

Candida glabrata: an emergent opportunist in vulvovaginitis

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Abstract

Background: *Candida* genus has various species. The incidence of *C. glabrata* has presented itself with more frequency over the past years with clinical importance.

Methods: A case study was made to determine the frequency of *C. glabrata* in 468 patients who presented clinical symptomatology for vulvovaginal candidiasis and the *in vitro* response for fluconazole using two methods: diffusion in agar plates and microdilution in liquid medium [NCLSI (NCCLS) method].

Results: The frequency for this specie was 12.6%, almost double the frequency observed 10 years ago. The resistance of *C. glabrata* to fluconazole treatment was confirmed in this study, representing 68.2% resistance in all strains on test plates and 51.2% on NCLSI method with a MIC of 16 µg/ml.

Conclusions: The frequency of *Candida glabrata* has increased over the past years. It presents resistance to usual treatments, which promotes the persistence and recurrence of genital and systemic infections.

Key words: candidiasis, *Candida glabrata*, resistance, vulvovaginitis.

Introduction

Infections by *Candida* genus fungi (candidiasis) have increased their prevalence in the last three decades and have become a significant cause of morbimortality, especially when they evolve into hematic infections. Although *Candida albicans* is still the most commonly found species, there has been a significant increase in the prevalence of other species known as non-*albicans* *Candida*: *C. parapsilosis*, *C. tropicalis* and *C. glabrata*.^{1,2} The latter is commonly found in oral and vaginal cavities of healthy individuals as well as in the hands of healthcare personnel.¹ Infection from this yeast increases with extended hospital stays and clinical deterioration of patients, representing the first sign of several infections.

C. glabrata is now frequently identified in our hospitals as an agent of vaginal candidiasis or producing severe systemic mycosis and candidemia in critical and immunocompromised patients who present solid or hematologic neoplasms.³⁻⁶ *C. glabrata* is the second most frequently identified species (after *C. albicans*) in women with vaginitis and increased vaginal discharge, having a prevalence between 0.6% and 36% with a mid-frequency between 15% and 20%.⁷⁻¹⁰ Some epidemic outbreaks have been identified in ICUs and are regarded as nosocomial infections. Muriel et al.¹¹ conducted a study including 108 strains from gynecological samples (138 from neonatal ICUs and 71 from ICUs) identifying *C. glabrata* in 19.4% of vaginal samples (regarded as community-acquired infections), 27.5% of neonatal samples and 29.6% of ICU samples. These data demonstrate the prevalence of nosocomial infections over community-acquired infections for that species.^{1,3,7,12}

Because of the relevance and increase in vulvovaginal candidiasis, it is important to determine its specific etiology with special attention to *C. glabrata* identification, in order to have a precise idea of its frequency in Mexico as well as its therapeutic behavior. The purpose of our study was to establish the frequency of *C. glabrata* in vaginal discharge cultures from patients with vulvovaginal candidiasis signs and symptoms in three hospitals in Mexico City and to evaluate *C. glabrata* strain susceptibility to fluconazole, one of the most frequently used azoles against candidiasis.

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Materials and Methods

We included 468 female patients from three hospitals in Mexico City: Gynecology Service of the General Hospital of Mexico, Women's Hospital of Mexico City and Gynecology Service of November 20th Hospital (ISSSTE). Patients were informed in detail about the study and were willing to participate in the research. We included patients >18 years old who presented signs and symptoms of vulvovaginal candidiasis: erythema, edema, leukorrhea, excoriations, pruritus and dyspareunia.

We obtained a complete clinical history from each patient along with gynecological exploration and vaginal samples that were tested with 10% KOH to observe the following images: pseudohyphae, pseudohyphae + blastoconidias and only blastoconidias. We used a gram-positive criteria for all described mycological images and regarded as normal flora the presence of yeasts (blastoconidias) <2+ per field.

Mycological Culture

Two discharge samples were taken using cotton swabs and cultured in six media: two in Sabouraud dextrose agar, two in Sabouraud dextrose agar with antibiotics and two in BiGGY (Nickerson, Becton Dickinson, Franklin Lakes, NJ) agar, incubating them for 7 days at 28°C. They were observed macro and microscopically to corroborate presence of yeast.

Species were identified using the following methods: germination tubes in human serum at 37°C for 3 h, pseudohyphae and Chlamydia conidia in corn flour media + Tween 80, and zymogram in commercial API-yeast-20 medium. Once species were identified, antifungal qualitative sensitivity tests were carried out using fluconazole in agar plates and quantitative sensitivity using broth dilution protocol from NCLSI (National Clinical and Laboratory Standards Institute). Ethical and legal requirements were met according to the Declaration of Helsinki recommendations (updated in 1989, Hong Kong). Results were statistically analyzed using central trend, dispersion and percentage measures.

Results

A total of 468 women were included in the study and were diagnosed with genital candidiasis. The following general data were reported: average age 35.96 years (± 9.8 SD), 19 years old as the minimum age and 69 years as the maximum age. Of these patients, 97.6% were mixed-race, whereas 2.4% were Caucasian.

Of the patients, 227 (48.50%) reported having similar episodes in the last 12 months. Clinical manifestations during the current episode were 425 (90.8%) pruritus, 345 (69.4%) erythema, 451 (96%) leukorrhea, 269 (57.4%) edema, 143 (30.5%) excoriations and 275 (58.7%) dyspareunia.

Of the cases, 143 were regarded as negative; therefore, the analyzed group was reduced to 325 cases with a positive culture

Table 1. Results of direct exam and mycological culture ($n = 325$)

Total	Direct exam	Species	%
220	S + B	<i>C. albicans</i>	67.7
52	S + B	<i>C. tropicalis</i>	16.0
41	B ^{3&4+}	<i>C. glabrata</i>	12.6
8	S + B	<i>C. krusei</i>	2.5
4	S + B	<i>C. parapsilosis</i>	1.2

S, pseudohyphae; B, blastoconidias; B^{3&4+}, abundant blastoconidias (3 & 4 crosses per field).

and parasitic image as well as species identification. Mycological results are shown in Table 1. Of the negative cases (143), 95 presented negative direct tests and cultures, whereas 48 presented parasitic images but had positive cultures with few colonies. These were regarded as part of the usual flora and account for 10.2% (48/468) of all included patients.

As shown in Table 1, 41/325 (12.6%) patients presented *C. glabrata*. With obtained *C. glabrata* strains, we carried out fluconazole sensitivity tests using two methods: agar plates (to obtain resistance/susceptibility cut-off points) and broth dilution according to NCLSI protocol (to identify resistant strains from obtained MICs). Of the strains, 68.2% (28/41) presented resistance to fluconazole sensitivity test in agar plates because there were no visible inhibiting halos at the highest concentration (32 μ g); 51.2% (21/41) presented resistance to fluconazole in broth dilution tests with an average of 16 μ g/ml.

Discussion

A 1996 epidemiological study including a high prevalence of candidemia in patients with neoplasms reported that 6/1000 admissions presented candidemia and, of these, 79% occurred in ICU patients. This study confirmed the increase of non-*albicans* *Candida* sp. prevalence, demonstrating that *C. glabrata* was responsible for 11% of sepsis related to central venous catheter and fluconazole prophylaxis. Candidemia is not a frequent complication in AIDS patients and generally appears in late phases; however, although *C. albicans* is the most frequently identified species, *C. glabrata* has also been isolated.^{7,12,13}

C. glabrata is found in candiduria cases with an increasing frequency, especially in diabetic patients, patients who receive multi-antibiotic treatments or those patients who have a urinary catheter. A retrospective study evaluating risk factors of nosocomial infections from *C. glabrata* and *C. albicans* reported that fluconazole and quinolones were specifically associated with *C. glabrata* candiduria. It has been questioned if the resistance to this antifungal drug presented by several strains produces *C. albicans* replacement or if this is an independent phenomenon.

Table 2. Characteristics of *C. glabrata* infections

• Community-acquired infection	Vaginitis
• Nosocomial infection	Immunosuppressed and weakened patients Admission to ICUs
• Associations	Frequently with other yeasts
• Origin of the infection	Frequently exogenous
• Risk factors for systemic infection	Urinary catheter (candiduria) Vascular catheter (candidemia) Broad-spectrum antibiotics
• Specific risk factors	Previous fluconazole administration Prolonged hospitalization

Because of the above, this yeast is considered as an emergent opportunist in most publications.⁵ Table 2 specifies the most relevant factors related to *C. glabrata* epidemiology infections.⁵

species-characteristic antigenic components have been described such as factor 34, which is the basis of the commercial identification system Candida Check[®].^{11,14,15}

Taxonomical Classification

C. glabrata belongs to Ascomycetes class, Saccharomycetales order, Saccharomycete family, *Candida* genus, *glabrata* species. *Torulopsis* genus was created to differentiate it from *Candida* because it lacks blastoconidias capable of forming pseudomycele or true hyphae either in infected tissues or in cultures. Currently, these characteristics are considered insufficient to differentiate both genera, and its integration into *Candida* genus has been proposed since 1978, although both are regarded as synonymous.

C. glabrata antigenic structure has been determined by several authors, demonstrating there is a certain cross-reactivity level with other more virulent species such as *C. albicans*, *C. tropicalis*, *C. guilliermondii*, *C. kefyr* and *C. parapsilosis*. However, some

Physiological Considerations

C. glabrata strains do not assimilate inositol and do not contain carotenoid pigments and are inhibited by cycloheximide 0.01%. Maximum temperature for growth is 43-45°C and optimal temperature for clinical strains is 35-37°C. Differential characteristics are shown in Table 3.

With 11 chromosomes and, because of its haploid character, *C. glabrata* is considered to have a greater chance for mutations than other diploid species such as *C. albicans*.^{2,16}

Several studies that have isolated *C. glabrata* in different populations and areas report a higher prevalence in elderly patients (27%) and in those patients with stomatitis due to dental prostheses (22-25%). *C. glabrata* has also been isolated in 5-25% of stomach

Table 3. *C. albicans* and *C. glabrata* differential characteristics

Characteristic	<i>Candida albicans</i>	<i>Candida glabrata</i>
Blastoconidias	3-7 × 3-14 µm	2.5-4.5 × 4-6 µm
Pseudohyphae	+	-
Chlamydia conidia	+	-
Chromosome number	7-9, diploid	11, haploid
Assimilation of sugars	G, S, M, T, Gal	G, T, A
Color of chromogenic media		
• CHROMagar [®]	Green	Lilac-purple
• Candida ID [®]	Blue	White
Growth in cycloheximide 0.1%	±	-
Experimental virulence	+++	+
Triazole resistance	+	+++

G, glucose; S, saccharose; M, maltose; T, trehalose; GAL, galactose; A, arabinose; +, positive; -, negative; ±, occasional.

samples and in 5-30% of gynecological samples from women without vaginitis. This species is seldom found in normal skin (1-2%), but is found in up to 36% of urine samples from hospitalized patients.^{3,4,11,14,15,17}

Virulence Factors

The absence of some virulence factors such as pseudohyphae (that increases fungus adherence and its ability to penetrate tissues) leads us to think *C. glabrata* is less virulent than other species such as *C. albicans* or *C. tropicalis*. This is true when using experimental laboratory animal models; however, there are evidences that demonstrate a rapid spread of *C. glabrata* infections in immunosuppressed patients who also present a high mortality rate. Although knowledge of virulence markers in this species is limited, some studies have confirmed that *C. glabrata* produces proteinases and that its cell surface hydrophobia is similar to *C. albicans*, which ensures its adherence ability in host cells.

Some host alterations that contribute to *C. glabrata* infections development are a decrease of vaginal secretory IgA, low inflammatory response and a quantitative/qualitative decrease of T-cells, which explains its higher prevalence in patients with AIDS, transplants or neoplasms.^{5-6,18-20}

In vitro Resistance

Molecular mechanisms of resistance to antifungal drugs are not yet well understood. *C. glabrata* resistance to azoles is due to an increased P450 cytochrome-dependent ergosterol synthesis and the existence of an active fluconazole flow pump. When referring to antifungal drug resistance, two concepts are often confused: on one hand, absence of a clinical response for therapeutic dosages and, on the other hand, the presence of high minimum inhibitory concentrations (MIC). In the first scenario, lack of therapeutic response may be associated with patient immunosuppression or an insufficient drug bioavailability. In the second scenario, antifungal drug resistance may be primary (innate) or secondary (acquired). MIC results can vary according to the method used for their determination.

C. glabrata is generally sensitive to polyenes such as nystatin and amphotericin B. However, because of commercialization and intensive use of fluconazole and itraconazole, there have been reported cases of *in vitro* resistance and lack of response in patients with candidiasis treated with these antifungal drugs. Several studies have been conducted in HIV-positive patients with oropharyngeal and esophageal candidiasis, either mixed or exclusive of *C. glabrata*. *C. glabrata* strains resistant to fluconazole are prevalent among HIV-positive patients, especially in those affected with oropharyngeal and esophageal candidiasis, although there are reported cases of resistant strains in vaginitis and systemic infections of critical patients with or without neutropenia. Although primary fluconazole resistances are described, most are acquired.

Because *C. glabrata* is a haploid yeast, this may favor the development of secondary resistances. Cross-resistance with other azoles such as itraconazole, ketoconazole and voriconazole is frequent. However, contrary to other yeast genera, *C. glabrata* is usually very sensitive to 5-fluorocytosine.

Using the Fungitest kit (Sanofi Diagnostics Pasteur, Paris, France), which classifies strains as sensitive, intermediate and resistant, 17.6% fluconazole-resistant strains have been isolated in community-acquired vaginal infections, whereas samples from ICU discharged adults reported 21% resistance and neonatal ICU patients reported 1% resistance. Itraconazole resistances were higher: 23.5%, 26.3% and 1%, respectively. There were no cases of amphotericin B resistance.¹¹ A previous study reported 13 *C. glabrata* cases with MIC >16 µg/ml for fluconazole, and 12 presented MIC >2 µg/ml for itraconazole. All strains were sensitive to amphotericin B with MIC <0.5 µg/ml. For 5-fluorocytosine, MIC presented a range <0.06-0.125 µg/ml, confirming its high sensitivity to this antifungal drug.¹⁸

The clinical interpretation of *C. glabrata in vitro* resistance is controversial. However, it is considered that resistance contributes to therapeutic failure, and management of patients with resistant strains is frequently unsatisfactory. The next group of antifungal drugs may contribute to improve prognosis for these infections.^{8-9,11,13,16,18,21-23}

Candida genus includes several species, with *C. albicans* and non-*albicans Candida* being prevalent. The latter have increased their prevalence as clinically significant opportunist infections.^{6,8} Non-*albicans Candida* sp. constitute a heterogeneous group with >200 biological differences. Only some are associated with human infections, which has become a challenge for diagnosis and treatment of candidiasis.¹⁹ One hundred years ago *C. albicans* was considered the only medically important *Candida* species, and *C. parapsilosis*, *C. tropicalis* and *C. guilliermondii* were considered occasional opportunists.^{7,19} Different species other than *C. albicans* have been isolated in infections since 1980 and, nowadays, the following are regarded as opportunists: *C. glabrata*, *C. krusei* and *C. lusitania*.^{8,19}

C. glabrata incidence has increased during the last 40 years³ because of diverse factors such as the use of new treatments and procedures for neoplastic diseases and other pathologies, invasive procedures for diagnosis, extended usage of broad-spectrum antibiotics, HIV and AIDS. In our study, *C. glabrata* frequency (12.6%) is twice that reported in similar studies 10 years ago.² This agrees with the increased frequency and resistance to antifungal drugs reported in several publications.

Fluconazole is one of the most widely used antifungal drugs used to treat systemic and superficial mycoses. *C. glabrata* has the ability to quickly develop resistance to this drug.¹⁷ Our study shows a high fluconazole resistance percentage from *C. glabrata* (68.2% in plates and 51.2% in microdilutions).

Resistance shown by *C. glabrata* is related to its increased prevalence and has converted it into an important factor for

nosocomial infections in ICUs. This favors the increase of *C. glabrata* systemic infections during the last 10 years as well as the persistence and recurrence of vulvovaginal infections.^{10,11,24} Hospital epidemiological studies have recently demonstrated that *C. tropicalis* is being replaced by *C. glabrata* and *C. parapsilosis*.²⁵

Results from our study show that *C. albicans* is responsible for 67.6% of cases, whereas *C. glabrata* has a prevalence of 12.6%. The widespread use of azoles may have contributed to the increase of *C. glabrata* incidence because it presents a very limited *in vitro* resistance to these antifungal drugs. In the U.S. it is the second most frequently isolated species after *C. albicans* to cause systemic candidiasis and candiduria. *C. glabrata* and *C. tropicalis* are non-*albicans* species that show a frequent prevalence in vaginal, oral and gastrointestinal samples, whereas *C. guilliermondii* and *C. parapsilosis* are frequently found on skin.^{16,25}

The most frequently found species in our study related to vulvovaginitis were *C. albicans* (67.9%), *C. glabrata* (12.6%) and *C. tropicalis* (16%). It is important to establish the relationship between recurrence of vulvovaginal candidiasis and non-*albicans* *Candida* species. Mycological tests should be carried out to determine the species. If results report *C. glabrata*, long-term treatment will be justified to eradicate the mycosis or to perform microbiological sensitivity tests to provide a specific treatment.

In conclusion, our study shows a significant increase of vulvovaginitis from *C. glabrata* compared with reports from previous years. Therefore, we consider this an emergent change for this species. Fortunately, available techniques to identify this species are accessible and tests to determine antifungal drug susceptibility allow us to establish the presence of fluconazole-resistant strains. Our results show that 50% of *C. glabrata* strains were resistant to fluconazole. This suggests an opportune therapeutic change to avoid treatment failures and the development of resistant strains.

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