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ORIGINAL ARTICLE

Supra-gingival microbiota in Sjögren's syndrome

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Abstract The aim of this study was to investigate the microbiota of noncaries associated supra-gingival plaque (SGP) microbiology in Sjögren's syndrome (SS). Stimulated whole saliva (SWS) and full-mouth SGP on intact tooth surfaces were collected from 26 primary (p) SS, 27 secondary (s) SS, and 29 control subjects for selective culture of lactobacilli, mutans streptococci, and aerobic and facultatively anaerobic gram-negative rods (AGNR). Predominant cultivable anaerobes from SGP of 11 randomly selected subjects from each group were investigated. Clinical and sialometric data were also collected. SS sufferers had significantly lower SWS flow rate and higher mean DMFT (decayed, missing, filled teeth), while pSS subjects also had lower SWS pH and fewer standing teeth. *Lactobacillus acidophilus* levels in SWS (P=0.012) and SGP (P<0.0001) were significantly elevated in pSS sufferers compared with sSS individuals and controls. AGNR isolation was uncommon. SS sufferers had significantly lower proportion of gram-negative species (P=0.047). Non-oral species were isolated in greater proportions from pSS SGP (P=0.007). Subjects with pSS harbored increased levels of *L. acidophilus* and non-oral species, while SS sufferers generally had lower proportions of gram-negative species. The microbial composition of noncaries associated SGP indicates a potential source of increased caries risk in SS.

Keywords Saliva · Sjögren's syndrome · Dental plaque · Xerostomia · Microorganism

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Introduction

Sjögren's syndrome (SS) is one of the most common autoimmune diseases that impairs oral health. It affects 3–4% of adults, typically middle-aged or older women [35]. Key pathological changes in SS are infiltration of the lacrimal, salivary, and other exocrine glands by lymphocytes and plasma cells, with progressive acinar destruction. The primary form of the syndrome (pSS) has characteristic inflammatory cell involvement of the salivary and lacrimal glands, while secondary SS (sSS) involves various systemic autoimmune disorders and connective tissue diseases, in addition to exocrine gland involvement [28]. Oral features of SS are caused predominantly by reduced salivary flow and associated qualitative changes in saliva and their sequelae. Notable features include increased dental caries especially root caries, a greater number of missing teeth, and early tooth loss [4, 17, 31]. Oral candidiasis and angular cheilitis are well-recognized sequelae of SS-induced xerostomia [1, 13]. Oral conditions associated with SS can have a profound impact on oral health-related quality of life [29].

A shift in oral microbial composition of plaque is associated with diminished salivary flow [37]. Recent work on the oral microbiota in oral rinse samples of people with hyposalivation showed a marked increase in *Lactobacillus* spp. and *Candida albicans* after head and neck irradiation therapy, whereas in people with pSS, 85% of them had high

numbers of *Streptococcus mutans* [3]. The total bacterial count was, however, similar between subjects with hyposalivation and controls, and there was no increase in common periodontopathogens, i.e., *Fusobacterium nucleatum* and *Prevotella intermedia*/*P. nigrescens.* Similar findings were obtained when salivary samples were used [14, 24]. Microbial analyses of samples obtained from various oral sites of pSS subjects indicated that a higher number and frequency of *S. mutans, Lactobacillus* spp. and *C. albicans* were harbored in supra-gingival plaque (SGP) [2]; whereas samples obtained from mucosa and tongue displayed an increased frequency of *C. albicans*, *Staphylococcus aureus*, enterics, and enterococci. In the gingival crevice, lower proportions of *F. nucleatum* and *P. intermedia*/*P. nigrescens* were detected. Two earlier investigations on the oral ecology of patients with SS using swab culture from four different oral sites found increased numbers and frequencies of *C. albicans*, *S. aureus*, and coliforms but reduced numbers of *Streptococcus salivarius*, *Neisseria pharyngis*, *Veillonella* spp., and *Micrococcus mucilagenosus* [25, 26].

Most of the microbiological studies of SS patients so far have been based only on soft tissue or saliva samples and have selectively focused on a few microorganisms. However, to our knowledge, the predominantly cultivable microbiological profile of noncaries associated SGP in patients with SS has not been comprehensively described. An understanding of the microbial composition of saliva as well as the intact tooth surface SGP in SS sufferers may help explain the dental problems experienced by this medically compromised group of patients. Therefore, this study aimed to determine comprehensively *Streptococcus*, *Lactobacillus*, AGNR, and the predominant microflora cultivated from SGP on intact tooth surfaces in individuals with SS. Concurrent salivary levels of *Lactobacillus* and *S. mutans* were also assessed.

Materials and methods

The present report is part of a project studying the oral health condition of southern Chinese SS sufferers in Hong Kong [17]. Fifty-three SS (26 pSS, 27 sSS) subjects and 29 controls, all under regular dental care, participated in this study. All participants were southern Chinese. SS sufferers were recruited in the University Rheumatology Clinic, Faculty of Medicine, the University of Hong Kong, Queen Mary Hospital, Hong Kong. The diagnosis of pSS or sSS was established using the American–European Consensus Criteria for the Classification of Sjögren's syndrome [38]. All participants had attended regular dental review at 6-month intervals for at least 12 months before study commencement. None of them were under active dental treatment during the study period. The control group comprised medically clear patients attending the Prince Philip Dental Hospital. Exclusion criteria included prior radiotherapy in the head and neck region and any concurrent condition other than SS in test subjects that might cause altered saliva flow. Participants in the control group were selected for similar age and gender using the age/gender characteristics of the first 29 SS subjects. The ethical principles defined by World Medical Association Declaration of Helsinki were followed in this study. The Institutional Review Board of the University of Hong Kong approved the study. Written informed consent was obtained from each participant prior to admission to the study.

Clinical examination

All clinical and laboratory procedures were carried out by one trained examiner (KCML). The examination procedures and diagnostic criteria for caries followed those recommended by the World Health Organization [39]. The DMFT index was used to measure tooth condition including root caries. Oral hygiene was assessed using the Plaque (PI) [33] and Calculus Indices (CI) [12]. Probing pocket depth (PPD) was measured at six sites per tooth [30]. Subjects with PPD≥4 mm were noted.

Sialometry

All subjects were provided with 10 ml of sterile phosphate-buffered saline (0.1 M, pH=7.2) for rinsing (60 s) before sampling. Stimulated whole saliva (SWS) and parotid saliva (SPS) were then obtained as mentioned in an earlier report [17]. In brief, each subject was instructed to chew on a piece of sterilized silicone rubber tubing for 5 min and to spit all SWS into a sterile, preweighed plastic cup. Immediately following SWS sampling, SPS, conveniently sampled from the right parotid gland of all subjects, was collected by stimulating dorsal surface of tongue with 0.1 ml of 2% citric acid at 3-min interval for 15 min. The SPS produced was collected using a Lashley's cup with one end attached to the opening of the Stenson's duct and the other end to a preweighed vial. The pH was measured (Sentron 501 Pocket FET pH meter, Sentron, WA, USA) and the flow rate was determined and expressed as milliliter per minute. The buffer capacity was graded as low, medium, or high using CRT® buffer (Vivadent, Liechtenstein).

Microbiological sampling

SGP was removed with a sterile sickle scaler above the gingival margins of all intact teeth at buccal and lingual surfaces after drying and cotton wool isolation. It was dispersed into a preweighed screw-capped bottle containing 1 ml reduced transport fluid (RTF) [34]. Intact tooth

surfaces were defined as buccal or lingual coronal tooth surfaces free from restorative material and/or any restorative margins. Care was taken to avoid sampling plaque subgingivally and at sites covered by removable denture components or teeth with open carious lesions. Plaque net wet weight (grams) was measured, and the bottle was then transported to the laboratory where the specimen was processed within 30 min.

Fifty microliters of the SWS samples collected for sialometry were serially diluted in tenfold increments for selective culture experiments while full-mouth SGP samples collected from all participants were subjected to the total viable count (TVC) enumeration, selective culture for AGNR, mutans streptococci, and *Lactobacillus*. Predominant cultivable anaerobic SGP flora of a random sample of 11 subjects from each group was also studied.

Anaerobic culture and quantification of plaque isolates

In the laboratory, the SPG samples were dispersed by vortexing (Autovortex Mixer SA2, Stuart Scientific, London, UK) at maximum setting for 15 s. They were then serially diluted in tenfold increments and spiral plated on to Columbia blood–agar base (Difco) supplemented with 5% defibrinated horse blood, 5 mg/l haemin, and 500 g/l menadione (CBABS) using a spiral plater (Model DU, Spiral Systems, Cincinnati, OH). The plates were incubated for 5–7 days at 37°C in an anaerobic chamber (Forma Scientific, Marietta, OH) supplied with a gas mixture of 80% N_2, 10% H_2 and 10% CO_2.

After incubation, plates with appropriate number of colonies that were well-separated and evenly dispersed with 30–300 colony-forming units (c.f.u.) were chosen for counting. TVC per gram wet weight SGP was calculated with reference to the number of colonies, inoculum size, and dilution factor. The percentage proportion of mutans streptococci, *Lactobacillus*, and AGNR from selective culture over the corresponding total anaerobic viable count was then calculated.

Predominant cultivable anaerobic bacteria isolation and identification was done on 33 randomly selected subjects, 11 from the each group. One edentulous pSS subject was excluded from this part of the study for obvious reasons. Sample size determination was based on the capacity of the available anaerobic culture facilities, i.e., the capacity of approximately 30 specimens at one particular time point. A stainless steel template was used to subdivide the agar surface of the selected plates into sectors with a defined area. Representative colonies were picked as described previously [22], subcultured on CBABS plates to obtain pure isolates, and Gram-stained for direct microscopy using a light microscope (Nikon 104, Nikon Corporation, Tokyo, Japan) at ×1,000 magnification. The c.f.u. of colonies with different morphotypes and the respective dilution factor of the inoculum were recorded for later quantification. All dispersion, plating, and subculturing were performed on a laboratory bench.

Selective culture

Appropriately diluted SWS and SGP samples were inoculated by spiral plating onto duplicated mitis salivarius-bacitracin agar (MSB; for mutans streptococci) [10] and Rogosa agar (for *Lactobacillus* spp.) [32]. The plaque samples were also spiral plated onto duplicated MacConkey agars (Oxoid, Hampshire, UK; for AGNR) [21]. The MSB and Rogosa plates were incubated at 37°C for 48 h anaerobically. The MacConkey plates were incubated at 37°C for 18 h in air. Cultures were examined, and the c.f.u. of representative colonies with different morphotypes were recorded and subcultured on their respective culture media to obtain pure isolate and the dilution factor of the inoculum was noted.

Identification of isolates

Pure isolates were identified according to their colonial and cell morphology, Gram-stain reaction, ability to grow in air supplemented with 10% CO_2, and presence of catalase and oxidase activities. Commercially available identification systems including RapID-STR, ANA II, and NH system and API Staph and Strep kits (Analytical Profile Index; Bio Mérieux SA, Marcy-l'Etoile, France) were used. Additional tests for the AGNR were glucose fermentation and motility in a semisolid medium [21]. Isolates that could not be identified in this manner and all lactobacilli other than *Lactobacillus acidophilus* were further characterized using MicroSeq 500 16S ribosomal DNA (rDNA)-based identification system (Applied Biosystems, Foster City, CA). The validity of the above biochemistry-based identification system was randomly confirmed by subjecting one out of every ten identified species through MicroSeq 500 16S rDNA-based identification. The corresponding percentage proportion data (percentage proportion of the identified species per total sample count) of individual species isolated were then calculated.

Statistical analysis

Differences in categorical variables among the three groups were tested using Chi-squared test or Fisher's exact test, whichever appropriate. One-way analysis of variance (ANOVA) or Kruskal–Wallis test was performed to evaluate differences in continuous variables among the three groups. Multiple comparisons were carried out with Bonferroni correction of P values. Data from the three

groups were considered significantly different from each other if Mann–Whitney *U* test or Fisher's exact test showed $P<0.017$ between any two of the three groups tested. Spearman's rank correlation coefficient was used to explore associations between the level of lactobacilli or *L. acidophilus*; streptococci or *S. mutans* and SWS flow, lower SWS pH, DT, or DMFT. A *P* value of <0.05, unless stated otherwise, was considered statistically significant. All data were analyzed using SPSS for Windows 14.0.

Results

Subjects

The study group consisted of 26 pSS (one male; aged 33–74 years) and 27 sSS (one male; aged 26–66 years) patients. The mean time since diagnosis was 7.2 (SD=7.1) and 3.9 (SD=4.0) years, respectively, for pSS and sSS groups. The control group had 29 participants (two men; aged 27–75 years). The demographic characteristics, sialometric, and dental data of the subjects are shown in Table 1. Duration of last dental recall visit was from 5.5–6.5 months for all participants. All subjects were dentate except one pSS patient who was excluded from the plaque culture study. Other than fluoridated toothpaste, no participant used homecare fluoride products. None of the SS patients were taking systemic or locally acting medication/saliva stimulants for dry mouth symptoms. They had significant problems associated with subjective symptoms of dry mouth, generally, and dry mouth when eating and speaking. They also had problems of sticky saliva and coughing [29]. Seven (27%) pSS and eight (30%) sSS patients were taking hydroxychloroquine as part of the management of extra-glandular manifestations of SS. None of the patients were receiving anticholinergic therapeutic agents. Eighteen sSS patients had SLE, four had RA, two had sicca symptoms, two had SLE-like disease, and one had autoimmune hepatitis. Regarding the 33 subjects randomly selected for the anaerobic plaque culture, the demographic, sialometric, and dental characteristics were similar to their parent groups except that all were female, and the pSS subjects had a higher DT score than the controls (3.4±3.4/1.6±2.0/0.1±0.3 for pSS/sSS/control groups, $P=0.033$).

Clinical findings and sialometry

As shown in Table 1, SS sufferers had significantly lower SWS flow and higher mean DMFT scores than controls

Table 1 Age, sex, sialometric, and dental conditions of subjects

	pSS (*n*=26)	sSS (*n*=27)	Control (*n*=29)	*P* value	Post hoc analyses
Mean age (year)	51.4±14.3	42.7±10.7	43.9±10.7	0.072	
Percent of female subject	96.2	96.3	93.1	0.821	
Sialometric parameters					
SWS					
Flow (ml/min)	0.3±0.3	0.4±0.4	0.9±0.5	0.003	pSS/sSS < control
pH	7.0±0.5	7.2±0.6	7.4±0.3	0.004	pSS < control
Buffer capacity[a] (%)	9.1/72.7/18.2	9.1/54.5/36.4	9.1/27.3/63.6	0.208	
SPS					
Flow (ml/min/gland)	0.1±0.1	0.1±0.1	0.1±0.1	0.395	
pH	6.7±0.6	6.9±0.6	6.4±0.6	0.007	sSS > control
Buffer capacity[a] (%)	50.0/20.0/30.0	70.0/10.0/20.0	45.5/36.4/18.2	0.693	
Dental status					
Number of standing teeth	21.5±10.1	26.3±4.6	27.8±2.1	0.048	pSS < control
DT	2.0±2.8	1.5±2.2	0.8±1.1	0.332	
D-root	0.2±0.4	0.4±1.2	0.1±0.4	0.933	
FT	3.7±3.3	4.0±3.6	3.0±2.8	0.550	
DMFT	16.1±9.7	11.1±6.1	8.1±3.2	0.004	pSS/sSS > control
Percent of denture wearer	30.8	0	6.9	0.001	pSS > sSS/control
Periodontal status[b]					
Percent of subject with PI≤1	88.7±22.5	90.7±16.2	95.7±6.0	0.931	
Percent of subject with CI=0	80.0±17.5	78.8±21.8	77.3±14.2	0.861	
Percent of subject with PPD≥4 mm	44.0	33.3	51.7	0.374	
Percent of subject with PPD≥6 mm	8.0	3.7	3.4	0.682	

SWS Stimulated whole saliva; *SPS* stimulated parotid saliva

[a] Low/medium/high, one pSS and one sSS subjects were excluded for SPS analysis because SPS=0.

[b] Excluded one completely edentulous pSS subject

while pSS subjects had lower SWS pH and number of standing teeth than controls. Two pSS, three sSS, and one control subjects had root caries, but the differences in both prevalence and number between groups were not statistically significant. Significantly more pSS subjects were removable denture wearers. Oral hygiene condition and periodontal status were similar among the three groups.

Salivary microbiology

Compared to controls, pSS subjects harbored significantly higher amount of *Lactobacillus* species (namely *L. acidophilus*, *L. fermentum*, *L. minutis*), *L. acidophilus* in particular (Table 2). However, no difference was observed among the three groups regarding prevalence and quantity of *S. mutans*. Spearman's rank correlation showed that mean SWS lactobacilli notably *L. acidophilus* was significantly negatively associated with SWS flow rate (r_s=−0.356, P=0.001) while positively associated with DT and DMFT (r_s=0.539 and r_s=0.409, respectively, P<0.001).

Selective SGP culture

Regarding prevalence of *Lactobacillus* species (namely *L. acidophilus*, *Lactobacillus fermentum*, *Lactobacillus minutis*), *S. mutans*, and AGNR in plaque, the situation was similar to that observed in SWS. The level of the *Lactobacillus* species and in particular *L. acidophilus* in SGP and their percentage proportion were significantly higher in pSS subjects (Table 3). Spearman's rank correlation showed that mean plaque lactobacilli and *L. acidophilus* counts were negatively associated with SWS flow rate (r_s=−0.225, P=0.043; and r_s=−0.298, P=0.007, respectively) and positively associated with DMFT (r_s=0.353, P=0.001; and r_s=0.309, P=0.005, respectively). The prevalence, quantity, and percentage proportions of *S. mutans* and AGNR were similar among the groups. AGNR were detected at low levels in SGP.

Anaerobic culture of SGP

A total of 311 microbial isolates (9.4±1.6 isolates per sample) were obtained and 305 (98%) were successfully subcultured, purified, and identified. The mean colony-forming units per gram wet weight plaque for control, pSS, and sSS subjects were $0.5\times10^9\pm0.3\times10^9$ (range, 0.1×10^9 to 1.3×10^9), $0.8\times10^9\pm0.9\times10^9$ (range, 0.1×10^9 to 3×10^9), and $3.2\times10^9\pm6.0\times10^9$ (range, 0.2×10^9 to 16×10^9), respectively, with no difference between groups. From the anaerobic culture, SS subjects appeared to harbor more gram-positive species while controls had approximately equal proportions of gram-positive and gram-negative species in their SGP (Fig. 1). Gram-positive facultative anaerobic cocci were detectable from all pSS specimens. In the sSS group, gram-negative anaerobic rods were the most prevalent isolated species (n=10) whereas gram-positive facultative anaerobic cocci and gram-negative facultative anaerobic fusiforms were the most prevalent isolated species (n=10) in controls. The most predominantly isolated microbes observed in both pSS and sSS groups were gram-positive facultative anaerobic cocci, which comprised 27 and 36%, respectively, of the total number of isolated microorganisms; whereas gram-negative anaerobic rods were the most predominant microbes (26%) detected from controls. When the data of the two SS groups were combined and compared to the controls, SS subjects had significantly lower proportion of gram-negative species (34 vs 52%, P=0.047).

The most prevalent microbial genera isolated from pSS, sSS, and control subjects were *Streptococcus* (n=10), *Streptococcus* and *Capnocytophaga* (n=7), and *Capnocytophaga*. (n=10), respectively, with no difference between groups. *Actinomyces* and *Capnocytophaga* were the microbial genera with the highest mean percentage proportion from the SS (18.4±22.0% and 20.9±29.3% for pSS and sSS, respectively) and control (22.9±20.5%) subjects, respectively, with no difference between groups.

Table 2 Selective culture of *Lactobacillus* species and *Streptococcus mutans* from stimulated whole saliva

	pSS (n=26)	sSS (n=27)	Control (n=29)	P value	Post hoc analyses
Lactobacillus species[a]					
Prevalence (%)	15 (57.7)	10 (37.0)	8 (27.6)	0.072	
Count ($\times10^4$ c.f.u./ml)[a]	18.5±39.5	4.1±14.3	0.1±0.2	0.011	pSS > control
Lactobacillus acidophilus					
Prevalence (%)	13 (50.0)	8 (29.6)	5 (17.2)	0.036	pSS > control
Count ($\times10^4$ c.f.u./ml)	13.9±28.1	4.0±14.4	0.1±0.2	0.012	pSS > control
Streptococcus mutans					
Prevalence (%)	9 (34.6)	8 (29.6)	5 (17.2)	0.330	
Count ($\times10^5$ c.f.u./ml)[a]	0.8±2.1	6.2±18.9	0.9±3.0	0.347	

[a] Included all identified oral lactobacilli, namely, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, and *Lactobacillus minutis*

Table 3 Total anaerobic viable counts (TVC), selective culture of aerobic and facultatively anaerobic gram-negative rods (AGNR), *Lactobacillus* species, and *Streptococcus mutans* from noncaries associated supra-gingival plaque

	pSS (n=25)[a]	sSS (n=27)	Control (n=29)	P value	Post hoc analyses
TVC ($\times 10^9$ c.f.u./g)	1.0±2.5	2.0±0.4	1.1±3.1	0.488	
AGNR					
Prevalence (%)	1 (4.0)	1 (3.7)	2 (6.9)	1.000	
Count ($\times 10^2$ c.f.u./g)	7.0±34.8	9.3±48.1	2.9±10.9	0.780	
Percent of proportion/TVC ($\times 10^{-4}$)	4.4±22.2	0.6±3.0	1.2±13.0	0.516	
Lactobacillus species[b]					
Prevalence (%)	9 (36.0)	4 (14.8)	0 (0.0)	0.001	pSS > control
Count ($\times 10^6$ c.f.u./g)	2.0±6.1	0.5±2.5	0.0±0.0	0.001	pSS/sSS > control
Percent of proportion/TVC ($\times 10^{-1}$)	4.5±11.6	0.0±0.1	0.0±0.0	<0.0001	pSS > sSS/control
Lactobacillus acidophilus					
Prevalence (%)	8 (32.0)	3 (11.1)	0 (0.0)	0.001	pSS > control
Count ($\times 10^6$ c.f.u./g)	1.3±4.2	0.0±0.0	0.0±0.0	0.001	pSS > sSS/control
Percent of proportion/TVC ($\times 10^{-1}$)	4.5±11.6	0.0±0.1	0.0±0.0	<0.0001	pSS > sSS/control
Streptococcus mutans					
Prevalence (%)	10 (40.0)	9 (33.3)	7 (24.1)	0.479	
Count ($\times 10^6$ c.f.u./g)	1.2±4.8	2.5±6.9	0.1±0.5	0.344	
Percent of proportion/TVC ($\times 10^{-1}$)	3.0±7.0	10.0±32.3	0.3±1.2	0.283	

[a] Excluded one completely edentulous female subject
[b] Included all identified oral lactobacilli, namely, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, and *Lactobacillus minutis*

A total of 86 different bacterial species were identified (Table 4, for species with prevalence >15%). Fifty two species were detected in low frequency (prevalence < 15%) representing 60% of the total identifiable species and a mean proportion of 16.9/35.1/16.6% per SGP TVC, respectively, for pSS/sSS/control subjects.

Actinomyces naeslundii was the species with the highest mean percentage proportion per TVC of pSS subjects; whereas in sSS group and controls, *Capnocytophaga* spp. had the highest mean percentage isolation (Table 4). *Capnocytophaga* spp. was also the most prevalent species among the three groups (63.6% for both pSS and sSS groups, and 81.8% for controls). No significant difference was detected for percentage isolation and prevalence in any of the bacterial isolates, between groups.

Approximately 4% of the isolates were regarded as uncommon or non-oral species, including *Aerococcus viridans* 2, *Bacteroides stercoris*, *Collinsella aerofaciens*, *Lactobacillus jensenii*, *Mobiluncus mulieris*, *Mobiluncus* spp., and *Tisserella praeacuta*, which were mostly isolated from SS subjects only. pSS sufferers had significantly higher percentage proportion isolation of non-oral species than the controls (P=0.012).

Discussion

Salivary acidogenic microbial counts are considered an important indicator of caries risk. A high salivary *S. mutans* count indicates the presence of a strong cariogenic challenge

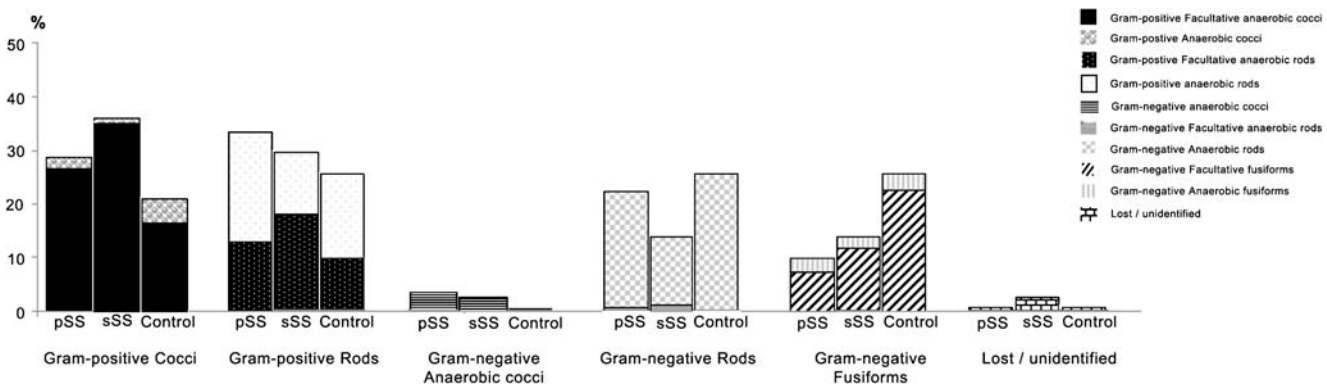

Fig. 1 Percentage proportion of predominant cultivable species from anaerobic culture of noncaries associated supra-gingival plaque according to morphology and Gram-stain characteristics

Table 4 Percentage proportion of predominantly cultivable microbial species[a] from anaerobic culture of noncaries associated supra-gingival plaque

	pSS (n=11)	sSS (n=11)	Control (n=11)
Gram-positive species			
Facultative anaerobic cocci			
Aerococcus viridans 2[b]	0.8±1.9	0.0±0.0	0.0±0.0
Gemella haemolysans	3.6±7.7	0.0±0.0	0.5±1.7
Gemella morbillorum	3.5±9.9	7.3±16.1	4.6±7.0
Staphylococcus capitis	0.0±0.0	1.4±4.5	3.0±6.2
Streptococcus anginosus	1.5±3.6	2.6±6.7	0.0±0.0
Streptococcus constellatus	0.7±1.6	0.7±1.6	0.2±0.5
Streptococcus intermedius	3.2±8.8	0.0±0.0	0.7±2.2
Streptococcus mitis 1	0.9±3.0	1.9±5.2	1.7±3.0
Streptococcus oralis	0.2±0.5	2.1±5.7	1.6±5.2
Streptococcus sanguinis	6.3±18.3	0.0±0.0	0.2±0.5
Anaerobic cocci			
Anaerococcus prevotii	2.2±4.2	0.2±0.7	4.4±9.3
Facultative anaerobic rods			
Actinomyces odontolyticus	1.4±2.4	2.2±6.2	5.0±14.5
Actinomyces naeslundii	9.7±17.7	5.1±12.5	4.0±9.3
Anaerobic rods			
Actinomyces israelii	2.7±8.3	5.6±18.6	0.0±0.0
Actinomyces turicensis	0.0±0.0	0.0±0.0	2.2±5.5
Actinomyces sp. oral clone	4.6±15.3	0.3±1.0	1.6±3.7
Bifidobacterium spp.	1.8±6.0	2.3±5.3	2.7±8.9
Collinsella aerofaciens[b]	4.8±13.1	0.1±0.2	0.6±1.9
Eubacterium sp. oral clone	2.1±5.2	0.2±0.5	1.9±4.9
Mobiluncus spp.[b]	3.6±8.2	1.5±3.3	0.0±0.0
Tissierella praeacuta[b]	0.0±0.0	1.5±4.9	2.9±9.4
Gram-negative species			
Anaerobic cocci			
Veillonella spp.	4.0±11.0	3.0±9.8	0.0±0.0
Anaerobic rods			
Bacteriodes stercoris[b]	2.6±8.7	0.6±1.5	0.0±0.0
Campylobacter gracilis	1.4±2.9	0.9±2.9	8.3±12.9
Campylobacter spp.	3.6±6.5	1.0±2.5	0.9±3.1
Leptotrichia buccalis	0.0±0.0	1.4±3.2	0.5±1.7
Leptotrichia sp. oral isolate	2.9±6.1	2.9±6.1	6.9±9.7
Prevotella corpois	1.4±3.2	0.0±0.0	6.2±13.0
Prevotella intermedia	0.5±1.6	1.3±2.6	0.3±1.0
Prevotella melaninogenica	5.8±18.4	2.1±7.0	0.0±0.0
Prevotella nigrescens strain 9-PNIG	0.0±0.0	1.1±2.6	0.1±0.2
Facultative fusiforms			
Capnocytophaga spp.	7.4±11.3	11.7±23.8	20.5±21.5
Anaerobic fusiforms			
Fusobacterium necrophorum	0.5±1.7	1.9±5.7	0.9±3.0
Fusobacterium nucleatum	2.1±4.8	0.2±0.5	0.6±0.2
Non-oral species[c]	14.8±14.9	2.2±3.8	0.6±1.9

[a] Only species with prevalence >15% in any one group listed
[b] Non-oral species
[c] P=0.012, pSS > control

to the teeth [6]. High lactobacilli counts are detected in subjects with active caries lesions or who consume diets with high sucrose content [7, 23]. With reference to the currently accepted cut-off for salivary lactobacilli- and streptococci-related caries risk, which is $\geq 10^5$ c.f.u./ml [5, 9], our pSS subjects showed high salivary lactobacillus count while sSS subjects had high *S. mutans* count. Furthermore, the increased SWS and intact tooth surface SGP *L. acidophilus* level in these subjects and its significant correlations with reduced SWS flow and the DT and DMFT scores confirmed

the association of the microbe with dental caries risks in oral niches of regularly maintained SS subjects.

High levels of oral AGNR have been reported in subjects with salivary hypofunction after head and neck irradiation [20] and in individuals with poor oral hygiene [21]. It is perhaps not surprising, therefore, to observe low prevalence as well as low level of oral AGNR in our SS subjects who had regular dental care, despite the significantly reduced SWS flow.

Dental plaque is a natural microbial biofilm. The bacterial composition of plaque remains relatively stable despite regular exposure to minor environmental perturbations [27]. This stability is due to a dynamic balance of both synergistic and antagonistic microbial interactions. Therefore, when the environment is modestly perturbed, self-regulatory mechanisms come into force to restore the original balance. On occasions, however, homeostasis does break down and disease can occur. This can be due to deficiencies or dysfunction of host defenses, or it can arise due to a change in local environmental conditions, for example, hyposalivation.

It is well documented that SS patients show a tendency to harbor increased levels of *S. mutans*, lactobacilli, and *Candida*. However, most of the previous microbial reports have reflected salivary or mucosal levels of the microorganisms and microbial cultures were obtained from selective media [3, 14, 24]. Owing to the differences in local environmental conditions, microflora of mucosal and tongue surfaces and microbes recoverable from saliva may differ in their compositions to that of dental plaque. Only one study, as far as we are aware, obtained speciated microbial samples from dental plaque cultured in selective medium [2]. Unfortunately, all their pSS subjects were using a saliva-stimulating agent, which complicated the interpretation of the results. Nonetheless, significantly higher proportions of *S. mutans* and *C. albicans* were observed. A tendency towards a higher proportion of *Lactobacillus* spp. was also noticed.

The present study describes, for the first time, the comprehensive profile of noncaries associated SGP microbiology in SS. The data available clearly demonstrated an alteration of microbial flora beyond the previously reported specific caries-related bacteria such as *S. mutans*, *Lactobacillus*, *Actinomyces*, or the aciduric opportunistic mucosal pathogen *C. albicans*. A higher proportion of SWS or SGP *Lactobacillus* especially *L. acidophilus* and increased proportion of SGP non-oral species were observed in pSS, while significantly lower proportion of gram-negative species were found in SGP of SS subjects. A recent report by our team has described an increased quantity of yeast in the SGP of SS sufferers [18]. The alteration of microbial flora places those with SS at an increased risk of caries, opportunistic infection, and candidiasis as reported earlier.

Our earlier report has described that dental attendance of these three groups of subjects was similar [17]. The fact that SS sufferers had significantly higher DMFT seems to indicate that dental caries and its complications probably develop at an increased rate. Moreover, the increased proportion of *L. acidophilus* in noncaries associated SGP as seen from the present SS subjects may suggest a theoretically increased likelihood of a flora shift to cariogenic composition, if dental decay is to be developed.

In this study, all SS subjects appeared to have similar and acceptable periodontal conditions when compared to controls. The prevalence and proportions of the anaerobic bacteria commonly associated with periodontal disease such as *F. nucleatum* and *P. intermedia/P. nigrescens* were low. This observation is in accord with some previous reports [3, 14, 15, 24] but in contrast to the others [8]. The present data seem to favor the notion that SS subjects who had significantly reduced salivary output are at no greater risk of developing periodontal disease.

Colonization of subgingival plaque by non-oral species has been reported in special patient groups, including renal transplant recipients, HIV-infected individuals, and those who have received head and neck irradiation [19, 21, 36]. The prevalence of such species has been reported to be as high as 50% and, in some instances, even higher [23], which is similar to our findings, although the sample sites are different. These species have also been isolated, albeit in low proportions, from healthy subjects [36]. Some of the bacterial species do not belong to the normal oral flora, such as *C. aerofaciens*, and genera *Tissierella* (previously known as a member of *Clostridium*), which are occasionally found in or associated with root canal infections [11, 16].

The study group exhibited a wide age range, which had the potential to reduce the power of the study. Age inevitably affects oral microflora. However, categorizing the results into different age groups was not considered appropriate due to the overall small number of subjects in the study. Only stimulated saliva was collected in the study, it would have been more comprehensive if unstimulated saliva is included because it is often very helpful in diagnosing SS and to explain caries prevalence and periodontal status. Similarly, investigation of the anaerobic flora in subgingival plaque would provide useful data for this special group of patients. These limitations should be taken into account when interpreting the present data.

Sjögren's syndrome sufferers displayed a distinct intact tooth surface, noncaries associated SGP flora in terms of reduced proportion of facultative anaerobic gram-negative rods, and an increased proportion of *Lactobacillus* especially *L. acidophilus* and non-oral microbes. While the implication of this finding is presently uncertain, such flora composition is potentially more susceptible to transformation into cariogenic biofilm and, should it occur, would

expose the affected individual to a higher risk of dental caries.

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References

1. Abraham CM, al-Hashimi I, Haghighat N (1998) Evaluation of the levels of oral *Candida* in patients with Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endo 86:65–68
2. Almståhl A, Wikström M, Kroneld U (2001) Microflora in oral ecosystems in primary Sjögren's syndrome. J Rheumatol 28:1007–1013
3. Almståhl A, Wikström M, Stenberg I, Jakobsson A, Fagerberg-Mohlin B (2003) Oral microbiota associated with hyposalivation of different origins. Oral Microbiol Immunol 18:1–8
4. Baudet-Pommel M, Albuisson E, Kemeny JL, Falvard F, Ristori JM, Fraysse MP, Sauvezie B (1994) Early dental loss in Sjögren's syndrome. Histologic correlates. European Community Study Group on Diagnostic Criteria for Sjögren's syndrome (EEC COMAC). Oral Surg Oral Med Oral Pathol 78:181–186
5. Bratthall D, Hänsel Petersson G (2005) Cariogram- a multifactorial risk assessment model for a multifactorial disease. Community Dent Oral Epidemiol 33:256–264
6. Carlsson P, Olsson B, Bratthall D (1985) The relationship between the bacterium *Streptococcus mutans* in the saliva and dental caries in children in Mozambique. Arch Oral Biol 30:265–268
7. Crossner C (1981) Salivary lactobacillus counts in the prediction of caries activity. Community Dent Oral Epidemiol 9:182–190
8. Çelenligil H, Eratalay K, Kansu E, Ebersole J (1998) Periodontal status and serum antibody responses to oral micro-organisms in Sjögren's syndrome. J Periodontol 69:571–577
9. Featherstone JD, Adair SM, Anderson MH, Berkowitz RJ, Bird WF, Crall JJ, Den Besten PK, Donly KJ, Glassman P, Milgrom P et al (2003) Caries management by risk assessment: consensus statement, April 2002. J Calif Dent Assoc 31:257–269
10. Gold OG, Jordan HV, van Houte J (1973) A selective medium for *Streptococcus mutans*. Arch Oral Biol 18:1357–1364
11. Gomes BP, Lilley JD, Drucker DB (1996) Associations of endodontic symptoms and signs with particular combinations of specific bacteria. Int Endod J 29:69–75
12. Greene J, Vermillion JR (1960) Oral hygiene index. J Am Dent Assoc 61:172–179
13. Kindelan SA, Yeoman CM, Douglas CWI, Franklin C (1998) A comparison of intraoral *Candida* carriage in Sjögren's syndrome patients with healthy xerostomic controls. Oral Surg Oral Med Oral Pathol Oral Radiol Endo 85:162–167
14. Kolavic SA, Gibson G, Al-Hashimi I, Guo IY (1997) The level of cariogenic micro-organisms in patients with Sjögren's syndrome. Spec Care Dent 17:65–69
15. Kuru B, McCullough MJ, Yilmaz S, Porter SR (2002) Clinical and microbiological studies of periodontal disease in Sjögren's syndrome patients. J Clin Periodontol 29:92–102
16. Lana M, Ribeiro-Sobrinho A, Stehling R, Garcia GD, Silva BKC, Hamdan JS, Nicoli JR, Carvalho MAR, Farias Lde M (2001) Microorganisms isolated from root canals presenting necrotic pulp and their drug susceptibility in vitro. Oral Microbiol Immunol 16:100–105
17. Leung KCM, McMillan AS, Leung WK, Wong MCM, Lau CS, Mok TMY (2004) Oral health condition and saliva flow in southern Chinese with Sjögren's syndrome. Int Dent J 54:159–165
18. Leung KCM, McMillan AS, Cheung BPK, Leung WK (2007). Sjögren's syndrome sufferers have increased oral yeast levels despite regular dental care. Oral Dis published online (DOI 10.1111/j.1601-0825.2007.01368.x)
19. Leung WK, Jin LJ, Samaranayake LP, Chiu GKC (1998) Subgingival microbiota of shallow periodontal pockets in individuals after head and neck irradiation. Oral Microbiol Immunol 13:1–10
20. Leung WK, Jin LJ, Yam WC, Samaranayake LP (2001) Oral colonization of aerobic and facultatively anaerobic gram-negative rods and cocci in irradiated, dentate, xerostomic individuals. Oral Microbiol Immunol 16:1–9
21. Leung WK, Yau JY, Cheung BP Jin LJ, Zee KY, Lo EC, Samaranayake LP, Corbet EF (2003) Oral colonization of aerobic and facultatively anaerobic gram-negative rods and yeasts in Tibetans living in Lhasa. Arch Oral Biol 48:117–123
22. Leung WK, Jin L, Yau JYY, Sun Q, Corbet EF (2005) Microflora cultivable from minocycline strips placed in persisting periodontal pockets. Arch Oral Biol 50:39–48
23. Loesche W, Schork A, Terpenning M, Chen Y, Stoll J (1995) Factors which influence levels of selected organisms in saliva of older individuals. J Clin Microbiol 33:2550–2557
24. Lundström IMC, Lindström FD (1995) Subjective and clinical oral symptoms in patients with primary Sjögren's syndrome. Clin Exp Rheumatol 13:725–731
25. MacFarlane TW, Mason DK (1974) Changes in the oral flora in Sjögren's syndrome. J Clin Path 27:416–419
26. MacFarlane TW (1984) The oral ecology of patients with severe Sjögren's syndrome. Microbios 41:99–106
27. Marsh PD (1994) Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 8:263–271
28. Mavragani CP, Moutsopoulos NM, Moutsopoulos HM (2006) The management of Sjögren's syndrome. Nat Clin Pract Rheumatol 2:252–261
29. McMillan AS, Leung KCM, Leung WK, Wong MCM, Lau CS, Mok TMY (2004) Impact of Sjögren's syndrome on oral health-related quality of life in southern Chinese. J Oral Rehabil 31:653–659
30. Ng SKS, Leung WK (2006) A community study on the relationship between stress, coping, affective dispositions and periodontal attachment loss. Community Dent Oral Epidemiol 34:252–266
31. Pedersen AM, Reibel J, Nordgarden H, Bergem HO, Jensen JL, Nauntofte B (1999) Primary Sjögren's syndrome: salivary gland function and clinical oral findings. Oral Dis 5:128–138
32. Rogosa M, Mitchell JA, Wiseman RF (1951) A selective medium for the isolation and enumeration of oral lactobacilli. J Dent Res 30:682–689
33. Silness J, Löe H (1964) Periodontal disease in pregnancy: II correlation between oral hygiene and periodontal condition. Acta Odontol Scand 22:121–135
34. Syed SA, Loesche WJ (1972) Survival of human dental plaque flora in various transport media. Appl Microbiol 24:638–644
35. Thomas E, Hay E, Hajeer A, Silman A (1998) Sjögren's syndrome: a community-based study of prevalence and impact. Br J Rheumatol 37:1069–1076
36. Tsang CSP, Samaranayake LP (2001) Predominant cultivable subgingival microbiota of healthy and HIV-infected ethnic Chinese. APMIS 109:117–126
37. van Houte J (1994) Role of micro-organisms in caries etiology. J Dent Res 73:672–681
38. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS et al (2002) Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 61:554–558
39. World Health Organization (1997) Oral health surveys. Basic methods, 4th edn. WHO, Geneva