ORIGINAL RESEARCH

A Preliminarily Investigation on Oral Colonization and Counts of *Streptococcus mutans* and *Streptococcus mitis* in a Group of Predentate Infants in Relation to Some Maternal and Infant Factors (A Longitudinal Observational Study)

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Abstract

Aim: Bacterial colonization of infants' oral cavities is a key factor for future caries risk. This study sought to longitudinally assess oral bacterial colonization in a group of predentate infants in relation to some mother and infant factors.

Materials and methods: A total of 50 mother-infant pairs were enrolled. When infants were 3-month-old, data were collected about some infant and mother factors; additionally, maternal Decayed, Missing, and Filled Teeth (DMFT) scores and salivary samples of mothers and infants were obtained. At 6 months of infant's age, another infants' salivary samples were obtained. Saliva was cultured to detect and quantify *Streptococcus mutans* (*S. mutans*) and *Streptococcus mitis* (*S. mitis*).

Results: *Streptococcus mitis* (*S. mitis*) was detected in all infants at 3 months. 74 and 96% of infants acquired *S. mutans* at 3 and 6 months, respectively. *S. mutans* detection was significantly higher with higher maternal DMFT scores, salivary *S. mutans* counts, and lower *S. mitis* counts, as well as when infants were given sugar-containing complementary foods/drinks. At 3–6 months, infants' *S. mutans* counts were significantly positively correlated with maternal *S. mutans* counts and DMFT scores and negatively correlated with maternal *S. mitis* counts. The opposite was evident for infants' *S. mitis* counts. Regression analysis showed that increased maternal DMFT scores and *S. mutans* counts, and Cesarean delivery were strong predictors for increased infant's *S. mutans* counts. While increased DMFT scores and maternal *S. mutans* counts were strong predictors for reduced infant's *S. mitis* counts.

Conclusion: Poor maternal oral health, early introduction of sugars in the diet, and probably Cesarean delivery can negatively impact infants' oral bacterial colonization and possibly future caries risk.

Clinical significance: Understanding factors associated with oral colonization of both caries-producing and protective flora in infants of different populations is important for caries prevention. This, in turn, can aid tailoring oral health promotion programs for expectant mothers.

Keywords: Longitudinal study, Oral colonization, Predentate infants, *Streptococcus mitis*, *Streptococcus mutans*.

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Introduction

Early childhood caries (ECC) is a special type of dental caries occurring in children younger than 6-year-old. ECC can have significant negative impacts on children's and parents' quality of life as well as children's growth and development. It is characterized by early onset and rapid progression. In some instances, it can affect infants soon after the eruption of primary incisors leading to significant damage to these teeth even before the first year of age; and though it affects primary teeth, it also increases the risk of dental caries in permanent dentition.[1]

Streptococcus mutans (*S. mutans*) is the main microorganism associated with caries initiation due to a wide array of virulence factors. The earlier the oral colonization and the higher the counts of *S. mutans*, the higher is the risk of ECC.[2] On the other hand, *S. mitis* is thought to have an antagonistic effect on *S. mutans*. Thus, its acquisition and counts may act as indicators of a healthy oral environment.[3]

Mothers are the main source of colonizing microorganisms; however, close individuals such as siblings and peers can also be potential sources. Many maternal, child, and environmental factors can influence the colonization process and subsequent growth of colonizing bacteria, such as the available microorganisms in the surrounding environment, their virulence, exposure frequency, the introduction of dietary carbohydrates, oral hygiene practices, presence of teeth and infant's immunological and hereditary

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status. The metabolic activity of initial colonizers of the oral cavity can influence the future colonization by other species paving the road for either a future healthy or pathogenic oral environment.[4,5]

Streptococci are the predominant species of oral flora during the 1st year of life, constituting more than 90% of the total microflora in the first 48 hours. *S. mutans* was thought to colonize the oral cavity only after teeth eruption, as it could not be detected in newborns in early studies.[6] Later, several studies reported *S. mutans* detection in infants before teeth eruption and as early as 34 days after birth, highlighting the importance of adopting strict hygienic methods in perinatal and early postnatal periods to reduce the possibility of early colonization.[7–9]

As an infant's close environment, culture, and habits, including dietary and oral hygiene, differ from one population to the other, bacterial acquisition may also differ towards either healthy or a more pathogenic pattern. Accordingly, the aim of the present study was to investigate some factors related to the acquisition and counts of *S. mutans* and *S. mitis* at 3–6 months of age in a group of predentate infants in relation to the different child and maternal factors.

Materials and Methods

This longitudinal observational study was approved by the Institutional Research Ethics Committee with an approval number (FDASU-Rec IM111213) and complied with the ethical principles of the Declaration of Helsinki for medical research involving human subjects. Around 120 mother-infant pairs were screened for eligibility from January 2016 to March 2016. A total of 50 mother-infant pairs were selected according to the delivery mode; cesarean delivery (n = 25) and vaginal delivery (n = 25), and the following inclusion criteria—infants aged 3-month-old at recruitment with a complete gestational period, medically free mothers, and infants and no history of antibiotic intake by either mothers or infants for four weeks before saliva collection. Children should not have any erupted primary teeth at both examinations. Finally, mothers should agree to sign consent at recruitment.

Participants were recruited from a governmental family healthcare center that serves a low socioeconomic status (SES) residential area. Mothers were first interviewed by a single interviewer to collect data on the mother's age and infants' gender, delivery method (cesarean or vaginal), nursing method (breast, formula, or both), the introduction of sugar-containing complementary foods/drinks, the use of a sweetened pacifier, the presence of siblings, and initiation of oral hygiene practices for infants.

Clinical examination and bacteriological sampling were done by a single examiner. At the beginning of the study, the mother's caries experience was recorded using the DMFT index.[10] Maternal salivary samples were collected at the beginning of the study only to assess maternal oral bacterial counts, while infants' salivary samples were collected at the age of three and 6 months to assess changes in oral bacterial acquisition and counts.

Unstimulated whole saliva samples were collected from mothers and infants using sterile polypropylene transfer pipettes. Around 50 µL of saliva was diluted in 2 mL sterile saline to give a dilution of 1/40 of the original saliva sample. Further dilutions were made up to 1/400 from the original salivary samples. Using standard biological loops, 0.01 mL of each dilution was cultured on Mitis salivarius agar (Difico, Georgia, United States of America) anaerobically for 72 hours at 37°C to detect and quantify *S. mitis* and *S. mutans* colonies.[11] Bacterial colonies were characterized based on their morphology under direct visualization and light microscopic observation and expressed in colony-forming units (CFU).

Statistical Analysis

Data were assessed for normality using the Kolmogorov–Smirnov test and were found to be non-parametric. Fisher's exact Chi-squared test and Mann–Whitney *U* test were used for pairwise comparison of qualitative and quantitative variables, respectively. The Kruskal–Wallis test was used to compare bacterial counts in relation to the three nursing methods (breastfeeding, formula, or both). Spearman's correlation was used to assess the association between quantitative variables. A multiple linear regression model was used to assess the effect of different predictor variables on infants' oral bacterial counts. Statistical Package for the Social Sciences for windows (IBM, New York—United States of America, version 24) was used for statistical analysis at $p \leq 0.05$.

Results

The age of participating mothers ranged between 25–35 years, with a mean age of 29.38 ± 3.521. All mothers reported not initiating any infants' oral hygiene practices.

Detection Frequency of *S. mutans* and *S. mitis* at 3–6 months of Infants' Age

All infants exhibited *S. mitis* at 3 months, regardless of other variables. A total of 37 infants (74%) and 48 infants (96%) acquired *S. mutans* at 3 and 6 months, respectively. All infants delivered by cesarean section (C-section) exhibited *S. mutans* at 3 months. The number of children having *S. mutans* was significantly higher in those delivered by C-section compared to vaginal delivery only at 3 months but not at 6 months of infant's age, Table 1.

Maternal *S. mutans* counts were significantly higher in children showing salivary *S. mutans* at both 3 and 6 months, whereas maternal DMFT scores were higher in infants with salivary *S. mutans* at 3 and 6 months, but a significant difference was evident at only 3 months. Maternal *S. mitis* counts were higher in children who were free from salivary *S. mutans* at 3 and 6 months, with a significant difference at 3 months (Table 1).

At 3 months, *S. mutans* was significantly detected in children whose mothers reported feeding their infants sugar-containing complementary foods or drinks. The detection frequency of *S. mutans* was significantly higher in children who were not given sweetened pacifiers. However, at 6 months, this difference was not significant (Table 1).

More males showed acquisition of *S. mutans* at 3 and 6 months compared to females; infants with siblings have more frequencies of *S. mutans* detection at 3 and 6 months, while detection frequency of *S. mutans* was in the ascending order of breastfeeding < bottle feeding > mixed feeding. Yet, no significant differences were evident in *S. mutans* detection frequency in relation to these variables at either 3 or 6 months of age (Table 2).

Counts of *S. mutans* and *S. mitis* at 3 and 6 Months of Infants' Age

Significant differences in bacterial counts were evident only in relation to the mode of delivery, the introduction of sugar-containing complimentary food, and the use of a sweetened pacifier.

Children delivered by C-section had significantly higher counts of *S. mutans* compared to vaginal delivery at both 3 and 6 months. While the opposite was true for *S. mitis,* where the counts were significantly higher in children delivered by vaginal delivery. The introduction of complementary food and the use of a sweetened

Table 1: Acquisition according to delivery mode, maternal oral bacterial counts, maternal DMFT, sugar consumption, and sweetened pacifiers

		Child and maternal factors													
		Delivery mode			*Maternal S. mutans mean counts x 10^5*		*Maternal S. mitis mean counts x 10^5*		*Maternal DMFT mean score*	*Sugar containing complementary foods/beverages*			*Sweetened pacifier*		
Bacterial acquisition		*Vaginal n = 25*	*C-section n = 25*	*p-value*		*p-value*		*p-value*		*p-value*	*No n = 12*	*Yes n = 38*	*p-value*	*No n = 39*	*Yes n = 11*	*p-value*
S. mutans																
3 months	No *n* = 13	13 (52%)	0 (0%)	<0.001*	1.65 ± 1.33	<0.001€	40.0 ± 0.0	<0.001€	0.69 ± 0.95	<0.001€	12 (100%)	1 (2.6%)	<0.001*	13 (33.3%)	0 (0%)	0.046*
	Yes *n* = 37	12 (48%)	25 (100%)		16.64 ± 12.37		20.30 ± 12.20		5.46 ± 1.95		0 (0%)	37(97.4%)		26 (66.7%)	11 (100%)	
6 months	No *n* = 2	2 (8%)	0 (0%)	0.49	0.12 ± 0.06	0.017€	40.0 ± 0.0	0.124	1.00 ± 1.41	0.094	2 (16.7%)	0 (0%)	0.054	2 (5.1%)	0 (0%)	1.0
	Yes *n* = 48	23 (92%)	25 (100%)		13.27 ±12.51		24.81 ± 13.75		4.35 ± 2.70		10 (83.3%)	38 (100%)		37 (94.9%)	11 (100%)	
S. mitis																
3 months	No *n* = 0	a	a	a	a	a	a	a	a	a	a	a	a	a	0 (0%)	a
	Yes *n* = 50	25 (100%)	25 (100%)		12.74 ± 12.58		25.42 ± 13.62		4.22 ± 2.74		12 (100%)	38 (100%)		39 (100%)	11 (100%)	
6 months	No *n* = 0	a	a	a	a	a	a	a	a	a	a	a	a	a	0 (0%)	a
	Yes *n* = 50	25 (100%)	25 (100%)		12.74 ± 12.53		25.42 ± 13.62		4.22 ± 2.74		12 (100%)	38 (100%)		39 (100%)	11 (100%)	

*Fisher's exact Chi-squared test; significant at $p \leq 0.05$; €Mann–Whitney *U* Test; significant at $p \leq 0.05$. a, the test cannot be computed on empty groups

Table 2: Frequency of *S. mutans* and *S. mitis* in relation to gender, nursing pattern, and presence of siblings

		Gender			*Nursing pattern*				*Siblings*		
Bacterial acquisition		*Female n = 22*	*Male n = 28*	*p-value*	*Breast (n = 33)*	*Formula (n = 5)*	*Both (n = 12)*	*p-value*	*No n = 11*	*Yes n = 39*	*p-value*
S. mutans											
3 months	No *n* = 13	9 (40.9%)	4 (14.3%)	0.051	10 (30.3%)	1 (20%)	2 (16.7%)	0.784	3 (27.3%)	10 (25.6%)	0.01*
	Yes *n* = 37	13 (59.1%)	24 (85.7%)		23 (69.7%)	4 (80%)	10 (83.3%)		8 (72.7%)	29 (74.4%)	
6 months	No *n* = 2	2 (9.1%)	0 (0%)	0.189	2 (6.1%)	0 (0%)	0 (0%)	1.0	1 (9.1%)	1 (2.6%)	0.395
	Yes *n* = 48	20 (90.9%)	28 (100%)		31 (93.9%)	5 (100%)	12 (100%)		10 (90.9%)	38 (97.4%)	
S. mitis											
3 months	No *n* = 0	a	a	a	a	a	a	a	a	a	a
	Yes *n* = 50	222 (100%)	28 (100%)		33 (100%)	5 (100%)	12 (100%)		11 (100%)	39 (100%)	
6 months	No *n* = 0	a	a	a	a	a	a	a	a	a	a
	Yes *n* = 50	22 (100%)	28 (100%)		33 (100%)	5 (100%)	12 (100%)		11 (100%)	39 (100%)	

*Fisher's exact Chi-squared test; significant at $p \leq 0.05$; a, test cannot be computed on empty groups

pacifier were significantly associated with higher counts of *S. mutans* and lower counts of *S. mitis* at 3–6 months (Table 3).

No significant differences were evident in *S. mutans,* and *S. mitis* counts in relation to the infant's gender, presence of siblings, or nursing patterns (breast milk, formula, or both) at either 3 or 6 months of age (Tables 3 and 4). However, *S. mutans* counts were highest in mixed feeding, followed by bottle and breastfeeding. While *S. mitis* counts were highest in breastfeeding, followed by bottle feeding and mixed feeding (Table 3). A positive correlation was found between the infant's *S. mutans* counts and maternal DMFT scores and *S. mutans* counts. While a negative correlation was found between the infant's *S. mutans* counts and maternal *S. mitis* counts. The opposite was true regarding infants' *S. mitis* counts (Table 5).

Multiple linear regression showed that only Cesarean delivery, sugar-containing complementary food, mixed feeding pattern, maternal *S. mutans* counts, and DMFT added significantly to the model regarding predicting high infants' *S. mutans* counts at 3 and 6 months. While sugar containing complementary food and maternal DMFT added significantly to reduced counts of infants' *S. mitis* counts (Table 6) (supplementary file).

Discussion

In the present study, *S. mutans* and *S. mitis* detection and counts were assessed with regard to some maternal and infant characteristics in a population of a low SES. Salivary *S. mutans* were detected in 74 and 96% of predentate infants at 3 and 6 months, respectively, agreeing with previous studies.[7–9] However, population factors related to colonization frequency and counts of colonizing bacteria are also important to consider, since they may vary according to the study population.

Infants delivered by C-section showed a higher acquisition of *S. mutans* compared to vaginal delivery. However, this difference was significant only at 3 months of age and not at 6 months, indicating that most children, regardless of delivery method, will eventually acquire *S. mutans* during their first 6 months of age. The evidence regarding the association of delivery modes with dental caries is contradictory. It is suggested that C-section delivery accelerates the initial acquisition of *S. mutans* probably due to reduced exposure to maternal microbiota at birth, causing an atypical microbial environment to prevail, which provides more potential biological binding niches for *S. mutans*.[11] Another explanation is that the immune defense system of newborns is reinforced by exposure to maternal microorganisms through the birth canal during vaginal delivery.[12]

On the contrary, other studies showed that a higher fraction of vaginally born children were colonized with high levels of *S. mutans* when compared to C-section delivery, possibly due to a closer mother-infant relationship in vaginal delivery[13] or a higher SES of mothers delivering by elective C-section combined with lower bacterial taxa.[14,15] However, counts of *S. mutans* were significantly higher in C-section-delivered infants at both 3 and 6 months supporting that C-delivery may increase caries risk in children through enhancing oral pathogenic bacterial growth in counts and not through enhancing their earlier acquisition.

Streptococcus mitis (S. mitis) was detected in all infants at 3 months of age regardless of any other factor supporting that it is one of the pioneers and most abundant bacteria that colonize the oral cavity as early as 1–3 days postdelivery.[6,16,17] *S. mitis* counts were significantly higher in infants delivered vaginally. Similar results were reported where lower counts of *S. mitis* were found in C-sections than vaginally delivered infants at 1, 3, and 6 months.[18,19] *S. mitis* has been reported to be dominating in caries-free individuals[3,19] probably due to having an inhibitory effect on *S. mutans*,[20] or due to being intolerant to acid produced by *S. mutans* and other acidogenic bacteria in subjects with active caries.[21]

Our results showed that maternal *S. mutans* counts and DMFT scores were significantly higher in children with *S. mutans* detection at both 3 and 6 months. Additionally, a positive correlation existed between maternal *S. mutans* counts and DMFT scores and infants' *S. mutans* counts at 3 and 6 months. This supports a plethora of studies that reported a maternal-to-child transfer of caries-causing oral bacteria. Some of these studies revealed identical *S. mutans* strains in mother-infants pairs. Others demonstrated that the higher the counts of maternal *S. mutans*, the earlier the colonization of their infants with *S. mutans* and in high counts, and that maternal salivary *S. mutans* level of more than 10^5 CFU/mL saliva was significantly associated with *S. mutans* transmission in predentate infants.[4,10,12,22–24] Additionally, one study showed that maternal *S. mutans* counts were directly related to DMFT scores in mothers as well as caries experience and *S. mutans* counts in dental plaque of their children at 2.5 years of age.[25] In the same context, we found that maternal *S. mutans* counts were the most significant predictor of infants' *S. mutans* counts in the regression model, followed by maternal DMFT score. While increased maternal DMFT score was found to be a strong predictor for reduced infants', *S. mitis* counts suggesting that maternal factors play a significant role in determining children's oral health by virtue of the intimate contact between mother and child.

Our results support previous studies that found an association between *S. mitis* and a healthy oral environment and a subsequent low caries risk where maternal *S. mitis* counts were higher in children without *S. mutans* detection.[3,19] Furthermore, an inverse correlation existed between maternal *S. mitis* counts and infants' *S. mutans* counts as well as between maternal *S. mutans* counts and their DMFT scores and infants' *S. mitis* counts. While a positive correlation was evident between maternal *S. mitis* counts and infants' *S. mitis* counts at both examinations supporting that microbial resemblance between mother and child includes a variety of oral bacterial and not only *S. mutans*.[18]

Horizontal transmission of salivary bacteria from other children with similar age groups, such as siblings or children in daycare centers, is also likely to occur.[24,26,27] However, our results showed that neither detection nor counts of both types of bacteria showed significant differences between infants with or without siblings agreeing with Jain et al. in 2015[28] and Namal et al. in 2005.[29]

A total of 38 mothers (76%) reported the introduction of sugar (mainly with beverages) to infants' diet as early as 3 months of infants' age. This was significantly associated with a higher frequency of *S. mutans* acquisition and counts as well as a lower frequency of *S. mitis* counts, agreeing with studies that showed an association of sweetened beverages with *S. mutans* infection at different age groups.[27,12] Wan et al. in 2001[8] found that infants infected with *S. mutans* consumed sugar at double the rate of the noninfected infants at the age of 6 months. Unfortunately, the introduction of sugary drinks and confectionery at an early stage is consistent with low SES and is known to establish a habit that persists even after children get older and puts them at risk of dental caries.[30]

Although the majority of mothers reported not introducing sweetened pacifiers to their infants, yet detection frequency of

Table 3: *S. mutans* and *S. mitis* counts according to delivery mode, feeding pattern, sugar consumption, and sweetened pacifiers

Mean bacterial log Counts x 10^5	*Delivery mode*			*Feeding pattern*			*Complementary food*			*Sweetened pacifier*		
	Vaginal	*C-section*	*p-value*	*Breastfeeding*	*Formula*	*Both*	*No*	*Yes*	*p-value*	*No*	*Yes*	*p-value*
S. mutans (3 months)												<0.001*
Mean ± SD	0.15 ± 0.20	5.71 ± 8.32	<0.001*	1.6 ± 2.61	2.78 ± 4.74	6.65 ± 11.74	0	3.86 ± 7.19	<0.001*	0.67 ± 0.87	10.9 5 ± 10.56	
Median (range)	0 (0–0.64)	2.56 (0.64–31.60)		0.40 (0–11.20)	0.89 (0–11.20)	2.0 (0–31.60)	0 (0–0)	1.20 (0–31.60)		0.32 (0–3.20)	6.40 (3.20–31.60)	
6 months												
Mean ± SD	0.42 ± 0.36	4.44 ± 3.47	<0.001*	1.84 ± 2.39	2.70 ± 3.49	3.93 ± 4.50	0.13 ± 0.14	3.15 ± 3.33	<0.001*	1.03 ± 0.96	7.38 ± 3.35	<0.001*
Median (range)	0.40 (0–1.20)	2.80 (1.20–14)		0.80 (0–8.80)	1.2 (0.32-8.80)	2.68 (0.04–14)	0.08 (0-40)	2.0 (0.04–14)		0.80 (0–3.20)	6.40 (3.20–14)	
S. mitis (3 months)												
Mean ± SD	8.37 ± 5.80	1.47 ± 1.12	<0.001*	5.84 ± 6.10	3.63 ± 2.84	2.93 ± 3.40	11.93 ± 6.80	2.70 ± 1.99	<0.001*	6.15 ± 5.53	0.54 ± 0.37	<0.001*
Median (range)	5.60 (4–28)	1.20 (0.12–4)		4.80 (0.40–28)	3.20 (0.40–7.60)	1.40 (0.12–12)	12.0 (5.60–28)	2.20 (0.12–5.60)		5.20 (1.20–28)	0.4 0 (0.12–1.20)	
6 months												
Mean ± SD	20.38 ± 11.06	2.69 ± 1.71	<0.001*	13.61 ± 12.87	8.69 ± 9.41	7.0 ± 8.71	29.39 ± 8.61	5.89 ± 5.38	<0.001*	14.47 ± 11.90	1.11 ± 0.60	<0.001*
Median (range)	20.40 (5.60–40)	2.40 (0.35–5.60)		8.4 (0.68–40)	5.40 (0.40–24.4)	3.02 (0.36-25.6)	25.02 (20.40-40)	4.0 (0.36-20.40)		9.60 (2.40–40)	1.20 (0.36–2.08)	

*Mann–Whitney *U* test; significant at $p \leq 0.05$

Table 4: Counts of infants' *S. mutans* and *S. mitis* in relation to gender and presence of siblings.

Mean bacterial log Counts x 10^5	*Gender*			*Siblings*		
	Female	*Male*	*p-value*	*No*	*Yes*	*p-value*
S. mutans (3 months)						
Mean ± SD	3.71 ± 7.12	2.32 ± 5.96	0.929	2.44 ± 3.333	3.07 ± 7.14	0.539
Median (range)	0.76 (0–31.60)	0.64 (0–31.60)		0.80 (0–11.20)	0.56 (0–31.60)	
6 months				2.56 ± 2.73	2.39 ± 3.32	0.614
Mean ± SD	2.86 ± 3.59	2.09 ± 2.83	0.899	1.68 (0–8.80)	0.80 (0–14)	
Median (range)	1.40 (0–12)	1.20 (0.04–14)				
S. mitis (3 months)						
Mean ± SD	5.99 ± 7.31	4.08 ± 3.13	0.875	4.86 ± 6.37	4.94 ± 5.20	0.487
Median (range)	3.60 (0.16–28)	4.0 (0.12–13.20)		2.40 (0.40–21.20)	4.0 (0.12–28)	
6 months						
Mean ± SD	13.50 ± 13.99	9.99 ± 9.92	0.891	10.29 ± 12.70	11.89 ±11.79	0.550
Median (range)	6.80 (0.40–40)	5.60 (0.36–40)		4.80 (0.40–40)	6.80 (0.36–40)	

*Mann–Whitney *U* test; significant at $p \leq 0.05$

Table 5: Correlation between maternal DMFT score, *S. mutans,* and *S. mitis* counts, and infants' *S. mutans* and *S. mitis* counts

		Infant's S. mutans mean counts		*Infant's S. mitis mean counts*	
Risk factor		*3 months*	*6 months*	*3 months*	*3 months*
Maternal DMFT	R	0.984	0.968	–0.987	–0.991
	p-value	<0.001*	<0.001*	<0.001*	<0.001*
Maternal *S. mutans* mean counts	R	0.989	0.997	–0.969	–0.983
	p-value	<0.001*	<0.001*	<0.001*	<0.001*
Maternal *S. mitis* mean counts	R	–0.973	-0.960	0.970	0.966
	p-value	<0.001*	<0.001*	<0.001*	<0.001*

r, Spearman correlation coefficient; * significant; [a] $p \leq 0.05$

Table 6: Liner regression model for infants *S. mutans* and *S. mitis* counts at 3–6 months

	S. mutans log counts						*S. mitis log counts*					
	3 months			*6 months*			*3 months*			*6 months*		
Predictor variables	*Standard error (SE)*	*Beta*	*p-value*	*SE*	*Beta*	*p-value*	*SE*	*Beta*	*p-value*	*SE*	*Beta*	*p-value*
Gender	1.240	0.007	0.942	0.302	0.001	0.978	1.017	–0.066	0.489	1.253	–0.003	0.957
Cesarean delivery	2.571	0.484	0.021*	0.626	0.234	0.024*	2.108	0.001	0.997	2.599	–0.024	0.827
Siblings presence	1.462	–0.001	0.995	0.356	–0.020	0.668	1.199	–0.006	0.953	1.478	0.028	0.592
Use of sweetened pacifier	3.044	–0.225	0.260	0.741	–0.064	0.518	2.496	0.141	0.470	3.078	0.134	0.224
Sugar-containing complementary food	2.634	0.403	0.027*	0.641	0.211	0.020*	2.160	–0.128	0.463	2.663	–0.283	0.006*
Mixed nursing pattern	0.737	0.199	0.049*	0.179	0.105	0.036*	0.604	–0.032	0.741	0.745	–0.038	0.489
Maternal *S. mutans* counts	0.173	0.899	0.011*	0.042	0.948	<0.001*	0.142	–0.013	0.969	0.175	0.177	0.344
Maternal *S. mitis* counts	0.185	0.184	0.640	0.045	0.106	0.585	0.152	–0.303	0.433	0.187	0.092	0.670
Maternal DMFT	0.899	0.841	0.033*	0.219	0.462	0.019*	0.737	–1.070	0.007*	0.909	–0.866	<0.001*

* Significant at $p \leq 0.05$

S. mutans was higher in children that are not using sweetened pacifiers. This unusual finding may be a result of self-reporting or may indicate that in this study group, other major factors may have contributed to the acquisition of bacteria rather than pacifier use, whether sweetened or not.

On the other hand, *S. mutans* counts were significantly higher in those using sweetened ones. Moreover, infants who used sweetened pacifiers had significantly lower *S. mitis* counts at 3–6 months, indicating that sugar use by infants enhances the growth of pathogenic bacteria and suppression of health-related ones, but it does not seem to have an impact on initial bacterial acquisition in the studied group.

Although no specific feeding pattern was significantly associated with increased bacterial acquisition or counts, breastfeeding showed the least acquisition frequency and *S. mutans* counts, as well as the highest *S. mitis* counts. Several reports indicated that the oral microbial profile could discriminate breastfed from formula-fed infants, with a potentially more health-associated oral flora in breastfed infants. Additionally, breastfeeding has been identified as a major factor in gut microbiota maturation, and this seems applicable also to the oral cavity, possibly through lectin interactions with bacterial adhesins, innate immunity, immunoglobulin effects, and interspecies interactions by milk bacteria such as lactobacilli and bifidobacteria.[31] Lactobacilli isolated from breastmilk were found to inhibit the growth of some oral pathogens, especially *S. mutans*. Growth inhibition of such species could be a mechanism for beneficial oral bacteria biofilm modulation.[31,32]

Although breastmilk offers the best health benefits to children, its cariogenicity is still a matter of debate where multiple and/or on-demand nocturnal feeding past the age of 1 year was reported to increase caries risk up to double or triple.[33] Wan et al. in 2001[8] found that breastfed, compared to bottle-fed children, were likely to be at a higher risk for predentate colonization of *S. mutans*. The authors speculated that the increased maternal contact associated with this feeding method is likely to lead to a greater chance of infection from mother to child.

Milk formulas are manufactured to closely mimic breastmilk in order to be used as a substitute or an adjunct to breastfeeding. The evidence about their cariogenicity is still inconclusive.[34]

To our knowledge, no studies assessed the association between type of infant's feeding and oral bacterial colonization in infants. However, caries experience and severity were found to be significantly lower in Italian toddlers who were exclusively breastfed compared to formula-fed toddlers.[35] In India, a significant reduction in saliva and plaque pH, as well as a significant elevation of salivary *S. mutans* counts, were evident when 1–2-year-old toddlers were

given infant formulas for 21 days.[36] This cariogenic potential may be related to the non-milk extrinsic sugar content or due to the deprivation of "breastmilk naturally occurring bioactive molecules."[34]

Conclusion

Results suggest that in infants of low socioeconomic background, early acquisition and higher counts of *S. mutans* as well as lower counts of *S. mitis* are associated with poor maternal oral health as indicated by increased maternal DMFT scores, high maternal *S. mutans* counts, and Low maternal *S. mitis* counts. Early introduction of sugars is also associated with early acquisition and higher counts of *S. mutans*. Mode of delivery may not affect the bacterial acquisition, but Cesarean delivery might facilitate an increase in *S. mutans* counts and thus future caries risk if inappropriate oral-health-related habits are advocated.

Maternal high *S. mutans* counts and DMFT scores are strong predictors of unhealthy oral bacterial flora in infants.

Clinical Significance

Primary health care providers such as gynecologists and pediatricians should encourage mothers to improve their oral health status through oral hygiene practices and treatment of any decayed teeth in the prenatal and neonatal period, avoid salivary sharing activities with their infants, avoid sugar in infants' diet and initiate infant's oral hygiene practices even before tooth eruption to help establish and maintain a healthy oral environment.

Study Limitations

Due to a lack of funding, this study is considered a preliminary investigation, and a study with a large sample size is still recommended.

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