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Susceptibility and molecular characterization of *Candida* species from patients with vulvovaginitis

Gheniffer Fornari[a], *Vania Aparecida Vicente*[a], *Renata Rodrigues Gomes*[a], *Marisol Dominguez Muro*[b], *Rosangela Lameira Pinheiro*[b], *Carolina Ferrari*[c], *Patricia Fernanda Herkert*[a], *Marcos Takimura*[c], *Newton Sérgio de Carvalho*[c], *Flavio Queiroz-Telles*[a,b,*]

[a] *Graduate Program in Microbiology, Parasitology and Pathology, Department of Basic Pathology, Laboratory of Microbiology and Molecular Biology-LabMicro, Federal University of Paraná, Curitiba, Paraná, Brazil*
[b] *Support and Diagnosis Unit, Mycology Laboratory, Federal University of Paraná, Brazil*
[c] *Clinical Hospital Federal University of Paraná, Brazil*

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ABSTRACT

Vulvovaginal candidiasis affects women of reproductive age, which represents approximately 15–25% of vaginitis cases. The present study aimed to isolate and characterize yeast from the patients irrespective of the presentation of clinical symptoms. The isolates were subjected to *in vitro* susceptibility profile and characterization by molecular markers, which intended to assess the distribution of species. A total of 40 isolates were obtained and identified through the CHROMagar, API20aux and by ITS and D1/D2 regions sequencing of DNAr gene. *Candida albicans* strains were genotyped by the ABC system and the isolates were divided into two genotypic groups. The identity of the *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. kefyr* and *Saccharomyces cerevisiae* isolates was confirmed by the multilocus analysis. The strains of *Candida*, isolated from patients with complications, were found to be resistant to nystatin but sensitive to fluconazole, amphotericin B and ketoconazole, as observed by *in vitro* sensitivity profile. The isolates from asymptomatic patients, *i.e.*, the colonized group, showed a dose-dependent sensitivity to the anti-fungal agents, fluconazole and amphotericin B. However, the isolates of *C. albicans* that belong to distinct genotypic groups showed the same *in vitro* susceptibility profile.

* *Corresponding author.*
E-mail: Queiroz.telles@uol.com.br (F. Queiroz-Telles).
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Introduction

Vulvovaginal candidiasis (VVC) is a primary opportunistic mycosis or secondary with endogenous or exogenous characteristics. It is also classified as a sexually transmitted disease (STD) and is caused by different species of *Candida*.[1,2] The disease is characterized by inflammation of the genital mucosa as a response to the yeast proliferation.[3]

The genus *Candida* includes approximately 300 heterogeneous species with different morphological and functional features, and is currently found as a part of the normal flora in skin, digestive tract and mucous membrane, including the human genito-urinary tract.[4] Predominantly, VVC is caused by *C. albicans* and its prevalence can reach 85–95%.[1] However, infections caused by other species such as *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. kefyr* and *C. lusitaniae* have been reported as well.[1,4,5] According to literature these species are part of the vaginal mucous microbiota and they are present in 20–80% of healthy adult population, with clinical manifestations in 10% of pre-menopausal patients, 5–10% in menopausal and 30% of pregnant women.[6,7]

Vulvovaginal infection, caused by *Candida* spp., affects women of reproductive age representing approximately 15–25% of the vaginitis cases.[8] These microorganisms usually remain hosted in the vaginal mucous only as colonizers; however, under inappropriate conditions the yeast reproduction increases inducing expression of virulence factors, which subsequently affects the mucous membrane, characteristic of the symptomatic VVC.[9]

Identification of strains that are isolated from VVC is crucial to clarify the distribution of *C. albicans* in relation to other species of *Candida* genus in different populations with manifestations of the infection. In clinical practice, the yeast identification is based on morphological and biochemical markers, including the automated methods.[10,11] However, not all the species are precisely identified by such procedures. Therefore, molecular markers based on the sequencing of variable domain (D1/D2) from the 26S region and internal transcribed spacers (ITS) of the RNA gene were utilized in the present study to enable identification and detection of various strains.[12]

VVC is not a notified disease and generally the drug treatment is recommended based on the clinical diagnosis. Epidemiological molecular studies are relevant in context of establishment of species prevalence, elucidation of virulence factors and mechanisms of drug resistance so as to support the treatment protocols.[13]

A few studies have focused on the correlation of antifungal susceptibility with clinical results in VVC.[14] In spite of a considerable enhancement in the resistance profile among the various *Candida* species, fluconazole is still widely used for treatment of VVC.[1] Since it has been noticed that *C. albicans* displays a variable sensitivity to azoles derivatives, it seems crucial to identify its sensitivity profile against various drugs for a better therapeutic conduct.[15]

In view of such grounds, the current work aimed to evaluate *in vitro* susceptibility and molecular characterization of yeast from genus *Candida* that were isolated from the patients with infection and the patients with no clinical symptoms, for elucidation of epidemiological aspects of vulvovaginal candidiasis.

Materials and methods

Test organism

The present study analyzed vaginal material isolates from the patients assisted by an outpatient clinic of Toco-gynecology at the Clinical Hospital/UFPR, Paraná (Table 1). The study was conducted from November 2011 to October 2012. The research work was approved by the Ethical Committee of Federal University of Paraná Clinical. Samples from 133 women were collected with their consent.

Casuistry

The study enrolled women, who were aged between 18 and 56 years, with or without VVC clinical symptoms, and who had not been administered any drug treatment in the last six months before collection of the samples. The patients were divided into two groups: colonized patients (without clinical symptoms) and infected patients.[1] The infected patients presented three or more of the following clinical symptoms: typical discharge, vaginal itching, vulvovaginal burning, dysuria and dyspareunia. Infected patient group was subdivided into two sub-groups: (i) complicated – which included women with a history of recurrence infection; and (ii) uncomplicated – patients with sporadic episodes of the infection. The exclusion criteria were age (under 18 and over 56), pregnancy and women with immunosuppressive diseases and under treatment.

Collection, isolation and phenotypic identification

The samples were collected by swabs, and each sample was sowed on Sabouraud Dextrose Agar medium followed by incubation at ±30 °C for a period of 48–120 h as per the growth parameters of each isolate. A presumptive identification of isolates was done by CHROMagar at 37 °C for 48 h.[16] Some of the isolates were identified by the API 20 AUX system (BioMérieux, France).

Molecular characterization of Candida isolates

DNA from the isolates was extracted by physical maceration of the samples in a mixture of silica/celite (2:1) in CTAB (cetyltrimethylammonium bromide). The isolated DNA was precipitated by CIA (acidic solution of chloroform-isoamyl alcohol) followed by sequencing on ABI3500 sequencer.[17] For ITS sequencing, the following primers were used: ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and the reaction conditions of sequencing were as follows[18]: one cycle at 94 °C for 2 min, followed by 30 cycles at (94 °C for 30 s, 56 °C for 1 min, 72 °C for 1 min) and a final extension at 72 °C for 3 min.[17] For amplification of D1/D2 region, the primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4

Table 1 – List of reference strains and the clinical isolates.

Name	Number of reference	Substrate	Geographical indication	GenBank access	
				ITS	D1/D1
Candida albicans	CBS 562	Clinical isolate	Australia	EF567995	U45776
Candida dubliniensis	CBS 7987	Clinical isolate	Japan	AB035589	U57685
Candida inconspícua	CBS 180	Clinical isolate	Australia	AJ853766	U71062
Candida intermedia	CBS 572	Clinical isolate	Australia	EF568011	U44809
Candida glabrata	CBS 138	–	Ireland	AY198398	U44808
Candida haemulonii	CBS 5149	–	Spain	JX459660	U44812
Candida tropicalis	CBS 94	Clinical isolate	Japan	AB437068	U45749
Clavispora lusitaniae	CBS 6986	Urine	Bulgaria	EF568049	U44817
Debaryomyces carsonii	CBS 2285	Clinical isolate	USA	AJ853767	U45743
Kluyveromyces marxianus	CBS 712	Clinical isolate	Australia	EF568057	U94924
Kluyveromyces lactis	CECT1961	Environmental	USA	AJ401704	U94922
Meyerozyma guilliermondii	CBS 2030	Clinical isolate	Australia	EF568003	U45709
Pichia fermentans	L1B	Environmental isolates	UK	FJ713081	U75726
Pichia membranifaciens	23	–	Spain	JQ410476	AJ508586
Pichia segobiensis	CECT 10-210	–	Spain	DQ409166	U45742
Torulaspora delbrueckii	CBS 1146	Clinical isolate	Australia	EF568083	AJ508558
Saccharomyces cerevisiae	NRRL Y-12632	–	USA	AM900404	AY048154
Schizosaccharomyces pombe	NRRL Y-12796	–	USA	AY251633	U40085
Candida glabrata	HC01	Cervicovaginal contents	Brazil	KJ651873	KJ624025
Saccharomyces cerevisiae	HC02	Cervicovaginal contents	Brazil	–	KJ624026
Candida albicans	HC03	Cervicovaginal contents	Brazil	KJ651903	KJ624027
Candida albicans	HC04	Cervicovaginal contents	Brazil	KJ651904	KJ624028
Candida albicans	HC05	Cervicovaginal contents	Brazil	KJ651905	KJ624029
Candida albicans	HC06	Cervicovaginal contents	Brazil	KJ651906	KJ624030
Candida glabrata	HC07	Cervicovaginal contents	Brazil	KJ651907	KJ624031
Candida guilliermondii	HC08	Cervicovaginal contents	Brazil	KJ651908	KJ624032
Candida kefyr	HC09	Cervicovaginal contents	Brazil	KJ651909	KJ624033
Candida glabrata	HC10	Cervicovaginal contents	Brazil	KJ651910	KJ624034
Candida albicans	HC11	Cervicovaginal contents	Brazil	KJ651874	KJ624035
Candida albicans	HC12	Cervicovaginal contents	Brazil	KJ651875	KJ624036
Candida albicans	HC13	Cervicovaginal contents	Brazil	–	KJ624037
Candida albicans	HC14	Cervicovaginal contents	Brazil	KJ651876	KJ624038
Candida albicans	HC15	Cervicovaginal contents	Brazil	KJ651877	KJ624039
Candida albicans	HC16	Cervicovaginal contents	Brazil	KJ651878	KJ624040
Candida albicans	HC17	Cervicovaginal contents	Brazil	KJ651879	KJ624041
Candida albicans	HC18	Cervicovaginal contents	Brazil	KJ651880	KJ624042
Candida albicans	HC19	Cervicovaginal contents	Brazil	KJ651881	KJ624043
Candida albicans	HC20	Cervicovaginal contents	Brazil	KJ651882	KJ624044
Candida albicans	HC01IC	Cervicovaginal contents	Brazil	KJ651883	KJ624045
Candida albicans	HC02IC	Cervicovaginal contents	Brazil	KJ651884	KJ624046
Candida dubliniensis	HC03IC	Cervicovaginal contents	Brazil	KJ651885	KJ624047
Candida albicans	HC04IC	Cervicovaginal contents	Brazil	KJ651886	KJ624048
Candida albicans	HC05IC	Cervicovaginal contents	Brazil	KJ651887	KJ624049
Candida albicans	HC06IC	Cervicovaginal contents	Brazil	KJ651888	KJ624050
Candida albicans	HC07IC	Cervicovaginal contents	Brazil	KJ651889	KJ624051
Candida albicans	HC08IC	Cervicovaginal contents	Brazil	KJ651890	KJ624052
Candida albicans	HC09IC	Cervicovaginal contents	Brazil	KJ651891	KJ624053
Candida albicans	HC10IC	Cervicovaginal contents	Brazil	KJ651892	KJ624054
Candida albicans	HC11IC	Cervicovaginal contents	Brazil	KJ651893	KJ624055
Candida albicans	HC01INC	Cervicovaginal contents	Brazil	KJ651894	KJ624056
Candida albicans	HC02INC	Cervicovaginal contents	Brazil	KJ651895	KJ624057
Candida albicans	HC03INC	Cervicovaginal contents	Brazil	KJ651896	KJ624058
Candida albicans	HC04INC	Cervicovaginal contents	Brazil	KJ651897	KJ624059
Candida albicans	HC05INC	Cervicovaginal contents	Brazil	KJ651898	KJ624060
Candida albicans	HC06INC	Cervicovaginal contents	Brazil	KJ651899	KJ624061
Candida albicans	HC07INC	Cervicovaginal contents	Brazil	KJ651900	KJ624062
Candida albicans	HC08INC	Cervicovaginal contents	Brazil	KJ651901	KJ624063
Candida albicans	HC09INC	Cervicovaginal contents	Brazil	KJ651902	KJ624064

(–) data not provided; HC: clinical hospital/UFPR; C: colonized; IC: complicated infection; INC: non-complicated infection.

(5′-GGTCCGTGTTTCAAGACGG-3′) were used following the same reaction conditions, as listed above.[19]

For ABC genotyping of *C. albicans*, the primers CA-int-L (5′-ATAAGGGAAGTCGGCAAAATAGATCCGTAA-3′) and CA-int-R (5′-CCTTGGCTGTGGTTTCGCTAGATAGTAGAT-3′) were used.[20] The genotyping was based on the presence or absence of a DNA insert, which codes for the ribosomal 26S RNA, dividing *C. albicans* in four groups[20]: A (*C. albicans* – 450 bp), B (*C. albicans* – 840 bp), C (*C. stellatoidea* – 840 bp) and D (*C. dubliniensis* – 1080 bp).

Alignment and phylogenetic construction

The obtained sequences were edited using the Staden program version 1.6, and were compared by the BLAST program for detection of the similarities using reference sequences available in the data bank (NCBI, National Center for Biotechnology Information – http://www.ncbi.nlm.nih.gov/).[21,22] The Mafft program (http://mafft.cbrc.jp/alignment/server/) was used for the alignment; and visual inspection was done by MEGA 5.1 version.[23] Forty sequences of *Candida* isolates were submitted for phylogenetic analysis using *Schizosaccharomyces pombe* strain U40085 as outgroup.[20] The Maximum Likelihood phylogenetic tree was built with 100 bootstraps, based on the evolutionary model Tamura-3 parameters with using 5.1 version of the MEGA software for final editing.[23]

In vitro susceptibility tests

The *in vitro* susceptibility tests were done by micro-dilution method of broth, as per the Norm M27-A3 recommendations provided by the Clinical and Laboratory Standards Institute.[24] The antifungals used were amphotericin B (Sigma–Aldrich 110 Química, Madrid, Spain), ketoconazole (Pharma Nostra, Brazil), itraconazole (Fragon), fluconazole (Pfizer, Madrid, Spain) and nystatin (Pharma Nostra, Brazil). The samples were diluted in RPMI (Roswell Park Memorial Institute Medium)-1640 medium (Sigma) and incubated at 37 °C for 48 h. According to the CLSI criteria, the sensitivity profile is classified as sensitive, dose-dependent sensitivity and resistant.

Results

A total of 40 isolates were obtained from 133 cervicovaginal samples, which were previously identified by CHROMagar and API 20AUX systems. On the basis of ITS and D1/D2 sequences, the isolates could be attributed to the genera *Candida* and *Saccharomyces* (Table 1). Among the isolates studied, 20 belonged to the colonized group, 11 were from the complicated infection group and 9 were from the uncomplicated infection group.

A tree was constructed using maximum likelihood analysis and the evolutionary model Kimura 2-parameter with 100 *bootstraps*. A total of 1959 sites were evaluated, of which, 786 were conserved sites, 1092 were variable sites, 712 sites provided parsimonious information (pi), and 361 were unique sites. The empirical basis frequencies were pi (A): 0.225836 pi (C): 0.283009 pi (G): 0.238533, pi (t) 0.252622. The phylogenetic tree was generated by using 18 strains as references, which included various types of strains of *Candida* species, *Kluyveromyces marxianus*, *K. lactis*, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, keeping *Schizosaccharomyces pombe* as an outgroup.

The evaluated VVC isolates were identified to be *C. albicans*, *C. dubliniensis*, *C. guilliermondii*, *C. kefyr*, *Saccharomyces cerevisiae* and *C. glabrata* and were found to be distributed into six clades supported by bootstrap values (Fig. 1). The phylogenetic data corroborated with the biochemical data, except for the HC03IC isolate that was identified as *C. albicans* by the API 20AUX system, but as *C. dubliniensis* by the phylogenetic analysis (Fig. 1).

According to the tree, most of the analyzed clinical isolates were identified to be *C. albicans*, with 33 isolates clustered in Albicans clade (bs, 100%). Analysis revealed that *C. albicans* isolates could not be separated according to the studied groups, *i.e.*, colonized, complicated, and uncomplicated infection groups. In Guilliermondii clade (bs, 99%), the clinical isolate (HC08C) and *P. guilliermondii* (NRRL Y-2075) type strain were grouped. The isolate (HC02C) from the colonized group and *Saccharomyces cerevisiae* type were clustered in Saccharomyces clade (bs, 100%). Kefyr clade consisted of *Kluyveromyces marxianus* (NRRL Y-8281), *Candida kefyr* teleomorph strain CBS 712, *K. marxianus var. Kluyveromyces lactis* strain NRRL Y-8279 and HC09C isolate of *C. kefyr*. Three isolates were classified into the Glabrata clade (HC01C, HC07C and HC10C), belonging to the colonized group and *C. glabrata* type (5478) with 100% bootstrap.

Based on the molecular data, amidst the 40 isolates that were obtained from vaginal samples, the most prevalent species was *C. albicans* (82.5%), followed by *C. glabrata* (7.5%), *C. guilliermondii* (2.5%), *C. kefyr* (2.5%), *C. dubliniensis* (2.5%) and *Saccharomyces cerevisiae* (2.5%). Among the colonized group alone, a total of 20 isolates belonging to five different species *C. albicans* (60%), *C. glabrata* (25%), *C. guilliermondii* (5%), *C. kefyr* (5%) and *Saccharomyces cerevisiae* (5%) were identified. A total of 9 isolates obtained from the uncomplicated infection group were *C. albicans* (100%). In the complicated infection group, 11 isolates were from two different *Candida* species: *C. dubliniensis* (9.1%) and *C albicans* (90.9%).

Regarding the ABC genotyping of *C. albicans*, at least two different genotypes (A and B) were observed, although 25 isolates belonged to type A and 7 isolates to type B, it was not possible to establish a correlation amidst the genotypes identified and the susceptibility profile of the tested drugs (Fig. 2).

The susceptibility testing results of the studied isolates from different patient groups are summarized in Table 2. In the colonized group (I), all isolates of *C. albicans* ($n = 14$) showed a dose-dependent sensitivity (SDD) to nystatin (8.0 μg/mL) and sensitivity (S) to itraconazole (0.0625 μg/mL), fluconazole (0.125 μg/mL), amphotericin B (0.03–1.0 μg/mL) and ketoconazole (0.0625 μg/mL). Three *C. glabrata* isolates (HC01C, HC02C and HC07C) were resistant (R) to itraconazole (4.0 μg/mL), SDD for the fluconazole (4.0–16 μg/mL), nystatin (8.0 μg/mL) and sensitive to amphotericin B (0.03–1.0 μg/mL) and ketoconazole (1.0–4.0 μg/mL). The isolate of *C. guilliermondii* (HC16C) showed SDD to nystatin (8.0 μg/mL), resistance to amphotericin B (2.0 μg/mL), sensitivity to itraconazole (0.0625 μg/mL), fluconazole (0.125 μg/mL), and ketoconazole (0.0625 μg/mL). *C. kefyr* (HC09C) presented SDD to itraconazole (0.25), nystatin (4.0 μg/mL), and sensitivity for fluconazole (0.25 μg/mL), amphotericin B (1.0 μg/mL) and ketoconazole (0.0625 μg/mL).

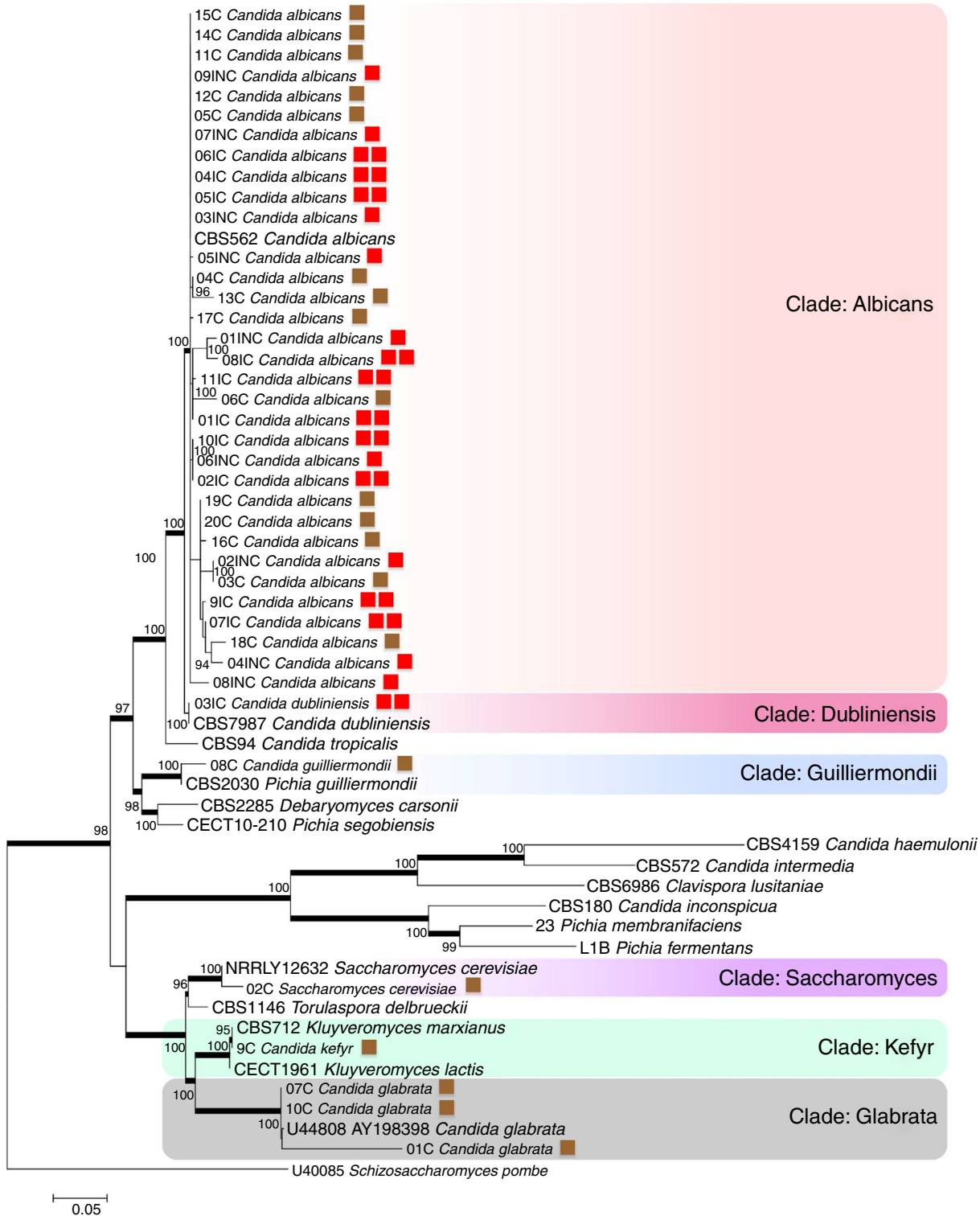

Fig. 1 – The phylogenetic tree of maximum likelihood based on the alignment of the entire region of its1/its2 and D1/D2 was built using 100 bootstrap, using the evolutionary model Tamura-3 parameters with program Mega version 5.1. *Schizosaccharomyces pombe* was used as an outgroup. The tree showed 6 clades (Albicans; Dubliniensis; Guilliermondii, Saccharomyces; Kefyr; Glabrata) diversified according to the isolated species. For a thorough understanding, the evaluated groups in this study are represented by colored squares for discernment: the brown square refers to the colonized group; one red square indicates isolates from the uncomplicated infection group; the two red squares represent the group with complicated infection.

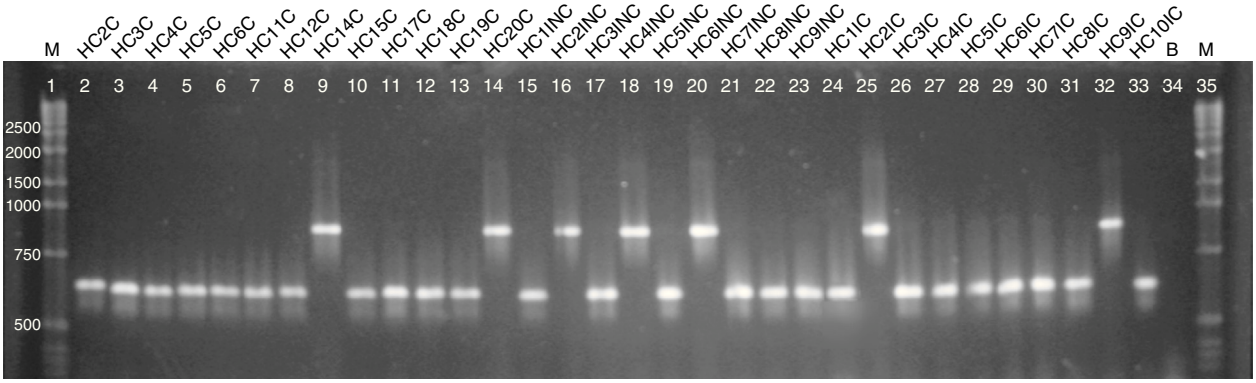

Fig. 2 – Agarose electrophoresis ABC genotyping of the *C. albicans* from the different studied groups, genotype A (*C. albicans* – 450 bp) and B (*C. albicans* – 840 bp). The lanes 2–14 correspond to the colonized group; 15–23 to the uncomplicated infection group and 24–33 to the complicated infection group. Lane 34 represents a blank; the lanes 1 and 35 indicate standard 1 kb molecular weight markers (Invitrogen, Carlsbad, Ca, USA).

Furthermore, *S. cerevisiae* isolate (HC02C) was SDD to nystatin (8.0 μg/mL) and sensitive to itraconazole (0.0625 μg/mL), fluconazole (0.125 μg/mL), amphotericin B (0.03–1.0 μg/mL) and ketoconazole (0.0625 μg/mL).

In the complicated infection group (II), one strain of *C. albicans* isolate (HC01IC) was found to be SDD to itraconazole (0.0625–0.25 μg/mL); all isolates ($n = 10$) were resistant to nystatin (≥64 μg/mL) and sensitive to fluconazole (0.125–2.0 μg/mL), amphotericin B (1.0 μg/mL) and ketoconazole (0.0625 μg/mL). *C. dubliniensis* isolate (HC03IC) was resistant to nystatin (≥64 μg/mL) and presented sensitivity toward the itraconazole (0.0625 μg/mL), fluconazole (0.125–2.0 μg/mL), amphotericin B (0.5–1.0 μg/mL) and ketoconazole (0.0625 μg/mL). Finally, in the uncomplicated infections group (III), all the isolates ($n = 9$) of *C. albicans* were SDD to nystatin (8.0 μg/mL) and sensitive toward itraconazole (0.0625 μg/ml), fluconazole (0.125–2.0 μg/mL), amphotericin B (0.5–1.0 μg/mL) and ketoconazole (0.0625 μg/mL).

Discussion

Identification of *Candida* species that causes VVC is highly desirable in microbiological practice, as it may help in clarifying the prevalence and incidence of species that affects the susceptible population. Moreover, determination of susceptibility of *Candida* to the antifungal drugs may be crucial in context of the recurrent clinical forms of VVC. Several studies have demonstrated the occurrence of vulvovaginitis due to *Candida* species, indicating heterogeneity among isolates from different geographical regions. In the present study, the prevalent species were[3,25–27] *C. albicans*, followed by *C. glabrata*, *C. guilliermondii*, *C. kefyr*, *C. dubliniensis* and *Saccharomyces cerevisiae*, thereby suggesting an increase of infection by non-albicans *Candida*. An increase in infections that are caused by non-albicans *Candida* has been registered, although *C. albicans* is still the most isolated species in VVC clinical cases.[13,28–31] Furthermore, the cultural and ethnic differences may also influence the isolation rate of yeast from vulvovaginitis samples.[32,33] The lack of data on epidemiology and genetic variability reinforces the importance of epidemiological studies by molecular methods.[6,27,34–36]

The colonized group investigated in the current study presented a wide diversity of species such as *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. kefyr* and *Saccharomyces cerevisiae*. In addition, *C. albicans* was found to be prevalent ($n = 19$) in the infection group and *C. dubliniensis* ($n = 1$) isolates were observed only in the complicated infection group (Table 2). The microbiota species found in colonized women are the same as reported in VVC.[1,4,27,30,35] Vaginitis caused by

Table 2 – Variations in the minimum inhibitory concentration (MIC) of antifungals for the different study groups.

Isolate species	Total of samples	Itraconazole	Fluconazole	Nystatin	Amphotericin B	Ketoconazole
C. albicans (I)	14	0.0625–0.0625	0.125–0.125	8.0–8.0 (SDD=14)	0.03–1.0	0.0625–0.0625
C. albicans (II)	10	0.0625–0.25 (SDD = 1)	0.125–2.0	≥64 (R = 10)	0.5–1.0	0.0625–0.25
C. albicans (III)	09	0.125–0.125	0.125–0.125	8.0–8.0 (SDD = 9)	1.0–1.0	0.0625–0.0625
C. glabrata (I)	03	2.0–4.0 (R = 3)	4.0–16.0 (SDD = 1)	8.0–8.0 (SDD = 3)	0.03–1.0	1.0–4.0
C. guillermondii (I)	01	0.0625–0.0625	0.125–8.0	8.0 (SDD = 1)	0.25–2.0 (R = 1)	0.0625–2.5
C. Kefyr (I)	01	0.25 (SDD = 1)	0.25	4.0 (SDD = 1)	1.0	0.0625
S. cerevisiae (I)	01	0.0625–0.0625	0.125–0.125	8.0–8.0 (SDD = 1)	0.03–1.0	0.0625–0.0625
C. dubliniensis (II)	01	0.0625–0.0625	0.125–2.0	≥ 64 (R = 1)	0.5–1.0	0.0625–0.25

SDD: sensitivity dose dependent; R: resistant; S: sensitivity; I: colonized group; II: complicated infection group; III: uncomplicated group.

S. cerevisiae is rare and it has been isolated from an asymptomatic patient.[37,38] This corroborates with the findings of the present study.

The VVC *Candida albicans* isolates, analyzed by us, were clustered into a single clade, indicating a monophyletic group (Fig. 1), which is in concordance with the data already reported by several authors.[31,39] The strain, identified as *C. albicans* (HC03IC) by biochemical test, proved to be *C. dubliniensis* according to the phylogenetic analysis. A lack of correlation between the phenotypic and molecular identification of the samples can be justified by the limitations of the commercial identification system, which do not allow distinguishing the yeast species, which have minor phenotypic differences.[40,41] Therefore, multilocus analyses are needed in order to identify *Candida* species.[31] In the ABC genotyping, two different genotypes among *C. albicans* isolates were detected. The genotype A was observed in 75.7% of the isolates, and the isolates of genotype B were present in all the analyzed groups. However, it had a higher occurrence in the uncomplicated infected group (Fig. 2). It has been reported that the candidiasis that is caused by *C. albicans* of genotype B has a higher tendency for persistent infections, though further studies with larger populations for a better assessment are required.[36]

In the current analysis, all the *C. albicans* isolates from the complicated group, regardless of genotype, were resistant to nystatin and susceptible to other tested antifungals, except for the isolate HC04IC that showed SDD to fluconazole. In the uncomplicated infections group, the isolates of *C. albicans* were SDD to nystatin and susceptible to other tested antifungals. The same was observed in the isolates from the group of colonized patients. Concerning the non-albicans *Candida* species, the isolate of *C. dubliniensis* was resistant only to nystatin, the *C. glabrata* isolates were resistant to itraconazole and SDD to fluconazole and nystatin. These results contrast from the previous reports that regarded *C. glabrata* isolates as resistant and SDD to fluconazole.[42] Besides, *C. kefyr* strain presented SDD to itraconazole and nystatin, and a similar susceptibility profile was previously reported.[10] In other studies, similar results were obtained, reporting that VVC species strains of genus *Candida* presented resistance and also a high frequency of SDD for nystatin and sensitivity to others tested drugs.[26,35,43]

The isolate of *C. guilliermondii* showed SDD to nystatin and resistance to amphotericin B. According to the literature, the treatment is problematical due to a low sensitivity for some antifungal classes, especially for fluconazole, itraconazole and amphotericin B; and VVC infections caused by *C. guilliermondii* are rare.[2] Furthermore, we obtained an isolate of *S. cerevisiae* SDD to nystatin, which differed from the data previously reported for this species, which demonstrated that *S. cerevisiae* isolates were resistant to fluconazole, posaconazole, and itraconazole.[44]

There are several factors that can influence the clinical response to treatment of VVC, as evident from different reports showing variation in the *in vitro* susceptibility and *in vivo* response to the drug.[39,45] Through *in vitro* susceptibility testing, it was observed that all the isolates were sensitive to ketoconazole, although fluconazole remains the drug of choice for VVC treatment. Such results indicate that the susceptibility profile for the isolates may not be a factor related to the recurrence of the disease. Therefore, it may be concluded that the molecular analysis provides accurate identification of *Candida* species isolated from patients with VVC. Hence, our findings demonstrated the importance of molecular tools for identification of the isolates and also to elucidate the epidemiology of VVC.

Conflicts of interest

The authors declare no conflicts of interest.

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