The prevalence of *Staphylococcus aureus*, *Enterobacteriaceae* species, and *Candida* species and their relation to oral mucosal lesions in a group of 79-year-olds in Göteborg*

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A subject sample comprising 100 persons (47 men and 53 women) 79 years of age and selected on a statistical basis (representing all persons of that age living in Göteborg) was the object of a general medical, clinical, and microbiologic study of the prevalence of microorganisms in the oral cavity known to cause opportunistic infections. A high prevalence of diseases and frequent medications were recorded among the participants. *Staphylococcus aureus* was present in five patients and *Enterobacteriaceae* species in only one individual. *Candida albicans* was not found in any samples from the palatal mucosa of the 25 individuals without dentures. Of 36 healthy denture wearers *C. albicans* was found in 9 (25%). In 39 persons with denture stomatitis *C. albicans* was obtained in 11 (28%) of the samples from the mucosa, 29 (74%) from the dentures, and 10 (26%) from the angulus oris. The prevalence of *S. aureus*, enteric rods, and *C. albicans* was low in the elderly population and, when present, correlated with the presence of dentures. No association with the patients' general health or drug use was obtained. □ *Elderly people, epidemiology; elderly people, oral microflora; denture stomatitis; tongue pathology*

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Staphylococcus aureus, *Enterobacteriaceae* species, and *Candida* species are microorganisms that may cause nosocomial and opportunistic infections, suggesting that they can, when present in a compromised host, be of a potential risk for oral infections (1–3).

S. aureus colonization has been demonstrated by culture on gingiva, tongue, saliva, mucosal surface, and removable dentures (4–6). *S. aureus* has also been associated with acute dentoalveolar infections, oral mucosal lesions, and denture-induced stomatitis. *Enterobacteriaceae* spp have previously been found in various oral sites and have been associated with oral mucosal lesions (4, 7, 8). The relationship between *C. albicans* and denture-induced stomatitis has been shown in numerous studies (9, 10). Despite this strong relationship, *C. albicans* is normally present in the resident oral flora in every second or third individual dentate or denture wearer (11, 12).

There is limited knowledge, however, about to what extent these microorganisms with a potential risk of being involved in oral mucosal lesions may occur in the transient flora of various age groups.

The elderly are considered to be at risk for an increased incidence and severity of certain infections (13). This is explained by altered immune functions or local defense factors due to physiologic aging per se and to the contribution of medical conditions such as underlying diseases, pharmacologic agents, nutritional deficiencies, and environmental exposure to infectious diseases. Additionally, broad-spectrum antibiotics promote a microbial shift of the mucosal flora, enabling colonization by opportunistic pathogens (5, 7, 8).

Recent studies have shown that age-related microbial changes can be registered after the age of 70 years (14, 15). However, there is still limited information on the occurrence of these opportunistic pathogenic microorganisms in the oral cavity of elderly people and their association with general health and oral mucosal conditions.

The aim of this study was to determine the prevalence of *S. aureus*, *Enterobacteriaceae*, and *Candida* species in the oral cavity in a consecutively selected group of elderly people subjected to a thorough medical examination. The microbial findings were related to general health and medications and to pathologic changes of the mucous membrane.

Materials and methods

The study population belonged to the longitudinal study

* From the gerodontologic and geriatric population studies in Göteborg, Sweden. Project leader before 1988, Alvar Svanberg; from 1988, Bertil Steen.

'70-Year-Old People in Gothenburg' and consisted originally of a sample of 973 70-year-old men and women. The sampling procedure, the study design, and the representativity of the subjects have been described previously (16–18). At the age of 79 years 537 men and women were medically and sociologically reexamined (16, 17). Subsamples were chosen for, for example, dental, psychologic, and nutritional examinations, and the subsample selected for the present study consisted of 100 consecutive subjects. These subjects did not differ from the others in the subsample with regard to factors such as dental status, prevalence of removable dentures, and salivary secretion rate.

Medical examination

At a home visit a registered nurse interviewed the participants about their drug consumption, and the drugs were grouped in accordance with the international classification (19). The number of drugs and the use of cardiovascular and psychopharmacologic drugs were tested against changes in the mouth. The intake of antibiotics during the previous 6-month period was registered. Diseases were defined in accordance with internationally used criteria and previously used definitions in this population (20), with special reference to diabetic mellitus, congestive heart failure, coronary heart disease, chronic bronchitis, stroke, rheumatoid arthritis, and anemia.

Venous blood samples were collected from the forearm after an overnight fast, and blood (B) and serum (S) were analyzed at the hospital laboratory. B-hemoglobin, B-folate, S-protein, S-thyroid-stimulating hormone (TSH), S-iron/transferrin iron-binding capacity (Fe/TIBC), B-glucose, and S-cobalamin (S-B_{12}) were used in this study.

Dental and oral examination

The patients were examined clinically for their dental status, the status of the oral mucosa, and the existence of complete or partial dentures. The oral mucosa including the lips was examined with particular regard to denture stomatitis, angular cheilitis including redness or ulcerations of tissue, and white lesions (including leukoplakia or perichymal keratosis and lichen planus).

The recorded tissue changes of the tongue were hypertrophy and atrophy of the tongue papillae and extensive fissurating or other alterations of the mucosa. Inflammatory changes of the palatal mucosa were classified in accordance with Newton (21) as follows: type I: red points mainly around the ducts of the salivary glands; type II: generalized erythema; and type III: a nodular hyperemic surface involving more or less of the denture-bearing tissues. Photographs were taken of the lips, the tongue, and pathologic changes of the oral mucosa.

The secretion rate of whole stimulated saliva was recorded. The stimulation was performed with paraffin chewing (22).

Microbiologic sampling and examination

Samples were taken for cultivation from the palatal mucosa and the right angulus oris. Additionally, in denture wearers one sample was taken from the fitting denture surface.

The sampling procedures were performed by scrapings (6), and samples were transferred in transport medium VMGA III (23).

Dispersion of the samples, cultivation, and analysis were performed using methods and techniques previously described (4). Each sample was inoculated on i) a *Brucella* agar plate (BBL, Microbiology Systems, Cockeysville, Md., USA), enriched with 5% defibrinated horse blood, 0.5% hemolyzed blood, and 5 μg/ml menadione on a blood agar plate, ii) a *Staphylococcus* agar plate (Medium 110, Difco 0297, Difco Laboratories, Detroit, Mich., USA), iii) a Gc-Cl plate (gonococcal-colistimate plate) (4), and iv) a Sabouraud-T-plate (Sabouraud glucose agar, Difco 0109 with 0.01% tetrazolium chloride). The *Brucella* agar plate was prereduced in an anaerobic glove box for at least 2 days. After inoculation the plates were incubated in a thermostated (37°C) chamber of the anaerobic glove box for 7 days. The blood agar plate and *Staphylococcus* agar plate were incubated aerobically for 3 days, and the Gc-Cl plate was incubated in an atmosphere of 10% carbon dioxide in air at 37°C for 5 days. The plates were examined for the presence of staphylococci, enteric rods, and fungi in relation to the normal flora and semiquantified in accordance with a scale given by Dahlén et al. (4). *S. aureus* was identified as being DNase- and coagulase-positive. *Enterobacteriaceae* spp were identified on the basis of API 20E (API System S.A., La Balme les Grottes, Montalieu, France), and fungi on the basis of API 20C. In the normal flora special attention was paid to the presence of anaerobic genera such as *Prevotella* (formerly *Bacteroides*) and *Fusobacterium*, shown by colony morphology, Gram-staining procedures, and gas chromatography (24).

Statistical methods

When testing for independence between microbial findings and general health, medication, changes of the mucous membrane, and salivary secretion, nonparametric tests were used (Fisher's test (25) and Pitman's test (26)).

Results

Medical examination

There was a high prevalence of diseases in this age group. Cardiovascular diseases were most common.

Table 1. The clinical status of the palatal mucosa in accordance with Newton (21) and its relation to the dental status of male and female patients

Dental status	Total		Healthy		Newton type I		Newton type II		Newton type III	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
No removable dentures*	14	11	14	11	0	0	0	0	0	0
Complete dentures	26	38	13	15	8	13	4	9	1	1
Partial acrylic dentures	6	3	5	2	0	0	1	1	0	0
Partial metallic (CoCr) dentures	1	1	1	0	0	0	0	1	0	0
Total	47	53	19	17	8	13	5	11	1	1

* Three subjects (one man and two women) were edentolous without dentures.

Table 2. Distribution of subjects on the basis of different pathologic changes of the lingual mucosa

Status of lingual mucosa	No. of subjects
Normal lingual mucosa	21
Partial atrophy	39
Total atrophy	9
Fissurated lingual mucosa	12
Partial hypertrophy of the filiform papillae	25
Hypertrophy of the filiform papillae	21
Lingua geografica	2

Coronary heart disease was present in 35% of the men and in 29% of the women and hypertension in 19% of the men and 39% of the women. Eleven per cent of the men and 8% of the women had diabetes, and 3% and 14%, respectively, had hyperthyroidism. Vitamin B_{12} deficiency was present in 8% and 6% and anemia in 10% and 6% among men and women, respectively.

The high prevalence of disease was reflected in a frequent medication. Seventy-nine per cent of the men and 89% of the women used on an average 3.5 and 4.3 medications, respectively. Among men and women 25% and 23% used digitalis, 27% and 42% diuretics, 17% and 10% beta-blockers, and 29% and 40% sedatives, respectively. The use of antibiotics was low (4%).

Oral examination

Twenty-five patients had no removable dentures (Table 1), 9% had partial acrylic dentures, and 2% partial metallic dentures. The rest wore complete dentures.

The patients without removable dentures had no stomatitis. Denture stomatitis was present in 36 (56%) of the patients with complete dentures and was equally distributed between the sexes.

Twenty-one patients had Newton type-I denture stomatitis, 16 had Newton type-II, and 2 patients had Newton type-III stomatitis.

Ten patients had angular cheilitis. Three of these wore complete dentures and had denture stomatitis corresponding to Newton type II. Two patients had their own teeth and no removable prosthesis. The last five patients were denture wearers with healthy oral mucosa. No intraoral mucosal lesions were diagnosed in these patients.

The clinical appearance of the lingual mucosa differed considerably between the patients (Table 2). Twenty-one patients (39%) were considered to have normal appearance of the lingual mucosa. Nine patients showed total atrophy and 39 partial atrophy of the taste buds. Forty-six patients had different degrees of hypertrophy of the filiform papillae. Twenty-three patients showed both hypertrophy of the filiform papillae and partial atrophy of the taste buds. There was no correlation among changes of the lingual mucosa, sex, and presence of dentures. Other mucosal changes had a very low incidence. One patient had leukokeratosis nicotina palati, two had lichen planus, and one had leukoplakia.

Microbiologic examination

S. aureus was present in four patients. Three had denture stomatitis (NII) simultaneously (Table 3). One patient with lung cancer showed heavy growth of *S. aureus* from the palatal mucosa, the denture, and the angles of the mouth. In two patients *S. aureus* were isolated from angular cheilitis lesions. One of the healthy patients showed sparse growth of *S. aureus* in the samples from the denture and from the angles of the mouth. No association was found between *S. aureus* and the status of lingual mucosa. Only one patient of the 100 examined showed growth of enteric rods—a healthy dentulous patient with sparse growth of *E. coli* in the angles of the mouth.

In the 75 denture wearers the frequency of *C. albicans* was 40%. *C. albicans* was not found in any of the samples from the palatal mucosa in the group of patients with their own teeth and no removable dentures. In healthy denture wearers *C. albicans* was found in 9 of 36 and only in the samples from the fitting surface of the denture (Table 4). In the 39 patients with inflammatory reactions in the palatal mucosa, a high frequency of *C.*

Table 3. Microbial findings (%) in 75 denture wearers and 2 dentate patients with angular cheilitis on the palatal mucosa and/or fitting surface of the dentures in relation to angular cheilitis and stomatitis

Site	Microbial finding	Angular cheilitis*† (n = 10)	Healthy angulus oris and stomatitis (*n* = 31)	Healthy angles of the mouth and no stomatitis (*n* = 36)
Palatal mucosa and/or fitting surface of the denture	*Staphylococcus aureus*	10	0	3
	Enteric rods	0	0	0
	Candida albicans	80	74	25
	No finding	10	26	72
Angulus oris	*S. aureus*	30	0	3
	Enteric rods	0	0	0
	C. albicans	50‡§	10	5
	No finding	30	90	92

* Including two subjects with their own teeth, no removable prothesis, and no intraoral lesions. *Candida* was not found intraorally. The others all had stomatitis.

† The microbial findings in angles of the mouth were in two cases quantitated as moderate and in the others as sparse.

‡ *C. albicans* and *S. aureus* were obtained concomitantly in one case.

§ Three subjects, all classified as Newton type II, showed *Candida* on the palatal mucosa and/or fitting surface of the dentures.

albicans was seen in the samples from the mucosa (28%) and the fitting surface of the dentures (74%) (Table 4). *C. albicans* was found in samples from both locations in nine patients. In three cases the quantity of *C. albicans* in the denture was recorded as heavy. In 10 patients *C. albicans* occurred in the samples from the angle of the mouth (Table 3). Five of these showed angular cheilitis. The remaining five patients with *C. albicans* had this microorganism also in at least one of the samples taken intraorally, of which three concomitantly had denture stomatitis (Table 3). *C. glabrata* was the only fungal species that occurred besides *C. albicans*. In the samples taken intraorally *C. glabrata* was found in four cases, all showing denture stomatitis corresponding to Newton type II. *C. glabrata* was isolated simultaneously with *C. albicans* in three of four cases.

Gram-negative anaerobic rods—that is, *Prevotella* and *Fusobacterium*—occurred in the predominant flora of 19 patients in the samples from the palatal mucosa. In nine cases Gram-negative anaerobic rods were found in the samples from both the mucosa and the fitting surface of the dentures. All these patients had complete or extensive partial upper dentures. Gram-negative anaerobic rods were present in angulus oris in five patients, three with their own teeth without removable prosthesis and two with complete dentures. Gram-negative anaerobic rods were registered in the predominant flora only simultaneously with *S. aureus* or *C. albicans* and stomatitis ($p < 0.05$).

Anemia, diabetes mellitus, renal disease, hepatic disease, thyroid disease, and data from blood and serum analysis were tested against the prevalence of investigated fungi and bacterial groups in the oral mucosa. Apart from a statistically significant correlation between low S-creatinine level in serum ($p < 0.05$), low blood folate level ($p < 0.05$), and *C. albicans* no associations were found.

The mean value of stimulated salivary secretion rate was 1.05 ± 0.65 ($\bar{x} \pm$ SD) in women and 1.62 ± 0.93 ($\bar{x} \pm$ SD) in men. No correlation was found between microbial findings and salivary secretion rate.

Discussion

A population study of 70-year-old people in Göteborg was carried out, and individuals still alive were reexamined 9 years thereafter. The study comprised an extensive examination of medical, odontologic, socioeconomic, and nutritional variables (16, 17, 19). The prevalence of medical diseases and medication was high in this age group. The present study showed a low prevalence of microorganisms known to cause opportunistic infections—that is, *S. aureus*, *Enterobacteriaceae* species, and *C. albicans*. The presence of these microorganisms could not be correlated to medical diseases, drug intake, or salivary secretion rate. Only one patient, who was severely immunocompromised because of a neoplasia, showed heavy growth of *S. aureus*.

The correlation with low blood folate level might indicate an increased prevalence of fungi and nonoral bacteria among individuals with a poor nutritional state. The lack of associations with the other tested variables may be explained by the fact that the oral functional capacity in general was sufficiently preserved, and signs of serious diseases were uncommon.

In a previous study on the same population group at the age of 70 years (27), a high percentage of oral lesions was found to be related to local factors. This may favor the establishment of microorganisms from the transient flora as secondary invaders, which in turn makes the lesions more severe or compromises the healing (13). However, except for atrophy and hypertrophy of the lingual mucosa, oral mucosal lesions in elderly people without dentures were rare in the present study, and no correlation to the microorganisms could be obtained.

Table 4. Frequency (%) of *Candida* in 79-year-olds with extensive partial or complete upper denture

Sample source	Condition of palatal mucosa			
	Healthy palatal mucosa ($n = 36$)	Newton type I ($n = 21$)	Newton type II ($n = 16$)	Newton type III ($n = 2$)
Palatal mucosa	0	4(19)	7(44)	0
Fitting surface of the dentures	9(25)	16(76)	11(63)	2(100)
Total	9(25)	16(76)*	13(81)†	2(100)

* Presence of *Candida* in healthy palatal mucosa and Newton type I is statistically significant ($p < 0.001$).
† Presence of *Candida* in healthy palatal mucosa and Newton type II is statistically significant ($p < 0.001$).

However, as could be expected from other studies (9, 10), there was a high prevalence of *C. albicans* in the denture wearers with inflammatory changes.

In contrast to the findings of Budtz-Jörgensen (28, 29), who suggested that the localized simple type of inflammatory lesions (Newton type I) probably was a result of trauma, the present study showed a statistically significant relationship with *C. albicans*. Furthermore, the subjects with healthy mucosa and removable upper partial or complete dentures showed the presence of *C. albicans* only in 25% of cases and only in samples taken from the fitting surface of the dentures. The close association between *Candida* and denture wearers contrasted with some recent studies (14, 15). This is explained by the difference in sampling methods and the fact that the dentures were sampled in the present study.

Fungi were the predominant microorganisms in angular cheilitis, which is in agreement with other studies (6, 9, 28, 29). *S. aureus* was present in oral cavity of four patients. Three had generalized denture stomatitis and *C. albicans* as well. The importance of *S. aureus* in different kinds of oral lesions has been shown in several studies (4–6, 28). The frequency of enteric rods was low, which confirms some studies (18, 19) but contrasts with others (4, 7). In the study of Dahlén et al. (4) the patients were all selected by oral clinical symptoms of the mucosa and included a higher frequency of hospitalized and immunocomprised patients. In the study by Slots et al. (7) the high frequency of microorganisms known to cause opportunistic infection was explained by a high antibiotic intake. Only a few persons in the present study used antibiotics.

Gram-negative anaerobic rods were present only in the intraoral samples in patients with removable dentures, which seems predisposing at least to plaque accumulation and a more anaerobic flora in comparison with an uncovered mucosal surface (30). The significance of these bacteria for the development and maintenance of mucosal lesions is not proved. They were always found concomitantly with *C. albicans*, often associated with denture stomatitis. It is likely that these bacteria participate in plaque formation on the dentures and therefore are indicators of poor denture hygiene.

This study has shown the importance of a denture in inducing a shift of the microbial flora towards fungi and Gram-negative anerobic rods. The upper denture and fungi are the predisposing factors for a palatal mucosal inflammation. The amount of *Candida* or the local resistance seems to be the determining factor for the development of the lesions. *S. aureus* or enterobacteria were seen only in a few cases, and an opportunistic infection may be considered when they appear. It is concluded that in the elderly, despite frequent general diseases and medications, there is a low prevalence of *S. aureus* and enterics. The high prevalence of *Candida* in this population group was mainly related to the presence of dentures.

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