Prospective study of potential sources of *Streptococcus mutans* transmission in nursery school children

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Transmission of *Streptococcus mutans*, a major dental caries pathogen, occurs mainly during the first 2.5 years of age. Children appear to acquire *S. mutans* mostly from their mothers, but few studies have investigated non-familial sources of *S. mutans* transmission. This study prospectively analysed initial *S. mutans* oral colonization in 119 children from nursery schools during a 1.5-year period and tracked the transmission from child to child, day-care caregiver to child and mother to child. Children were examined at baseline, when they were 5–13 months of age, and at 6-month intervals for determination of oral levels of *S. mutans* and development of caries lesions. Levels of *S. mutans* were also determined in caregivers and mothers. A total of 1392 *S. mutans* isolates (obtained from children, caregivers and mothers) were genotyped by arbitrarily primed PCR and chromosomal RFLP. Overall, 40.3% of children were detectably colonized during the study, and levels of *S. mutans* were significantly associated with the development of caries lesions. Identical *S. mutans* genotypes were found in four nursery cohorts. No familial relationship existed in three of these cohorts, indicating horizontal transmission. Despite high oral levels of *S. mutans* identified in most of the caregivers, none of their genotypes matched those identified in the respective children. Only 50% of children with high levels of *S. mutans* carried genotypes identified in their mothers. The results support previous evidence indicating that non-familial sources of *S. mutans* transmission exist, and indicate that this bacterium may be transmitted horizontally between children during the initial phases of *S. mutans* colonization in nursery environments.

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INTRODUCTION

Streptococcus mutans, a major pathogen of dental caries, can colonize the mouth of children at an early age, depending on the presence and intensity of factors that favour its transmission and establishment in the oral cavity. Control of this cariogenic organism is difficult once the bacteria achieve significant levels in the oral cavity at early ages (Köhler *et al.*, 1983, 1988; Emilson *et al.*, 1987). Strategies that interfere with the initial establishment of *S. mutans* are desirable (Köhler *et al.*, 1983). The transmission of *S. mutans* among adults or older children is apparently difficult (Saarela *et al.*, 1993; Emanuelsson *et al.*, 1998; Redmo Emanuelsson & Thornqvist, 2001). The initial

colonization by *S. mutans* was investigated in a prospective study of 46 US children from birth to 5 years of age whose mothers carried high levels of *S. mutans* (Caufield *et al.*, 1993). From that study, a window of infectivity was defined as the period from 19 to 31 months of age, when the risk of *S. mutans* acquisition was apparently high. The establishment of a competitive, complex microbial community after complete eruption of teeth (by 30 months of age) and after maturation of IgA antibody responses to *S. mutans* antigens may partially explain the decreasing risk of colonization after this initial window of infectivity (Caufield *et al.*, 2000; Nogueira *et al.*, 2005, 2007). However, several factors might influence the initial age of *S. mutans* acquisition, for example the extent of sucrose intake (van Houte *et al.*, 1982) and the frequency of salivary contact with *S. mutans*-colonized subjects (Köhler *et al.*, 1983). Mothers have been defined as

Abbreviations: AP-PCR, arbitrarily primed PCR; EMEI, nursery school (Escola Municipal de Ensino Infantil).

the main source of *S. mutans* transmission to children (Berkowitz & Jordan, 1975; Rogers, 1977; Köhler *et al.*, 1983; Li & Caufield, 1995; Li *et al.*, 2000; Redmo Emanuelsson & Thornqvist, 2001), but other familial and non-familial sources of transmission may also exist (Kozai *et al.*, 1999; Mattos-Graner *et al.*, 2001b; Klein *et al.*, 2004). The identification and understanding of the sources and course of *S. mutans* transmission are important to define strategies to control the initial colonization. Several studies suggest that pathogenic species of the upper respiratory tract, such as *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*, are more prevalent and persistent, and are transmitted horizontally among children in day-care centres (Pons *et al.*, 1996; Yano *et al.*, 2000; Peerbooms *et al.*, 2002; Sá-Leão *et al.*, 2008). The salivary contact between nursery children during a phase of initial establishment of their oral microbiota and maturation of the immune response might also contribute to the transmission of oral micro-organisms. A significant proportion of young child populations regularly attend day-care centres worldwide. In this study, we investigated potential sources of *S. mutans* transmission in day-care nurseries in a prospective study of infants from nursery cohorts, their respective day-care caregivers and their mothers.

METHODS

Population and study design. The study population comprised 160 children aged 5–13 months at baseline and 91 caregivers from 28 municipal day-care nursery schools [Escolas Municipais de Ensino Infantil (EMEIs)] in the city of Piracicaba, São Paulo, Brazil. These children were enrolled in a previous study to analyse patterns of salivary IgA antibody response to *S. mutans* antigens (Nogueira *et al.*, 2005). As a positive control for *S. mutans* transmission, 15 mothers of a subset of 16 children who carried high levels of *S. mutans* at the end of the study were also analysed. Children spent a 10 h daily period, 5 days a week, in these EMEIs. All of the EMEIs followed the same policy for care provision; thus a nearly homogeneous sucrose-rich diet was provided to children. Exposure to fluoride, habits of oral hygiene and dietary schedules were similar among these EMEIs, as described in detail elsewhere (Mattos-Graner *et al.*, 1998, 2001a).

Clinical and microbiological examinations were performed on children at baseline (T_0), and after 6 (T_6), 12 (T_{12}) and 18 (T_{18}) months of follow-up, for determination of caries development and oral levels of *S. mutans*. Microbiological examinations were also performed in 91 caregivers at T_6 or T_{12} , when initially colonized children were observed. Genotypic identities of *S. mutans* were compared among isolates from children who attended the same nursery and among isolates from children and their respective caregivers. All of the clinical examinations and sample collections were performed by one of the authors (A.C.A) after calibration (with R.D.N.). At the end of the follow-up period (T_{18}), 119 children (74.3 %) remained in the study. Forty-one children did not continue in the study because they were not present in the EMEIs at the follow-up examinations or because they no longer lived in the city. Only children whose mothers provided informed consent were enrolled in the study, which was previously approved by the Ethical Committee of the Piracicaba Dental School, University of Campinas (protocol no. 110/2002).

Clinical examinations. Clinical examinations were performed using a sterile mouth mirror and portable light to evaluate the presence of

erupted teeth and/or caries lesions, as described in detail elsewhere (Mattos-Graner *et al.*, 2000, 2001a). The mothers of children who showed caries lesions were informed and treatment was arranged at the Dental Clinic for Baby Treatment of the Centro de Atendimento de Pacientes Especiais (CEPAE), at the Piracicaba Dental School, University of Campinas. All of the transport costs for attending the CEPAE were provided to the families.

Microbiological analysis. Levels of *S. mutans* were determined in oral samples collected with sterile tongue blades, which were introduced into the mouth, moistened with saliva and pressed on both sides against the dorsum of the tongue. Each side of the tongue blade was then pressed onto the surface of mitis-salivarius agar containing 15 % sucrose and 3.3 mg bacitracin l^{-1} in a RODAC plate (Mattos-Graner *et al.*, 2000). The plates were incubated in candle jars at 37 °C for 48 h. The number of c.f.u. of *S. mutans* was determined with the aid of a stereomicroscope and expressed as number of colonies in a pre-determined area of the tongue blade impression (c.f.u. per area). Approximately eight colonies representative of the observed colonial morphologies were then cultivated in Todd–Hewitt broth and pure streaked on mitis-salivarius agar. All pure culture samples were frozen at –70 °C in skimmed milk (Difco).

Analysis of the genetic diversity of *S. mutans* by arbitrarily primed PCR (AP-PCR). *S. mutans* isolates were analysed by AP-PCR as described previously (Mattos-Graner *et al.*, 2001b). Briefly, genomic DNA was purified from *S. mutans* cultures using a MasterPure kit (Epicentre Technologies). The concentration and integrity of the samples were evaluated by spectrophotometry (A_{260}) and analysis in 1 % agarose gels stained with ethidium bromide. PCRs were performed with primer OPA02 (5'-TGCCGAGCTG-3') and OPA03 (5'-AGTCAG-CCAC-3') (Invitrogen). Thermal conditions were initial denaturation at 95 °C for 5 min, followed by 45 cycles of 95 °C for 30 s, 36 °C for 30 s and 72 °C for 1 min. Amplicons were electrophoresed at 5 V cm^{-1} in 1.5 % agarose in Tris/borate/EDTA and stained with ethidium bromide. Digital images of stained gels were captured with a Gel Logic 100 Imaging System (Pharmacia Biotech).

A total of 1392 *S. mutans* isolates were analysed during the study (819 from children, 448 from caregivers and 125 from mothers). After initial visual screening of all AP-PCR profiles for each subject, AP-PCRs were rerun with isolates representative of each AP-PCR profile detected in each subject in order to perform side-by-side comparisons between child–child, child–caregiver and child–mother pairs. Gel images of these second sets of AP-PCR were obtained as described above.

Chromosomal DNA RFLP. Isolates identified in different subjects but representing the same AP-PCR profile were subjected to chromosomal DNA RFLP analysis to confirm genotypic identity. RFLP was performed as described elsewhere (Mattos-Graner *et al.*, 2001b). Briefly, genomic DNAs were purified using phenol/chloroform and samples of 10 µg chromosomal DNA were digested for 3 h with *Hae*III (Invitrogen) at 37 °C. DNA fragments were then resolved on a 0.5 % agarose gel in Tris/borate/EDTA at 1.4 V cm^{-1} . After staining of the gel with ethidium bromide, digital images were captured under UV light as described above.

Data analyses. AP-PCR profiles were analysed using Fingerprinting II Informatix software version 3.0 (Bio-Rad) for calculation of the Dice coefficients of similarity. Isolates were considered to be the same genotype when the AP-PCR profiles showed a Dice coefficient equal to or greater than 95 %. This cut-off value has been shown to provide high agreement between AP-PCR profiles and RFLP fingerprinting (Li & Caufield, 1998). Consistencies between AP-PCR profiles generated in replicate assays were also checked by visual analysis. RFLP profiles were visually compared between strains representing identical AP-PCR genotypes (Dice coefficient ≥ 95 %). Comparisons of the

frequencies of children with detectable levels of *S. mutans*, the number of *S. mutans* genotypes and caries development over time were evaluated using a χ^2 test. Spearman's correlation analysis was applied to analyse associations between levels of *S. mutans* and the number of caries lesions that developed during the study. Associations between age and number of erupted teeth with *S. mutans* levels and caries lesions were also analysed at each phase of the study, as the studied children were within an age range of 8 months. The levels of *S. mutans* and number of genotypes were compared between children who remained caries-free or who developed caries lesions during the study by a Mann-Whitney U-test.

RESULTS AND DISCUSSION

S. mutans colonization, genotypic diversity and caries development in nursery school children

At baseline (T_0), *S. mutans* was detected in only 5.6 % of children. Two pre-dentate children showed detectable levels of *S. mutans*, indicating that some of the isolates analysed in this study might be from the tongue or other mucosal surfaces. No *S. mutans* strains could be recovered in further examinations in these two children, suggesting that the strains initially detected were transitory. The prevalence of *S. mutans* at each phase of the study is depicted in Table 1. Caries lesions (cavities) were detected at baseline and affected 20.1 % of children ($n=24$) at the end of the study (T_{18}). Except for one child, all other 23 caries-affected children carried detectable levels of *S. mutans*. These 23 children represented 47.9 % of all of the children detectably colonized with *S. mutans* ($n=48$) at T_{18} . No significant differences were observed in mean age and mean number of teeth between the groups of *S. mutans*-positive and *S. mutans*-negative children at each study phase (data not shown). Levels of *S. mutans* (c.f.u. per area) were associated with the number of caries lesions at T_{12} and T_{18} (Spearman correlation: $r=0.53$ and $r=0.55$, respectively, at T_{12} and T_{18} ; $P<0.001$), but did not correlate with age ($r=0.14$ and $r=0.02$ at T_{12} and T_{18} , respectively; $P>0.05$) or number of erupted teeth ($r=0.17$ and $r=0.10$; $P>0.05$). Median oral levels of *S. mutans* were significantly higher among children with cavities

when compared with caries-free children at T_{12} (82.5 vs 0 c.f.u. per area) and T_{18} (96 vs 0 c.f.u. per area) (Mann-Whitney; $P<0.001$). High levels of *S. mutans* (≥ 100 c.f.u. per area) were detected in 0.6, 5.0, 8.5 and 16 % of children at T_0 , T_6 , T_{12} and T_{18} , respectively.

Between 20 and 35 % of the detectably colonized children carried more than one *S. mutans* genotype during the study. The frequencies of children with more than one genotype in the caries-free and caries-affected subsets are shown in Table 1. The number of genotypes per child did not increase significantly during the study (Table 1), although the levels of *S. mutans* colonization increased from T_0/T_6 to T_{12}/T_{18} in several children (data not shown). In addition, the frequencies of more than one genotype were not associated with oral levels of *S. mutans* in each phase of the study (χ^2 test, $P>0.05$; data not shown), in agreement with our previous observations in a similar nursery population (Mattos-Graner *et al.*, 2001b). Several *S. mutans* genotypes identified in the early phases of this study (22.5 %) could still be recovered at the 1 year follow-up (T_{12}), as shown in Fig. 1, supporting the notion that *S. mutans* clones can successfully colonize the oral cavity at a very early age. However, fluctuations were also observed in the detection of specific genotypes.

S. mutans transmission in nursery school cohorts

Previously, we reported a case of two children with high levels of *S. mutans* attending one public nursery school who carried the same *S. mutans* genotype (Mattos-Graner *et al.*, 2001b). Sharing of *S. mutans* strains among nursery children was later reported in a day-care nursery in Japan (Tedjosasongko & Kozai, 2002). Among the 28 EMEIs studied, 19 (67.8 %) were shown to have at least two children who were colonized with *S. mutans* during the study. In seven of these 19 EMEIs, at least two children were found to share similar AP-PCR profiles (Dice coefficient $\geq 95\%$). Four of these pairs (21.1 %) shared identical *S. mutans* RFLP profiles (Fig. 2). Except for one pair of children who were siblings, the other three cohorts (15.8 %) had no contact outside the EMEIs. Interestingly,

Table 1. *S. mutans* colonization, caries development and genotypic diversity in children during the prospective study

Study phase	No. (%) of children with <i>S. mutans</i>	No. of isolates tested	No. of genotypes	No. (%) of caries-free children colonized by:*		No. (%) of children with caries lesions colonized by:*	
				1 genotype	>1 genotype	1 genotype	>1 genotype
T_0 ($n=160$)	9 (5.6)†	25	8	5 (100.0)	0	0	1 (100.0)
T_6 ($n=141$)	22 (15.6)	162	34	14 (66.7)	7 (33.3)	0	1 (100.0)
T_{12} ($n=131$)	42 (32.1)	287	62	17 (63.0)	10 (37.0)	10 (66.7)	5 (33.3)
T_{18} ($n=119$)	48 (40.3)	345	62	21 (84.0)	4 (16.0)	15 (65.2)	8 (34.8)

*No significant associations were detected between the presence of caries lesions and the frequency of detection of more than one genotype at each phase of the study (χ^2 test, $P>0.05$).

†Isolates from three children could not be recovered.

Fig. 1. Recovery of *S. mutans* genotypes (AP-PCR profiles) of three representative children at each phase of the study (T_0 , T_6 , T_{12} and T_{18}). The genotype identities of each isolate are indicated by letters above each lane. UA, Reference strain UA130; M, marker.

two to four distinct genotypes were identified per child in two of these cohorts, which may indicate that these children were challenged with multiple *S. mutans* genotypes. The genotypes shared by nursery cohorts were detected at T_{12} and T_{18} , when children were within the window of infectivity. In a preliminary study of one child pair, the shared *S. mutans* genotype continued to be detected 10 months after the T_{18} examinations (data not shown). No similar *S. mutans* genotypes were detected among children from different EMEIs (data not shown).

S. mutans transmission between day-care caregivers and children

There are two possible explanations for detection of matching of *S. mutans* genotypes among nursery children cohorts. One is that the shared *S. mutans* genotypes were transmitted laterally between children by direct or indirect salivary contact. Alternatively, the co-colonized children could have been exposed to a common transmission source in the nursery environment, e.g. their day-care caregivers. Among the 66 caregivers who provided care to the *S. mutans*-colonized children, 92.4% showed detectable levels of *S. mutans*, and more than half of these caregivers ($n=34$) carried high levels of these bacteria (≥ 100 c.f.u. per area), indicating the potential for *S. mutans* transmission. Thirty caregivers were colonized by only one *S. mutans* genotype. The frequency of colonization by more than one genotype was not associated with oral levels of *S. mutans* in these subjects ($\chi^2=2.26$, $P=0.322$). Despite the high prevalence of *S. mutans* in the caregivers, no genotype matching was found between caregivers and the respective children.

S. mutans transmission between mothers and children

Because mothers are recognized as the main source of *S. mutans* transmission (Berkowitz & Jordan, 1975; Rogers, 1977; Emanuelsson *et al.*, 1998; Li *et al.*, 2000), we investigated the similarities of genotypes between mother and child in a subset of 16 children with high levels of *S. mutans* (≥ 100 c.f.u. per area) at the end of the study (T_{18}). All of the mothers analysed carried detectable levels of *S. mutans*. Eleven mothers (68.8%) carried high levels of *S. mutans* (≥ 100 c.f.u. per area), and nine of them (56.3%) carried more than one *S. mutans* genotype. However, matching genotypes were detected in only half of the mother-child pairs ($n=8$). Among the mothers who shared *S. mutans* genotypes with their child, seven carried high

Fig. 2. Representative DNA RFLP profiles detected in eight isolates from four nursery cohorts (indicated by square brackets). Child identities (numbers) are indicated above each lane. M, Marker.

levels of *S. mutans* (≥ 100 c.f.u. per area) and one showed low levels of *S. mutans* (1–20 c.f.u. per area). The low frequency of genotype matching among the mother–child pairs suggests that these nursery children were exposed to diverse sources of *S. mutans*. It was reported previously that maternal *S. mutans* genotypes are observed less frequently in nursery children (Li *et al.*, 2000; Tedjosasongko & Kozai, 2002) than in children who stay at home (Li & Caufield, 1995). Although genotypes detected in children but not in mothers or other family members have been repeatedly reported in several populations (Emanuelsson *et al.*, 1998; Redmo Emanuelsson & Wang, 1998; Kozai *et al.*, 1999; Li *et al.*, 2000; Tedjosasongko & Kozai, 2002; Klein *et al.*, 2004), there is still limited information about non-familial sources of *S. mutans* transmission (Tedjosasongko & Kozai, 2002; Mattos-Graner *et al.*, 2001b).

The findings of this study indicate that *S. mutans* strains may be transmitted from child to child during the initial stages of oral colonization in nursery environments. Although horizontal transmission was not a major event, the low frequency of detection of maternal genotypes in the studied children further indicates various sources of *S. mutans* initial transmission. There is suggestive evidence that initial *S. mutans* colonization may be controlled through antimicrobial suppression of *S. mutans* levels in mothers before or during their child's window of infectivity (Köhler *et al.*, 1983; Gripp & Schlagenhauf, 2002). This approach may be less effective in susceptible child populations who are exposed to diverse sources of *S. mutans*.

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REFERENCES

- Berkowitz, R. J. & Jordan, H. V. (1975). Similarity of bacteriocins of *Streptococcus mutans* from mother and infant. *Arch Oral Biol* **20**, 725–730.
- Caufield, P. W., Cutter, G. R. & Dasanayake, A. P. (1993). Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. *J Dent Res* **72**, 37–45.
- Caufield, P. W., Dasanayake, A. P., Li, Y., Pan, Y., Hsu, J. & Hardin, J. M. (2000). Natural history of *Streptococcus sanguinis* in the oral cavity of infants: evidence for a discrete window of infectivity. *Infect Immun* **68**, 4018–4023.
- Emanuelsson, I. R., Li, Y. & Bratthall, D. (1998). Genotyping shows different strains of mutans streptococci between father and child and within parental pairs in Swedish families. *Oral Microbiol Immunol* **13**, 271–277.
- Emilson, C. G., Lindquist, B. & Wennerholm, K. (1987). Recolonization of human tooth surfaces by *Streptococcus mutans* after suppression by chlorhexidine treatment. *J Dent Res* **66**, 1503–1508.
- Gripp, V. C. & Schlagenhauf, U. (2002). Prevention of early mutans streptococci transmission in infants by professional tooth cleaning and chlorhexidine varnish treatment of the mother. *Caries Res* **36**, 366–372.
- Klein, M. I., Florio, F. M., Pereira, A. C., Höfling, J. F. & Goncalves, R. B. (2004). Longitudinal study of transmission, diversity, and stability of *Streptococcus mutans* and *Streptococcus sobrinus* genotypes in Brazilian nursery children. *J Clin Microbiol* **42**, 4620–4626.
- Köhler, B., Bratthall, D. & Krasse, B. (1983). Preventive measures in mothers influence the establishment of the bacterium *Streptococcus mutans* in their infants. *Arch Oral Biol* **28**, 225–231.
- Köhler, B., Andreen, I. & Jonsson, B. (1988). The earlier the colonization by mutans streptococci, the higher the caries prevalence at 4 years of age. *Oral Microbiol Immunol* **3**, 14–17.
- Kozai, K., Nakayama, R., Tedjosasongko, U., Kuwahara, S., Suzuki, J., Okada, M. & Nagasaka, N. (1999). Intrafamilial distribution of mutans streptococci in Japanese families and possibility of father-to-child transmission. *Microbiol Immunol* **43**, 99–106.
- Li, Y. & Caufield, P. W. (1995). The fidelity of initial acquisition of mutans streptococci by infants from their mothers. *J Dent Res* **74**, 681–685.
- Li, Y. & Caufield, P. W. (1998). Arbitrarily primed polymerase chain reaction fingerprinting for the genotypic identification of mutans streptococci from humans. *Oral Microbiol Immunol* **13**, 17–22.
- Li, Y., Wang, W. & Caufield, P. W. (2000). The fidelity of mutans streptococci transmission and caries status correlate with breast-feeding experience among Chinese families. *Caries Res* **34**, 123–132.
- Mattos-Graner, R. O., Zelante, F., Line, R. C. & Mayer, M. P. (1998). Association between caries prevalence and clinical, microbiological and dietary variables in 1.0 to 2.5-year-old Brazilian children. *Caries Res* **32**, 319–323.
- Mattos-Graner, R. O., Smith, D. J., King, W. F. & Mayer, M. P. (2000). Water-insoluble glucan synthesis by mutans streptococcal strains correlates with caries incidence in 12- to 30-month-old children. *J Dent Res* **79**, 1371–1377.
- Mattos-Graner, R. O., Correa, M. S., Latorre, M. R., Peres, R. C. & Mayer, M. P. (2001a). Mutans streptococci oral colonization in 12–30-month-old Brazilian children over a one-year follow-up period. *J Public Health Dent* **61**, 161–167.
- Mattos-Graner, R. O., Li, Y., Caufield, P. W., Duncan, M. & Smith, D. J. (2001b). Genotypic diversity of mutans streptococci in Brazilian nursery children suggests horizontal transmission. *J Clin Microbiol* **39**, 2313–2316.
- Nogueira, R. D., Alves, A. C., Napimoga, M. H., Smith, D. J. & Mattos-Graner, R. O. (2005). Characterization of salivary immunoglobulin A responses in children heavily exposed to the oral bacterium *Streptococcus mutans*: influence of specific antigen recognition in infection. *Infect Immun* **73**, 5675–5684.
- Nogueira, R. D., Alves, A. C., King, W. F., Goncalves, R. B., Hofling, J. F., Smith, D. J. & Mattos-Graner, R. O. (2007). Age-specific salivary immunoglobulin A response to *Streptococcus mutans* GbpB. *Clin Vaccine Immunol* **14**, 804–807.
- Peerbooms, P. G., Engelen, M. N., Stokman, D. A., van Benthem, B. H., van Weert, M. L., Bruisten, S. M., van Belkum, A. & Coutinho, R. A. (2002). Nasopharyngeal carriage of potential bacterial pathogens related to day care attendance, with special reference to the molecular epidemiology of *Haemophilus influenzae*. *J Clin Microbiol* **40**, 2832–2836.
- Pons, J. L., Mandement, M. N., Martin, E., Lemort, C., Nouvellon, M., Mallet, E. & Lemeland, J. F. (1996). Clonal and temporal patterns of nasopharyngeal penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae* strains in children attending a day care center. *J Clin Microbiol* **34**, 3218–3222.

- Redmo Emanuelsson, I. M. & Thornqvist, E. (2001).** Distribution of mutans streptococci in families: a longitudinal study. *Acta Odontol Scand* **59**, 93–98.
- Redmo Emanuelsson, I. M. & Wang, X. M. (1998).** Demonstration of identical strains of mutans streptococci within Chinese families by genotyping. *Eur J Oral Sci* **106**, 788–794.
- Rogers, A. H. (1977).** Evidence for the transmissibility of human dental caries. *Aust Dent J* **22**, 53–56.
- Saarela, M., Troil-Linden, B., Torkko, H., Stucki, A. M., Alaluusua, S., Jousimies-Somer, H. & Asikainen, S. (1993).** Transmission of oral bacterial species between spouses. *Oral Microbiol Immunol* **8**, 349–354.
- Sá-Leão, R., Nunes, S., Brito-Avo, A., Alves, C. R., Carrico, J. A., Saldanha, J., Almeida, J. S., Santos-Sanches, I. & de Lencastre, H. (2008).** High rates of transmission of and colonization by *Streptococcus pneumoniae* and *Haemophilus influenzae* within a day care center revealed in a longitudinal study. *J Clin Microbiol* **46**, 225–234.
- Tedjosasongko, U. & Kozai, K. (2002).** Initial acquisition and transmission of mutans streptococci in children at day nursery. *ASDC J Dent Child* **69**, 284–285.
- van Houte, J., Gibbs, G. & Butera, C. (1982).** Oral flora of children with “nursing bottle caries”. *J Dent Res* **61**, 382–385.
- Yano, H., Suetake, M., Kuga, A., Irinoda, K., Okamoto, R., Kobayashi, T. & Inoue, M. (2000).** Pulsed-field gel electrophoresis analysis of nasopharyngeal flora in children attending a day care center. *J Clin Microbiol* **38**, 625–629.