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Microbiological composition of whole saliva and caries experience in minority populations

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Saliva has various functions in the oral environment: it contributes to clearing of the oral cavity of food debris and bacteria; it has a buffering capacity on tissue-damaging strong bases and acids; it provides a saturated solution of calcium, which is needed in the remineralization of the teeth; it has antibacterial, antifungal, and antiviral capacity; and it has additional properties beneficial to the oral environment [1–4]. Saliva is also an important device for transmitting pathogenic bacteria from host to host via "vertical" and "horizontal" relationships between people [5–7]. Saliva carries substantial numbers of streptococci (S) and lactobacilli (L). Mutans

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streptococci (MS) have been strongly implemented in the initiation of carious lesions [7–9], whereas L are believed to be involved in their progression [10,11]. The quantity of MS in saliva is a strong indicator of its infectivity [5]. Intake of certain foods, especially in different ethnic groups, seems to gives rise to a specific oral ecology [12–14].

The objective of this study was to determine the levels of the major potentially cariogenic "target" organisms in whole saliva, obtained from participants belonging to four ethnic groups: Asians, blacks, whites, and Hispanics. The number of colony forming units (CFU) of total cultivable bacteria, S, MS, and L in whole saliva were compared within the four ethnic groups studied. Moreover, the bacteriological factors in saliva were examined for possible relations to socioeconomic status (SES), gender, smoking habit, and caries index (decayed, missing, filled surfaces [DMFS]).

Materials and methods

Human participants

Whole saliva samples were obtained from at least 50 caries-positive participants of each of the four major ethnic racial groups studied: Asians (and Pacific Islanders) (n=50), blacks (n=74), whites (n=46), and Hispanics (n=54). These participants were also characterized in other respects, such as gender (male, female), smoking habit (smoker, nonsmoker), SES (no high school, high school degree, college degree), and oral health, DMFS score [15]. Informed consent was obtained from all participants. The research complied with all relevant federal guidelines and institutional policies.

Saliva collection

Whole saliva samples were obtained from each participant. Each participant was given a sterile 50-mL wide-mouth test tube, and was asked to collect unstimulated saliva over a time period of approximately 20 minutes. The saliva sample, of at least 10 mL, was immediately refrigerated and analyzed within hours on the same day. Saliva samples obtained at field sites within the community were placed in an icebox until refrigeration became accessible. Reduced transport fluid (RTF) [16] was not used.

Traditional microbiological assay

For the microbiological assay, 1 mL of each individual saliva sample, freed of coarse debris by sedimentation, was used. A 10-fold serial dilution was prepared over five tubes containing 9 mL of sterile 0.85% potassium chloride saline with a repeat homogenization on a Vortex Genie mixer (Scientific Products, American Hospital Supply Co., Evanston, IL) for 10 sec-

onds at the start and between successive dilutions. Using the Drigalski technique, 0.2 mL of each dilution was spread on the following agar media: blood agar, consisting of trypticase soy agar (Baltimore Biological Laboratories [BBL], Cockeysville, MD) with 5% sheep blood (Scott Laboratories, Fiskeville, RI) for enumeration of CFU of T; *Mitis-salivarius* agar (Difco Laboratories, Detroit, MI), for enumeration of CFU of S; MSB agar [17], for enumeration of CFU of MS; MSFA agar [18], for enumeration of CFU of MS, acid producers, and yeasts; and *Rogosa* SL agar (Difco Laboratories), for enumeration of CFU of L. The inoculated *Rogosa* SL agar plates were incubated in candle jars at 36°C. The inoculated MSB agar plates were incubated anaerobically under an atmosphere of 80% N_2, 10% H_2, and 10% CO_2, using an anaerobic jar (BBL or Oxoid: Unipath, Ltd., Basingstoke, UK) equipped with a gas-generating envelope (BBL or Oxoid). The other agar plates were placed in jars with a loose cover to avoid drying out of the agar media during prolonged incubation. Jars were placed in a 36°C incubator for 10 to 15 days depending on growth of the bacteria. After the appropriate incubation period, the number of CFU of the target bacteria was determined, using agar plates with less than 250 colonies, with the aid of a dissection microscope (10 to 20×). Typical colonial morphology was detected on the specialized agar media utilized: blood agar (total CFU of all bacteria [T] in saliva), MS agar (total CFU of S), MSB agar (total CFU of MS), MSFA agar (total CFU of MS, acid producers, and yeasts), and Rogosa SL agar (total CFU of L). S, L, and MS were expressed as number of CFU per mL saliva. For the MS, the highest CFU count with any of the media employed was used.

Statistical analysis

Student *t* test and Tukey-Kramer honestly significant difference were performed according to the established procedures, using a statistical IBM software package (JMP by SAS).

Results

Saliva samples were obtained from 224 participants from four different ethnic groups (Asians, blacks, whites, and Hispanics). The ethnic and gender profiles are summarized in Table 1. The black group (n = 74) was the largest group studied, followed by the Hispanic group (n = 54), the Asian group (n = 50), and the white group (n = 46). Gender was almost evenly distributed in numbers among the ethnic groups studied (Table 1). The age profile of all studied participants is summarized in Table 2. The mean age (female/male) was highest in the Hispanic group (31.7/38.4) and lowest in the white group (27.6/27.8). The youngest participant in the study was 18 and the oldest was 63; however, both ages were considered statistically as

Table 1
Ethnic and gender profiles of participants

Ethnic group	Female	Male	Total
Asian (and Pacific Islander)	19	31	50
Black	38	36	74
White	19	27	46
Hispanic	27	27	54
Total number of participants	103	121	224

outliers. The microbiological data obtained for T, S, MS, and L are summarized in Table 3. The studywide mean of T, S, MS, and L are summarized in Fig. 1a–d. As shown in Fig. 1A, the black group had the highest mean CFU of T in saliva. Results were significantly lower for the Asian group, but only slightly lower for the other ethnic groups in comparison with the black group. The highest mean CFU of S in saliva was found in the black group (Fig. 1B), and the lowest mean CFU again was found in the Asian group. The highest mean CFU of MS in saliva was found in the Hispanic group, followed by the black, Asian, and white groups (Fig. 1C). The mean CFU of L (Fig. 1D) in saliva was numerically highest in the white group, followed by the black, Asian, and Hispanic groups.

The largest number of CFU of highly acid-producing bacteria was found in the saliva of the black participants (Table 4). The highest number of CFU of yeasts (*Candida*, etc.) in saliva also was observed among the black participants (Table 4).

Statistical analysis of the microbiological data for saliva (T, S, MS, and L) did not indicate any fundamental differences among the ethnic groups studied. In the female subgroup, however, black females had the highest mean CFU of T, which differed significantly from the Hispanic and Asian females, who had the lowest mean CFU of T ($P < 0.05$). In the male subgroup, Hispanic males had the highest mean CFU of MS, which differed significantly from the other three ethnic male subgroups ($P < 0.05$). There were also statistically significant differences among the socioeconomic groups with regard to the presence of MS in saliva: As shown in Table 4, the subgroup with a high school degree differed significantly from the other two

Table 2
Age (years) profile of participants

	Mean/median/range	
Ethnic category	Female	Male
Asian	26.3/25/24–42	28/26/24–52
Black	30.2/30/18–47	33.7/34/19–51
Caucasian	27.6/25/22–43	27.8/27/22–41
Hispanic	31.7/30/18–55	38.4/37/24–63

Table 3
Pathogenic bacteria in whole saliva by ethnicity

	Mean/median/range	
Ethnic category	Female	Male
Total cultivable bacteria		
CFU per mL saliva ($n \times 10^7$)		
Asian	1.44/1.14/0.02–5.80	2.10/1.41/0.03–6.40
Black	4.65/2.28/0.17–18.8	2.82/2.38/0.23–9.00
White	2.91/1.19/0.01–11.43	3.30/3.00/0.02–9.40
Hispanic	2.28/1.25/0.01–18.23	3.38/2.21/0.001–16.4
Streptococci		
CFU per saliva ($n \times 10^6$)		
Asian	6.24/5.60/0.01–19.60	6.59/4.70/0.001–29.40
Black	10.16/5.40/0.04–59.40	7.14/6.70/0.25–35.00
White	6.92/4.80/0.07–25.80	9.00/6.00/0.12–43.40
Hispanic	6.91/4.20/0.05–24.00	8.82/7.30/0.005–20.00
Mutans streptococci		
CFU per mL saliva ($n \times 10^5$)		
Asian	2.89/0.29/0–40.00	1.74/0.48/0–11.60
Black	3.28/1.02/0–25.00	2.12/1.50/0.11–8.52
White	1.99/1.37/0.01–9.17	1.98/0.70/0–12.50
Hispanic	2.05/2.00/0–6.75	5.70/1.28/0–59.50
Lactobacilli		
CFU per mL saliva ($n \times 10^4$)		
Asian	5.36/0.41/0–42.00	4.65/0.63/0–70.00
Black	8.21/0.59/0–90.00	5.16/0.42/0–72.00
White	12.61/0.07/0–94.00	5.03/0.75/0–59.00
Hispanic	3.57/0.52/0–28.00	3.89/0.44/0–28.13

Abbreviation: CFU, colony forming units.

subgroups—without a high school degree and with a college degree ($P < 0.05$). With increased SES, there was a statistically nonsignificant increase observed in mean CFU of L in saliva studywide: no high school (12,800 L/mL) < high school degree (48,200 L/mL) < college degree (63,000 L/mL). No significant statistical differences were found with regard to the presence of these bacteria in saliva and smoking habit (Table 4) of the participants examined: approximately the same mean CFU of T per mL of saliva were observed in both smokers and nonsmokers. Although the mean CFU of S and MS per mL of saliva were lower in nonsmokers than in smokers, the mean CFU of L per mL of saliva was higher in nonsmokers than in smokers.

The DMFS data are summarized in Table 5. The studywide mean DMFS score of the examined ethnic groups was found to be in the following order: (highest) Hispanic > black > white > Asian (lowest). For the female subgroup, the mean DMFS score was found to be in the following order (highest) Hispanic > Asian > black > white (lowest); the order for the male

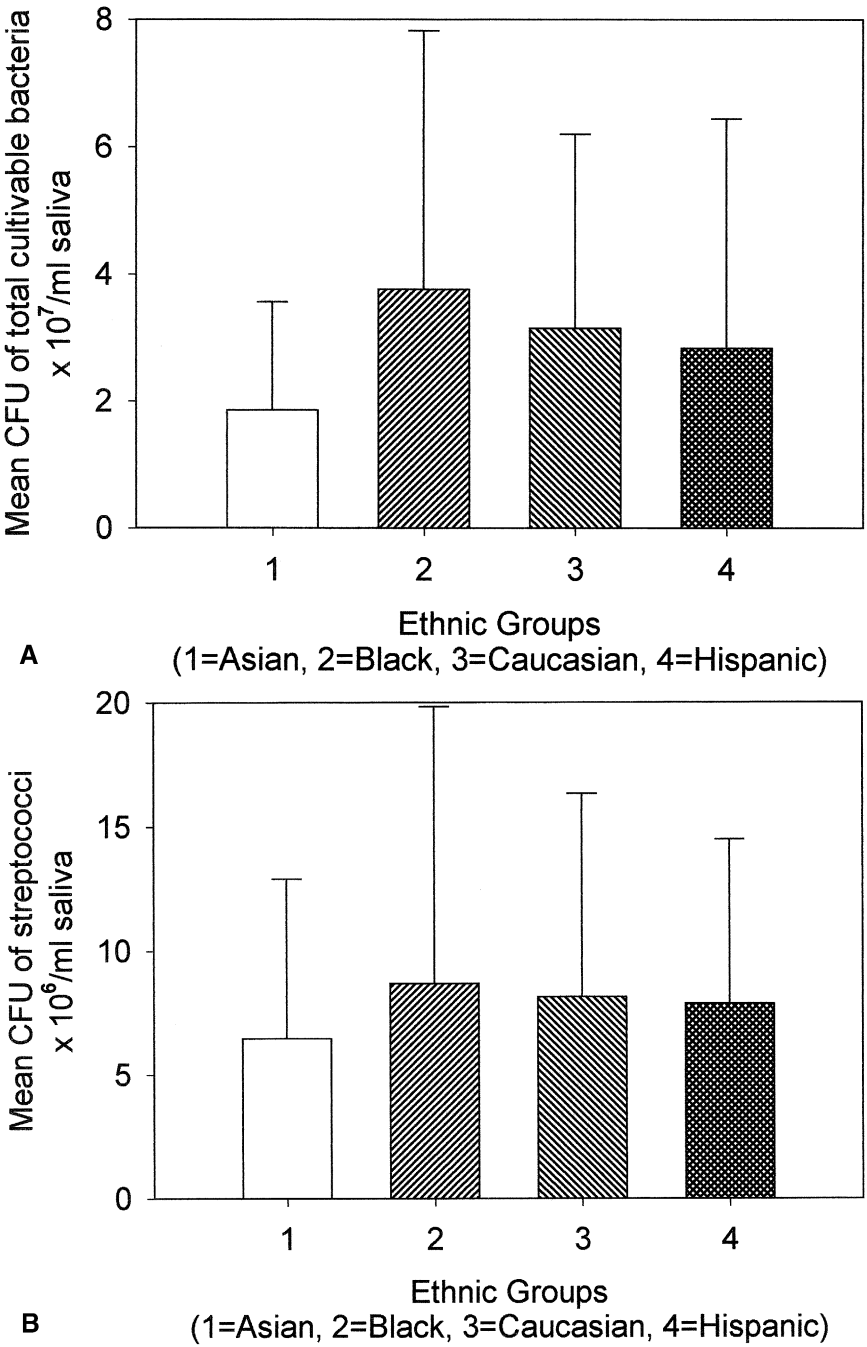
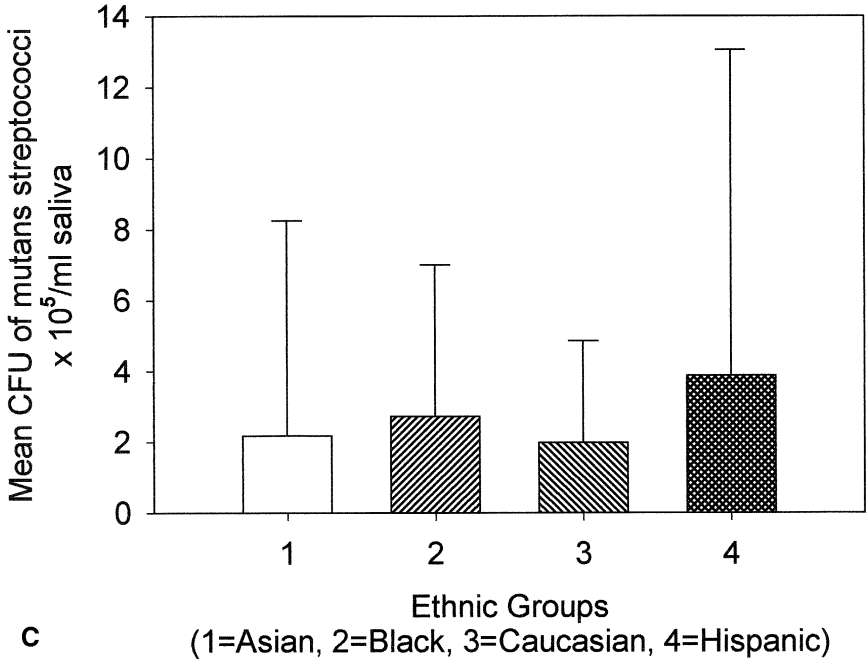


Fig. 1. Studywide mean and standard deviation according to ethnic group of (A) colony forming units (CFU) of total cultivable bacteria per mL of saliva, (B) CFU of streptococci per mL of saliva, (C) CFU of mutans streptococci per mL of saliva (three outliers ignored), and (D) CFU of lactobacilli per mL of saliva (four outliers ignored).



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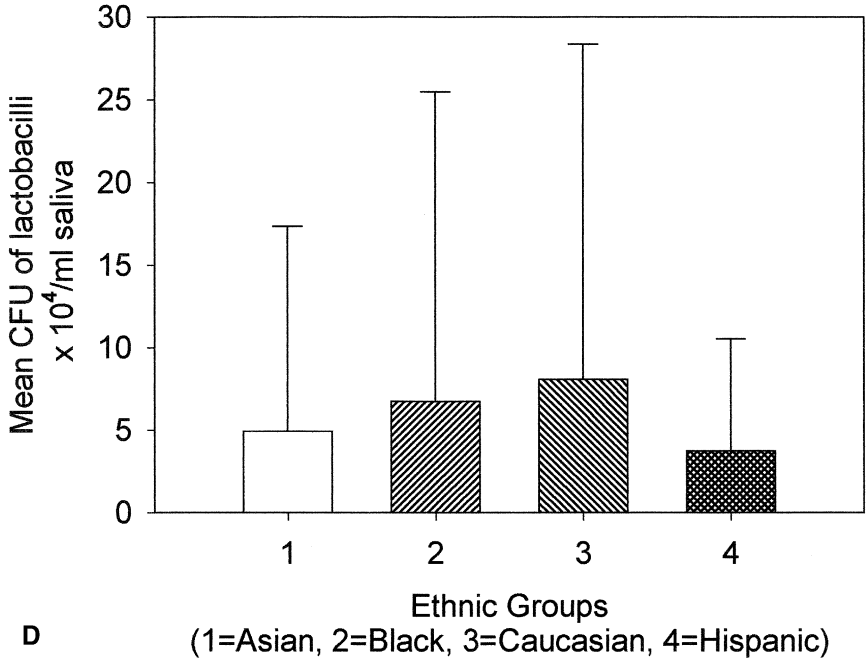

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Fig. 1 (*continued*)

Table 4
Participant distribution[a] according to smoking habit and socioeconomic status and number of patients with strong acid-producing bacteria and yeasts in their saliva

	Asian	Black	White	Hispanic
Nonsmokers	34	36	33	35
Smokers	12	22	7	13
No high school	1	2	a	16
High school degree	1	31	a	24
College degree	41	19	38	8
Acid producers	14	21	13	16
Yeasts	21	24	23	16

[a] Some patient data not available.

subgroup was the same as for the studywide group. Statistically (Student t test), the studywide mean DMFS score of the Hispanic group differed significantly from that of the black ($P < 0.05$), white ($P < 0.001$), and Asian ($P < 0.001$) groups. While the male subgroup reflected these statistical differences with regard to ethnicity, no differences were found in the female subgroup (Table 5). Studywide, in the subgroup with a DMFS ≤ 10, the mean DMFS score of the Asian and white groups differed significantly from that of the black group ($P < 0.05$). In the subgroup with a DMFS > 10, however, the mean DMFS score of the Asian and white groups differed significantly from the Hispanic ($P < 0.001$) and black ($P < 0.05$) groups.

Discussion

Saliva, usually neglected by dentists and ignored by physicians, is the least known and least appreciated of all body fluids. Nevertheless, saliva plays a vital role in the integrity of the oral soft and hard tissues [19]. Whereas unstimulated (resting) saliva functions in maintaining the integrity of the oral tissues, stimulated saliva functions in relation to food intake and mastication. Saliva is a complex secretion, and contains a number of constituents of nonsalivary origin, such as crevicular fluid, serum and blood cells,

Table 5
DMFS profile of participants

	Mean/median/range		
Ethnic category	All Patients	Female	Male
Asian	13.7/12/0–66	16.1/14/0–66	12.5/11/0–55
Black	23/15.5/0–136	16/4.5/0–102	30.4/20.5/0–136
White	14.3/12/0–72	15.6/13/0–72	13.4/11/0–41
Hispanic	34.4/24/0–138	21.7/15/0–115	47.1/34/0–138

DMFS, decayed, missing, filled surfaces.

a wide variety of bacteria, viruses and fungi, desquamated epithelial cells and cellular components, food debris, and expectorated bronchial secretions [4]. There is a significant difference in chemical composition of plaque fluid and saliva. A study comparing plaque fluid with saliva [20] found that plaque fluid contained higher concentrations, among others, of Na (35.1 mM), K (61.5 mM), Ca (6.5 mM), Pi (14.2 mM), glucose (13.9 mM), and protein (1.49 g%) than did saliva, which contained, among others, Na (13 mM), K (20.5 mM), Ca (1.45 mM), Pi (5.4 mM), glucose (0.06 mM), and protein (0.28 g%). Saliva, however, contained higher concentrations of H^+ and F^- than did plaque fluid [20]. Saliva plays a major role in the regulation of (1) the exposure of tooth surfaces to carbohydrates due to dilution and clearance of dietary sugars, (2) plaque acidity due to neutralizing and buffering of the acids in plaque, and (3) the provision of ions (calcium) for remineralization. Therefore, saliva affects the microbial composition, pH lowering, and cariogenic potential of dental plaque [21].

The focus of this investigation was the study of microbiological factors in whole saliva that contribute to dental caries formation. Caries etiology has a multifactorial nature; therefore, it is expected that multivariate approaches, rather than the use of a single parameter, may improve caries risk assessment for single participants or groups of participants. Caries predictors include carbohydrate consumption (eg, sugars and cooked starches), counts of S and MS in saliva, DMFS score increments, age of participant, sampling frequency, type of bacteriological growth media used, and use of simplified standardized test kits [22]. The goal of this study was to find potential differences in salivary microbiology related to ethnicity, as well as socioeconomic status (SES), gender, smoking habit, and caries index (DMFS). Most oral bacteria prefer to utilize different sugars and starches. Various types of starches are metabolized in a distinguished pattern of acid production [14], which eventually leads to demineralization of the tooth enamel and dentin. Because ethnic people adhere to diversified and region-specific diets, mainly consuming potatoes, rice, corn, and wheat starches, one would expect to observe a distinguished variation in the profile of oral bacteria related to ethnic groups.

Within the mean CFU of T of saliva, there were no statistical differences between the male subgroups of the four ethnic groups studied; the mean CFU was found in the following order: (highest) Hispanic > white > black > Asian (lowest). There were, however, significant differences found in the female subgroup. The mean CFU of T was found in the following order for females: (highest) black > white > Hispanic > Asian (lowest); and the black females differed significantly from the Hispanic and Asian females ($P < 0.05$). Studywide, the mean CFU of T was found to be in the following order: (highest) black > white > Hispanic > Asian (lowest).

Studywide, the mean CFU of S in saliva was similar within the black, white, and Hispanic groups (Fig. 1B); however, the mean CFU of S was much lower in the Asian group. This finding, however, was not statistically

significant. In the female subgroup, the mean CFU of S was high in the black group, but much lower in the other three ethnic groups (see Table 3). Again, the difference was not statistically significant. In the male subgroup, the following order of the mean CFU of S was obtained: (highest) white > Hispanic > black > Asian (lowest), with no statistically significant differences. Another clinical study [23] evaluated the number of total S in stimulated whole saliva of 16 participants and found 1.4×10^7 S per mL of saliva—a finding that is compatible with the mean CFU of S found in black males in the present study.

The mean CFU of MS studywide was found to be in the following order: (highest) Hispanic > black > Asian > white (lowest). The Hispanic male subgroup differed significantly from the other three ethnic male subgroups ($P < 0.05$), whereas the female subgroup showed no statistically significant differences. Another clinical study [23] evaluated the number of MS in stimulated whole saliva of 16 participants and found 3.5×10^4 MS per mL of saliva, which was slightly lower than the results found in the present study. The analytical and physiological variability of salivary microbial counts was evaluated on stimulated whole saliva samples from 10 healthy adult participants (3 males, 7 females), ranging in age from 18 to 43 years [24]. The obtained saliva samples were found to be generally stable over a wide temperature range for at least 72 hours; however, there was some loss of viability of L on prolonged storage at room temperature. Saliva samples collected upon arising yielded higher counts of MS and L than did samples collected after breakfast and toothbrushing. Day-to-day variability was significant; with 95% confidence limits exceeding 1 log in 28% of datasets for MS count and 39% of datasets for L.

A preferable protocol for caries prediction in borderline cases might be to perform salivary microbial analysis on more than one sample, collected on separate days [24]. Intraday variability of salivary bacteria (MS, L, and yeasts) was investigated by serial analysis of 16 individuals who gave eight saliva samples at 2-hour intervals on a single day [25]. Interday variations were studied by analyzing the morning saliva values of 24 individuals on 3 consecutive days. This study found that the variation in the morning was significantly higher than at noon or at later sampling times of the day. The variation was considerable in participants with high sucrase levels. The day-to-day variability of the salivary microbes was considerable for L, but relatively small for MS and yeasts. It was suggested that salivary samples should be collected in the morning because of the higher accumulation of bacteria at that time of the day [25].

A clinical study was conducted on 273 participants age 46 to 64 years [26]. Forty-three participants (group I) had no root-surface caries or restorations, 110 participants (group II) had one or more root-surface caries lesion with or without root-surface restorations, and 120 participants (group III) had root-surface restorations only. Stimulated whole saliva was obtained from most of the participants and analyzed for presence of MS and

L. The following mean/median concentrations per mL of saliva (MS and $L \times 10^5$) were obtained: group I (53 participants), MS = 2.0/9.0, L = 1.5/0.4; group II (55 participants), MS = 7.7/64.0, L = 1.6/3.0; and group III (53 participants), MS = 7.4/12.0, L = 2.0/0.9. A tendency toward higher median salivary concentrations of MS and L was noted for the participants with root-surface caries, as compared with participants without root-surface caries. There was no evidence for a progressive tendency toward higher median salivary concentrations with increasing number of caries lesions. Saliva populations of MS specifically were correlated positively with the presence of root-surface caries [26].

A clinical study [27] investigated the distribution and predictors of tooth loss in elderly blacks (n = 263) and whites (n = 228) in North Carolina over 3 years. During the 3-year follow-up, 53% of blacks and 29% of whites lost at least one tooth. Blacks lost 13% of their remaining teeth compared with whites, who lost 4%. One of the many factors related to tooth loss for blacks was more MS in stimulated saliva; whereas for whites, one of many factors was more L in stimulated saliva. The study showed that older blacks were at greater risk of tooth loss than were older whites [27].

MS can interact with saliva, and this is an important mechanism in initiation of dental caries. An in-vivo study [28] examined the relationship between oral implantation of exogenous *Streptococcus mutans* (strain IB1600) and aggregation of these cells in whole saliva from infected participants. A similar relationship could not be demonstrated for *Streptococcus sobrinus* (OMZ65). With strain IB1600, low levels of infection were associated with high levels of aggregation. Strain OMZ65 did implant well, but did not aggregate to any measurable degree. The ability of whole saliva to aggregate *S mutans* may influence the ability of these microorganisms to infect the mouth; however, sucrose-mediated glucan binding cannot be excluded as an important mechanism for colonizing teeth [28].

In the studywide mean of the present study, CFU of L was found to be in the following order: (highest) white > black > Asian > Hispanic (lowest); no statistical differences were found in the female or male ethnic subgroups (see Table 3). Another clinical study evaluated the number of total L in stimulated whole saliva of 16 participants and found 3.0×10^2 L per ml of saliva [23]. In a microbiological study of saliva (72 samples) and plaque samples taken from 93 patients [29], 25% L spp., 8.7% S spp., and 20.1% yeast spp. were found in 104 isolates among unidentified bacteria. The caries predictive value of salivary counts of L and yeasts was evaluated in a 3-year xylitol field study in 298 Hungarian children from 6 to 11 years of age [30]. The count of L was determined using a commercial dip slide kit (Dentocult, with $\geq 10^4$ to 10^5 CFU as risk; Orion Diagnostica, Helsinki); yeasts were detected using a similar test kit (Oricult-N, with $\geq 10^3$ to 10^6 CFU as risk). This study concluded that the combined information of L and yeasts predicted the 3-year caries increment acceptably. The best sensitivity and specificity of yeasts were 74% and 75%, of the L at 10^4 (10^5) CFU as risk

95% (74%) and 23% (40%), and the combined information of L and yeasts, 69% and 83%, respectively. The authors hypothesized that the presence of salivary yeasts can be useful in caries prediction, either as a sole test or together with salivary L [30].

In Scottish infants, a complex relationship was found between social deprivation and the isolation frequency of caries-associated bacteria [31]. In contrast to yeasts, which only showed an association when the infants were 1 and 2 years old, L developed an association in infants who were 3 to 4 years old. MS counts were associated with social deprivation when the infants were 2 years or older, but were dependent on caries status when the infants were 3 and 4 year olds [31].

In a group of 270 home-dwelling elderly in Helsinki [32], researchers compared stimulated salivary flow rates with the numbers of salivary MS, L, and yeasts over a 5-year period. Although the stimulated whole saliva flow rate significantly decreased with age over the 5-year interval (−0.16 mL/min), and the salivary L counts decreased (−0.44 CFU/mL of saliva), no changes were found in the saliva levels of other bacteria tested. Denture wearers had higher microbial counts in saliva than did those with natural dentition. The salivary factors did not correlate with the root caries incidence or the number of teeth lost during the 5-year follow-up [32].

The secretion rate, buffering capacity, and cariogenic microorganisms of resting and stimulated whole saliva were examined in 208 Swedes aged 55, 65, and 75 years [33]. The secretion rate for both resting and stimulated saliva decreased with age. Men had higher secretion rates than did women ($P < 0.05$). The number of MS and L increased with age; however, this age-related finding was significant only for L ($P < 0.05$). The number of these microorganisms was lower in resting than in stimulated saliva ($P < 0.0001$). The mean CFU ($n \times 10^5$) in resting (and stimulated) saliva per mL were as follows: at age 55, MS = 0.7 (4.9), L = 0.7 (1.3); at age 65, MS = 3.0 (9.1), L = 1.0 (2.7); at age 75, MS = 3.6 (16.1), L = 0.6 (4.2); and studywide all ages, MS = 2.6 (8.8), L = 0.8 (2.4). It was concluded that the number of MS and L in stimulated saliva strongly correlated with the proportions of these bacteria in plaque from root surfaces [33].

With regard to smoking habit, no significant differences were found between the salivary bacteria tested and ethnicity. Smokers and nonsmokers harbored approximately the same mean CFU of T per mL of saliva. The mean CFU of S and MS per mL of saliva appeared to be lower in nonsmokers than in smokers, whereas the mean CFU of L per mL of saliva appeared to be higher in nonsmokers than in smokers. No significant statistical relations were found in the studied plaque microorganisms of smokers and nonsmokers. One of the reasons that no significant statistical relations were found in the studied bacteria of saliva of smokers and nonsmokers could be that this study examined only gram-positive microflora. It is a well-known fact that the gram-negative microflora that contribute to periodontal disease are very much affected by smoking habit [34]. In an in-vivo

study [35], the effect of varying amounts of nicotine on growth of MS was investigated. Concentrations of 10^{-1} to 10^{-2} M nicotine caused total inhibition of bacterial growth, whereas concentrations of 10^{-3} to 10^{-4} M nicotine produced more colonies than did the control. Concentrations of 10^{-6} and 10^{-7}M nicotine again produced a significant reduction in the mean number of colonies. These results suggested a biphasic, dose-dependent effect of nicotine on the growth of MS. Depending on smoking habit, concentrations of 10^{-3} M nicotine could stimulate growth of MS and possibly place the user at increased risk for dental caries [35].

The present study also examined whether a relationship existed between SES and salivary microbiology. Three socioeconomic subgroups were studied: no high school, high school degree, and college degree. The socioeconomic subgroup with a high school degree differed significantly in their mean CFU of MS in saliva from the other two subgroups ($P < 0.05$). Studywide, with increased SES there was a statistically nonsignificant increase observed in the mean CFU of L per mL of saliva in the following order: (lowest) no high school degree < high school degree < college degree (highest). The data discussed represent a reduced number of studywide participants (see Table 4), because SES data was not obtained from patients at the outset of this study. A study of 1393 one-year-old consented infants in Scotland [36] found that infants who lived in areas of high deprivation had higher levels of caries as compared with those from more affluent areas. Socioeconomic background was not significantly related to the isolation frequencies of any of the caries-associated microorganisms, such as MS and L, however [36]. Saliva samples from 200 children aged 6 to 8 years old, representing five socioeconomic categories, were analyzed for the presence of MS and L [37]. A significant number of the children, particularly in the two lower socioeconomic categories, were considered at high risk for developing dental caries because of their high number of MS and L [37]. The prevalence of dental caries was also studied in 76 Uruguayan children, 3 to 5 years old, living in two areas with different socioeconomic and cultural conditions [38]. The occurrence of MS and L was determined in whole unstimulated saliva. MS were detected in 42% of the children, with significant correlation to their caries experience; L were recovered less frequently (18%). Children from the low socioeconomic areas had a higher incidence of caries (68%) than did children from the middle-class to high-class neighborhoods (19%), with a statistical significance of $P < 0.05$. The former children also had poorer oral hygiene than did the latter children [38].

Some ethnic data of our study were related to caries index or DMFS score [15]. The relationship between caries scoring using DMFS or DMFT index are discussed elsewhere [39]; another similar index is DFS, which represents the baseline caries experience [40]. Studywide, there were statistically significant differences among ethnic groups with regard to DMFS. The studywide mean DMFS score of the Hispanic group differed significantly from the black ($P < 0.05$), white ($P < 0.001$), and Asian ($P < 0.001$)

groups. Although these statistical differences were reflected in the male subgroups, there were no differences detected within the female subgroups. There were significant differences found studywide, however, when the DMFS score was examined in the two subgroups, "DMFS ≤ 10" and "DMFS > 10." In the studywide subgroup with a DMFS ≤ 10, the mean DMFS score of the Asian and white groups differed significantly from that of the black group ($P < 0.05$). In the studywide subgroup with a DMFS > 10, the mean DMFS score of the Asian and white groups differed significantly from the Hispanic ($P < 0.001$) and black ($P < 0.05$) groups. Although these results do not necessarily relate to the presence of pathogenic bacteria in saliva, they may be related to oral hygiene or access to professional health care. The latter two factors were not incorporated into the present study.

Using multiple regression analysis, a study of 472 British white, black, and Asian children, aged 15 to 17 years [41], found that ethnic origin had a significant influence on plaque and DMFT score, as well as on other parameters (eg, subgingival calculus). Another study [42] investigated 12-year-old children from Porto Alegre, Brazil; 91 who were from a poor background and 89 who were from a rich background. This study found that the poor children had a higher DMFS than did the rich children. It was concluded that the unequal access to dental care within the two socioeconomic groups had influenced the DMFS values. Filled surfaces, however, comprised 96% (rich) and 50% (poor) of the DMFS values, and caries experience for the first molars were similar in both groups.

A study of 641 white and black children, 3 to 4 years of age, living near London [43] found that the black children had a lower DMFT score than did the white children. The mean DMFT scores of the 3- and 4-year-old black children were 0.36 and 0.51, respectively, compared with 0.80 and 1.48 for the same-age white children ($P < 0.001$). MS and L were recovered less frequently from the black children than from the white children, but in both groups significant correlations were found between the plaque and salivary levels of MS and caries experience. In addition, in both groups, children from whom both L and MS were isolated also had the greatest mean DMFT scores, but these were not significantly different. Other investigators [44] studied the relationship between MS and dental caries experience within mothers and children of three ethnic groups: black, white, and Hispanic. Hispanic children had a greater caries incidence and more untreated caries lesions than did the black and white groups. No significant racial differences were found for mothers' mean age, DMFT, DMFS, or MS levels. White and Hispanic mother–child correlations for caries experience were moderately strong and significant, but black mother–child correlations were not significant. Results suggested important mother–child associations in caries experience, but not MS levels [44].

In the present study, no significant relations were found between the socioeconomic groups studied and plaque microbiology. In a study comparing clinical data regarding oral status (DMFS/DMFT index) and counts of MS

and L in stimulated whole saliva in 172 children aged 6 and 8 years [45], statistical significant differences were found for both MS and L counts between those children with and without caries in the primary teeth ($P < 0.05$). The different L counts were also significant in relation to caries in permanent teeth in the older children [45]. A clinical investigation [46] examined associations between caries experience (DMFS) and the number of MS in stimulated saliva in 2700 South African children, 4 to 5 years of age. The children in the lower MS classes generally had low DMFS scores ($P < 0.001$), whereas the children in the higher MS classes had DMFS scores distributed over the entire range [46].

The progression of dental caries in older adults (age 60+) was studied over a 3-year period [47]. The significant factors associated with high coronal caries incidence rate were high baseline root DMFS ($P < 0.001$), high salivary counts of MS and L ($P < 0.036$), male gender ($P < 0.007$), and Asian ethnicity ($P < 0.002$). This study affirmed the value of baseline DMFS and salivary variables to modeling caries incidence, and introduced ethnicity as a variable useful for the study of dental caries in older adults [47].

The prevalence and levels of MS and L in saliva and its possible correlation with dental caries was investigated in 473 Italian school children, 9 and 13 years of age [48]. Thirty-five percent of the children were caries free, with a mean DMFT of 1.9 at age 13. Salivary MS and L were identified in 52% and 21% of the children, respectively. The prevalence of MS was higher among the 13-year-olds than the 9-year-olds, whereas no differences were found with regard to the L ($P < 0.01$). MS and L prevalence in saliva were significantly ($P < 0.01$) correlated to caries [48].

A comparative clinical study was conducted on 2728 South African 4- to 5-year-old children to confirm whether MS or L in saliva better explained the variations of caries. Sixty-eight percent of the children had detectable L in their saliva, and 74% harbored MS. Children with a DMFS > 6 scored higher for MS than L; however, because of variations in DMFS the question of whether the L values were a better predictor remained [49]. In another experiment, baseline caries experience (DFS) and salivary counts of MS and L were compared with each other for the ability to predict 3-year caries increment in a group of 122 teenagers [40]. The L test was slightly more sensitive than was the DFS test when individuals were selected on the basis of moderate or high L levels (57% of the participants). The DFS score was a more sensitive predictor than was the MS count at the screening levels studied. The results indicated that the DFS score was better than, or as powerful as, the salivary factors in predicting future caries increments [40].

A clinical study was conducted on 30 Brisbane schoolgirls from whom stimulated saliva was collected at monthly intervals for 24 months. The count of L, buffering capacity, and Snyder test (acid-producing potential of saliva) of saliva were determined and correlated with DMFS score. Count of L and Snyder test showed a close similarity to each other; 74% of the tests

showed complete or moderate agreement with clinical assessment of caries increments. DMFS increment was higher when the Snyder test was strongly positive and the buffering capacity was low, but not when the Snyder test was positive and the buffering capacity was high. There was a correlation between increments of DMFS score and L count: +, DMFS 0 and L count per mL of saliva 1 to 1000; ++, DMFS 1 and L count per mL of saliva 1001 to 5000; +++, DMFS 2 or 3 and L count per mL of saliva 5001 to 100,000; and ++++, DMFS 4 or more and L count per mL of saliva > 100,000. There were also correlations observed between the Snyder test and the buffering capacity of saliva [50].

Salivary counts of MS and L and DMFS scores were obtained from 372 Scottish adolescents [51]. Counts of MS and L (and *Candida*) were consistently and significantly associated with the DMFS score, and buffering capacity of saliva was consistently inversely related to the DMFS score. Salivary flow rate was not correlated with caries prevalence [51].

Various factors were studied with respect to predicting dental caries on 68 patients with an average age of 56 years. They were examined once a year during a 2-year period with respect to, among other variables, number of new carious lesions, DMFS, number of salivary MS and L, and buffering capacity of saliva. The median values of all studied variables, as found at the baseline examination, were more favorable in the caries inactive ($n = 30$) than in the caries active ($n = 38$) group, but only the DMFS scores ($P < 0.001$) and number of MS in saliva ($P < 0.05$) differed significantly between the two groups [52].

Considering the complexity of salivary sampling, cultivation of salivary bacteria, and the ethnic diversity of the patients examined in the present study, one can be satisfied with the results obtained. Basically, this study agreed well with the data obtained by other investigators. An important factor not considered in this investigation, however, was the widespread availability and use of fluoride by most people, which could have had an effect on the obtained results.

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