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Species Distribution and Virulence Factors of *Candida* spp. Isolated from the Oral Cavity of Kidney Transplant Recipients in Brazil

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Abstract Although yeasts belonging to the genus *Candida* are frequently seen as commensals in the oral cavity, they possess virulence attributes that contribute for pathogenicity. The aims of the present study were to study the prevalence of *Candida* spp. isolated from the oral cavity of renal transplant recipients and to analyze strains virulence factors. We isolated a total of 70 *Candida* strains from 111 transplant recipients, and *Candida albicans* was the most prevalent species (82.86 %). Oral candidiasis was diagnosed in 14.4 % kidney transplant patients, while 11 isolates (15.7 %) corresponded to non-*Candida albicans Candida* (NCAC) species. *C. albicans* adhered to a higher extension than NCAC strains. Some isolates of *Candida tropicalis* were markedly adherent to human buccal epithelial cells and highly biofilm-forming strains. Regarding proteinase activity, *Candida orthopsilosis* was more proteolytic than *Candida metapsilosis*. *Candida glabrata* and *Candida dubliniensis* showed very low ability to form biofilm on polystyrene microtiter plates. We have demonstrated here diverse peculiarities of different *Candida* species regarding the ability to express virulence factors. This study will contribute for the understanding of the natural history and pathogenesis of yeasts belonging to the genus *Candida* in the oral cavity of patients who were submitted to kidney transplant and are under immunosuppressive therapies.

Keywords *Candida* spp. · Kidney transplant recipients · Epidemiology · Virulence factors

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Introduction

The genus *Candida* comprises yeasts that colonize the human oral cavity as commensal organisms in 20–80 % of adults without evidences of infection [1]. Nevertheless, under certain circumstances, specifically

among immunosuppressed individuals, such as AIDS patients and transplant organ recipients, they may cause opportunistic diseases ranging from superficial infections of mucous membrane to systemic infections leading to life-threatening diseases [2].

Oral candidiasis is an opportunistic infection commonly observed among renal transplant recipients [3]. The prevalence of this disease for this group of patients ranges from 9.4 to 46.7 % [3]. *Candida albicans* is the most frequent species associated with oral lesions, but other less virulent *Candida* species such as *Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida krusei* and *Candida dubliniensis* have also been isolated from the saliva of patients with or without oral candidiasis [4].

Kidney transplant is the treatment of choice for treating patients with chronic renal insufficiency. The main oral manifestations cited in the literature of kidney transplant patients under immunosuppressive therapy are the following: oral candidiasis, gingival hyperplasia, herpetic infections and pilous leukoplakia [5].

The transition of opportunistic yeasts from commensalism to infection is related to the virulence factors as well as to host susceptibility [6]. Several putative virulence factors contribute to *Candida* spp. pathogenicity, including the ability to adhere to epithelial and endothelial cells, yeast-to-hypha transition, phenotypic switching and the production of hydrolytic enzymes, specifically phospholipases and proteinases and biofilm formation [7–9].

Although several factors may contribute for the development of oral candidiasis among kidney transplant recipients, virulence factors of *Candida* species isolated from the oral cavity of this group of patients were not yet determined in this specific clinical scenario. Therefore, the objective of the present study was to evaluate the prevalence of yeasts isolated from the oral cavity of kidney transplant recipients and some in vitro virulence factors of the isolates, including the ability to adhere to human buccal epithelial cells (HBEC), biofilm formation and proteinase production. This study will contribute to the discussion of the essential pathogenicity factors important for the transition from commensalism to infection of *Candida* spp. during oral candidiasis in renal transplant patients.

Materials and Methods

Patients

We evaluated a total of 111 kidney transplant recipients followed up at the Onofre Lopes University Hospital, Natal, Brazil, from April to July 2008. The patients were subjected to clinical examination of the oral cavity for candidiasis diagnosis, according to the criteria established for oral lesions in HIV patients recommended by the EC-Clearinghouse and World Health Organization classifications [10]. Only patients who agreed to take part on a surveillance confidential study, in accordance with the Local Research Ethics committee from the referred Hospital, were enrolled in this study. Patients were under immunosuppressive therapy during the whole period of the study.

Samples Collection and Yeasts Identification

Samples containing 2 mL of saliva were collected from all patients, by previous stimulation with chewing gums [11]. Subsequently, 100 µL of cell suspensions was inoculated on the surface of Sabouraud Dextrose Agar (SDA; Oxoid, UK) added 300 µg/mL of chloramphenicol (Park–Davis), using a Drigalsky loop. The plates were incubated at 37 °C for 48 h. Yeast colonies were plated on CHROMagar Candida® (CHROMagar Microbiology, Paris, France) to check for purity and screening for different color colonies. Species identification was based on the characteristics of the cells observed microscopically after cultivation on cornmeal agar added Tween 80, as well as assimilation and fermentation testing and ID32C System (bioMérieux Marcy l'Etoile, France), whenever it was necessary [12]. *C. dubliniensis* presumptive identification was based on its inability to grow on Sabouraud dextrose broth with 6.5 % NaCl (Sigma-Aldrich, Brazil), after 72 h of incubation at 37 °C [13].

Molecular Identification

We performed molecular methods to identify the cryptic species *C. dubliniensis* and the species of the *C. parapsilosis* species complex (*C. parapsilosis*, *Candida orthopsilosis* and *Candida metapsilosis*).

DNA Extraction

Candida albicans cells were grown overnight in YPD liquid medium (dextrose 20 g/L, peptone 20 g/L, yeast extract 10 g/L) incubated at 30 °C and rotated at 200 rpm in a gyratory shaker (TE-420, Tecnal® Piracicaba, Brazil). DNA was extracted using the PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Genomic DNA concentration and purity were checked with a NanoDrop instrument (Thermo Scientific; Amersham Pharmacia Biotech, Wilmington, DE, USA).

Differentiation Among *C. albicans* and *C. dubliniensis*

In order to double-check the *C. dubliniensis* presumptive identification, the isolates previously identified by phenotypic methods as *C. dubliniensis* were identified with ABC genotyping, which is based on the amplification of an intron present in the 26 S rDNA, where *C. dubliniensis* is considered to belong to the former *C. albicans* genotype D [14]. *C. albicans* ATCC 90028 and *C. dubliniensis* CBS 7987 were used as control strains. The ABC identification was performed with the following primers: CA–INT–L (5′-ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3′) and CA–INT–R (5′-CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3′; [14]. Briefly, 1.0 µL of DNA 40 ng/µL was added to 2× PCR Master Mix (Promega) to a final volume of 25 µL. The samples were amplified in a Thermocycler (Amplitherm TX 96, USA) using the following cycling parameters: one initial cycle of 94 °C for 3 min followed by 30 cycles of 1 min at 94 °C, 1 min at 57 °C, 1 min at 72 °C and a final cycle of 5 min at 72 °C. PCR products were size-separated by agarose gel electrophoresis, and the gel was stained in a 0.5 µg/mL ethidium bromide buffer solution (1× TAE).

Differentiation of the Species Belonging to *C. parapsilosis* Species Complex

To differentiate the cryptic species belonging to the *C. parapsilosis* species complex, we have used the RAPD primer RPO2 validated in combination with DNA sequencing by Ge et al. [15]. For RAPD reactions, 1.0 µL of DNA 40 ng/µL, 2.5 µL of 10× PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 3.5 mM MgCl2), 5 µL of dNTPmix (100 mM each dNTP), 1.0 µL of primer (50 pmol/µL), 0.13 µL of Tween 20 and 1.0 unit of Taq DNA polymerase were added to a final volume of 25 µL. Amplifications were performed in a thermal circler Amplitherm TX 96 (USA) with the following three-step program: 2 min at 94 °C followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C, 75 s at 72 °C and then 10 min at 72 °C. PCR products were size-separated by agarose gel electrophoresis at 100 V for 30 min followed by 55 V for 5.5 h in a Tris–acetate buffer (TAE). The gel was stained in a 0.5 µg/mL ethidium bromide buffer solution (1× TAE) for 10 min and distained twice for 15 min in 400 mL of water.

Candida spp. Virulence Factors

For the *Candida* spp. virulence factors tested in vitro, *C. albicans* ATCC90028, *C. tropicalis* 13803, *C. dubliniensis* CBS797, *C. glabrata* ATCC2001, *C. orthopsilosis* ATCC96143 and *C. metapsilosis* ATCC96139 were used as reference controls. For these experiments, we randomly selected 26 isolates of *C. albicans* and included all non-*Candida albicans Candida* (NCAC) strains isolated ($n = 11$).

Candida spp. Adherence to Human Buccal Epithelial Cells

Candida cells were grown overnight to stationary phase in NGY 0.1 % (Neopeptone [Difco], 0.4 % glucose and 0.1 % yeast extract [Difco]) at 37 °C and were mixed with HBEC from healthy volunteers at a ratio of 10 yeast cells per HBEC. The mixtures were incubated at 37 °C for 45 min with shaking; then cells were vortexed, formalin-fixed and transferred to a microscope slide. The number of *Candida* cells adhering to 150 HBEC was determined with the operator blinded to the nature of the material on the slide. Tests were done in triplicate [16].

Candida spp. Proteinase Activity

Proteinase activity was determined by a method of Macdonald and Odds [17]. Fifty microliter samples from NGY cultures were grown in 5 mL YCB + BSA medium (11.7 g/L Yeast Carbon Base [Difco]; 10 g/L glucose; 5 g/L bovine serum albumin, fraction V,

Batch 08k0560 [Sigma]) rotated in a rotator shaker at 30 °C for 72 h, 200 rpm. Proteolytic activity was determined by measuring the increase in trichloroacetic acid-soluble products absorbing at 280 nm in triplicate after 1-h incubation of the culture supernatant with BSA substrate at 37 °C. Specific activity was expressed as OD280/OD600 of the culture.

Candida spp. Biofilm Formation

Biofilms were performed according to Melo et al. [18]. At first, 100 μL aliquots of a standardized cell suspension (10^7 cells/mL) were transferred to flat-bottom 96-well microtiter plates and incubated for 1.5 h at 37 °C in a shaker at 75 rpm. As controls, eight wells of each microtiter plate were handled in an identical fashion, except that no *Candida* suspensions were added. Following the adhesion phase, the cell suspensions were aspirated and each well was washed twice with 150 μL of PBS to remove loosely adherent cells. A total of 100 μL of RPMI-1640 medium buffered with MOPS was added to each of the washed wells and incubated at 37 °C in a shaker at 75 rpm. The biofilms were allowed to develop for 72 h, and then the yeasts were quantified by the crystal violet assay. Briefly, the biofilm-coated wells of microtiter plates were washed twice with 150 μL of PBS and then air-dried for 45 min. Then, each of the washed wells was stained with 110 μL of 0.4 % aqueous crystal violet solution for 45 min. Afterward, each well was washed four times with 350 μL of sterile distilled water and immediately destained with 200 μL of 95 % ethanol. After 45 min of destaining, 100 μL of destaining solution was transferred to a new well and the amount of the crystal violet stain in the destaining solution was measured with a microtiter plate reader (SpectraMAX 340 Tunable Microplate Reader; Molecular Devices Ltd.) at 595 nm. The absorbance values for the controls were subtracted from the values for the test wells to minimize background interference.

Statistical Analysis

Data were analyzed using the statistical software "GraphPad," version 3.0. Results were presented as mean ± standard deviation, and differences were analyzed by the Mann–Whitney test. For all the analyses, *P* was considered a default value of 0.05 and the confidence interval of 95 %.

Results

Patients Demographic Data

Patient's clinical and demographic data are summarized in Table 1.

Microbiological Profiling

Yeasts were isolated from the oral cavity of 70 out of 111 kidney transplant patients (63.1 %). *C. albicans* was the most prevalent species with 58 isolates (82.86 %), while 11 isolates (15.7 %) corresponded to NCAC, such as *C. tropicalis* (3 isolates; 4.35 %), *C. orthopsilosis* (2 isolates; 2.89 %), *C. metapsilosis*

Table 1 Demographic data of kidney transplant recipients, according to gender, age, time between transplant and saliva collection, reason that lead to transplantation and kind of donor

Variables	*N*	%
Sex		
Female	47	42.3
Male	64	57.7
Total	111	100
Age		
12–18 years old	6	5.4
19–34 years old	44	39.6
35–64 years old	59	53.2
65 years old or more	2	1.8
Total	111	100
Time after TX		
Up to 1 year	32	28.8
1–5 years	45	40.6
5 years or more	34	30.6
Total	111	100
TX reason		
Diabetic nephropathy	8	7.2
Arterial hypertension	47	42.3
Chronic glomerulonephritis	23	20.7
Polycystic kidney diseases	6	5.5
Other	27	24.3
Total	111	100
Donor type		
Cadaveric	46	41.4
Alive	65	58.6
Total	111	100

TX transplant

(2 isolates; 2.89 %), *C. glabrata* (2 isolates; 2.89 %) and *C. dubliniensis* (2 isolates; 2.89 %).

From the 111 kidney transplant recipients, we were able to diagnose oral candidiasis in 16 (14.4 %) of them, while 54 (48.6 %) individuals were only colonized by *Candida* spp. On the other hand, 41 (36.9 %) patients had negative *Candida* culture. In the patients diagnosed with oral candidiasis, atrophic candidiasis was the most prevalent clinical symptom (supplementary material). Interestingly, from the 70 kidney transplant patients whose yeasts were isolated from the oral cavity (colonization or infection), 33 had denture prosthesis. Twelve out of 16 patients with oral candidiasis had dental prosthesis. Therefore, the relationship between oral candidiasis and dentures prosthesis wearing was considered significant ($P = 0.01$). Patients only colonized by *Candida* spp. did not show any symptoms compatible to oral candidiasis. It seems that the specific immunosuppressive regimen adopted did not influence the positivity of cultures, because the percentage of patients with positive cultures ranged from 55.5 to 68 % independently of the immunosuppressors combination adopted (Table 2).

Adhesion to HBEC

Due to our limited number of NCAC strains representative for each species, we compared in vitro attributes of virulence among two groups: 26 *C. albicans* isolates versus 11 NCAC isolates. The ability of the *Candida* spp. isolates to adhere to HBEC was expressed by the number of blastoconidia adhered to 150 HBEC, determined with optical microscopy. In general, *C. albicans* isolates were highly capable to adhere to HBEC with an average of 237.38 blastoconidia/150 HBEC (Table 4). Nevertheless, an enormous variation among the isolates was observed. The number of *C. albicans* cells adhered to buccal epithelia ranged from 60 ± 3 to 379 ± 29/150 HBEC (Tables 3, 4). NCAC strains were statistically significant less adherent to HBEC then *C. albicans* isolates ($P = 0.01$; Table 4). Interestingly, an isolate of *C. tropicalis* was highly adherent to HBEC (335 ± 20/150 HBEC; Table 3).

Proteinase Activity

All the strains evaluated were proteinase producers. Proteinase activity was determined by growing cells in the presence of albumin as a sole nitrogen source. The enzymatic activity ranged from 0.01 to 0.08 (Table 3). We could not detect differences for proteinase production between *C. albicans* versus NCAC strains. However, a comparison within *C. parapsilosis* species complex showed that all *C. orthopsilosis* strains produced higher amounts of proteinase than *C. metapsilosis* isolates, including reference strains (mean of 0.06 ± 0.01 and 0.03 ± 0.01, respectively; $P = 0.02$.)

Biofilm Formation

Biofilms were formed when cells were grown on microtiter plates at 37 °C for 72 h of incubation. Once again, we could not detect a statistically significant difference between the two groups evaluated. Nevertheless, we noticed a *C. tropicalis* strain that was a high biofilm producer (1.82 ± 0.21); *C. glabrata* and *C. dubliniensis* were in general low biofilm producers both with a mean equal to 0.02 ± 0.01).

Virulence Factors of *Candida* Species Versus the Presence of Oral Candidiasis

We could not find a statistically significant correlation for any of the virulence factors evaluated and patient's clinical condition (colonization × infection) when all the strains for both groups were evaluated.

Table 2 Immunosuppressive drugs used by kidney transplant recipient, according the presence of oral candidiasis colonization and negative culture

Immunosuppressive regimens	Oral candidiasis	Colonization	Negative culture	Total
CSA + Mmf + Pred	4	24	13	41
Tac + Mmf + Pred	10	21	20	51
Sir + Mmf + Pred	1	4	4	9
Other	1	5	4	10
Total	16	54	41	111

CSA Cyclosporin A, *Mmf* Mycophenolate mofetil, *Pred* Prednisone, *Tac* Tacrolimus, *Sir* Sirolimus

Table 3 Virulence factors of *Candida* spp. clinical isolates obtained from the oral cavity of kidney transplant recipients

Isolates	Clinical condition	No of *C. albicans* cells adhered to 150 HBEC	Proteinase activity (OD_{280nm}/OD_{600nm})	Biofilm formation (OD_{595nm})
C. albicans ATCC 90028	Reference	229 ± 25	0.04 ± 0.00	0.04 ± 0.01
C. albicans SC5314	Reference	102 ± 14	0.01 ± 0.01	0.09 ± 0.05
C. albicans 1	Colonization	167 ± 15	0.03 ± 0.01	0.06 ± 0.01
C. albicans 2	Colonization	82 ± 17	0.05 ± 0.01	0.02 ± 0.01
C. albicans 3	Colonization	60 ± 5	0.03 ± 0.01	0.02 ± 0.00
C. albicans 5	Infection	221 ± 28	0.02 ± 0.01	0.06 ± 0.01
C. albicans 6	Colonization	162 ± 23	0.02 ± 0.01	0.10 ± 0.02
C. albicans 10	Infection	269 ± 25	0.01 ± 0.00	0.05 ± 0.00
C. albicans 11	Colonization	257 ± 23	0.05 ± 0.01	0.02 ± 0.00
C. albicans 12	Infection	327 ± 27	0.02 ± 0.00	0.04 ± 0.01
C. albicans 17	Colonization	150 ± 15	0.04 ± 0.01	0.03 ± 0.00
C. albicans 20	Colonization	189 ± 8	0.03 ± 0.00	0.12 ± 0.02
C. albicans 21	Colonization	379 ± 29	0.04 ± 0.01	0.09 ± 0.01
C. albicans 23	Colonization	338 ± 6	0.02 ± 0.01	0.02 ± 0.01
C. albicans 24	Infection	309 ± 26	0.02 ± 0.01	0.06 ± 0.02
C. albicans 28	Infection	190 ± 27	0.06 ± 0.03	0.02 ± 0.00
C. albicans 30	Colonization	207 ± 27	0.08 ± 0.02	0.06 ± 0.01
C. albicans 31	Colonization	362 ± 14	0.04 ± 0.01	0.12 ± 0.01
C. albicans 32	Colonization	366 ± 29	0.03 ± 0.01	0.08 ± 0.01
C. albicans 34	Colonization	236 ± 27	0.05 ± 0.00	0.25 ± 0.03
C. albicans 37	Colonization	280 ± 31	0.02 ± 0.01	0.05 ± 0.01
C. albicans 40	Infection	245 ± 34	0.05 ± 0.00	0.03 ± 0.01
C. albicans 44	Infection	312 ± 29	0.03 ± 0.01	0.06 ± 0.01
C. albicans 53	Colonization	219 ± 31	0.04 ± 0.01	0.04 ± 0.02
C. albicans 60	Colonization	238 ± 28	0.07 ± 0.01	0.02 ± 0.00
C. albicans 70	Colonization	165 ± 29	0.03 ± 0.02	0.07 ± 0.01
C. albicans 82	Colonization	280 ± 28	0.04 ± 0.00	0.21 ± 0.01
C. albicans 85	Colonization	162 ± 31	0.07 ± 0.01	0.03 ± 0.00
C. tropicalis ATCC 13803	Reference	96 ± 17	0.04 ± 0.01	0.05 ± 0.00
C. tropicalis 43	Colonization	335 ± 20	0.03 ± 0.01	0.35 ± 0.02
C. tropicalis 77	Colonization	68 ± 9	0.05 ± 0.01	1.82 ± 0.21
C. tropicalis 49	Colonization	74 ± 11	0.04 ± 0.01	0.01 ± 0.01
C. orthopsilosis ATCC 96139	Reference	89 ± 11	0.05 ± 0.01	0.01 ± 0.00
C. orthopsilosis 101	Infection	57 ± 14	0.08 ± 0.01	0.08 ± 0.01
C. orthopsilosis 79	Colonization	162 ± 20	0.06 ± 0.01	0.23 ± 0.06
C. metapsilosis ATCC 96143	Reference	47 ± 3	0.03 ± 0.01	0.01 ± 0.00
C. metapsilosis 97	Colonization	210 ± 22	0.03 ± 0.00	0.11 ± 0.03
C. metapsilosis 47	Colonization	193 ± 28	0.03 ± 0.01	0.11 ± 0.03
C. glabrata ATCC 2001	Reference	65 ± 9	0.03 ± 0.01	0.03 ± 0.00
C. glabrata 48	Colonization	112 ± 22	0.04 ± 0.00	0.01 ± 0.01
C. glabrata 33	Colonization	181 ± 19	0.03 ± 0.01	0.01 ± 0.01
C. dubliniensis CBS 7987	Reference	115 ± 26	0.05 ± 0.01	0.02 ± 0.02
C. dubliniensis 19	Infection	175 ± 20	0.06 ± 0.01	0.02 ± 0.01
C. dubliniensis 68	Colonization	99 ± 2	0.05 ± 0.02	0.02 ± 0.02

Table 4 Average virulence factors comparisons of *C. albicans* and non-*Candida albicans Candida* clinical isolates obtained from the oral cavity of kidney transplant recipients

	Number of *C. albicans* cells adhered to 150 HBEC	Proteinase activity (OD_{280nm}/OD_{600nm})	Biofilm formation (OD_{595nm})
C. albicans versus non-*Candida albicans Candida* species	237.38 ± 23.54 versus 151.45 ± 17*	0.04 ± 0.01 versus 0.05 ± 0.01	0.07 ± 0.01 versus 0.25 ± 0.04

* Statistically significant, $P < 0.05$

Discussion

Here, we evaluated the prevalence of oral candidiasis as well as colonization rates among patients submitted to renal transplant. We observed that 14.4 % of our patients showed clinical signs and symptoms compatible with oral candidiasis, while 48.6 % of them were colonized by *Candida* spp. The high number of patients colonized in the oral cavity with *Candida* spp. is threatening, because previous colonization may lead to infection. The prevalence of oral candidiasis in this specific group of patients ranges from 9.4 to 46.7 % [3, 5]. This noticeable variation may be related to the different procedures applied in the different studies, regarding the collection and processing of saliva samples and the different levels of immunosuppression.

Several studies have demonstrated that erythematous candidiasis is the major clinical manifestation of oral candidiasis [3, 19–21]. Nevertheless, our investigation showed chronic atrophic candidiasis as the main clinical manifestation. This fact might be related to the high frequency of our denture-wearing population associated with poor hygiene habits favoring the development of this manifestation of oral candidiasis. We also found a statistically significant association between wearing dentures and the presence of oral candidiasis ($P = 0.01$), corroborating the investigation of Darwazeh et al. [22], who reported that the presence of prosthetic dentures is a risk factor for developing oral candidiasis.

We found that *C. albicans* was the main species isolated from the oral cavity of our patients, corresponding to 82.86 %. *C. albicans* is still the most frequently isolated species from the oral cavity among immunosuppressed patients [23, 24]. Nevertheless, other *Candida* species have been implicated as etiological agents of oral candidiasis, such as *C. glabrata* [23, 25].

Most of the studies have demonstrated that *C. albicans* is the most adherent *Candida* species to HBEC [26, 27]. This trend was also observed in our study. In fact, we also observed a highly adherent *C. tropicalis* strain. *C. tropicalis* isolates' adherence to laminin and fibronectin was described as significantly higher than that obtained for the *C. albicans* isolates [28].

Regarding proteinase production, we have found that *C. orthopsilosis* isolates were lower proteinase producers than *C. metapsilosis* strains. In fact, Sabino et al. [29] showed that *C. orthopsilosis* were SAP producers, whereas *C. metapsilosis* were not.

We found a tremendous variation regarding the ability of the strains to form biofilm. An interesting finding is that we could detect a very strong *C. tropicalis* biofilm-forming strain. *C. tropicalis* clinical isolates have been classified as abundant biofilm formers [21], and their mature biofilms consist of a dense network of yeast cells in addition to evident filamentous morphologies [30].

In the present study, *C. dubliniensis* showed lower ability to form biofilm on polystyrene microtiter plates, suggesting clear differences between the two phenotypically undistinguishable species *C. albicans* and *C. dubliniensis*. Biofilm formation by *C. albicans* isolates has been reported as statistically significant higher than by *C. dubliniensis* [31]. In addition, all *C. glabrata* strains were low biofilm producers. This fact is also in agreement with the findings of Silva et al. [21] and Shin et al. [32] who reported that biofilm formation by *C. glabrata* is lower than in other NCAC species.

Studies regarding oral candidiasis in kidney transplant recipients are mostly concerning the epidemiology of *Candida* spp. and clinical manifestations. In respect of that, we could find that chronic atrophic candidiasis is the main clinical manifestation in our specific clinical scenario. Nevertheless, we also describe here some peculiarities of *Candida* spp. virulence factors. For instance, *C. albicans* was very adherent to HBEC, but some strains of *C. tropicalis* may be also highly adherent to buccal epithelia and

biofilm producer. On the other hand, *C. glabrata* and *C. dubliniensis* may be less effective in biofilm formation. Further investigations with a higher number of NCAC strains should be performed in order to confirm whether our findings follow the same trend in larger population studies.

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