Clinical Microbiology

Isolation of Candida spp. from denture-related stomatitis in Pará, Brazil

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ABSTRACT

The aim of this study was to isolate and identify Candida species from the oral cavity of denture wearers with denture-related stomatitis who were attended at the University Federal of Pará (Belém City, Pará State, Brazil). A total of 36 denture wearers with denture-related stomatitis were included, and type I (50%), type II (33%) and type III (17%) stomatitis were observed. Candida spp. were isolated from 89% of the cases and included five different Candida species. C. albicans was the most frequently recovered species (78% of the cases), followed by C. famata and C. tropicalis. We observed a significant association between Candida species isolation and unsatisfactory denture condition (p = 0.0017). Our results demonstrated the highly frequency of Candida species isolation in denture wearers with denture-related stomatitis and showed the relationship between these species and poor denture maintenance.

Introduction

Approximately 200 Candida species are known, and 10% can cause infections in humans. C. albicans is the most frequently described species in cases of hospital infections, followed by C. parapsilosis, C. tropicalis and C. glabrata.1 The frequency at which these species are observed has significant implications for human infections. These species are commensal organisms that constitute part of the normal oral microbiota, and they are present in 30–60% of healthy individuals and 60–100% of patients with dentures.2 The long-term use of dentures is the most important risk factor for Candida species colonization of the mucosa surface and may be sufficient for the development of oral candidiasis.3 Oral candidiasis is associated with mucosal trauma caused by poor denture fit, the increasing age of the denture wearers, the increased age of the dentures, fungal infections (primarily C. albicans) and...
poor dental hygiene. In this context, the adherence of 
*Candida* to the surface of denture materials, such as polymethyl 
methacrylate, facilitates colonization by *Candida*. The 
mechanisms by which *C. albicans* adhere to polymeric surfaces (e.g., 
dentures) primarily include biofilm formation and morpho-
logical switching, which facilitate the colonization of these 
materials by the fungus. Colonization is the main risk 
factor for the development of denture-related stomatitis (DRS), 
which is the most common clinical manifestation of *Candida* 
infection in denture wearers.

Infection by *Candida* spp. is frequently observed in patients 
with dentures and may lead to secondary oral lesions, such as 
lichen planus, leukoplakia and carcinoma. *Candida* species 
colonization and infections in the oral cavity of denture 
wearers have been reported worldwide, and *C. albicans* is 
particularly prevalent. Moreover, the isolation of *Candida* 
species other than *C. albicans* has been increasing, which 
is likely because of the misuse of antifungals. In Brazil, 
few studies have demonstrated the profile of *Candida* species 
related to colonization of the denture surface or oral mucosa 
and the incidence of these lesions in denture wearers. Therefore, 
we focused this study on the isolating and identifying 
*Candida* species from the surface of dentures and the oral 
mucosa of denture wearers with DRS.

### Materials and methods

**Ethical aspects**

The study was approved by the Ethics Committee of Evan-
dro Chagas Institute (CEP/IEC 032/10) and conducted between 
March and October 2012. All patients were informed about 
the study and provided written informed consent.

**Population**

Thirty-six (n = 36) patients fitted with acrylic-based dentures 
who presented with denture-related stomatitis were included. 
The patients were fitted with complete dentures (n = 32) or 
partially removable dentures (n = 4). All of the participants 
were attended at the dental school clinic at the University 
Federal of Pará (Belém, Pará, Brazil). Analyses were performed 
of the patient demographic data, which included age, gen-
der, hygiene habits (poor or not), mouthwash use, present 
denture condition (satisfactory or unsatisfactory) and 
qualitative characteristic (new or old dentures). The presence 
of DRS was assessed according to a modified version of New-
ton’s classification. The severity of the palatal inflammation 
was classified as (1) no stomatitis, which included no evidence 
of palatal inflammation or slight color change of the palatal 
mucosa; (2) stomatitis type I, which included petechiae dis-
persed throughout all or any part of the palatal mucosa in 
contact with the denture; (3) type II, which included mac-
ular erythema without hyperplasia; and (4) type III, which 
included diffuse or generalized erythema with papillary 
hyperplasia.

The patient exclusion criteria included the presence of diabetes or 
autoimmune disease and the use of corticosteroids.

### Isolation and identification

After an examination of the oral cavity, denture and mucosal 
specimens were harvested by scraping sterile swabs across the 
inner surface of the denture (basis of prosthesis, BP) and the 
oral mucosa (palatal mucosa, PM) in contact with the denture. 
Subsequently, the specimens were cultured in Sabouraud dext-
rose agar (Difco, Laboratories, Detroit, MI, USA), incubated 
at 35 °C and observed daily for 7 days. When the growth of 
yeast colonies was observed, the Gram stain method was used 
to verify the absence of bacterial contamination. The yeasts 
were identified via carbohydrate assimilation profiles using 
the Vitrek 2 System (BioMerieux E’toile, France) according to 
the manufacturer’s instructions.

Yeasts identified as *C. dubliniensis* were subjected to 
molecular confirmation because of the close phenotypical 
relationship of this species with *C. albicans*. Briefly, genomic 
DNA was extracted as previously described, and when neces-

sary, molecular identification was performed as described by 
Mannarelli and Kurtzman. *C. dubliniensis* (forward: CDU2 − 5’-AGT TAG TCT TTC GGG GGT GCC CT-3’; *C. dublini-
ensis* (reverse: NL4CAL – 5’-AAC ATC ATT AGG CCA ACA TCG TAG GTA AA-3’) and by Luo and Mitchell. *C. albicans* (forward: 
CALB1 – 5’-TTT ATC AAC TGG TTG TCA CAG CAG A-3’; *C. albi-
cans* (reverse: CALB2 – 5’-ATC CCG CCT TAC TAC CG-3’). 
The mix was prepared to a final volume of 25 μL as follows: 
10× MgCl2 (2 μL), 10 mM dNTP (1 μL), 10× PCR buffer (2.5 μL), Q 
solution (2 μL), Taq DNA polymerase (1 U; Invitrogen Life Tech-
nologies, Carlsbad, Calif.) and genomic DNA template (2 μL). 
Amplification was performed in a thermal cycler (T96 plus, 
Amplitatherm, Axigen) as follows: for *C. dubliniensis*: 98 °C for 
3 min; followed by 35 cycles of 95 °C for 1 min, 52 °C for 1.5 min, 
and 72 °C for 10 min; and then 72 °C for 10 min; and for *C. albi-
cans*: 96 °C for 5 min; followed by 40 cycles of 94 °C for 30 s, 58 °C 
for 30 s, and 72 °C for 30 s; and then 72 °C for 15 min. The PCR 
products were submitted to horizontal electrophoresis. The 
amplified fragments were 175 bp and 273 bp for *C. dubliniensis* 
and *C. albicans*, respectively.

### Statistical analysis

Statistical inferences of the descriptive results were per-
formed based on non-parametric tests, such as the G 
adherence independence test, using BioEstat version 5.3 (Insti-
tuto Maumirauá, Belém, Pará, Brazil). Statistical significance 
was considered at *p* ≤ 0.05.

### Results

Thirty-six (n = 36) denture wearers with DRS were included 
in this study. The patients ranged in age from 40 to 83 years 
(mean age = 62 years) and included 12 males (33%) and 24 
females (67%). According to Newton’s classification, the DRS 
cases were distributed as follows: Type I (50%), Type II (33%), 
and Type III (17%) among the cases. *Candida* species were 
isolated from the BP only (17%), FM only (5%) and BP and FM 
simultaneously (67%). In four cases (11%), *Candida* species 
were not isolated. Based on biochemical or biochemical and 
molecular identification, we observed five different *Candida*
species. C. albicans was the most frequently observed species (78% of the cases), and it was observed alone or in simultaneous isolations with C. famata, C. tropicalis or C. parapsilosis. The restricted isolation of Candida species other than C. albicans was observed in 11% of the cases. All of these results are summarized in Table 1.

According to the demographics data, an unsatisfactory denture condition was an influencing factor for the isolation of Candida species (p=0.0017); however, gender (p=0.7015), mouthwash use (p=0.6514), hygiene habits (p=0.3897), continuous denture use (p=0.4011) and old dentures (p=0.2502) were not related to Candida species isolation.

**Discussion**

DRS exhibits a multifactorial etiology and is associated with denture use, indicating that disease presentation can be affected by both endogenous and exogenous factors. A critical risk factor, is the colonization of the oral mucosa by Candida species. In the present study, Candida spp. were isolated in 89% of the cases of denture wearers with DRS (according to Newton’s classification), thereby reinforcing the relationship between Candida spp. and DRS. In Brazilian denture wearers, DRS was previously observed as the major lesion in the oral cavity, which is consistent with our results. In the present study, the factor that influenced whether Candida was isolated was an unsatisfactory denture condition. The frequent maintenance of dentures allows for improvements to certain functional characteristics, such as the RVD (rest vertical dimension) and VDO (vertical dimension of occlusion) of the patient, the adhesion of the denture to the mucosa and the diminution of the resin roughness. Improving these factors may decrease microorganism contamination of the surface denture, which will influence the oral health of denture wearers. In this context, Pereira-Cenci et al. observed that the colonization of dentures by Candida species is caused by surface roughness, which facilitates the adhesion of the yeast to resin dentures. Because the adherence of C. albicans to acrylic surface resins is related to the surface porosity and roughness, denture surfaces can be considered an infection source, and these findings are consistent with the significant colonization observed in denture wearers presenting with DRS because all patients were fitted with acrylic resin dentures. Moreover, the results support the relationship between unsatisfactory denture condition and Candida species isolation.

Others factors have been frequently reported as influential in the development of DRS. For example, Bulad et al. mentioned that the continuous use of dentures facilitated denture stomatitis because extended mucosal exposure to dental plaques may intensify certain injuries. Additionally, Darwazeh et al. observed a strong association between poor dental hygiene and Candida colonization and suggested that plaque accumulation on the denture surface may create an ideal environment for yeast. However, an association between poor hygiene, continuous denture use or old dentures and the isolation of Candida species was not observed in this study.

Among the colonized denture wearers in our study, C. albicans was the major isolated species, followed by C. famata and C. tropicalis, which were the most frequent non-C. albicans species. These results corroborated the findings of other studies, including other Brazilian studies. Additionally, Sant’Ana et al. reported that the presence of two or more species in the same patient can predispose the patient to recurrent stomatitis, and double isolations were also observed in denture wearers with DRS in the present study. Moreover, C. famata was observed in the PM of denture wearers who exhibited DRS and found in association with C. albicans. These results demonstrated the potential for emergent pathogens in cases of DRS.

We observed a significant frequency of DRS in Brazilian patients colonized by Candida species, and the infection was primarily caused by C. albicans. The results indicate that in the observed patients, DRS developed along a pathway that was dependent on the unsatisfactory state of the prosthesis and the colonization of the oral cavity by Candida species.
Conflicts of interest
The authors declare no conflicts of interest.

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REFERENCES


