doi: 10.5216/rpt.v49i2.63481

ORIGINAL ARTICLE

MULTICENTER STUDY OF *CANDIDA* SPECIES IN ORAL MUCOSA OF DIFFERENT PATIENTS: ANALYSIS OF 711 STRAINS AND LITERATURE REVIEW

Rennan Luiz Oliveira dos Santos[1], Luciana da Silva Ruiz[2], Marcos Ereno Auler[3], Jorge Kleber Chavasco[4], Débora Moreira[5], Regina Teixeira Barbieri Ramos[1], Bruna Rossini Lara[2,6] and Claudete Rodrigues Paula[1]

ABSTRACT

This study aimed to identify *Candida* spp. collected from oral mucosa and maintained in culture collections, correlating the findings with the medical history of patients and comparing with data from the literature over the past five years. Seven hundred and eleven oral *Candida* spp. isolates, collected between 2013 and 2017, were selected and identified using traditional and molecular methods. In addition, a literature review was performed with the key words: "Oral", "*Candida*" and "Yeast". Seven species of the genus *Candida*: were identified *C. albicans* (73.3%); *C. tropicalis* (9.3%); *C. parapsilosis* (8.2%); *C. glabrata* (3.9%); *C. guilliermondii* (2.8%); *C. krusei* (1.7%) and *C. lusitaniae* (0.3%). The strains identified as *C. albicans* were submitted to molecular methods using specific primers and of these, 5.8% were identified as *C. dubliniensis* strains. The greatest diversity of strains was found in patients presenting no systemic diseases or HIV +, while the highest percentage of strains of *Candida non-albicans* were observed in cancer patients. This study reports a representative distribution of *Candida* species among individuals exhibiting distinct clinical conditions, in order to contribute to the design of future research on details of aspects involved in the infections caused by these microorganisms. The correct identification of oral *Candida* strains contributes to a realistic epidemiological approach and future clinical protocols against these pathogens.

KEY WORDS: *Candida;* oral candidiasis; dentistry; yeasts; HIV; co-infection.

INTRODUCTION

Disorders in the human organism can negatively affect the mouth, presenting one only, the first, or more serious characteristic of systemic diseases, such as infections, hematological and gastrointestinal disorders

1. Department of Stomatology, School of Dentistry, University of São Paulo (USP), São Paulo, Brazil.
2. Adolfo Lutz Institute, Section of Biomedical Sciences, Mycology Laboratory, Bauru, São Paulo, Brazil.
3. Department of Clinical Analysis, University of Unicentro, Paraná, Brazil.
4. Department of Microbiology and Immunology, University of Alfenas, Minas Gerais, Brazil.
5. Department of Gynecology and Obstetrics, Medical School, USP, São Paulo, Brazil.
6. Institute of Biosciences, Univ. Estadual Paulista, Botucatu, São Paulo, Brazil.

Corresponding author: Claudete Rodrigues Paula. E-mail: crpmicol@uol.com.br

Received for publication: 18/5/2020. Reviewed: 22/6/2020. Accepted: 25/6/2020.

among others. Many diseases may be initially present in the oral mucosa, and oral mucosal disorders may arise as a result of systemic diseases. Among these, the most noteworthy is oral candidiasis, the most common fungal infection of the oral cavity in humans (Millsop & Fazel, 2016; Porter et al., 2017; Hu et al., 2019).

Yeasts of the genus *Candida* are described as commensal microorganisms of the oral microbiota. However, in patients with several local conditions, such as prosthetic devices and hyposalivation, or systemic conditions that affect their immunity, namely cancer, diabetes mellitus and AIDS, these microorganisms proliferate due to their opportunistic character, causing candidiasis (Lewis & Williams, 2017).

These fungi are, therefore, considered one of the primary indicators of immunodeficiency (Santos et al., 2018), and monitoring and knowledge of the different species are essential for the establishment of therapeutic and prophylactic drug protocols to be recommended against the disease already detected (Leon et al., 2006; Badiee et al., 2017). Moreover, in recent years there has been an increase in the resistance of these yeasts to antifungal agents used in dental practice (Silva et al., 2002).

Candida albicans is the most frequent species related to oral fungal infections in human adults and children, and is one of the most virulent species of the genus. As well as *C. albicans*, other species are described in oral mucosa, such as *C. parapsilosis, C. tropicalis, C. guillermondii, C. dubliniensis*, among others (Batista et al., 2014; Loster et al, 2016; Aslani et al., 2018). Currently, studies in different countries have shown a change in the epidemiology of *Candida* spp. (Goulart et al., 2018).

Candida dubliniensis, described by Sullivan et al. in 1995, has attracted considerable attention because of its association with HIV+ individuals. There is some difficulty in identifying and phenotypically differentiating *C. albicans* from *C. dubliniensis*, only possible through molecular identification methods (Chavasco et al., 2006; Livério et al., 2017). Mycological studies in the field of dentistry that target the genus *Candida*, often focus on *C. albicans*, while other species remain neglected. In addition, it should be noted that *C. dubliniensis* strains may be misidentified as *C. albicans*, explaining the controversial literature data and the clinical impact of oral candidiasis (Al-Ahmad et al., 2016).

Considering that oral candidiasis is a significant health problem in terms of morbidity and economic effort, the purpose of this study was to identify collection strains of the genus *Candida* isolated from the buccal vestibule of pediatric and adult patients, correlating the findings with their medical history and comparing the data with a literature review covering the last five years.

MATERIAL AND METHODS

Multicenter study and strains

Seven hundred and eleven strains from culture collections in four different Brazilian centers were studied. Of these, 350 were obtained from the Pathogenic Yeast Laboratory in the Faculty of Dentistry at the University of São Paulo, 150 from the Laboratory of Clinical Analysis at the Unicentro University, 124 from the Mycology Sector in the Adolf Lutz Institute and 87 from the Microbiology and Immunology department in the University of Alfenas.

These strains were isolated from the oral mucosa of children and non-smoking adults, without prosthesis and without clinical signs of candidiasis, from 2013 to 2017. Each strain was seeded on Petri dish surfaces with Sabouraud dextrose agar medium (SDA DIFCO®, Detroit, MI, USA) plus chloramphenicol (100 mg/L-1) and incubated at 25°C for 96 hours. In addition, the medical data of patients were obtained to correlate with the strains studied.

Phenotypic identification

The isolated yeasts were studied for their macroscopic, microscopic, reproductive and physiological characteristics, according to the methods recommended by Kurtzman & Fell (2011).

Molecular identification

The samples identified phenotypically as *C. albicans* underwent molecular characterization for differentiation from the *C. dubliniensis* species. The DNA was extracted, quantified and amplified following the protocol of Chavasco et al. (2006). Two pairs of primers were used: one for *C. dubliniensis* (CDU2 – 5'AGT TAC TCT TTC GGG GGT GGC CT 3'/NL4CAL – 5'AAG ATC ATT ATG CCA ACA TAG TAG GTA AA 3') and another for *C. albicans* (CAL5 – 5'TGT TGC TCT CTCGGG GGC GGC CG 3'/NL4CAL – 5'AAG ATC ATTATG CCA ACA TAG TAG GTA AA 3'). The presence or absence of an amplified fragment was analyzed by agarose gel electrophoresis visualized in UV transilluminator. Standard strains ATCC64548 (*C. albicans*) and 777 (*C. dubliniensis*) were used.

Literature Review

A literature review covering the last five years was carried out regarding the prevalence of Candida species in oral mucosa. The research was conducted in August 2018 using the terms "oral", "*Candida*", "yeast". The

inclusion criteria were studies on the prevalence of oral *Candida* in humans and studies in English. The exclusion criteria were studies that did not report the prevalence of oral *Candida* in humans, reviews on the subject, studies in languages other than English, studies that restricted the species studied and studies with unavailable complete texts. The electronic database PubMed (National Library of Medicine) was examined to identify studies that could be included. The articles were checked regarding the eligibility criteria, all duplicated articles were removed and, finally, selected. The following data were extracted from the articles: number of strains identified, species identified, methods used to identify these strains and groups of patients evaluated.

RESULTS

Phenotypic identification

Seven different species of the genus *Candida* were identified, among which the most prevalent was *C. albicans* with 521 (73.3%) strains identified, followed by *C. tropicalis* with 70 (9.9%) and *C. parapsilosis* with 58 (8.2%). (Figure 1).

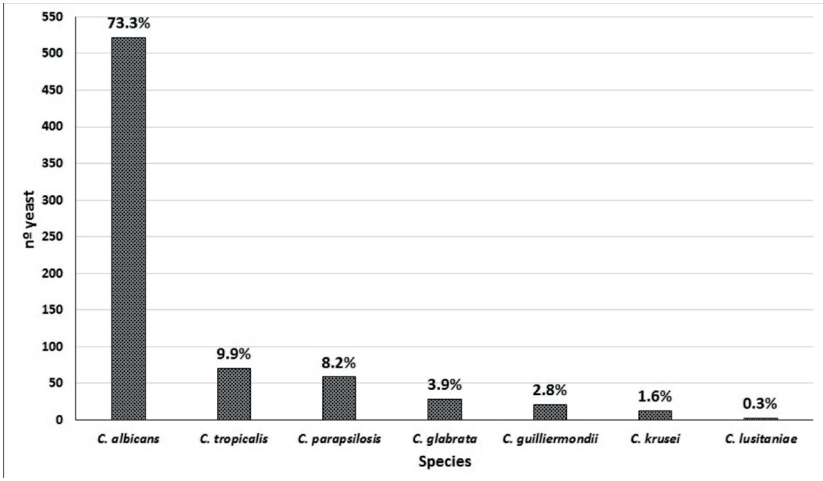

Figure 1. Distribution of the species of yeasts isolated from the oral mucosa of children and non-smoking adults, without prosthesis and without clinical signs of candidiasis, from 2013 to 2017.

Molecular identification

Since the phenotypic isolates from *C. albicans* and *C. dubliniensis* behave identically, the 521 strains initially identified as *C. albicans* underwent molecular methods for the differentiation between these species, where 491 (94.2%) were maintained as *C. albicans* and 30 (5.8%) were identified as *C. dubliniensis* (Figure 2).

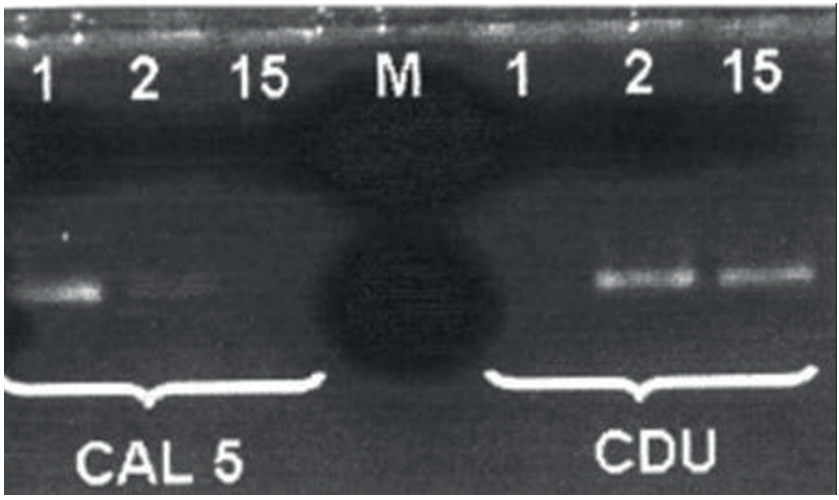

Figure 2. Eletrophoretic analysis of the products obtained through the amplification of the genomic DNA of the isolate studied using the primers CAL5 and NL4CAL, and CDU2 and NL4CAL. (1) - standard strain of *C. albicans*; (2) - standard strain of *C. dubliniensis*; (15) - sample strain (Chavasco et al., 2016). Photo courtesy of Prof. Dr. Jorge Kleber Chavasco.

Correlation with clinical data

Patient age ranged from 2 to 40 years, with a mean age of 23 years. All these strains were analyzed by discussing the medical history of the donor. The samples came from patients without systemic diseases (PWSD) and from patients compromised with systemic diseases: HIV+ patients, oncological and diabetic patients, as shown in Table 1.

Table 1. Prevalence of species of the genus *Candida* in relation to the group of patients.

PATIENTS	PREVALENCE OF YEAST SPECIES OF THE GENUS CANDIDA - N (%)								TOTAL n (%)
	C. albicans n (%)	*C. tropicalis* n (%)	*C. parapsilosis* n (%)	*C. dubliniensis* n (%)	*C. glabrata* n (%)	*C. guillermondii* n (%)	*C. kruse* n (%)	*C. lusitanie* n (%)	
PWSD*	233 (66.9)	31 (8.9)	30 (8.6)	21 (6.0)	17 (4.9)	11 (3.2)	4 (1.1)	1 (0.3)	348 (100)
HIV+	176 (81.5)	13 (6.0)	5 (2.3)	9 (4.2)	3 (1.4)	5 (2.3)	4 (1.9)	1 (0.5)	216 (100)
Oncological	48 (45.3)	25 (23.6)	23 (21.7)	-	3 (2.8)	4 (1.9)	3 (2.8)	-	106 (100)
Diabetic	34 (82.9)	1 (2.4)	-	-	5 (12.2)	-	1 (2.4)	-	41 (100)
Total	491 (69.1)	70 (9.8)	58 (8.2)	30 (4.2)	28 (3.9)	20 (2.8)	12 (1.7)	2 (0.3)	711 (100)

*PWSD: Patients without systemic diseases; n: number of samples

Literature Review

Electronic database Pubmed was researched and 212 articles were evaluated according to Figure 3. Of these, 29 articles were selected (Table 2), of which 22 reported the amounts of strains studied, totaling 2,691 identified strains. Some articles reported the colony-forming variable units and/or did not report the number of strains. The studies came from four continents, Asia being the most frequent with 11 (37.9%) published studies, followed by America with 9 (31%), Europe with 8 (27.6%) and Oceania with 1 (3.5%).

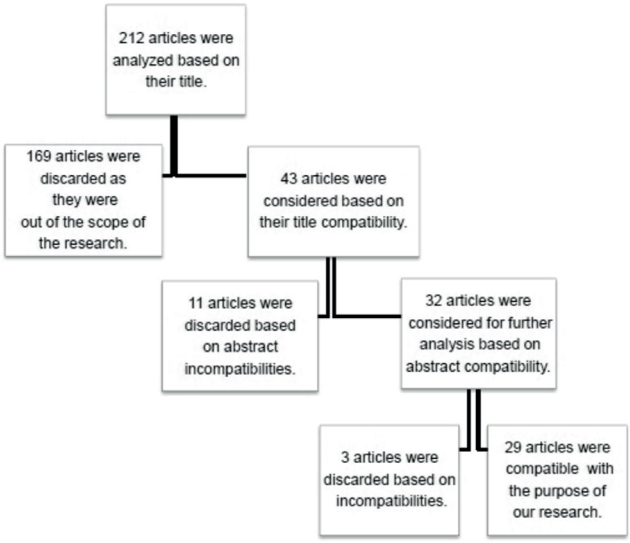

Figure 3. Planning the search of articles through the PUBMED database.

Regarding the prevalence of *Candida* species, *C. albicans* species stands out being identified in all the studies. On the other hand, *C. non-albicans* species presented a proportion: *C. glabrata*, *C. parapsilosis* in 22 (78.6%), *C. tropicalis* in 21 (75%), *C. krusei* in 15 (53.6%), *C. dubliniensis* in 14 (50%), *C. guilliermondii* in 9 (32.1%), *C. famata* in 6 (21.4%), *C. lusitaniae*, *C. kefyr* in 5 (17.8%), *C. inconspicua, C. lipolytica, C. rugosa, C. norvegiensis, C. intermedia* in 3 (10.7%), *C. pelliculosa, C. sphaerica* and *C. quercitrusa* in 2 (7.1%) and *C. zeylanoides, C. railenensis, C. sake, C. pulcherrima, C. colliculosa, C. lambica, C. fukuyamaensis, C. africana, C. orthoparapsilosis, C. metaparapsilosis* in 1 (3.6%). Three of the articles did not identify the non-albicans species.

Regarding the methodologies used for the identification of the strains, presumptive techniques and commercial tests were the most used with reports in 16 (57.1%) articles. Conventional, molecular, MALDI-TOF MS, automatic system and methods were used in 15 (53.5%), 11 (39.3%), 2 (7.14%), 2 (7.14%), and 1 (3.6%), respectively.

Regarding patient systemic diseases, 11 (37.9%) studies reported patients with no systemic problem, 6 (20.7%) with diabetic and pre-diabetic conditions, 5 (17.2%) with HIV+, 2 (6.9%) presented oncological patients and other diseases were pointed out in 10 (34.5%) articles.

Table 2. Literature review of studies related to the isolation of yeasts of the genus *Candida* from the oral vestibule of patients.

Autor (year of publication)	Country	Number of yeasts identified	Species identified	Methods used for identification	Systemic diseases of the assessed patient groups
Aslani et al. (2018)	Iran	162	*C. albicans, C. glabrata, C. tropicalis, C. dubliniensis.*	Conventional, Commercial test and MALDI-TOF MS	Oncological
Santos et al. (2018)	Brazil	19	*C. albicans, C. non-albicans*	Conventional	Transplanted
Loster, Wieczorek and Loster (2018)	Poland	22	*C. albicans, C. glabrata, C. tropicalis, C. krusei, C. parapsilosis, C. inconspicua*	Presumptive, automatic system and commercial test	Patients who did not report systemic diseases
Spalanzani et al. (2018)	Brazil	45	*C. albicans*	Presumptive and molecular	HIV+
Costa et al. (2017)	Portugal	Not informed	*C. albicans, C. dubliniensis, C. zeylanoides, C. lipolytica, C. pelicullosa, C. railenensis, C. guilliermondii.*	Molecular	Diabetics and patients who did not report systemic diseases
Imabayashi et al. (2016)	Japan	18	*C. albicans, C. dubliniensis, C. tropicalis, C. parapsilosis, C. krusei*	Presumptive and molecular	Patients who did not report systemic diseases
Pieralisi et al. (2016)	Brazil	Not informed Used UFC	*C. albicans, C. glabrata, C. tropicalis, C. parapsilosis*	Conventional and Presumptive	Transplanted
Gulcan et al. (2016)	Turkey	42	*C. albicans, C. glabrata, C. krusei, C. kefyr, C. parapsilosis*	Presumptive, Conventional and Commercial test	Transplanted
Benedito-Cruz et al. (2016)	Mexico	113	*C. albicans, C. glabrata, C. krusei, C. kefyr, C. sake, C. sphaerica, C. dubliniensis, C. pulcherrima, C. tropicalis*	Conventional and presumptive	Diabetics and hypertensives

Lydia et al. (2016)	India	81	*C. albicans, C. glabrata, C. tropicalis, C. krusei, C. parapsilosis.*	Conventional, presumptive and molecular.	Diabetics and patients who did not report systemic problems
Das et al. (2016)	India	61	*C. albicans, C. dubliniensis, C. parapsilosis, C. glabrata, C. famata*	Conventional, presumptive and commercial test	HIV + and patients who did not report systemic problems
Aurora et al. (2016)	Malaysia	26	*C. albicans, C. dubliniensis, C. parapsilosis, C. glabrata, C. krusei.*	Conventional	Lichen planus
De Souza et al. (2016)	Brazil	46	*C. albicans, C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C. lusitaniae.*	Conventional	Oncological
De-la-Torre et al. (2016)	Spain	Not informed	*C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. rugosa, C. quercitrusa, C. guilliermondii, C. lipolytica, C. dubliniensis, C. inconspicua, C. norvegensis, C. krusei, C. collicullosa.*	Conventional, presumptive, commercial test.	Patients did not report systemic diseases
Hertel, Schmidt-Westhausen and Strietzel (2016).	Germany	958	*C. albicans, C. tropicalis, C. glabrata, C. krusei, C. dubliniensis, C. famata, C. parapsilosis, C. kyfer, C. guilliermondii, C. sphaerica, C. lipolytica, C. lusitaniae, C. intermedia, C. inconspicua, C. rugosa, C. lambica*	Presumptive and MALDI-TOF MS	Several systemic diseases
Przybyłowska et al. (2016)	Poland	40	*C. albicans, C. glabrata, C. tropicalis, C. parapsilosis*	Presumptive and commercial test	Chronic obstructive pulmonary disease
Mun et al. (2016)	Australia	Not informed	*C. albicans, C. krusei and C. non-albicans*	Presumptive.	Several systemic problems
Menezes et al. (2015)	Brazil	111	*C. albicans, C. dubliniensis, C. parapsilosis, C. glabrata, C. krusei, C. kefyr, C. famata, C guilliermondii, C. lusitaniae, C. pelliculosa, C. tropicalis.*	Automatic system	HIV+

Ribeiro Ribeiro et al. (2015)	Brazil	103	*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis e C. famata.*	Conventional and presumptive.	HIV+
Pieralisi et al. (2015)	Brazil	26	*C. albicans, C. glabrata, C. famata, C. parapsilosis, C. tropicalis.*	Conventional and presumptive	Diabetics
Astvad et al. (2015)	Denmark	71	*C. albicans, C. glabrata, C. tropicalis, C. krusei, C. dubliniensis, C. parapsilosis, C. norvegiensis*	Commercial test, presumptive and molecular.	Terminal patients in palliative treatments
Pan et al. (2014)	China	93	*C. albicans, C. glabrata, C. krusei, C. guilliermondii, C. africana.*	Presumptive and molecular	No systemic diseases have been reported
Ho et al. (2014)	Taiwan	149	*C. albicans, C. dubliniensis, C. tropicalis, C. glabrata, C. guilliermondii, C. famata, C. intermedia, C. parapsilosis.*	Presumptive, commercial test and molecular	HIV+
Da Silva-Rocha et al. (2014)	Brazil	88	*C. albicans, C. tropicalis, C. dubliniensis, C. glabrata, C. tropicalis, C. ortopsilosis, C. metapsilosis*	Presumptive, conventional and molecular	Diabetics and hypertensives
Javed et al. (2014)	Saudi Arabia	Not informed	*C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. guilliermondii, C. krusei, C. lusitaniae*	Commercial test and molecular	Pre-diabetics
Kilic et al. (2014)	Turkey	29	*C. albicans, C. glabrata, C. kefyr e C. norvegensis*	Commercial test	Patients who did not report systemic diseases
Wang et al. (2013)	China	415	*C. albicans, C. tropicalis, C. glabrata, C. parapsilosis, C. krusei, C. metapsilosis, C. guilliermondii, C. dubliniensis, C. orthopsilosis, C. intermedia, C. fukuyamaensis, C. quercitrusa, C. rugosa.*	Molecular	No systemic diseases have been reported
Javed et al. (2013)	Saudi Arabia	Not informed	*C. albicans, C. tropicalis, C. parapsilosis, C. krusei, C. lusitaniae, C. glabrata, C. guilliermondii.*	Commercial test and molecular	Patients who did not report systemic diseases

DISCUSSION

Colonization by *Candida* is common in humans. Several species of *Candida* are part of the oral microbiota (Lynch et al., 2016). In the present work, we highlight the considerable number of isolates from oral mucosa correctly identified using phenotypic and molecular methods.

Eight different yeast species of the genus *Candida* were identified, and these findings corroborate the global data shown in the literature review. The authors draw attention to the number of studies noted in the literature identifying *C. non-albicans* strains without correct conventional and/or molecular identification methods, which are necessary, as demonstrated by Jafari et al. (2017) and Livério et al. (2017). These findings, therefore, may be false positives and false negatives due to the lack of specificity against some of these strains.

In PWSD patients, eight yeast species of *Candida* genus were isolated, presenting a great diversity of strains in these patients, among which 21 were *C. dubliniensis*. Although this species is prevalent in HIV+ patients, we consider the occurrence of this pathogen relatively high among the PWSD patients investigated, almost twice as high as that of HIV+. Recently, studies have reported the appearance of this pathogen in HIV- patients (Imabayashi et al., 2016; De-la-Torre et al., 2016), while others report no findings (Keten et al., 2015; Rajakumari et al., 2016). New controlled studies with the use of molecular identification techniques are desirable to clarify this aspect, distinguishing between identification failures in previous studies or confirming a characteristic of our population.

Candida lusitaniae was found in the oral mucosa of PWSD and HIV+ patients, a non-routine finding, but already reported in the studies of Javed et al. (2013) and Thanyasrisung et al. (2014). As the occurrence of *C. lusitaniae* isolates was limited to only two cases, it is necessary to consider the possibility of a transient micobiota, which could become a permanent micobiota, or the interference of socio-environmental factors still unknown in these individuals.

In HIV+ patients studies have demonstrated the prevalence of several *Candida* species, such as *C. albicans, C. tropicalis, C. parapsilosis, C. krusei, C. glabrata, C. tropicalis, C. lusitaniae* and *C. guilliermondii* (Ribeiro et al. 2015; Menezes et al. 2015; Das et al. 2016; Osaigbovo et al., 2017; Spalanzani et al. 2018; Goulart et al., 2018) corroborating our findings.

When *Candida non-albicans* species are considered, there is great prevalence variation in these patients in the several published studies. In the same country, studies in different cities presented different results (Back-Brito et al., 2009; Junqueira et al., 2012; Spalanzani et al., 2018). Therefore, special attention must be paid to a correct identification of the *Candida* species present, as well as monitoring the prevalence of these pathogens which may cause serious infections in susceptible patients (Maheshwari et al., 2016).

Cancer patients represent a high-risk group for the acquisition of candidemia due to their overall condition, such as neutropenia, disease severity, presence of disseminated disease and comorbidities (Jayachandran et al., 2016; Soni et al., 2017). In the literature, *C. albicans* and *C. tropicalis* seem to compete which is the most prevalent species in these patients (Suryawanshi et al., 2012; Yogitha et al., 2015) corroborating our findings. This variation in prevalence seems to be dependent on the type of cancer as well as the general condition of the patient and the therapeutic protocol in use (Maheshwari et al., 2016).

In diabetic patients, *Candida* colonization may be due to the ability of this pathogen to attach to epithelial cells in conjunction with the reduction of tissue resistance against infection (Belazi et al., 2005). Rajakumari et al. (2016) identified *C. albicans, C. glabrata, C. tropicalis* and *C. krusei* corroborating with the data in the present study. However, Zomorodian et al. (2016) did not verify the presence of *C. krusei* and *C. dubliniensis* without associations with other species in this population.

Comparing the groups studied here, diabetics exhibited the smallest variety of *Candida* species, while PWSD and HIV+ patients presented all eight species identified in this study. Diabetes is a frequent universal disease and, in relation to the identification of *Candida* in the oral mucosa, suggests that the alterations caused by the disease in the body of the affected individual, follow a similar pattern, favoring certain species of the fungus that adapt to such local and systemic conditions, as opposed to the diversity of factors that probably occur in the group of cancer patients.

The data presented in Table 1 show a clear predominance of *C. albicans* species in these studied strains, and the groups of diabetic and HIV+ patients presented similar percentages. The group of PWSD patients showed an intermediate number and the group of cancer patients presented a number below 50% for *C. albicans*. Another interesting fact that merits investigation is the expressive presence of *C. tropicalis* and *C. parapsilosis* in the group of cancer patients. This group of patients may be the most complex with predisposing conditions to diverse fungal infections, depending on the cancer type, location, comorbidities and therapeutics proposed for each patient.

The group of HIV+ patients presented the same variety of species as the group of PWSD patients, with small variations in incidence among *C. non-albicans* species. This fact is somewhat understandable since immunosuppression is quite variable in this group of individuals and this aspect was not analyzed.

In conclusion, this study provides an interesting panel of distribution of *Candida* species among individuals exhibiting distinct clinical conditions, in order to contribute to the design of future research on the details of the conditions involved in the infections caused by this microorganism. With this research, a protocol of management and communication, regarding to the most effective prophylactic and therapeutic measures, can be defined.

ACKNOWLEDGEMENTS

The authors are grateful for the funds provided by “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq), “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) and by “Fundação de Amparo à Pesquisa do Estado de São Paulo” (FAPESP).

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Al-Ahmad A, Auschill TM, Dakhel R, Wittmer A, Pelz K, Heumann C, Hellwig E, Arweiler NB. Prevalence of *Candida albicans* and *Candida dubliniensis* in caries-free and caries-active children in relation to the oral microbiota-a clinical study. *Clin Oral Investig 20*: 1963-1971, 2016.
2. Aslani N, Janbabaei G, Abastabar M, Meis JF, Babaeian M, Khodavaisy S, Boekhout T, Badali H. Identification of uncommon oral yeasts from cancer patients by MALDI-TOF mass spectrometry. *BMC Infect Dis 18*: 24, 2018.
3. Back-Brito GN, Mota AJ, Vasconcellos TC, Querido SM, Jorge AO, Reis AS, Balducci I, Koga-Ito CY. Frequency of *Candida* spp. in the oral cavity of Brazilian HIV-positive patients and correlation with CD4 cell counts and viral load. *Mycopathologia 167:* 81-87, 2009.
4. Badiee P, Choopanizadeh M, Moghadam AG, Nasab AH, Jafarian H, Shamsizadeh A, Soltani J. Antifungal susceptibility patterns of colonized *Candida* species isolates from immunocompromised pediatric patients in five university hospitals. *Iran J Microbiol 9*: 363-371, 2017.
5. Batista GC, Krebs VL, Ruiz LS, Auler ME, Hahn RC, Paula CR. Oral colonization: a possible source for candidemia in low-weight neonates. *J Mycol Med 24*: 81-86, 2014.
6. Belazi M, Velegraki A, Fleva A, Gidarakou I, Papanaum L, Baka D, Daniilidou N, Karamitsos D. Candidal overgrowth in diabetic patients: potential predisposing factors. *Mycoses 48*: 192-196, 2005.
7. Chavasco JK, Paula CR, Hirata MH, Aleva NA, Melo CE, Gambale W, Ruiz LS, Franco MC. Molecular identification of *Candida dubliniensis* isolated from oral lesions of HIV-positive and HIV-negative patients in São Paulo, Brazil. *Rev Inst Med Trop São Paulo 48*: 21-26, 2006.
8. Das PP, Saikia L, Nath R, Phukan SK. Species distribution & antifungal susceptibility pattern of oropharyngeal *Candida* isolates from human immunodeficiency virus infected individuals. *Indian J Med Res 143:* 495-501, 2016.
9. De-la-Torre J, Marichalar-Mendia X, Varona-Barquin A, Marcos-Arias C, Eraso E, Aguirre-Urizar JM, Quindós G. Caries and *Candida* colonization in adult patients in Basque Country (Spain). *Mycoses 59*: 234-240, 2016.
10. Goulart LS, Souza WWR, Vieira CA, Lima JS, Olinda RA, Araújo C. Oral colonization by *Candida* species in HIV-positive patients: association and antifungal susceptibility study. *Einstein (Sao Paulo) 16*: eAO4224, 2018.
11. Hu L, He C, Zhao C, Chen X, Hua H, Yan Z. Characterization of oral candidiasis and the *Candida* species profile in patients with oral mucosal diseases. *Microb Pathog 134*: 103575, 2019.

12. Imabayashi Y, Moriyama M, Takeshita T, Ieda S, Hayashida JN, Tanaka A, Maehara T, Furukawa S, Ohta M, Kubota K, Yamauchi M, Ishiguro N, Yamashita Y, Nakamura S. Molecular analysis of fungal populations in patients with oral candidiasis using next-generation sequencing. *Sci Rep 6*: 28110, 2016.
13. Jafari Z, Motamedi M, Jalalizand N, Shokoohi GR, Charsizadeh A, Mirhendi H. Comparison of CHROMagar, polymerase chain reaction-restriction fragment length polymorphism, and polymerase chain reaction-fragment size for the identification of *Candida* species. *Curr Med Mycol 3:* 10-15, 2017.
14. Javed F, Yakob M, Ahmed HB, Al-Hezaimi K, Samaranayake LP. Oral *Candida* carriage among individuals chewing betel-quid with and without tobacco. *Oral Surg Oral Med Oral Pathol Oral Radiol 116*: 427-432, 2013.
15. Jayachandran AL, Katragadda R, Thyagarajan R, Vajravelu L, Manikesi S, Kaliappan S, Jayachandran B. Oral Candidiasis among Cancer Patients Attending a Tertiary Care Hospital in Chennai, South India: An Evaluation of Clinicomycological Association and Antifungal Susceptibility Pattern. *Can J Infect Dis Med Microbiol 2016:* 8758461, 2016.
16. Junqueira JC, Vilela SFG, Rossoni RD, Barbosa JO, Costa ACBP, Rasteiro VM, Suleiman JMAH, Jorge AOC. Oral colonization by yeasts in HIV-positive patients in Brazil. *Rev Inst Med Trop São Paulo 54*: 17-24, 2012.
17. Keten HS, Keten D, Ucer H, Yildirim F, Hakkoymaz H, Isik O. Prevalence of oral *Candida* carriage and *Candida* species among cigarette and maras powder users. *Int J Clin Exp Med 8:* 9847-9854, 2015.
18. Kilic K, Koc AN, Tekinsen FF, Yildiz P, Kilic D, Zararsiz G, Kilic E. Assessment of *Candida* species colonization and denture-related stomatitis in bar- and locator-retained overdentures. *J Oral Implantol 40:* 549-556, 2014.
19. Kurtzman CP, Fell JW. The Yeasts: *A Taxonomic Study*. 4th ed. Elsevier Science, Burllington, MA, EUA, 2011. 176p.
20. León C, Ruiz-Santana S, Saavedra P, Almirante B, Nolla-Salas J, Álvarez-Lerma F, Garnacho-Montero J, León MA, EPCAN Study Group. A bedside scoring system ("*Candida* score") for early antifungal treatment in nonneutropenic critically ill patients with *Candida* colonization. *Crit Care Med 34*: 730-737, 2006.
21. Lewis MAO, Williams DW. Diagnosis and management of oral candidosis. *Br Dent J 223*: 675-668, 2017.
22. Livério HO, Ruiz LDS, Freitas RS, Nishikaku A, Souza AC, Paula CR, Domaneschi C. Phenotypic and genotypic detection of *Candida albicans* and *Candida dubliniensis* strains isolated from oral mucosa of AIDS pediatric patients. *Rev Inst Med Trop São Paulo 59*: e14, 2017.
23. Loster JE, Wieczorek A, Loster BW. Correlation between age and gender in *Candida* species infections of complete denture wearers: a retrospective analysis. *Clin Interv Aging 11*: 1707-1714, 2016.
24. Lynch MA, Brightman VJ, Greenberg MS. *Burkets oral medicine, diagnosis and treatment*. JB Lippincott Co. Philadelphia, 2008. 396-399p.
25. Maheshwari M, Kaur R, Chadha S. *Candida* species prevalence profile in HIV seropositive patients from a Major Tertiary Care Hospital, in New Delhi, India. *J Pathog 2016:* 6204804, 2016.
26. Menezes RP, Borges AS, Araujo LB, Pedroso RS, Röder DV. Related factors for colonization by *Candida* species in the oral cavity of hiv-infected individuals. *Rev Inst Med Trop São Paulo 57*: 413-419, 2015.
27. Millsop JW, Fazel N. Oral candidiasis. *Clin Dermatol 34*: 487-494, 2016.
28. Osaigbovo II, Lofor PV, Oladele RO. Fluconazole Resistance among Oral *Candida* Isolates from People Living with HIV/AIDS in a Nigerian Tertiary Hospital. *J Fungi (Basel) 3*: 69, 2017.

29. Porter SR, Mercadante V, Fedele S. Oral manifestations of systemic disease. *Br Dent J 223*: 683-691, 2017.

30. Rajakumari ML, Kumari OS. Prevalence of *Candida* species in the buccal cavity of diabetic and non-diabetic individuals in and around Pondicherry. *J Mycol Med 26*: 359-367, 2016.

31. Ribeiro ALR, Menezes TOA, Alves-Junior SM, Menezes SA, Marques-da-Silva SH, Rosário Vallinoto AC. Oral carriage of *Candida* species in HIV-infected patients during highly active antiretroviral therapy (HAART) in Belém, Brazil. *Oral Surg Oral Med Oral Pathol Oral Radiol 120*: 29-33, 2015.

32. Santos SBD, Sabadin CES, Mario DN, Rigo L, Barbosa DA. Presence of *Candida* spp. and candidiasis in liver transplant patients. *An Bras Dermatol 93*: 356-361, 2018.

33. Silva MR, Costa MR, Miranda AT, Fernandes OF, Costa CR, Paula CR. Evaluation of Etest and macrodilution broth method for antifungal susceptibility testing of *Candida* sp strains isolated from oral cavities of AIDS patients. *Rev Inst Med Trop São Paulo 44*: 121-125, 2002.

34. Soni P, Parihar RS, Soni LK. Opportunistic Microorganisms in Oral Cavity According to Treatment Status in Head and Neck Cancer Patients. *J Clin Diagn Res 11*: DC14-DC17, 2017.

35. Spalanzani RN, Mattos K, Marques LI, Barros PFD, Pereira PIP, Paniago AMM, Mendes RP, Chang MR. Clinical and laboratorial features of oral candidiasis in HIV-positive patients. *Rev Soc Bras Med Trop 51:* 352-356, 2018.

36. Sullivan DJ, Westerneng TJ, Haynes KA, Bennett DE, Coleman DC. *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. *Microbiology 141*: 1507-1521, 1995.

37. Suryawanshi H, Ganvir SM, Hazarey VK, Wanjare VS. Oropharyngeal candidosis relative frequency in radiotherapy patient for head and neck cancer. *J Oral Maxillofac Pathol 16*: 31-37, 2012.

38. Thanyasrisung P, Kesakomol P, Pipattanagovit P, Youngnak-Piboonratanakit P, Pitiphat W, Matangkasombut O. Oral *Candida* carriage and immune status in Thai human immunodeficiency virus-infected individuals. *J Med Microbiol 63*: 753-759, 2014.

39. Yogitha PPV, Lakshmi N, Lakshmi KR, Murali Krishna PB, Cheemala SS. Isolation and speciation of genus *Candida* in patients undergoing chemotherapy and radiotherapy for head and neck tumours. *Int J Res Med Sci 3:* 1189-1194, 2015.

40. Zomorodian K, Kavoosi F, Pishdad GR, Mehriar P, Ebrahimi H, Bandegani A, Pakshir K. Prevalence of oral *Candida* colonization in patients with diabetes mellitus. *J Mycol Med 26*: 103-110, 2016.