

# The predominant microflora of the palatal mucosa in an elderly island population

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The prevalence and microbiology of macrophotographically documented denture stomatitis were studied in denture wearers participating in an interdisciplinary health-monitoring project (Koster Health Project) on the Koster islands, Sweden. Upper dentures were used by 26.6% of the adult population, and 59.2% of the denture wearers had stomatitis. Denture stomatitis type I was identified as sialadenitis. The more severe forms of denture stomatitis (types II and III) demonstrated increased recovery of microorganisms in the palatal mucosa in addition to sialadenitis. Only one proband showed increased growth of fungi. *Hemophilus spp.* and *Bacteroides spp.* were the predominating microorganisms in stomatitis types II and III. Shifts in the normal oral flora are suggested to be an important factor for the development of denture stomatitis. It is concluded that bacterial colonization on the palatal mucosa may play an important role in denture stomatitis in this relatively healthy population. □ *Denture stomatitis; general health; oral health; oral microflora; sialadenitis*

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The normally keratinized palatal mucosa in healthy individuals may respond with inflammation (denture stomatitis or sialadenitis) if loaded with a denture plate. The biologic thresholds of the mucosa against possible chemical, mechanical, and microbial insults from the denture-wearing may be influenced generally by the health of the individual and locally by the denture hygiene and denture-wearing habits (for a review, see Bergendal (1)). Environmental, socio-psychologic, nutritional, and medical factors as well as medications and abuses of drugs may determine the magnitude of the oral tissue reactions (2). The function and fitness of upper dentures have been claimed to be of less importance to the development of denture stomatitis (3).

The prevalence of stomatitis among denture wearers in different populations has been estimated to be 38-65% (4-7). Newton (8) classified denture stomatitis into three different types: type I, with localized, simple inflammatory reactions; type II, with generalized, simple inflammatory reactions, and type III, with generalized, granular inflam-

ation. Similar classifications have also been proposed by other authors (1, 9, 10). A type-I stomatitis has been suggested to be induced by mechanical occlusion of the excretory ducts of the minor palatal salivary glands or local denture-induced trauma (8). Denture stomatitis of types II and III are suggested to be conditioned by increased growth of some microorganisms, especially *Candida albicans* (9, 11-17). The local environment underneath the denture plate is considered to predispose to fungal growth (18).

*C. albicans* belongs to the normal oral microflora in man and may, therefore, be found both in denture stomatitis and in clinically healthy mucosa (4, 15). Pathologic colonization of *C. albicans* seems to be related to tobacco use and particularly to deteriorated general health involving a compromised immune defence system in denture-wearing individuals (for a review, see Budtz-Jørgensen (19)). The decreased nutritional status of elderly people may also favor fungal growth (20). Although *C. albicans* has been claimed to be the major pathogenic component in denture stomatitis, it has been

demonstrated that treatment of denture stomatitis with chlorhexidine (17, 21, 22) and penicillin (23) might be successful, which indicates that bacteria may play an important role in the development of denture stomatitis. *Staphylococcus aureus*, enterobacteria, and shift of the oral flora to higher proportions of gram-negative and anaerobic bacteria have been associated with denture stomatitis, suggesting that fungal growth is not the only etiologic factor (14, 24). Additionally, it has been demonstrated that some antifungal drugs may also have antibacterial effects (25).

The aim of the present study was to estimate the prevalence of denture stomatitis, discuss the role of sialadenitis, and analyze the microbial colonization on the palatal mucosa in an isolated, rather homogeneous island population submitted to careful environmental, sociopsychologic, nutritional, and medical examinations yearly, since 1987 (the Koster Health Project (26)).

## Materials and methods

The annual oral examination of 184 adult probands (102 women and 82 men; mean age, 56.2 years), participating during June–August 1989 in the Koster Health Project (26), included standardized series of macrophotographs of the oral mucosa and any skin lesions present (15–20 color slides of each individual; magnification, 1:1.5 or 1:1) (27). The condition of the palatal mucosa was documented in two to four photographs. The color slides were evaluated by the dentists in the project. As color reproduction may vary in different slides, mainly owing to the development procedures, the evaluations were based on existing differences between clinically healthy and inflamed areas in subjects with denture stomatitis types I and II and on structural changes of the mucosal surface in subjects with denture stomatitis type III. Our criteria for sialadenitis have been reported earlier (28).

Individual protocols with environmental (air and water pollution), sociopsychologic, nutritional (including drinking water

quality), general medical, and oral medical data were used for the health evaluations.

Pilot studies with smears of the palatal mucosa for direct microscopy were made during 1988. Probands with denture stomatitis showed little or no presence of *C. albicans* in the oral lesions. In 1989 all probands with denture stomatitis ( $n = 29$ ; mean age, 70.4 years) were submitted to microbiologic sampling and examinations as described below. A control group consisting of 8 upper-denture wearers (mean age, 75.6 years) without stomatitis and 14 probands (mean age, 62.2 years) with natural teeth and clinically healthy palatal mucosa were selected randomly among the total number of probands participating in the Koster Health Project.

### Sampling technique

Before the sampling, the mouth was rinsed with water. Samples were taken by scraping deep in the palatal mucosa with a sterile Ward's carver over an area of approximately 20 mm<sup>2</sup>, aiming to obtain the microflora colonizing on and within the superficial epithelial cell layer. The samples were taken from the most inflamed areas in the denture stomatitis subjects and from corresponding areas in the healthy subjects. One sample from each test area was introduced into VMGA III transport medium (VMG III, in accordance with Möller (29), modified by anaerobic preparation and sterilization) in 3-ml glass bottles with 10 glass beads 1–2 mm in diameter. The medium was overlaid with oxygen-free nitrogen. Samples from the same test areas were also evenly spread in a drop of saline on two separate glass slides. All samples were sent to the Department of Oral Microbiology at the University of Göteborg and, as a rule, processed within 24 h.

### Laboratory procedures

The sampling bottles containing the transport medium were warmed to 37°C and shaken in a whirling mixer for 20 sec. A volume of 0.1 ml of the medium was stroked on the following agar plates: one Brucella Agar (11 086 BBL, Microbiological System,

Cockeysville, Md., USA) plate with the addition of 0.3% Bacto-Agar (Difco Laboratories, Detroit, Mich., USA), 5% defibrinated horse blood, 0.5% hemolyzed human erythrocytes, and  $5 \times 10^{-5}$  mg/l menadione incubated in an anaerobic jar (the hydrogen combustion method of Möller & Möller (30)) at 37°C for 6–8 days; one Blood Agar (4% Blood Agar Base No. 2, CM 271, Oxoid, Basingstoke, UK) plate with 5% defibrinated horse blood and 0.5% sodium-lactate for incubation in air with 10% CO<sub>2</sub> at 37°C for 2–3 days; one HI (*Haemophilus influenzae*) plate (31), for selective culturing of *Haemophilus spp.*, incubated in air with 10% CO<sub>2</sub> at 37°C for 2–3 days; one Gc-Cl plate (Difco) with colistinate, 7.5 mg/l (Lundbeck, Copenhagen, Denmark), lincomycin (Lincocin®, 4.0 mg/l; Upjohn, Kalamazoo, Mich., USA) and a vitamin supplement (Isovitalex®, 1% (BBL)), was incubated at 37°C for 3–5 days (24). This medium, selective primarily for gonococci and meningococci, was used also because of its selectivity for fungi. Additionally, one Sabouraud Dextrose Agar (Difco) plate with 1% tetrazolium chloride was incubated at 25°C for 5–7 days for differentiation of fungal colony types. Finally, 1 ml of the transport medium was inoculated in a test tube containing the semifluid medium HCMG-Sula (29), anaerobically prepared and sterilized. After inoculation it was flowed with oxygen-free CO<sub>2</sub> and incubated for 14 days.

The plates were examined in a plate microscope. The quantities of the different colony types in the strokes was semi-quantitatively estimated as very heavy, heavy, moderate, sparse, or very sparse growth, corresponding approximately to  $> 10^4$ ,  $10^{3-4}$ ,  $10^{2-3}$ ,  $10^{1-2}$ , and  $< 10^1$  cells/ml (24).

The tubes were examined every 2nd day for growth. When growth was registered, 0.1 ml of the turbid part of the medium was inoculated on the plates described above. When no growth was obtained on the plates, growth and isolation of colonies were quantified from the semifluid medium. In such cases, the growth was estimated to be very sparse.

Pure cultures were made from each colony type, and strain identification as to genus

and main bacterial groups was performed by means of morphology, Gram staining, and biochemical tests and, for the anaerobic bacteria, also gas chromatography. The principles outlined in *Bergey's Manual of Systematic Bacteriology* (32) and by Holdeman & Moore (33) were followed. For identification of some strains of streptococci, enterobacteria, and fungi the API system (Montalieu Vercieu, France) was used.

#### *Staining and examination of directly prepared slides*

One slide was fixed by heat and stained in accordance with Gram. The other was fixed with methanol for 5 min and stained by the method of Giemsa (rapid method, described by Mackie & McCartney (34)). The slides were analyzed by means of a light microscope at  $\times 1200$  magnification for morphology and presence of yeast hyphae.

#### *Statistical analyses*

Differences were tested for statistical significance by Student's *t* test, and *p* values lower than 0.05 were considered statistically significant.

## Results

The prevalence of upper dentures in the Koster population in 1989 ( $n = 184$ ) was 26.6%, and the prevalence of stomatitis among the denture wearers was 59.2% (67.7% in women and 44.4% in men; the difference was not statistically significant). No denture stomatitis was found in the mandibular mucosa, and none of the probands was wearing a denture in the lower jaw only.

Oral streptococci were recovered in all four categories (Table 1) and semiquantified as moderate growth or more in 90% of the individuals.

*Neisseria spp.* and *Haemophilus spp.* were also found in moderate growth in healthy control individuals with or without dentures. No significant differences were observed between healthy controls with and without

Table 1. Number of patients (%) with recovery of indicated microorganisms semiquantified as moderate growth or more

	Healthy controls without denture (n = 14)	Healthy controls with denture (n = 8)	Patients with denture stomatitis type I (n = 10)	Patients with denture stomatitis type II and III (n = 19)
Oral microorganisms				
Gram-positive spp.				
Oral streptococci	13 (92.9)	8 (100)	9 (90)	17 (89)
<i>Corynebacterium spp.</i>	1 (7.1)	0 (0)	0 (0)	0 (0)
Gram-negative spp.				
<i>Neisseria spp.</i>	3 (21.4)	2 (25)	0 (0)	2 (10.5)
<i>Haemophilus spp.</i>	3 (21.4)	1 (12.5)	1 (10)	7 (36.8)
<i>Bacteroides</i> (non-pigm.)	0 (0)	1 (12.5)	1 (10)	4 (21.0)
Fungi				
<i>Candida albicans</i>	0 (0)	0 (0)	0 (0)	1 (5.3)
Other <i>Candida spp.</i>	0 (0)	0 (0)	0 (0)	1 (5.3)
Non-oral microorganisms				
<i>Staphylococcus aureus</i>	0 (0)	0 (0)	0 (0)	1 (5.3)
<i>Haemophilus influenzae</i>	0 (0)	0 (0)	0 (0)	1 (5.3)
<i>Klebsiella pneumoniae</i>	1 (7.1)	0 (0)	0 (0)	1 (5.3)
Total gram-negative spp., fungi and non-oral microorganisms	5 (35.7)*	3 (37.5)*	2 (20)	10 (52.6)*

\* Gram-negative spp., fungi, and non-oral microorganisms were found in the predominant flora concomitantly in few patients.

dentures or between healthy controls and patients with denture stomatitis type I.

Except for one proband with *C. albicans* and *C. glabrata*, the dominant difference between patients with denture stomatitis types II and III and the other individuals was increased presence of *Haemophilus spp.* (*H. parainfluenzae*;  $p < 0.05$ ) and *Bacteroides spp.* (non-pigmented species; statistically not significant) in the types II–III group. Black-pigmented *Bacteroides spp.* and *Fusobacterium spp.* were not found in the predominant flora in any of the patient groups. Additionally, non-oral bacteria were found

in three subjects in the denture stomatitis II and III group; one patient with heavy growth of *Staph. aureus*, one with *H. influenzae*, and one with *Klebsiella pneumoniae* were recorded (Table 1). Examinations of the glass slides showed presence of hyphae and yeast cells in the sample that was *Candida*-positive in culture.

Among the probands showing denture stomatitis of types II and III, two individuals had upper partial prostheses covering mainly the edentulous alveolar ridges. These probands did not show any sialadenitis in the palatal mucosa, whereas all wearers of full upper dentures in the denture stomatitis groups demonstrated presence of clinically/photographically diagnosed sialadenitis. The difference in prevalences of sialadenitis between the probands ( $n = 184$ ) with and without denture stomatitis was statistically significant ( $p < 0.001$ ). A survey of the medical protocols of the probands showed no ongoing treatment with antibiotics, nor could any differences in general health or drinking water quality be confirmed between

Table 2. Prevalence of sialadenitis and use of tobacco and diuretics among the islanders ( $n = 184$ )

	No denture stomatitis, %	Denture stomatitis, %
Sialadenitis	35.5	93.1
Tobacco use	26.5	13.8
Diuretics	9.7	20.7

the stomatitis group and the controls. The prevalence of tobacco use, medication with diuretics, and sialadenitis in the minor palatal salivary glands is presented in Table 2. The differences between the groups, with regard to the use of diuretics, were not statistically significant. However, the lower prevalence of tobacco use among the probands with denture stomatitis, as compared with those without denture stomatitis, was statistically verified ( $p < 0.05$ ).

## Discussion

The prevalence of denture-wearing in the present study is similar to that presented in other Swedish investigations (35–38). The prevalence of stomatitis among the denture wearers is in agreement with data from earlier studies (4–7). Inflammatory reactions localized around the excretory ducts of the minor palatal salivary glands are usually diagnosed as type-I stomatitis, in accordance with Newton (8). This type of denture stomatitis may, therefore, be regarded as predominantly a palatal sialadenitis. Mechanical occlusion of the excretory ducts and other insults provoked by the denture material are believed to be the main etiologic factors of this type of denture stomatitis (3, 7, 8). As the effects of tobacco and diuretics on the development of sialadenitis in the palatal mucosa have been demonstrated also among non-denture wearers in the Koster population (28), it seems experimentally supported to regard sialadenitis as a separate entity. The clinically/photographically documented inflammatory reactions have been found to include histopathologically verified degenerative changes in the salivary gland parenchyma (28). Long-standing sialadenitis may, thus, result in alterations in the quality and quantity of saliva and in irreversible loss of gland tissue. It should be stressed that the use of tobacco among the islanders was lower in the denture stomatitis group than among the other probands, as indicated earlier (35).

The more severe forms of denture stomatitis (types II and III, in accordance with Newton (8)) are also characterized by sialadenitis in denture-bearing mucosa, being

partly masked by widespread inflammatory reactions in the soft tissues. Such tissue reactions are, however, seen also beyond mucosal areas containing salivary glands—for example, on denture-bearing alveolar ridges. The etiology of denture stomatitis of types II and III includes microbial infection of the oral soft tissues. Growth of *C. albicans* is usually suggested to be involved. Recent studies concerning fungal infection in denture stomatitis have, however, been performed on institutionalized elderly persons and on individuals with deteriorated general health (20, 39). In a compromised health situation with immunologic changes, commensalism may be transformed into parasitism, and fungi from the denture plaque may colonize the mucosa. Only one proband had *C. albicans* in the predominant flora, and hyphae were demonstrated in the smears for direct microscopy. Thus mucosal colonization of *C. albicans* did not appear to be a main etiologic factor for development of denture stomatitis types II and III in this population of otherwise healthy individuals.

Non-oral microorganisms have been recognized in denture stomatitis and other oral infections. Many of these studies have been focused on the association between opportunistic infections and compromised individuals (40, 41). Despite the low frequency of denture stomatitis associated with a heavy growth of non-oral bacteria, this study illustrated, in accordance with other investigations (24, 42, 43), that non-oral bacteria (*Staph. aureus*, *H. influenzae*, and *K. pneumoniae*) may also establish themselves on mucosal membranes even in non-compromised individuals and thus be related to the clinical symptoms.

The palatal mucosa is normally colonized by oral streptococci. This is also the case for the plaque on the fitting surface of upper full dentures (12). Only four probands in our study showed less than moderate or heavy growth of oral streptococci. Two of these showed a very sparse total microbial recovery, suggesting a very low microbial density on the mucosal surface. Two other individuals with low oral streptococcal recovery showed growth of *Klebsiella*. One of these probands, a denture wearer, had type II-III

stomatitis. A shift of the mucosal flora to high proportions of *Klebsiella* and low proportions of oral streptococci as well as a shift within the normal flora towards higher proportions of gram-negative bacteria might constitute a risk for development of denture stomatitis. This hypothesis needs to be studied further.

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