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Isolation of *Candida dubliniensis* in denture stomatitis

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ABSTRACT

Objective: To describe the isolation of *Candida dubliniensis* from a patient with denture stomatitis and to compare with the presence of yeasts in the oral cavities of denture wearers.

Design: One hundred and fifty-two *Candida* isolates were recovered through oral swabs from denture as well as the underlying mucosa from 100 patients wearing denture. For detection and identification of fungal isolates, standard phenotypic and genotypic methods were used.

Results: Forty-five of 100 denture wearers suffered from denture stomatitis. Seventy-three *Candida* isolates were recovered from 38 denture wearers without denture stomatitis. In this group, *Candida albicans* was the predominant species (58.9%), followed by *Candida tropicalis* (15.1%), *Candida guilliermondii* (13.7%), *Candida glabrata* (9.6%), and *Candida parapsilosis* (2.7%).

Seventy-nine isolates were yielded from 40 patients suffering from denture stomatitis. *C. albicans* was also the most frequently isolated species (58 isolates, 73.4%), followed by *C. glabrata* and *C. tropicalis* (7 isolates each, 8.9%), and *Saccharomyces cerevisiae* (2 isolates, 2.5%). One isolate was yielded of the following species: *Candida famata, Candida krusei, C. parapsilosis* and *C. guilliermondii*. Moreover 1 isolate was phenotypic and genotypic identified as *C. dubliniensis* genotype 1.

Conclusions: C. albicans is the predominant fungal species isolated from denture wearers. *C. dubliniensis* could be isolated from adults with denture stomatitis.

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1. Introduction

Denture stomatitis is an inflammatory process of the oral mucosa associated to the presence of *Candida* or other microorganisms and several local and systemic factors, such as denture wearing, an acid salivary pH, a high carbohydrate ingestion, a long-term antibiotic therapy, hormonal therapy, diabetes mellitus or arterial hypertension which have a direct repercussion in the environment of the oral cavity.[1–3] *Candida albicans* represents the most common causative agent of oral candidiasis; however, other species of *Candida*, have begun to be isolated from oral specimens.[4] *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *Candida parapsilosis* have been isolated with relative frequency from patients with denture stomatitis.[5,6] He et al. have observed that denture base composition influences significantly in the adhesion of

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Candida to denture and produces differences in the species of *Candida* attached.[7]

C. albicans shares many phenotypic and genotypic characteristics with closely related species like *Candida dubliniensis* and some oral clinical isolates identified as *C. albicans* could be actually misidentified isolates of *C. dubliniensis*. The latter species was first described in 1995 associated with pseudomembranous candidiasis in HIV-infected patients.[8] More recently, a limited number of reports have associated this species to colonisation or disease in other anatomical sites in HIV-infected patients, in patients suffering from diabetes and also in healthy persons.[9–11] Epidemiological studies have demonstrated that prevalence of *C. dubliniensis* in the oral cavity of HIV-infected patients ranges from 1.5 to 32%.[12] One important issue of *C. dubliniensis* is the rapid development of resistance to fluconazole, a commonly used antifungal for the treatment of mycoses.[13,14] Mosca et al.[15] reported the isolation of *C. dubliniensis* from the oral specimens from an Argentinean non-HIV infected teenager with denture stomatitis and no other description of *C. dubliniensis* as coloniser or co-pathogen in healthy patients with denture stomatitis has been done.

In the present study we described the first *C. dubliniensis* isolation in Europe from a 69-year-old woman, non-HIV infected, with denture stomatitis.

2. Materials and methods

2.1. Patients

A total of 100 denture wearers (mean age: 65.17 years, range: 40–87 years) attending the Odontology clinics at the Universidad del País Vasco-Euskal Herriko Unibertsitatea, Bilbao (Spain) were studied. Among them, 45 patients (45%) (mean age: 59.66 years, range: 40–87 years) suffered from different degrees of denture stomatitis according to the Newton's classification. A complete medical and dental history was recorded from each patient after being informed and consent document signed by all of them. Patients with other underlying conditions than denture (such as diabetes, corticosteroids or immunosuppressant therapy, HIV infection, leukoplakia, oral lichen) were excluded from the study.

2.2. Collection, isolation and counting methods

Oral swabs were collected from the denture of each patient and from the underlying mucosa, and these specimens were grouped by the site of isolation (prosthesis or mucosa). When denture stomatitis lesions were present a specimen was collected from the lesion. All specimens were cultured on the ChromID Candida (previously Candida ID2) chromogenic medium (bioMérieux, Marcy l'Étoile, France)[16,17] and incubated at 37 °C for 48 h. The number of colony-forming units was quantified and the different isolates were subcultured on the same chromogenic medium in order to obtain pure cultures.

2.3. Yeast identification

Isolates were identified by conventional mycological methods, such as the germ tube test in serum, microscopic morphology, chlamyconidia production in corn meal agar with Tween 80, and carbon source assimilation with the commercial kit ID 32 C (bioMérieux).[18–20] Additionally, all those yeasts identified as *C. albicans* were screened for their ability to grow on Sabouraud dextrose agar at 45 °C for 48 h, their reactivity with a specific polyclonal anti-*C. dubliniensis* antibody by immunofluorescence[21] and other morphological characteristics, such as growth on CHROM-Pal's medium[22] and ChromID Candida chromogenic medium,[16,17] and the reactivity with Bichro–Dubli latex agglutination test (Fumouze Diagnostics, Levallois-Perret, France).[23] The isolates classified as *C. dubliniensis* were definitely identified by a polymerase chain reaction (PCR) with specific primers (CDBF28-f: 5′-AAA TGG GTT TGG TGC CAA ATT A-3′, and CDBR110-r: 5′-GTT GGC ATT GGC AAT AGC TCT A-3′) as described by Kanbe et al.,[24] which amplifies topoisomerase II gene, giving a DNA product size of 816 bp.

2.4. *C. dubliniensis* genotyping

Characterisation of *C. dubliniensis* genotypes was performed by PCR with the primers G1F/G1R (G1F: 5′-TTG GCG GTG GGC CCC TG-3′, and G1R: 5′-AGC ATC TCC GCC TTA TA-3′), G2F/G2R (G2F: 5′-CGG TGG GCC TCT ACC-3′, and G2R: 5′-CAT CTC CGC CTT ACC-3′), G3F/G3R (G3F: 5′-TTG GTG GTG GGC TTC TG-3′, and G3R: 5′-GCA ATC TCC GCC TTA CC-3′) and G4F/G4R (G4F: 5′-GGC CTC TGCC TGC CGC CAG AGG ATG-3′, and G4R: 5′-AGC AAT CTC CGC CTT ACT-3′),[25] as described by Brena et al.[26]

2.5. Antifungal susceptibility testing

The antifungal susceptibility of the *C. dubliniensis* isolate to common antifungal agents (5-fluorocytosine, amphotericin B, fluconazole, ketoconazole, itraconazole, voriconazole, posaconazole and caspofungin) was tested by the Sensititre YeastOne (Trek Diagnostic System Ltd., Imberhorne Lane, East Grinstead, West Sussex, England) colorimetric method.[27] Sensititre YeastOne contains: 5-fluorocytosine 0.03–64 μg/ml, amphotericin B 0.008–16 μg/ml, caspofungin 0.008–16 μg/ml, fluconazole 0.125–256 μg/ml, itraconazole 0.008–16 μg/ml, ketoconazole 0.008–16 μg/ml, posaconazole 0.008–16 μg/ml, and voriconazole 0.008–16 μg/ml. Briefly, 20 μl of a 0.5 McFarland standard yeast suspension was transferred into 11 ml of the RPMI broth tube and each well of the panel was inoculated with 100 μl of the diluted inoculum. The plates were visually read after 24 h of incubation at 37 °C according to the manufacturer's instructions.

2.6. Quality control

Quality control was performed using *C. albicans* serotype A (NCPF 3153) and *C. dubliniensis* CD36 (NCPF 3949, genotype 1) obtained from the National Collection of Pathogenic Fungi (NCPF, Bristol, United Kingdom) and *C. albicans* serotype B (ATCC 90028), *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). For the typing of *C. dubliniensis* strains CBS 2747 (genotype 2) from Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands), p6265 (genotype 3) and p7718 (genotype 4) were also used.[26]

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