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Differentiation of *Candida albicans* complex species isolated from invasive and non-invasive infections using *HWP1* gene size polymorphism

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A B S T R A C T

Background and Purpose: Taxonomy of *Candida* is controversial and has changed due to the investigation of the novel species. *Candida africana* and *Candida dubliniensis* are new members of the *C. albicans* complex that are currently gaining both clinical and epidemiologic significance. This study aimed to report the prevalence of *C. africana* among the strains isolated from patients using hyphal wall protein 1 (*HWP1*) gene size polymorphism.

Materials and Methods: In total, 235 yeasts confirmed as *C. albicans* complex based on chromogenic media and internal transcribed spacers sequencing isolated from various clinical forms of invasive and non-invasive candidiasis mainly candidemia were re-identified using *HWP1* gene polymorphisms. The *HWP1*-polymerase chain reaction amplicons were re-confirmed by sequencing and BLAST analysis.

Results: Based on the *HWP1* gene size polymorphism, 223 strains were identified as *C. albicans* (94.89%) from which 7 isolates produced two DNA fragments (850 and 941 bp). The *C. dubliniensis* (n=4, 1.7%), *C. africana* (n=1, 0.42%), and mix of *C. albicans* and *C. africana* (n=7, 2.97%) were also identified.

Conclusion: It can be said that *C. albicans* remains the most common *Candida* species, while *C. dubliniensis* and *C. africana* are rarely found among the patient isolates. Due to limited information on the molecular epidemiology of this novel yeast, more studies using molecular methods are recommended.

Keywords: *Candida africana*, *Candida albicans* species complexes, *Candida dubliniensis*, *HWP1* gene

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Introduction

Incidence rate of infections caused by various yeasts species has increased considerably in the past decades [1]. Classification of *Candida albicans* as the most common cause of invasive fungal infections has been subjected to significant changes describing new species, such as *Candida dubliniensis* and *Candida africana*, as the cryptic species complexes [2, 3]. According to the limited number of studies performed to date, *C. africana* is reported to have a strong association with human genitals, and it is rarely isolated from other body sites [4]. Accordingly, *C. dubliniensis* and *C. africana* have received less attention, compared to *C. albicans* and there is a lack of experimental and clinical evidence about their pathogenic potential.

According to the previous studies, *C. dubliniensis* and *C. africana* are inherently susceptible to azole and polyene antifungal drugs. However, some reports have shown that the antifungal susceptibility patterns of *C. africana* and *C. dubliniensis* are slightly different from those of *C. albicans* [5, 6]. Moreover, based on previous studies, some *C. africana* isolates have been classified as resistant to itraconazole, fluconazole, voriconazole, clotrimazole, 5-flucytosine, and Terbinafine [4, 7, 8]. Echinocandins is the first-line antifungal drug for the treatment of *Candida* infections and has shown prolonged post antifungal effect and concentration-dependent killing activity against the majority of *Candida* species, including the *C. albicans* complex [9, 10].

Phenotypic characteristics do not allow differentiation between the members of closely related *C. albicans* complex species. More reliable tests are

based on molecular techniques, such as specific polymerase chain reaction (PCR) amplification of the hyphal wall protein 1 (*HWP1*) gene [11]. The *HWP1* gene has been proposed as the molecular target for discriminating between *C. albicans* species complex based on its size polymorphism as it has 941/850 base pair (bp) for *C. albicans*, 569 bp for *C. dubliniensis*, and ~700 bp for *C. africana* [11].

Although epidemiological and clinical data suggest that *C. africana* has a worldwide distribution, little is known about the frequency of *Candida* isolated from systemic candidiasis in Iran [12]. Hence, this study was carried out to investigate the microbial epidemiology of *C. albicans* complex species among different clinical specimens, especially those strains isolated from systemic candidiasis.

Materials and Methods

Candida albicans complex isolates and strains

The majority of *Candida* samples had already been isolated from the patients with systemic candidiasis admitted to the neonatal and pediatric ICUs of Children's Medical Centre, Tehran, Iran, and identified as *C. albicans* mostly by internal transcribed spacers sequencing and/or matrix-assisted laser desorption ionization-time of flight [13]. In addition, a part of the samples was isolated from vulvovaginal candidiasis and candiduria from the patients in Al-Zahra Hospital, Isfahan, Iran. The *C. albicans* (ATCC 64553), *C. dubliniensis* (ATCC 2018), and two isolates of *C. africana* (GenBank accession number: MG434677 and MG434680) were used as the positive controls.

Molecular identification

The colonies conserved at -20 ºC freezer were subcultured on CHROMagar Candida, and DNA was extracted from a single colony by boiling method [14]. A fragment of the *HWP1* gene was amplified using CR-f (5'- GCT ACC ACT TCA GAA TCA TCATC-3') and CR-r (5'- GCA CCT TCA GTC GTA GAG ACG-3') primers [11] in the following thermal conditions: 5 min at 95 ºC, followed by 35 cycles of 40 s at 94 ºC, 45 s at 60 ºC, and 60 s at 72 ºC as well as a final extension of 5 min at 72 ºC. The reaction mixture contained 7.5 µL of 2× master mix (Ampliqon, Denmark), 0.33 µM of each primer, and 2 µl of DNA in a total volume of 15 µl. It should be mentioned that appropriate positive and negative controls were used for each PCR run.

An aliquot of 5 µl of each sample was added to 1.5% agarose gel containing 0.5 µg/ml of ethidium bromide. It was electrophoresed for 2 h in 100 V and visualized under UV light documentation. Species identification was performed based on the size polymorphism of the *HWP1* gene in different species, i.e. *C. albicans* (~940/850 bp), *C. dubliniensis* (~570 bp), and *C. africana* (~700 bp) [11, 15]. The *HWP1*-PCR product identified as *C. africana* was subjected to sequencing with the above-mentioned forward primer and the result was analyzed by Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast).

Results

In this study, a total of 235 *Candida albicans* isolates were re-identified based on *HWP1* gene polymorphisms. The isolates were collected from patients with systemic candidiasis (n=150), vulvovaginal candidiasis (n=60), and candiduria (n=25). The age of patients with candidiasis ranged from 1 to 78 years and the majority of them were female (n=154, 65.53 %).

Based on *HWP1* gene amplification, the species distribution was as follows: *C. albicans* (n=223, 94.89%), from which 7 isolates produced two different DNA fragments (850 and 941 bp), *C. dubliniensis* (n=4, 1.7%), *C. africana* (n=1, 0.42%), and the mix of *C. albicans* and *C. africana* (n=7, 2.97%) (Figure 1). The amplicon of the single pure *C. africana* isolate was subjected to PCR-sequencing. The obtained sequences showed 99.71% identity with an isolate of *C. africana* (MN817936.1) with an E-value of 99.42 and 100% coverage and the sequence was inserted in GenBank under accession numbers MZ578437. It is noteworthy that BLAST analysis of the obtained sequence confirmed the identity.

This *C. africana* was obtained from the urine specimen of a 45-year-old female with diabetes. All isolates of *C. dubliniensis* and all samples with a mix of *C. albicans* and *C. africana* were collected from the patients with systemic candidiasis. The results of the molecular analysis are shown in Table 1.

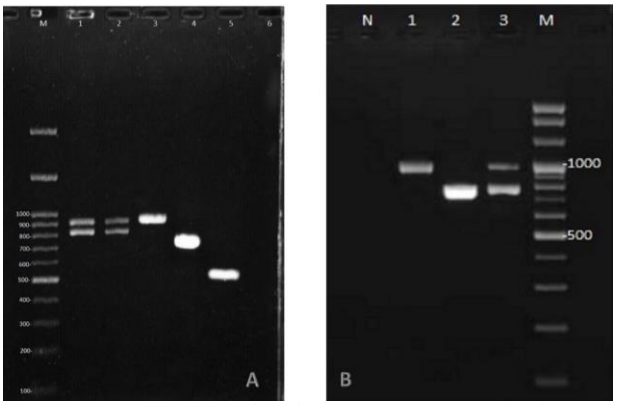

Figure 1. Electrophoretic profile of hyphal wall protein 1 amplification in some clinical isolates. A) Lane M: 100 bp molecular size marker, lanes 1 and 2: heterozygous isolates of *Candida albicans* (~850 and 940 bp), lane 3: homozygous isolate of *C. albicans* (~940 bp), lane 4: *Candida africana* (~700 bp), lane 5: *Candida dubliniensis* (~570 bp), and lane 6: negative control. B) Lane 1: negative control, lane 2: homozygous isolate of *C. albicans* (~940 bp), lane 3: *C. africana* (~700 bp), *C. albicans*, and *C. africana* (dual bands), and lane M: 100 bp molecular size marker.

Table 1. Distribution of *Candida albicans* species complexes in this study.

	Candida albicans	***Candida dubliniensis***	***Candida africana***	**Mix of *C. albicans* and *C. africana***
Systemic candidiasis	139	4	-	7
Candiduria	24	-	1	-
Vulvovaginal candidiasis	60	-	-	-
Total	223	4	1	7

Discussion

The incidence of mild to severe fungal infections has dramatically increased worldwide in the last several decades. Fungal species distribution varies owing to the hospital, hospitalization unit, and geographical area [16]. Invasive candidiasis is a considerable cause of morbidity and mortality, especially amongst patients suffering from immunodeficiency [17]. The *C. albicans* complex is one of the major fungal groups, which is involved in more than 50% of *Candida* infections, pointing out their significant prevalence among human beings [18].

The differences in adherence ability, pathogenicity, and biofilm formation observed between *C. albicans* and *C. africana* highlight the necessity of discriminating them in clinical laboratories [19]. Therefore, this study aimed to identify the archived cryptic specimens belonging to the *C. albicans* complex isolated from clinical samples of hospitalized patients to demonstrate the existence of species that are not routinely identified and reported.

The *HWP1* is a particular target for differentiation of the *C. albicans* complex species i.e. *C. albicans*, *C. dubliniensis*, and *C. africana* [7]. In the present study, *C. albicans* (94.89%) was the predominant species among the 235 isolates, which is in line with the results of some previous studies [12, 15, 20-23]. In the present research, the majority (97.3 %) of *C. albicans* with 941 bp DNA fragments were homozygous, while 2.97% (n=7) of them produced two DNA fragments of 850 and 941 bp, demonstrating heterozygosity at *HWP1* locus. The 850 bp DNA fragment is considered a novel allele of the *HWP1* gene [21].

Nouraei et al. [24] evaluated the exoenzyme activity of 60 *C. albicans* species consisting of 30 homozygous and 30 heterozygous strains. They found that the homozygous strains of *C. albicans* had more phospholipase and proteinase exoenzyme activity than heterozygous strains in different ranges, while no significant statistical differences were observed between the strains in terms of virulence factors. Further studies are needed to clarify the probabilistic pathogenic role of these homozygous or heterozygous strains.

In this study, *C. africana* (3.4%) had a higher prevalence rate than *C. dubliniensis* (1.7%). This result corroborates those of the previous research performed in Iran [7, 12, 25-27]. However, the higher isolation rate of *C. dubliniensis* over *C. africana* has also been reported in other studies [20, 28, 29]. In a study conducted by Romeo et al. [30], the frequency of *C. africana* (7.2%) was higher than that of *C. dubliniensis* (2.9%) among the *Candida* strains isolated from 498 clinical specimens collected from various patient groups [30]. The *C. dubliniensis* is less prevalent than *C. albicans* and shows phenotypic similarities with *C. albicans*, which may invade sterile body sites, such as mucosal surfaces, blood, central nervous system, and pleural fluid, with mortality rates similar to *C. albicans* [31-34].

Although *C. africana* has a worldwide distribution, an epidemiological meta-analysis showed that its overall prevalence rates in Iran and Honduras were higher, compared to other countries worldwide [5]. Shokoohi et al. [25] reported that one of the largest clusters of *C. africana* isolates was from Iran with a prevalence rate similar to those reported from some other countries indicating that this yeast may be more locally or regionally prevalent [25]

Hana et al. [20] reviewed the global epidemiological status of *C. africana* reported between 2010 and 2019 from more than 11 different countries (Senegal, Nigeria, Cameroon, Algeria, United Kingdom, Argentine, Colombia, USA, Iran, China, and Turkey). They found that the majority of *C. africana* strains were identified in America (35/90-38.8%), followed by Asia (27/90-30%), Europe (15/90-16.6%), and Africa (13/90-14.4%). Despite its worldwide distribution, the majority of *C. africana* isolates have been isolated from vulvovaginal candidiasis (60/90-66.6%) followed by nosocomial origins (11/90), balanoposthitis (5/90), blood (1/90), cerebral liquid (1/90), buccal (1/90), and urine (1/90) [20].

Based on the results of global epidemiological studies, most of the *C. africana* strains have been isolated from vulvovaginal specimens [35]. However, in agreement with the study conducted by Yazdanpanah et al. [36] and Gumral et al. [37], the results of our assay revealed that no *C. africana* was recovered from the vaginal specimens. The distribution of *C. africana* may be partially based on geographical variation, although a larger number of vulvovaginal samples are needed to confirm this hypothesis. We also identified *C. africana* among the patients with candiduria, suggesting that this fungus can also be associated with a wider clinical spectrum [30].

In this investigation, molecular identification demonstrated seven co-infections by *C. africana* and *C. albicans* in patients with systemic candidiasis. An attempt to discriminate species in mixed infection/colonization, especially in children, is important for clinicians as they could differ both in virulence and spectrum of antifungal. Consequently, the lack of specific microbiological data could force physicians to empirically treat life-threatening mycoses with broad-spectrum antifungal medications, which would impact the existing issues with antifungal resistance.

Conclusion

While *HWP1* size polymorphisms are a simple and cost-effective method for the differentiation of *C. africana* and *C. dubliniensis* from *C. albicans*, *C. africana* was detected in 3.4% of the isolates. This means that this species is not uncommon in Iranian patients.

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Authors' contribution

H.M. designed the study. A.C. and B.A. provided the isolates. S.A. and K.S. performed the experiments. S.A. and H.M. prepared the draft of the paper. All authors assisted in the edition and revision of the manuscript.

Conflicts of interest

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

Financial disclosure

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References

1. Aydin M, Kustimur S, Kalkanci A, Duran T. Identification of medically important yeasts by sequence analysis of the internal transcribed spacer and D1/D2 region of the large ribosomal subunit. Rev Iberoam Micol. 2019; 36(3):129-38.
2. Nnadi NE, Ayanbimpe GM, Scordino F, Okolo MO, Enweani IB, Criseo G, et al. Isolation and molecular characterization of *Candida africana* from Jos, Nigeria. Med Mycol. 2012; 50(7):765-7.
3. 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 (Reading). 1995; 141(Pt 7):1507-21.
4. Romeo O, Criseo G. *Candida africana* and its closest relatives. Mycoses. 2011; 54(6):475-86.
5. Gharehbolagh SA, Fallah B, Izadi A, Ardestani ZS, Malekifar P, M Borman A, et al. Distribution, antifungal susceptibility pattern and intra-*Candida albicans* species complex prevalence of *Candida africana*: a systematic review and meta-analysis. PLoS One. 2020; 15(8):e0237046.
6. Moran GP, Sullivan DJ, Henman MC, McCreary CE, Harrington BJ, Shanley DB, 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; 41(3):617-23.
7. Farahyar S, Izadi S, Razmjou E, Falahati M, Roudbary M, Ashrafi-Khozani M, et al. Low prevalence of antifungal resistant *Candida africana*, in the C. albicans complex causing vulvovaginal candidiasis. Heliyon. 2020; 6(3):e03619.
8. Lotfali E, Mardani M, Abolghasemi S, Darvishnia D, Rabiei MM, Ghasemi R, et al. Isolation of *Candida africana* in oral candidiasis: first report among cancer patients in Iran. Curr Med Mycol. 2020; 6(2):58-62.
9. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the infectious diseases society of America. Clin Infect Dis. 2016; 62(4):e1-50.
10. Perlin DS. Echinocandin resistance, susceptibility testing and prophylaxis: implications for patient management. Drugs. 2014; 74(14):1573-85.
11. Romeo O, Criseo G. First molecular method for discriminating between *Candida africana*, *Candida albicans*, and *Candida dubliniensis* by using hwp1 gene. Diagn Microbiol Infect Dis. 2008; 62(2):230-3.
12. Nikmanesh B, Ahmadikia K, Getso MI, Gharehbolagh SA, Aboutalebian S, Mirhendi H, et al. *Candida africana* and *Candida dubliniensis* as causes of pediatric candiduria: a study using HWP1 gene size polymorphism. AIMS Microbiol. 2020; 6(3):272-9.
13. Mirhendi H, Charsizadeh A, Eshaghi H, Nikmanesh B, Arendrup MC. Species distribution and antifungal susceptibility profile of *Candida* isolates from blood and other normally sterile foci from pediatric ICU patients in Tehran, Iran. Med Mycol. 2020; 58(2):201-6. .
14. Aboutalebian S, Mahmoudi S, Okhovat A, Khodavaisy S, Mirhendi H. Otomycosis due to the rare fungi *Talaromyces purpurogenus*, *Naganishia albida* and *Filobasidium magnum*. Mycopathologia. 2020; 185(3):569-75.
15. Ngouana TK, Krasteva D, Drakulovski P, Toghueo RK, Kouanfack C, Ambe A, et al. Investigation of minor species *Candida africana*, *Candida stellatoidea* and *Candida dubliniensis* in the *Candida albicans* complex among Yaoundé (Cameroon) HIV-infected patients. Mycoses. 2015; 58(1):33-9.
16. Pemán J, Cantón E, Quindós G, Eraso E, Alcoba J, Guinea J, et al. Epidemiology, species distribution and in vitro antifungal susceptibility of fungaemia in a Spanish multicentre prospective survey. J Antimicrob Chemother. 2012; 67(5):1181-7.
17. Gil-Alonso S, Quindós G, Eraso E, Jauregizar N. Postantifungal effect of anidulafungin against *Candida albicans*, *Candida dubliniensis*, *Candida africana*, *Candida parapsilosis*, *Candida metapsilosis* and *Candida orthopsilosis*. Rev Esp Quimioter. 2019; 32(2):183-8.
18. Njunda LA, Assob JC, Nsagha SD, Kamga HL, Ndellejong EC, Kwenti TE. Oral and urinary colonization of *Candida* species in HIV/AIDS patients in Cameroon. Basic Sci Med. 2013; 2(1):1-8.
19. Romeo O, De Leo F, Criseo G. Adherence ability of *Candida africana*: a comparative study with *Candida albicans* and *Candida dubliniensis*. Mycoses. 2011; 54(4):e57-61.
20. Hana S, Latifa M, Camilia C, Boutheina J. Characterization of the '*Candida albicans* Complex': first report of *Candida africana* in Tunisia. J Med Microb Diagn. 2020; 9(307):2.
21. Mucci MJ, Cuestas ML, Landanburu MF, Mujica MT. Prevalence of *Candida albicans*, *Candida dubliniensis* and *Candida africana* in pregnant women suffering from vulvovaginal candidiasis in Argentina. Rev Iberoam Micol. 2017; 34(2):72-6.
22. Gajdács M, Dóczi I, Ábrók M, Lázár A, Burián K. Epidemiology of candiduria and *Candida* urinary tract infections in inpatients and outpatients: results from a 10-year retrospective survey. Cent European J Urol. 2019; 72(2):209-14.
23. Naeimi B, Mirhendi H, Khamisipour G, Sadeghzadeh F, Ahmadi B. *Candida africana* in recurrent vulvovaginal candidiasis (RVVC) patients: frequency and phenotypic and genotypic characteristics. J Med Microbiol. 2018; 67(11):1601-7.
24. Nouraei H, Sheykhi S, ZareShahrabadi Z, Khodadadi H, Zomorodian K, Pakshir K. Comparative analysis of virulence factors of homozygous and heterozygous strains of *Candida albicans* vaginal isolates. Int J Microbiol. 2020; 2020:8889224.
25. Shokoohi G, Javidnia J, Mirhendi H, Rasekh-Jahromi A, Rezaei-Matehkolaei A, Ansari S, et al. Molecular identification and antifungal susceptibility profiles of *Candida dubliniensis* and *Candida africana* isolated from vulvovaginal candidiasis: a single-centre experience in Iran. Mycoses. 2021; 64(7):771-9.
26. Fakhim H, Vaezi A, Javidnia J, Nasri E, Mahdi D, Diba K, et al. *Candida africana* vulvovaginitis: prevalence and geographical distribution. J Mycol Med. 2020; 30(3):100966.
27. Hashemi SE, Shokohi T, Abastabar M, Aslani N, Ghadamzadeh M, Haghani I. Species distribution and susceptibility profiles of *Candida* species isolated from vulvovaginal candidiasis, emergence of C. lusitaniae. Curr Med Mycol. 2019; 5(4):26-34.
28. Theill L, Dudiuk C, Morano S, Gamarra S, Nardin ME, Méndez E, et al. Prevalence and antifungal susceptibility of *Candida albicans* and its related species *Candida dubliniensis* and *Candida africana* isolated from vulvovaginal samples in a hospital of Argentina. Rev Argent Microbiol. 2016; 48(1):43-9.
29. Rezazadeh E, Moazeni M, Sabokbar A. Use of cost effective and rapid molecular tools for identification of Candida species, opportunistic pathogens. Curr Med Mycol. 2016; 2(3):1-4.
30. Romeo O, Criseo G. Molecular epidemiology of *Candida albicans* and its closely related yeasts *Candida dubliniensis* and *Candida africana*. J Clin Microbiol. 2009; 47(1):212-4.
31. Petty LA, Gallan AJ, Detrick JA, Ridgway JP, Mueller J, Pisano J. *Candida dubliniensis* pneumonia: a case report and review of literature. Mycopathologia. 2016; 181(9-10):765-8.

32. Khan Z, Ahmad S, Joseph L, Chandy R. *Candida dubliniensis*: an appraisal of its clinical significance as a bloodstream pathogen. PLoS One. 2012; 7(3):e32952.
33. Coleman DC, Sullivan DJ, Bennett DE, Moran GP, Barry HJ, Shanley DB. Candidiasis: the emergence of a novel species, *Candida dubliniensis*. AIDS. 1997; 11(5):557-67.
34. van Hal SJ, Stark D, Harkness J, Marriott D. *Candida dubliniensis* meningitis as delayed sequela of treated *C. dubliniensis* fungemia. Emerg Infect Dis. 2008; 14(2):327-9.
35. Tavanti A, Davidson AD, Fordyce MJ, Gow NA, Maiden MC, Odds FC. Population structure and properties of *Candida albicans*, as determined by multilocus sequence typing. J Clin Microbiol. 2005; 43(11):5601-13.
36. Yazdanpanah A, Khaithir TM. Issues in identifying germ tube positive yeasts by conventional methods. J Clin Lab Anal. 2014; 28(1):1-9.
37. Gumral R, Sancak B, Guzel AB, Saraçlı MA, Ilkit M. Lack of *Candida africana* and *Candida dubliniensis* in vaginal *Candida albicans* isolates in Turkey using HWP1 gene polymorphisms. Mycopathologia. 2011; 172(1):73-6.