

# *Streptococcus mutans* and *Streptococcus sanguinis* Colonization Correlated with Caries Experience in Children

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## Key Words

Early childhood caries · *Streptococcus mutans* · *Streptococcus sanguinis*

## Abstract

The aim of this study was to examine the colonization of *Streptococcus mutans* and *Streptococcus sanguinis* in the oral cavity and the association with severe early childhood caries (S-ECC). Saliva and plaque samples were collected from 14 S-ECC children and 8 caries-free (CF) children. All S-ECC children were *S. mutans* positive; 100% of CF children and 93% of S-ECC children were *S. sanguinis* positive. The children's caries severity was positively correlated with levels of *S. mutans* ( $p < 0.001$ ), total oral streptococci ( $p < 0.01$ ), total cultivable oral bacteria ( $p < 0.05$ ), and children's age ( $p < 0.05$ ). Logistic regression analysis showed that the interaction of *S. sanguinis* with *S. mutans* was a significant factor associated with the caries status in children, suggesting that the relative levels of these two microorganisms in the oral cavity play an important role in caries development.

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Severe early childhood caries (S-ECC), a particularly aggressive form of dental caries affecting young children, is strongly linked to the mutans streptococci, most notably *Streptococcus mutans* and *Streptococcus sobrinus* [Caufield et al., 1993; Berkowitz, 1996; Becker et al., 2002; Beighton et al., 2004]. A number of studies, however, report that the mere presence of *S. mutans* is not sufficient to predict the formation of dental caries in children [Burt et al., 1985; Loesche, 1986]. The presence of other oral streptococcus species in the oral cavity may also moderate caries outcome in children. *Streptococcus sanguinis*, one of the predominant species of the indigenous oral biota colonizing saliva and dental plaque, is usually associated with tooth surfaces free of caries [Marchant et al., 2001; Becker et al., 2002]. In the 1980s, Loesche and others proposed that the *S. mutans* to *S. sanguinis* ratio might be indicative of caries outcome or risk for caries [Loesche et al., 1984; Burt et al., 1985; Alaki et al., 2002]. In fact, several investigators using conventional culture methods have deduced from their studies that *S. sanguinis* may play an antagonistic role against *S. mutans* colonization [De Stoppelaar et al., 1969; Loesche et al., 1984; Caufield et al., 2000]. However, the determination and significance of this ratio is complicated by the nonhomogeneous distribution of *S. mutans* to *S. sanguinis* in the oral microbiota and the cumbersome cultivation meth-

**Table 1.** Comparison of the mean levels (log<sub>10</sub> value) and prevalence of oral bacteria examined between the S-ECC and CF children

Microorganism	Mean log <sub>10</sub> value (SD)			Prevalence, %		
	S-ECC (n = 14)	CF (n = 8)	p value <sup>1</sup>	S-ECC (n = 14)	CF (n = 8)	p value <sup>2</sup>
<i>S. mutans</i> <sup>3</sup>	6.0 (1.5)	2.2 (0.8)	0.000	100	37.5	0.002
<i>S. sobrinus</i> <sup>3</sup>	2.5 (1.3)	2.1 (1.2)	0.457	35.7	12.5	0.351
<i>S. sanguinis</i> <sup>3</sup>	6.4 (1.3)	6.6 (0.6)	0.432	92.9	100	1.000
Total oral lactobacilli	4.1 (1.5)	4.3 (1.3)	0.785	100	100	
Total oral streptococci	8.0 (0.4)	7.4 (0.4)	0.005	100	100	
Total cultivable count	8.2 (0.3)	7.9 (0.5)	0.133			

<sup>1</sup> Nonparametric Mann-Whitney U test. <sup>2</sup> Fisher's exact test. <sup>3</sup> Zero value was replaced with detection limit.

ods necessary to simultaneously grow the two species from saliva and/or dental plaque samples.

The objective of the present study was to determine the presence and relative amounts of different oral bacteria, including *S. mutans*, *S. sobrinus*, *S. sanguinis*, and lactobacilli, and their effect on the S-ECC status in children.

## Subjects and Methods

### Study Cohort

Twenty-two children (11 boys and 11 girls, ages 2.5–8.6 years) were included in this study. Fourteen of the 22 children (8 boys, 6 girls) were diagnosed with S-ECC. The mean decayed, missing, and filled teeth (dmft) score was  $10.9 \pm 3.8$  (mean  $\pm$  SD) and the mean decayed, missing, and filled tooth surfaces (dmfs) score was  $19.3 \pm 8.5$  (mean  $\pm$  SD). The S-ECC children were selected from a list of children who were scheduled for extensive dental treatment at Bellevue Hospital (New York, N.Y., USA) from April 2003 to April 2004. Eight children (3 boys, 5 girls) were diagnosed as being free of detectable caries. They were comparable in gender and age to the children with S-ECC. The study protocol was approved by the Institutional Review Board of New York University School of Medicine and Bellevue Hospital for human subjects. All children were from low-income, Hispanic families residing in New York City. Informed consent was obtained from all children's parents or guardians prior to the study.

### Bacterial Sample Collection

Bacterial samples from 14 S-ECC children were collected in the operating room of Bellevue Hospital under general anesthesia. Bacterial samples from the 8 caries-free (CF) children were obtained at the NYU College of Dentistry pediatric dental clinic in a routine dental setting. Nonstimulated whole salivary samples were collected from all children by carefully swabbing the children's mouths around sublingual and mucosal surfaces with sterile and absorbent cotton swabs until the swabs were saturated. Pooled supragingival plaque samples were collected from all approximal tooth surfaces and along the gingivobuccal surfaces

with a sterile Gracey curette. The salivary and pooled plaque samples were placed immediately into a 1-ml prerduced transport fluid centrifuge tube, transferred on ice to a microbiological lab, and processed within 2 h.

### Microbiological Processing

The saliva samples were vortex-mixed followed by a 30-second sonication; 25- $\mu$ l aliquots of 10-fold diluted samples were plated on the following selective media: mitis salivarius with bacitracin, mitis salivarius, Rogosa, and MM10 sucrose blood agar using a Spiral Autoplate 4000 (Spiral Biotech, Inc., Bethesda, Md., USA). After 72 h of incubation at 37°C in an anaerobic atmosphere of 85% N<sub>2</sub>, 10% H<sub>2</sub>, and 5% CO<sub>2</sub>, colony-forming units (CFU) were enumerated for the estimation of *S. mutans* and *S. sobrinus* levels on mitis salivarius with bacitracin, *S. sanguinis* on MM10, total oral streptococci on mitis salivarius, total lactobacilli species on Rogosa, and total cultivable count in the oral cavity on MM10, according to the manufacturer's recommendations. Typical *S. mutans*, *S. sobrinus*, and *S. sanguinis* colonies were identified, tested biochemically and enzymatically, pure-streaked and stored at -70°C. The detailed procedures have been published elsewhere [Caufield et al., 2000; Li et al., 2001; Pan et al., 2001].

### Statistical Analyses

Data were managed and analyzed using SPSS 13.0 software (SPSS Inc., Chicago, Ill., USA). The enumeration of microorganisms per milliliter of saliva for *S. mutans*, *S. sobrinus*, *S. sanguinis*, total lactobacilli species, and total oral streptococci were based on previously described culture methods [Dasanayake et al., 1995]. All bacterial data were compiled and logarithmically transformed in SPSS to normalize the variance distribution. For statistical analyses, where no bacterium was detected, the levels of detection limit were 50 CFU for each bacterial species. The number was determined by the intermediate value between 0 (no growth) and 1 (the lowest actual possible count) multiplied by 100 (the dilution factor).

Differences in mean bacterial counts (log<sub>10</sub> value) and prevalence of each bacterial species between S-ECC and CF children were evaluated by using the nonparametric Mann-Whitney U test and Fisher's exact test. Correlations between mean bacterial levels

**Table 2.** Correlation of bacterial levels in the saliva with the children's age and caries status

	Age		dmft		dmfs	
	correlation coefficient <sup>1</sup>	p value <sup>1</sup>	correlation coefficient	p value	correlation coefficient	p value
<i>S. mutans</i> <sup>2</sup>	-0.384	0.078	0.764	<0.001	0.755	<0.001
<i>S. sobrinus</i> <sup>2</sup>	-0.185	0.409	0.121	0.295	0.093	0.340
<i>S. sanguinis</i> <sup>2</sup>	-0.246	0.269	0.202	0.184	0.160	0.238
Total oral lactobacilli	0.386	0.076	-0.184	0.206	-0.172	0.222
Total oral streptococci	-0.370	0.090	0.579	0.002	0.627	0.001
Total cultivable count	-0.115	0.611	0.422	0.025	0.467	0.014

<sup>1</sup> Spearman correlation coefficients. <sup>2</sup> Zero value was replaced with detection limit.

and mean dmft/dmfs scores were analyzed by using the nonparametric Spearman correlation coefficient. A logistic regression model (level of significance to enter the model  $\leq 0.05$ ) was used to identify which bacteria colonies were significant factors producing S-ECC. All p values presented are 2-tailed.

## Results

Comparisons for each bacterium examined between S-ECC and CF children are given in table 1. *S. mutans* was detected for all S-ECC children (100%) and 3 out of 8 (37.5%) CF children ( $p = 0.002$ ; Fisher's exact test). S-ECC children also had significantly higher levels of *S. mutans* ( $p < 0.001$ ) and total oral streptococci ( $p < 0.01$ ) than CF children. 35.7% of S-ECC children and 12.5% of CF children were *S. sobrinus* positive, and 83.3% of children who were both *S. mutans* and *S. sobrinus* positive were in the S-ECC group, although these differences were not statistically significant. In addition, lactobacilli were present in the saliva of all children, and no detectable differences in mean levels of lactobacilli were found between the two groups. Children's age and gender were not significantly correlated with any of the bacterial levels listed in table 1.

The study also evaluated the correlation between bacterial colonization in the saliva and caries severity of the children. We found that the children's caries severity did not differ between boys and girls, but positively correlated with levels of *S. mutans* ( $p < 0.001$ ), total oral streptococci ( $p < 0.01$ ), total cultivable oral bacteria ( $p < 0.05$ ) (table 2) and children's age ( $p < 0.05$ ). Although the univariate analysis did not show a significant correlation between *S. sanguinis* level and caries status, the logistic regression analysis showed that the interaction of *S. sanguinis*

**Table 3.** Logistic regression analyses to predict the presence or absence of caries in the children

Variables	Score statistic	d.f.	p value
Age	5.9	1	0.015
<i>S. mutans</i>	14.9	1	<0.001
<i>S. sanguinis</i>	0.05	1	0.816
<i>S. mutans</i> $\times$ <i>S. sanguinis</i>	11.5	1	0.001
Overall statistics	17.4	4	0.002

Model:  $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{23} X_2 \times X_3$ ;  $Y =$  caries (yes, no);  $X_1 =$  age;  $X_2 = S. mutans$  ( $\log_{10}$  value);  $X_3 = S. sanguinis$  ( $\log_{10}$  value); model  $\chi^2 = 28.841$ ;  $r^2 = 0.730$ ;  $p < 0.001$ .

*nis* with *S. mutans* was significantly associated with the caries status in the children. The association persisted in an age-adjusted model (table 3).

## Discussion

Previously, we reported that 63–90% of 2- to 3-year-old children who were *S. mutans* positive (even with a high level of *S. mutans*) were CF [Li et al., 2000]. In addition, early colonization of *S. sanguinis* was significantly correlated with delayed colonization of *S. mutans* in children [Caufield et al., 2000]. Others have demonstrated that *S. sanguinis* is associated with healthy tooth surfaces but not with caries [Loesche et al., 1984; Becker et al., 2002; Corby et al., 2005]. A number of hypotheses have been raised by Loesche and others in the early 1980s. Perhaps the best-known hypothesis is the antagonistic colonization correlation between *S. mutans* and *S. sanguinis*

in the oral cavity. It is also suggested that *S. sanguinis* may serve a protective role against *S. mutans*. However, investigations of the correlation between the two microorganisms remain unsatisfactory because the level of *S. sanguinis* is always in excess of the level of *S. mutans* in saliva, resulting in non-normally distributed negative ratios for appropriate data analysis or meaningful clinical interpretation.

Our study aimed to investigate the relationship between *S. mutans* and *S. sanguinis* colonization associated with caries in children. Previously, De Stoppelaar et al. [1969] and then Loesche et al. [1973] proposed converting the absolute values of *S. mutans* to *S. sanguinis* counts based on the MM10 agar enumerations. In the present study, the two microorganisms were enumerated on two different media. *S. mutans* comprised, on average, less than 2% of the total cultivable microflora, and the proportions of *S. sanguinis* in the total cultivable count were 10- to 100-fold higher, particularly in CF children. The distribution of absolute CFU was skewed but, after testing several data transformations, this problem was addressed quantitatively by logarithmic transformation of the CFU values of *S. sanguinis* and *S. mutans*. Statistical analyses and correlation of these two bacteria against other variables were then possible since the distribution of the  $\log_{10}$  values was approximately normal. Furthermore, for nondetectable counts, 50 CFU/ml were used as the detection limit value. The determination was based on empirical data for the detection of oral bacteria using conventional culture methods [Dasanayake et al., 1995; Li and Caufield, 1995; Li et al., 2005] and permitted us to avoid a  $\log_{10}$  transformation of 0 in data analyses.

Results of this study showed that *S. sanguinis* was detected in almost all children. All CF children had higher relative levels of *S. sanguinis* to *S. mutans* than all S-ECC children, suggesting that CF children were colonized by an absolutely high count of *S. sanguinis* over the level of *S. mutans*. Additionally, results from the logistic regression analysis showed that the interaction between *S. sanguinis* and *S. mutans* was significantly associated with caries outcome. These findings not only support the notion that the presence of *S. mutans* alone may not be the sole indicator for increased risk for caries, but also suggest that an interactive effect of *S. mutans* and *S. sanguinis* may play an important role in children's caries experiences.

Considerable epidemiological evidence has established a positive correlation between *S. mutans* and early childhood caries [Loesche, 1986; Matee et al., 1992; Li et al., 1994, 2000; Berkowitz, 1996; Babaahmady et al., 1998;

Becker et al., 2002; Beighton et al., 2004]. Our results confirmed that *S. mutans* is associated with S-ECC in terms of both prevalence (more S-ECC children were positive) and amount of the bacterium (S-ECC children had more *S. mutans*). We also found that the severity of S-ECC measured by the dmft/dmfs index was positively correlated with high levels of total oral streptococci counts, which could be primarily explained by the high level of *S. mutans* in S-ECC children as a contributing factor to the total counts. The positive correlation between caries and total cultivable bacterial counts suggests that other oral bacterial species may also contribute to the difference in caries development since on average *S. mutans* comprises less than 5% of total oral streptococci and total streptococci accounted for less than 10% of the total counts of the plaques [Thurnheer et al., 2001].

In this study, we observed that more S-ECC children were *S. sobrinus* positive compared to CF children; 83.3% of children who were both *S. mutans* and *S. sobrinus* positive were in the S-ECC group. The results support the hypothesis that mixed colonization by *S. mutans* and *S. sobrinus* may increase children's risk for S-ECC [Hamada and Slade, 1980; Babaahmady et al., 1998; Okada et al., 2002, 2005; Lindquist and Emilson, 2004; Seki et al., 2006]. Though no significant correlation between dmft/dmfs and *S. sobrinus* was found, one explanation could be that the overall number of children in the study who were *S. sobrinus* positive was low (27.3%) given the wide age range and small sample size. As children's age increased, the proportion of children positive for *S. sobrinus* may increase [Kohler et al., 1988; Seki et al., 2006]. Unexpectedly, the study did not find a positive correlation between the children's age and caries status, which could be due to a 14-month difference in mean age between the S-ECC group and the CF group. Investigations with more defined age groups and a larger sample size of children are needed to further test the hypothesis of the role of *S. sanguinis* in caries development.

In conclusion, this study demonstrated that S-ECC is associated not only with increased levels of *S. mutans* but also with elevated levels of total streptococci and total cultivable bacteria in the oral cavity. The caries lesions might serve as retentive sites for the additional bacterial load. Our results also indicated that the use of logarithmic transformation to evaluate the relative level of *S. sanguinis* and *S. mutans* could facilitate the assessment of the shift in ecology resulting from S-ECC as previously suggested [Li et al., 2007]. Further studies will be necessary to determine the reliability and the accuracy of the use of

relative levels of *S. sanguinis* and *S. mutans* in predicting caries formation in children, and the mechanisms by which the two major indigenous microorganisms compete with each other to result in disease or health of the oral cavity.

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