Microflora in Oral Ecosystems in Primary Sjögren's Syndrome

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ABSTRACT. ***Objective.*** Knowledge of the effect of primary Sjögren's syndrome (pSS) on the microbial flora in the different predilection sites for oral disorders is needed for planning preventive treatment. We carried out microbial analysis of samples from the dorsum of the tongue, smooth mucosa, supragingival tooth surfaces, and the gingival crevice region of 20 patients with pSS.

Methods. A clinical oral examination was performed and whole unstimulated and stimulated secretion rates were measured.

Results. Compared with healthy controls, subjects with pSS harbored higher numbers and frequencies of *Streptococcus mutans*, *Lactobacillus* spp., and *Candida albicans* in the supragingival plaque. On the smooth mucosa and tongue, the pSS subjects displayed an increased frequency of *C. albicans*, *Staphylococcus aureus*, enterics, and enterococci. *C. albicans* was detected about twice as frequently in the supragingival plaque as it was on the tongue. In the gingival crevice region, the pSS group harbored slightly lower proportions of *Fusobacterium nucleatum* and *Prevotella intermedia*/*Prevotella nigrescens* than controls. The clinical and microbial differences were mainly due to the pSS subjects with a stimulated secretion rate of < 0.5 ml/min. *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* were not detected in any subject with pSS.

Conclusion. The microbial flora in the different ecosystems reflected the status of oral disorders in the subjects with pSS. Specific site sampling and analysis in subjects with pSS revealed further differences compared with controls, and is therefore preferable to saliva sampling for oral treatment planning and for the evaluation of the effect of oral treatment and of preventive measures implemented in individuals with pSS. (J Rheumatol 2001;28:1007–13)

Key Indexing Terms:
SJÖGREN'S SYNDROME ECOSYSTEM MICROORGANISM HYPOSALIVATION

Due to differences in surface structure, availability of nutrients, and oxygen tension, for example, 4 major microbial ecosystems can be discerned in the mouth[1]. They are the dorsum of the tongue, the smooth mucosa, the supragingival tooth surfaces, and the gingival crevicular region. The composition of microbial flora is specific in each of these areas. In healthy conditions, microorganisms associated with oral disorders are not present or are only present in low proportions[2]. Factors that might lead to a shift in the microbial flora are hyposalivation, decreased host defence, frequent intake of carbohydrates, and antibiotic treatment[2-4]. Other factors that affect the microbial composition of these ecosystems are dental status, age, and oral hygiene habits[5,6].

Subjects with primary Sjögren's syndrome (pSS) have hyposalivation[7]. Moreover, the output of organic and inorganic components, important factors in the regulation of the microbial flora, has been shown to be affected[8,9]. Tapper-Jones, *et al*[10] studied the presence of *Candida albicans* in subjects with pSS and described an increased frequency and number on the dorsum of the tongue, the buccal mucosa, the palate, and in the vestibulum. This is the only study we have found in which results from different oral ecosystems in subjects with pSS have been documented. MacFarlane and Mason[11] and MacFarlane[12] cultivated samples from different ecosystems — the dorsum of the tongue, the buccal mucosa, the palate, and the tonsillar area — but presented the results as a total score for all 4 sites. They found increased numbers and frequencies of *C. albicans*, *Staphylococcus aureus*, and coliforms and reduced numbers of *Streptococcus salivarius*, *Veillonella* spp., *Neisseria pharyngis*, and *Micrococcus mucilagenosus*. Studies in which the microbial flora in supra- or subgingival plaque in subjects with pSS have been analyzed are lacking. Results from studies analyzing saliva samples, however, indicate increased numbers of mutans streptococci and *Lactobacillus* spp.[7,13,14], but no difference in the numbers of *Fusobacterium nucleatum* and *Prevotella intermedia*/ *Prevotella nigrescens*[15].

More comprehensive knowledge of the effect of pSS on microbial flora in the different predilection sites for oral

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disorders would be of great value for effective treatment planning in pSS. We sampled the dorsum of the tongue, the smooth mucosa, the supragingival tooth surfaces, and the gingival crevice region. Samples were analyzed for the following microorganisms: mutans streptococci, *Lactobacillus* spp. and *Actinomyces* spp., associated with the development of caries[16], *F. nucleatum* and *P. intermedia*/*P. nigrescens*, associated with plaque accumulation and gingivitis[17], *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, frequently found in elevated numbers in periodontitis sites[18], and *C. albicans, S. aureus*, enterics, and enterococci, associated with mucosal infections[19]. The total microbial count was also registered, together with the total number of streptococci, mitis streptococci, and *S. salivarius* associated with good oral health[5].

MATERIALS AND METHODS

This study was approved by the Ethics Committee at Göteborg University. Twenty dentate subjects with pSS diagnosed according to the Copenhagen criteria[20] 7 ± 5 years previously were included. The subjects were recruited from the Department of Rheumatology, Sahlgrenska University Hospital, in the order they showed up for yearly controls. The mean age of the subjects, 19 women and one man, was 56 ± 8 years (range 38–69). Subjects with removable prosthetic constructions or implants were not included. No subject had a history of radiotherapy in the head and neck region or of chemotherapy. Each pSS subject was matched according to age, sex, and number of teeth with a healthy control with a normal unstimulated and stimulated salivary secretion rate. The controls were recruited among patients registered at the Students' Clinic, Faculty of Odontology, and from private dentists. Both groups had given their informed consent. The appointments were between 8 AM and 12 PM. Duplicate measurements of secretion rates and collections of samples for microbial analysis were performed 9 ± 7 days apart (range 2–39 days). A clinical oral examination was performed on the first appointment. All measurements and examinations were performed by author AA. Clinical variables that were registered were: status of the mucosal membranes, number of teeth, fillings, crowns and bridges[15,21], presence of plaque along the gingival margin, bleeding following probing, and periodontal pocket probing depths of > 4 mm at 4 sites on each tooth[15,21]. The unstimulated secretion rate was measured for 15 min and the paraffin wax stimulated secretion rate for 5 min. The pH and buffer capacity were measured in the stimulated saliva with a pH meter (Metrohm 632, Herisau, Switzerland). With the assay used, "normal" pH range is between pH 6.3 and 7.2 and "normal" buffer capacity between pH 5.0 and 7.0[22]. A detailed description of methods for measuring secretion rates, pH, and buffer capacity has been given[21].

Microbial sampling. Microbial sampling was performed after determination of the unstimulated secretion rate and before measurement of the stimulated secretion rate and the clinical examination. Before sampling the dorsum of the tongue, the buccal mucosa, and the vestibulum, saliva was wiped off with sterile compresses to equalize the 2 groups. Sterile cotton sticks immersed in VMGI[23] were used to collect the samples. The samples collected from the left and right buccal mucosa, centrally in the molar region, were pooled, as were samples collected from the upper right and lower left vestibulum in the molar region. Supragingival plaque was collected with sterile toothpicks. The sites that were pooled were between the upper right first and second molar and between the lower left first and second molar, and buccally along the gingival margin on the upper right and the lower left first molar. Four gingival crevicular sites were sampled using the paper point technique[18]. The sites that were sampled and pooled were mesially on the upper right first molar, distally on the first premolar, mesially on the lower left molar, and distally on the lower left premolar. Two paper points were used at each site. All the microbial samples were transported to the laboratory in a bottle containing 3.3 ml of transport medium, VMGA III[23], and were processed within 4 h.

Laboratory analysis. Dilutions of the samples were performed in VMG I[23]. Brucella agar plates were incubated using the hydrogen combustion technique[24] at 36°C for 5–7 days. Plates with Mitis-Salivarius-agar, Mitis-Salivarius-Bacitracin agar, Rogosa agar, CFAT agar, and TSBV agar were incubated in an atmosphere of 10% CO_2 and 90% N_2 at 36°C for 3–5 days. Plates with Sabouraud T agar, Staphylococcus agar, Drigalski agar, and Enterococcus agar were incubated aerobically at 36°C for 3–5 days.

Microbial species identified at the specific sites are shown in Tables 2–6. The detection limit was 20 colony forming units (cfu)/ml for all species except *A. actinomycetemcomitans,* where it was 10 cfu/ml. If possible, the number of the different microorganisms in a sample was calculated from their number on a plate giving 30–300 colonies. The total number of bacteria growing anaerobically and the number of *P. intermedia*/*P. nigrescens*, *P. gingivalis*, and *F. nucleatum* were calculated from their growth on Brucella agar plates. *P. intermedia*/*P. nigrescens* was identified as black, indole-positive colonies showing dark red fluorescence in longwave (360 nm) UV light. *P. gingivalis* was identified as greenish-black colonies not showing fluorescence. *F. nucleatum* was identified as grey colonies with a nacreous appearance of Gram-negative long and slender cells with tapering ends. Representative colonies were subjected to carbohydrate fermentation tests. *S. salivarius* and mitis streptococci were identified on Mitis-Salivarius agar plates. *S. salivarius* was identified as large, light blue colonies of gram-positive cocci, and mitis streptococci were identified as firm, adherent colonies. *S. mutans* and *S. sobrinus* were identified on Mitis-Salivarius-Bacitracin agar plates. *S. mutans* was identified as small, mucoid, and irregular colonies and *S. sobrinus* as creamy marzipan-like colonies. *Lactobacillus* spp. were calculated from their growth on Rogosa agar plates and identified as Gram-positive rods. *Actinomyces* spp. were identified as small, yellow and high colonies of Gram-positive rods on CFAT agar plates. *S. aureus* was calculated from its growth on Staphylococcus agar plates. Colonies of *S. aureus* were distinguished from *Staphylococcus epidermidis* by their ability to degrade DNA on DNA agar plates (Difco). The number of *C. albicans* was calculated from its growth on the Sabouraud T agar plates. It was identified as lustreless and creamy whitish-pink or pink colonies. Enterics were identified as large yellow or green colonies of Gram-negative rods on Drigalski agar plates, and enterococci on Enterococcus agar plates as small brown colonies of Gram-positive cocci surrounded by a black zone. *A. actinomycetemcomitans* was identified on TSBV agar on the basis of a star-shaped colony morphology and a positive catalase reaction. In doubtful cases, further identification was performed with API biochemical tests (API Products, Biomerleux, Lyon, France).

Statistical methods. To normalize the microbial data, the numbers were logarithmically transformed. Zero counts were treated as 1 colony forming unit/ml. For the analysis of possible differences between the 2 groups, Student's 2 sample (unpaired) t test was used.

RESULTS

Two subjects in the pSS group and one control were smokers. Sixteen pSS subjects and 2 controls were taking medication. The groups of medicines most frequently used in the pSS group were estrogen (10 subjects), antiinflammatory drugs (5 subjects), and antirheumatic drugs (3 subjects). Six subjects had taken antibiotics < 3 mo prior to the examination. The antibiotics were broad spectrum (3 subjects), penicillin (2 subjects), and antifungal (one subject). The 2 controls taking medication both used antihypertensives and estrogen and one also took a blood-thinning medicine. In the pSS group, all the subjects frequently used saliva-stimu-

lating agents and/or fluoride containing products. No subject in either group showed clinically visible signs of *Candida* infection. As shown in Table 1, the pSS subjects had higher numbers of crowned teeth and filled surfaces than the controls. The proportion of surfaces with plaque along the gingival margin tended to be higher in the pSS group. Six pSS subjects and 7 controls had sites with periodontal pocket probing depths of > 4 mm (data not shown). For these subjects, the number of sites with pocket probing depths of > 4 mm varied between one and 6. The mean pocket probing depths of these sites were 5.5 ± 0.8 mm in the pSS group and 5.8 ± 1.5 mm in controls.

The highest unstimulated secretion rate measured in the pSS group was 0.06 ml/min. Seven pSS subjects had a stimulated secretion rate of > 0.5 ml/min and 13 subjects had a stimulated secretion rate ≤ 0.5 ml/min. The saliva pH was significantly lower in the pSS group, but only 3 pSS subjects had pH lower than normal, pH 6.3. The buffer capacity was also lower in the pSS group than in controls. A buffer capacity below normal, pH 5.0, was found in 10 pSS subjects and 4 controls. For the 7 pSS subjects with a secretion rate > 0.5 ml/min, a lower pH was found compared with the controls ($p < 0.05$). The other differences in clinical status between the pSS group and controls (Table 1) were mainly due to the results for the 13 subjects with a stimulated secretion rate ≤ 0.5 ml/min.

When we calculated the total microbial count per cm^2 in the sampled sites, 17 pSS subjects and all the controls displayed a higher microbial density on the tongue than on the buccal mucosa and in the vestibulum. In the pSS group, the median total counts were 3×10^6 on the tongue, 7×10^4 on the buccal mucosa, and 1×10^5 in the vestibulum. The corresponding numbers for controls were 4×10^6, 3×10^4, and 9×10^4.

Dorsum of the tongue. The total microbial count on the dorsum of the tongue was similar in the pSS and the control group (Table 2), while the proportions of streptococci and *S. salivarius* of the total count were higher in the pSS group. *F. nucleatum* and *P. intermedia*/*P. nigrescens* were frequent findings in both groups. *F. nucleatum* was detected in 19 pSS subjects and 17 controls and *P. intermedia*/*P. nigrescens* in 14 pSS subjects and 15 controls. The pSS group harbored a lower proportion of *F. nucleatum* ($p = 0.004$) and tended to harbor a lower proportion of *P. intermedia*/ *P. nigrescens*.

Table 1. Salivary secretion rates (mean of 2 measurements) and clinical features in 20 pSS subjects and 20 controls matched for age, sex, and number of teeth.

Subjects	Unstimulated Secretion Rate, ml/min	Stimulated Secretion Rate, ml/min	pH	Buffer Capacity	No. of Teeth	No. of Crowned Teeth	No. of Filled Surfaces	Surfaces with Plaque along Gingival Margin, %	Surfaces with Bleeding on Probing, %
Mean ± SD									
pSS	0.02 ± 0.02	0.47 ± 0.38	7.0 ± 0.8	4.9 ± 1.1	24 ± 4	10 ± 6	70 ± 24	55 ± 20	30 ± 14
Controls	0.32 ± 0.16	2.01 ± 0.70	7.6 ± 0.1	5.8 ± 0.9	26 ± 4	4 ± 5	53 ± 21	41 ± 26	24 ± 14
p	< 0.001	< 0.001	< 0.01	< 0.01		< 0.01	< 0.05	NS	NS
Median									
pSS	0.01	0.40	7.3	5.0	26	8	67	60	28
Controls	0.28	1.9	7.6	6.2	28	3	48	40	20
Range									
pSS	0.00–0.06	0.06–1.45	5.0–7.7	2.8–6.6	13–29	0–24	14–111	26–100	10–60
Controls	0.14–0.71	0.73–3.89	7.3–8.1	3.8–6.7	16–31	0–15	17–85	6–91	5–57

Table 2. Total count of microorganisms on the dorsum of the tongue (sampled area about 7 cm^2), expressed as log_{10}, and proportions (%) of total count of specific microorganisms (mean of 2 samples) in 20 pSS subjects and 20 controls matched for age, sex, and number of teeth.

	Total Count, log_{10}	Proportion of Total Count, %							
		Streptococci	*S. salivarius*	*F. nucleatum*	*P. intermedia*/ *P. nigrescens*	*C. albicans*	*S. aureus*	Enterics	Enterococci
Mean ± SD									
pSS	7.14 ± 0.75	62 ± 37	26 ± 32	0.20 ± 0.35	0.08 ± 0.20	0.006 ± 0.019	0.02 ± 0.09	0.004 ± 0.013	0.36 ± 0.94
Controls	7.31 ± 0.47	39 ± 29	7.6 ± 8.5	0.96 ± 1.3	0.28 ± 0.65	0.0001 ± 0.0005	0.0001 ± 0.0005	0.00 ± 0.00	0.0002 ± 0.0009
p	NS	0.04	0.02	0.02	NS	NS	NS	NS	NS
Median									
pSS	7.37	60	5.9	0.018	0.008	0.00	0.00	0.00	0.03
Controls	7.44	26	4.7	0.19	0.01	0.00	0.00	0.00	0.00
Range									
pSS	5.13–8.03	6.3–100	1.3–100	0.00–1.2	0.00–0.89	0.00–0.08	0.00–0.41	0.00–0.05	0.00–2.9
Controls	6.29–7.94	5.4–100	0.2–34	0.00–3.9	0.00–2.5	0.00–0.002	0.00–0.002	0.00–0.00	0.00–0.004

Table 3. Total count of microorganisms on the buccal mucosa (sampled area about 6 cm^2), expressed as log_{10}, and proportions (%) of specific microorganisms (mean of 2 samples) in 20 pSS subjects and 20 controls matched for age, sex, and number of teeth.

	Total Count, log^{10}	Proportion of Total Count, % Streptococci	Mitis Strep	*P. intermedia/ P. nigrescens*	*C. albicans*	*S. aureus*
Mean ± SD						
pSS	5.64 ± 0.46	64 ± 30	3.6 ± 6.5	0.15 ± 0.48	0.002 ± 0.004	0.01 ± 0.04
Controls	5.30 ± 0.48	59 ± 27	7.0 ± 7.3	0.37 ± 1.05	0.0002 ± 0.0007	0.0009 ± 0.004
p	0.03	NS	NS	NS	NS	NS
Median						
pSS	5.61	65	1.5	0.00	0.00	0.00
Controls	5.30	60	4.0	0.03	0.00	0.00
Range						
pSS	4.72–6.35	10–100	0.00–29	0.00–2.13	0.00–0.015	0.00–0.19
Controls	4.48–6.19	17–100	0.00–28	0.00–4.67	0.00–0.003	0.00–0.019

C. albicans was more frequently isolated in the pSS group, in 7 subjects compared with 2 controls. Fourteen pSS subjects and 4 controls harbored at least one of the following species: *C. albicans*, *S. aureus*, enterics, and enterococci. The number of enterococci was higher in the pSS group than in controls (p = 0.008).

Buccal mucosa. The total microbial count and the number of streptococci were higher in the pSS group than in the controls (p = 0.03 and p = 0.02, respectively). The mean proportion of mitis streptococci of the total microbial count displayed a tendency to be lower in the pSS group than in controls (Table 3). Moreover, the proportion of mitis streptococci of the total number of streptococci tended to be lower in the pSS group than in the controls, 5.4 ± 7.7% and 10 ± 7.9%, respectively (p = 0.05). The mean proportion of *P. intermedia*/*P. nigrescens* of the total count was slightly lower in the pSS group. *C. albicans* was detected in 4 pSS subjects and one control and *S. aureus* in 3 subjects in each of the 2 groups.

Vestibulum. The total microbial count was similar in the 2 groups (Table 4). *F. nucleatum* was detected in 16 pSS subjects and 18 controls and *P. intermedia/ P. nigrescens* in 9 pSS subjects and 10 controls. The proportions of *F. nucleatum* and *P. intermedia*/*P. nigrescens* of the total count were somewhat lower in the pSS group than in controls. Nine pSS subjects, but no control, harbored at least one of the following species: *C. albicans*, *S. aureus*, enterics, and enterococci. *C. albicans* was found in 6 pSS subjects, *S. aureus* in 2 subjects, enterics in 2 subjects, and enterococci in 8 subjects.

Supragingival plaque. The 2 groups had a similar total microbial count (Table 5). Mitis streptococci, *S. mutans*, and *Actinomyces* spp. were frequent findings in both groups. Mitis streptococci were detected in 19 pSS subjects and in all controls, *S. mutans* in 19 pSS subjects and 15 controls, and *Actinomyces* spp. in 17 pSS subjects and 19 controls. *S. sobrinus* was only detected in 2 pSS subjects. The proportion of mitis streptococci and *Actinomyces* spp. of the total count was similar in the 2 groups, but the proportion of *S. mutans* was significantly higher in the pSS group than in the controls. Moreover, the number of *S. mutans* was higher in the pSS group (p = 0.0007). *Lactobacillus* spp. were detected in 14 pSS subjects and 2 controls and *C. albicans* in 12 pSS subjects and 5 controls. The pSS group tended to

Table 4. Total count of microorganisms in the vestibulum in the molar region (sampled area about 5 cm^2), expressed as log_{10}, and proportions (%) of specific microorganisms (mean of 2 samples) in 20 pSS subjects and 20 controls matched for age, sex, and number of teeth.

	Total Count, log_{10}	Proportion of Total Count, % Streptococci	*F. nucleatum*	*P. intermedia/ P. nigrescens*	*C. albicans*	*S. aureus*	Enterics	Enterococci
Mean ± SD								
pSS	5.90 ± 0.67	47 ± 33	0.13 ± 0.25	0.31 ± 0.62	0.007 ± 0.017	0.0006 ± 0.003	0.02 ± 0.09	0.13 ± 0.28
\ Controls	5.54 ± 0.61	53 ± 31	0.33 ± 0.94	0.21 ± 0.49	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
p	NS	NS	NS	NS	NS	NS	NS	NS
Median								
pSS	5.84	40	0.04	0.00	0.00	0.00	0.00	0.00
Controls	5.65	37	0.06	0.005	0.00	0.00	0.00	0.00
Range								
pSS	4.87–7.48	3.1–100	0.00–1.12	0.00–2.43	0.00–0.07	0.00–0.01	0.00–0.4	0.00–1.1
Controls	4.35–6.49	12–100	0.00–4.31	0.00–1.99	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00

Table 5. Total count of microorganisms in supragingival plaque, expressed as $\log_{10}$, and proportions (%) of specific microorganisms (mean of 2 samples) in 20 pSS subjects and 20 controls matched for age, sex, and number of teeth.

	Total Count $\log_{10}$	Proportion of Total Count, %					
		Streptococci	Mitis strep	*S. mutans*	*Lactobacillus* spp	*Actinomyces* spp	*C. albicans*
Mean ± SD							
pSS	6.45 ± 0.49	35 ± 28	16 ± 29	11 ± 21	7.6 ± 24	3.3 ± 5.0	0.04 ± 0.07
Controls	6.30 ± 0.72	36 ± 33	13 ± 22	0.8 ± 2.6	0.025 ± 0.11	3.3 ± 6.8	0.005 ± 0.015
p	NS	NS	NS	0.03	NS	NS	NS
Median							
pSS	6.47	30	7.3	1.8	0.02	0.4	0.003
Controls	6.54	19	8.7	0.04	0.0	1.4	0.0
Range							
pSS	4.85–7.17	6.2–100	0.0–100	0.0–74	0.0–100	0.0–15	0.0–0.26
Controls	5.08–7.35	2.4–100	0.2–100	0.0–11	0.0–0.49	0.0–31	0.0–0.06

have higher proportions of *Lactobacillus* spp. and *C. albicans* compared with controls. However, the numbers of *Lactobacillus* spp. and *C. albicans* were significantly higher in the pSS group than in controls (p = 0.001 and p = 0.04, respectively). Eleven pSS subjects and 2 controls harbored both *S. mutans* and *Lactobacillus* spp. as well as *C. albicans*.

Gingival crevicular region. The total microbial count was similar in the 2 groups (Table 6). *F. nucleatum* was detected in 16 subjects in each group and *P. intermedia*/*P. nigrescens* in 14 pSS subjects and 13 controls. The proportions of *F. nucleatum* and *P. intermedia*/*P. nigrescens* were slightly lower in the pSS group than in controls. *P. gingivalis* was not detected in any subject and *A. actinomycetemcomitans* was detected in one control.

Of the specific microorganisms analyzed in samples collected from more than one site, *F. nucleatum*, analyzed at 3 sites, was most widespread. Fourteen subjects in each of the 2 groups harbored *F. nucleatum* at all 3 sites. *P. intermedia*/*P. nigrescens* was detected at all 4 sites analyzed in 6 pSS subjects and 4 controls. In 13 of the pSS subjects and 7 controls, *C. albicans* was found at at least one of the 4 sites analyzed. Two pSS subjects harbored *C. albicans* at all 4 sites. In both groups, *C. albicans* was detected about twice as frequently in the samples from the supragingival plaque as it was on the dorsum of the tongue. *S. aureus* was detected at at least one of the 3 sites analyzed in 4 pSS subjects and at one site in 4 controls. Enterics were detected only in the pSS group, where 2 subjects harbored enterics at both sites analyzed and 2 subjects at one site. Eight pSS subjects harbored enterococci at both sites analyzed and one pSS subject and 2 controls harbored them at only one site.

The 6 pSS subjects who had been treated with antibiotics < 3 months before the examination showed an oral microbial flora similar to the pSS subjects not recently treated with antibiotics.

Further analysis of the microbial results revealed that the significant differences in the oral flora were mainly due to the results obtained in the 13 pSS subjects with a stimulated secretion rate of ≤ 0.5 ml/min.

Table 6. Total count of microorganisms in the gingival crevicular region, expressed as $\log_{10}$, and proportions (%) of specific microorganisms of the total count (mean of 2 samples) in 20 pSS subjects and 20 controls matched for age, sex, and number of teeth. *A. actinomycetemcomitans* was detected in only one of the controls and the proportion of the total count was 0.15%. *P. gingivalis* was not detected in any subject in either group.

	Total Count, $\log_{10}$	Proportion of Total Count, %		
		Streptococci	*F. nucleatum*	*P. intermedia*/ *P. nigrescens*
Mean + SD				
pSS	5.55 ± 0.60	34 ± 27	0.5 ± 1.2	1.4 ± 2.1
Controls	5.60 ± 0.58	31 ± 27	0.7 ± 1.3	3.3 ± 10
p	NS	NS	NS	NS
Median				
pSS	5.76	25	0.09	0.3
Controls	5.65	24	0.3	0.2
Range				
pSS	4.00–6.13	4.6–100	0.0–5.3	0.0–7.7
Controls	4.40–6.90	4.4–94	0.0–5.6	0.0–43

DISCUSSION

Several studies describe an increased incidence of both mucosal infections[7,25,26] and caries[7,27-29] in subjects with pSS. With some exceptions[30,31], the periodontal conditions have been found to be comparable in subjects with pSS and healthy controls[7,14,15,32-35].

In our study, clinically visible caries lesions were rare findings in the pSS group. In a study by Ravald and List[14], the caries frequency was only slightly higher in the pSS group than in controls. In contrast, a high frequency of caries lesions was reported by Lundström and Lindström[7] and by Soto-Rojas, *et al*[29] in pSS subjects. One explanation of the discrepancies might be that the pSS subjects in the study by Lundström and Lindström[7] had a lower mean stimulated secretion rate than our pSS group, 0.21 ± 0.27 and 0.47 ± 0.38 ml/min, respectively. The subjects in the study by Soto-Rojas, *et al*[29] only went to the dental clinic when they felt oral discomfort or pain, while pSS subjects in our

study visited the dental clinic at least once a year.

In supragingival plaque from healthy subjects aged 40–59 years, mutans streptococci were detected[36] in 52% of subjects, *Lactobacillus* spp. in 9%, *Actinomyces naeslundii* in 65%, and yeasts in 22%. These results are in accord with our findings in the healthy group. In clinical studies, the regular use of fluoride gel has been found to reduce the frequency of caries[37] and frequent use of xylitol sweetened gum has been shown to reduce the acidogenic potential of dental plaque[38] and the numbers of *S. mutans*[39]. In *in vitro* experiments performed by Bradshaw, *et al*[40] and Bradshaw and Marsh[41] with the growth of mixed cultures under controlled conditions in a chemostat, it was shown that the presence of fluoride and xylitol interfered negatively with the growth of *S. mutans*, while the growth of *Lactobacillus rhamnosus* was unaffected. According to the API system manual, 90% of *C. albicans* strains are able to ferment xylitol. The majority of our pSS subjects reported frequent use of fluoride and xylitol-containing products and their prevalence of mutans streptococci, *Lactobacillus* spp., *Actinomyces* spp., and *C. albicans* was 95%, 70%, 85%, and 60%, respectively. The most prominent change in detection frequency was found for *Lactobacillus* spp., which were found 7 times more frequently in the pSS group than in the healthy controls. The proportion of *Lactobacillus* spp. of the total count tended to be higher in the pSS group and the proportion of *S. mutans* was significantly increased. Further, *Lactobacillus* spp. are favored by retention sites such as filling and crown joints[41a], which were frequent in the pSS group.

Tapper-Jones, *et al*[10] suggested that one explanation for the higher density of microorganisms on the smooth soft surfaces in pSS subjects compared with healthy controls could be the diluting effect of saliva in healthy subjects. In this study, to equalize the pSS and the control group, saliva was wiped off the sampling sites. In spite of this, the total microbial count on the buccal mucosa was higher in the pSS group than in controls. The proportions of mitis streptococci and *P. intermedia*/*P. nigrescens*, both acid sensitive[42], tended to be lower in the pSS group than in the controls, which is in accord with results obtained by Bradshaw, *et al*[40] and Bradshaw and Marsh[41]. Tapper-Jones, *et al*[10] found *C. albicans* on smooth soft surfaces in all 5 of their dentate subjects with SS. In contrast, we detected *C. albicans* in 8 of the 20 pSS subjects. Tapper-Jones, *et al*[10] used imprint sampling and culture, which is a more sensitive technique than the one used in our study.

Samples were collected from both the buccal and vestibular mucosa, because of different exposure to mechanical cleansing and oxygen in the 2 regions. As might be expected, both the pSS group and the controls harbored a higher total number of microorganisms in the vestibulum than on the buccal mucosa. Bradshaw, *et al*[40] showed that *P. nigrescens*, when grown in a mixed culture, was less sensitive than *F. nucleatum* to a low pH. We found the proportion of *P. intermedia*/*P. nigrescens* in the vestibulum to be somewhat higher and the proportion of *F. nucleatum* to be slightly lower in the pSS group than in controls (Table 4).

On the dorsum of the tongue, the pSS group harbored a higher proportion of streptococci and lower proportions of *F. nucleatum* and *P. intermedia*/*P. nigrescens* compared with their controls, as might be expected from the results of Bradshaw, *et al*[40]. The pSS group had a low saliva pH and buffer capacity (Table 1). Moreover, their good oral hygiene habits could have been a contributory factor to the lower proportions of *F. nucleatum* and *P. intermedia*/*P. nigrescens*. Hyposalivation and a low pH have been shown to predispose for the presence of *Candida*[43,44]. It should be noted, however, that *C. albicans* was detected twice as frequently in the supragingival plaque than on the tongue in the pSS group.

In accord with most previous studies, we found the prevalence of sites with plaque, gingival bleeding, and periodontal probing depths > 4 mm to be similar in the pSS group and in the healthy control group[14,33-35]. Although the proportion of surfaces with supragingival plaque was somewhat high in the pSS group, the plaque layers were generally thin (data not shown) and their proportions of *F. nucleatum* and *P. intermedia*/*P. nigrescens* were also somewhat lower compared with the controls. Elastase activity in the crevicular fluid is positively correlated to *P. gingivalis* (unpublished results) and destruction of the tooth-supporting tissues[45]. Tervahartiala, *et al*[34] found the crevicular fluid elastase activity in subjects with SS to be comparable to that of healthy controls. In the pSS subjects in our study, *P. gingivalis* and *A. actinomycetemcomitans* could not be detected. A pH < 7.0 is unfavorable for the growth of *P. gingivalis* and *A. actinomycetemcomitans*[46,47] and for elastase activity.

Compared with saliva sampling for microbial analysis[15], specific site sampling and analysis in pSS subjects revealed further differences from healthy controls. Moreover, the microbial flora in the different ecosystems reflected the clinical oral status of microbial disorders in the pSS subjects. Microbial analysis of samples collected from specific predilection sites in subjects with pSS could be an important tool in risk assessment and control of the treatment effect.

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