Aerobic bacteria in the oral cavity of patients with removable dentures

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Abstract

Purpose: Determination of bacterial composition in the oral cavity of patients with removable dentures and with own dentition (without dentures).

Material and methods: Bacteriological investigations were performed in 55 patients from the department of internal medicine (32 diabetic patients) and 40 patients treated in surgical department (25 patients with malignancy). Palate mucosa and tongue dorsa swabs were collected from two groups of patients, and additionally swabs from mucosal part of denture surfaces in prosthetic patients. Cultures in oxygenic and microaerophilic (5% CO_2) conditions were conducted on solid non-selective and selective media as well as media enriched with 5% sheep blood. Standard procedures of bacterial culture and identification were applied.

Results: Among 95 of examined patients, 57 (60.0%) with removable dentures and 38 (40.0%) had their own dentition. As far as prosthetic patients were concerned, the rate of bacterial isolations from palate, tongue dorsa and denture plaque swabs were generally comparable ($p>0.05$); in number and species compositions. Statistically significant differences were observed in the bacterial composition of denture plaques, palate and tongue dorsa in patients with and without abdominal cancers. Patients without cancer did not reveal staphylococci and enteric bacteria in the samples from a various sites of their oral cavities. These bacteria were most common in cancer patients. Similar (in number and species) composition of bacteria occurred in palate and tongue swabs in patients without dentures ($p>0.05$). The incidence rate of aerobic bacteria in denture plaques and palatal mucosa of patients with (37/57; 64.9%) and without (20/57; 35.1%) denture associated stomatitis were comparable (except for *Neisseria* spp.).

Conclusions: 1) Generally, there were no statistically significant differences in species composition of bacteria isolated from the hard palate and tongue dorsa in patients with and without removable dentures. 2) *Staphylococcus* spp. and Gram-negative enteric bacilli were isolated more often from denture plaque, palate and tongue dorsa of cancer patients than from patients without cancer ($p<0.05$). 3) *Staphylococcus* spp. was isolated more frequently from denture plaques of diabetic patients compared with non-diabetic patients ($p<0.05$). 4) No significant differences observed in isolation frequencies (%) of aerobic bacteria in denture plaques and palatal mucosa of patients with and without denture associated stomatitis.

Key words: bacterial composition, denture plaque, diabetic patients, patients with malignancy, patients with removable dentures, non-denture patients, denture associated stomatitis.

Introduction

Wearing removable dental prosthesis causes an alteration in the oral microflora [1]. For certain individuals, this new environment is responsible for the development of a particular condition: dental prosthetic, stomatitis or denture associated stomatitis.

Denture stomatitis is characterized by mucosal inflammation and redness underneath a denture [2]. It is caused by the microbial biofilm on the fitting surface of the denture rather than on the mucosal surface of, for example, the palate [3].

Denture associated stomatitis (DAS) or chronic atrophic candidosis is one of the most common clinical presentations of oral candidosis [4], affecting 24-60% of otherwise well denture wearers [5]. Nearly 90% of cases are thought to be caused by

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Received 11.05.2006 Accepted 15.05.2006

Table 1. **Isolation frequency (%) of bacteria in hard palate and tongue dorsa of denture wearers and non-denture wearing patients**

Bacterial species	Isolation frequency (%)			
	Denture (n=57)		**No-denture** (n=38)	
	Palate	Tongue	Palate	Tongue
Gram-positive bacteria				
Gram-positive cocci				
Staphylococcus spp.	21.1	22.8	21.1	34.2
S. aureus	8.8	8.8	0	5.3
Coagulase-negative	12.3	14.0	21.1	28.9
Streptococcus spp.	93.1	91.2	97.4	89.5
S. mitis	15.8	19.3	23.7	26.3
S. sanguis	15.8	22.8	21.1	13.2
S. oralis	8.8	8.8	7.9	10.5
S. salivarius	24.6	21.1	23.7	13.2
S. vestibularis	26.3	14.0	18.4	10.5
S. anginosus	1.8	1.8	2.6	5.3
S. intermedius	0	3.5	0	10.5
Gram-negative bacteria				
Gram-negative cocci				
Neisseria spp.	71.9	73.7	65.8	78.9
N. mucosa	8.8	12.3	13.2	7.9
N. sicca	14.0	17.5	13.2	18.4
N. subflava	28.1	26.3	23.7	34.2
N. flavescens	21.1	17.5	15.8	18.4
Gram-negative rods				
Haemophilus parainfluenzae	10.5	26.3	13.2	13.2
Enterobacteriaceae spp.	17.5	12.3	5.3	10.5
Escherichia coli	3.5	1.8	2.6	2.6
Klebsiella pneumoniae	8.8	7.0	2.6	5.3
Morganella morganii	0	0	0	2.6
Enterobacter cloacae	3.5	1.8	0	0
Citrobacter freundii	1.8	1.8	0	0
Pseudomonas aeruginosa	8.8	7.0	2.6	5.3
Acinetobacter spp.	0	0	2.6	2.6

yeasts [3,6], typically *Candida albicans*, although lesions have also been associated with variety of other *Candida* spp. [4-7] as well as bacteria from several genera [2,4,8,9]. Hence, the aim of the present study was to determine the bacterial composition in the oral cavity of patients with removable dentures and with own dentition (without dentures).

Material and methods

Bacteriological investigations were performed in 55 patients (24 male and 31 female) from the Department of Endocrinology, Diabetology and Internal Medicine (32 diabetic patients) and 40 patients (18 male and 22 female) treated in the II Department of General Surgery and Gastroenterology (25 abdominal cancer patients). Palate mucosa and tongue dorsa swabs and additionally swabs of mucosal part of denture surfaces in prosthetic patients were collected. Cultures in oxygenic and microaerophilic (5% CO_2) conditions were conducted on agar non-selective and selective media as well as media enriched with 5% sheep blood. Standard procedures of culture bacteria and identification were applied [10,11].

The Chi-squared test with Yates correction was used for analysis. Significance was established at 5% ($p \leq 0.05$).

Approval for the study protocol was obtained from the Ethics Committee of the Medical University of Białystok.

Results

Tab. 1 presents the results of bacterial composition isolated from the hard palate and dorsum of tongue in 57 denture wearer patients aged 40-83 (mean 66.4 ±11.7) and 38 patient non- wearing dentures, 19-76-year-old (mean 49.9 ±16.0). Isolation frequency (%) in relation to number of patients in the two groups was comparable for all Gram-positive and Gram-negative bacteria with the exception of *Enterobacter cloacae* and *Citrobacter freundii*. These species were isolated only from patients without dentures. No growth of *Streptococcus intermedius* species observed in the mucosal palate swabs of the patients in both groups (*Tab. 1*). Significant difference in the growth rate was seen only for *Haemophilus parainfluenzae*, which isolated most frequently from the tongue (15/57; 26.3%) as compared to palate mucosa (6/57; 10.5%) ($p<0.05$); in patients with dentures.

Table 2. **Isolation frequency (%) of bacteria in denture plaque according to clinical status of patients**

Bacterial species	Isolation frequency (%)			
	Cancer patients (n=16)	Non-cancer patients (n=6)	Diabetic patients (n=19)	Non-diabetic patients (n=16)
Gram-positive bacteria				
Gram-positive cocci				
Staphylococcus spp.	56.3	0	52.6	6.3
S. aureus	37.5	0	0	0
Coagulase-negative	18.8	0	52.6	6.3
Streptococcus spp.	81.3	100.0	84.2	100.0
S. mitis	25.0	16.7	21.0	18.8
S. sanguis	18.8	33.4	10.5	18.8
S. oralis	6.3	0	5.3	0
S. salivarius	12.5	33.4	15.8	37.5
S. vestibularis	18.8	16.7	31.6	25.0
Gram-negative bacteria				
Gram-negative cocci				
Neisseria spp.	25.0	33.4	57.9	62.5
N. mucosa	12.5	0	0	12.5
N. sicca	0	16.7	10.5	12.5
N. subflava	12.5	16.7	31.8	37.5
N. flavescens	0	0	15.8	0
Gram-negative rods				
Haemophilus parainfluenzae	6.3	33.4	5.3	18.8
Enterobacteriaceae spp.	43.8	0	15.8	25.0
Escherichia coli	6.3	0	15.8	6.3
Klebsiella pneumoniae	31.3	0	0	12.5
Citrobacter freundii	6.3	0	0	0
Enterobacter cloacae	0	0	0	6.3
Pseudomonas aeruginosa	18.8	16.7	0	6.3

The aerobic bacteria were isolated with the same frequencies ($p>0.05$) from the oral cavity of patients with and without dentures. Gram-positive cocci (*Staphylococcus* spp. and *Streptococcus* spp.) more often isolated than Gram-negative cocci (*Neisseria* spp.) from both the palate and tongue of patients with and without dentures ($p<0.05$). Among *Streptococcus* spp., *S. mitis* species predominate in the tongue and *S. salivarius* and *S. vestibularis* in the palate of both the groups of patients ($p<0.05$). Bacteria isolated from denture plaques were frequently equal to that isolated from the palate mucosa of 57 patients with dental prosthetic (see *Tab. 1*), with the exception of *Neisseria* spp. which isolated more often from the palate (41/57; 71.9%) than from denture plaque (27/57; 47.4%) ($p=0.05$).

Thorough analysis of isolation frequency of different bacterial species from denture plaques in diabetic and non-diabetic patients, and in patients with and without gastrointestinal cancer shown in *Tab. 2*. Significant difference seen in the composition of bacteria isolated from the denture plaques in patients with and without gastrointestinal cancer. Either no staphylococci nor enteric bacilli isolated from patients without cancer. These bacteria were most frequently isolated from patients with cancer (*Tab. 2*). *Staphylococcus* spp. were more frequently isolated from diabetic patients (10/19; 52.6%) compared to non-diabetic patients (1/16; 6.3%) ($p=0.0032$).

The denture associated stomatitis was observed in 37/57 (64.9%) patients with dental prosthetics. Whole analysis of bacterial composition concerned the number and percent of isolated bacteria from palatal mucosa and denture plaques show no significant difference in patient with and without denture associated stomatitis with the exception of *Neisseria* spp. (*Tab. 3*). *Neisseria* spp. strains more frequent were isolated from palate (73.0%) than denture plaque (51.4%) ($p<0.05$).

Candida albicans was also isolated in 29/37 (78.4%) patients with denture associated stomatitis (data not shown). Among 8/37 (21.6%) patients with denture stomatitis without *Candida albicans*, *Streptococcus* spp. (7/8; 87.5%), *Neisseria* spp. (5/8; 62.5%) and only one species of each (12.5%) *Staphylococcus aureus*, *S. epidermidis*, *Haemophilus parainfluenzae* and *Pseudomonas aeruginosa* were detected.

Discussion

A significant proportion of the adult population wears complete or partial dentures. The factors associated with tooth loss-dental caries, loss of periodontal support, a history of dentoalveolar trauma, a history of dental care – are additive over time, thus denture wearing is more associated with an older population [12]. The oral conditions particularly associated with the wearing of dentures is denture associated stomatitis (DAS)

Table 3. **Incidence rate of aerobic bacteria in denture plaque and palatal mucosa of patients with denture associated stomatitis**

	Number and (%) of bacterial strains			
	Stomatitis (37)		**No-stomatitis** (20)	
	Denture	Palate	Denture	Palate
Gram-positive bacteria				
Staphylococcus spp.	14 (37.8)	9 (24.3)	6 (30.0)	3 (15.0)
Streptococcus spp.	34 (91.9)	34 (91.9)	17 (85.0)	19 (95.0)
Gram-negative bacteria				
Neisseria spp.	*19 (51.4)	*27 (73.0)	8 (40.0)	14 (70.0)
Haemophilus parainfluenzae	5 (13.5)	4 (10.8)	2 (10.0)	2 (10.0)
Enterobacteriaceae	8 (21.6)	5 (13.5)	6 (30.0)	5 (25.0)
Pseudomonas aeruginosa	5 (13.5)	4 (10.8)	1 (5.0)	1 (5.0)

*$p<0.05$

[1-8] or denture related stomatitis [13]. According to Nikawa et al. [13], the term „denture related stomatitis" would be preferable to „denture induced stomatitis" [9,12], since the inflammation of (palatal) mucosa is not induced by the denture, but by wearing the denture or by plaque on the denture. The term "plaque on denture" should by used instead of the term "denture plaque" which used throughout the literature [2,4,12,13], because the microbial flora and its pathogenicity of denture plaque resembles those of plaque formed on the tooth surface, so called dental plaque [13].

Denture plaque has not been studied to the same extent as dental plaque, and although there are many similarities in microbial composition, there are some significant differences [12]. There have been relatively few studies on denture plaque microbiology, the bulk being carried out in the 1980s. Images of denture plaque from the upper fitting surface reveal the presence of a pellicle, a typical biofilm morphology of columnar microcolonies, surmounted by occasional epithelial cells from the maxilla [12,14]. The composition has been deemed similar to dental plaque particularly that on the occlusal surfaces [12,15].

Recent studies are few [12,16]. In published works, the isolates obtained are often a direct consequence of the isolation media used. Many publications focus on *Candida* [12,13]. Thus other groups of organism may be overlooked. This is particularly true for the obligate anaerobes, which are important if oral malodour is the focus of a study [12,16,17].

Some microorganisms which are unusual in the oral microbiota but have been isolated from dentures include respiratory pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *H. parainfluenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* [16-20]. In some studies, 48% of dentures sampled harboured members of *Enterobacteriaceae* [19,20]. Inhalation pneumonia is a common cause of death amongst the elderly debilitated, thus the role of the denture in harbouring such potential pathogens may be significant.

A variety of potential respiratory pathogens had colonized the dentures of our examined patients, the predominant one being *Staphylococcus* spp. (35.1%), among them *S. aureus* contribute to 30.0%. The other putative respiratory pathogens were as follows: *H. parainfluenzae* and *K. pneumoniae* (12.3% each), *S. aureus* (10.5%), *E. coli* and *P. aeruginosa* (8.8% each) and *Enterobacter cloacae* (1.8%). However, no *H. influenzae*, *S. pneumoniae* or *Proteus* spp. were detected. Potential respiratory pathogens were found to have colonized the denture surfaces in 13/57 (22.8%) of the patients.

Our study demonstrated that *Staphylococcus* spp. was isolated more frequently from denture plaques of diabetic patients (52.6%) compared with non-diabetic patients (6.3%) ($p<0.05$). *Staphylococcus* spp. and Gram-negative enteric bacilli were isolated more often from denture plaque (56.3% and 43.8%, respectively) and also from tongue dorsa (50.0% and 31.2%) and palate mucosa (56.3% and 37.5%) in cancer patients than in non-cancer patients (staphylococci and enteric bacilli were not detected) ($p<0.05$). We are suggesting that denture plaque and tongue dorsa can function as a reservoirs of potential respiratory pathogens.

Sumi et al. [21] concluded that denture plaque can function as a reservoir of potential pathogens to facilitate colonization in the pharynx, and it is suggested that denture hygiene status is a significant factor in promoting pharyngeal bacterial colonization. It has been suggested that the tongue surface may also constitute an additional, and possibly more stable, reservoir of respiratory pathogens [22].

The results of this study showed no significant differences in incidence rate of aerobic bacteria in denture plaques and palatal mucosa of patients with and without denture associated stomatitis (DAS). Among our wearer patients 64.9% were with DAS. *Candida albicans* was the most common species associated with DAS (78.4%; data not shown). Our previous study showed that DAS was associated with 94% of yeast isolated, while in wearer subjects and healthy adults (without denture) yeasts have been detected in 75% and 41%, respectively [6]. In 8/37 (21.6%) of our patients with DAS free *Candida albicans* we isolated bacteria such as *Staphylococcus aureus*, *S. epidermidis*, *Haemophilus parainfluenzae*, *Pseudomonas aeruginosa*, *Streptococcus* spp. and *Neisseria* spp. In addition to these bacteria *Enterobacteriaceae* with *C. albicans* were isolated from denture and palate of DAS patients and patients with removable dentures without DAS.

Other authors also showed that DAS was caused by extra-oral species, such as *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp. [1,2,8,9,16]. However, only a strong correlation has been shown for *Candida albicans* and *Staphylococcus aureus* [8]. Moreover, *Streptococcus* spp. and several genera

of anaerobic bacteria (e.g. *Veillonella*, *Lactobacillus*, *Prevotella* and *Actinomyces* spp.) have also been isolated from lesions of patients with DAS [4,9].

There have been relatively few studies on mixed bacterial-fungal biofilms generated in vitro [4]. Different microbial species have frequently been reported to be associated with DAS, however the interactions between bacteria and yeast in the oral cavity have been recognised for several years [24]. Interestingly, co-aggregation studies have shown that *C. albicans* colonization can be aided by primary colonizers such as streptococci [25].

Dorko et al. [7] showed *Candida*-associated denture stomatitis demonstrated in 71.25% examined patients with partial or total dentures. Diabetes mellitus, malignant diseases, chemotherapy, radiotherapy, and broad-spectrum antibiotic therapy were identified by the authors [7] as some of the large number of factors predisposing patients to stomatitis prothetica. Shulman et al. [23] showed that denture stomatitis prevalence is associated with the amount of tissue covered by dentures, low vitamin A levels, cigarette smoking, and constant denture wear. These conclusions were found from a large USA probability sample from the National Health and Nutrition Examination Survey, 1988-1994 (NHANES III); oral examinations (without microbiology) were performed on 3450 individuals 18-90+ years of age [23].

Denture associated stomatitis is usually caused by poor denture hygiene [12], although may be worsened by immunosuppression (e.g. HIV disease) [4,12]. The presence of a denture on the oral mucosa by itself alters the local environmental conditions due to the inaccessibility of saliva and lack of mechanical cleaning by the tongue. Hence, dentures act as reservoirs that harbour *Candida* spp. within a mixed species of bacterial biofilm.

In conclusion, denture hygiene is the obvious method for ensuring that the denture remains clean. There are several oral hygiene products available for use by denture wearers.

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