Microbiological Screening for Cariogenic Bacteria in Children 9 to 36 Months of Age

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Abstract

Purpose: The objective of this study was to evaluate sampling methods for recovery of *mutans streptococci* (MS) and *lactobacilli* (LB) in children 9 to 36 months of age.
Methods: Tongue and plaque specimens collected on cotton swabs and stimulated saliva were diluted and plated on selective and nonselective media. Tongue specimens on a swab and mouth mirror were inoculated directly on selective agar media (MS only). Sampling methods were compared by frequency of recovery of MS or LB, correlation of microbial counts with dmfs scores, and potential of specific microbial counts to predict caries presence or absence.
Results: The mean dmfs score of 87 subjects was 6.3; 48 subjects were caries free. Levels of MS and LB were consistently higher in plaque than in other sampling techniques ($P<.001$), and frequencies of recovery of MS were highest in plaque ($P<.041$) and tongue ($P<.006$). Frequency of LB recovery did not differ significantly between sampling methods. Counts of MS or LB in total subjects and subjects aged 9 to 24 months correlated positively with dmfs scores ($P<.028$). Threshold levels of MS which were predictive of presence of caries were: (1) plaque=>2×10^5; (2) tongue=>10^4; (3) saliva=>10^5; (4) mirror=>50; and (5) swab=>50. Comparable levels of LB were: plaque, >10^3; tongue, >10^2 and saliva, >10^3. Specificities associated with these predictions were higher than sensitivities for all sampling methods.
Conclusions: (1) All sampling methods were adequate for microbial risk assessment tests in children under 3 years of age; (2) MS was a stronger indicator of caries status than LB. (*Pediatr Dent.* 2004;26:231-239)

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Microbial techniques for dental caries risk assessments have been examined extensively in adults and older children, and parameters for sampling sites and levels of target bacteria have been established. Levels accepted as risk for caries in adults and older children per ml stimulated saliva are between 10^5 and 10^6 for *mutans streptococci* (MS) and between 10^4 and 10^5 for *lactobacilli (*LB),[1-4] but in children younger than 3 years of age these parameters have not been well defined. Due to differences in dental profiles, possible non, or delayed acquisition of MS,[5] and sampling difficulties, different protocols for microbial assessments may be needed for young children.

Although many microbial studies of young children have been conducted,[6-15] few have compared the sampling and culture methods for clinical application in patients younger than 3. An investigation of children up to 36 months of age by Dasanayake et al[16] found pooled plaque specimens and swabs of the oral mucosa to be superior to unstimulated saliva for recovery of MS. The present study was designed to determine clinically useful approaches for microbial sampling of young children. The authors' findings were evaluated by frequency of recovery of cariogenic bacteria, sensitivity and specificity of recovery level vs caries status, correlations between dmfs scores and recovery levels, and subjectively by ease of specimen collection.

Methods

Patient selection and examination

A convenience sample of Maryland children was recruited from individuals who attended a well baby clinic at the University of Maryland Pediatric Ambulatory Center (PAC) in Baltimore's inner city or were patients or younger siblings of patients attending the pediatric dentistry clinic at the University of Maryland Dental School. The sample inclusion criteria included children between 6 months and 3 years of age for whom parental consent could be obtained. The sample exclusion criteria included children older than 36 months, systemic antibiotics within 14 days, and professional fluoride treatment within 48 hours of the microbial specimen collection. The investigation received approval from the Institutional Review Board of the University of Maryland at Baltimore.

With parental (or legal guardian) consent, the children received an oral examination by a trained and calibrated examiner using mouth mirrors and knee-to-knee technique,[17] with the aid of a headlight at the PAC or in a conventional dental operatory at the pediatric dentistry clinic. Radiographs were not used. Calibration of the examiner was done by means of blinded, repeated child examinations by the examiner and a calibrator under similar dental exam environment conditions. Both individuals examined 8 children at the beginning of the study, and Kappa scores for diagnosis agreement of dental cavitation, presence of opacities, and white-spot lesions (WSL) were calculated for the total dentition. There was an interexaminer agreement (kappa statistic) of 93% between the 2 examiners for presence of dental cavitation and WSL.

The NIDCR dental caries diagnostic criteria[18], was used in the assessment of the number of decayed and filled (or crowned) primary teeth (dft) or surfaces (dfs). Teeth prematurely lost due to caries and/or teeth indicated for extraction were also recorded (m). Data were recorded by the examiner onto a standardized data entry form for subsequent entry into a computer spreadsheet. A child was considered to have early childhood caries (ECC) if 1 or more tooth surfaces were decayed, as suggested by Drury et al.[19]

Sampling methods

The sampling methods, referred to as "mirror," "swab," "tongue," "plaque," and "saliva," are described as follows.

Mirror

A tongue imprint sample was obtained by placing the back of a sterile mouth mirror on the dorsum of the tongue for 5 or 10 seconds until it became covered by saliva. It was then placed directly on the surface of *Mitis salivarius kanamycin bacitracin* agar (MSKB)[20] for recovery of MS. This medium was comprised of *Mitis salivarius* agar (Difco, Detroit, Mich), to which 1% potassium tellurite (Difco), sorbitol (Sigma, St. Louis, Mo), kanamycin sulfate (Sigma), and bacitracin (Sigma) were added.

Swab

Two sterile cotton swabs were wiped simultaneously across the dorsum of the tongue. The one designated as "swab" was rolled directly onto the surface of the MSKB plate for recovery of MS.

Tongue

The other cotton swab was placed in 1 ml of sterile 0.85% saline solution in a screw-capped vial.

Plaque

A pooled plaque specimen was collected on 2 cotton swabs. One was rubbed across facial and lingual surfaces of maxillary and mandibular anterior teeth and the other across occlusal surfaces of maxillary and mandibular molars. Both swabs were placed in 1 ml of saline in a screw-capped vial.

Saliva

Stimulated saliva was collected in a cotton roll which the child chewed for 1 or 2 minutes. Having the caregiver model the procedure with a cotton roll enhanced saliva collection with very young (<2 years) or anxious children. The cotton roll was placed in the barrel of a 5-ml disposable syringe and the saliva expressed by the syringe plunger into a sterile screw-capped vial.

Vials containing tongue, plaque, and saliva were dispersed by vortexing for 10 seconds, serially diluted 1:10 in saline, and plated in duplicate on MSKB, Rogosa SL agar (Difco, Detroit, Mich) for recovery of MS and total LB, respectively, and Trypticase Soy agar (Difco) for total viable counts (TVC). These were included to provide a measurement of oral microbial load and which might relate to oral hygiene status. Dilution and plating were conducted by microtechniques described by Westergren and Krasse (1978).[21] All inoculated plates were incubated at 37°C in air containing 5% carbon dioxide for 72 hours. After incubation, colony-forming units (CFUs) on each plate were enumerated. MS and LB were identified by gram stain and colony morphology with the use of a stereo microscope. Counts were conducted by one investigator who had no knowledge of the clinical status of the subject.

Statistical evaluations

The analyses included tabulation of ungrouped data to examine frequency distributions and univariate analyses. Means and standard deviations of the levels of microorganisms were calculated for each microbiological method used (mirror, swab, tongue, plaque, and saliva,) and presented for the population divided by age (9 to 24 months and 25 to 36 months) and caries status (ECC or caries-free). In addition, means and standard deviations of dmfs scores were calculated for subjects according to the age and caries status groups mentioned above, and for teeth affected by caries (ie, maxillary incisors, mandibular incisors, and posterior teeth). Group comparisons were made for caries-free (CF) children as compared to children with ECC, as

Table 1. Clinical Data of Children Ages 9 to 36 Months

	ECC* plus caries-free	ECC	Caries-free
Total subjects			
Number	87	39	48
dmfs			
Total	6.3 (±12.5)†	14.1 (±15.4)	0
Range	0-78		
Maxillary incisors	4.2 (±6.9)	9.4 (±7.5)	0
Mandibular incisors	1.59 (±5.17)	3.54 (7.51)	
Posterior teeth	0.48 (±0.62	1.08 (3.86)	
Age (months)	24.4 (±7.5)	28.2 (±5.3)	21.3 (±7.7)
Teeth present	15.6 (4.9)	18 (2.9)	13.7 (5.4)
Age 9 to 24 months			
Number	41	9	32
dmfs			
Total	1.88 (4.89)	8.56 (±7.45)	
Range	0-21		
Maxillar incisors	1.49 (4.11)	6.78 (6.65)	
Mandibular incisors	0.05 (0.31)	0.22 (0.67)	
Posterior teeth	0.44 (1.07)	1.56 (1.67)	
Age (months)	17.46 (4.13)	20.11 (2.20)	16.72 (4.26)
Teeth present	12.02 (4.74)	15.56 (3.13)	11.03 (4.67)
Age 25 to 36 months			
Number	46	30	16
dmfs			
Total	10.3 (±15.2)	15.8 (±16.8)	
Range	0-78		
Maxillary incisors	6.65 (7.91)	10.2 (7.72)	
Mandibular incisors	0.87 (3.57)	1.33 (4.37)	
Posterior teeth	2.69 (6.92)	4 (8.28)	
Age (months)	30.61 (3.02)	30.63 (3.02)	30.56 (3.12)
Teeth present	18.8 (2.15)	18.73 (2.38)	18.94 (1.69)

*ECC=Early Childhood Caries.
†Mean (standard deviation).

well as for children with different levels of CFUs of MS or LB (eg, <50 CFUs, or ≥50 CFUs for mirror). In addition, associations between dmfs scores, age, and number of teeth vs microbial values were examined. CFU values of diluted specimens were transformed into $\log_{10}$ values to facilitate data management in some statistical analyses.

Data analyses were performed using the program Sigma Stat 2.0 (Jandel Corporation) and included Mann-Whitney Rank Sum, Signed Rank, Linear Regression and Pearson Correlation tests, and the *z* test for differences in sample proportions. For grouped data, univariate odds ratios (ORs) were used to assess the magnitude of the associations and Fisher exact tests were used to assess associations involving small cell sizes. To determine the validity of the different microbiological methods in the differentiation of children with ECC from caries-free children, sensitivity and specificity were used.

Results

Eighty-seven children ranging in age from 9 to 36 months were included in the investigation. Clinical data of the population is presented in Table 1. In the total population, 39 children presented with ECC and 48 were caries free. The mean dmfs score of ECC subjects was 14.1, most of which (9.4 dmfs) was due to unrestored lesions or extractions of maxillary incisor teeth. Four ECC children had dmfs scores above 30; 2 ECC children presented with restorations and 5 with extractions due to caries. Among ECC subjects in the total population, the mean dmfs values of 10.6, 2.7, and 0.77 represented unrestored lesions, extracted teeth, and filled teeth, respectively. Forty-six subjects were females and 41 were males. Dmfs scores between males and females were not significantly different ($P<.05$, *t* test). Dmfs scores of subjects under 1 year of age, aged 12 to 24 months and aged 25 to 36 months, were: 0 (only 3 subjects), 2 (±5.1), and 10.3 (±15.2), respectively. In the same age groups, prevalence of CF subjects (CF/number) was 3/3, 32/41, and 16/46, respectively. Mean values and standard deviations of dental surfaces affected by caries in children 9 to 24 months and children 25 to 36 months are shown in Table 1, as are dmfs scores represented by maxillary incisors, mandibular incisors, and posterior teeth. In all groups (total subjects, ages 9 to 24 months and ages 25 to 36 months), dmfs scores were highest for maxillary incisors.

Mean values and standard deviations for microbial counts of the 3 groups which were further separated into ECC plus CF, ECC, or CF subjects are presented in

Table 2. Microbiological Findings According to Caries Status, Sampling Method, and Age

	ECC* plus caries-free	ECC	Caries-free	ECC vs caries-free
Total (N=87)				
Mutans streptococci				
Mirror	294 (±550) †	538 (±589)	101 (±433)	0.001‡
Swab	201 (±404)	381 (±472)	59 (±271)	0.001
Plaque ($\times 10^4$)	105 (±265)	179 (±247)	46 (±266)	0.001
Tongue ($\times 10^4$)	6 (±22)	7 (±14)	4 (±27)	0.001
Saliva ($\times 10^4$)	20 (±42)	34 (±48)	8 (±32)	0.001
Lactobacilli				
Plaque ($\times 10^3$)	44 (±167)	70 (±185)	23 (±150)	0.001
Tongue ($\times 10^3$)	12 (±47)	12 (±34)	11 (±55)	0.001
Saliva ($\times 10^3$)	7 (±32)	13 (±47)	2 (±9)	0.001
Total viable count				
Plaque ($\times 10^6$)	25 (±36)	36 (±49)	16 (±16)	0.001
Tongue ($\times 10^6$)	6 (±16)	8 (±24)	4 (±4)	NS§
Saliva ($\times 10^6$)	12 (±24)	18 (±33)	7 (±12)	0.001
9 to 24 months (N=41)				
Mutans streptococci				
Mirror	200 (±507)	375 (±410)	150 (±526)	0.002
Swab	145 (±387)	349 (±516)	88 (±330)	0.002
Plaque ($\times 10^4$)	72 (±290)	86 (±110)	68 (±320)	0.006
Tongue ($\times 10^4$)	7 (±31)	10 (±20)	0.6 (±3)	0.003
Saliva ($\times 10^4$)	17 (±46)	30 (±0.7)	11 (±40)	0.006
Lactobacilli				
Plaque ($\times 10^3$)	34 (±183)	35 (±43)	34 (±18)	0.008
Tongue ($\times 10^3$)	14 (±60)	5 (±9)	17 (±68)	NS§
Saliva ($\times 10^3$)	9 (±39)	32 (±84)	3 (±11)	0.028
Total viable count				
Plaque ($\times 10^6$)	20 (±17)	34 (±15)	17 (±16)	0.001
Tongue ($\times 10^6$)	4 (±4)	3 (±3)	4 (±4)	NS§
Saliva ($\times 10^6$)	11 (±17)	21 (±26)	8 (±13)	0.011
25 to 36 months (N=46)				
Mutans streptococci				
Mirror	588 (±632)	588 (±631)	1 (±3)	0.001
Swab	391 (±467)	391 (±467)	1 (±2)	0.001
Plaque ($\times 10^4$)	206 (±271)	206 (±271)	0.7 (±2)	0.001
Tongue ($\times 10^4$)	6 (±9)	6 (±9)	0.01 (±0.03)	0.001
Saliva ($\times 10^4$)	34 (±43)	34 (±43)	0.2 (±0.9)	0.001
Lactobacilli				
Plaque ($\times 10^3$)	81 (±209)	81 (±209)	0.6 (±2.5)	0.001
Tongue ($\times 10^3$)	14 (±38)	14 (±38)	0	0.001
Saliva ($\times 10^3$)	8 (±31)	8 (±31)	0.006 (±0.03)	0.001
Total viable count				
Plaque ($\times 10^6$)	28 (±47)	36 (±55)	13 (±15)	0.001
Tongue ($\times 10^6$)	7 (±22)	9 (±27)	4 (±2)	NS§
Saliva ($\times 10^6$)	13 (±29)	17 (±36)	5 (±8)	0.002

*ECC=Early Childhood Caries.
†Mean (standard deviation).
‡*P* value, Mann-Whitney Rank Sum test, $P<.05$.
§NS=Not significant.

Table 2. With the exception of TVC counts for tongue in the 3 groups and LB counts for tongue in the 9 to 24 month group, ECC values were higher than CF values ($P<.001$, Mann-Whitney Rank Sum test; Table 2). Plaque values were higher than tongue, saliva, mirror, or swab ($P<.001$, signed rank test) in all groups and saliva harbored higher MS counts than tongue in the total and 25 to 36 month groups ($P<.026$, signed rank test). MS counts of plaque, tongue, saliva, mirror, and swab in the combined ECC plus CF data of the 25 to 36 month age group were significantly higher than the corresponding data of the 9 to 24 month group ($P<.011$, Mann-Whi-tney Rank Sum test). All other microbial categories of the 2 age or caries-status groups of ECC were not significantly different.

Frequency of recovery of MS or LB by the different sampling methods was compared and evaluated statistically. Differences in recoveries of MS are presented in Table 3. Discordance in recovery rates between all combinations of individual sampling methods was expressed as a percent of the samples positive for either MS or LB recovery by one particular method (plaque, tongue, saliva, mirror, or swab) when the opposite method did not recover the microorganism. Significance of discordance was tested by the *z* test for comparison of proportional differences. Plaque showed higher recovery of MS than mirror, swab, or saliva in the total and 9 to 24 month groups ($P<.041$),

Table 3. Discordance in Recovery of *Mutans Streptococci* (MS) Between Sampling Methods

Percent recovery of MS-positive samples* when the opposite method was MS-negative†															
	Total subjects ages 9 to 36 months (N=87)					Ages 9 to 24 months (N=41)					Ages 25 to 36 months (N=46)				
	M	S	P	T	Sal	M	S	P	T	Sal	M	S	P	T	Sal
Total															
M	X	3	**17‡**	18	2										
S	6	X	**18**	18	2										
P	3	5	X	5	0										
T	6	6	8	X	2										
Sal	8	6	**17**	**17**	X										
24															
M						X	0	**33**	26	11					
S						6	X	**33**	30	12					
P						7	4	X	4	4					
T						7	4	8	X	0					
Sal						21	18	**37**	**33**	X					
36															
M											X	6	6	9	0
S											6	X	9	11	6
P											0	6	X	5	0
T											6	8	8	X	6
Sal											3	10	9	11	X

Mirror (M), Swab (S), Plaque (P), Tongue (T), Saliva (Sal).
*Top headings.
†Side headings.
‡Bold figures represent significant discordance vs the opposite sampling method (side column); $P<.05$, z test for difference in sample proportions. For example, the referenced value indicates that plaque recovered MS in 17% of the specimens when mirror did not recover MS. Mirror recovered MS in 3% of the samples when plaque did not recover MS. The 2 proportions were compared by the z test.

and tongue showed higher recovery than saliva in the same groups ($P<.004$). All other comparisons of MS or LB recoveries by the various sampling methods were not significantly different.

Microbial values compared with dental caries status

Univariate associations between cross-sectional caries status (dependent variable) and microbial counts (independent variable) were evaluated by the linear regression test (Table 4). In the total population, recovery of MS and LB by the diluted specimens (plaque, tongue, saliva) showed significant correlation with dmfs ($P<.002$), however the direct inoculation methods (mirror, swab) did not. TVC vs dmfs showed a significant association with the saliva method ($P<.018$), but not with the plaque or tongue methods. In the 9 to 24 month age group, all microbial categories showed significant association with dmfs scores ($P<.028$) except for the tongue TVC data. With subjects aged 25 to 36 months, there were no significant associations.

Table 5 shows the statistical comparisons of caries status vs the selected levels of counts for MS or LB, using dmfs=0, or dmfs ≥1 as the outcome categories, and specific threshold levels of MS or LB in each specimen as risk categories. Threshold values for specific counts of MS/ml of collection fluid (diluted specimens), above which dmfs ≥1 is predicted, or below which dmfs=0 is predicted, were as follows for each sampling method: 50 (mirror); 50 (swab); 2×10^5 (plaque); 10^4 (tongue); and 10^5 (saliva). Comparable values for LB were: 10^3 (plaque); 10^2 (tongue); and 10^3 (saliva). Results were expressed as sensitivity and specificity to predict caries status and evaluated by ORs and the Fisher exact test (Table 5). Each evaluation of the 3 groups except for LB data of plaque, tongue, or saliva in the 9 to 24 month group, was significant ($P<.008$, Fisher exact test). Specificity was higher than sensitivity in all comparisons. Specificities were 1 with plaque, tongue, and saliva data in the 25 to 36 month group. The highest ORs were 56 for swab and 50 for plaque in the total subject group, and 58 for saliva data in the 9 to 24 month group (MS recovery). The highest ORs for LB data was saliva in the total group (OR=15).

In an effort to evaluate the effect of age and number of teeth on the microbial findings, Pearson correlation tests were conducted for age or number of teeth vs each microbial variable of the 3 population groups and those

of CF subjects only. Statistically significant correlations were found for:

1. plaque, tongue, and saliva MS counts (total subjects) vs both age and number of teeth ($P<.003$);
2. mirror and swab MS counts (total subjects) vs age ($P<.05$).

All other comparisons (ie, age and number of teeth vs TVC counts, LB counts, microbial categories of all other age groups, and microbial categories of CF subjects) were not significant.

Discussion

The aim of this investigation was to evaluate and compare 5 oral sampling methods for recovery of MS and LB in children younger than 3 years of age which could be readily obtained in the clinical setting (ie, plaque, tongue, saliva, mirror, and swab). Although the sampling methods were not standardized for quantity of specimen collection, all methods adequately recovered MS and LB, and with the exception of LB recovery in the 9 to 24 month group, the specific threshold values for MS and LB counts predicted caries status ($P<.008$, Table 5). MS and LB counts of the diluted samples (plaque, tongue, and saliva) in the total population ($P<.002$) and of all sampling methods in the 9 to 24 month group ($P<.028$) were positively associated with dmfs scores (Table 4). The latter findings agreed with previous studies of children less than 3 years old, despite differences in caries and infection status of the populations.[4,8,12,13]

Associations between MS and LB counts and dmfs scores in the 25 to 36 month group were not significant. This may be due, in part, to treated subjects who all were included within this group. Removal of

Table 4. Linear Regression Analysis of dmfs Scores Versus Microbiological Values Using Different Sampling Methods

	R^2	SD (±)	ß coefficient	Probability value
Total (N=87)				
Mutans streptococci				
Mirror	0.038	0.0024	0.0044	NS*
Swab	0.042	0.0033	0.0063	NS*
Plaque	0.187	0.449	1.983	0.001
Tongue	0.11	0.611	1.985	0.002
Saliva	0.154	0.481	1.892	0.001
Lactobacilli				
Plaque	0.137	0.604	2.218	0.001
Tongue	0.133	0.708	2.561	0.001
Saliva	0.179	0.739	3.167	0.001
TVC				
Plaque	0.037	3.187	5.746	NS*
Tongue	0.007	3.096	2.441	NS*
Saliva	0.064	2.427	5.866	0.018
9 to 24 months (N=41)				
Mutans streptococci				
Mirror	0.328	0.612	2.669	0.001
Swab	0.315	0.659	2.795	0.001
Plaque	0.248	0.281	1.007	0.001
Tongue	0.251	0.366	1.324	0.001
Saliva	0.262	0.294	1.094	0.001
Lactobacilli				
Plaque	0.196	0.389	1.199	0.004
Tongue	0.117	0.435	0.989	0.028
Saliva	0.238	0.457	1.574	0.001
Total viable count				
Plaque	0.17	1.671	4.724	0.007
Tongue	0.083	1.768	3.33	NS*
Saliva	0.267	1.272	4.789	0.001
25 to 36 months (N=46)				
Mutans streptococci				
Mirror	0.004	0.391	0.172	NS*
Swab	0.012	0.413	0.303	NS*
Plaque	0.011	0.180	0.128	NS*
Tongue	0	0.236	0.016	NS*
Saliva	0.013	0.193	0.145	NS*
Lactobacilli				
Plaque	0.012	0.220	0.162	NS*
Tongue	0.016	0.280	0.234	NS*
Saliva	0.002	0.297	0.083	NS*
Total viable count				
Plaque	0.027	1.103	-1.212	NS*
Tongue	0.02	1.024	-0.98	NS*
Saliva	0.003	0.823	-0.322	NS*

*NS=not significant; $P>.05$.

Table 5. Prediction of Caries Status by Specific Threshold Counts of MS or LB

Microbial variable; threshold count	Sensitivity	Specificity	Odds ratio	Fisher exact (*P*)
Total (N=87)				
Mutans streptococci **(MS)**				
Mirror; 50*	0.79	0.92	41	0.001
Swab; 50	0.79	0.94	56	0.001
Plaque; 2×10^5	0.82	0.92	50	0.001
Tongue; 10^4	0.74	0.94	42	0.001
Saliva; 10^5	0.67	0.94	30	0.001
Lactobacilli **(LB)**				
Plaque; 10^3	0.51	0.9	9	0.001
Tongue; 10^2	0.67	0.88	11	0.001
Saliva; 10^3	0.67	0.94	15	0.001
9 to 24 months (N=41)				
Mutans streptococci **(MS)**				
Mirror; 50	0.77	0.91	34	0.001
Swab; 50	0.67	0.91	19	0.001
Plaque; 2×10^5	0.78	0.88	25	0.001
Tongue; 10^4	0.75	0.91	29	0.001
Saliva; 10^5	0.86	0.91	58	0.001
Lactobacilli **(LB)**				
Plaque; 10^3	0.62	0.79	6	NS†
Tongue; 10^2	0.56	0.75	4	NS†
Saliva; 10^3	0.63	0.9	6	NS†
25 to 36 months (N=46)				
Mutans streptococci **(MS)**				
Mirror; 50	0.79	0.92	41	0.001
Swab; 50	0.79	0.94	56	0.001
Plaque; 2×10^5	0.9	1	ND‡	0.001
Tongue; 10^4	0.77	1	ND‡	0.001
Saliva; 10^5	0.73	1	ND‡	0.001
Lactobacilli **(LB)**				
Plaque; 10^3	0.5	0.94	15	0.003
Tongue; 10^2	0.6	1	ND‡	0.001
Saliva; 10^3	0.37	1	ND‡	0.008

*Sampling method; threshold microbial count of the microorganism above which dmfs≥1 is predicted, and below which dmfs=0 is predicted.
†NS=Not significant; *P*>.05.
‡ND=Not Determined

carious teeth or restoration of open lesions may have reduced quantity of cariogenic bacteria at the sampled sites. In the total and 9 to 24 month groups (Table 3), plaque recovered MS more frequently than saliva, mirror, or swab (*P*<.017) and tongue recovered MS more frequently than saliva (*P*<.006). Recoveries of LB and all other intermethod comparisons were not significantly different. The plaque vs saliva findings were in accordance with those of Dasanyake et al[16] who reported higher recovery of MS in pooled plaque than in saliva of children younger than 3 years. All sampling methods of the present investigation, except the saliva method, were rapid and well tolerated by the subjects and easy to conduct. This was based on subjective appraisal by the authors and was not evaluated statistically.

Threshold levels of MS/ml or LB/ml in stimulated saliva, above which caries presence is predicted or below which not predicted for MS/ml and LB/ml, were 10^5 and 10^3, respectively. These were significant for MS recovery in the 3 groups (*P*<.001, Table 5) and for LB in the total population and 25 to 36 month group (*P*<.008, Table 5). The latter findings differed from stimulated saliva values reported for older children, which were 2×10^5 for MS and 10^5 for LB.[1,2] Differences may be attributed to more teeth being present in the older populations. Threshold counts for MS by the direct inoculation methods (mirror, swab), agreed with previous studies using this technique (ie, 50 CFUs),[22,23] but those of MS or LB of plaque and tongue specimens could not be compared to previous investigations because identical sampling and culture methods were not found reported in the dental literature.

TVC counts which may reflect hygiene status of the subjects, showed significant association with dmfs scores for saliva in the total group and for plaque and saliva in the 9 to 24 month group (*P*<.018, Table 4). As TVC levels were higher in plaque and saliva of ECC vs CF subjects (*P*<.011, Table 2), a link between oral hygiene status and dmfs scores may be suggested. However, routine oral microbial tests for TVC may not be indicated for routine clinic use due to lack of

established threshold values for TVC counts. TVC data was also employed in the present investigation, which was not case controlled for age and number of teeth, to examine the effect of the latter variables on choice or validity of a particular microbial screening test. As TVCs of all microbial categories did not show significant positive association with age or number of teeth, MS, and LB, which are part of TVC, may not naturally increase as the age or number of teeth increase.

An additional argument against a natural increase in MS or LB with increase in age or number of teeth is that CF subjects showed no significant correlations between the microbial and clinical variables. A stronger determinant of MS or LB levels may have been dmfs scores which correlated positively with these bacteria in both the total group (diluted specimens, $P<.001$) and the 9 to 24 month group (all specimens except tongue LB, $P<.028$). Although MS in diluted specimens did correlate positively with age and number of teeth ($P<.002$), dmfs scores did as well ($P<.0005$).

The sensitivity/specificity and ORs of microbial tests with specific cut-off values showed the value of the different methods in predicting caries status. Specificities of the tests were high indicating good identification of children not at risk. High specificity values for microbial tests indicating caries status were reported previously.[24,25] The lower sensitivity values of specific MS or LB levels, in relation to presence or absence of caries in the present study (Table 5), may reflect data from children without clinical caries who harbored cariogenic bacteria. It is also possible that some of these children were caries active but harbored undetected interproximal lesions (radiographs were not employed), or that the disease had not expressed itself as visual demineralization. In 2 studies of children younger than 2 years,[9,15] MS as a single variable correlated more closely with caries at the subsequent year than with caries at the first sampling. The possibility that high MS levels may appear on pre-carious teeth of young children underscores the need for longitudinal study designs in evaluations of microbial screening methods.

Recent investigations have challenged and expanded conventional concepts about the microbiology of ECC and signify that microbiological predictive or diagnostic tests for dental caries may undergo changes in the future.[11,26,27] A comprehensive cultural study of 14 caries-active and 15 CF children aged 2 to 8 years by Marchant et al[11] found plaque levels of *Veillonella spp*, *Candida albicans,* and *Actinomyces israelii*, in addition to MS and LB, to be associated with dental caries.

Culture-independent microbiological identification studies based on molecular techniques also have addressed ECC[26,27] and have identified nonconventional bacterial species as possible indicators of caries status. Tanner et al[26] using 38 DNA probes to oral species, evaluated 171 children 9 to 36 months of age and found *Streptococcus mutans* and *A israelii* to be significantly higher in caries-susceptible vs CF children after 19 months of age. In another investigation of children by Becker et al,[27] using amplification of 16s rDNA primers to 23 species, *S mutans* was found to be associated with caries, along with *Actinomyces gerencseria, and Bifidobacterium* species.

Conclusions

1. Both direct inoculation (mirror and swab) and diluted sampling methods (plaque, tongue, and saliva) recovered sufficient MS and LB for use in microbial screening of young children.
2. MS was a stronger indicator of caries status than LB or TVC.
3. The diluted specimen methods showed strongest association of MS with dmfs scores, perhaps due to higher recovery of target bacteria, while direct inoculation methods and diluted specimen methods with specific cut-off values all performed well as caries diagnostic tests.

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