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Original article

Denture contamination by yeasts in the elderly

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Denture contamination by yeasts in the elderly

Objectives: The aim of this study was to investigate yeast carriage in healthy denture wearers by swabbing and to evaluate the effect of denture hygiene habits.
Materials and methods: Denture wearers (n = 87) without evidence of denture stomatitis or any other oral disease were investigated by separately swabbing the fitting surface of the upper denture and the corresponding palatal mucosa in contact with the appliance. In a group of volunteers, a gel without any active compound was spread on the palatal side of the denture once in every morning for 2 weeks.
Results: Screening showed *Candida* colonisation of upper prosthesis in 75.9% of individuals. The most frequent species isolated were *Candida albicans* (77.9% of the positive cultures), *Candida glabrata* (44.1%) and *Candida tropicalis* (19.1%). Carriage of more than one yeast species was found in 48.5% of the contaminated dentures. There was a statistically significant association between denture contamination and palatal mucosa colonisation (chi-squared test: $p < 0.0001$). Repeated swabbings after 1 week as well as during a weekly follow-up for 1 month confirmed the denture contamination and its degree of severity. A daily gel application produced a yeast-count decrease to 10% of the initial value after 2 weeks (chi-squared test: $p = 0.0134$ and $p = 0.2841$ for prosthesis and palatal mucosa, respectively).
Conclusion: This study documented the reliability of oral swabbing when investigating yeast carriage in healthy denture wearers. Moreover, just a diagnostic tool, sampling upper dentures for *Candida* could be the opportunity to verify the patient's compliance to hygiene advice.

Keywords: *Candida*, dentures, hygiene, yeast.

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Introduction

Candida species are commensal yeasts isolated from the oral environment in a large proportion of healthy individuals[1–4]. *Candida albicans* is the yeast species most frequently found in isolates; the other yeasts encountered are: *Candida tropicalis*, *Candida krusei*, *Candida guilliermondii*, *Candida stellatoidea*, *Candida parapsilosis* and *Candida glabrata*[5]. Yeast infections often affect the elderly[5–8]. Moreover, some authors[9] have shown a significant association between mouth colonisation and infection by yeasts. Denture wearing and deficient oral hygiene are recognised as two local factors predisposing to *Candida* overgrowth as well as to oral infections[10–13]. *Candida albicans* on denture material is also considered as the major cause of denture-associated oral mucosa erythema[14]. Though *Candida* oral pathologies originate in commensal yeasts, contamination from person to person (elderly patients and healthcare workers) has been demonstrated[15]. Detection of denture contamination by yeasts can thus be useful to promote better oral hygiene. Several sampling methods (mouth rinsing, denture imprint and swabbing) are available to detect oral yeast carriage; swabbing is particularly easy in elderly subjects. The aim of this study in healthy denture wearers was to report mycological data related to the swabbing

procedure, to analyse the variability of results due to the sampling technique itself and to describe the distribution of yeast carriage between the denture and the adjacent mucosa.

Patients and methods

Patients

This study was carried out collaboratively by the departments of Geriatrics and Stomatology in the Brugmann Hospital (Université Libre de Bruxelles, Brussels, Belgium) over a period of 6 months (November 2005–April 2006). A total of 87 healthy denture wearers (61 females and 26 males; median age: 83 years; range: 55–97 years) without evidence of denture stomatitis or any other oral disease were investigated by separately swabbing the fitting surface of the upper denture and the corresponding palatal mucosa in contact with the appliance. Inclusion criterion was wearing a maxillary removable acrylic prosthesis and exclusion criteria were the presence of stomatitis, acute illness, chronic infection and the use of oral hygiene care products with antimicrobials or antifungals. At the point of enrolment in the study and through the investigation, the absence of exposure to medication susceptible to upset the oral microbiological balance was assessed by checking the medical files. During the follow-up time (mainly 1 week, sometimes up to 4 weeks), only one individual developed, 2 weeks after the initial screening, a yeast infection of the mouth, with painful erythema, thrush and glossitis and subsequently received antimycotics: this patient was included for the contaminated-denture rate determination but not for the hygiene follow-up. All subjects or their tutors were informed about the purpose and procedures to be used in this survey and signed an informed consent form; this investigation was approved by the local ethical committee. Medical anamnesis, oral examination and swab samplings of all subjects were conducted by a team of two dentists accompanied by one biologist who organised all the laboratory procedures. The studied denture wearers ($n = 87$) had a mean age of 82.3 ± 8.9 years (median: 83.0 years) with no significant difference (unpaired t-test: $p = 0.0610$; Mann–Whitney test: $p = 0.1753$) between females (83.2 ± 8.5 years; median: 84.0 years) and males (80.3 ± 9.7 years; median: 81.5 years); 92% of the tested population was over 70 years of age. Table 1 documents the prosthetic status of the recruited subjects. The superior removable acrylic denture was complete in 82.8% of cases and partial in others (17.2% of cases), with (10.3% of cases) or without metallic framework (6.9% of all patients). The maxillary denture was free of damage in 53.7% of cases. Among the subjects with a maxillary prosthesis, 78.2% also wore mandibular dentures. Palatal mucosa was healthy in all subjects but a borderline redness of the mucosa (without any complaints or other signs of disease) was observed in 25.3% of cases. During the same period of time, 44 appliance-free young volunteers (26 females and 18 males; median age: 23 years; range: 20–25 years) were investigated by swabbing separately the tongue and the corresponding palate mucosa.

Table 1 Prosthetic status of the investigated edentulous patients.

	All patients (n = 87)	*Yeast positive (n = 66)*	*Yeast negative (n = 21)*
Superior removable prosthesis status			
Partial	15	11	5
Complete	72	55	30
Superior prosthesis damage			
Not damaged (<5 years)	36	25	11
Damaged (>10 years)	51	41	10
Inferior removable prosthesis status			
Absence	19	13	6
Presence	68	53	15
Palatal mucosa aspect			
Healthy	65	49	16
Redness	22	17	5

Sampling

In the elderly group, the presence of yeast was assessed at least two hours after the last meal on both the palatal mucosa and the acrylic surface of the removable prosthesis; positive denture wearers were controlled 1 week later. Moreover, 11 subjects among these (eight females and three males aged from 77 to 94 years; median age: 82 years) were tested during two additional weeks and 10 others (four females and six males aged from 57 to 94 years; median age: 78 years) were submitted to daily care involving the application of a gel without any active compounds against *Candida*: a controlled amount of gel was spread once on the palatal side of the acrylic surface every morning for 2 weeks and a swab sampling of the prosthesis and palate was carried out after the 2-week hygiene care programme in addition to the swabbing performed before the care programme. The gel consisted of a

polyglycerol methacrylate polymer, starch hydrogenated hydrolysate and hydroxyethylcellulose and water. This oral gel was conditioned in monodose bags (5 g/bag); each bag was weighed before and after use in order to control the administered amount of gel. In young people, the presence of yeasts was assessed at least 2 h after the last meal, on the tongue and palatal mucosa using a similar material and procedure for sample collection as in the denture wearers.

Mycological investigations

All swabs were inoculated on CHROMagar plates (BD Diagnostics™, Erembodegem, Belgium) which were incubated for 2 days at 37°C. Cultivatable *Candida* species were then identified from the macroscopic morphology of colonies[16] and from additional tests after subculture on Sabouraud agar with chloramphenicol and gentamycin (BD Diagnostics™). The latter involved namely germ tube formation in human serum, chlamydoconidia formation on rice agar tween (RAT) medium and API™ yeast identification system. Green colonies on CHROMagar with germ tubes and chlamydoconidia formation were considered as *C. albicans*; other strains were identified on the basis of their carbohydrate assimilation pattern using the ID32C API system (bioMérieux™, Marcy-l'Etoile, France). *Candida* insensitivity to oral care gel was assessed *in vitro*. *Candida albicans* ATCC 10231 (Culti-Loops™, Oxoïd™, Basingstoke, UK) was grown at 37°C on Sabouraud–gentamycin–chloramphenicol agar (BD Diagnostics™, Belgium); all the experiments were performed on the third subculture. A yeast suspension in Sabouraud broth (OXOID™ CM147, Basingstoke, UK) was adjusted to 8 blastoconidia/µl. Inocula (20 µl) of the suspension were spread onto the surface of a Sabouraud plate and then covered by 2 g of oral care gel. Colonies were counted after 2 days incubation at 37°C and controls were performed in absence of gel covering.

Statistics

Data were analysed using the GraphPad Prism version 5.00 (GraphPad Software™, San Diego, CA, USA). Paired and unpaired *t*-tests of Student, Wilcoxon signed-rank tests, Mann–Whitney, chi-squared, Fischer's exact tests, risk factor analysis and odds ratio calculations were performed using the same software. Mean values were expressed with their related standard deviation if not indicated otherwise.

Results

Reliability of Candida count from swabs

Successive counts (n = 6) of four different swabbed Petri dishes facilitated calculation of a coefficient of variation, which ranged between 0.27% (mean ± SD: 75 ± 1 colony-forming unit (CFU)/dish) and 1.38% (912 ± 3 CFU/dish). *Candida* counts performed by two independent observers (Fig. 1) provided data that were not statistically different (paired *t*-test of Student: p = 0.7403, n = 8); interobserver difference was 1.0 ± 0.4% for counts ranging between 77 and 948 CFU/dish (n = 6) and 10.2 ± 2.1% for two counts above 2000 CFU/dish (2021 and 2212 CFU/dish). Figure 2a illustrates the repeatability of *Candida* count from two successive swabs of the same material (prosthesis or mucosa): variability

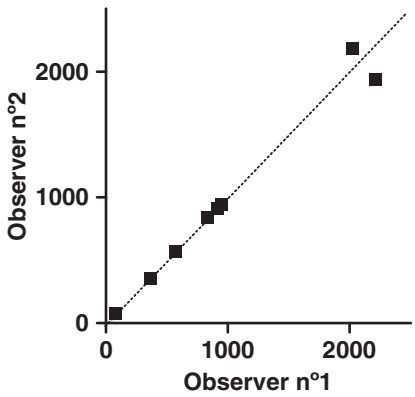

Figure 1 Interobserver correlation for *Candida* counts.

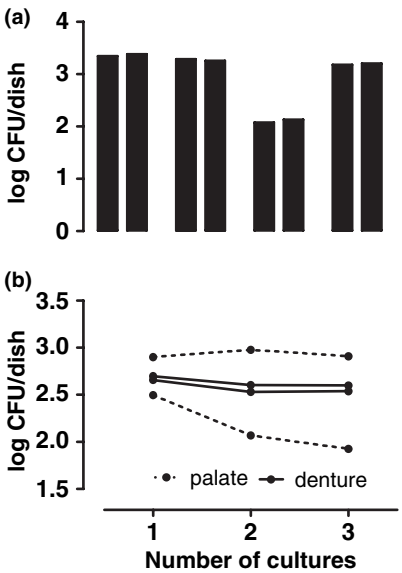

Figure 2 (a) Variability of yeast counts between two successive swabbings. (b) Variability of yeast counts in three different cultures originating from the same swab.

Table 2 Strength of association between palatal mucosa colonisation and maxillary prosthesis contamination.

Maxillary prosthesis contamination	*Palatal mucosa colonisation*	
	Yes	*No*
Yes	61 (a)	5 (b)
No	2 (c)	19 (d)

Relative risk [a/(a + b)]/[c/(c + d)] = 9.705 (relative risk equals 1 in absence of association).
Difference between proportions [(a/(a + b)] – [c/(c + d)] = 82.9% (it equals 0 in absence of association).
Odds ratio (a/b)/(c/d) = 115.9 (odds ratio equals 1 in absence of association).

Table 3 Prevalence of the different species identified in 68 maxillary denture wearers.

	Yeast carrier (n = 68)	
	(Number)	*(%)*
Candida albicans	53	77.9
Candida glabrata	30	44.1
Candida tropicalis	13	19.1
Candida lusitaniae	2	2.9
Candida sake	2	2.9
Candida dattila	1	1.5
Candida kefyr	1	1.5
Candida rugosa	1	1.5
Geotrichum capitum	1	1.5
Saccharomyces cerevisiae	2	2.9

ranged within an interval of 0.1 logarithmic unit. Figure 2b shows the variability range of data for three successive cultures obtained from the same swab: this was inside an interval of 0.5 logarithmic unit.

Mycological data

Swabbing of the palatal side of the prosthesis was yeast-positive in 66 out of 87 subjects (75.9%), while the adjacent mucosal surface was positive in 63 of 87 cases (72.4%); 68.2% of subjects simultaneously showed contamination of both denture and mucosa. The contaminated maxillary dentures were heavily colonised (more than 50 CFU) in 90.9% of cases and more than one yeast species was found in 50.0% of samples. There was a statistically significant association between denture contamination and palatal mucosa colonisation (chi-squared test: $p < 0.0001$): denture wearing was found to be a significant risk factor for yeast colonisation (Table 2). Indeed, 92.4% of contaminated denture wearers also carried *Candida* on their mucosa, while only 9.5% of yeast-free denture wearers were *Candida*-positive on their mucosa: the difference between the proportions was 82.9% (it equals 0 in absence of association). The strength of association was appreciated by calculating the relative risk, with odds ratios of 9.705 and 115.9, respectively. The median value in yeast count ratio for palatal mucosa over denture was 4.8% in subjects wearing heavily contaminated dentures (that is more than 50 CFU and less than 10^4 CFU – a range where this ratio can be calculated). Identified yeast species in isolates are listed in Table 3. Taking all subjects into account, either with denture contamination or with mucosal colonisation, *Candida albicans* was the main species encountered, occurring in 77.9% of yeast-positive subjects. *Candida glabrata* and *Candida tropicalis* were isolated from 44.1 and 19.1% of swabs, respectively. Other *Candida species* (*Candida lusitaniae*, *Candida sake*, *Candida dattila*, *Candida kefyr and Candida rugosa*) and additional (*Geotrichum capitum* and *Saccharomyces cerevisiae*) were detected at a lower rate (1.5–2.9%). An identical species or association was found simultaneously on dentures and palatal mucosa in 95.1% ($n = 61$) of cases: 100.0% of the mono-colonised ($n = 38$) and 87.0% of the pluri-colonised subjects ($n = 23$). In subjects followed for 4 weeks without gel application, one new species appeared during the investigation in two out of 11 cases (18.2%), while one species disappeared in one case (9.1%). Data management of all samples (dentures and palatal mucosa) was conducted separately in patients with presumed (or doubtful) mucosal redness and in patients with an indisputably healthy palatal mucosa. Statistical discrepancy, if any (chi-squared test and Fischer's exact test), could then be demonstrated in terms of overall yeast carriage, *C. albicans* carriage, heavy yeast carrier, heavy *C. albicans* carrier or number of different *species* ($p > 0.05$). In young people without orthodontic appliances ($n = 44$), yeast colonisation of tongue or palatal mucosa was observed in 25.0 and 27.3% of cases, respectively; the only heavy yeast carrier (>50 CFU) admitted the use of antimicrobials during the previous days. The yeast count was always inferior to 10 CFU except in two cases. The tongue and palatal mucosa were simultaneously colonised in 64.3% of yeast-positive volunteers.

Effect of the professional health care

Table 4 shows the results of performing two swabbings of both dentures and palate at an interval of 1 week in 46 subjects. The second yeast count represented 100.0 and 53.2% (median values) of the

Table 4 Comparison between two swabbings performed at a 1-week interval in 46 subjects.

	Number of cases
Yeast count from denture swabs	
Remains in the range 'initial count ± 1 logarithmic unit'	41
Decreases under the limit 'initial value − 1 logarithmic unit'	5
Increases above the limit 'initial value + 1 logarithmic unit'	0
Yeast count from palatal mucosa swabs	
Remains in the range 'initial count ± 1 logarithmic unit'	39
Goes below the limit 'initial value − 1 logarithmic unit'	6
Exceeds the limit 'initial value + 1 logarithmic unit'	1
	Median value
Second over 1st swabbing count ratio from denture	1.00
Second over 1st swabbing count ratio from palatal mucosa	0.53

initial count obtained from prosthesis and palatal mucosa respectively: the Mann–Whitney test ($p = 0.3088$, NS) and the Wilcoxon signed-rank sum test ($p = 1.0000$, NS) did not show any statistically significant discrepancy. Taking into account, the range of ±1 logarithmic unit, 5 out of 46 (10.9%) vs. 6 (13.0%) subjects presented a CFU decrease under the limit 'initial value minus 1 logarithmic unit' from their dentures and palatal mucosa, respectively; only one patient showed an increased yeast count in the second swab from palatal mucosa. These results minimised the hygiene-stimulation effect produced by the investigators themselves.

A group of 11 subjects was followed for two additional weeks. For 10 other denture wearers, an oral gel conditioned in a 5-g bag was spread on the palatal side of the acrylic surface once in every morning for 2 weeks. The administered amount per patient averaged 2.49 ± 0.72 g/day ($n = 143$ weighted bags). This oral gel has been shown ineffective *in vitro* against typed strain *Candida albicans* ATCC 10231. Indeed, the recovery percentage in the presence of the oral gel averaged 110.1 ± 8.6% ($n = 7$) of the CFU count found in absence of gel through seven independent *in vitro* experiments conducted every 2 months during the course of the clinical trial (Table 5). Nevertheless, this gel application protocol was observed to produce a yeast-count reduction to 10% of the initial value for prosthesis contamination after 2 weeks (chi-squared test: $p = 0.0134$), but not for palatal mucosa colonisation (chi-squared test: $p = 0.2841$). Table 6 summarises these data.

Table 5 CFU counts on Sabouraud plates sowed with *Candida albicans* ATCC 10231 and then recovered by an oral gel.

	Oral gel % of control (n)	*Control, i.e. no gel CFU/plate (n)*
Time $_0$	124.8 ± 5.4 (6)	262 ± 8 (6)
Time $_{2\ months}$	101.6 ± 8.0 (6)	63 ± 3 (6)
Time $_{4\ months}$	112.4 ± 9.3 (6)	89 ± 2 (6)
Time $_{6\ months}$	116.0 ± 11.4 (6)	150 ± 12 (6)
Time $_{8\ months}$	100.0 ± 6.6 (6)	86 ± 4 (6)
Time $_{10\ months}$	104.8 ± 5.8 (6)	83 ± 2 (6)
Time $_{12\ months}$	111.1 ± 7.8 (6)	81 ± 5 (6)

Discussion

In order to be able to colonise and infect the oral environment, yeasts must first adhere to oral surfaces (host mucosa or prosthetic materials) or coaggregate with oral bacteria. Initial attachment is followed by proliferation and biofilm formation which enhances yeast resistance to salivary host defences and antimicrobial agents[17]. Roughness of biomaterials such as acrylic polymers and hydrophobicity of yeast cells could explain the high prevalence of *Candida* contamination on dentures[18]. *Candida* detection on upper dentures could be useful to follow the progress of yeast colonisation on the one hand and to investigate the benefit of antifungal prophylaxis by drugs or natural antifungal proteins/systems on the other. In this perspective, the procedures used to evaluate yeast biomass in the oral environment need to be validated and the effect of the investigation design on patients' hygiene must be specified.

Reliability of Candida count from swabs

Procedures to quantify yeast biomass *in vitro* are not applicable *in vivo* for epidemiological studies and hygiene purposes, particularly as denture wearers

Table 6 Palate vs. prosthesis yeast counts: effects of a daily gel application on the palatal surface of dentures for 2 weeks.

	Number of cases	
	gel	*Nil*
Yeast count from denture swabbings		
Remains in the range 'initial count ± 1 logarithmic unit'	3	8
Goes below the limit 'initial value − 1 logarithmic unit'	6	1
Exceeds the limit 'initial value + 1 logarithmic unit'	1	2
Total	10	11
Yeast count from palatal mucosa swabbings		
Remains in the range 'initial count ± 1 logarithmic unit'	5	7
Goes below the limit 'initial value − 1 logarithmic unit'	5	3
Exceeds the limit 'initial value + 1 logarithmic unit'	0	1
Total	10	11

are not always compliant. Yeast extraction from the oral environment can be carried out by rinsing, imprinting or swabbing. Swabs and imprints are more suitable for accessing yeasts adherent to surfaces; swabbing is technically easier for studies on a larger scale. Yeast counts after swab culture reflect the biomass present on the oral surfaces, but the number of yeast cells included in oral biofilms and their extraction by swabbing should be more efficient. Data in Fig. 2 suggested that CFU counted on the agar medium represented only a small part of the cells harvested, as assessed by three successive spreadings of the same cotton that provided similar data in the range interval of 0.1 logarithmic unit. Furthermore, two successive swabs of the same oral surface yielded similar quantities of yeast cells (in the range interval of 0.5 logarithmic unit) indicating that the extracted fungal cells by one swab from the oral surface represented only a small part of the fungal cells present on this surface. This double low extraction rate contributes to decrease the variability due to the sampling itself. Repeated sampling (at an interval of 1 week) of 46 healthy denture wearers showed yeast counts remaining in the same range ±1 logarithmic unit for 89.1% of the denture swabs and 84.7% from the mucosal samples. Exceeding the upper limit (+1 logarithmic unit) was incidental (n = 1 for palate) and moving below the lower limit (−1 logarithmic unit), occurred in 10.9 and 13.0% of denture and mucosa swabs, respectively: this could be attributed to behavioural change in hygiene practice following the first visit.

The palatal vs. denture yeast counts

These findings confirmed that dentures are often contaminated by yeasts (75.9% of 87 prostheses) and that *C. albicans* is the species predominantly found in the mouth. These data confirm several previous studies in hospitalised patients[19], especially those from geriatric units for long-term care[9,11]. Applying the 50-CFU breakpoint for oral swabs taken from dentures, 90.9% of the contaminated subjects were considered heavy carriers. Despite a statistically significant association between denture contamination and palatal mucosa colonisation, the palatal mucosa over denture yeast count ratio showed a preferential attachment of yeasts onto dentures rather than on mucosa. Further investigations on a larger group of denture wearers are needed to ascertain the predictive value of the *Candida* count ratio from the biomaterial surface and adjacent mucosa to develop oral candidosis.

Hygiene effect

A second swabbing taken in 46 patients after an interval of 1 week demonstrated only minor variations, thus minimising the hygiene-stimulation effect produced by the investigators themselves. In contrast, the hygiene programme with an oral gel prescription led to a decrease of yeast carriage after 2 weeks even in the absence of any active compounds in the gel. It could be hypothesised that the gel not only stimulated hygiene practice, but also modified yeast attachment conditions and biofilm formation by changing the physical properties of the potential attachment surfaces and making the support less hydrophobic. Clinical trials testing antifungals (or other antimicrobials) should therefore take these factors into consideration as well as the hygiene effect produced by the investigators' intervention when reporting on the beneficial effects of oral gels.

Conclusions

This study documented the reliability of oral swabbing when investigating yeast carriage in healthy denture wearers. Sampling upper dentures for *Candida* could be more than just a diagnostic tool and provide the opportunity to verify the patient's compliance to hygiene advice as well as the efficiency of new topical antifungals. The influence of the investigators on the hygiene behaviour and the galenic formulation of products, without any active antimicrobial, are the two important biases that have to be taken into account when evaluating oral health care products.

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