LETTER TO THE EDITOR

OCCURRENCE OF *CANDIDA* SPECIES COLONIZATION IN A POPULATION OF DENTURE-WEARING IMMIGRANTS

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Infection of the oral cavity and dentures by *Candida* species are frequent in denture wearers. *C. albicans* is the most common pathogen; however, other emerging *Candida* species are also responsible for this condition. Few data are available about the occurrence of *Candida* species in the oral cavities of denture-wearing immigrants to Italy. In this study, we compare the *Candida* species found in the oral mucosa and on dentures from a population of denture wearing immigrants to Italy to a matched Italian group. Oral swabs were collected from dentures and the underlying mucosa of patients enrolled in the study and were then cultured to test for the presence of *Candida* species in each sample. Out of 168 patients enrolled (73 Italians and 95 immigrants), 51 Italians (69.8%) and 75 immigrants (78.9%) tested positive for the presence of *Candida*. *Candida albicans* was the most frequently observed species overall; however, we found a higher occurrence of *C. glabrata* among immigrants than among Italians. In addition, immigrants displayed a higher incidence of *Candida* – associated stomatitis and a lower mean age than *Candida*-positive individuals from the Italian group. Immigrants are more prone to longer colonization of the oral mucosa and dentures by *Candida*. In these patients, dentures must be checked periodically to prevent the presence of *Candida*.

The alterations of the oral mucosa by dentures might result from mechanical irritation or inflammatory responses induced by denture materials (1, 2). In addition, biofilm formation on the denture surface might contribute to the altered nature of the oral micro-environment among denture wearers (3). *Candida* species, which comprise 25–50% of the oral cavity microbiota from healthy individuals and roughly 80% from denture wearers, are a primary cause of microbial biofilm formation on medical devices (1).

Until recently, *Candida albicans* was considered the most important opportunistic pathogen in this genus. However, other *Candida* species, such as *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis*, have also emerged as causative agents of infection (1).

Candida glabrata is an emerging fungal pathogen that accounts for 15% of mucosal and systemic candidoses and is associated with severe

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inflammation in denture wearers (1).

Candida dubliniensis has recently been isolated from the oral cavity of human immunodeficiency virus (HIV)-infected patients, leading to its consideration as a novel, emerging, opportunistic pathogen (4). *Candida* colonization and biofilm formation on dentures may depend on oral hygiene practices, such as overnight denture removal, denture cleanser use, smoking and specific denture characteristics (5).

Denture-related stomatitis (DRS) is an inflammatory process of the mucosa underlying a removable partial or total dental prosthesis or appliance (6). DRS has been reported in more than 60% of denture wearers, and although it is typically asymptomatic, it occasionally associates with leukoplakia, pseudomembrane formation, erythema and angular cheilitis (4, 6, 7).

Key factors that can dramatically increase the risk of DRS are loose denture fit, poor denture hygiene and *Candida albicans* colonization of the denture surface and oral mucosa that contact the denture fitting surfaces (5). Denture materials themselves can contribute to the risk of denture stomatitis, as areas of surface roughness and the hydrophobicity of denture surfaces can promote the attachment of microorganisms and biofilm development (8, 9).

The pathogenesis of the *Candida*-associated denture stomatitis includes local and systemic factors related to the host and to the ability of *Candida* to adhere and proliferate in the host epithelial tissues (8). *Candida*-associated denture stomatitis usually occurs when conditions of the oral micro-environment are favorable for the growth and the adhesion of yeast and when systemic factors lead to a systemic immunodepression (10-12).

To date, no data are available about the occurrence of *Candida* species in the oral cavity of denture-wearing immigrants to Italy. In this study, we compared the colonization of the oral mucosa and dentures from a population of denture-wearing immigrants to Italy with a matched Italian group.

MATERIALS AND METHODS

Patients

All denture-wearing patients attending the outpatient department of Social Dentistry Department at National Institutes of Health, Migration and Poverty during the period of June 2011-June 2012 were enrolled. A complete medical and dental history was recorded for each patient, including age, gender, drug use, smoking habits, systemic diseases, DRS symptoms and hygienic habits (modality and frequency) (13).

Patients receiving antifungal or antibacterial treatment within 30 days of enrollment were excluded from the study as well as patients who had undergone immunosuppressive therapies or were affected by an immunosuppressive disease (diabetes, kidney failure, HIV infection). All patients affected with xerostosmy of idiopatic or iatrogenic origin were also excluded.

To identify and characterize the different presentations of DRS, we used the Newton Classification described by Budtz–Jorgensen and Bertram (14):

DRS type I – localised inflammation or hyperaemia points (pin point hyperaemia).

DRS type II – diffuse erythema.

DRS type III – pseudomembrane formation.

Sample collection and isolation

After examination of the oral cavity, oral swabs were collected from the denture of each patient and from the underlying mucosa according to the procedure described by Marcos-Arias et al. (15). When denture-related stomatitis lesions were evident, a specimen was collected from the lesion. All oral swabs were cultured within 2 h of collection on CHROMagar *Candida* medium (Becton Dickinson GmbH, Germany) as well as on Sabouraud dextrose agar plates containing chloramphenicol (Becton Dickinson GmbH, Germany) and were incubated at 37° C for 48 h. We considered a *Candida*-associated denture stomatitis to be an isolation of > 10 *Candida* colonies (16).

The plates were scored based on the number of colonies and then subcultured on the same chromogenic medium and Sabouraud dextrose agar to obtain pure cultures.

Characterization of Candida species

Isolates were identified by conventional mycological methods such as color formation in CHROMagar *Candida* medium, germ tube tests in calf serum at 37°C for 2 days, and microscopic morphology. Additionally, all yeast identified as *Candida albicans* were screened for their ability to grow at 45°C on Sabouraud dextrose agar for 3 days and for chlamydoconidia formation on Casein agar at 30°C for 10 days (16).

In these cases, isolates highly suspected to be *Candida dublinensis* were definitively identified using polymerase chain reaction (PCR). PCR identification of *Candida dublinensis* with the *Candida dublinensis*-specific primer pair DUBF and DUBR (17) was carried out in a 50-µl final volume containing 10 pmol each of the forward and reverse primers, 2.5 mM MgCl2, 10 mM Tris-HCl (pH 9.0 at 25°C), 10 mM KCl, 0.1% (vol/vol) Triton X-100, 2.5 U

of Taq DNA polymerase (Promega), and 25 µl of template DNA-containing cell supernatant. Cycling conditions consisted of 6 min at 95°C, followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C, followed by 72°C for 10 min. *Candida* template DNA for use in PCR experiments with the *Candida dubliniensis*-specific primer pair DUBF (5'GTATTTGTCGTTCCCCTTTC-3') and DUBR (5'-GTGTTGTGTGCACTAACGTC-3') was prepared as described by Donnelly et al. (17).

Statistical analysis

For the statistical analysis, SPSS software vers.13 was used (IBM, Armonk, NY, USA). A $p < 0.05$ was considered significant.

RESULTS

We enrolled a total of 190 patients with removable dentures, and 22 were excluded from the study for the following reasons: 5 were affected with diabetes mellitus, 1 was affected with kidney failure, 11 were suffering from xerostomy (2 because of Sjogren syndrome and 9 as consequence of anti-hypertensive or anti-depressive therapy) and 5 had received antimycotic or antibiotic therapy within 30 days of enrollment.

Of the remaining 168 patients, 73 were Italians (described as group 1) and 95 were immigrants (described as group 2), who were defined as people born in a country other than Italy that came to Italy within three years of enrollment in the study.

The demographics of the patients in group 1 (Italian patients) and group 2 (immigrant patients) are listed in Table I.

In total, 51 Italians (69.8%) and 75 immigrants (78.9%) tested positive for contamination by *Candida* species ($p=0.12$, *chi*-squared test). The patients displaying *Candida*-colonization of the oral mucosa were younger in group 2 than group 1 (71.5±11 *vs* 49.3±11.2 years ± ds $p<0.05$, Student's *t*-test).

Italian patients colonized by *Candida* species other than albicans had a younger mean age than those colonized by *C. albicans* (58±8.1 *vs* 75±9.2 $p<0.05$ *chi*-squared test). Fig.1 shows the percentage of patients positive for the colonization of dentures and oral mucosa, divided according to *Candida* species. Table II shows the frequency of *Candida* species isolated from Italian patients (group 1) and immigrant patients (group 2) based on culture and molecular methods. The most frequently isolated species from oral mucosa and dentures from group 1 were *Candida albicans* 33 (64.7%), *Candida glabrata* 5 (9.8%), *Candida dubliniensis* 2 (3.9%), and *Candida tropicalis* 2 (3.9%). From group 2, the most frequently isolated species were *Candida albicans* 52 (69.3%), *Candida glabrata* 10 (13.3%), *Candida dubliniensis* 1 (1.3%), *Candida tropicalis* 1 (1.3%) and *Candida krusei* 1 (1.3%).

Simultaneous colonization by two yeast species was found in 6 (11.7%) oral mucosa and denture samples from patients in group 1, particularly *Candida albicans* and *Candida krusei* 4 (7.8%), *Candida albicans* and *Candida parapsilosis* 1 (1.9%) and *Candida albicans* and *Candida tropicalis* 1 (1.9%). In group 2, we found simultaneous colonization in 13 (17.3%) oral mucosa and denture samples, particularly *Candida albicans* and *Candida glabrata* 5 (6.6%), *Candida albicans* and *Candida krusei* 4 (5.3%) and *Candida albicans* and *Candida tropicalis* 4 (5.3%).

The frequency of *Candida albicans* colonization

Table I. *Demographic characteristic of Italian (group 1) and immigrant (group 2) patients.*

	Group 1	**Group 2**
Gender (F:M)	39:34	40:55
Mean age	69.2 (range 43-86)	49.8 (range 22-68)

Table II. *Presence of different species of Candida in positive oral and denture specimens in Italian (group 1) and immigrant (group 2) patients.*

Yeast growth	*Group 1*	*Group 2*
C. albicans	33 (64.7%)	52 (69.3%)
C. glabrata	5 (9.8%)	10 (13.3%)
C. dubliniensis	2 (3.9%)	1 (1.3%)
C. tropicalis	2 (3.9%)	1 (1.3%)
C. krusei	0 (0%)	1 (1.3%)
C. albicans + C. krusei	4 (7.8%)	4 (5.3%)
C. albicans + C. glabrata	0 (0%)	5 (6.6%)
C. albicans + C. parapsilosis	1 (1.9%)	0 (0%)
C. albicans + C. tropicalis	1 (1.9%)	4 (5.3%)
Total	**51**	**75**

Table III. *Gravity of lesion of oral mucosa in patients of our series resulted positive to colonization by other species than Candida albicans alone and in association.*

Other than Candida Albicans		
lesion gravity	**Italians**	**Immigrants**
I type	4	2
II + III type	4+1	5+6*
Association		
lesion gravity	**Italians**	**Immigrants**
I type	5	3*
II + III type	2+2	3+4*

.* *p < 0.05 calculated by chi square.*

of the oral mucosa and dentures was significantly ($p<0.05$) higher than that of other *Candida* species in both groups 1 and 2. The colonization by *Candida glabrata* was more frequent among patients from group 2 than those from group 1 ($p<0.05$).

Table III shows the severity of oral lesions (according to the classification in degree 1-3 $p< 0.05$) observed from the two groups of patients. Immigrants whose dentures and oral mucosa tested positive for *Candida* species other than *Candida*, both alone and in association, displayed oral lesions that were clinically more severe (type II and III)

Table IV. *Denture soaking and brushing habits (Panel A) and frequency of denture cleaning (Panel B) in Italians (group 1) and immigrants (group 2).*

A

	Group 1		Group 2	
	POS n=51	NEG n=22	POS n=75	NEG n=20
Brushing only	10	5	20	5
Soaking in solution only	14	5	24	7
Soaking in water only	13	7	13	5
Brushing and soaking	13	4	16	2
Nothing	2	1	2	1

B

	Group 1		Group 2	
	POS n=51	NEG n=22	POS n=75	NEG n=20
< 1 time / day	4	4	49	10
1 time / day	13	7	14	5
2 or > times / day	34	11	12	5

than those of Italian denture wearers with the same colonization.

In Table IV (A and B), we describe the oral and denture hygienic habits of the patients included in the study. No difference existed between the groups of patients divided according to the type and frequency of oral and denture cleaning (test U, p=0.16) and the colonization by *Candida* species (Mann Whitney, p= 0.25).

DISCUSSION

Our results show that the mean age of enrolled patients in group 2 was significantly lower than that of patients in group 1. One explanation for this finding is that, generally, immigrant populations primarily consist of young individuals. Despite their younger age, the immigrant group had severe dental problems that could lead to tooth loss and denture placement. Early denture placement in immigrant patients may cause premature, chronic colonization of the denture by *Candida* species and an early onset of denture stomatitis.

Our data corroborated that *Candida albicans* is the most frequently isolated species, followed by *Candida glabrata, Candida dubliniensis* and *Candida tropicalis* in the oral mucosa as well as on dentures in both group 1 and group 2. We also reported, in accordance with previous studies, that among non-*albicans Candida* species, the most frequent yeast isolated from the oral mucosa and dentures is *Candida glabrata*; however, we newly

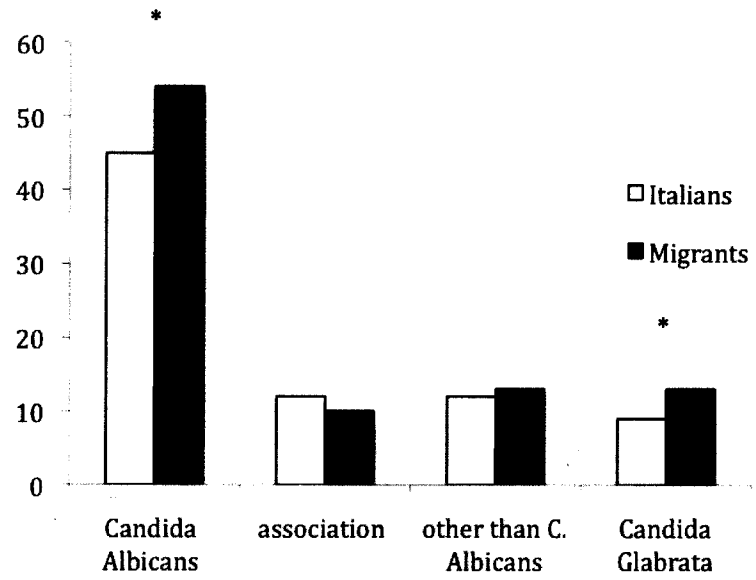

Fig. 1. *Percentage of patients positive to the different species of yeast in our series, out of 51 Italians and 75 migrants with a contamination of oral mucosa and dentures. *p<0.05 Italians versus immigrants calculated by chi-square test).*

report that *Candida glabrata* is more frequently found in immigrant patients than in native Italians.

Denture use has been demonstrated as a predisposing condition for oral candidiasis (18, 19). Compared with *Candida albicans*, *Candida glabrata* demonstrated a two-fold greater tendency to adhere to denture acrylic surfaces *in vitro* (20). With this high propensity to adhere to denture surfaces, it is not surprising that *Candida glabrata* has been identified as the predominant yeast isolated from dentures of elderly persons with chronic atrophic candidiasis (21). Due to the fact that *Candida glabrata* is frequently co-isolated with other *Candida* species from oral lesions (22), the role of this organism in pathogenesis is still obscure. *Candida glabrata* is most frequently co-isolated from mucosal lesions with *Candida albicans* (23). It has been reported that mixed infections with *Candida glabrata* and *Candida albicans* can cause more severe symptoms and are more difficult to treat (23, 24). Among the clinical manifestations seen in our patients, we found a correlation of *Candida* species with the type of oral lesion; *Candida albicans* correlated with the absence of lesions or pin point heperemia/erythema, whereas *Candida glabata, Candida dublinesins* and *Candida tropicalis* correlated with pseudomembrane formation. Pseudomembrane formation is likely to be linked to the dramatic histopatological tissue alteration induced by *Candida tropicalis, Candida dublinensis* and *Candida glabrata*, as reported previously (26).

Notably, the oral lesions of denture-stomatitis related to non-*albicans Candida* are clinically more severe in immigrants than in Italians. In our opinion, this finding is due to the fact that immigrants become denture wearers at a younger age, and consequently, the denture is exposed to potential pathogens for a longer period of time. This leads immigrant patients to have more severe denture stomatitis. Additionally, denture materials are often not as reliable as those available in Italy and thus are more prone to colonization.

Moreover, immigrants have limited access to the health care system, and thus, they rarely undergo periodic clinical check-ups of the dentures and the oral mucosa.

We also found that hygienic habits did not impact *Candida* colonization. This finding differs from previous reports in the literature that describe a

correlation between hygienic habit and the frequency of oral and denture colonization.

In our opinion, colonization mainly relies upon the denture materials, particularly the micro-porosity of the denture base and the micro-irregularity of the denture surface. These features have a pivotal role in the development of biofilm (26), although hygienic habits might contribute to maintaining the biofilm once established. Further studies evaluating the resistance of different types of resins to different *Candida* species colonization are required to confirm this assumption.

Interestingly, we found simultaneous colonization by two *Candida* species in the oldest Italian patients. Therefore, we assumed that age-related immunosuppression may play a major role in the colonization and maintenance of *Candida* species in the oral cavity as well as in dentures. Several factors, in our opinion, can account for this, such as the chronic use of dentures and the varied composition of oral microbiota and diets.

Because biofilm formation is a risk factor for *Candida* infection in denture wearers, it is advisable to periodically screen denture wearers for *Candida* presence. Nevertheless, a prompt treatment is strictly required in patients with non-*albicans Candida,* as these are the yeast with the highest tendency to invade and destroy the underlying oral mucosa.

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