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IDENTIFICATION OF *CANDIDA* SPP. BY PHENOTYPIC TESTS AND PCR

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ABSTRACT

The correct identification of *Candida* species is of great importance, as it presents prognostic and therapeutical significance, allowing an early and appropriate antifungical therapy. The purpose of this study was to identify isolates of *Candida* spp. from oral mucosa of 38 patients with oral candidosis evaluated in 2004 by phenotypic methods and PCR, discriminating *C. albicans* from the other *Candida* species. The tests used for phenotypic analysis were germ-tube and chlamydoconidia production, culture in CHROMAgar™ *Candida*, carbohydrate assimilation test, growth at 45ºC and culture in Tween 80 agar. Genotypic confirmation was performed by PCR. Phenotypic tests showed that 63.2% strains formed germ-tubes, 73.7% produced chlamydoconidia, and 63.2% showed green colonies in chromogenic medium, presumptively indicating *C. albicans* or *C. dubliniensis*. The carbohydrate assimilation test confirmed these results. A total of 21% strains were identified as *C. krusei* and 13.2% were indicative of *C. tropicalis*. Of these later strains, three produced chlamydoconidia. The association of other phenotypic tests with culture in Tween 80 agar identified 95.8% of strains as *C. albicans* and 4.2% as *C. dubliniensis*. All 24 strains indicative of *C. albicans* and *C. dubliniensis* were confirmed by PCR as *C. albicans*.

Key words: *Candida* spp. - identification – PCR - phenotypic tests

INTRODUCTION

Human pathogenic yeasts are ubiquitous in the environment (1), and some species belong to the normal human microbiota (2, 3). *Candida* species are usually opportunistic organisms (4), and oral candidosis ranks high in terms of incidence among the many opportunist infections observed in human immunodeficiency virus (HIV)-infected patients. *C. albicans* has long been considered the predominant etiologic agent of oral candidosis (26). Over the last decade, however, there has been an increase in the incidence of candidosis in immunocompromised individuals caused by other *Candida* species, such as *C. glabrata, C. krusei, C. tropicalis* and *C. parapsilosis* (6). In 1995, *C. dubliniensis*, which is a species very closely related to *C. albicans*, was identified in cases of oral candidosis in HIV-infected individuals (28). It is likely that *C. dubliniensis* strains have been and will continue to be identified as *C. albicans*, because both species share similar

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phenotypic characteristics such as formation of germ-tubes, production of chlamydoconidia in rice agar, biochemical pattern and same color of colonies on differential media CHROMagarTM Candida. *C. dubliniensis* is a species that can rapidly develop resistance to antifungal therapy. Therefore, patients who have received multiple treatments for fungal infections may be at increased risk of harboring *C. dubliniensis* as the predominant species in their oral cavities (15). If this ability of *C. dubliniensis* to develop stable fluconazole resistance *in vitro* also occurs rapidly *in vivo*, this may explain, at least in part, its recent emergence as an opportunistic pathogen in the oral cavities of HIV-infected individuals and AIDS patients, who are often treated with this drug (27).

Research in many laboratories is carried out to develop new drugs or drug delivery systems, but the development of approaches that allows quick and accurate identification of disease-causing yeasts is also necessary, especially because the incidence of human disease caused by the less common *Candida* species has increased.

Thus, the identification of *Candida* species is very important in the diagnostic laboratory, because such identification shows prognostic and therapeutical significance, allowing the early and correct antifungical therapy (11, 21). The purpose of this study was to identify isolates of *Candida* spp. by phenotypic and genotypic methods, discriminating *C. albicans* from other species of *Candida*.

MATERIAL AND METHODS

Samples

Samples were obtained in 2004 from 38 adult patients, female or male, with oral candidosis assisted by the Stomatology Service of São Lucas Hospital of PUCRS. The diagnosis of candidosis was established according Holmstrup and Axell (12). Patients who have used antifungal drugs were excluded from the sample. All the patients signed an informed consent form to participate in the study, which was approved by the local Ethics Committee.

The samples were collected from the oral mucosa with sterile swabs, which were rubbed on the candidosis lesion. All samples were seeded on Sabouraud dextrose agar (SDA, Merck) with chloramphenicol (16 mg/mL, Neo Química). The plates were incubated at 30ºC for 48 h. Each morphological colony isolate was characterized according to Milan and Zaror (21). Three colonies with characteristics of *Candida* sp. were inoculated individually in yeast peptone dextrose (YPD, Sigma) broth and incubated at 30ºC for 48 h. After this period, each culture was individually stored at -20ºC with 20% glycerol. Only one sample of each isolate was used for all characterizations.

Germ-tube test

Yeast cells were inoculated into 0.5 mL of fetal bovine serum and incubated at 37°C for 2.5 h. After this period, aliquots were removed for microscopic examination (25). Germ tube was considered as a slender tube with straight walls, without septum and without constriction at the junction between the cells (10, 21). Germ-tube was indicative of *C. albicans* or *C. dubliniensis*. *C. abicans* ATCC 28367, *C. dubliniensis* CBS 7987 and *C. krusei* ATCC 6250 were used as reference strains.

Chlamydoconidia production test

Chlamydoconidia production test was performed using rice agar medium (10 g rice, 10 g bacteriological agar, and distilled water to a final adjusted volume of 1000 mL) supplemented by 8 mL of Tween 80. The samples previously grown in SDA were seeded as 3 parallel streaks in a rectangular piece of rice agar placed between two slides, incubated in wet chamber (8) at 30°C for 72 h and visualized in an optical microscope (10X and 40X magnification) (10). The formation of rounded spores with double-wall isolates was observed as chlamydoconidia, and was indicative of *C. albicans* or *C. dubliniensis* (22).

Chromogenic agar culture

Each isolate was cultured on SDA at 30°C for 48 h. After this, they were seeded on CHROMAgarTM Candida (Chromagar Microbiology) and incubated at 30°C for 48 h. The CHROMAgar™ allows selective yeast isolation, identifying

colonies of *C. albicans*, *C. dubliniensis*, *C. tropicalis* and *C. krusei* by morphology and color reaction (13). The strains were identified according to the manufacturer's instructions, which define *C. albicans* or *C. dubliniensis* as green colonies, *C. tropicalis* as steel blue colonies, *C. krusei* colonies as showing rose color and rough aspect, and the other species as developing colonies from white to rose.

Carbohydrate assimilation test

The carbohydrate assimilation test determines the ability of a yeast isolate to use a particular carbohydrate substrate as its sole carbon in a medium. Yeasts were cultured in YPD for 24 h at 30°C. After, the cultures were centrifuged for 5 min at 2790 g and the pellets were washed 3 times successively, suspended in sterile saline and adjusted to 5 McFarland standard. A volume of 300 µL of Yeast Nitrogen Base (YNB, Difco) and 1.5 mL of *Candida* spp. suspension were mixed to 30 mL of sterile bacteriological agar in Falcon tubes. The suspension was placed in 15 cm Petri dishes and after solidification of the medium, discs with the 2% carbohydrates (maltose, trehalose, xylose, galactose, lactose, sucrose and glucose) were placed following a guide. The suspension was incubated in 30°C for 96 h, and observed daily. Glucose was used as positive control, since all the species of *Candida* assimilate this carbohydrate (21). The carbohydrate assimilation was observed with a presence of a halo of growth around each carbohydrate.

Growth at 45°C

Growth at 45ºC has been considered a useful test for the differentiation of *C. dubliniensis* (no growth) from *C. albicans* (growth) (23). This test was used in the 24 positive samples for the germ-tube test, for chlamydoconidia production and that developed green colonies in CHROMAgarTM.

The temperature test was performed using YPD, Brain Heart infusion (BHI, Merck), SDA and Emmons medium (2% SDA). All samples were incubated at 45ºC, and growth was assessed daily for 10 days.

Tween 80 agar

Tween 80 agar (10 g peptone, 5 g NaCl, 0.1 g CaCl2, 1000 mL distilled water, 5 mL Tween 80, pH 6.8) was used to evaluate the lypolityc activity of the strains (24). The samples previously cultured in SDA at 30ºC for 24 h, which were indicative of *C. albicans* or *C. dubliniensis*, were transferred to Tween 80 agar by softly touching the colony with a sterile swab perpendicularly oriented towards the agar. The cultures were incubated at 30°C and evaluated daily, for 5 days. Esterase production was evidenced as the presence of a halo around the site of inoculation, observed with transmitted light after 2 to 3 days, being indicative of *C. albicans* (24).

Polymerase Chain Reaction (PCR)

The pair of primers (5'TGTTGCTCTCTCGGGGGCGGC CG3' and 5' AGATCATTATGCCAACATCCTAGGTTAAA 3') specific for the amplification of a fragment of the RNAr gene of *C. albicans* described by Mannarelli and Kurtzman (18) was used to confirm phenotypic identification of *C. albicans* and *C. dubliniensis*.

For *Candida* DNA extraction, an aliquot of pure culture was spread on SDA and a colony was collected and inoculated into 20 µL of TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0). The mixture was heated for 10 min in water bath at 100ºC and, after this period, heated in microwaves at high power for 2 min.

PCR amplifications were performed in a final volume of 25 µL containing 1 µL of lysed yeast cell, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.0), 5 mM KCl, 1.25 mM of each nucleotide (Amersham Biosciences), 0.02 mM of each primer (Integrated DNA Technologies), and 1.0 U of *Taq* DNA polymerase (Promega). Amplifications were carried out in a MinicyclerTM Termocycler (MJ Research) using conditions modified from Mannarelli and Kurtzman (18). Reactions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 1 min and extension at 72°C for 30 s, with a final extension at 72°C for 4 min. Amplification products were separated by

electrophoresis on 2% agarose gel containing 5 µg/mL ethidium bromide using a 100-bp ladder (Amersham Biosciences) as molecular weight marker. All PCR used *C. albicans* ATCC 28367 as positive control and *C. dubliniensis* CBS 7987 as negative control.

RESULTS

A total of 63.2% (24/38) of strains were able to produce germ-tubes. Chlamydoconidia production was observed in 28/38 (73.7%) of the tested strains, and 24/38 (63.2%) showed green colonies in chromogenic medium, indicating *C. albicans* or *C. dubliniensis* (Table 1). To confirm these tests, the carbohydrate assimilation test was used in all 38 strains, and 24/38 (63.2%) were indicative of *C. albicans* or *C. dubliniensis* (Table 1). A total of 8 strains (21%), which were able neither to form germ-tube, neither to produce chlamydoconidia, were identified as *C. krusei* by CHROMagarTM Candida and carbohydrate assimilation tests. Five strains in 38 (13.2%) were indicative of *C. tropicalis* by the same tests. Of these 5 strains, 3 were positive for chlamydoconidia (7A, 34A and 44A), but they failed to produce germ-tube or to develop blue colonies in chromogenic agar, being thus incompatible with *C. albicans*. These results associated with carbohydrate assimilation tests indicated *C. tropicalis* as the probable species. One strain (8A) showed the ability to form germ-tube, and developed green colonies on chromogenic medium, but did not produce chlamydoconidia and was therefore classified as *C. albicans.* Moreover, its behavior in the carbohydrate assimilation test was compatible with this species (Table 1).

Table 1. Identification of *Candida* spp. by germ-tube test (GT), chlamydoconidia production test (CH), culture in CHROMAgar™ Candida and carbohydrate assimilation test (CA)

Strain	GT	CH	CHROMAgar ™	CA							Presumptive identification
				TRE[1]	XYL	GLU	GAL	MAL	LAC	SUC	
5A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
6A	-	-	rose	-	-	+	-	-	-	-	*C. krusei*
7A	-	+	steel blue	+	+	+	+	+	-	+	*C. tropicalis*
8A	+	-	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
12A	-	-	rose	-	-	+	-	-	-	-	*C. krusei*
13A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
15A	-	-	rose	-	-	+	-	-	-	-	*C. krusei*
27B	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
28B	-	-	rose	-	-	+	-	-	-	-	*C. krusei*
33A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
34A	-	+	steel blue	+	+	+	+	+	-	+	*C. tropicalis*
35B	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
38A	-	-	rose	-	-	+	-	-	-	-	*C. krusei*
40B	-	-	rose	-	-	+	-	-	-	-	*C. krusei*
41A	-	-	white to rose	+	+	+	+	+	-	+	Other species

42A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
43A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
44A	-	+	steel blue	+	+	+	+	+	-	+	*C. tropicalis*
45A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
46A	-	+	steel blue	+	+	+	+	+	-	+	*C. tropicalis*
47B	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
48A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
49A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
51A	-	-	rose	-	-	+	-	-	-	-	*C. krusei*
55A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
57A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
58A	-	-	rose	-	-	+	-	-	-	-	*C. krusei*
59A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
60A	-	+	steel blue	+	+	+	+	+	-	+	*C. tropicalis*
61A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
62A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
63A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
64A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
66A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
67A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
68A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
69A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
71A	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
C. albicans ATCC 28367	+	+	green	+	+	+	+	+	-	+	*C. albicans* or *C. dubliniensis*
C. dubliniensis CBS 7987	+	+	green	+	+	+	+	+	-	+	*C. dubliniensis* or *C. albicans*
C. krusei ATCC 6250	-	-	rose	-	-	+	-	-	-	-	*C. krusei*

[1]TRE: Trehalose; XYL: Xylose; GLU: Glucose; GAL: Galactose; MAL: Maltose; LAC: Lactose; SUC: Sucrose

The results obtained for reference strains using growth at 45ºC test showed that this test was not reproducible, because a strain that grew in a culture medium at 45ºC did not grow in a subculture in the same medium. Also, a strain grew in a culture medium at 45ºC and did not grow in another one incubated at the same temperature. Thus, the strains isolated from patients were not tested by this assay.

Culture in Tween 80 agar identified 23/24 (95.8%) strains as *C. albicans*, and only one strain (64A) as *C. dubliniensis*.

The PCR produced a DNA fragment of 175 bp when tested with *C. albicans* ATCC 28367, and did not yield amplification products when the lysed cells of *C. dubliniensis* CBS 7987 were used. All 24 strains characterized phenotypically as *C. albicans* or *C. dubliniensis* were tested by PCR, being identified as *C. albicans*.

The phenotypic tests used in this study were not enough to identify strain 41A.

DISCUSSION

The phenotypic methods for the identification of *Candida* spp., although simple and inexpensive, impose some limitations. These methods are time-consuming and/or more often unable to discriminate *C. albicans* and *C. dubliniensis*. Therefore, the most reliable tests for rapid identification of *Candida* spp. and, especially, for differentiation between *C. albicans* and *C. dubliniensis* are based on molecular techniques. In the present study, all strains identified as *C. albicans* by PCR produced also germ-tubes. The germ-tube production test has the advantage to be simple and efficient in the economical and fast identification of *C. albicans* (8, 16). However, some results are liable to subjective interpretation, which makes necessary many repetitions, training and experience to discriminate germ-tube from pseudohyphae (6). Furthermore, although the germ-tubes cannot be quantified, a large amount is needed in a material to afford a diagnosis of *C. albicans*. Some authors evaluated sensitivity and specificity of the germ-tube test, finding results between 93 and 98.8%, and between 73.3 and 100%, respectively (5, 7, 10).

Chlamydoconidia production was observed in 73.7% of strains, with three strains (7A, 34A and 44A) not being identified as *C. albicans* by other phenotypic tests and PCR. These three strains developed blue color colonies in chromogenic agar and did not produce germ-tubes, being identified as *C. tropicalis*, since some strains of this species can produce chlamydoconidia. The production of chlamydoconidia by isolates from *C. dubliniensis* is unusual, but when produced, chlamydoconidia are observed in abundance and often in triplets or in contiguous pairs, which can also be observed in *C. albicans* (28, 29). The strain 8A showed the ability to form germ-tubes, behaved as *C. albicans* in CHROMagar™ medium, and showed a behavior characteristic of *C. albicans* in carbohydrate assimilation tests, but did not produce chlamydoconidia. The confirmation of this strain as *C. albicans* was obtained by PCR, and corroborates what described in the literature, as 10% of the strains of this species do not produce chlamydoconidia (21).

The strains that developed green colonies on CHROMagarTM Candida were identified as *C. albicans* by PCR. However, it is possible that if *C. dubliniensis* had been isolated, it would not have been discriminated from *C. albicans* by culture on chromogenic agar, since both species develop colonies of the same color. According to Milan and Zaror (21), there is a difference in the intensity of color of colonies between these species; *C. albicans* colonies are green-blue-pale, and *C. dubliniensis* dark-green. Yet, it is important to observe that this ability is lost after freezing of the samples, and it did not show reproducible results (21). Furthermore, some authors described that the dark-green color on CHROMagarTM Candida as phenotypic marker for *C. dubliniensis* cannot be generally adopted as criterion, and may be limited to primary cultures [Mähnß *et al.* (17); Tintelnot *et al.* (29). Moreover, *C. albicans* colonies can grow sharing a variation of green color, ranging from light-green to dark-green, depending on growth density and incubation period (10), often with the periphery of the colonies having a color distinctly different from that of the rest of the colony (26). However, Mesa *et al.* (20) observed that among 55 strains

identified as *C. albicans* tested by CHROMagar™ medium, none showed the dark-green color typical of *C. dubliniensis*, indicating this medium as a good phenotypic criterion to differentiate both species.

The pattern of carbohydrate assimilation is considered a reliable test and is generally used for the correct identification of yeasts of clinical interest (14). The results obtained corroborated the identification of *C. albicans* or *C. dubliniensis*, *C. tropicalis* and *C. krusei*. The assimilation pattern obtained would not be useful to differentiate between *C. dubliniensis* and *C. albicans*, if the former had been isolated from patients, since the xylose was assimilated by all strains suspected of being *C. albicans* or *C. dubliniensis*.

As the phenotypic tests mentioned above were not able to discriminate *C. albicans* and *C. dubliniensis*, the growth at 45ºC test and the observation of lipolytic activity was performed. The lipolytic activity test is simple, economical to conduct, and is easy to interpret, but it showed discrepancies as compared to the results obtained with PCR. One isolate (64A) was presumptively identified as *C. dubliniensis*, but the PCR identified this strain as *C. albicans*. An explanation for this could be the low viability of that sample, which presented problems to grow in subcultures after successive thawing. Moreover, the culture in Tween 80 agar can show lower sensitivity than PCR. The present results do not support the report of Slifkin (24), who describe that this test permitted the clear differentiation of the strains of *C. albicans* from the strains of *C. dubliniensis* within 3 days of incubation on Tween 80. The differentiation of *C. albicans* from *C. dubliniensis* by growth at 45ºC was not reproducible and showed conflicting results; therefore, the test could not be used for the differentiation between these species in the present study, as already indicated by Gales *et al.* (9). These authors showed that none of 66 *C. dubliniensis* isolates was able to grow at 45ºC, and 23 out of 100 *C. albicans* isolates were not able to grow at this temperature. This demonstrates that this test offers low specificity, and that *C. albicans* isolates could be falsely identified as *C. dubliniensis*. However, Pinjon *et al.* (23) describe this test as simple, reliable, inexpensive, reproducible, and readily applicable to large numbers of isolates.

The differentiation between *C. albicans* and *C. dubliniensis* remains a challenge for clinical microbiology laboratories. The most pronounced differences between these species are genetic, where *C. dubliniensis* is approximately 2.5% divergent from the *C. albicans* DNA sequence, as determined by DNA fingerprinting, karyotype analysis, and DNA sequence analysis of rRNA genes (17, 27). Sequence differences between *C. albicans* and *C. dubliniensis* have been potentially exploited in the design of oligonucleotide primers used in PCR, an approach which is increasingly being used in clinical laboratories. In this study, all 24 *C. albicans* or *C. dubliniensis* presumptively identified by phenotypic methods were confirmed as *C. albicans* by PCR using oligonucleotide primers specific for this species. The advantages of PCR include reproducibility, high sample volume throughput (27), relatively short processing time, high sensitivity and specificity, ability to detect low levels of yeasts from minimal sample volumes (30), ease to perform and it is available to most laboratories (18). The PCR procedure described here allowed the identification of *C. albicans* in less than 1 day, using a simple technique for obtaining DNA, using boiling and heating in microwaves, which helps to turn this method inexpensive, as commercial kits for DNA extraction are not necessary.

No single phenotypic test has proven to be highly effective in the distinction between *C. albicans* and *C. dubliniensis,* and genotypic tests may be necessary for definitive identification. The combination of some phenotypic methods can be useful for the presumptive identification of these species, but they would require a pure culture and 3-5 days or longer for a differentiation between *C. albicans* and *C. dubliniensis* isolates. Molecular methods can give definitive identification with one-day results and do not need previous cultures. Thus, the diagnostic can be made directly from the material collected from patients, providing valuable information for patient management.

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REFERENCES

1. Aheam, D.G. (1998). Yeasts pathogenic for humans. In: Kurtzman, C.P. & Fell, J.W. (eds) *The yeasts, a taxonomic study*. Amsterdam: Elsevier Science, 9-14.
2. Beighton, D.; Ludford, R.; Clark, D.T. *et al.* (1995). Use of CHROMagar Candida medium for isolation of yeasts from dental samples. *J Clin Microbiol* 33: 3025-7.
3. Budtz-Jörgensen, E. (1990). Etiology, pathogenesis, therapy, and prophylaxis of oral yeast infections. *Acta Odontol Scand* 48: 61-9.
4. Budtz-Jörgensen, E. (1990). Histopathology, immunology, and serology of oral yeast infections. Diagnosis of oral candidosis. *Acta Odontol Scand* 48: 37-43.
5. Campbell, C.K.; Holmes, A.D.; Davey, K.G.; Szekely, A.; Warnock, D.W. (1998). Comparison of a new chromogenic agar with the germ tube method for presumptive identification of *Candida albicans*. *Eur J Clin Microbiol Infec Dis* 17: 367-8.
6. Carrillo-Muñoz, A.J.; Quindós, G.; Cárdenes, C.D. *et al.* (2001). Evaluation of Chromalbicans Agar for presumptive identification of *Candida albicans*. *Rev Iberoam Micol* 18: 105-8.
7. Conceição, G.C.; Coelho, P.P.; Sousa Junior, M.A.; Pereira, M.L.; Miguel, D.S.C.G.; Toralles, M.B.P. (2005). Avaliação do teste tubo germinativo em secreção vaginal a fresco para triagem de *Candida albicans*: um teste rápido. *Newslab* 73: 106-12.
8. Fisher, F.; Cook, N. (1998). Reagents, stains, media and methods. In: Fisher, F. & Cook, N. (eds) *Fundamentals of diagnostic mycology*. Saunders, 320-340.
9. Gales, A.C.; Pfaller, M.A.; Houston, A.K. *et al.* (1999). Identification of *Candida dubliniensis* based on temperature and utilization of xylose and ß-methyl -D-glucoside as determined with the API 20C AUX and Vitek YBC systems. *J Clin Microbiol* 37: 3804-8.
10. Gatica, J.L.M.; Goic, I.B.; Martinez, M.A.T. *et al.* (2002). Utilidad del agar cromocandida para el diagnostico diferencial de *Candida* spp aisladas de muestras vaginales. *Rev Chil Obstet Ginecol* 67: 300-4.
11. Godoy, P.; Almeida, L.P.; Colombo, A.L. (2001). Identificación de *Candida albicans* utilizando el medio cromogénico Albicans ID. *Rev Iberoam Micol* 18: 197-9.
12. Holmstrup, P.; Axéll, T. (1990). Classification and clinical manifestations of oral yeast infections. *Acta Odontol Scand* 48: 57-9.
13. Hospenthal, D.R.; Beckius, M.L.; Floyd, K.L.; Horvath, L.L.; Murray, C.K. (2006). Presumptive identification of *Candida* species other than *C. albicans*, *C. krusei* and *C. tropicalis* with the chromogenic medium CHROMagar Candida. *Ann Clin Microbiol Antimicrob* 5: 1-5.
14. Hussain Qadri, S.M.; Nichols, C.W. (1978). Tube carbohydrate assimilation method for the rapid identification of clinically significant yeasts. *Med Microbiol Immunol* 165: 19-27.
15. Jabra-Rizk, M.A.; Baqui, A.; Kelley, J.I.; Falkler, W.A.Jr.; Merz, W.G.; Meiller, T.F. (1999). Identification of *Candida dubliniensis* in a prospective study of patients in the United States. *J Clin Microbiol* 37: 321-6.
16. Lacaz, C.S.; Porto, E.; Martins, J.E.C. *et al.* (2002). Leveduras de interesse médico. In: Lacaz, C.S., Porto, E., Martins, J.E.C, Vaccari-Heins, E.M., De Melo, N.T. (eds) Tratado de Micologia Médica. São Paulo: Sarvier, 123-73.
17. Mähnß, B.; Stehr, F.; Schäfer, W.; Neuber, K. (2005). Comparison of standard phenotypic assays with a PCR method to discriminate *Candida albicans* and *C. dubliniensis*. *Mycoses* 48: 55-61.
18. Mannarelli, B.M.; Kurtzman, C.P. (1998). Rapid identification of *Candida albicans* and other human pathogenic yeasts by using short oligonucleoides in a PCR. *J Clin Microbiol* 36: 1634-41.
19. Martins, C.A.P.; Koga-Ito, C.Y.; Jorge, A.O.C. (2002). Presence of *Staphylococcus* spp. and *Candida* spp. in the human oral cavity. *Braz J Microbiol* 33: 236-40.
20. Mesa, L.M.; Arcaya, N.; Cañas, O.; Machado, Y.; Calvo, B. (2004). Evaluación de los caracteres fenotípicos para diferenciar *Candida albicans* de *Candida dubliniensis*. *Rev Iberoam Micol* 21: 135-8.
21. Milan, E.P.; Zaror, L. (2004). Leveduras: identificação laboratorial. In: Sidrim, J.J.C. & Rocha, M.F.G. (eds) *Micologia Médica à luz de autores contemporâneos*. Rio de Janeiro: Guanabara Koogan, 89-101.
22. Paula, C.C. (1998). Candidíases. In: Zaitz, C., Campbell, I., Marques, A.S., Ruiz, L.R., Souza, V.M. (eds) *Compêndio de micologia médica*. Rio de Janeiro: Medsi, 99- 107.
23. Pinjon, E.; Sullivan, D.; Salkin, I.; Shanley, D.; Coleman, D. (1998). Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from *Candida albicans*. *J Clin Microbiol* 36: 2093-95.
24. Slifkin, M. (2000). Tween 80 test responses of various *Candida* species. *J Clin Microbiol* 38: 4626-28.
25. Souza, V.M. (1998). Técnicas laboratoriais utilizadas em micologia médica. In: Zaitz, C.; Campbell, I.; Marques, A.S.; Ruiz, L.R.; Souza, V.M. (eds) *Compêndio de micologia médica*. Rio de Janeiro: Medsi 51-63.
26. Stenderup, A. (1998). Oral mycology. *Acta Odontol Scand* 1990; 48: 3-10.
27. Sullivan D, Coleman D. *Candida dubliniensis*: characteristics and identification. *J Clin Microbiol* 36: 329-34.
28. Sullivan, D.; Westerneng, T.J.; Haynes, K.A.; Bennett, D.E.; Coleman, D.C. (1995). *Candida dubliniensis*:sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. *Microbiology* 141: 1507-21.
29. Tintelnot, K.; Haase, G.; Seibold, M. *et al.* (2000). Evaluation of

phenotypic markers for selection and identification of *Candida dubliniensis*. *J Clin Microbiol* 38: 1599-608.

30. Wahyuningsih, R.; Freisleben, H.J.; Sonntag, H.G.; Schnitzler, P. (2000). Simple and rapid detection of *Candida albicans* DNA in serum by PCR for diagnosis of invasive candidiasis. *J Clin Microbiol* 38: 3016-21.