Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

Journal homepage: http://www.e-jmii.com

Original Article

Comparison of Phenotypic Methods for the Identification of *Candida dubliniensis*

Julia Pasligh, Clarissa Radecke, Michael Fleischhacker*, Markus Ruhnke

Division of Oncology, Department of Medicine, Charité Universitätsmedizin Berlin, Charité Campus Mitte, Berlin, Germany.

BACKGROUND/PURPOSE: Mixed infections caused by different *Candida* species are the rule rather than the exception. The discrimination between the two closely related species *Candida albicans* and *Candida dubliniensis* is not trivial. Therefore, there is a need for fast, reliable, and inexpensive methods with high specificity for the identification and differentiation of these two *Candida* species, which are frequently detected in the oral cavities of patients with a human immunodeficiency virus infection.

METHODS: We applied several phenotypic identification methods (growth on Rice-agar, Bird-seed agar, CHROMagar® Candida, API ID 32C; growth at 42°C and 45°C) and compared them with genotyping by arbitrarily primed-polymerase chain reaction.

RESULTS: A sensitivity of 44% for the identification of *C. dubliniensis* was achieved for growth on Rice-agar, 97% for discrimination on Bird-seed agar, 95% with the assimilation profile index API ID 32C, and 97% when grown at 45°C. We found two API codes not described for *C. dubliniensis* so far. Additionally, 88% of our *C. dubliniensis* isolates assimilated palatinose, in contrast to the 1% described in the API reference manual.

CONCLUSION: According to our results, cultivation of *Candida* isolates on Bird-seed agar after screening on CHROMagar® Candida is a very sensitive, simple, and cost-effective method for discriminating *C. dubliniensis* from *C. albicans* in routine practice.

KEYWORDS: API ID 32C, AP-PCR, Bird-seed agar, *Candida dubliniensis*, CHROMagar® Candida

Introduction

Invasive candidosis, especially catheter-related infections, still present major diagnostic and therapeutic challenges to the clinician.[1,2] These infections are seen in patients in intensive care units,[3] in immunocompromised hospitalized patients,[4] and in patients with central venous catheters.[5] The definite diagnosis of these infections is not only important from an epidemiological point of view, but the strains involved differ in their susceptibility to antifungal agents.[6] Additionally, mixed infections caused by different *Candida* species can be identified from the same

*Corresponding author. Charité-Universitätsmedizin Berlin, Med. Klinik m. S. Onkologie u. Hämatologie, Charité Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany.

E-mail: michael.fleischhacker@charite.de

Article History:
Received: Sep 2, 2008
Revised: Oct 8, 2008
Accepted: Nov 7, 2008

patient upon diagnosis of an infection[7]. Therefore, the need for fast, reliable and cost-effective methods with high specificity for the identification and differentiation of *Candida* species is of utmost importance.[8–10]

Candida dubliniensis has been detected in various clinical samples, but mostly from specific risk groups such as human immunodeficiency virus (HIV)-infected individuals.[11–15] The highest prevalence of *C. dubliniensis* was found in the oral cavity of HIV-infected individuals,[11,16] in whom the rates varied between 17% and 25%.[10,17] In contrast to these figures, the incidence of *C. dubliniensis* in the oral rinses of asymptomatic healthy individuals was 3%.[18] In addition, *C. dubliniensis* is rarely isolated from clinical samples such as bronchoalveolar lavage, sputum, vaginal, anal, and intestinal samples, from heart valves or from blood cultures in non-HIV-infected individuals.[19–23]

C. dubliniensis may be found in addition to other *Candida* species when isolated from an oral cavity.[18,24] The increased clinical relevance of *C. dubliniensis* infection, and an unambiguous identification of this organism is still a problem. Using routine diagnostic methods such as the API ID 32C coding system, we frequently observed the assimilation of palatinose in our *C. dubliniensis* isolates. This is in contrast to the API ID 32C coding system for the identification of *C. dubliniensis*. Therefore, we examined a large group of molecularly identified *C. dubliniensis* and *Candida albicans* isolates for their assimilation characteristics and compared the results with other phenotypic identification methods commonly used in routine mycological laboratories.

Methods

Clinical specimens

All isolates were obtained from the oral cavity of HIV-infected patients, who have a history of recurrent oropharyngeal candidosis. The patients were asked to clean their mouth with tap water before rinsing their oral cavity with 10 mL sterile isotonic sodium chloride solution. From each sample, 100 µL were plated onto Sabouraud-Dextrose-Agar or CHROMagar® Candida for further diagnostic work-up.

Media and reference strains

Rice-agar and Bird-seed agar were purchased as ready to go plates (Heipha, Eppelheim, Germany). The ingredients for Sabouraud-Dextrose-Agar (DIFCO Laboratories, Detroit, MI, USA) and CHROMagar® Candida (Mast Diagnostica, Reinfeld, Germany) were purchased and prepared according to the manufacturer's instructions. *C. albicans* ATTC 90028 and *C. dubliniensis* CBS 8500 were used as reference strains for all experiments.

Cultivation on CHROMagar® Candida

All clinical specimens were analyzed by parallel cultivation on 2% Sabouraud-Dextrose agar, and on CHROMagar® Candida. The plates were incubated at 37°C for 2 days, and for 1 additional day at room temperature, before they were examined. The colonies were identified according to their shade of green. The dark green was used as a potential indicator for *C. dubliniensis*, whereas *C. albicans* colonies developed a light green appearance. [18,25–27] All green colonies were analyzed by arbitrarily primed-polymerase chain reaction (AP-PCR), which served as the reference method for discrimination of *C. dubliniensis* from other *Candida* species, *C. albicans* in particular.

AP-PCR

AP-PCR was originally described by Welsh and McClelland and uses one primer with an arbitrary sequence.[28] The amplification in a PCR yields a band pattern that is specific for the corresponding template DNA, able to differentiate between different species, and is therefore used as the reference method. In our experiments, the RP02 primer described by Akopyanz et al, which has the sequence 5′-GCG ATC CCC A-3′, was used.[29] The use of this primer in an AP-PCR for the characterization of *C. dubliniensis* was reported by Sullivan et al.[16] The PCR was set up in a final volume of 50 µL, with 100 ng template DNA. The cycling conditions were as follows: 5 minutes at 94°C, 5 minutes at 36°C, and 5 minutes at 72°C for 4 cycles; followed by 30 cycles of 1 minute at 94°C, 1 minute at 36°C and 2 minutes at 72°C, and a final extension at 72°C for 10 minutes. PCR products (7 µL) were loaded on a 1.2% agarose gel containing ethidium bromide and the band patterns were documented with a digital camera.

Carbohydrate assimilation assay (API ID 32C)

The API ID 32C assay was performed according to the manufacturer's recommendations (AB bioMérieux, Marcy-l'Etoile, France). Reading was performed visually after

incubating the plates for 24–72 hours at 29°C. For test interpretation, the software api-web™ 2006 (AB bioMerieux) was used.

Rice-agar

One colony was transferred onto a Rice-agar plate and incubated under reduced oxygen tension (a cover slip was put on top of the smeared cells) for 24–72 hours at room temperature. The colonies were then inspected microscopically. Under these growth conditions, *C. dubliniensis* produced many thick-walled chlamydospores at the end of short branched pseudohyphae, which appeared frequently as duplets, triplets, or in larger groups.[23]

Bird-seed agar (Cryptococcus Agar according to Staib)

One colony was transferred from Sabouraud-Dextrose agar onto Bird-seed agar and incubated at 30°C in the dark. The growth of *C. dubliniensis* was characterized microscopically by rough colony morphology, caused by pseudomycelia and chlamydospores, which could be easily differentiated from *C. albicans* cells that form smooth colonies after a 2-day cultivation on this agar as described previously.[30]

Growth at increased temperature

Yeast cells were plated onto Sabouraud-Dextrose-agar plates and incubated at 42°C and 45°C according to the protocol of Pinjon et al.[31] The plates were examined after 24 hours and 48 hours.

Results

The yeast isolates from oral samples were grown on different types of growth media (summarized in Table 1). From these isolates, 111 were identified as *C. dubliniensis* by AP-PCR. To calculate the specificity of the tests, we also analyzed 107 *C. albicans* isolates, which were tested with all methods. Interestingly, we also identified 48 non-*C. albicans*/non-*C. dubliniensis* isolates from the oral rinses, including 30 *C. glabrata*, 10 *C. krusei*, 3 *C. tropicalis*, 2 *C. lambica*, and 3 *Saccharomyces cerevisiae* (data not shown). Almost half of the *C. dubliniensis* isolates (48/111) were isolated from mixed cultures, together with up to three other *Candida* species. Since our main focus was the differentiation between *C. dubliniensis* and *C. albicans*, all other non-*C. albicans* and non-*C. dubliniensis* isolates were spared.

Species identification with AP-PCR

All green colonies grown on CHROMagar® Candida were subjected to the AP-PCR analysis. The banding patterns for all strains were reproducible and distinct from each other for all species (Figure). A total of 111 isolates were identified as *C. dubliniensis* and 107 isolates were identified as *C. albicans*. All *Candida* isolates were studied with the test methods listed above. Additionally, all *C. dubliniensis* isolates were later hybridized with the species specific Cd25 probe,[32] and these results confirmed our AP-PCR studies (data not shown).

Table 1. Summary of the data for cultivation methods used for the identification of *C. dubliniensis*[a]. The cultivation on Bird-seed agar, the API ID 32C method, and the incubation at 45°C demonstrate a high sensitivity and specificity for the identification of both species. While the majority of the *C. dubliniensis* isolates grew at 42°C (resulting in the poor sensitivity of 3%), only the strongly restricted growth of *C. dubliniensis* in comparison to *C. albicans* at 45°C (i.e. 97% sensitivity) is able to differentiate between the two *Candida* species

Cultivation method	*C. dubliniensis*–Sensitivity ($n=111$)	*C. albicans*–Specificity ($n=107$)
CHROMagar® Candida	14 (13)	102 (95)
Rice agar	49 (44)	104 (97)
Bird-seed agar	108 (97)	105 (98)
Growth at 42°C	108 (97)	107 (100)
Growth at 45°C	3 (3)	107 (100)
API ID 32C	106 (95)	106 (99)

[a]Data presented as *n* (%).

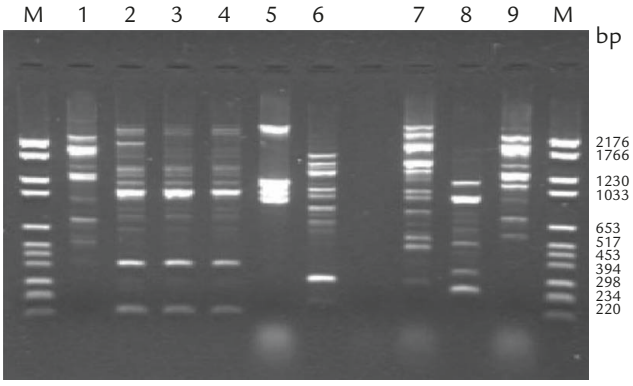

Figure. Species discrimination with arbitrarily primed-polymerase chain reaction. M = Molecular weight marker VI, Roche Applied Science (154–2176 bp, the 220 bp and 234 bp bands can not be discriminated); 1 = *C. albicans* ATCC 90028; 2 = *C. dubliniensis* CD 36; 3 = *C. dubliniensis* CBS 8500; 4 = *C. dubliniensis* CBS 8501; 5 = *C. glabrata* DSM 90050; 6 = *C. parapsilosis*; 7 = *C. krusei*; 8 = *C. kefyr* DSM 11954; 9 = *C. albicans* ATCC 76615.

Strain identification with API ID 32C assay

The following API ID 32C codes were detected: 7142.1400.15, 7142.1000.15, 7143.1400.15, 7042.1000.15, and 7142.1400.11. Of the 111 isolates identified as *C. dubliniensis* by AP-PCR, 104 revealed an API ID 32C code which had been described for this species, but seven isolates showed an API ID 32C code which identified the isolates as *C. albicans*. The API ID 32C code 7142.1400.15 was found in 92 *C. dubliniensis* isolates, code 7142.1000.15 in 11 isolates, code 7147.3400.15 in five isolates, code 7147.1400.15 in two isolates, and code 7042.1000.15 in one isolate. Interestingly API ID 32C code 7147.3400.15 and API ID 32C code 7147.1400.15, found in seven *C. dubliniensis* isolates, had previously been described for *C. albicans*.

All *C. dubliniensis* isolates assimilated D-galactose, actidione (cycloheximide) D-maltose, D-saccharose, potassium 2-ketogluconate, D-sorbitol, D-mannitol and D-glucose, while none of the isolates was able to assimilate L-arabinose, D-cellobiose, D-raffinose, D-ribose, L-rhamnose, erythritol, D-melibiose, sodium-glucuronate, D-melezitose, potassium-gluconate, levulinate, D-lactose, inositol or L-sorbose. Eighty-eight percent of our *C. dubliniensis* isolates assimilated palatinose, which is in contrast to only 1% as described in the API database. The sensitivity of the method was 95%. One of the 107 *C. albicans* isolates showed an API ID 32C code which refers to *C. dubliniensis*. The results of all API ID 32C tests are summarized in Table 2.

CHROMagar® Candida

Of 111 colonies picked from CHROMagar® Candida, 14 isolates were dark green (i.e. *C. dubliniensis*). This leads to a test sensitivity of 12.6%. From the 107 isolates identified as *C. albicans* by AP-PCR, 102 yielded colonies with a lighter shade of green (i.e. *C. albicans*).

Rice-agar results

All of the 111 isolates produced pseudomycelia and chlamydospores on rice-agar, but only 49/111 isolates showed a set of features characteristic for *C. dubliniensis*. These features included an exceedingly high number of chlamydospores, many branched and short pseudomycelia, and chlamydospores which were grouped in duplets or triplets. The sensitivity of this method for the identification of *C. dubliniensis* was 44.1%.

Cultivation on Bird-seed agar

Of the 111 isolates identified as *C. dubliniensis* according to AP-PCR, 108 demonstrated rough colonies on Bird-seed agar, leading to an assay sensitivity of 97.3%. Of the 107 *C. albicans* isolates tested, 105 showed colonies with smooth edges, leading to a test specificity of 98.1%.

Growth at elevated temperatures

Of the 111 *C. dubliniensis* isolates, 108 grew at 42°C and 3 grew at 45°C, which corresponds to a test sensitivity of 3% (growth at 42°C) and 97% (growth at 45°C). All 107 *C. albicans* isolates grew at both temperatures.

Discussion

The development of a fast, specific, and sensitive method for the identification of *Candida* species is crucial for accurate mycological diagnosis and therapy. In addition, the method should be inexpensive and easy to use for application in routine laboratories. The cultivation of yeasts on solid growth media, and its phenotypic characterization, is easy to perform and frequently the method of choice.

Growth on special agar plates, such as CHROMagar® Candida, is a very useful tool for the determination of

Table 2. Comparison of data obtained with the API ID 32C assay with the API® database from AB bioMerieux, France

Carbohydrate source	Assimilation (%)			
	Present study		API® database	
	C. dubliniensis	*C. albicans*	*C. dubliniensis*	*C. albicans*
D-Galactose	100	100	100	98
Actidione (Cycloheximide)	100	100	100	99
D-Saccharose	100	100	100	100
N-Acetyl-Glucosamine	99.5	100	90	100
Lactic acid	0.5	3.6	10	96
L-Arabinose	0	0	0	0
D-Cellobiose	0	0	0	0
D-Raffinose	0	0	0	1
D-Maltose	100	100	100	100
D-Trehalose[a]	6.6	100	0	97
Potassium 2-Ketogluconate	100	100	100	100
Methyl-αD-Glucopyranoside[a]	6.1	98.6	0	98
D-Sorbitol	100	100	100	99
D-Xylose[a]	4.7	97.1	0	98
D-Ribose	0	0	0	1
Glycerol	0.5	0	0	10
L-Rhamnose	0	0	0	0
Palatinose[b]	88.2	100	1	100
Erythritol	0	0	0	0
D-Melibiose	0	0	0	0
Sodium-Glucuronate	0	0	0	0
D-Melezitose	0	0	0	0
Potassium-Gluconate	0	0	0	2
Levulinate	0	0	0	1
D-Mannitol	100	100	100	100
D-Lactose	0	0	0	1
Inositol	0	0	0	1
D-Glucose	100	100	100	98
L-Sorbose	0	0	0	0
Glucosamine	99.5	100	60	99

[a]Best suited for the identification of *C. dubliniensis*; [b]a new observation.

whether clinical isolates grow as monocultures, or in mixed cultures.[24] Our observation that 48/111 of the *C. dubliniensis* clinical isolates obtained from oral samples of HIV-infected individuals grew as mixed cultures underscores this fact. Interestingly, in our samples, *C. dubliniensis* was most frequently associated with *C. glabrata* (25%) and not with *C. albicans* as described before.[18,24] About 75% of our patients were either continuously, or temporarily, exposed to fluconazole, which could have led to the selection of *C. glabrata*, which is less sensitive to azole antimycotics.

The aim of this paper was to identify the most specific conventional phenotypic method from a panel of commonly used testing systems that could yield comparable results as a genotyping tool for the discrimination of the closely related species *C. albicans* and *C. dubliniensis*. In our

study, all green colonies grown on CHROMagar® Candida were identified to be either *C. albicans* or *C. dubliniensis*. However, in contrast to other studies demonstrating that it is possible to use this method for a differentiation between these two species,[33] we found that the assay yields a low sensitivity for the discrimination between *C. dubliniensis* and *C. albicans*. The shade of green (either light or dark) could not reliably discriminate between the two *Candida* species.[34–38] In addition, the incubation temperature has an influence on the results obtained with this assay,[39] and *C. dubliniensis* isolates which are revived after storage at low temperatures (–20°C or less) can lose their ability to form dark green colonies, and develop a light green color only.[24] We used this assay as a screening tool to differentiate between *C. albicans* plus *C. dubliniensis* (as one group) and other *Candida spp*., and obtained a specificity of 100% (data not shown).

Another chromogenic medium, Candida ID2, has been successfully used for the detection of mixed cultures, and led to the differentiation between *C. dubliniensis* and *C. albicans* with very good sensitivity and specificity.[40] Sahand et al mixed CHROMagar® Candida with Pal's medium,[36] and observed that the two species could be easily differentiated from each other according to the color and colony morphology.

In addition to chromogenic media, there are several agar plate cultivation methods described, discriminating between *C. dubliniensis* and *C. albicans* due to abundant chlamydospore formation of the former species. In contrast, *C. albicans* produces fewer chlamydospores at the end of pseudohyphae under these growth conditions.[16,23,30,41,42] Our results demonstrate that the colony morphology of cells grown on Bird-seed agar is not only a useful method for the identification of *C. dubliniensis*, but shows the highest sensitivity and specificity of all the agar cultivation methods. In addition, cultivation on Bird-seed agar is easy to perform.

We observed a high test sensitivity for the differentiation between the two species when the cells were grown at 45°C, but not when they were grown at 42°C, which is in contrast to others who demonstrated highly restricted growth, or no growth at all, when *C. dubliniensis* was incubated at 42°C.[31,43] According to these conflicting results, the discrimination of *C. dubliniensis* from *C. albicans* by growth at increased temperature may not be reliable. Also, the method used for growth at increased temperatures requires additional equipment.

The identification of *C. dubliniensis* with the API ID 32C system is seen as a specific and sensitive assay, and one of its advantages is the ease of the determination, i.e. the appearance of turbidity in the cultivation vessels.[44] If the identification of *C. dubliniensis* depends on a single assimilation reaction, the time at which the results are determined is of crucial importance. It is known that the API ID 32C code 7143.1400.15, which identifies *C. dubliniensis,* develops after a cultivation period of 48 hours and, therefore, we determined all API ID 32C codes after this time. In our study, 104/111 *C. dubliniensis* isolates identified by AP-PCR showed an API ID 32C code which had been published before.[11,16,26] Interestingly, we also detected seven *C. dubliniensis* isolates showing two different API ID 32C codes, which have been associated with *C. albicans*. The most striking observation is the assimilation of palatinose in 88% of our *C. dubliniensis* isolates, in contrast to the 1% described in the API reference manual. Our observation is in agreement with data obtained by Sullivan et al in a small number of isolates.[16] The assimilation data for trehalose, methyl-αD-glucopyranoside and xylose show the largest differences between *C. dubliniensis* and *C. albicans* and, therefore, a panel of these carbohydrates seems to be best for the differentiation of these species. In contrast, Tintelnot et al concluded that the observed pattern for the assimilation of xylose, lactic acid and methyl-αD-glucopyranoside is not able to discriminate between *C. dubliniensis* and *C. albicans*.[43] Pincus et al found that lactic acid, methyl-αD-glucopyranoside, xylose, and trehalose are the best assimilation markers for discrimination between *C. dubliniensis* and *C. albicancs*,[26] but assimilation of lactic acid by the majority of *C. dubliniensis* isolates had also been described.[24,43] We observed that methyl-αD-glucopyranoside and xylose are assimilated by 6.1% and 4.7%, respectively, of our *C. dubliniensis* isolates, while others reports show that none of these isolates were able to metabolize these carbohydrates.[12,16,26,45] An even higher percentage of cells able to assimilate xylose (up to 57%) has also been described.[24,43,46] While, in some reports, the discriminatory power of the API ID 32C assay was described as poor,[37,38,45] our results suggest that the specificity of the API ID 32C method is rather high, and useful for the reliable identification of *C. dubliniensis*.[47]

A useful extension of the available methods for the identification of *C. dubliniensis* might be the recently developed antibody based latex agglutination test,[48–50] but apart from the higher costs of this assay, there are so far not enough data available about how this method performs in the routine laboratory.

In summary, we conclude that using the Bird-seed agar method after screening on chromogenic agars such as CHROMagar® Candida for green colonies is a very sensitive, simple and cost-effective method to discriminate *C. dubliniensis* from *C. albicans* in routine practice. Other phenotypic identification methods do not add further relevant discriminative information, or should be used in special cases only.

Acknowledgments

We would like to thank Dr Tintelnot and Mrs Antweiler (Robert Koch-Institut, Berlin, Germany) for helping us with the api-web database and Dr G. Moran (Trinity College Dublin, Dublin) for helpful discussion.

References

1. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007;20: 133–63.
2. Pappas PG. Invasive candidiasis. *Infect Dis Clin North Am* 2006; 20:485–506.
3. Kauffman CA. Candiduria. *Clin Infect Dis* 2005;41(Supp l): S371–6.
4. Sims CR, Ostrosky-Zeichner L, Rex JH. Invasive candidiasis in immunocompromized hospitalized patients. *Arch Med Res* 2005; 36:660–71.
5. Donelli G. Vascular catheter-related infection and sepsis. *Surg Infect (Larchmt)* 2006;7(Suppl):S25–7.
6. Ellepola AN, Morrison CJ. Laboratory diagnosis of invasive candidiasis. *J Microbiol* 2005;43:65–84.
7. Jensen J, Munoz P, Guinea J, Rodriguez-Creixems M, Pelaez T, Bouza E. Mixed fungemia: incidence, risk factors, and mortality in a general hospital. *Clin Infect Dis* 2007;44:e109–14.
8. Campanha NH, Neppelenbroek KH, Spolidorio DM, Spolidorio LC, Pavarina AC. Phenotypic methods and commercial systems for the discrimination between *C. albicans* and *C. dubliniensis*. *Oral Dis* 2005;11:392–8.
9. Giammanco GM, Pizzo G, Pecorella S, Distefano S, Pecoraro V, Milici ME. Identification of *Candida dubliniensis* among oral yeast isolates from an Italian population of human immunodeficiency virus-infected (HIV+) subjects. *Oral Microbiol Immunol* 2002;17: 89–94.
10. Kirkpatrick WR, Revankar SG, Mcatee RK, Lopez-Ribot JL, Fothergill AW, McCarthy DI, et al. Detection of *Candida dubliniensis* in oropharyngeal samples from human immunodeficiency virus-infected patients in North America by primary CHROMagar® Candida screening and susceptibility testing of isolates. *J Clin Microbiol* 1998;36:3007–12.
11. Sullivan D, Coleman D. *Candida dubliniensis*: an emerging opportunistic pathogen. *Curr Top Med Mycol* 1997;8:15–25.
12. Salkin IF, Pruitt WR, Padhye AA, Sullivan D, Coleman D, Pincus DH. Distinctive carbohydrate assimilation profiles used to identify the first clinical isolates of *Candida dubliniensis* recovered in the United States. *J Clin Microbiol* 1998;36:1467.
13. Alves SH, Milan EP, Branchini ML, Nishimura K, Fukushima K, Oliveira LO, et al. First isolation of *Candida dubliniensis* in Rio Grande do Sul, Brazil. *Diagn Microbiol Infect Dis* 2001;39: 165–8.
14. Al MA, Sullivan DJ, Polacheck I, Shaheen FA, Soliman O, Al HS, et al. Novel 5-flucytosine-resistant clade of *Candida dubliniensis* from Saudi Arabia and Egypt identified by Cd25 fingerprinting. *J Clin Microbiol* 2005;43:4026–36.
15. Meis JF, Ruhnke M, De Pauw BE, Odds FC, Siegert W, Verweij PE. *Candida dubliniensis* candidemia in patients with chemotherapy-induced neutropenia and bone marrow transplantation. *Emerg Infect Dis* 1999;5:150–3.
16. 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* 1995;141:1507–21.
17. Meiller TF, Jabra-Rizk MA, Baqui A, Kelley JI, Meeks VI, Merz WG, et al. Oral *Candida dubliniensis* as a clinically important species in HIV-seropositive patients in the United States. *Oral Surg Oral Med Oral Pathol Oral Radio Endod* 1999;88:573–80.
18. Coleman D, Sullivan D, Harrington B, Haynes K, Henman M, Shanley D, et al. Molecular and phenotypic analysis of *Candida dubliniensis*: a recently identified species linked with oral candidosis in HIV-infected and AIDS patients. *Oral Dis* 1997;3(Suppl 1): S96–101.
19. Fotedar R, Al Hedaithy SS. Prevalence of *Candida dubliniensis* among germ tube-positive yeasts recovered from the respiratory specimens in HIV-negative patients. *Mycoses* 2004;47:150–5.
20. Polacheck I, Strahilevitz J, Sullivan D, Donnelly S, Salkin IF, Coleman DC. Recovery of *Candida dubliniensis* from non-human immunodeficiency virus-infected patients in Israel. *J Clin Microbiol* 2000;38:170–4.
21. Kim JO, Garofalo L, Blecker-Shelly D, McGowan KL. *Candida dubliniensis* infections in a pediatric population: retrospective identification from clinical laboratory isolates of *Candida albicans*. *J Clin Microbiol* 2003;41:3354–7.
22. Carr MJ, Clarke S, O'Connell F, Sullivan DJ, Coleman DC, O'Connell B. First reported case of endocarditis caused by *Candida dubliniensis*. *J Clin Microbiol* 2005;43:3023–6.

23. Sullivan D, Coleman D. *Candida dubliniensis*: characteristics and identification. *J Clin Microbiol* 1998;36:329–34.
24. Schoofs A, Odds FC, Colebunders R, Ieven M, Goossens H. Use of specialised isolation media for recognition and identification of *Candida dubliniensis* isolates from HIV-infected patients. *Eur J Clin Microbiol Infect Dis* 1997;16:296–300.
25. Odds FC, Bernaerts R. CHROMagar® Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *J Clin Microbiol* 1994;32:1923–9.
26. Pincus DH, Coleman DC, Pruitt WR, Padhye AA, Salkin IF, Geimer M, et al. Rapid identification of *Candida dubliniensis* with commercial yeast identification systems. *J Clin Microbiol* 1999; 37:3533–9.
27. Willinger B, Hillowoth C, Selitsch B, Manafi M. Performance of candida ID, a new chromogenic medium for presumptive identification of *Candida* species, in comparison to CHROMagar® Candida. *J Clin Microbiol* 2001;39:3793–35.
28. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res* 1990;18:7213–8.
29. Akopyanz N, Bukanov NO, Westblom TU, Kresovich S, Berg DE. DNA diversity among clinical isolates of Helicobacter pylori detected by PCR-based RAPD fingerprinting. *Nucleic Acids Res* 1992;20:5137–42.
30. Staib P, Morschhauser J. Chlamydospore formation on Staib agar as a species-specific characteristic of *Candida dubliniensis*. *Mycoses* 1999;42:521–4.
31. Pinjon E, Sullivan D, Salkin I, Shanley D, Coleman D. Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from *Candida albicans*. *J Clin Microbiol* 1998;36:2093–5.
32. Joly S, Pujol C, Rysz M, Vargas K, Soll DR. Development and characterization of complex DNA fingerprinting probes for the infectious yeast *Candida dubliniensis*. *J Clin Microbiol* 1999;37:1035–44.
33. Jabra-Rizk MA, Brenner TM, Romagnoli M, Baqui AA, Merz WG, Falkler WA, Jr., et al. Evaluation of a reformulated CHROMagar® Candida. *J Clin Microbiol* 2001;39:2015–6.
34. Momani OM, Qaddoomi A. Identification of *Candida dubliniensis* in a diagnostic microbiology laboratory. *East Mediterr Health J* 2005;11:366–71.
35. Hospenthal DR, Beckius ML, Floyd KL, Horvath LL, Murray CK. Presumptive identification of *Candida* species other than *C. albicans*, *C. krusei*, and *C. tropicalis* with the chromogenic medium CHROMagar® Candida. *Ann Clin Microbiol Antimicrob* 2006;5:1.
36. Sahand IH, Moragues MD, Eraso E, Villar-Vidal M, Quindos G, Ponton J. Supplementation of CHROMagar® Candida medium with Pal's medium for rapid identification of *Candida dubliniensis*. *J Clin Microbiol* 2005;43:5768–70.
37. Mesa LM, Arcaya N, Canas O, Machado Y, Calvo B. Phenotypic evaluation to differentiate *Candida albicans* from *Candida dubliniensis*. *Rev Iberoam Micol* 2004;21:135–8.
38. Mahnss B, Stehr F, Schafer W, Neuber K. Comparison of standard phenotypic assays with a PCR method to discriminate *Candida albicans* and *C. dubliniensis*. *Mycoses* 2005;48:55–61.
39. Odds FC, Davidson A. "Room temperature" use of CHROMagar® Candida. *Diagn Microbiol Infect Dis* 2000;38:147–50.
40. Eraso E, Moragues MD, Villar-Vidal M, Sahand IH, Gonzalez-Gomez N, Ponton J, et al. Evaluation of the new chromogenic medium Candida ID 2 for isolation and identification of *Candida albicans* and other medically important *Candida* species. *J Clin Microbiol* 2006;44:3340–5.
41. Al Mosaid A, Sullivan D, Salkin IF, Shanley D, Coleman DC. Differentiation of *Candida dubliniensis* from *Candida albicans* on staib agar and caffeic acid-ferric citrate agar. *J Clin Microbiol* 2001;39:323–7.
42. Sancak B, Colakoglu S, Acikgoz ZC, Arikan S. Incubation at room temperature may be an independent factor that induces chlamydospore production in *Candida dubliniensis*. *Diagn Microbiol Infect Dis* 2005;52:305–9.
43. Tintelnot K, Haase G, Seibold M, Bergmann F, Staemmler M, Franz T, et al. Evaluation of phenotypic markers for selection and identification of *Candida dubliniensis*. *J Clin Microbiol* 2000; 38:1599–608.
44. Caniaux I, Villard J, Gayral JP. Détection automatisée de l'assimilation des sources de carbone par les levres. *Bulletin de la Société Francaise de Mycologie Medicale* 1985;14:269–76.
45. Alves SH, Horta JA, Milan EP, Scheid LA, Vainstein MH, Santurio JM, et al. Carbohydrate assimilation profiles of Brazilian *Candida dubliniensis* isolates based on ID 32C system. *Rev Inst Med Trop Sao Paulo* 2005;47:109–11.
46. Kurzai O, Korting HC, Harmsen D, Bautsch W, Molitor M, Frosch M, et al. Molecular and phenotypic identification of the yeast pathogen *Candida dubliniensis*. *J Mol Med* 2000;78: 521–9.
47. Xu J, Millar BC, Moore JE, McClurg R, Walker MJ, Evans J, et al. Comparison of API20C with molecular identification of *Candida spp* isolated from bloodstream infections. *J Clin Pathol* 2002; 55:774–7.
48. Marot-Leblond A, Beucher B, David S, Nail-Billaud S, Robert R. Development and evaluation of a rapid latex agglutination test using a monoclonal antibody to identify *Candida dubliniensis* colonies. *J Clin Microbiol* 2006;44:138–42.
49. Sahand IH, Moragues MD, Robert R, Quindos G, Ponton J. Evaluation of Bichro-Dubli Fumouze to distinguish *Candida dubliniensis* from *Candida albicans*. *Diagn Microbiol Infect Dis* 2006; 55:165–7.
50. Chryssanthou E, Fernandez V, Petrini B. Performance of commercial latex agglutination tests for the differentiation of *Candida dubliniensis* and *Candida albicans* in routine diagnostics. *APMIS* 2007;115:1281–4.