, enabling the toothbrushes to dry before being sampled. Each toothbrush was transferred to a test tube containing 50 ml transport medium (VMG I (14)) with 10 glass beads 5 mm in diameter. The tube was vigorously shaken for 2 min. The samples were transported to the laboratory and were processed within 1 h.

Microbiologic examination

The samples were inoculated on each of the following media: 1) *Brucella* agar (BBL, Microbiological Systems, Cockeysville, Md., USA) enriched with 5% defibrinated horse blood, 0.5% hemolyzed blood, and 5 mg/ml menadione; 2) blood agar (BBL) with 5% defibrinated horse blood; 3) *Haemophilus* agar (15), *Staphylococcus* agar (Difco 0297, Medium 110, Difco Laboratories, Detroit, Mich., USA); 4) trypticase-soy agar plates with 75 mg/l bacitracin and 5 mg/l vancomycin (Difco TSBV agar; 16); 5) Mitis-Salivarius (MS) agar (17); 6) Rogosa agar; and 7) Sabouraud-T agar (Sabouraud glucose agar, Difco 0190 with 0.01% tetrazolium chloride). The *Brucella* agar plate was pre-reduced in an anaerobic glove box for at least 2 days. Aliquots of 0.1 ml of the sample were plated on all six media with a spiral plater (Spiral System, Spiral Systems Inc., Cincinnati, Ohio, USA).

The *Brucella* agar plate was incubated anaerobically (5% H_2, 10% CO_2, and 85% N_2) in a chamber (37°C) of an anaerobic glove box for 7 days. The blood agar and *Staphylococcus* agar plates were incubated in air for 3 days. The *Haemophilus* agar, TSBV agar, MS agar, and Rogosa agar plates were incubated in 10% CO_2 in air at 37°C for 5 days. The Sabouraud-T agar plates were incubated in air at 25°C for 5 days.

The total viable count (TVC) was determined as the total number of colony-forming units (CFU) obtained on *Brucella* agar plates. Streptococci were counted on the MS agar plate, and the percentage TVC was calculated. Species were distinguished by the use of colony morphology, and the predominant morphotype was subjected to API-strep (API-system, S. A. La Balme les Grottes, Montallieu, France). No distinction was made between *S. sanguis* I and *S. gordonii*, *S. salivarius* and *S. vestibularis*, and *S. sanguis* II was referred to as *S. oralis*. Further, streptococci were identified, and strains suspected of beta-hemolysis were tested in accordance with Cowan & Steel (18). The number of lactobacilli was calculated on the Rogosa agar plate but was not further speciated.

Actinobacillus actinomycetemcomitans was identified on the TSBV agar plate, usually on the basis of colony morphology and a positive catalose reaction. Colonies growing on *Haemophilus* agar were tested for their dependence on X and V factors (Biodisc AB, Solna, Sweden). *Staphylococcus aureus* was identified from *Staphylococcus* agar plates as being DNase- and coagulase-positive. Enteric organisms were identified in accordance with API 20E (API System) and yeasts on the basis of API 20C and criteria described by Lodder (19). Further, on the *Brucella* blood agar plate special attention was paid to the anaerobic genera, such as *Prevotella* (formerly *Bacteroides*) and *Fusobacterium*.

The black-pigmented gram-negative rods were further classified on the basis of the following criteria. *Porphyromonas gingivalis* exhibits no long-wave ultraviolet fluoresence and ferments no carbohydrates; *Prevotella intermedia* fluoresces red, produces indole, and ferments glucose but not lactose; and species belonging to the *Pr. melaninogenica* group fluoresces red, does not produce indole, and ferments lactose. Other *Prevotella* isolates were confirmed by gas chromatography. *Fusobacterium* spp were identified on the basis of their nacreous-appearing colonies, their gram-negative spindle-shaped cell morphology, and their production of butyric acid. Colonies of gram-negative rods having a pitted appearance were identified, and distinction was made between non-motile strains in phase contrast microscopy.

Table 1. Frequency, mean, median, and range for various groups of microorganisms isolated from toothbrushes at daycare centers 2 h after use

	Total	*Strepto-coccus* spp.	*Lacto-bacillus* spp.	*Haemo-philus* spp.	Anaerobes	*Staphy-lococcus* spp.	Enterics*			Fungi		
							A. calcoaceticus	*Ps. luteola*	Others†	*Candida*	Others‡	Molds
No. of positive samples	44	40	18	36	36	38	18	9	1	14	7	4
Percentage	100	91	41	82	82	86	41	20	2	32	16	9
Mean of positive samples (CFU × 10^3)	1960	960	5.8	121	630	211	190	61	100	20	13	4.5
Median of positive samples (CFU × 10^3)	1900	1050	0.8	45	400	9	95	15	100	1.5	10	3.5
Range (CFU × 10^3)	50–3400	20–2850	0.5–30	1–1108	10–1.700	0.5–3000	1–1000	6–200	100	0.5–110	1–30	1–10

* *A* = *Acinetobacter*; *Ps.* = *Pseudomonas*.
† Including *Edwardsiella*.
‡ Including *Cryptococcus* and *Rhodutorula* spp.

The motile forms that could not ferment glucose usually showed a spreading tendency ("dry gliders") on the agar surface, a characteristic common for most strains of *Campylobacter* (formerly *Wolinella*) *rectus*. Bacterial strains with a "wet gliding" motility and no signs of corroding or pitting of the agar surface were grouped as *Capnocytophaga* spp if they showed no motility in phase contrast but were long and slender gram-negative rods after gram staining. Their presence as a dominant species was confirmed on the TSBV agar plate. Peptostreptococci were identified as gram-positive cocci not growing in air.

Results

Forty-four toothbrushes were analyzed, and the results are given in Table 1. The sampling solution contained a mean TVC of 2×10^6 microorganisms/ml (range, 5×10^4–3.4×10^6). Two samples had a low total numbers of microorganisms ($<10^5$). The TVC was calculated on the anaerobic *Brucella* agar medium, except for 13 samples for which the aerobic blood agar plate constituted the basis for calculation of the TVC owing to the presence of higher number of microorganisms on the plate. Five of these showed heavy growth of enteric organisms, seven of *Staph. epidermidis*, and one of *Staph. aureus*.

Streptococci constituted the most frequently recovered group of microorganisms and generally contained the greatest part of the flora (on average, 50%). Only four samples did not show the presence of streptococci, and all four contained large numbers of enteric organisms (all from day-care center A). In all samples oral streptococci, predominantly *S. salivarius, S. sanguis*, or *S. mitis*, were found. In no sample were betahemolytic streptococci found. Only 41% of the samples showed lactobacilli, never constituting any major part of the flora.

Haemophilus species were found in 36 samples (82%), at a mean level of 1.2×10^5 microorganisms/ml (range, 1×10^3–1.1×10^6). In one sample *Haemophilus* was not identified owing to heavy growth of enteric organisms. *H. parainfluenzae* was the most

frequently found species. *H. influenzae* was identified in one sample. *A. actinomycetemcomitans* was detected in two samples, both at less than 10^3 microorganisms/ml.

Next to the streptococci, anaerobes or microaerophils formed the most commonly found group (82% of the samples) and constituted on average one-third of the flora. Six samples with no anaerobes detected showed heavy growth of enteric organisms, and two samples had a very low TVC. There was a remarkable similarity between the samples with regard to anaerobic and microaerophobic species. Among the dominant bacteria on the *Brucella* agar plate, *Prevotella, Fusobacterium, Campylobacter, Peptostreptococcus*, and *Capnocytophaga* species were identified. Only one sample contained black-pigmented gram-negative rods, which were identified as *Pr. intermedia*.

Staphylococci were identified in 38 samples (86%) in various amounts, from single colonies to a total predominance in the sample (median value, 0.9×10^6; mean, 2.1×10^5; range, 0.5×10^3–3×10^6). In all samples showing staphylococci, *Staph. epidermidis* were identified as the dominant species, though *Staph. aureus* dominated in two samples with lower counts.

Enteric organisms were identified in almost half of the samples (45%). *Acinetobacter calcoaceticus* showed the highest prevalence (41%) and constituted in the positive samples a mean count of 1.9×10^5, but in several samples it was the totally dominant species. *Pseudomonas luteola* was recovered from nine samples (20%), of which seven were in coexistence with *Acin. calcoaceticus*. In one sample *Edvardsiella* was identified.

Fungi, including molds, were found in 50% of the samples. *Candida* was the most common species (32%)—however, in comparison with bacteria often in low numbers (mean, 2×10^4; median, 1.5×10^3; range, 0.5×10^3–1.5×10^5). *Candida albicans* and *C. quillierimondii* were among the predominant microorganisms in one sample each. Other fungi were isolated in seven samples and constituted only minor proportions of the TVC (range, 1×10^3–3×10^4). In four samples *Cryptococcus uniguttulatus* was identified, in one sample *Crypt. laurentii*, and in one sample *Crypt. terreus*. In one sample, *Rhodotorula* were identified. Molds were found in four samples but in small amounts, and further identification was not undertaken.

The frequency of the recovered microorganisms from the four day-care centers is shown in Table 2. Day-care center A differed from the other three by having a markedly lower frequency of streptococci, *Haemophilus*, anaerobes, and fungi—that is, all typical oral microorganisms—whereas the frequency of enteric organisms was higher. Six of nine samples having a high median value for *Acin. calcoaceticus* were obtained from center A. In four of these six samples no streptococci were found; in all six no anaerobes were found, and in four samples no *Haemophilus* were shown. From center A no sample had growth of staphylococci higher than the median value (Table 1). The frequency of fungi was higher from day-care center C.

Table 2. Percentage frequency of various groups of microorganisms isolated from toothbrushes in relation to the four investigated daycare centers (A–D)

Day-care center	Total	*Strepto-coccus* spp.	*Lacto-bacillus* spp.	*Haemo-philus* spp.	Anaerobes	*Staphylo-coccus* spp	Enterics	Fungi	Molds
A	8	50	63	38	25	63	86	13	0
B	12	100	42	92	92	83	17	42	17
C	10	100	20	90	100	90	60	80	0
D	14	100	43	93	93	100	21	43	14

Discussion

This study has shown that toothbrushes are heavily contaminated with microorganisms for up to 2 h after use. These microorganisms are of oral as well as environmental origin. The use of non-supervised toothbrushing at day-care centers has been considered a possible route for transmission of microorganisms between children, perhaps explaining outbreaks of upper respiratory infections (10, 20). The moist toothbrush may hypothetically harbor potential pathogens from one child, which are transferred to another child's mouth directly by use of the same brush, contact between two brushes, or indirectly by exchange of brushes between the children from one occasion to another. The transmission of oral bacteria from one child to another is therefore likely to occur, as shown in some previous studies (11–13).

There was no clinical infection outbreak among the children at the day-care centers at the time for investigation. The children's carrier state of these pathogens was not considered in the present study, but it is known that potential pathogens of upper respiratory infection, such as group A streptococci, pneumococci, *Branhamella catarrhalis*, and *H. influence*, primarily colonize the pharynx and tonsil region and may occasionally occur in the saliva and around the teeth (21, 22). In only one case was *H. influenzae* found in the present study. The low recovery rate of potential upper respiratory pathogens does not mean that transmission through toothbrushes does not occur. When they are present in the oral cavity, they would probably be present on the toothbrushes simultaneously with the oral species of the same genera.

The level of microorganisms on toothbrushes is probably closely related to the dryness achieved after use, which is dependent on time and how they are stored. It might be argued that 2 h is a short period for drying and that overnight storage may give a markedly lower number of microorganisms. A free-hanging storage in a vented room appears to be the preferred method. The number of bacteria on toothbrushes stored in room air after use decreases more quickly than on brushes in containers (11). This implies that as long as the toothbrushes are not dry, they will harbor microorganisms from the oral cavity, which may stay viable for longer times.

The moist toothbrush as a place for potential microbial growth was further substantiated by the surprising finding of heavy growths of enterics, yeasts, and molds, which only occur as transients in the oral cavity (20, 21). Unsupervised toothbrushing gives the children opportunities to play with the toothbrushes, which may contaminate the brushes with environmental microorganisms. Consulting the day-care centers' personnel also confirmed that brushing their toys, the washstand, and even the lavatory is common among the children. Even if the potential risk for infection by these microorganisms must be considered low, it is not acceptable hygienically, and the value of unsupervised toothbrushing must be questioned. On the other hand, if toothbrushing is supervised by trained personnel and fluoridated toothpaste is used, and if the toothbrushes are carefully rinsed after use and allowed to dry, free-hanging in vented rooms, it may be argued that gains in dental care are present and should be further encouraged.

Acknowledgements.—This study was supported by grants from Förstamajblommans Riksförbund and Oral Microbiological Diagnostic Service, Göteborg, Sweden. We thank Mrs. Eva-Monica Paulsson for her technical assistance.

References

1. Glass RT, Lare MM. Toothbrush contamination: a potential health risk? Quintessence Int 1986;17:39–42.
2. Petersson C, Håkansson A. A prospective study of infectious morbidity and antibiotic consumption among children in different forms of municipal day-care. Scand J Infect Dis 1989;21:449–57.
3. Söderström M, Hovelius B, Schalén C. Decreased absence due to infectious disease in children at two day-care centres over an eight-year interval (1979/80–1987/88). Acta Pediatr Scand 1990;79:454–60.
4. Cars H, Petersson C, Håkansson A. Infectious diseases and day care centre environment. Scand J Infect Dis 1992;24:525–8.
5. Belfrage-Sanzén I, Bygren PG, Christensen P, Holm S, Hevatius B, Scharlén C. Förekomst och

handläggning av streptokockepidemier på daghem. Lakartidningen 1980;77:1929–33.
6. Bygren PG, Cegrell L, Christensen P, Johansson B, Nermark J. En streptokockepidemi på förskolor i Lund våren 1976. Lakartidningen 1977;74:2987–91.
7. Gran B. Streptokockepidemi på ett daghem i Luleå. Lakartidningen 1980;77:4018–20.
8. Smith TD, Wilkinson V, Kaplan EL. Group A Streptococcus-associated upper respiratory tract infections in a day-care center. Pediatrics 1989; 83:380–4.
9. Holmström L, Nyman B, Rosengren M, Wallander S, Ripa T. Outbreaks of infections with erythromycin-resistant group A streptococci in child day care centres. Scand J Infect Dis 1990;22:179–85.
10. Falck G, Kjellander J. Outbreak of group A streptococcal infection in a day-care center. Pediatr Infect Dis J 1992;11:914–9.
11. Dayoub MB, Rusilko D, Gross A. Microbial contamination of toothbrushes. J Dent Res 1977;56: 706.
12. Svanberg M. Contamination of toothpaste and toothbrush by *Streptococcus mutans*. Scand J Dent Res 1978;86:412–4.
13. Kozai K, Iwai T, Miura K. Residual contamination of toothbrushes by microorganisms. J Dent Child 1989;May–June:201–4.
14. Möller ÅJR. Microbiol examination of root canals and periapical tissues of human teeth [thesis]. Odontol Tidskr 1966;74:1–380.
15. Chapin KC, Doern GV. Selective media for recovery of *Hemophilus influenzae* from specimens contaminated with upper respiratory tract microbial flora. J Clin Microbiol 1983;17:1163–5.
16. Slots J. Selective medium for isolation of Actinobacillus actinomycetemcomitans. J Clin Microbiol 1982;15:606–9.
17. Hamada S, Slade HD. Biology, immunology and cariogenicity of *Streptococcus mutans*. Microbiol Rev 1980;44:331–84.
18. Cowan ST. Cowan and Steel's manual for the identification of medical bacteria. Cambridge: Cambridge University Press, 1974.
19. Lodder JL. The yeasts. A taxonomic study. Amsterdam: North-Holland Publishing Co, 1971.
20. Falck G, Kjellander J. Studies on the transmission of group A streptococci. II. The role of streptococcal contamination of the environment—recurrence rate of streptococcal pharyngitis related to hygienic measures. Paediatr Infect Dis J 1994. In press.
21. Tanner A, Lai C-H, Maiden M. Characteristics of oral gram-negative bacteria. In: Slots J, Taubman M, editors. Contemporary oral microbiology and immunology. St. Louis (MO): Mosby Year Book, 1992:299–341.
22. Maiden M, Lai C-H, Tanner A. Characteristics of oral gram-positive bacteria. In: Slots J, Taubman M, editors. Contemporary oral microbiology and immunology. St. Louis (MO): Mosby Year Book, 1992:342–72.

Received for publication 3 June 1993
Accepted 21 September 1993