

MEDICAL DIAGNOSTIC LABORATORIES, L.L.C.

Candida dubliniensis, *Candida kefyr*, *Candida krusei*, *Candida lusitanae*, and *Candida utilis*

The genus *Candida* includes approximately 154 species. Among these, eight are most frequently isolated in human infections. While *Candida albicans* is the most abundant and significant species, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida kefyr*, *Candida krusei*, *Candida dubliniensis* and *Candida lusitanae* are also isolated as causative agents of candidiasis infections (Table 1).

Candida species other than *C. albicans* have emerged as causes of human candidiasis. The variety of non-*albicans* *Candida* species involved in human pathology, their rising contribution to invasive infections and the unusual antifungal susceptibility profiles of some of these species makes their identification at the species level essential for epidemiological investigations and for optimizing therapy and patient management. The causes of this change in epidemiology are not entirely clear (1). The use of fluconazole in prophylactic regimens for severely immunosuppressed patients has been strongly associated with changes in the etiology of candidemia in this population (2, 3, 4) (Table 2). However, the same phenomenon has occurred in populations not exposed to this agent (5).

Candida dubliniensis

Candida dubliniensis was first described as a novel species in 1995. This organism is very closely related to the important human yeast pathogen,

Candida albicans. However, despite the very close phylogenetic relationship between



C. albicans and *C. dubliniensis* and the fact that they share a large number of phenotypic traits, epidemiological and virulence model data indicate that they differ in pathogenicity and pharmacology. *C. dubliniensis* has been implicated as an agent of oral candidiasis in HIV-positive persons but has also been recovered from HIV-negative persons with clinical signs of oral

candidiasis and from the genital tract of some women with vaginitis (6, 7, 8). First isolated from AIDS patients in Dublin, Ireland, *C. dubliniensis* has a worldwide distribution (9, 10). In a study of Irish subjects, *C. dubliniensis* was recovered from the oral cavities of 27% of HIV-infected individuals and 32% of AIDS patients presenting with symptoms of oral candidosis (7). The majority of *C. dubliniensis* clinical isolates tested to date are susceptible to fluconazole (MIC range, 0.125 to 1.0 g/ml) and to other commonly used

Table 1. *Candida* species commonly causing Candidiasis.

Species	Frequency (%)
<i>Candida albicans</i>	50
<i>Candida tropicalis</i>	15-30
<i>Candida glabrata</i>	15-30
<i>Candida parapsilosis</i>	15-30
<i>Candida krusei</i>	~2
<i>Candida lusitanae</i>	~1
<i>Candida dubliniensis</i>	~1
<i>Candida kefyr</i>	~1

antifungal drugs including ketoconazole, itraconazole and amphotericin B (11). A study by Moran *et al.*, reported an occurrence of fluconazole resistance in 20% of oral isolates of *C. dubliniensis* recovered from AIDS patients who had been treated previously with fluconazole. Furthermore, sequential exposure of fluconazole-susceptible clinical isolates of *C. dubliniensis* to increasing concentrations of fluconazole in agar medium resulted in the recovery of derivatives that expressed a stable fluconazole-resistant phenotype (12). It has been suggested that the ability of *C. dubliniensis* to rapidly develop resistance to fluconazole may contribute to its ability to successfully colonize the oral cavities of HIV-infected individuals who are receiving long-term therapy with this compound (12). Furthermore, this may, at least in part, explain the apparent recent emergence of this organism.

Molecular mechanisms of azole resistance in *C. dubliniensis* include increased drug efflux, modifications of the target enzyme and alterations in the ergosterol biosynthetic pathway (13). Its potential to cause deep or disseminated candidiasis is not known, largely because *C. dubliniensis* has rarely been isolated from sterile body sites (14); however, the phenotypic characteristics the organism shares with *C. albicans* (producing germ tubes and chlamydospores) suggest that some *C. dubliniensis* isolates may have been misidentified as *C. albicans*.

Traditional diagnosis of *Candida* infections is slow and complicated. The ability to diagnose and identify candidiasis may be enhanced by the use of molecular techniques, such as Polymerase Chain Reaction (PCR). In particular, the discrimination of *Candida albicans* from *Candida dubliniensis* is difficult to establish by classic biochemical methods, as these two species have almost identical phenotypes; yet, both species can be differentiated by their genetic profiles by the Real-Time PCR assay.

Table 2. Susceptibility of *Candida* species to antifungal drugs.

<i>Candida</i> species	Fluconazole	Itraconazole	Voriconazole (not standardized)	Amphotericin B	Caspofungin (not standardized)
<i>C. albicans</i>	S	S	S	S	S
<i>C. tropicalis</i>	S	S	S	S	S
<i>C. parapsilosis</i>	S	S	S	S	S (to I?)
<i>C. glabrata</i>	S-DD to R	S-DD to R	S to I	S to I	S
<i>C. krusei</i>	R	S-DD to R	S to I	S to I	S
<i>C. lusitanae</i>	S	S	S	S to R	S

Modified from Pappas, *et al. Clin Infect Dis* 2004, 38: 161-89. S=Susceptible S-DD=Susceptible dose-dependant I=Intermediate R=Resistant

Table 3. *Candida albicans* and *Candida krusei* susceptibilities to various antifungal drugs.

Antifungal Agent	<i>C. albicans</i> (n=20)			<i>C. krusei</i> (n=26)		
	MIC range, µg / mL	MIC ₅₀ , µg / mL	MIC ₉₀ , µg / mL	MIC range, µg / mL	MIC ₅₀ , µg / mL	MIC ₉₀ , µg / mL
Clotrimazole	0.006 – 0.50	0.010	0.06	0.030 – 0.50	0.125	0.25
Caspofungin	0.050 – 1.00	0.250	0.50	0.060 – 2.00	0.500	1.00
Fluconazole	0.130 – 8.00	0.130	2.00	32 to < 64	32	> 64
Itraconazole	0.0160 – 0.25	0.016	0.13	0.25 – 2.00	0.500	1.00
Voriconazole	0.006 – 0.13	0.030	0.03	0.25 – 1.00	0.250	1.00
Miconazole	0.010 – 0.13	0.013	0.03	1.00 – 4.00	2.000	4.00
Amphotericin B	0.030 – 0.50	0.120	0.25	0.25 – 1.00	1.000	1.00

Note: *C. albicans* isolates were recovered from women who experienced recurrent vaginal candidiasis. (Adopted with modification Singh *et al.*, 2002).

Candida krusei

Although *C. albicans* is the predominant etiologic agent of candidiasis, other *Candida* species that tend to be less susceptible to the commonly used antifungal drugs, such as *C. krusei*, *C. glabrata*, *C. lusitanae*, and the newest *Candida* species, *C. dubliniensis*, have emerged as substantial opportunistic pathogens.

Candida krusei is an opportunistic pathogen commonly implicated in urinary tract infections in immunocompromised patients and has emerged as a true, albeit uncommon, cause of fungal vaginitis (15, 16). Infections with *Candida krusei* have increased in recent years as a consequence of its intrinsic resistance to fluconazole, an antifungal azole widely used in immunocompromised individuals to suppress infections due to azole-susceptible *C. albicans*.

Since cultures are rarely performed, there is limited data regarding the antifungal susceptibility of yeast causing vulvovaginal candidiasis. In a study by Singh *et al.*, susceptibility testing was performed on vaginal yeast isolates from 593 patients with suspected vulvovaginal candidiasis. The results demonstrated the following infectious hierarchy: *Candida albicans* (n = 420), *Candida glabrata* (n = 112), *Candida parapsilosis* (n = 30), *Candida krusei* (n = 12), *Saccharomyces cerevisiae* (n = 9), *Candida tropicalis* (n = 8), *Candida lusitanae* (n = 1) and *Trichosporon* sp. (n = 1). Among the different species, elevated fluconazole MICs (> or = 16 microg/ml) were only observed in *C. glabrata* (15.2% resistant [R], 51.8% susceptible-dose dependent [S-DD]), *C. parapsilosis* (3.3% S-DD), *S. cerevisiae* (11.1% S-DD) and *C. krusei* (50% S-DD, 41.7% R, considered intrinsically fluconazole resistant). Resistance to itraconazole was observed among *C. glabrata* (74.1%), *C. krusei* (58.3%), *S. cerevisiae* (55.6%) and *C. parapsilosis* (3.4%). Among 84 patients with recurrent episodes, non-albicans species were detected more frequently (42% versus 20%) and treatment is further complicated by the fact that azole agents are less effective against these species (17).

C. krusei is predominately seen as a cause of vaginitis in comparatively older women. A possible pathophysiological explanation for the selection of *C. krusei* is that the older population may have been exposed to repeated episodes of vulvovaginal candidiasis and thus had been exposed to many courses of a wide array of antifungal therapy. The repeated exposure to azole-based antifungals, including topical agents, may cause a shift in the vaginal mycoflora from the more drug-susceptible *C. albicans* to the less drug-susceptible *Candida* species, such as *C. krusei* (18, 2).

In patients with chronic and recurrent fungal vaginitis, it should never be assumed that the yeast species responsible is invariably *C. albicans*. Signs and symptoms of vaginitis due to *C. krusei* appear to be indistinguishable from those of vaginitis due to other *Candida* species, an observation that emphasizes the need to obtain subspeciation of *Candida* prior to the initiation of antifungal therapy.

Prolonged, not abbreviated, therapy with either topical boric acid or topical clotrimazole or oral therapy with either ketoconazole or itraconazole should be considered as the first line therapy for patients with *C. krusei* vaginitis. Therapy with all active antifungal agents should also be prolonged (duration, usually 2 to 6 weeks), regardless of the agent used (16) (Table 3).

Candida lusitanae

Among the non-*Candida albicans* species, *Candida lusitanae* is of special interest owing to its uncommon susceptibility pattern (19, 20, 21). Rapidly acquired resistance to amphotericin B has been described or suspected, and some strains of *C. lusitanae* may be intrinsically resistant (22, 23); therefore, the detection of amphotericin B resistance is essential for treatment of *C. lusitanae*-associated infections (24).

The yeast *Candida lusitanae* was first described by van Uden and by Carmo-Sousa as a common organism in the gastrointestinal tracts of warm-blooded animals (25). *C. lusitanae* was found as a part of the mycoflora of the upper-respiratory, gastrointestinal and urinary tracts of hospitalized patients. This yeast species was recovered from both the skin and vagina of only one patient. Although an infrequent isolate overall (0.64% of 9,105 yeast isolates) (26), lately it has been recovered from a variety of clinical specimens including urinary tract infection and from vaginal candidiasis patients (27, 28).

In a study by Favel *et al.*, the antifungal susceptibility of thirty-five *Candida lusitanae* isolates was determined *in vitro* by the National Committee for Clinical Laboratory Standards (NCCLS) M27-P microdilution methodology. All the isolates were susceptible to ketoconazole, itraconazole and fluconazole. Of the thirty-five isolates, eight (23%) were resistant to flucytosine. For amphotericin B, M27-P yielded a narrow range of MICs (0.06-0.5 mg/L) (Table 4) (30).

Table 4. Antifungal susceptibility of *C. lusitaniae* (Adopted with modification from Favel *et al.*, 1997 [29])

Antifungal agent	MIC ₅₀ (µg/L)	MIC ₉₀ (µg/L)
Amphotericin B	0.25	0.5
Flucytosine	0.06	>=64
Econazole	0.12	0.12
Ketoconazole	0.03	0.06
Fluconazole	1	2
Itraconazole	0.12	0.5

Amphotericin B is the drug of choice for many systemic fungal infections (30). Amphotericin B susceptibility testing was recently performed and reported on 4,936 isolates of *Candida spp.* by the Etest methodology (31) (Table 5).

Table 5. Comparative amphotericin B susceptibility testing results for 4,935 isolates of *Candida spp.* (Adopted and modified from Pfaller MA, *et al.*, 2004).

Species (no. of isolates)	MIC ₅₀ ^a (µg/L)	MIC ₉₀ ^a (µg/L)
<i>C. albicans</i> (2,728)	0.5	0.5
<i>C. glabrata</i> (722)	1	2
<i>C. parapsilosis</i> (666)	1	2
<i>C. tropicalis</i> (528)	1	2
<i>C. krusei</i> (143)	4	8
<i>C. lusitaniae</i> (54)	0.25	1
<i>Candida spp.</i> ^b (95)	0.5	2
All <i>Candida</i> (4,936)	0.5	2

^a 50% and 90%, MICs at which 50 and 90% of isolates tested, respectively, are inhibited.

^b Includes *C. guilliermondii* (39 isolates), *C. pelliculosa* (17 isolates), *C. kefyr* (15 isolates), *C. rugosa* (11 isolates), *C. dubliniensis* (5 isolates), *C. zeylanoides* (4 isolates), *C. lipolytica* (3 isolates), and *C. famata* (1 isolate).

Candida utilis

This organism adds to the growing list of *Candida* species associated with human disease. *Candida utilis* was cultured from the blood of a patient with acquired immunodeficiency syndrome. The candidemia was apparently associated with catheter implantation. A report by Hazen KC, *et al.* describes the first demonstration and isolation of the industrially important yeast *C. utilis* from a urinary tract infection. In this present case, the organism was associated with chronic, symptomatic disease (32). In addition, *C. utilis* was also associated with fungal keratitis. The clinical features exhibited typical feather-like infiltration at the ulceration margin in this case. After treatment with topical fluconazole and amphotericin-B, the ulceration healed within 3 weeks (33).

Candida kefyr

Identified in 1931 and originally classified as *Endomyces pseudotropicalis*, *Candida kefyr* was considered a rarely isolated species that occasionally caused disease within immunocompromised individuals (34). Since then the organism has been reclassified several times and, most recently, has been deemed an emerging pathogen (35). Despite the limited literature documentation on *C. kefyr*, eight clinical studies and two case reports have established this organisms' ability to cause disease in humans (35). Though still a relatively rare cause of Candidiasis and fungemia, *Candida kefyr* has been isolated from a variety of body regions, including blood, urine, the esophagus and the cervical-vaginal tract in populations other than the immunocompromised (36,37). Geographical distribution studies of clinically relevant *Candida* strains demonstrates a relatively low prevalence rate within the United States (~0.5%) with higher rates reported within Europe (Table 6). Resistance of *C. kefyr* isolates has been observed in conjunction with amphotericin B therapy (38) and building resistance to common antifungal agents (38,39) (Table 7).

Table 6. Geographical distribution of infectious *Candida* species. Adapted with modification from Pfaller, MA *et al.*, 2006 [39]

Candida species	% of isolates					
	Asia (518)	Latin Amer. (548)	Europe (847)	Canada (156)	U.S. (587)	Total (2656)
<i>C. albicans</i>	60.2	48.9	63.5	64.1	44	55.6
<i>C. glabrata</i>	7.3	4.2	11.8	21.8	27.4	13.4
<i>C. kefyr</i>	0.2	0.4	1.3	0.0	0.5	0.6
<i>C. krusei</i>	0.8	1.8	4.1	1.3	2.0	2.4
<i>C. lusitaniae</i>	1.0	0.5	0.4	0.6	2.0	0.9
<i>C. parapsilosis</i>	16.2	19.7	10.6	9.0	14.8	14.4
<i>C. tropicalis</i>	12.5	16.4	7.6	2.6	7.8	10.1

Table 7. Susceptibility of *Candida kefyr* to common antifungal agents. Compiled with modification from Pfaller, MA *et al.*, 2006 and Pfaller, MA *et al.*, 2004.

Antifungal agent	No. of isolates	Cumulative % susceptible at MIC (µg / mL) values of:						
		0.007	0.015	0.03	0.06	0.12	0.25	0.5
Fluconazole	29	0	10	55	93	100	100	100
Ravuconazole	"	100	0	0	0	0	0	0
Flucytosine	"	31	66	66	72	90	90	100
Micafungin	17	0	0	41	100	0	0	0
Caspofungin	"	12	94	100	0	0	0	0

REFERENCES:

1. White MH. The contribution of fluconazole to the changing epidemiology of invasive candidal infections. *Clin Infect Dis*. 1997 Jun; 24(6):1129-30.
2. Abi-Said D, Anaissie E, Uzun O, et al. The epidemiology of hematogenous candidiasis caused by different *Candida* species. *Clin Infect Dis*. 1997 Jun;24(6):1122-8.
3. Wingard JR, Merz WG, Rinaldi MG, et al. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. *N Engl J Med*. 1991 Oct 31;325(18):1274-7.
4. Wingard JR, Merz WG, Rinaldi MG, et al. Association of *Torulopsis glabrata* infections with fluconazole prophylaxis in neutropenic bone marrow transplant patients. *Antimicrob Agents Chemother*. 1993 Sep; 37(9): 1847-1849.
5. Merz WG, Karp JE, Schron D, et al. Increased incidence of fungemia caused by *Candida krusei*. *J Clin Microbiol*. 1986 Oct; 24(4): 581-584.
6. Sullivan DJ, Westerneng TJ, Haynes KA, et al. *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. *Microbiology*. 1995 Jul;141 (Pt 7):1507-21.
7. Coleman DC, Sullivan DJ, Mossman JM. *Candida dubliniensis*. *J Clin Microbiol*. 1997 Nov; 35(11): 3011-3012.
8. Sullivan D, Coleman D. *Candida dubliniensis*: Characteristics and Identification. *J Clin Microbiol*. 1998 Feb; 36(2): 329-334.
9. Sullivan D, Haynes K, Bille J, et al. Widespread geographic distribution of oral *Candida dubliniensis* strains in human immunodeficiency virus-infected individuals. *J Clin Microbiol*. 1997 Apr; 35(4): 960-964.
10. Odds FC, Van Nuffel L, Dams G. Prevalence of *Candida dubliniensis* Isolates in a Yeast Stock Collection. *J Clin Microbiol*. 1998 Oct; 36(10): 2869-2873.
11. Loffler J, Kelly SL, Hebart H, et al. Molecular analysis of *cyp51* from fluconazole-resistant *Candida albicans* strains. *FEMS Microbiol Lett*. 1997 Jun 15;151(2):263-8.
12. Moran GP, Sullivan DJ, Henman MC, et al. Antifungal drug susceptibilities of oral *Candida dubliniensis* isolates from human immunodeficiency virus (HIV)-infected and non-HIV-infected subjects and generation of stable fluconazole-resistant derivatives in vitro. *Antimicrob Agents Chemother*. 1997 Mar; 41(3): 617-623.
13. Pinjon E, Jackson CJ, Kelly SL, et al. Reduced Azole Susceptibility in Genotype 3 *Candida dubliniensis* Isolates Associated with Increased CdCDR1 and CdCDR2 Expression. *Antimicrob Agents Chemother*. 2005 Apr; 49(4): 1312-1318.
14. Odds FC, Rinaldi MG, Cooper CR Jr, et al. *Candida* and *Torulopsis*: a blinded evaluation of use of pseudohypha formation as basis for identification of medically important yeasts. *J Clin Microbiol*. 1997 Jan; 35(1): 313-316.
15. Hepburn MJ, Pennick GJ, Sutton DA, et al. *Candida krusei* renal cyst infection and measurement of amphotericin B levels in cystic fluid in a patient receiving AmBisome. *Med Mycol*. 2003 Apr;41(2):163-5.
16. Singh S, Sobel JD, Bhargava P, et al. Vaginitis due to *Candida krusei*: epidemiology, clinical aspects, and therapy. *Clin Infect Dis*. 2002 Nov 1;35(9):1066-70. Epub 2002 Oct 10.
17. Singhi SC, Reddy TC, Chakrabarti A. Oral itraconazole in treatment of candidemia in a pediatric intensive care unit. *Indian J Pediatr*. 2004 Nov;71(11):973-7.
18. Wingard JR. The use of fluconazole prophylaxis in patients with chemotherapy-induced neutropenia. *Leuk Lymphoma*. 1992 Nov;8(4-5):353-9.
19. Wingard JR. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. *Clin Infect Dis*. 1995 Jan;20(1):115-25.
20. Blinkhorn RJ, Adelstein D, Spagnuolo PJ. Emergence of a new opportunistic pathogen, *Candida lusitanae*. *J Clin Microbiol*. 1989 Feb; 27(2): 236-240.
21. Fromtling RA, Galgiani JN, Pfaller MA, et al. Multicenter evaluation of a broth macrodilution antifungal susceptibility test for yeasts. *Antimicrob Agents Chemother*. 1993 Jan; 37(1): 39-45.
22. Peyron F, Favel A, Michel-Nguyen A, et al. Improved Detection of Amphotericin B-Resistant Isolates of *Candida lusitanae* by Etest. *J Clin Microbiol*. 2001 Jan; 39(1): 339-342.
23. van Uden N, Madeira-Lopes A. Concurrent exponential growth and death of cell populations of *Saccharomyces cerevisiae* at superoptimal growth temperatures. *Z Allg Mikrobiol*. 1970;10(7):515-26.
24. Merz WG. *Candida lusitanae*: frequency of recovery, colonization, infection, and amphotericin B resistance. *J Clin Microbiol*. 1984 Dec; 20(6): 1194-1195.
25. Baker JG, Nadler HL, Forgacs P, et al. *Candida lusitanae*: a new opportunistic pathogen of the urinary tract. *Diagn Microbiol Infect Dis*. 1984 Apr;2(2):145-9.
26. Silverman NS, Morgan M, Nichols WS. *Candida lusitanae* as an unusual cause of recurrent vaginitis and its successful treatment with intravaginal boric acid. *Infect Dis Obstet Gynecol*. 2001;9(4):245-7.
27. Favel A, Michel-Nguyen A, Chastin C, et al. In-vitro susceptibility pattern of *Candida lusitanae* and evaluation of the Etest method. *J Antimicrob Chemother*. 1997 May;39(5):591-6.
28. Kauffman CA, Carver PL. Antifungal agents in the 1990s. Current status and future developments. *Drugs*. 1997 Apr;53(4):539-49.
29. Hadfield TL, Smith MB, Winn RE, et al. Mycoses caused by *Candida lusitanae*. *Rev Infect Dis*. 1987 Sep-Oct;9(5):1006-12.
30. Drouhet E., Dupont, B., Improvisi, L. et al. Disc agar diffusion and microplate automatized techniques in vitro evaluation of antifungal agents on yeasts and sporulated pathogenic fungi. In *Vitro and In Vivo Evaluation of Antifungal Agents* (Iwata, K & Vanden Bossche, H., Eds). Elsevier Science Publishers, Amsterdam.
31. Pfaller MA, Boyken L, Messer SA, Tendolkar S, et al. Evaluation of the Etest Method Using Mueller-Hinton Agar with Glucose and Methylene Blue for Determining Amphotericin B MICs for 4,936 Clinical Isolates of *Candida* Species. *J Clin Microbiol*. 2004 Nov; 42(11): 4977-4979.
32. Azen KC, Theisz GW, Howell SA. Chronic Urinary Tract Infection Due to *Candida utilis*. *J Clin Microbiol*. 1999 Mar; 37(3): 824-827.
33. Shih MH, Sheu MM, Chen HY, et al. Fungal keratitis caused by *Candida utilis*—case report. *Kaohsiung J Med Sci*. 1999 Mar;15(3):171-4.
34. Hazen, KC. New and emerging yeast pathogens. *Clin Microbiol Rev*. 1995, Oct; 8:462-478.
35. Corpus K, Hegeman-Dingle H and Bajjoka I. *Candida kefyr*, an uncommon but emerging fungal pathogen: report of two cases. *Pharmacotherapy*. 2004 Aug; 24(8):1084-1088.
36. Abu-Elteen KH, Abdul Malek AM and Abdul Wahid NA. Prevalence and susceptibility of vaginal yeast isolates in Jordan. *Mycoses*. 1997 Oct; 40:179-185.
37. Listemann H, Schulz KD, Wasmuth R, Begemann F and Meigel W. Oesophagitis caused by *Candida kefyr*. *Mycoses*. 1998 Sep-Oct; 41:343-344.
38. Pfaller MA, Diekma, DJ, Messer SA, Boyken L, Hollis RJ and Jones RN. In vitro susceptibilities of rare *Candida* bloodstream isolates to ravuconazole and three comparative antifungal agents. *Diagn Microbiol Infect Dis*. 2004 Feb; 48:101-105.
39. Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S and Diekma DJ. Global surveillance of in vitro activity of micofungin against *Candida*: a comparison with Caspofungin by CLSI-recommended methods. *J Clin Microbiol*. 2006 Oct; 44(10): 3533-3538.

