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Factors Which Influence Levels of Selected Organisms in Saliva of Older Individuals

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The most commonly measured bacterial parameters in saliva are the levels of the mutans group streptococci and lactobacilli, which have diagnostic implications for the incidence of dental decay. Diagnostic guidelines which are applicable to children and young adults in whom most, if not all, teeth are present and in whom the rate of stimulated saliva is almost always greater than 0.5 ml/min have been developed. Dental decay is a potential health problem of considerable magnitude among elderly individuals. In elderly individuals, missing teeth, the presence of dentures, and a reduced salivary flow could confound the interpretation of salivary levels of cariogenic bacteria. In the present study, in which saliva was collected from more than 560 elderly individuals (average age, 70 ± 8 years), there was a significant positive relationship between the salivary levels of *Streptococcus mutans* and increased numbers of teeth. There was a positive association between the salivary levels of *S. mutans* and decay when the data were stratified for the presence of a complaint of xerostomia and the presence of dentures. However, a similar analysis indicated that lactobacilli and yeasts were more likely to be associated with decay. The various variables which could influence the bacterial counts per milliliter of saliva, e.g., independent or dependent living status, complaint of xerostomia, stimulated salivary flow, salivary pH, the presence of dentures, number of teeth, and decay, were analyzed simultaneously by using a multivariable linear model. In that analysis the number of decayed teeth was significantly associated with the presence of lactobacilli ($P = 0.0001$) and yeasts ($P = 0.025$) but not with the presence of *S. mutans*. Our findings indicate that salivary levels of lactobacilli and yeasts, as well as the salivary levels of *S. mutans*, should be monitored when seeking microbial indicators that might predict the incidence of caries in elderly individuals.

Dental decay is a potential health problem of considerable magnitude among elderly individuals, primarily because more elderly people are retaining their teeth, and these teeth may lose some of the protection from saliva because of a reduced salivary flow secondary to the "polypharmacia" that is used for the medical treatment of many of these individuals. Several studies have documented the salivary levels of the cariogenic species *Streptococcus mutans* in elderly individuals. However, unlike in children and young adults, in whom *S. mutans* was found to be uniquely associated with dental decay (13, 28), in elderly individuals lactobacilli and yeasts, in addition to *S. mutans*, can be associated with decay (9–11). This suggests that the types of microbes involved in dental decay may be somewhat different in elderly individuals compared with the types involved in dental decay in younger individuals.

The interpretation of these findings in elderly individuals is complicated by missing teeth, the presence of dentures, decreased salivary flow, and the dependent living status of some elderly people. The presence of partial or full dentures increases the levels of the mutans group streptococci (3, 10, 23), yeasts (3, 12, 17), and lactobacilli (11, 17). Several inconsistencies within these findings probably reflect the independent or dependent living status of the individual and, possibly, the use of medications (1).

In the present investigation, we examined the effects of dentures, independent or dependent living status, the rate of salivary flow, and other variables on the salivary levels of the aciduric organisms such as *S. mutans*, *Streptococcus sobrinus*, lactobacilli, and yeasts. We included for comparison purposes nonaciduric species such as *Streptococcus sanguis*, a tooth-associated species, and *Streptococcus salivarius*, a soft tissue-associated species. We used citric acid to stimulate salivary flow, thereby reducing the variability associated with chewing paraffin wax by edentate and partially edentate individuals. We also measured the volume of the saliva gravimetrically to minimize reading errors because of the presence of foam (5). We used a series of multivariable analyses to determine those factors which influenced the levels of these organisms in the saliva of elderly individuals.

MATERIALS AND METHODS

Subjects. In order to assess the possible effects of independent or dependent living status, we included subjects living both independently and dependently. Samples were obtained from the subjects living independently when they attended a dental outpatient clinic in a Veteran's Affairs (VA) medical center ($n = 234$) or when they were seen in a dental operatory in a private residential retirement community ($n = 141$). The subjects living dependently were residents of a long-term-care facility in the VA medical center ($n = 132$) or were recently admitted to an acute-care ward of the VA hospital with a diagnosis of a cerebral vascular accident or other neurological condition ($n = 92$). All subjects were older than 60 years of age (average age, 70.7 ± 7.9 years).

Questionnaire. All participants were interviewed by trained individuals who used a structured questionnaire to elicit information about demographic characteristics, medical and dental histories, oral hygiene habits, complaints of a dry mouth (xerostomia), and use of prescription medications. Some of the questions on xerostomia reportedly reflect actual salivary gland performance (4, 25), and these questions were again asked by the dentist when the subjects had their dental examination. The kappa statistic for the amount of agreement between the interview and clinical forms concerning questions about xerostomia ranged from 0.65 to 0.74, indicating substantial agreement between the two responses. For our analysis only those subjects who answered yes to the xerostomia questions on both occasions were considered to have xerostomia.

Dental examination. All patients in the VA medical center were examined by the same clinician-dental assistant team at the VA dental facility. All patients not seen at the VA medical center were examined by a second clinician-dental

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hygienist team. The number of teeth that were present and the number of decayed, missing, or filled teeth and surfaces were recorded by using the criteria described in the national survey of the oral health of U.S. adults (18). The presence of edentulousness and any fixed or removable prostheses, dentures, or implants was recorded. Complete or partial dentures were available for approximately 90% of the edentulous individuals. The patients were grouped according to the presence of teeth as follows: 0 natural teeth, 1 to 14 natural teeth, and 15 to 28 natural teeth (7). Third molars were not included in the analysis. This grouping assured that no full dentures would be present in the subjects with 15 to 28 teeth.

Saliva. Whole saliva was stimulated by swabbing the tongue with a 2% citric acid solution three times at 30-s intervals (16). During the next minute the patients swallowed the first flow of saliva containing the citric acid. Thereafter, the stimulated saliva was collected over a 3- to 5-min period by asking the patient to tilt the head forward and to spit the saliva into a graduated, preweighed, conical tube. If the patient was unable to provide about 1 ml of saliva after 5 min, he or she was given a 10-ml solution of sterile H_2O to rinse, and this was collected and used for the bacteriological studies. Saliva was collected from all hospitalized patients by a suction procedure (4), because it was not possible because of their medical condition for them to spit. The amount of saliva, as determined gravimetrically, the length of the collection period, and the time of day of the collection were recorded. All stimulated saliva samples and rinse solutions were immediately frozen in liquid nitrogen and were returned to the laboratory.

The output of the minor salivary gland was measured as the degree of wetness present in specially prepared standardized cellulose strips (24). These strips were individually placed on the hard palate, the buccal mucosa, and the inside lip and were allowed to absorb moisture for 30 s. The wetness was measured by using a Periotron instrument that was calibrated each day against a known volume of serum. The findings were reported in microliters per minute for each location, and the sum of the three intraoral locations was reported as the output of the minor salivary gland.

Bacteriology. The saliva samples were processed in batches of 10 each. They were removed from the liquid nitrogen and thawed at room temperature, the weight of the saliva was determined, and then the saliva was placed in an anaerobic chamber (15). A 0.5-ml portion of saliva was added to 4.5 ml of reduced transport fluid, which was then sonified for 20 s (Kontes sonifier), serially diluted, and automatically plated (Spiral Systems) on a variety of nonselective and selective media, as follows: enriched Trypticase soy agar (ETSA) which, when incubated anaerobically, provided an estimate of the total facultative and anaerobic flora; ETSA with 2% sucrose and 20 μg of metronidazole per ml, which, when incubated anaerobically, permitted, because of the sucrose, the enumeration of the unique colonies of *S. mutans*, *S. sanguis*, and *S. salivarius* (15); *Lactobacillus* selective agar, which permitted the enumeration of the lactobacilli; Trypticase soy sucrose bacitracin (TSSB) agar (26), which permitted the determination of *S. mutans* and *S. sobrinus* counts; and Sabouraud agar, which was incubated aerobically to obtain the yeast count. All agar plates except the Sabouraud plates were incubated anaerobically for 7 days; the Sabouraud plates were incubated aerobically overnight. The counts of *S. mutans* on TSSB medium tended to be higher than those obtained on ETSA-metronidazole-sucrose medium, and these TSSB counts were used in the statistical analysis. All bacteriological counts were normalized per milliliter of saliva. For those counts obtained from the rinse, 0.2 ml of saliva was used as the default value.

The remainder of each saliva sample was removed from the chamber, and the salivary pH was determined with a pH meter or, in cases of a very small residual volume, i.e., <0.1 ml, with pH paper.

Statistical analysis. The numbers of subjects available for the analysis varied because of missing clinical data for some subjects. All analyses were performed by using the SAS statistical package. The chi-square test was used to compare frequencies by independent or dependent living status (see Table 1). Comparisons for various outcome variables among levels of prediction were made on the basis of the Kruskal-Wallis rank sum nonparametric analog of the analysis of variance (8). This nonparametric method was used because of the skewed distributions for certain outcome variables, e.g., bacterial counts. For a number of analyses (see Tables 2 and 4 through 7), several predictors were combined to form subgroups. Pairwise differences were obtained by using a multiple comparison procedure based on the Kruskal-Wallis test. Multiple linear regression models (21) were developed to assess combined and partial effects of predictors for the outcomes (see Tables 8 and 9).

RESULTS

Among the monitored species, the prevalence of *S. mutans*, *S. salivarius*, and yeasts in saliva varied significantly according to living status (Table 1). *S. mutans* and *S. salivarius* were higher in the subjects living independently, whereas yeasts were more prevalent in the subjects living dependently (Table 1). There was no difference in the prevalence of these organisms within each of the living status groups with the exception that the prevalence of lactobacillus species was significantly higher in the nursing home group relative to that in the hospitalized group (72 versus 54%). *S. sobrinus* was rarely found in any of the groups, i.e., 3 to 7% prevalence.

TABLE 1. Prevalence of selected bacterial species in saliva of geriatric subjects

Species	Significance (P values)[a]	% Subjects living:	
		Independently (n = 361)	Dependently (n = 208)
Aciduric			
S. mutans	<0.001	64	38
S. sobrinus	NS	6	4
Lactobacillus spp.	NS	63	64
Yeasts	<0.001	64	79
Nonaciduric			
S. sanguis	0.06	53	45
S. salivarius	0.001	25	13

[a] Chi-square test. NS, not significant.

The low prevalence of *S. mutans* in the subjects living dependently could reflect the fact that about 50% of these individuals were edentulous (10). The data were stratified according to the presence of dentures and to the number of teeth. Under these conditions, on the basis of pairwise comparisons, there was an indication of a positive relationship between the salivary levels of *S. mutans* and increased number of teeth, especially when dentures were present (Table 2). The edentate individuals had minimal levels of *S. mutans* and *S. sobrinus*, and these levels were not affected by the presence of dentures. The levels of yeasts tended to be higher in the presence of dentures. The other aciduric species were not affected by either the number of teeth or the presence of dentures. *S. sanguis* levels increased both in the presence of dentures and with increased number of teeth. Higher salivary pH and higher stimulated salivary flow were associated with an increased number of teeth (Table 2).

The amount of saliva can greatly influence these bacteriological variables, especially if the flow rate is diminished. We examined this possibility by asking questions concerning complaints of xerostomia and by measuring the amount of saliva gravimetrically. The prevalence of xerostomia can vary with the type of question asked (6). About 48% (266 of 594) of our subjects reported having xerostomia in the morning, but only 11% (61 of 554) reported a dry mouth while eating, and 21% (122 of 566) complained that they had too little saliva. All complaints were associated with an increased percentage of decayed coronal surfaces, i.e., 5% ± 9% in those with a complaint of xerostomia in the morning versus 2.7% ± 8.2% in those with no complaint (data not shown) (P = 0.008, Wilcoxon rank sum test). A positive response to specific questions on dry mouth while eating and too little saliva was associated, on average, with a greater reduction in stimulated salivary flow and with significantly higher levels of total cultivable organisms (Table 3). This reflected the concentrating effect of the reduced salivary flow and would confound the interpretation of the significant increases in lactobacilli, yeasts, *S. sobrinus*, and *S. mutans* that were observed in subjects with these complaints. Among the subjects who complained of a xerostomia in the morning, there was a slight (i.e., 0.05 ml/min) but significant reduction in salivary flow. This complaint was not associated with a significant increase in the count of cultivable anaerobic bacteria. This suggested that the significant increases in the numbers of yeasts, lactobacilli, *S. salivarius*, and *S. sobrinus* that were observed in these individuals were reflective of the com-

TABLE 2. Effects of dentures and number of teeth on salivary levels of selected aciduric species

Species	Significance (P value)[a]	Median $\log_{10}$ CFU/ml of saliva					
		Without dentures			With dentures		
		0 teeth (n = 22)	1–14 teeth (n = 8)	15–28 teeth (n = 154)	0 teeth (n = 146)	1–14 teeth (n = 80)	15–28 teeth (n = 79)
Aciduric							
S. mutans	0.0001	2.0[b,c,d]	3.72	4.69[b,e]	1.97[e,f,g]	4.54[c,f]	4.7[d,g]
S. sobrinus	NS	1.89	2.11	1.8	1.88	1.83	1.8
Lactobacillus spp.	NS	4.62	2.16	4.21	4.05	4.52	4.33
Yeasts	0.003	3.0	3.98	2.72[b,c]	3.34[b]	3.33[c]	3.04
Nonaciduric							
S. sanguis	0.0001	1.89[b,c]	3.59	5.18[b,d]	2.0[d,e,f]	5.02[e]	5.38[c,f]
S. salivarius	NS	1.98	2.01	1.9	2.0	1.9	1.9
Anaerobic count	NS	7.99	8.04	8.07	7.91	8.07	8.04
Salivary							
pH	0.0001	6.4[b,c,d]	6.8[b]	6.9[c,e]	6.2[e,f]	6.7	7.0[d,f]
Flow rate[h]	0.0045	0.52[b,c]	0.40	0.66[b,d]	0.54[d,e]	0.63	0.66[c,e]

[a] Kruskall-Wallis test. NS, not significant.
[b–g] Values in the same row with the same superscript are significantly different; multiple comparisons are based on Kruskal-Wallis rank sums.
[h] Flow rate is in milliliters per minute.

plaint of xerostomia and not the confounding effect of increased bacterial numbers associated with a reduced salivary flow.

The levels of *S. mutans* and *S. sanguis* did not change in the presence of a complaint of a dry mouth in the morning (Table 3). Because the niche of these organisms in the oral cavity is the tooth surface (13), the absence of a selection for these organisms in these xerostomic individuals could reflect the confounding effect of missing teeth on the data. This possibility was examined by stratifying the data by the presence or absence of xerostomia in the morning and according to the number of teeth in the mouth, i.e., 0, 1 to 14, and 15 to 28 teeth (Table 4). The salivary levels of *S. mutans* and *S. sanguis* reflected the number of teeth, rather than xerostomia, whereas the levels of yeasts were lowest in the individuals with 15 to 28 teeth and no complaint of xerostomia (Table 4). The levels of lactobacilli showed no pattern with tooth number or xerostomia. An increased number of teeth was significantly associated with both increased salivary pH and increased salivary flow in individuals with and without a complaint of xerostomia (Table 4).

The lower salivary pH associated with a complaint of xerostomia (Table 4) could be a mechanism by which xerostomia selected for the aciduric species. We examined the effects of interactions between xerostomia and a low pH on the monitored species (Table 5). The pH and xerostomic status were associated with significant differences for yeasts, *S. sobrinus*, *S. salivarius*, and lactobacilli, but they had no significant effect on *S. mutans* or *S. sanguis*. The salivary flow in the group with low salivary pH-xerostomia was significantly lower than that seen in the other groups. This group had the highest levels of yeast and *S. sobrinus*. Conversely, a high pH and no complaint of xerostomia was associated with the lowest levels of yeasts and lactobacilli. The salivary flow in the group with high salivary pH and no xerostomia was significantly higher than that in the other groups.

TABLE 3. Ability of different questions on xerostomia to identify groups with high salivary levels of aciduric organisms

Question and response	Stimulated salivary flow (ml/min)	Salivary pH	Anaerobic count	Median $\log_{10}$ CFU/ml of saliva					
				Aciduric				Nonaciduric	
				S. mutans	*S. sobrinus*	*Lactobacillus* spp.	Yeasts	*S. sanguis*	*S. salivarius*
Dry mouth in morning									
No (n = 298)	0.64	6.8	8.0	3.74	1.82	4.16	2.92	4.92	1.89
Yes (n = 266)	0.59	6.6	8.15	4.13	1.92	4.48	3.42	2.3	2.06
Significance[a]	0.0001	NS	NS	NS	0.0001	0.0042	0.0001	NS	0.0055
Dry mouth while eating									
No (n = 493)	0.61	6.7	8.04	4.26	1.85	4.28	3.04	4.44	1.92
Yes (n = 61)	0.40	6.4	8.38	2.3	2.01	4.40	3.65	2.3	2.3
Significance[a]	0.0001	0.004	0.0145	NS	0.0001	NS	0.0042	NS	0.0004
Too little saliva									
No (n = 444)	0.63	6.8	8.0	4.13	1.84	4.2	2.99	4.63	1.91
Yes (n = 122)	0.42	6.4	8.18	4.35	1.98	4.58	3.54	2.3	2.3
Significance[a]	0.0001	0.0004	0.0075	0.0162	0.0001	0.0027	0.0001	NS	0.0005

[a] P values were determined by the Kruskall-Wallis test. NS, not significant.

TABLE 4. Effect of complaint of xerostomia and number of teeth on salivary levels of selected aciduric species

Species	Significance (P value)[a]	Median $\log_{10}$ CFU/ml of saliva for subjects with or without complaint of xerostomia in morning					
		No complaint			Complaint		
		0 teeth (n = 92)	1–14 teeth (n = 50)	15–28 teeth (n = 147)	0 teeth (n = 84)	1–14 teeth (n = 41)	15–28 teeth (n = 132)
Aciduric							
S. mutans	0.0001	1.92[b,c,d,e]	4.66[b,f]	4.57[c,g]	2.04[f,g,h,i]	4.50[d,h]	4.77[e,i]
S. sobrinus	0.0003	1.85	1.82	1.78[b,c,d]	1.97[b]	1.95[c]	1.89[d]
Lactobacillus spp.	0.02	4.3	3.97	4.05[b]	3.69	4.83	4.59[b]
Yeasts	0.0001	3.17[b]	3.23	2.72[b,c,d,e]	3.50[c]	3.74[d]	3.25[e]
Nonaciduric							
S. sanguis	0.0001	2.0[b,c,d]	3.92	5.38[b,e]	2.0[e,f,g]	4.97[c,f]	4.62[d,g]
S. salivarius	NS	1.91	1.86	1.87	2.14	2.06	1.97
Anaerobic count	0.02	7.90[b]	8.02	8.06	7.91	8.23	8.18[b,c]
Salivary							
pH	0.0001	6.4[b,c]	6.7[d]	6.9[b,e]	6.1[d,e,f]	6.6	6.9[c,f]
Flow rate[j]	0.0001	0.57[b]	0.66[c]	0.68[b,d,e,f]	0.44[c,d]	0.48[e]	0.55[f]

[a] Kruskal-Wallis test. NS, not significant.
[b–i] Values in the same rows with the same superscript are significantly different; multiple comparisons are based on Kruskal-Wallis rank sums.
[j] Flow rate is in milliliters per minute.

The influence of dental decay and a complaint of xerostomia on the levels of the monitored species was assessed in the dentate subjects (Table 6). The levels of *S. mutans* and lactobacilli were notably elevated in subjects with decay who complained of a dry mouth in the morning. The levels of *S. mutans*, and lactobacilli were also elevated in those subjects with decay who did not complain of xerostomia (Table 6). The levels of yeasts were significantly elevated in subjects with a complaint of xerostomia. The levels of the nonaciduric species were not affected by either the complaint of xerostomia or the presence of decay, although there was a tendency for *S. sanguis* levels to be higher in the presence of decay. The anaerobic count was significantly higher in those individuals with a complaint of xerostomia and with decayed teeth. The stimulated salivary flow was reduced in subjects with a complaint of xerostomia.

Among the dentate individuals, the presence of partial or full dentures was associated with elevated levels of *S. mutans* and *S. sanguis* (Table 2). When the presence of dentures was compared with the presence of decay, a nonsignificant association between elevated levels of *S. mutans* and decay was observed both in the presence and in the absence of dentures (Table 7). Lactobacillus levels were also elevated in the presence of dentures. The yeast levels tended to be higher in the presence of decay. This stratification had no effect on salivary flow or pH.

This series of two-way and three-way analyses showed the influence of dentures, decay, teeth, salivary pH, and other variables on the monitored flora. In the case of *S. mutans* it was difficult to determine whether the number of teeth (Table 2) or the number of decayed teeth (Tables 6 and 7) was more influ-

TABLE 5. Effect of complaint of xerostomia and salivary pH on median levels of selected aciduric bacterial species in saliva

Species	Significance[a]	Median $\log_{10}$ CFU/ml of saliva for xerostomia in morning			
		No xerostomia		Xerostomia	
		pH ≤ 6.4 (n = 101)	pH > 6.4 (n = 197)	pH ≤ 6.4 (n = 117)	pH > 6.4 (n = 149)
Aciduric					
S. mutans	NS	4.38	4.13	2.3	4.30
S. sobrinus	0.0001	1.86[b]	1.79[b,c]	2.05[d]	1.87[c]
Lactobacillus spp.	0.0001	4.85[b]	3.84[b,c]	4.98[c,e]	4.30[e]
Yeasts	0.0001	3.04	2.82[c]	4.70	3.14[c]
Nonaciduric					
S. sanguis	NS	2.3	5.18	2.3	2.3
S. salivarius	0.002	1.93	1.85[b]	2.3[b]	1.97
Anaerobic count	NS	7.98	8.02	8.13	8.17
Salivary flow rate[f]	0.0001	0.58	0.67	0.38	0.56

[a] Kruskal-Wallis test. NS, not significant.
[b, c, e] Values in the same row with the same superscript are significantly different ($P < 0.05$) by the Wilcoxon rank sum test.
[d] Values in boxes are significantly different ($P < 0.05$) from other values in row by (Wilcoxon rank sum test).
[f] Flow rate is in milliliters per minute.

TABLE 6. Effect of complaint of xerostomia and presence of decay on median levels of selected aciduric bacterial species in saliva

Species	Significance (P value)[a]	Median $\log_{10}$ CFU/ml of saliva for xerostomia in morning			
		No xerostomia		Xerostomia	
		No decay (n = 60)	Decay (n = 108)	No decay (n = 51)	Decay (n = 114)
Aciduric					
S. mutans	0.004	4.17[b]	4.71[c]	4.04[c,d]	5.04[b,d]
S. sobrinus	0.007	1.79[b]	1.79[c,e]	1.92[c]	1.90[b,e]
Lactobacillus spp.	0.0002	3.94[b]	4.21[b]	4.11	4.68[g]
Yeasts	0.0001	2.61	2.96	3.66	3.28
Nonaciduric					
S. sanguis	NS	5.17	5.40	4.47	5.04
S. salivarius	NS	1.95	1.83	2.15	1.96
Anaerobic count	0.03	8.05	8.06	8.07	8.32
Salivary					
pH	NS	6.9	6.8	6.7	6.8
Flow rate[h]	0.0001	0.68[b,f]	0.68[c,e]	0.50[c,f]	0.54[b,e]

[a] Kruskal-Wallis test. NS, not significant.
[b–f] Values in the same row with the same superscript are significantly different ($P < 0.05$) by the Wilcoxon rank sum test.
[g] Values in boxes are significantly different ($P < 0.05$) from all other values in the row (Wilcoxon rank sum test).
[h] Flow rate is in milliliters per minute.

ential in increasing their levels in the saliva. This issue was addressed by analyzing all of the measured variables simultaneously by using a multivariable linear model (Table 8). These overall models included the eight variables (predictors) listed in Table 8. This type of model was particularly informative for determining the factors influencing the numbers of *S. mutans* (r^2 = 0.2), the anaerobic bacteria count (r^2 = 0.18), and the yeast count (r^2 = 0.15). Reduced models, in which only the significant variables were retained, did not show significantly diminished r^2 values, indicating that the identified variables accounted for almost all of the variability encountered. In the case of *S. mutans* this meant that increased numbers of teeth, independent living status, and reduced salivary flow accounted for 90% of the variability found when all eight variables were used in the model (0.18/0.20). In the case of the yeasts, a reduced salivary flow, the presence of dentures and decay, and dependent living status accounted for 93% of the variability ascribed to the eight variables (Table 8). In the case of the lactobacilli, reduced salivary flow (both stimulated and in the minor salivary gland output), the presence of decay, and dependent living status accounted for 89% of the variability accounted for in the overall model. None of these variables was useful in explaining the levels of *S. sobrinus.*

Dependent living status was significantly associated with the anaerobic bacteria count and the levels of *S. mutans*, lactobacilli, and yeasts. However, the direction of the change varied

TABLE 7. Effects of dentures and presence of decay on median levels of selected aciduric bacterial species in saliva (unadjusted for multiple comparison)

Species	Significance (P value)[a]	Log_{10} CFU/ml of saliva for presence of dentures of any kind			
		No dentures		Dentures	
		No decay (n = 66)	Decay (n = 107)	No decay (n = 52)	Decay (n = 113)
Aciduric					
S. mutans	0.08	4.14	4.92	4.15	4.82
S. sobrinus	NS	1.87	1.82	1.84	1.83
Lactobacillus spp.	0.06	3.99	4.38	5.01	4.60
Yeasts	NS	2.75	2.88	2.92	3.23
Nonaciduric					
S. sanguis	0.02	5.45[b]	4.99	3.43[b,c]	5.53[c]
S. salivarius	NS	2.07	1.90	1.97	1.87
Anaerobic count	NS	8.05	8.20	7.99	8.14
Salivary					
pH	NS	6.8	6.8	6.9	6.8
Flow rate[d]	NS	0.59	0.64	0.64	0.65

[a] Kruskal-Wallis test. NS, not significant.
[b,c] Values in the same row with the same superscript are significantly different; multiple comparisons were based on Kruskal-Wallis rank sums.
[d] Flow rate is in milliliters per minute.

TABLE 8. Effects of various parameters on salivary levels of aciduric and nonaciduric species in the saliva of older individuals (dentate and edentulous)

Oral or salivary parameter	*P* value[a]						
	Anaerobic count	Aciduric species				Nonaciduric species	
		S. mutans	*S. sobrinus*	*Lactobacillus* spp.	Yeasts	*S. sanguis*	*S. salivarius*
Living status	0.0001	0.006	NS	0.001	0.004	NS	0.07
Complaint of xerostomia	NS	NS	NS	NS	NS	0.006	NS
Minor salivary gland	NS	NS	NS	0.04	0.09	NS	0.004
Salivary flow rate	0.0001	0.05	NS	0.0001	0.0001	NS	NS
Salivary pH	0.01[b]	NS	NS	NS	NS	NS	NS
Number of teeth	0.04	0.0001	NS	NS	NS	0.0001	NS
Presence of dentures	NS	NS	NS	0.08	0.008	NS	NS
Number of decayed teeth	NS	NS	0.08	0.0001	0.025	NS	NS
r^2	0.18[c]	0.20	0.02	0.13	0.15	0.11	0.05
r^2 reduced model	0.18[c]	0.18		0.11	0.14	0.08	0.04

[a] *P* value with all parameters in the multivariable linear model procedure. NS, not significant ($P \geq 0.1$ in the multivariable linear model procedure).
[b] Not significant when model was restricted.
[c] $P < 0.01$ overall.

with the environment: in the hospital setting the values decreased, whereas in the nursing home setting they increased (Tables 8 and 9). A reduced salivary flow, i.e., ≤0.5 ml/min, increased the anaerobic counts and the levels of *S. mutans*, lactobacilli, and yeasts (Table 9). The presence of dentures increased the levels of yeasts, whereas the presence of teeth increased the total count and the levels of *S. mutans* and *S. sanguis* (Table 9). The presence of decay increased the levels of lactobacilli and yeasts. A complaint of xerostomia was associated with decreased levels of *S. sanguis*. A reduction in the minor salivary gland output was associated with increased levels of *S. salivarius* and lactobacilli. About 20% of the patients not seen at the VA outpatient clinic or hospital and living independently provided rinse samples. We could see no effect of this collection process in the living status component of the model.

DISCUSSION

The most commonly sought after bacterial parameters in the saliva are the levels of *S. mutans* and lactobacilli, which have diagnostic implications for the incidence of dental decay (9, 13). The development of the diagnostic guidelines was based on data obtained from children and young adults, in whom the contribution of the teeth to the surface area would be relatively constant and in whom the rate of stimulated saliva is almost always greater than 0.5 ml/min (10). In elderly individuals, missing teeth would decrease the surface area, whereas dentures would increase the surface area and would introduce unknown variables in terms of the types of bacteria that would colonize these surfaces. Reduced salivary flow in elderly individuals, usually secondary to the usage of medications (14, 19, 27), could result in higher bacterial concentrations in the saliva, presumably because more time would be needed to collect a milliliter of saliva. This could confound the interpretation of bacteriological data reported as the numbers of organisms cultured per milliliter of saliva. Missing teeth and complaints of xerostomia would affect food choices and would further affect the bacteriological composition of the saliva. All of these considerations indicate that the diagnostic implications of salivary bacterial counts in the elderly could be different from those established for children and young adults.

If the surface area and shed rate of the oral surfaces remain constant and then if saliva is secreted at a reduced rate, reduced saliva secretion alone would increase the concentrations of most oral species present in the saliva. In the present investigation, a stimulated salivary flow of ≤0.5 ml/min was associated with a significant increase in anaerobic bacteria, *S. mutans*, lactobacillus, and yeast counts. This indicates that any reported increases in these species in the saliva of older individuals, which do not take into account the confounding nature of a reduced salivary flow, will be difficult to interpret.

The surface area of the oral cavity will vary according to the number of teeth and the presence of dentures. Individuals with

TABLE 9. Effects of various parameters on changes in the levels of selected aciduric and nonaciduric species in saliva of older individuals (reduced model)[a]

Parameter	Anaerobic count	Aciduric species				Nonaciduric species	
		S. mutans	*S. sobrinus*	*Lactobacillus* spp.	Yeasts	*S. sanguis*	*S. salivarius*
Living status[b]	−H	−H		−H, +N	+N		−H, ±N
Complaint of xerostomia						−	
Minor salivary gland output ≤ 2.5 μl/min				±			±
Stimulated saliva ≤ 0.5 ml/min	+	±		+	+		
Presence of dentures					+		
Presence of teeth	±	+				+[c]	
Presence of decay				+	+		

[a] +, levels increase ($P < 0.01$); ±, levels increase ($P = 0.01$ to 0.05); −, levels decrease ($P \leq 0.01$).
[b] H, hospital group; N, nursing home group; directional changes are relative to values observed in the group living independently and attending VA outpatient clinic.
[c] Presence of 15 to 28 teeth compared with 0 teeth.

more teeth had more bacteria per milliliter of saliva and more *S. mutans* and *S. sanguis* organisms. The latter finding is consistent with the fact that the teeth are the preferred surfaces for colonization by these species (13). Only yeasts were selected for in the presence of dentures; this finding has been noted by others (12, 17). The presence of decay, which would both change the flora on the tooth surface and possibly affect the surface area, was associated with significant increases in the levels of lactobacilli and yeasts. The absence of a significant association between decay and *S. mutans* was unexpected.

A low salivary pH was associated with a reduction in the anaerobic counts, but it otherwise had no effect on the monitored species. A complaint of xerostomia in the morning, which in the two-way model increased the levels of most of the monitored species, was found in the general model to be associated only with a decrease in the levels of *S. sanguis*. In the two-way model *S. sanguis* was not significantly associated with a complaint of xerostomia, a fact which illustrates the need to approach the salivary levels of various bacteria by using a multivariate model.

The living status of the subjects, especially if it was dependent, had a strong bidirectional influence on the levels of the monitored species. In the nursing home environment, the levels of yeasts, lactobacilli, and *S. salivarius* increased, probably as a result of a low salivary flow and the presence of both dentures and decay. However, in the hospital environment these same predisposing conditions were associated with decreased levels of total cultivable bacteria, *S. mutans*, lactobacilli, and *S. salivarius*. The decline in the lactobacillus levels runs counter to the increased prevalence of dentures and decay as well as a reduced salivary flow and suggests that other more dominant factors are operating on these hospitalized subjects. This was not likely to be a difference in the levels of oral hygiene, because both the nursing home and hospitalized groups had poor oral hygiene. This could be the usage of various medications, including antibiotics, as well as tube and intravenous feeding. It could reflect the fact that in the hospitalized patients, less saliva was collected by the suction method than by the spitting method.

Previous studies have shown great variability in the salivary levels of *S. mutans* in elderly individuals but have converged on the strong positive association of high salivary *S. mutans* levels with the wearing of either partial or complete dentures (17, 23) or with decay (1, 9, 10). When we used a two-way analysis, we observed a tendency for *S. mutans* levels to increase in our dentate subjects, who wore either a partial denture or one complete denture, but did not observe it in our edentulous subjects who wore dentures. These findings are consistent with those presented in the literature. However, no effect of the presence of either decay or dentures on the salivary levels of *S. mutans* could be found when other predictors were included in the model. Rather, the presence of teeth and a reduced salivary flow were the dominant factors affecting *S. mutans* levels. This finding raises the possibility that the diagnostic value of salivary *S. mutans* levels may be suspect if the rate of salivary flow and number of teeth are not considered.

When mechanical stimulation is used, the stimulated salivary flow rates are usually between 1 and 2 ml/min (10). The stimulated salivary flow rate when a gustatory stimulus is used is lower (12, 20) and is about the 0.5- to 0.6-ml/min value observed in the present study. We used a gustatory stimulus because some of our subjects, specifically those in the hospitalized group, were known aspirators, and we did not want the risk of them aspirating the wax. Also, among our subjects were individuals who were edentulous with and without dentures and individuals who had various numbers of teeth, with and without prosthetic replacements. Their ability to chew the wax would vary according to their number of teeth and the adequacy of their dentures or prosthetic replacements. This could introduce an uncontrolled variable on the amount of saliva secreted, because a positive correlation is found between the number of teeth and a masticatory stimulated salivary flow (22). We observed this phenomenon also, because the salivary volume increased significantly with increased numbers of teeth. Finally, the chewing of paraffin causes the shearing of bacteria from the tooth and other oral surfaces, and in the case of *S. mutans*, there may be a very large increase in its levels in paraffin-stimulated saliva compared with those in resting saliva (2). This means that the specific bacterial counts obtained per milliliter of saliva in studies that use a gustatory stimulus will not be directly comparable to those in studies which have used the chewing of paraffin wax to obtain their salivary samples (1, 5, 10, 23).

This analysis demonstrates how many confounders and effect modifiers affect the interpretation of salivary bacterial counts in geriatric individuals. Our findings indicate that the levels of yeasts and lactobacilli may be as important, if not more so, than the levels of mutans group streptococci when seeking microbial indicators that might predict the future incidence of caries in elderly individuals. In order to use the levels of these organisms as predictors of dental decay, it may be necessary to find more appropriate ways of expressing them rather than as the numbers of CFU per milliliter of saliva. We are exploring models which also take into consideration the rate of salivary flow and the number of teeth. It may be that a parameter such as the numbers of CFU of mutans group streptococci per tooth per milliliter of saliva could be more valuable in identifying the risk of caries in elderly individuals.

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