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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

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Candida dubliniensis **has been associated with oropharyngeal candidiasis in patients infected with human immunodeficiency virus (HIV).** ***C. dubliniensis*** **isolates may have been improperly characterized as atypical** ***Candida albicans*** **due to the phenotypic similarity between the two species. Prospective screening of oral rinses from 63 HIV-infected patients detected atypical dark green isolates on CHROMagar Candida compared to typical** ***C. albicans*** **isolates, which are light green. Forty-eight atypical isolates and three control strains were characterized by germ tube formation, differential growth at 37, 42, and 45°C, identification by API 20C, fluorescence, chlamydoconidium production, and fingerprinting by Ca3 probe DNA hybridization patterns. All isolates were germ tube positive. Very poor or no growth occurred at 42°C with 22 of 51 isolates. All 22 poorly growing isolates at 42°C and one isolate with growth at 42°C showed weak hybridization of the Ca3 probe with genomic DNA, consistent with** ***C. dubliniensis*** **identification. No** ***C. dubliniensis*** **isolate but only 18 of 28** ***C. albicans*** **isolates grew at 45°C. Other phenotypic or morphologic tests were less reliable in differentiating** ***C. dubliniensis*** **from** ***C. albicans*****. Antifungal susceptibility testing showed fluconazole MICs ranging from ≤0.125 to 64 μg/ml. Two isolates were resistant to fluconazole (MIC, 64 μg/ml) and one strain was dose dependent susceptible (MIC, 16 μg/ml). MICs of other azoles, including voriconazole, itraconazole, and SCH 56592, for these isolates were lower.** ***C. dubliniensis*** **was identified in 11 of 63 (17%) serially evaluated patients. Variability in phenotypic characteristics dictates the use of molecular and biochemical techniques to identify** ***C. dubliniensis*****. This study identifies** ***C. dubliniensis*** **in HIV-infected patients from San Antonio, Tex., and shows that** ***C. dubliniensis*** **is frequently detected in those patients by using a primary CHROMagar screen.**

Oropharyngeal candidiasis (OPC) continues to be a common opportunistic infection in patients infected with human immunodeficiency virus (HIV) or who have AIDS (8–11). *Candida albicans* is the most common causative agent; however, other species, such as *C. glabrata*, *C. tropicalis*, and *C. krusei* have become increasingly prominent pathogens (10–12, 19). A recently described species, *C. dubliniensis*, has been associated with the presence of OPC in HIV-infected patients and has also been recovered from healthy, non-HIV-infected patients (1, 16–18). *C. dubliniensis* has been reported from patients worldwide (17) including the United States (13), but the prevalence and antifungal susceptibility of this species in patients from the United States has not been reported.

C. dubliniensis is closely related to *C. albicans*, and many *C. dubliniensis* isolates may have been improperly characterized as atypical *C. albicans* due to the phenotypic similarity of the two species (4, 15, 16). For example, both species produce germ tubes and chlamydoconidia, typically used to identify *C. albicans*. Variation in the ability to differentiate the two species by using phenotypic characteristics has led researchers to investigate more reliable methods for proper differentiation of these isolates (15, 17) and dictates the use of a combination of mycological, biochemical, and molecular techniques to unequivocally identify *C. dubliniensis* (2, 7, 13, 16, 18).

C. dubliniensis has been reported to produce a distinctive dark green color on CHROMagar Candida (15). However, this atypical color may not persist after serial passage of the organism and may be less useful for identifying isolates (16). In the present study, atypical yeast isolates were prospectively identified by serial evaluation of primary CHROMagar Candida cultures from HIV-infected patients with OPC in San Antonio, Tex. These atypical isolates were then screened for *C. dubliniensis* by using phenotypic and genotypic assays, and the identification and prevalence of *C. dubliniensis* in this population was established.

Little information is available on susceptibility patterns of clinical isolates of *C. dubliniensis*. Fluconazole susceptibility testing by a modified microbroth dilution method on 20 *C. dubliniensis* isolates, including 15 oral isolates from HIV-infected patients, demonstrated MICs ranging from ≤1 to 32 μg/ml, with MICs of ≤1 μg/ml being determined for 80% of isolates tested (4). In this study antifungal susceptibilities to amphotericin B and four azole agents were tested for one *C. dubliniensis* type strain and 22 clinical isolates of *C. dubliniensis* from HIV-infected patients with oropharyngeal candidiasis.

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MATERIALS AND METHODS

Specimen collection. Clinical samples were obtained from HIV-infected patients enrolled in a longitudinal study of OPC at the University of Texas Health Science Center at San Antonio and the South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio, Tex. (9, 10). These patients had advanced AIDS with mean CD4 cell counts of $<50/mm^3$. Samples were obtained weekly during therapy and quarterly as surveillance cultures by patients swishing and spitting 10 ml of normal saline to be used for culture (6, 9). One-hundred microliters of swish solution was plated on media with and without fluconazole at concentrations of 8 and 16 μg/ml and incubated at 30°C for 48 h before growth was assessed. CHROMagar Candida (CHROMagar Company, Paris, France) with fluconazole was used to improve detection of non-*C. albicans* species and resistant isolates (6). Colonies with a light-green color on CHROMagar Candida were considered typical *C. albicans*, whereas those with a dark-green color were considered atypical *C. albicans* (10, 11). Isolate color was recorded, and three to five yeast colonies from each culture were stored on Sabouraud dextrose slants (BBL, Cockeysville, Md.) at −70°C. Forty-eight atypical clinical isolates from 23 patients were detected and were further characterized by phenotypic and genotypic identification tests.

Reference strains. Three control strains, including the *C. albicans* reference strain 3153A, generously provided by D. R. Soll (University of Iowa, Iowa City, Ia), the *C. dubliniensis* type strain NCPF 3949, kindly provided by J. R. Naglik (UMDS Guy's Hospital, London, England), and a laboratory control strain, fluconazole-resistant *C. albicans* 279, were included in this study.

Phenotypic identification tests. All dark green atypical *C. albicans* isolates were further characterized by analysis of germ tube formation in human serum at 37°C for 3 h, growth at 37, 42, and 45°C on Sabouraud dextrose agar (BBL), identification by API 20C (bioMérieux, Marcy-l'Etoile, France), fluorescence on methyl-blue 93 Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) with illumination at 365 nm, and degree of chlamydoconidium production on cornmeal agar (Difco Laboratories, Detroit, Mich.) supplemented with 1% Tween 80 (Sigma, St. Louis, Mo.).

Genotypic identification tests. Organism identification was further investigated by restriction fragment length polymorphism (RFLP) and DNA fingerprinting with the moderately repetitive Ca3 probe, which as previously described is specific for *C. albicans* (14). Chromosomal DNA from each isolate was prepared in agarose plugs and separated by pulsed-field gel electrophoresis (Bio-Rad, Hercules, Calif.). Pulsed-field gel electrophoresis was performed using ramped switch times of 5 to 35 s for 18 h at 3 V/cm. RFLP patterns were obtained by digestion of chromosomal DNA with *Eco*RI (Boehringer-Mannheim, Indianapolis, Ind.). Digested DNA present in the RFLP gels was transferred to nylon membranes (Nytran; Schleicher and Schuell, Keene, N.H.) and hybridized under stringent conditions with a Ca3 probe radioactively labeled by random priming (Random Primers DNA Labeling System; GibcoBRL, Gaithersburg, Md.) (14). After being washed, the membranes were exposed to autoradiography film (Du Pont, Wilmington, Del.). Films were scanned and imported to Adobe Photoshop (Adobe Systems, Mountain View, Calif.).

Antifungal susceptibility testing. Susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards macrobroth method M27-A (5). Inocula were standardized at 85% transmittance at 530 nm by using a spectrophotometer. Antibiotic medium 3 (Difco Laboratories) was used for testing amphotericin B (Bristol-Myers Squibb, Princeton, N.J.) (0.03 to 16 μg/ml), and RPMI-1640 buffered with morpholinepropanesulfonic acid (MOPS) (American Biorganics, Niagara Falls, N.Y.) was used for testing fluconazole (Pfizer, Inc., New York, N.Y.) (0.125 to 64 μg/ml), itraconazole (Janssen Pharmaceutica, Beerse, Belgium) (0.015 to 16 μg/ml), voriconazole (Pfizer, Inc., Sandwich, United Kingdom) (0.125 to 64 μg/ml), and SCH 56592 (Schering Plough, Kenilworth, N.J.) (0.03 to 16 μg/ml). Amphotericin B and fluconazole were prepared from pharmaceutical solutions in sterile water. Itraconazole, voriconazole, and SCH 56592 were prepared from powder to stock solutions of 1,600 μg/ml in 100% polyethylene glycol. Tubes were incubated at 35°C and read at 24 and 48 h.

RESULTS

Twenty-three of 63 (37%) HIV-infected patients had atypical, dark green colonies on CHROMagar Candida in 48 episodes of OPC. Colonies with light green color (Fig. 1, left side of plate) were considered typical for *C. albicans*, whereas those with a dark green color on primary isolation were initially considered atypical *C. albicans* (Fig. 1, right side of plate) and were subjected to further investigation.

Results of other phenotypic and genotypic identification assays are shown in Table 1. All isolates produced germ tubes.

FIG. 1. Color comparison of typical *C. albicans* (left) and atypical *C. albicans* (right) on CHROMagar Candida following 48 h of incubation at 30°C.

Abundant chlamydoconidium production was seen in only 17 of 51 (33%) isolates. Overall, of the isolates ultimately identified as *C. dubliniensis* (see below), 16 of 23 (70%) had abundant chlamydoconidium production, indicative of *C. dubliniensis*, while only 1 of 28 (4%) of those identified as *C. albicans* had abundant chlamydoconidium production (Table 1). With API 20C, 25 of 28 (89%) *C. albicans* isolates were identified as *C. albicans*, while 8 of 23 (35%) *C. dubliniensis* isolates were misidentified as *C. albicans* (Table 1). Xylose utilization was negative in all 23 isolates identified as *C. dubliniensis* and was positive in all 28 *C. albicans* strains. A total of 42 of 51 (82%) isolates were positive for fluorescence (with illumination at 365 nm) on methyl-blue 93 Sabouraud dextrose agar. Fluorescence was seen in 25 of 28 (89%) *C. albicans* isolates and in 17 of 23 (74%) *C. dubliniensis* isolates (Table 1).

Differential temperature growth is shown in Table 1. Fifty of 51 (98%) isolates grew well at 37°C. Very poor or no growth occurred at 42 and 45°C in 22 of 51 (43%) and 33 of 51 (65%) isolates, respectively. One isolate grew poorly at all temperature settings. Twenty-eight of 29 (97%) isolates that grew well at 42°C showed good hybridization of the moderately repetitive Ca3 probe with genomic DNA, indicative of *C. albicans* (Fig. 2). All 22 isolates with negligible growth at 42°C and 1 strain with growth at 42°C showed weak hybridization of genomic DNA with the Ca3 probe, consistent with *C. dubliniensis* identification. All 23 *C. dubliniensis* strains demonstrated negligible or no growth at 45°C, but growth was seen in only 18 of 28 (64%) *C. albicans* isolates.

Overall, 22 clinical *C. dubliniensis* isolates were identified from 11 of 63 (17%) serially evaluated patients. *C. dubliniensis* was associated with infection in 16 of 22 episodes but was detected on surveillance cultures from the remaining 6 episodes. Isolation occurred after 2.9 ± 0.2 episodes (mean ± standard error; range, 1 to 8), although isolation occurred in the initial episode for five patients. Isolation of *C. dubliniensis* occurred in mixed yeast cultures including *C. albicans* and other yeasts in 11 of 22 (50%) cases (Table 2).

TABLE 1. Phenotypic and genotypic characteristics of clinical isolates identified as atypical *C. albicans* upon primary isolation on CHROMagar Candida[a]

Isolate no.	Chlamydoconidium formation	API-20C identification	Xylose utilization	Fluorescence on methyl-blue (18 h)	Growth after 72 h at:			Ca3 probe hybridization[b]	Identification
					37°C	42°C	45°C		
903	+	No ID	−	+	+	−	−	Poor	*C. dubliniensis*
1029	−	No ID	−	+	+	−	−	Poor	*C. dubliniensis*
1154	+	No ID	−	+	+	−	−	Poor	*C. dubliniensis*
1231	+	No ID	−	+	+	−	−	Poor	*C. dubliniensis*
1232	+	*C. albicans*	−	+	+	−	−	Poor	*C. dubliniensis*
1439	+	No ID	−	−	−	−	−	Poor	*C. dubliniensis*
1478	−	No ID	−	+	+	+	−	Poor	*C. dubliniensis*
1680	+	*C. albicans*	−	+	+	−	−	Poor	*C. dubliniensis*
1721	−	No ID	−	+	+	−	−	Poor	*C. dubliniensis*
1770	+	*C. albicans*	−	+	+	−	−	Poor	*C. dubliniensis*
1869	+	*C. albicans*	−	+	+	−	−	Poor	*C. dubliniensis*
2419	+	No ID	−	+	+	−	−	Poor	*C. dubliniensis*
2696	−	No ID	−	+	+	−	−	Poor	*C. dubliniensis*
2929	−	*C. albicans*	−	+	+	−	−	Poor	*C. dubliniensis*
3163	+	No ID	−	−	+	−	−	Poor	*C. dubliniensis*
3418	+	No ID	−	−	+	−	−	Poor	*C. dubliniensis*
3698	+	No ID	−	−	+	−	−	Poor	*C. dubliniensis*
3744	−	No ID	−	+	+	−	−	Poor	*C. dubliniensis*
3949[c]	+	*C. albicans*	−	+	+	−	−	Poor	*C. dubliniensis*
3973	−	*C. albicans*	−	+	+	−	−	Poor	*C. dubliniensis*
4516	+	*C. albicans*	−	+	+	−	−	Poor	*C. dubliniensis*
4572	+	No ID	−	−	+	−	−	Poor	*C. dubliniensis*
4712	+	No ID	−	−	+	−	−	Poor	*C. dubliniensis*
279[c]	−	*C. albicans*	+	+	+	+	−	Good	*C. albicans*
1019	−	*C. albicans*	+	+	+	+	−	Good	*C. albicans*
1582	−	*C. albicans*	+	+	+	+	−	Good	*C. albicans*
1593	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
1683	−	*C. albicans*	+	+	+	+	−	Good	*C. albicans*
2144	−	No ID	+	−	+	+	+	Good	*C. albicans*
2579	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
2759	−	*C. albicans*	+	+	+	+	−	Good	*C. albicans*
3153A[c]	−	*C. albicans*	+	+	+	+	−	Good	*C. albicans*
3505	−	*C. albicans*	+	+	+	+	−	Good	*C. albicans*
3510	−	*C. albicans*	+	+	+	+	−	Good	*C. albicans*
3748	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
3777	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
3786	−	No ID	+	+	+	+	+	Good	*C. albicans*
4016	−	No ID	+	−	+	+	−	Good	*C. albicans*
4077	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4105	+	*C. albicans*	+	−	+	+	+	Good	*C. albicans*
4221	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4350	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4369	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4372	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4413	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4487	−	*C. albicans*	+	+	+	+	−	Good	*C. albicans*
4665	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4667	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4724	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4730	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*
4812	−	*C. albicans*	+	+	+	+	+	Good	*C. albicans*

[a] "+" and "−" indicate presence or absence of characteristic, respectively, except in the case for growth at 72 h, where such symbols indicate normal and none to slight, respectively.
[b] Poor Ca3 probe hybridization is consistent with *C. dubliniensis* identification; good Ca3 probe hybridization is consistent with *C. albicans* identification.
[c] Lab control strain.

Antifungal susceptibility testing was performed on the 22 isolates plus a *C. dubliniensis* type strain according to the National Committee for Clinical Laboratory Standards method M-27A with amphotericin B, fluconazole, itraconazole, voriconazole, and SCH 56592 (Table 2). Isolates were all susceptible to amphotericin B, with the MIC for 16 of 23 isolates being 0.125 μg/ml (range 0.125 to 0.5 μg/ml) at 48 h. The azoles demonstrated a wide range of MICs. Fluconazole MICs ranged from ≤0.125 to 64 μg/ml. The results for the other azoles were as follows: SCH 56592, MICs of ≤0.03 to 0.5 μg/ml; itraconazole, MICs of ≤0.03 to 1 μg/ml; and voriconazole, MICs of ≤0.125 to 2 μg/ml. Two isolates were resistant to fluconazole (MICs of 64 μg/ml at 48 h), and one showed dose-dependent susceptibility (MIC = 16 μg/ml). MICs of the other azoles for these isolates were lower: SCH 56592, MICs of 0.25 to 0.5 μg/ml; itraconazole, MICs of 0.25 to 1 μg/ml; and voriconazole, MICs of 0.5 to 2 μg/ml.

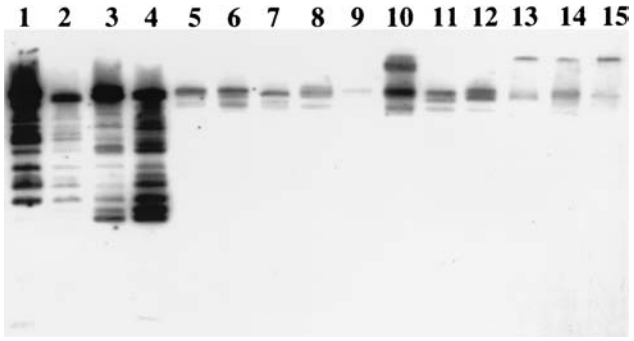

FIG. 2. Southern hybridization fingerprinting of restriction endonuclease *Eco*RI-digested whole-cell DNA with ^{32}P-labeled moderately repetitive Ca3 probe. Fingerprinting patterns of *C. albicans* reference strains 3153A and 279 (lanes 1 and 2), *C. dubliniensis* reference strain 3949 (lane 8), clinical isolates 1683, 4413, 1680, 1770, and 4516 (lanes 3 to 7, respectively) and clinical isolates 1439, 2419, 1231, 2696, 3163, 1154, 1478 (lanes 9 to 15, respectively) are shown. Lanes 3 and 4 show hybridization patterns typical of *C. albicans*; lanes 5 to 7 and 9 to 15 show poor hybridization patterns characteristic of *C. dubliniensis*.

DISCUSSION

Although *C. dubliniensis* has been reported in association with oropharyngeal infection from patients in the United States (13), the prevalence and antifungal susceptibility of *C. dubliniensis* from patients in the United States has not been reported. In this study, the detection and prevalence of *C. dubliniensis* was established in a cohort of patients from San Antonio, Tex., with recurrent OPC and advanced AIDS who were serially evaluated. The prevalence of *C. dubliniensis* in this population was similar to that reported in HIV-infected patients in Ireland (32%) and confirms the widespread geographic distribution of this species (1, 17). However, it should be noted that the 22 clinical *C. dubliniensis* isolates identified in this study represent <1% of the more than 5,500 isolates collected from these 63 patients in this ongoing serial evaluation (9, 10), suggesting that these isolates appear to remain uncommon in HIV-infected patients with oropharyngeal infection.

Isolates were screened for further study by selecting for atypical, dark green color on primary CHROMagar Candida cultures. Variable results using CHROMagar Candida to identify *C. dubliniensis* have been reported (15, 16), which may be due to the fact that color may vary after serial passage. In this study, 22 of 48 (46%) clinical isolates initially considered to be atypical *C. albicans* were subsequently identified as *C. dubliniensis*. Since only atypical *C. albicans* isolates were screened for this study, it is possible that the reported prevalence could be an underestimate of the true prevalence in this population. However, more than 50 typical light green CHROMagar Candida isolates were confirmed to be *C. albicans* by phenotypic assays and the Ca3 probe in other studies (data not shown). These results suggest that primary isolation of atypical *C. albicans* colonies on chromogenic media could be used as a screening assay for additional identification studies.

Phenotypic assays demonstrated variable utility in identifying *C. dubliniensis*, but trends appear to exist. The use of chlamydoconidium production to identify *C. dubliniensis* isolates produced highly variable results and did not fully distinguish these isolates. However, of the isolates identified as *C. dubliniensis*, 16 of 23 (70%) had abundant chlamydoconidium production, indicative of *C. dubliniensis*, whereas only 1 of 28 (4%) of those identified as *C. albicans* had abundant chlamydoconidium production. In addition, most isolates tested were positive for fluorescence, which stands in contrast to data presented by Schoofs et al. (15) where all *C. dubliniensis* isolates failed to fluoresce. In the present study, 6 of 23 (29%) of the isolates showing poor Ca3 hybridization did not fluoresce, but of the isolates with Ca3 probe banding patterns indicative of *C.*

TABLE 2. Antifungal susceptibility of *C. dubliniensis* isolates from HIV-infected patients

Patient	Isolate	MICs of the following compound at 24/48 h (μg/ml):					No. of OPC episodes	Prior fluconazole treatment (g)	Other species present
		Amphotericin B	Fluconazole	Itraconazole	Voriconazole	SCH 56592			
2	1029	.125/.25	.25/.5	≤.015/.03	≤.125/≤.125	≤.03/≤.03	2	0.7	*C. albicans*
2	1231	.06/.25	.25/.5	≤.015/.03	≤.125/≤.125	≤.03/≤.03	Surveillance[a]	1.4	None
2	1232	.125/.125	1/2	.06/.125	≤.125/≤.125	≤.03/≤.03	Surveillance	1.4	None
9	1439	.25/.25	1/1	≤.015/.03	≤.125/≤.125	.125/.125	4	3.5	None
21	903	.125/.25	≤.125/.≤.125	≤.015/.03	≤.125/≤.125	≤.03/.06	1	0.7	*C. glabrata*
26	1154	.125/.25	1/2	.06/.125	≤.125/≤.125	.25/.25	1	None	*C. albicans*
32	1680	.06/.125	≤.125/.25	.03/.06	≤.125/≤.125	≤.03/≤.03	2	2.1	None
32	1721	.06/.125	≤.125/1	.06/.06	≤.125/≤.125	≤.03/≤.03	2	3.5	None
32	1869	.125/.125	≤.125/.5	.06/.06	≤.125/≤.125	≤.03/≤.03	3	4.9	None
36	1478	.25/.5	.25/.5	.06/.06	≤.125/≤.125	.125/.25	1	None	None
46	1770	.06/.125	≤.125/≤.125	≤.015/.03	≤.125/≤.125	≤.03/≤.03	1	0.7	*C. krusei*
46	2419	.06/.125	≤.125/.25	≤.015/≤.015	≤.125/≤.125	≤.03/.06	4	2.8	*C. krusei*
46	3744	.06/.125	≤.125/.25	.03/.06	≤.125/≤.125	.125/.125	Surveillance	1.4	*C. krusei*
46	3973	≤.03/.125	≤.125/≤.125	≤.015/.03	≤.125/≤.125	.125/.125	8	24.2	*C. krusei*
59	4572	≤.03/.125	32/64	.5/.5	1/1	.125/.25	6	10	None
60	2696	.06/.25	.25/.5	.03/.06	≤.125/≤.125	.06/.25	1	None	None
60	3163	.06/.125	≤.125/.25	≤.015/.03	≤.125/≤.125	.016/.125	4	3	None
60	3418	.06/.125	.25/.5	.03/.06	≤.125/≤.125	.125/.25	5	3.8	None
61	2929	.06/.125	.25/1	≤.015/.03	≤.125/≤.125	≤.03/.125	2	2.3	*C. krusei*
64	3698	≤.03/.125	16/16	.125/.25	.25/.5	.25/.5	Surveillance	3.2	*C. glabrata*
64	4516	≤.03/.125	4/4	.03/.125	≤.125/.25	≤.03/≤.03	Surveillance	11.2	*C. glabrata*
64	4712	.06/.125	32/64	.5/1	1/2	.125/.25	Surveillance	12	*C. glabrata*
	3949[b]	.06/.125	≤.125/.25	.06/.06	≤.125/≤.125	≤.03/≤.03			

[a] Surveillance culture was used.
[b] Lab strain.

albicans, only 3 of 28 (11%) did not fluoresce. Variability in the results obtained by using these phenotypic methods limit their utility for identifying these isolates.

API 20C was useful only when identification was confirmed as *C. albicans*. Fifteen of 23 (65%) of the isolates subsequently identified as *C. dubliniensis* were assigned "no ID" by API 20C, yet only 3 of 28 (11%) isolates identified as *C. albicans* by the Ca3 probe hybridization were not identified by API 20C. However, as reported by Salkin and colleagues previously (13), xylose assimilation was present in all 28 *C. albicans* isolates and in none of the 23 *C. dubliniensis* isolates. However, expense prohibits the routine use of API 20C in identifying germ tube-positive yeasts.

Differential temperature was useful in distinguishing *C. albicans* from *C. dubliniensis*. At 42 and 45°C, *C. dubliniensis* demonstrated negligible or no growth. All isolates identified as *C. albicans* grew well at 37 or 42°C. All twenty-two (100%) isolates with negligible growth at 42°C and one with growth at 42°C were confirmed as *C. dubliniensis* by using the Ca3 probe. However, in this study it should be noted that *C. albicans* did not grow as well at 42°C and that slight growth of *C. dubliniensis* can occur at 42°C, although growth was significantly reduced compared to that of *C. albicans*.

Pinjon and colleagues showed that all *C. dubliniensis* strains tested failed to grow at 45°C compared to growth of all but one strain of *C. albicans* at that temperature (7). However, in the present study, while none of the *C. dubliniensis* strains grew at 45°C, only 18 of 28 (64%) *C. albicans* isolates grew at that temperature, indicating that as a screening method, 45°C would falsely suggest the presence of *C. dubliniensis* in some cases.

Identification of *C. albicans* and *C. dubliniensis* was confirmed with fingerprinting of genomic DNA with the Ca3 probe. Twenty-eight of 29 isolates that grew well at 42°C had distinct hybridization patterns characteristic of *C. albicans* while 22 of 22 (100%) of the isolates that grew poorly at 42°C hybridized poorly and in a distinctive pattern, indicative of *C. dubliniensis*. Probes have recently been developed which are specific for *C. dubliniensis*, although use of these methods would be limited to a research setting (2, 3).

Although the pathogenicity of this yeast has not been established, *C. dubliniensis* was associated with clinical oropharyngeal infection in 16 of 22 (73%) cases and was present as the sole yeast isolate in 11 of 22 (50%) episodes. Risk factors and significance of this organism in oropharyngeal disease have also not been established. Isolation frequently occurred after several episodes of oropharyngeal infection but was found in the initial episode of oropharyngeal infection in five patients. Most strains remained susceptible to fluconazole although resistance or dose-dependent susceptibility occurred for three isolates. Increased susceptibility for the resistant strains was seen with itraconazole and the newer azoles, voriconazole and SCH 56592. Some isolates are resistant to fluconazole and may be more susceptible to other azole compounds.

In summary, the presence of atypical colonies on primary CHROMagar Candida cultures was used to identify isolates for further study. Differential temperature screening was extremely useful as a simple and inexpensive method for presumptively identifying *C. dubliniensis* from atypical *C. albicans* isolates for additional molecular studies (7). Twenty-two clinical isolates from 48 episodes of OPC in 63 patients were confirmed as *C. dubliniensis* by using a primary CHROMagar screening method followed by differential temperature growth and molecular studies to establish identity. Late-stage HIV-infected patients were found to have a *C. dubliniensis* prevalence rate of 17% in oropharyngeal samples by this approach. These isolates may show decreased susceptibility to fluconazole and may have increased susceptibility to newer azoles. Additional studies are needed to establish the epidemiology and significance of this organism in recurrent OPC.

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