Colonization of Mutans Streptococci in Costa Rican Children from a High-risk Population

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ABSTRACT

Purpose: The aim of the study was to determine the colonization of mutans streptococci (MS) in dental biofilm and saliva of children from a high-risk population with low socio-economic status from San José, Costa Rica.

Methods: A total of 68 healthy babies from 8 to 20 months of age (mean age=13 months), with caries-free status and cariogenic feeding habits (eg, nursing bottle- or breast-feeding on demand) were examined. Children divided in two groups (<13 months and 14-20 months). Samples from dental biofilm and saliva from each subject were processed and plated in blood agar (BA) and Mitis-Salivarius-Bacitracin agar (MSB) to determine MS percentage. Statistical analysis included the analysis of variance test, and stratification by age of MS levels.

Results: Detectable MS levels in dental biofilm and saliva were found in 75% and 72% of children, respectively. Counts higher than 20000 colony forming units (CFU) per ml were found in 4% of saliva and in 35% of biofilm samples. The two groups showed significant differences in MS levels for dental biofilm (P=.036) and saliva (P=.009). Children older than 17 months presented higher MS levels (P<.05). Analysis by MS levels (<0.1%; 0.1-1.0%; >1.0% of MS) The two groups showed an insignificant association with increasing mean age. MS density was associated with mean number of erupted primary molars.

Conclusions: Mutans streptococci colonization of dental biofilm and saliva from high caries risk infants is associated with age and dental development. (J Dent Child 2007;74:36-40)

KEYWORDS: MUTANS STREPTOCOCCI, COLONIZATION, DENTAL CARIES, DENTAL BIOFILM, SALIVA

In Costa Rica, Early Childhood Caries (ECC) afflicts 36% of the children between 12 and 24 months of age, with a mean dt (decayed teeth) of 4.1±3.6 and a mean percentage of decayed to present teeth of 26.8±21.3. Currently, no effective program has been established to address this issue, although the Salt Fluoridation Program was introduced in Costa Rica since 1987 for the benefit of the entire population.[1] Like most Latin American children, Costa Rican children commonly make their first dental visit after the age of 3, when it can be too late to prevent ECC.[2]

It has been demonstrated through evidence that dental caries is an infectious disease and that mutans streptococci (MS) are the primary etiologic bacterial agents in humans.[3-8] The pathogenicity of these micro-organisms is related to their: adherence characteristics; acidic nature; and resistance to low pH levels.[6] It has been shown that early colonization by MS is closely related to a high dental caries experience, although the mere presence of MS is not sufficient to develop dental caries.[9] Longitudinal studies have demonstrated that, as soon as MS bacteria are detected in an infant's mouth, they persist as resident micro-organisms

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Table 1. Presence of Mutans Streptococci (MS) in Dental Biofilm and Saliva of Costa Rican Children According to Age Group

Age group	n	Percentage of children with MS in:			
		Dental biofilm		Saliva	
		Detectable	Nondetectable	Detectable	Nondetectable
≤13 mos	33	54	46	58	42
14-20 mos	25	94	6	86	14
Total	**68**	**75**	**25**	**72**	**28**

among the normal oral microbiota.[3,4] Evidence-based dental studies regarding ECC suggest that, at present, there are 3 events for the development of carious lesions during early childhood:

1. primary infection and initial colonization by MS;
2. increase of the MS population in the mouth associated with the frequent and long exposure to a cariogenic diet rich in carbohydrates; and
3. demineralization and development of caries.[10-13]

It has also been suggested that bedtime feeding with bottle- or breast-feeding is associated with the presence of carious lesions in infants and children at early age.[14,15]

Several investigators have used MS counts in saliva and in dental biofilm to predict caries risk in children and, consequently, in the prevention of development of demineralization lesions or other signs of dental disease.[7] Other authors have stated that MS counts higher than 10,000 or 20,000 UFC/ml in saliva indicate a high risk of acquiring the dental caries disease.[16]

At present, there is no available information regarding the frequency of MS colonization in Costa Rican children or adults.

Therefore, the present study's aim was to:

1. isolate and identify representative strains of mutans streptococci (MS);
2. determine the percentage of colonization by MS in dental biofilm and saliva samples; and
3. establish associations to some epidemiological and biological factors in a sample of children between 8 and 20 months of age from a high-risk, low socioeconomic status population within San Jose, Costa Rica.

METHODS

STUDY POPULATION

The total sample consisted of 68 children (40 males, 28 females) 8 to 20 months old, selected at random while attending their routine medical appointment at the Healthy Babies Clinic of the Basic Team of Integral Health Attention (Equipo Básico de Atención Integral en Salud—EBAIS) in a low socioeconomic area of San José, Costa Rica, during the months of June to December, 2004. The mothers and/or caregivers were invited to participate in the study after they were explained the risks and benefits of participating in the study; following their acceptance, an informed written consent form approved by the Institutional Review Board of the University of Costa Rica, San Jose, Costa Rica, was given and signed by the mothers or caregivers.

Subsequently, a medical and dental clinical history of every child was performed by investigators who were residents of the Pediatric Dentistry Residency of the University of Costa Rica following the regulations and standard pediatric dentistry recommendations and guidelines for dental examination of young children and infants.

The inclusion criteria were the following:

1. a child between 8 and 20 months of age;
2. unremarkable medical history, assessed by the EBAIS-consulting pediatricians;
3. the presence of at least 2 primary incisors erupted. Erupted tooth was defined by at least two thirds of the tooth visible in the oral cavity;
4. absence of white spot lesions, caries, decalcification lesions or other hipoplastic defects on primary dentition; caries diagnosis was based on the criteria of Radike (1968), with the modification that enamel was not scraped from white spot lesions[17];
5. sustained cariogenic feeding habits, such as the use of a nursing bottle at bedtime or naptime with a liquid other than water, and breast-feeding on demand.

The use of a nursing bottle and/or breast-feeding habits was assessed via a questionnaire and by interviewing mothers or caregivers. Nursing bottle contents included cow milk, cow milk and sucrose, cow milk and cereals, milk formula, and fruit juices.

A pooled dental biofilm sample was obtained by swabbing the gingival third of the primary incisors with a sterile cotton swab. Saliva samples were collected by introducing a sterile cotton swab sublingually on the floor of the mouth for 15 seconds. Samples were dispersed in test tubes containing 2 ml of sterile phosphate-buffered saline solution (PBS, pH 7.4) and sent within 2 hours of collection to the Bacteriology Laboratory, School of Microbiology, University of Costa Rica.

LABORATORY PROCEDURES FOR BACTERIAL COUNTS

PBS samples were agitated using a vortex mixer for 30 seconds. Serial dilutions were performed for each sample at decimal levels in PBS. After agitation, aliquots of 0.1 ml were dispensed in mitis salivarius agar plates, supplemented with 1% sodium tellurite and 0.2 U/ml bacitracin (MSB),[18,19] and in blood agar plates (BA). After incubation for 48 hours at 37°C in a 6% CO_2 atmosphere, plates between 30 and 300 colony forming units (CFU) were chosen to determine the total number of CFU/ml of the initial suspension of each sample in 2 ml PBS. Representative colonies with morphological characteristics of MS were isolated and presumably confirmed by mannitol and sorbitol fermentation.[20] Mutans streptococci

density for each sample was expressed as a percentage of the CFU in MSB from the total CFU in BA. Statistical analysis included analysis of variance (ANOVA) test, and stratification by age and MS levels was performed.

RESULTS

The mean age of the children in the study sample was 13 months. For data analysis purposes, the study population was divided into 2 groups:

1. children ranging in age from 8 to 13 months; and
2. children 14 to 20 months old (Table 1).

Detectable MS levels were found in 75% of dental biofilm samples (95% confidence interval [CI]=64%-86%) and in 72% of saliva samples (95% CI=61%-83%). A comparison of the two age groups showed statistically significant differences for MS counts in dental biofilm and saliva (P=.036 and P=.009, respectively). The mean average density of MS detectable in saliva was 0.087% and in dental biofilm was 1.341%. A correlation of 0.982 (confidence level=99%) was found between MS populations in dental biofilm and saliva.

Detectable MS amounts were found in children as young as 8 months for dental biofilm and 9 months for saliva. In children younger than 12 months (N=32), dental biofilm and saliva samples with detectable MS were 63% and 56%, respectively. High MS quantities in dental biofilm and saliva were found in all children 17 months of age or older (N=15). In children with viable MS counts, counts higher than 20,000 CFU/ml were found in 4% of saliva samples and in 35% of dental biofilms. Older children (>17 months) showed significantly higher MS populations in dental biofilm and saliva (P<.005).

As shown in Table 2, children were stratified into 3 categories according to MS density in dental biofilm and saliva: (1) <0.1%; (2) 0.1%-1.0 %; and (3) >1.0%.[21] ANOVA test compared the colonization of dental biofilm and saliva per MS density category and mean age for each density group. A confidence level of 95% showed that density of infection does not vary considerably with age in dental biofilm or saliva samples (P=.602 and P=.255, respectively). The results showed, however, that children with MS densities higher than 1% are older than the group with less than 0.1% MS.

Table 3 shows the percentage of children with erupted molars and the mean number of molars with the density of MS in dental biofilm and saliva. Only 19 children from the total sample population presented the 4 primary molars as erupted. In the association between MS density, the number of children with primary molars and the mean number of erupted primary molars—a tendency whereupon MS levels increased proportionally to the number of molars present—was observed. This association held true for both dental biofilm and saliva samples, but the differences were not significant. Further, no significant relation was observed between the number of teeth present and MS density (P=.05).

Table 2. Distribution of Mutans Streptococci Density in Costa Rican Children With Relation to Oral Localization

MS density	Dental biofilm			Saliva		
	N (%)	Mean age (mos)	95% CI	N (%)	Mean age (mos)	95% CI
<0.1%	40 (59)	12.5	11.45-13.65	58 (85)	12.9	12.04-13.79
0.1%-1.0%	17 (25)	14.0	12.5-15.48	8 (12)	15.1	11.86-18.39
>1.0%	11 (16)	15.0	13.35-17.65	2 (3)	17.5	11.15-23.85

Table 3. Mutans Streptococci Density in Oral Samples of Costa Rican Children in Relation to Erupted Molars*

MS density	Dental biofilm		Saliva	
	n	Mean no. of erupted molars	n	Mean no. of erupted molars
<0.1%	9	3.3	14	2.9
0.1%-1.0%	5	2.2	3	4.0
>1.0%	5	4.0	2	4.0
Total	**19**	**3.2**	**19**	**3.2**

**** Children with ≥1 erupted molars.***

DISCUSSION

Age is an important factor in the colonization of mutans streptococci in preschool children. The mean age of the Costa Rican children with detectable MS levels in dental biofilm and saliva was significantly higher than the mean age with children without detectable MS levels in dental biofilm and saliva. This confirms the findings of the association of an increase in age and infection by mutans streptococci.[12,21]

It has been suggested that the oral flora of children with low caries activity contained MS levels less than 1% of cultivable flora.[22,23] In contrast, MS levels comprise more than 50% of the total cultivable flora in children with ECC.[24] The present study, performed in a caries-free, high-risk group of children based on a cariogenic diet, showed a lesser number of children with detectable MS levels in dental biofilm and saliva. A relatively high proportion of dental biofilm samples, however, presented MS levels greater than 20,000 CFU/ml. According to several authors, caries-free children with high MS counts present a higher biological risk for the development of dental caries, especially ECC.[16]

In a study performed with children from 6 to 36 months of age, it was demonstrated that acquisition and colonization occurs very early in life, given that detectable MS colonies were found in 25% of nondentate children.[14] This finding

appears to be in opposition to the proposal that:

1. at 12 months of age, the proportion of children with MS colonization is about 25%; and
2. previous to that age, MS are relatively absent from the oral cavity of infants.[2]

In the present study, it was possible to detect MS as early as 8 months for the dental biofilm and 9 months for saliva.

In Puerto Rican children, research studies using comparable sampling and microbiological procedures as the ones employed in this study have shown that the rate of MS colonization was 85% in a group 12 to 18 months old.[21] In concordance with these findings, the present data revealed a 100% rate of MS colonization after 17 months through 20 months of age. The particularly high rates of MS colonization suggest a potential racial or ethnic influence, which could be a new issue to be addressed in future research: risk determinants and risk factors for ECC in Hispanic children with low socioeconomic status.

In the present study population, the high MS density did not vary with age for dental biofilm or saliva. Possible explanations for this might be the:

1. small sample size;
2. relative homogeneity of children and their families;
3. increased susceptibility to MS infection of racial/ethnic origin;
4. poor oral hygiene habits;
5. potential cariogenic diets; and
6. oral health knowledge of the mothers.

With a larger study population, these issues should be included in future studies to determine their impact on MS infection in Costa Rican children before age 2.

The microbiological analysis showed that children with erupted molars have a tendency to yield higher MS counts in both dental biofilm and saliva. Therefore, it is feasible to assume that human saliva may act as a reservoir and transmission vehicle for MS infection in pit and fissures of newly erupted teeth that have nonmature enamel and are more prone to decalcification lesions. This provides a theoretical evidence-based support for improving oral hygiene habits and dietary counseling at an early age to prevent ECC, especially in children with low socioeconomic status.

CONCLUSIONS

Based on this study's results, the following conclusions can be made:

1. In a high-risk population, there is a higher probability of mutans streptococci infection in dental biofilm and saliva as age increases.
2. The density of infection does not significantly vary with age in this population.
3. MS densities in dental biofilm and saliva are not related to the total number of erupted teeth.
4. The saliva and dental biofilm samples account for similar results regarding MS counts and density.

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