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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

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poor dental hygiene.[4–6] In this context, the adherence of *Candida* to the surface of denture materials, such as polymethyl methacrylate, facilitates colonization by *Candida*.[7,8] The mechanisms by which *C. albicans* adhere to polymeric surfaces (e.g., dentures) primarily include biofilm formation and morphological 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.[9]

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.[10] *Candida* species colonization[11,12] and infections[9] in the oral cavity of denture wearers have been reported worldwide, and *C. albicans* is particularly prevalent.[13] Moreover, the isolation of *Candida* species other than *C. albicans* has been increasing, which is likely because of the misuse of antifungals.[14] 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.[15] 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 Evandro 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, gender, 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 Newton's classification.[16] 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 palate mucosa; (2) *stomatitis* type I, which included petechiae dispersed throughout all or any part of the palatal mucosa in contact with the denture; (3) type II, which included macular 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 dextrose 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 Vitek 2 System (BioMerieux I'Etoile, 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,[17] and when necessary, molecular identification was performed as described by Mannarelli and Kurtzman[18] (*C. dubliniensis*/forward: CDU2 – 5′-AGT TAC TCT TTC GGG GGT GGC CT-3′; *C. dubliniensis*/reverse: NL4CAL – 5′-AAG ATC ATT ATG CCA ACA TCC TAG GTA AA-3′) and by Luo and Mitchell[19] (*C. albicans*/forward: CALB1 – 5′-TTT ATC AAC TTG TCA CAC CAG A-3′; *C. albicans*/reverse: CALB2 – 5′-ATC CCG CCT TAC CAC TAC CG-3′). The mix was prepared to a final volume of 25 μL as follows: 10× $MgCl_2$ (2 μL), 10 mM dNTP (1 μL), 10× PCR buffer (2.5 μL), Q solution (2 μL), Taq DNA polymerase (1 U; Invitrogen Life Technologies, Carlsbad, Calif.) and genomic DNA template (2 μL). Amplification was performed in a thermal cycler (TX96 plus, Amplitherm, 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 *C. albicans*: 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 performed based on non-parametric tests, such as the G adherence independence test, using BioEstat version 5.3 (Instituto Maumirauá, Belém, Pará, Brazil). Statistical significance was considered at $p \leq 0.05$.

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.

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