



ORIGINAL ARTICLE

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



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Received 30 July 2015; accepted 13 October 2015

Available online 26 February 2016

KEYWORDS

Candida Africana;
Vulvovaginal
candidiasis;
Antifungal resistance;
Molecular
identification;
Argentina

Abstract *Candida africana* taxonomical status is controversial. It was proposed as a separate species within the *Candida albicans* species complex; however, phylogenetic analyses suggested that it is an unusual variety of *C. albicans*. The prevalence of *C. albicans*-related species (*Candida dubliniensis* and *C. africana*) as vulvovaginal pathogens is not known in Argentina. Moreover, data on antifungal susceptibility of isolates causing vulvovaginal candidiasis is scarce. The aims of this study were to establish the prevalence of *C. dubliniensis* and *C. africana* in vaginal samples and to evaluate the antifungal susceptibilities of vaginal *C. albicans* species complex strains. We used a molecular-based method coupled with a new pooled DNA extraction methodology to differentiate *C. dubliniensis* and *C. africana* in a collection of 287 strains originally identified as *C. albicans* isolated from an Argentinian hospital during 2013. Antifungal susceptibilities to fluconazole, clotrimazole, itraconazole, voriconazole, nystatin, amphotericin B and terbinafine were evaluated by using the CLSI M27-A3 and M27-S4 documents. Of the 287 isolates, 4 *C. dubliniensis* and one *C. africana* strains (1.39% and 0.35% prevalence, respectively) were identified. This is the first description of *C. africana* in Argentina and its identification was confirmed by sequencing the ITS2 region and the *hwp1* gene. *C. dubliniensis* and *C. africana* strains showed very low MIC values for all the tested antifungals. Fluconazole-reduced-susceptibility and azole cross-resistance were observed in 3.55% and 1.41% of the *C. albicans* isolates, respectively. These results demonstrate that antifungal resistance is still a rare phenomenon in this kind of isolates.

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PALABRAS CLAVE

Candida africana;
Candidiasis
vulvovaginal;
Resistencia a los
antifúngicos;
Identificación
molecular;
Argentina

Prevalencia y sensibilidad a los antifúngicos de *Candida albicans* y sus especies relacionadas, *Candida dubliniensis* y *Candida africana* aisladas de muestras vulvovaginales en un hospital de Argentina

Resumen La clasificación taxonómica de *Candida africana* está en discusión, es considerada una nueva especie dentro del complejo *C. albicans* o una variedad inusual de *C. albicans*. La prevalencia de las especies relacionadas a *C. albicans* (*C. dubliniensis* y *C. africana*) como agentes de vulvovaginitis en Argentina se desconoce. Los objetivos de este trabajo fueron determinar la prevalencia de *C. dubliniensis* y *C. africana* en muestras vaginales y evaluar la sensibilidad a los antifúngicos de aislamientos vaginales de las especies del complejo *C. albicans*. Para diferenciar *C. dubliniensis* y *C. africana* utilizamos un método molecular asociado a un nuevo método de extracción de ADN. Se utilizó una colección de 287 cepas originalmente identificadas como *C. albicans* aisladas durante 2013 en un hospital de Argentina. Se evaluó la sensibilidad a fluconazol, clotrimazol, itraconazol, voriconazol, nistatina, anfotericina B y terbinafina utilizando los documentos M27-A3 y M27-S4 del CLSI. De los 287 aislamientos, se identificaron 4 *C. dubliniensis* y 1 *C. africana* (1,39 y 0,35% de prevalencia, respectivamente). Esta es la primera descripción de *C. africana* en Argentina. Su identificación fue confirmada por secuenciación de la región ITS2 y del gen *hwp1*. Las cepas identificadas como *C. dubliniensis* y *C. africana* mostraron valores de CIM muy bajos para todos los antifúngicos probados. En los aislamientos de *C. albicans*, la sensibilidad reducida al fluconazol y la resistencia cruzada a todos los azoles se observó en el 3,55% y el 1,41%, respectivamente. Estos resultados demuestran que la resistencia a los antifúngicos es todavía un fenómeno raro en este tipo de aislamientos.

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Introduction

More than twenty different species of *Candida* have been reported as human pathogens^{14,20}. However, *Candida albicans* is the most common human fungal pathogen and the most studied of all *Candida* species^{8,10,15,17,20}. *C. albicans* has a great degree of variability and some of the firstly known as "atypical" *C. albicans* isolates were later considered as related species. That is the case with the well-known *Candida dubliniensis*³⁰. Other "atypical" *C. albicans* considered as separated species are now described as varieties (e.g. *C. albicans* var. *stellatoidea*) and included as one of the 173 *C. albicans* synonyms¹⁰. The most recently described *C. albicans* related species is *Candida africana*³¹. The first *C. africana* strain was isolated from African patients suffering from vulvovaginal candidiasis (VVC) and firstly considered "atypical" *C. albicans* strains³². Later, it was proposed as a new species based on morphological, biochemical and physiological differences which include its inability to assimilate glucosamine and N-acetylglucosamine and its impossibility to form chlamydo spores on corn meal agar^{24,31}. Odds *et al.* in 2007 included *C. africana* in *C. albicans* clade 13 and supported a varietal status (*C. albicans* var. *africana*)¹⁶. One year later, the same group included several *C. africana* strains in a group highly distinct from the majority of *C. albicans* together with *Candida stellatoidea* type I and other sucrose-negative atypical *C. albicans* isolates⁹. These facts support the controversial taxonomic status of these isolates.

The prevalence of these *C. albicans* related species as agents of VVC is not known in Argentina. Moreover, data on

antifungal susceptibility patterns of isolates causing VVC are scarce¹².

The aim of this study was to establish the prevalence of *C. dubliniensis* and *C. africana* in a collection of yeasts originally identified as *C. albicans* and isolated from VVC using a molecular-based method coupled with a pooled DNA extraction methodology. Additionally, we evaluated the antifungal susceptibility patterns of these strains.

Materials and methods

Yeast strains

A total of 287 strains were included in this study. All isolates were obtained from patients having vaginal infection symptoms attending the *José María Cullen Hospital* (Santa Fe – Argentina) during 2013. All the strains were identified as *C. albicans* by germ tube formation in human serum and their ability to form green color colonies on CHROMAgar *Candida*TM (Biomérieux – Medica-Tec SRL, Buenos Aires, Argentina). All yeast isolates were referred to the "Micología y Diagnóstico Molecular" Laboratory (CONICET Universidad Nacional del Litoral) where they were stored and identified in accordance with morphology and carbohydrate assimilation and fermentation¹¹ and by a molecular procedure as described below. *C. albicans* ATCC 90028 and *C. dubliniensis* NCPF 3949 were used as control strains.

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