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Prevalence of *Candida albicans*-closely related yeasts, *Candida africana* and *Candida dubliniensis*, in vulvovaginal candidiasis

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Abstract

Isolates of *Candida africana* and *C. dubliniensis* were recovered from patients with vulvovaginal candidiasis (VVC). The isolates were initially identified as *C. albicans* through use of the API Candida System. We retrospectively reexamined 1014 vaginal isolates presumptively determined to be *C. albicans* at the Department of Obstetrics and Gynecology of Peking University Shenzhen Hospital from 1 January 2003 through 31 December 2012. Our objective was to determine, via detection of the *HWP1* gene, if any of the isolates were *C. africana* or *C. dubliniensis.* One and a half percent of these isolates (15/1014) were found to be *C. africana,* whereas *C. dubliniensis* was not detected. The 15 *C. africana* isolates were susceptible to nystatin, fluconazole, itraconazole, miconazole, and clotrimazole. *Candida africana* could not be recovered from clinical vaginal specimens from the 15 patients at follow-up on days 7–14 and days 30–35 when treated with different antifungal agents. We conclude that *C. africana*, but not *C. dubliniensis*, was present in the vaginal samples of patients with VVC. The *C. africana* isolates were susceptible to the tested antifungal agents. VVC caused by *C. africana* appears to respond well to current therapies.

Key words: vulvovaginal candidiasis, *Candida africana*, *Candida dubliniensis*.

Introduction

Candida albicans remains the most common fungal pathogen isolated from clinical samples of patients with vulvovaginal candidiasis (VVC) [1,2]. *Candida africana* was first isolated from patients in Africa and Germany and studied by Tietz et al. [3]. In 2001, it was proposed as a new *Candida* species that produces a germ tube but not chlamydospores [4]. *Candida africana* has been reported as a cause of VVC in African, German, Spanish, and Italian patients [3–10]. *Candida dubliniensis* is a yeast species that is closely related to *Candida albicans* [11] and has been found in vaginal samples [11–23]. *Candida africana* and *C. dubliniensis* strains are often misidentified as *C. albicans*.

In 2008, Romeo and Criseo [7] described a specific molecular method for differentiating *C. albicans*, *C. africana*, and *C. dubliniensis* that involves use of a single pair of primers targeting the *HWP1* gene and its homologues in a polymerase chain reaction (PCR)–based assay. In the current study, we evaluated clinical samples from patients with VVC for the presence of *C. africana* and *C. dubliniensis*, which are closely related to *C. albicans*.

Materials and methods

Clinical Candida isolates and reference strains

Vaginal swabs were obtained from patients attending the clinic of the Gynecology Department, Peking University Shenzhen Hospital, from 1 January 2003 through 31 December 2012. The specimens were inoculated onto plates containing CHROM agar (Biocell Laboratory Ltd, Zhengzhou, China) and incubated for 24–48 hours at 37°C in ambient air, with all isolates stored in a medium containing 2% glucose, 2% peptone, and 20% glycerol at –70°C. The strains were identified using a standard API Candida system (bioMérieux, Marcy l'Etoile, France). *Candida albicans* isolates produce germ tubes when incubated in sterile test tubes that contain 0.5 ml of serum at 35°C for 2.5–3 h. All 15 *C. africana* isolates were inoculated on cornmeal agar plates supplemented with 1% Tween 80 for 24–72 h at 25°C in ambient air. Wet mounts of colonies were examined microscopically at 24, 48, and 72 h to detect the production of pseudohyphae and chlamydospores. The reference strains used as controls were *C. dubliniensis* (Centraalbureau voor Schimmelcultures [CBS] 7988) and *C. albicans* (American Type Culture Collection [ATCC] 90028, SC5314).

Molecular identification

For molecular studies, a single yeast colony of presumptively identified *C. albicans* isolates grown on a Sabouraud glucose agar plate were randomly selected and suspended in microcentrifuge tubes containing 50 μl of lysis buffer for direct PCR in order to detect the microorganisms. The contents were heated at 80°C for 15 min and centrifuged; 1 μl of the supernatant was removed for use in the PCR. The primers (CR-f 5-GCTAC CACTTCAGAATCATCATC-3 and CR-r 5-GCAC CTTCAGTCGTAGAGACG-3), the composition of the PCR mixture, and the PCR conditions were previously described by Romeo and Criseo [7]. The PCR products were electrophoresed on a 1.2% (w/v) agarose gel in Tris/borate/ethylenediaminetetraacetic acid buffer and then visualized using a gel-imaging system (UVItec Ltd, Cambridge, MA, USA) after staining with ethidium bromide (0.5 mg/ml).

Antifungal susceptibility testing

The *in vitro* antifungal susceptibility of 32 *C. albicans* isolates obtained through a randomized selection protocol and the 15 strains of *C. africana* were tested using a commercial agar diffusion test (A/S Rosco; Rosco Laboratory, Taastrup, Denmark). The Neo-Sensitabs tablet assay (A/S Rosco) was performed according to the manufacturer's instructions [24] and M44-A guidelines [25]. Briefly, the inoculum suspensions were standardized using a spectrophotometer in order to obtain a concentration of approximately 5×10^5 colony-forming units/ml. Plates were then flooded with yeast suspensions and excess fluid immediately removed with a pipette. The plates were dried for 15 min and 9-mm tablets containing 50 μg of nystatin, 10 μg of clotrimazole, 25 μg of fluconazole, 8 μg of itraconazole, and 10 μg of miconazole (provided by Rosco Laboratory) were then applied to the inoculated agar using forceps. The plates were incubated in ambient air at 35°C, and the diameter of the zones of inhibition were measured after 44–48 h of incubation. Plates displaying faint growth closer to the disc were discarded. The quality control isolates *C. albicans* ATCC 64548 and *C. albicans* ATCC 64550 were tested in the same manner. The endpoints for the antifungal agents were interpreted according to the manufacturer's instructions and were as follows: S, yeasts considered susceptible to fluconazole, miconazole, clotrimazole if zone diameter ≥20 mm; I, intermediate susceptibility if zone diameter were 12–19 mm; and R, resistant if zone diameter ≤11 mm. For nystatin and itraconazole isolates were as follows: S, zone diameter ≥15 mm; I, zone diameter 10–14 mm; and R, no zone.

Statistical methods

All values in the tables are expressed as the mean unless otherwise indicated. Each variable was tested for differences between groups using Student *t* test or χ^2 analysis where appropriate. Statistical significance was set at $P < 0.05$. Statistical analysis of the data was performed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA).

Results

During the study period, 3181 strains of *Candida* were obtained from specimens collected from 3141 patients with VVC. A combination of *C. glabrata*, *C. krusei*, and *C. albicans* was isolated from 40 patients with VVC (1.3%; 40/3141). *Candida albicans* was the predominant *Candida* species (2705 strains; 85.0%) in VVC.

Among the 1014 isolates presumptively identified as *C. albicans*, on the basis of the colony's appearance on

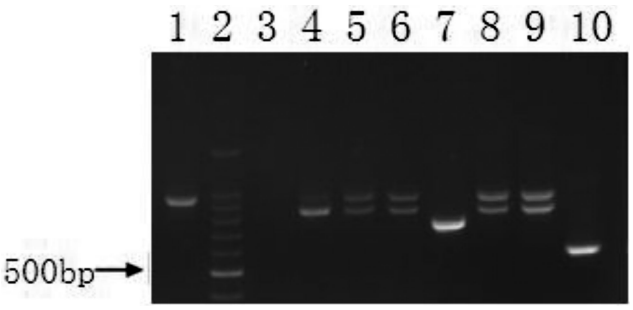

Figure 1. Molecular discrimination of *Candida albicans* (lanes 1, 4, 5, 6, 8, 9), *C. africana* (lanes 7), and CBS 7988 (*C. dubliniensis*; lanes 10) using a single pair of primers derived from the *HWP1* genes. Lane 2 contains molecular size markers.

CHROMagar, germ-tube test results, and identification codes in the API *Candida* system, 999 were found to be *C. albicans* (98.5%) and 15 to be *C. africana* isolates (1.5%). None of these presumptive *C. albicans* isolates were verified to be *C. dubliniensis*.

Figure 1 shows four DNA fragments produced by three *Candida* species: approximately 700 bp for *C. africana*, 839 bp and 941 bp for *C. albicans*, and 569 bp for *C. dubliniensis*. The API codes obtained from all 15 strains of *C. africana* examined were 7010 bp and formed pseudohyphae but not chlamydospores.

Table 1 shows some epidemiological and clinical characteristics of patients with infections caused by *C. africana* or *C. albicans*. There were no significant differences between the two groups in terms of mean age, use of antibiotic agents in the preceding month, or prevalence of diabetes mellitus.

All 15 *C. africana* isolates were susceptible to nystatin, fluconazole, itraconazole, miconazole, and clotrimazole (Table 2), and cultures inoculated with clinical samples were negative for all cases of VVC caused by this yeast at days 7–14 and days 30–35 follow-up when treated with different antifungal agents (Table 3).

Discussion

In most previous studies, *C. africana* was detected in small research samples [3,5,10,26]. Odds et al. [5] analyzed 1391 *C. albicans* isolates from all regions of the world and found

Table 1. Comparison of clinical characteristics of vulvovaginal candidiasis caused by *Candida africana* and *C. albicans*.

Characteristic	*Candida africana*	*Candida albicans*
Number of cases	15	30
Mean age (mean standard deviation, years)	29.40 ± 5.99	30.07 ± 6.79
Antibiotics (in prior 6 weeks)	1 (6.7)	5(16.7)
Previous VVC	3 (20.0)	15 (50.0)
RVVC	1 (6.7)	1 (3.4)
DM	0 (0)	1 (3.4)
Allergy to drugs or food	1 (6.7)	2 (6.7)
Symptoms and signs		
Pruritus		
0	1 (6.7)	1 (3.4)
1	4 (26.7)	9 (30.0)
2	5 (33.4)	13 (43.4)
3	5 (33.4)	7 (23.4)
Burning		
0	8 (53.4)	11 (36.7)
1	7 (46.6)	13 (43.4)
2	0	3 (10.0)
3	0	3 (10.0)
Erythema		
0	3 (20.0)	8 (26.7)
1	3 (20.0)	5 (16.7)
2	3 (20.0)	14 (46.7)
3	6 (40.0)	3 (10.0)
Discharge		
0	3 (20.0)	0 (0)
1	3 (20.0)	4 (13.4)
2	3 (20.0)	14 (46.7)
3	6 (40.0)	12 (40.0)

DM, diabetes mellitus; RVVC, recurrent vulvovaginal candidiasis; VVC, vulvovaginal candidiasis.

Table 2. Antifungal activities against *Candida albicans* and *C. africana*.

Antifungal agent	*Candida africana* Number of strains (%)	*Candida albicans* Number of strains (%)
Fluconazole		
S	15 (100.0)	25 (83.4)
I	0 (0)	5 (16.6)
R	0 (0)	0 (0)
Itraconazole		
S	14 (93.3)	21 (70.0)
I	1 (6.7)	9 (30.0)
R	0 (0)	0 (0)
Miconazole		
S	12 (80.0)	26 (86.7)
I	3 (20.0)	3 (10.0)
R	0 (0)	1 (3.30)
Clotrimazole		
S	14 (93.4)	30 (100.0)
I	1 (6.6)	0 (0)
R	0 (0)	0 (0)
Nystatin		
S	15 (100.0)	30 (30.0)
I	0 (0)	0 (0)
R	0 (0)	0 (0)

S, susceptible; I, intermediate susceptible; R, resistant.

15 to be *C. africana*. These included 12 vaginal isolates, 2 from the penis, and 1 was from a blood culture. Nine of the 15 isolates were of African origin, one was from Japan, two were from the United Kingdom, two were from Germany, and one (the blood isolate) was from Chile. In 2011, Gumral et al. [26] evaluated 195 *C. albicans* vaginal isolates using *HWP1* gene polymorphisms to detect the presence of *C. africana* and *C. dubliniensis*; however, none of the isolates were found to be either *C. africana* or *C. dubliniensis*. In 177 *Candida* isolates obtained from women presenting with symptoms of vulvovaginal inflammation, 84 were presumptively identified as being *C. albicans* by phenotypic methods and two were confirmed as *C. africana* based on the use of *HWP1* gene amplification [10]. In 2013 Borman et al. [27] evaluated the occurrence of *C. africana* and *C. dubliniensis* in clinical samples in the United Kingdom in 2013 by pyrosequencing all presumptive isolates of *C. albicans* submitted to the Mycology Reference Laboratory during a 9-month period. The *C. albicans* complex constituted 44.9% (826/1839) of yeast isolates received during the study period, which included 783 isolates of *C. albicans*, 28 of *C. dubliniensis*, and 15 of *C. africana*. *Candida africana* constituted 6% (15/251) of the "*C. albicans*" isolates from female genital specimens during the study period. In the current study, no *C. dubliniensis* isolates were recovered from our clinical samples. This may, in part, be due to the fact that *C. dubliniensis* generally has a low incidence in vaginal samples [10]. Although *C. africana* was originally reported in Africa [5] and some European countries [4,6], follow-up studies have demonstrated that it appears in most regions of the world, including Japan, Germany, the United Kingdom, and Chile [5,27]. The current study found *C. africana* in 1.5% of samples of "*C. albicans*" presumptive isolates from patients with VVC, but no *C. dubliniensis* isolates were recovered from these patients.

In our study, *C. albicans* produced two DNA fragments, 839 bp and 941 bp. The 839 bp DNA fragment could be the novel allele of the *HWP1* gene *(HWP1–2;* GenBank: EU044787.1) of *C. albicans* [28]. The two DNA fragments demonstrate that the *C. albicans* isolates were heterozygous at *HWP1* locus.

Among the 15 *C. africana* isolates, all were susceptible to the antifungal agents tested, which is in agreement with previously published evaluations [5,27] and suggests the yeasts would be susceptible to all antifungal agents that are appropriate for treating vaginal candidiasis. In our retrospective therapy analysis, VVC caused by *C. africana* appeared to respond well to the current treatment regimens.

Table 3. Therapeutic efficacy in patients with vulvovaginal candidiasis caused by *Candida africana*.

Treatment regimen	Number of cases	Cured cases at follow-up (%)	
		Days 7–14	Days 30–35
Oral fluconazole: 150 mg, 2 doses	3	3	3
Oral itraconazole: 200 mg, twice daily for 1 day	3	3	3
Oral itraconazole: 200 mg, twice daily for 2 days	2	2	1
Miconazole nitrate vaginal suppository: 1200 mg, 2 doses	2	2	2
Miconazole nitrate vaginal suppository: 1200 mg, 1 dose	1	1	1
Oral fluconazole: 150 mg, 1 dose	1	1	1
Miconazole nitrate vaginal suppository: 200 mg, daily for 7 days	1	1	1
nystatin vaginal suppository: 20 mIU, daily for 14 days	1	1	1
Oral fluconazole: 150 mg, on days 1, 4, 7, and 150 mg/week	1	1	1
Total	15	15(100.0)	14(93.4)

In conclusion, *C. africana* was detected in vaginal samples from patients with VVC, whereas we did not identify *C. dubliniensis* in these patient samples. Additionally, the *C. africana* isolates were susceptible to nystatin, fluconazole, itraconazole, miconazole, and clotrimazole. VVC caused by *C. africana* appears to respond well to current therapies.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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