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ORIGINAL ARTICLE

A retrospective study on the microbiology in patients with oral complaints and oral mucosal lesions

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OBJECTIVE: The aim of this study was to microbiologically analyze oral mucosal samples collected during 2 years from patients with oral mucosal complaints.

MATERIALS AND METHODS: Mucosal scraping samples were taken from 297 patients and semiquantified by culture for detection of opportunistic microorganisms e.g. *Staphylococcus aureus*, enterococci, aerobic Gram-negative bacilli (AGNB) and yeasts. Antibiotic susceptibility test was performed.

RESULTS: Altogether 297 patients were sampled (mean age 56.8 ± 20.7). Among the 110 patients with known medical condition, 48 were systemically immunocompromised, 35 had systemic diseases, and 27 had only local oral complaints. Opportunists in moderate growth or more were present commonly in all three groups and most frequent in the immunocompromised patients (66.7%). *Candida* species were the most frequent opportunist (68.8%), however, their level was low and combinations with bacterial opportunists were common (39.6%). All bacterial opportunists tested were antibiotic multiresistant. Follow-up samples were collected in 23 cases out of which seven showed still presence of opportunists in heavy growth despite repeated treatment with ciprofloxacin.

CONCLUSIONS: This study showed a frequent presence of bacterial and fungal opportunists in patients with oral mucosal complaints, which were most common in immunocompromised individuals, however, also frequent in patients with local oral complaints only. Systematic evaluation of different treatment strategies is needed.

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Keywords: *Staphylococcus aureus*; enterococci; enterics; *Candida*; oral mucosal lesions; oral infections

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Oral complaints are frequently occurring in all ages, even if they are more frequent in older ages (Jorge Júnior *et al*, 1991; Thomson *et al*, 1992; Samaranayake *et al*, 1995; Limeback, 1998; Peltola *et al*, 2004). They are more common among women and especially women in the menopause (Scala *et al*, 2003). The complaints consist of burning sensations and pain usually concentrated to the dorsum of the tongue (Samaranayake *et al*, 1989) with a concomitant loss of taste. Objective symptoms are inflammatory lesions, red and white mucosal changes, atrophia (especially of the papilla at the dorsum of the tongue), angular and lip cheilitis (Dahlén, 2009). In the past, many of these symptoms were referred to denture wearing and *Candida* colonization and infection (Dahlén *et al*, 1982; Samaranayake *et al*, 2009). It is still the opinion among many dentists that 'stomatitis' in general is synonymous with candidosis and that anti-fungal therapy is the treatment of choice. Denture wearing is steadily decreasing in most western countries and with that also the occurrence of denture stomatitis. Instead, due to drugs and systemic diseases, the number of immunocompromised patients is increasing especially among the older adults. Even if the lesions and complaints are mainly concentrated on the tongue of these patients, the inflammation and complaints often involves the whole mouth and the term general stomatitis can be used. Those individuals are the targets of classical opportunistic infections many of which are hospital acquired, and diagnosed and treated by the hospital dentist (Samaranayake *et al*, 1984; Wahlin and Holm, 1988; Bergman, 1991; Jobbins *et al*, 1992). The classical opportunistic microorganisms other than *Candida* reported to be present in patients in these studies are *Staphylococcus aureus*, enterococci, aerobic Gram-negative bacilli (AGNB) including *Pseudomonas* spp. and enteric rods e.g. *Escherichia coli*, *Enterobacter* spp. and *Klebsiella* spp. These bacteria are not usually present in the resident flora of the oral cavity. They appear in the resident flora in other body sites e. g the skin (*S. aureus*) or the intestine (enterococci and enteric rods) but are also present as contaminants in the human environment e.g. food, water, pet animals etc. There-

fore, they frequently appear in low amounts in the transient oral flora but sometimes they may be more constantly established (colonized) on the oral surfaces. They do not necessarily cause any harm and these patients are referred to as healthy carriers. If the host suffers from local or general compromising conditions the microbial homeostasis (Dahlén, 2009) prevailing under healthy conditions is disturbed. During such conditions these opportunistic microorganisms are favored and may increase in number and cause infection (classical opportunistic infections). Classical opportunistic infection is an infection caused by pathogens that usually do not cause disease in an individual with healthy immune system. On the other hand infections in many other compromised conditions with a weakened host defense but with an unclear role of the immune system would also be included in the term opportunistic infection. An important distinction between infection and colonization follows by the occurrence of the opportunistic microorganism in the *predominant* flora when infection is likely. Another distinction is whether the patient has symptoms or not. The presence of complaints or lesions of the oral mucosa reflects most likely a mucosal infection. Such infections should be microbiologically diagnosed and treated accordingly against the actual infectious agent. Other cases may start primarily as a mucositis with ulceration due to cytotoxic drugs, virus infection, chemical burns, trauma etc., that may secondary become colonized and infected with bacteria or fungi (Scully *et al*, 2006). Surprisingly, few studies that address the question on microbiological diagnosis in dentistry in general and mucosal lesions in particular are found in the literature (Roy *et al*, 1999; Dahlén, 2006). Therefore our knowledge on mucosal infections that may appear in the oral cavity is poor. Since many years the Laboratory of Oral Microbiology, Sahlgrenska Academy at University of Gothenburg runs a microbiological service for private and hospital dentists as well as for dentists in the public dental health system and university clinics. This paper reports retrospectively the microbiological outcome during 2 years (2006–2007) of the diagnostics of samples sent to this laboratory. These samples were taken from patients with both subjective and objective symptoms from the oral mucosa.

Materials and methods

Patients and samples

Altogether 297 samples from as many patients were sent in, 143 during 2006 and 154 during 2007 (Table 1).

Table 1 Age, gender and total number of samples obtained from patients with oral mucosal lesions during 2006–2007

Variable	*2006*	*2007*	*2006–2007*
Mean age (years ± s.d.)	58.0 ± 20.3	55.8 ± 20.9	56.8 ± 20.7
Females	100 (69.9%)	98 (63.6%)	198 (66.7%)
Males	43 (30.1%)	55 (36.4%)	98 (33.3%)
Total	143	154	297

Table 2 Systemic and local conditions of 110 patients with known medical background and with oral complaints

Patient condition	*Number of patients*
Immuncompromised	48
Transplantation	20
Radiation	9
Leukemia	4
Cancer	14
HIV positive	1
Systemic diseases	35
Diabetes mellitus	4
Cardiovascular diseases	3
Rheumatoid arthritis	2
Mb Chron	2
Chronic obstructive pulmonary disease	2
Bone disease	2
Skin disease	3
Dislabeled, demens	7
'Multi-diseased'	10
Sjögrens syndrome	2
Medically healthy, only oral complaints	27
Total	110

Dentists took the samples mostly from patients living in the western region of Sweden and with a majority coming from dentists working in or close to hospitals. The reason for taking a sample was the patient's complaints or the dentist's clinical diagnosis of a general stomatitis; a not normal appearance of the mucosa or localized white or red mucosal lesions of the mucosa.

The medical background of the patients was only available for 110 patients and is shown in Table 2. Due to the great variation in the medical background, the patients were grouped as (i) immuncompromised, (ii) systemic diseases, and (iii) medically healthy with local complaints. In the immunocompromised group, we included also those that were radiated and HIV positive even if they were not diagnosed as immunocompromised. The site of sampling was given for 254 samples (Table 4). The remaining 43 samples (14.5%) were referred to as unspecified mucosal samples.

All samples were taken as recommended in written instructions from the laboratory. Before sampling, the mouth was rinsed with water. Samples were taken by scraping deep in the mucosa with a sterile Wards carver over an area of ca 20 mm^2 at the site of the lesion, aiming to obtain the microflora present on and within the superficial epithelial layer. The samples were taken at the most inflamed areas of the mucosa. In the case of more general stomatitis, the tongue was sampled. The sample was transferred to a bottle (3.3 ml) with VMGA III transport medium (Möller, 1966 as modified by Dahlén *et al*, 1993) and sent immediately to the Department of Oral Microbiology, Institute of Odontology, Sahlgrenska Academy at University of Gothenburg.

Microbiological processing

The samples reached the laboratory within 24 h. The sample bottles containing the transport medium were warmed to 37°C and shaken with a whirly mixer for 20 s. A volume of 0.1 ml of the sample was placed and

stroked in a standardized fashion on the following plates: One enriched Brucella agar plate (BBL; Microbiological System, Cockeysville, MD, USA) supplemented with 0.3% Bacto-agar (Difco Laboratories, Detroit, MI, USA), 5% defibrinated horse blood, 0.5% hemolyzed human erythrocytes, and 0.5 mg/l of menadione was incubated anaerobically in jars with the hydrogen combustion method (Möller and Möller, 1961) at 37°C for 6–8 days; one blood agar plate (4% Blood Agar Base No.2 CM 271; Oxoid, Basingstoke, UK) with 5% defibrinated horse blood and 0.5% sodium-lactate for incubation in air with 10% CO_2 at 37°C for 2–3 days; one MacConkey agar plate (Phillips and Nash, 1985); one chocolate agar (Phillips and Nash, 1985) for selective culturing of *Haemophilus* spp., incubated in air with 10% CO_2 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, MI, USA) and a vitamin supplement (Isovitalex, 1%, BBL), was incubated at 37°C for 3–5 days. This medium, selective primarily for gonococci and meningococci, was used also because of its selectivity for fungi. Additionally, one Saboraud dextrose agar (Difco) plate with tetrazolium-chloride 1%, was incubated at 25°C for 5–7 days for differentiation of fungi colony types.

The plates were examined for typical colony morphology and were semi-quantified according to a scale previously published (Dahlén *et al*, 1982). Very sparse growth was used for colonies <10, sparse growth for 10–100, moderate growth for 100–1000, heavy growth for 1000–10 000 and very heavy growth for >10 000 colonies. The amount was also compared proportionally with the presence of commensal viridans (alfa) streptococci.

Results

The frequency of samples was 143 and 154 for the 2 years respectively. Mean age of the patients was 58.0 and 55.9 years, and they were predominantly women (69% and 64% respectively) (Table 1). The clinical and microbiological sample profiles for the 2 years were similar and therefore merged in the following result description.

The total number of samples where the opportunistic microorganism was detected are shown in Figure 1 in comparison with the number of samples where they were found in moderate growth or more. It can be noted that the bacterial pathogens occurred commonly in moderate growth or more. *Candida* showed a 70% occurrence, but only 30% were in a moderate growth or more.

Table 2 shows the medical systemic condition for the 110 patients for which this information was available in the study. The group of immunocompromised patients due to chemotherapeutic drugs and/or radiation constituted the largest group, although it can be noted that medical background is highly variable. Twenty-seven (24.4%) patients did not report on any known systemic condition that could have explained a destruction of the microbial homeostasis or development of lesions that lead to mucosal complaints and infection. This group of

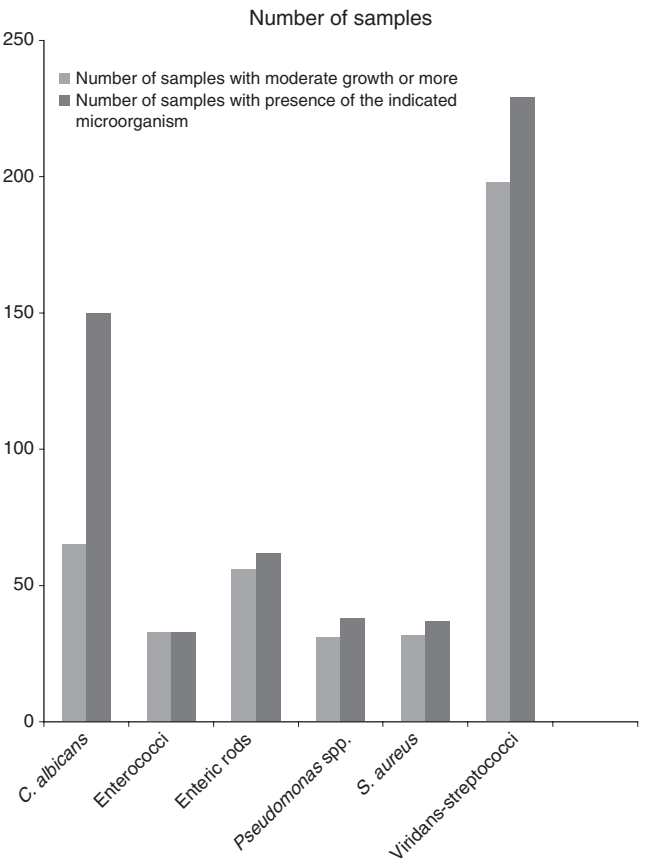

Figure 1 Number of samples with presence and number with high levels (moderate growth or more) in mucosal samples ($n = 297$)

local complaints included patients with burning mouth sensations.

Opportunists (e.g. *Candida* spp., enteric rods, *Pseudomonas* spp., enterococci and *S. aureus*) in moderate and heavy growth were found alone or in combinations in 49.8% of all samples (Table 3). In the group of 110 patients with known medical background this finding was more common among those that were immunocompromised (66.7%) or had systemic diseases (60.0%) than those with local complaints only (40.7%). *Candida* spp. was the most common finding (52.2%) of all 297 samples, whereas enteric rods were found in 22.2%, *Pseudomonas* spp. in 12.8%, enterococci in 11.1% and *S.aureus* in 12.5% of all 297 samples. Only 27.9% of all samples were microbiologically negative with respect to the opportunists. On the other hand viridans-streptococci were found in 76.8% of the samples, which means that in the remaining 69 samples (23.2%) streptococci were not detected and overgrown by the opportunists. In all these cases the opportunists were enteric rods, *Pseudomonas* spp. and enterococci alone or in combinations (data not shown).

The most common sample site was the tongue, followed by an unspecified mucosal sample and samples from the palatum (Table 4). A significant number of samples were also taken from the buccal mucosa, the gingiva, lips and angle of the mouth. The microbiological pattern of opportunists found alone or in various

Table 3 Number (*n*) and frequency (%)[a] of various microorganisms and combinations in relation to the background of the patients from which the samples were taken

Background	*No. samples* n	*Viridans-Streptococci* n *(%)*	*Opportunist negative* n *(%)*	*Opportunists in moderate growth or more* n[b] *(%)*	*Combination of Candida and bacterial opportunists* n *(%)*	Candida spp. n (%)	*Enteric rods*[c] n *(%)*	Pseudomonas *spp.* n (%)	*Entero cocci* n *(%)*	Staphylococcus aureus n *(%)*
Immune Compromised	48	34 (70.8)	8 (16.7)	32 (66.7)	19 (39.6)	33 (68.8)	13 (27.1)	8 (16.7)	11 (22.9)	5 (10.4)
Systemic Diseases	35	28 (80.0)	8 (22.9)	21 (60.0)	11 (31.4)	26 (74.3)	8 (22.9)	6 (17.1)	3 (8.6)	4 (11.4)
Local Complaints	27	20 (74.1)	7 (25.9)	11 (40.7)	5 (18.5)	10 (37.0)	9 (33.3)	3 (11.1)	3 (11.1)	2 (7.4)
Total	110	82 (74.5)	23 (20.9)	64 (58.2)	35 (31.8)	69 (62.7)	30 (27.3)	17 (15.5)	17 (15.5)	11 (10.0)
Remaining samples from patients with unknown systemic background	187	146 (78.1)	60 (32.1)	84 (44.9)	44 (23.5)	86 (46.0)	36 (19.2)	21 (11.2)	16 (8.6)	26 (13.9)
Grand total	297	228 (76.8)	83 (27.9)	148 (49.8)	79 (26.6)	155 (52.2)	66 (22.2)	38 (12.8)	33 (11.1)	37 (12.5)

[a]% denotes percent of total in each row.
[b]43 (20, 6 and 17 resp) samples with opportunists in sparse and very sparse growth are not included.
[c]Including coliforms, *Klebsiella* spp. and *Proteus* sp.

Table 4 Numbers of samples (% of total in each column) with various microbial findings in moderate growth or more in samples from the indicated samples sites

	Total samples	*Viridans-streptococci*	*Opportunist negative samples*	*Opportunist in moderate growth or more*	*Combinations of opportunists*	Candida *spp.*	*Enteric rods*[a]	Pseudomonas *spp.*	*Enterococci*	Staphylococcus aureus
Sample site	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*
Buccal	25 (8.4)	21 (9.2)	8 (12.0)	12 (5.4)	3 (3.8)	10 (6.5)	3 (4.5)	4 (10.5)	0	2 (5.4)
Gingiva	22 (7.4)	19 (8.3)	5 (7.5)	16 (7.2)	3 (3.8)	9 (5.8)	4 (6.1)	4 (10.5)	2 (6.1)	1 (2.7)
Palatum	39 (13.1)	30 (13.1)	8 (12.0)	24 (10.8)	8 (10.3)	20 (12.9)	12 (18.2)	5 (13.2)	3 (9.1)	2 (5.4)
Lips	19 (6.4)	14 (6.1)	3 (4.5)	11 (4.8)	2 (2.7)	11 (7.1)	1 (1.5)	0	1 (3.0)	5 (13.5)
Angle of the mouth	22 (7.4)	12 (5.3)	5 (7.5)	23 (10.3)	8 (10.3)	12 (7.7)	5 (7.6)	2 (5.3)	1 (3.0)	10 (27.0)
Dentures	4 (1.3)	4 (1.8)	0	4 (1.8)	0	4 (2.6)	0	0	0	0
Tongue	123 (41.4)	96 (42.1)	24 (35.8)	92 (41.3)	38 (48.7)	67 (43.2)	28 (42.4)	18 (47.4)	21 (63.6)	11 (30.0)
Mucosa unspecified	43 (14.5)	32 (14.0)	14 (20.9)	41 (18.4)	16 (20.5)	22 (14.2)	13 (19.7)	5 (13.2)	5 (15.2)	6 (16.2)
Total	297	228 (76.8)	67 (22.6)	223 (75.1)	78 (26.3)	155 (52.2)	66 (22.2)	38(12.8)	33 (11.1)	37 (12.5)

[a]Including coliforms, *Klebsiella* spp. and *Proteus* spp.

combinations on the various sample locations was generally the same as found for all 297 samples. The exceptions were the samples from lips and angle of the mouth where *S. aureus* was overrepresented and 40.5% of the positive *S. aureus* samples came from these locations. Viridans-streptococci were detected in 76.8% of the samples, and samples with undetected streptococci were noted for all sample sites.

In addition to viridans-streptococci most samples contained *Haemophilus* spp., and *Neisseria* spp. on smooth mucosal surface (data not shown). In presence of an ulcer, bite marks and always on the dorsum of the tongue the bacterial density was higher and the flora also contained high levels of anaerobes (*Prevotella* spp. and *Fusobacterium* spp.). *Haemophilus influenzae* was detected in only eight cases and usually together with *S. aureus*. Other *Haemophilus* spp. predominantly were *Haemophilus parainfluenzae*. No pathogenic *Neisseria* spp. (e.g. *Neisseria gonorrhoeae*) was found. None of the samples contained hemolytic streptococci (*Streptococcus pyogenes* or Group A streptococci). *Candida* spp. were found in 155 samples when also categories 'sparse' and 'very sparse' growth were included (Tables 3 and 4). The most frequent species were *Candida albicans* (93.5%), *Candida glabrata* (3.2%), *Candida tropicalis* (1.9%), and *Candida krusei* (1.3%). AGNB and enterococci were mostly found on the unspecified mucosal surface, palatum, and tongue. *Candida* spp., AGNB and/or enterococci were often seen in various combinations. Also combination of two different AGNB's e.g. coliforms and *Pseudomonas* were seen.

The result of the antibiotic susceptibility tests is shown in Table 5. It should be noted that susceptibility test was only performed on specific request from the dentist. Nineteen strains of enterics (*E. coli* 16, *Klebsiella* spp. 2 and *Proteus* spp.) showed a high degree of resistance. Ciprofloxacin (12) and cefotaxime (13) were the antibiotics that showed a significant effect (S) on most of the tested 16 coliform strains. Some coliform strains were also susceptible for tobramycin (seven strains) and gentamycin (seven strains). Out of 11 strains of *Pseudomonas* spp, five were sensitive to cefotaxime, nine to ciprofloxacin, five to tobramycin and six to gentamycin. Enterococcal and staphylococcal strains were tested only against eight commonly used antibiotics in dentistry including vancomycin (enterococci for VRE) and methicillin (*S. aureus* for MRSA). All 12 enterococcal strains were resistant against clindamycin, 11 against isoxapenicillin, 10 against doxycycline and nine against erythromycin. No VRE strains were found. Similarly, *S. aureus* showed low susceptibility for penicillin and ampicillin, while the sensitivity was higher for isoxapenicillin and clindamycin. No MRSA strains were detected.

Twenty-three cases were resampled within 3–6 months because the complaints still remained after treatment (Table 6). They had all been treated by the local hygiene measures, rinsing with various antiseptic solutions and systemically with that antibiotic for which the microorganism was susceptible. In case of microbial combinations, the primary treatment was directed against the fungi. No remaining opportunists were detected in 16 cases (Table 6) but showed presence of streptococci indicating that the microbial homeostasis has been restored. While treatment against *Candida*, enterococci and *S. aureus* showed a reduction in most

Table 5 Number of antibiotic susceptible strains of enteric rods, *Pseudomonas* spp., enterococci and *Staphylococcus aureus* isolated from oral mucosal lesions

	Coliforms (n = *16*)			Pseudomonas (n = *11*)			*Enterococci* (n = *12*)			S. aureus (n = *8*)		
Antibiotics	*R*	*I*	*S*	*R*	*I*	*S*	*R*	*I*	*S*	*R*	*I*	*S*
Benzylpenicillin	15		1	11			4	6	2	1	4	3
Fenoxypenicillin	16			11			4	6	2	2	3	3
Isoxapenicillin	15		1	11			11		1	1		7
Ampicillin/amoxicillin	15		1	11			2	5	5		5	3
Metronidazole	16			11			12			8		
Doxycycline	16			11			10	2		2	4	2
Erythromycin	15		1	11			9	3		1	5	2
Clindamycin	16			10	1		12			1		7
Amoxicillin	15		1	11								
Tetracykline	15		1	9	2							
Fusidic acid	16			11								
Cefaclor	13	3		11								
Cefadroxil	15	1		11								
Cefalexin	13	3		9	2							
Cefataxime	2	1	13	4	2	5						
Cefuroxime	9	7		8	1	2						
Ciprofloxacin	0	4	12		2	9						
Tobramycin	6	3	7	4	2	5						
Gentamycin	6	3	7	4	1	6						
Vancomycin									12			
Methicillin												8

R, resistant; I, intermediate susceptible; S, susceptible for serum concentration of each antibiotics.

Table 6 The microbiological outcome of 23 cases after treatment with the antibiotics selected after susceptibility test of the microorganism found in the first sample

Microorganism	*First sample (no. positives)*	*Antibiotics*	*Second sample (no. negatives)*	*Second sample (no. positives)*
Candida albicans	10	Fluconazole	7	7[a]
Enterococci	7	PcV, Amp, Amox	4	3
Staphylococcus aureus	2	IsoxaPc	2	0
coliforms	10	Ciprofloxacin	3	7
Pseudomonas	5	Ciprofloxacin	2	3
Streptococci	1	–	7	16
Total	23	–	16	7

PcV, penicillin V; Amp, ampicillin; Amox, amoxicillin.
[a]Four samples that were *Candida* negative in the first sample showed *Candida* in the second.

cases, such positive effects were noted only sporadically for the AGNB group. Seven samples showed presence of opportunists after treatment, all seven having coliforms in various combinations with fungi, enterococci and *Pseudomonas* spp. They were all treated with ciprofloxacin and in presence of *Candida* spp. also with fluconazole. Four cases were followed with a third and in two cases also with a 4th sample after retreatment with ciprofloxacin. In all these four cases coliforms in combinations with *Pseudomonas*, *Klebsiella* and/or *Candida* were persisting.

Discussion

This report shows that in patients complaining of various kinds of discomfort from the oral mucosa and with objectively detected symptoms such as inflammation and epithelial athrophia, microbiological sampling and analysis, could disclose an infection of classical opportunistic microorganisms in most cases (75.1%). The opportunists included *Candida* spp., *S. aureus*, enterococci and AGNB.

The analysis was performed on samples from patients for which the dentist had difficulties to set the clinical diagnosis and wanted a treatment guidance of the patients. The question raised was if a microbiological diagnosis could answer whether there was an infection or not. This means that a negative sample also gives important information as it will exclude the possibility of bacterial and fungal involvement and suggest the lesions to be of virus or non-infectious origin. Virus infections usually are quite obvious clinically due to typical manifestation often including ulcers. Other reasons for lesions may be trauma (bite marks), allergy, burns or chemicals. The high frequency of medically compromised patients was striking in this study but was of no surprise. It has long been known that cytotoxic drugs induce oral mucosal complaints and infections (Samaranayake *et al*, 1984; Wahlin and Holm, 1988; Bergman, 1991; Jobbins *et al*, 1992; Jacobson *et al*, 1997; Jackson *et al*, 1999; Sheehy *et al*, 2000; Leung *et al*, 2001; Pajukoski *et al*, 2001; Almståhl *et al*, 2008). Other compromising conditions are treatment with antibiotics and/or corticosteroids, xerostomia, disabilities and hospitalization. Often, there is a combination of several factors. Old age is thus not a factor *per se* but in combination with diseases and medication it explains why older people are overrepresented in these kinds of studies. Similarly, women were overrepresented in this study which does not necessarily mean that they suffer more frequently from oral mucosal complaints than men. It may reflect a difference in attitudes to oral health and that women seek treatment more often than men (Helldén *et al*, 1989). The overrepresentation may be explained by the menopause. The burning mouth syndrome is most common in women. Although, this is by definition no infection and the symptoms are not clinically and objectively present (Scala *et al*, 2003), microbiological studies on this patient group have revealed a higher frequency of enterics and yeasts (Samaranayake *et al*, 1989). It should therefore be emphasized that microbiological sampling in this group of patients with little or no clinical signs should be performed as it may disclose subclinical infections or disturbances in the oral microbial ecology.

The detection of opportunistic microorganisms is dependent on where and how the sample is taken. Oral rinse samples or saliva are frequently used and give adequate information in case of general oral complaints and heavy growth of the opportunistic pathogen (Samaranayake *et al*, 1986). In case of more localized lesions and if the pathogen is less abundant or a disturbance of the microbiological ecology should be disclosed, a scraping sample is recommended (Dahlén, 2006). All samples in this study were scraping samples. Scraping is also advantageous in combination with a specimen for microscopic examination for diagnosis of fungal infection. An invasive growth of hyphae into the epithelial layer of the mucosa is a criterium for infection rather than colonization by the fungi and should be treated as such. A disadvantage for scraping samples is that an absolute quantification of the microorganisms is not possible and proportional calculations must be performed. This was performed in this study where findings of streptococci were related to the findings of opportunists. Commensal viridanstreptococci should be present normally on the oral mucosa and, thus, is a marker for the microbial ecology in health. It was quite apparent in this study that in seriously compromised situations (25% of the cases) no streptococci could be detected due to a total overgrowth of the opportunistic microorganism(s). The chemotherapeutics function firstly as a cytotoxic drug against host cells and mucositis may develop due to epithelial destruction

(Scully *et al*, 2006). Secondly, cytotoxic drugs have an antimicrobial effect on some but not all microorganisms why mucosal infection due to overgrowth of resistant microorganisms may be induced (Renard *et al*, 1986). Common opportunistic microorganisms e.g. fungi, *S. aureus*, enterococci and AGNB exhibit a high degree of resistance against many antimicrobials, which explains the high frequency and level in these patients.

The interpretation of the laboratory results is in most cases not very difficult. Presence of the opportunistic pathogens in moderate growth or more should be regarded as infection. *Candida* constitutes a specific problem in this context. The study showed a frequent presence of *Candida* in sparse growth. It cannot be excluded that sparse growth of *Candida* may support the growth of bacteria in the infection. However, it can be questioned what role a limited number of *Candida* cells have in mucosal infections.

A high resistance against many antimicrobials was obvious for opportunistic microorganisms in this study. It illustrates that knowledge of the infectious agent or combinations is necessary to select an antimicrobial that at least have some chance to be effective. Especially for the AGNB group most antibiotics are not effective according to the laboratory susceptibility test. Also, even if ciprofloxacin was indicated to be the drug of choice for most AGNB infections, the repeated sampling showed that the clinical outcome was negative and the patients still had the bacteria in a heavy growth even after three times of ciprofloxacin administration. It should be noted that only four patients were resampled, which does not mean that the rest were successful. On the contrary, discussions with the dentists revealed that the outcome was disappointing for patients under chemotherapeutic treatment and who had an oral mucosal infection with especially AGNB, as long as the cytotoxic medication lasted. Systematic longitudinal evaluation of treatment strategies in this kind of patients is lacking in the literature. Treatment of infections with Gram-positive bacteria (enterococci and *S. aureus*) seems to be more efficient. These infections were less common in the heavy compromised patients than AGNB and more efficient antibiotics are available. *S. aureus* infections were treated generally with isoxapenicillin. No MRSA was involved. Similarly, enterococcal infections were treated with ampicillin/amoxicillin with good result unless they appeared in combination with AGNB. No VRE-isolates were found. Specification of the enterococcal isolates was not performed in this study. However, based on other studies on oral isolates most strains were plausibly *Enterococcus faecalis* even if *Enterococcus faecium* can be observed (Sedgley *et al*, 2004). The latter species is more commonly developing resistance for vancomycin (Linden, 2002). The presence of VRE strains in the clinic is of a general medical concern as vancomycin is regarded as the drug of last resistance for MRSA (Weinberg and Scheinfeld, 2003). A spread of this resistance between Gram-positive bacteria must therefore be prevented. It should also be noted that the frequency of both MRSA and VRE strains in the Swedish population is still low on an international basis, but an alarming increase is reported (Stenhem *et al* 2006). It is a major responsibility among dentists to diagnose and isolate potential infections that are caused by multiresistant microorganisms to increase the knowledge and prevent the spread of these strains in the population.

Despite the retrospective character of this study and lack of information about the patient's general health in most cases, some important conclusions can be drawn. A frequent finding (75.1%) of microbial opportunists in patients with oral complaints, burning sensations and mucosal lesions was found. This report emphasizes the frequent occurrence of oral mucosal bacterial infections along with the even more frequent fungal infections especially in patients that are systemically or locally compromised. It is of outmost importance to distinguish between infections of various microbial origins from non-infectious lesions and treat these conditions with adequate antibiotics or symptomatically. Systematical evaluation of various treatment strategies is needed.

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Author contributions

Dr Dahlén was the main writer of the manuscript and responsible for the microbiological analysis. Dr Blomqvist was responsible for microbiological culture, analysis, data collection and calculations. Dr Carlén was assistant writer and responsible for revisions, also assistant in microbiological analysis and data calculations.

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