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Species identification and antifungal susceptibility pattern of *Candida* isolates in cases of vulvovaginal candidiasis

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Abstract Vulvovaginal candidiasis (VVC) remains one of the most common infections of the female genital tract. Correct identification of the isolated *Candida* species is essential to direct the empirical antifungal therapy. *Objectives:* This local study was conducted to identify the spectrum of *Candida* species associated with VVC using different phenotypic and genotypic methods and assess their antifungal susceptibility pattern. *Materials and methods:* High vaginal swabs were collected from 125 patients presenting with a clinical picture suggestive of VVC. Swabs were subjected to Gram-stain and culture on Sabouraud dextrose agar. Species identification of *Candida* isolates was done using phenotypic methods including germ tube test, Rice Tween-80 agar, Chrom ID (CAN2) agar and API 20C AUX, while PCR-RFLP was used as the gold standard method. Antifungal susceptibility testing was done using the disk diffusion method. *Results:* Vaginal swab cultures yielded *Candida* growth in 63 cases (50.4%). *Candida albicans* was the predominant isolated species (60.3%) while the most common non-albicans species was *Candida glabrata* (12.7%). Forty-five (71.4%) and fifty-five (87.3%) *Candida* isolates were correctly speciated by Rice Tween-80 Agar and API 20C AUX, respectively, while fifty-seven isolates (90.5%) were correctly assigned into the 3 groups of yeasts identified by CAN2 agar. Amphotericin B was more effective than azoles against vaginal *Candida* isolates. *Conclusion: C. albicans* is the most common species associated with VVC. API 20C AUX was the most accurate phenotypic method for the proper identification of most *Candida* species whereas PCR-RFLP could properly confirm *Candida* species identification genotypically.

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1. Introduction

Vulvovaginal candidiasis (VVC) is a common disorder in women.[1,2] The majority of cases of VVC are caused by *Candida albicans*; however, episodes due to non-*albicans* species of *Candida* appear to be increasing in immunodeficient as well as healthy women.[3] The most commonly implicated non-albicans species include *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *Candida parapsilosis*.[4] Azoles are the treatment of choice for VVC; however, resistance has been reported especially in non-albicans *Candida* species.[5,6] Because of the different susceptibility of *Candida* species to antifungal agents, it is important to identify the causative *Candida* to the species level correctly[7]; however, conventional methods are time-consuming and may lead to misdiagnosis among closely related species. Therefore, molecular methods may provide a rapid and accurate alternative.[8–10]

2. Aim of the work

This local study was conducted to identify the spectrum of *Candida* species associated with VVC using different phenotypic and genotypic methods and assess their antifungal susceptibility pattern.

3. Materials and methods

The study has been approved by the Research and Ethical Committee of Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University. Informed consent was obtained from all individual participants included in the study.

3.1. Specimen collection

High vaginal swabs were collected from 125 married patients in the reproductive age period presenting to the Obstetrics and Gynecology outpatient clinics of Cairo University Hospitals during the period from January through June 2011 with a clinical picture suggestive of VVC. Patients who were non-married, outside the reproductive age period or using any systemic or local antifungal therapy in the previous month were excluded from the study.

3.2. Specimen processing

Vaginal swab specimens were subjected to direct Gram-stained smear examination as well as culture on Sabouraud dextrose agar (SDA) (Oxoid, UK) incubated at 37 °C for 24–48 h. Isolates on SDA were identified as *Candida* by colony morphology and Gram staining.

3.3. Phenotypic identification of Candida species

Candida isolates were speciated phenotypically by germ tube test (GTT),[11] Rice Tween-80 agar performed as described in previous studies,[12] in addition to Chrom ID Candida Agar (CAN2) (BioMérieux, France) and API 20C AUX (BioMérieux, France), which were performed according to manufacturers' instructions. The *Candida* isolates were then stored in glycerol broth at −70 °C for further processing by PCR-restriction fragment length polymorphism (PCR-RFLP).

3.4. Genotypic identification of Candida species

Genotypic identification by PCR-RFLP was used as the gold standard method for *Candida* species identification in the current study.[13,14] *C. albicans* standard strain (ATCC 10231) supplied by Egypt Microbial Culture Collection (EMCC), Faculty of Agriculture, Ain-Shams University, was used as a positive control in both PCR and RFLP.

3.4.1. DNA extraction

DNA extraction was performed using QIAamp DNA Mini kit (Qiagen) proceeded by cell disruption using tissue homogenizer.[15] Briefly, multiple fresh pure colonies of *Candida* were dissolved in 500 µL sterile distilled water in a sterile 1.5 mL microcentrifuge tube. Then, *Candida* cells were disrupted using tissue homogenizer (Qiagen) for 3 min followed by centrifugation at 13,000 rpm for 3 min. The sediment was then subjected to DNA extraction using QIAamp DNA Mini kit according to manufacturer's instructions.

3.4.2. PCR

The ITS-1 and ITS-2 regions of *Candida* spp. were amplified using universal primers; ITS-1(5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′).[16,17] The amplification was performed in Biometra T 3000 Thermal cycler as previously published[18] with modifications in the concentration of each primer (50 pmol/reaction) and DNA template (5 µL extracted DNA/reaction), in addition to change the annealing temperature (53 °C). Amplified PCR products were run on 2% agarose gel electrophoresis and visualized by UV transilluminator (BiometraTi 3).

3.4.3. RFLP analysis

1 µL *Msp*I enzyme 5000 units (BioLabs, England) and 2 µL enzyme buffer (NEB buffer 4) were added to 7 µL of each PCR product. Incubation at 37 °C for 16 h was done.[18] Restriction fragments were separated by 3% agarose gel electrophoresis and interpretation was done accordingly[17] as shown in Table 1.

3.5. Antifungal susceptibility testing

The *Candida* isolates were tested by disk diffusion method using Muller-Hinton agar supplemented with 2% glucose

Table 1 Size of ITS1-ITS4 PCR products before and after digestion with *Msp*I.[17]

Candida species	Size of ITS1-ITS4 (bp)	Size of restriction products (bp)
C. glabrata	871	557 and 314
C. guilliermondii	608	371,155 and 82
C. albicans	535	297 and 238
C. tropicalis	524	340 and 184
C. parapsilosis	520	520
C. krusei	510	261 and 249

Table 2 Interpretive break points of different antifungal drugs.

Drug	Susceptible (mm)	Susceptible dose dependent (SDD)	Resistant (mm)	Refs.
Fluconazole (25 μg)	⩾19	15–18 mm	⩽14	[19,21]
Voriconazole (1 μg)	⩾17	14–16 mm	⩽13	[6,19]
Ketoconazole (50 μg)	> 20	10–20 mm	< 10	[6]
Miconazole (50 μg)	> 20	10–20 mm	< 10	[6]
Clotrimazole (50 μg)	> 20	12–19 mm	⩽10	[20]
Amphotericin B (100 μg)	> 10	–	⩽10	[6]

and 0.5 μg of methylene blue/mL.[19] The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of 0.5 McFarland standard.[19] The following antifungal disks were used: fluconazole (25 μg), voriconazole (1 μg), ketoconazole (50 μg), clotrimazole (50 μg), miconazole (50 μg) and amphotericin B (100 μg) (BioRad). Inhibition zones were interpreted using validated CLSI interpretive break points for fluconazole and voriconazole, while for other drugs, the interpretive break points were adopted from published studies (Table 2).[6,20,21]

3.6. Statistical analysis

Data were statistically described in terms of frequencies and percentages. Comparison between the study groups was done using Chi square (χ^2) test. Exact test was used instead when the expected frequency is less than 5. Accuracy was represented using the terms sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy. P-value was considered statistically significant if less than 0.05. All statistical calculations were done using computer program Statistical Package for the Social Science (SPSS; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

4. Results

Culture on SDA was the gold standard method for the diagnosis of VVC in the current study.[22] Accordingly, out of the 125 symptomatic patients included in the present study, 63 (50.4%) were positive for VVC. Direct Gram-stained smear was positive in only 13.6% of specimens with sensitivity, specificity, PPV and NPV of 26.98%, 95.16%, 85% and 56.19%, respectively.

Considering PCR-RFLP as the gold standard test for *Candida* species identification,[13,14] *C. albicans* was the predominant isolated species (38 isolates, 60.3%), followed by *C. glabrata* (8 isolates, 12.7%), *C. krusei* and *C. parapsilosis*, (5 isolates for each species, 7.9%), *C. tropicalis* (4 isolates, 6.3%) and lastly, *Candida guilliermondii* (3 isolates, 4.8%). Methods used for species identification in this study were unable to differentiate between *C. albicans* and *Candida dubliniensis*. Sensitivity, specificity, PPV and NPV of the different methods used for speciation of *Candida* isolates are shown in Table 3.

4.1. Colony morphology on SDA

In the current study, it was observed that colony morphology on SDA may help in the identification of *Candida* species (Fig. 1). All *C. albicans* isolates grew as convex dome-shaped pearl-like colonies on SDA. Moreover, 7 of 8 *C. glabrata* isolates (87.5%) produced colonies with dark center and light periphery. All *C. krusei* isolates grew as irregular colonies with mycelial fringe. Three isolates of the 4 *C. tropicalis* (75%) produced umbilicated colonies. However, *C. parapsilosis* and *C. guilliermondii* had no specific colony morphology.

4.2. Germ tube test (GTT)

GTT was positive in 38 isolates; however, only 33 of them were identified as *C. albicans* by PCR-RFLP.

4.3. Subculture on Rice Tween-80 agar

Microscopic appearance of the different *Candida* species isolated in this study on Rice-Tween-80 agar was interpreted according to earlier studies.[23] Forty-five (71.4%) of the 63 *Candida* species isolates were correctly identified by Rice Tween-80 Agar.

4.4. Chrom ID Candida agar (CAN2)

This chromogenic medium can differentiate between 3 groups of *Candida*. The first group includes *C. albicans*; the second

Table 3 Comparison between the sensitivity, specificity, PPV and NPV of the different methods used in the study for *Candida* species identification using RFLP as the gold standard.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Colony morphology on SDA				
C. albicans	100	88	92.7	100
C. glabrata	87.5	100	100	98.2
C. krusei	100	94.8	62.5	100
C. tropicalis	75	84.8	25	98
Germ tube test[a]	86.8	80	86.8	80
Rice-Tween-80				
C. albicans	94.7	72	83.7	90
C. glabrata	62.5	96.4	71.4	94.6
C. krusei	20	96.6	33.3	93.3
C. tropicalis	50	91.5	28.6	96.4
C. parapsilosis	20	100	100	93.6
C. guilliermondii	0	98.3	0	95.2
API 20C AUX				
C. albicans	89.5	96	97.1	85.7
C. glabrata	62.5	100	100	94.8
C. krusei	100	96.6	71.4	100
C. tropicalis	100	93.2	50	100
C. parapsilosis	100	100	100	100
C. guilliermondii	66.7	98.3	66.7	98.3

[a] For *C. albicans* and *C. dubliniensis*.

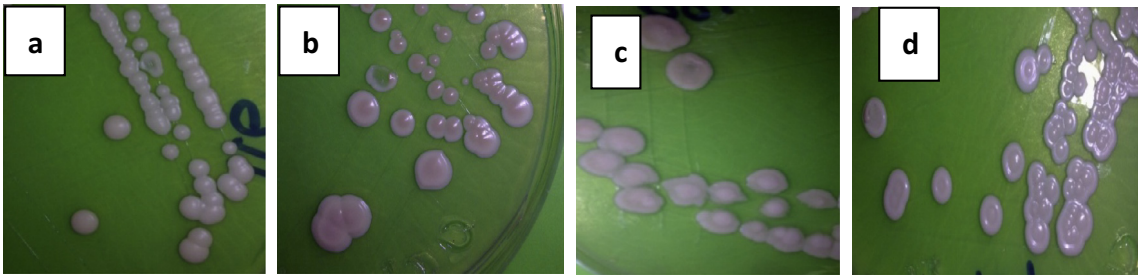

Figure 1 Colony morphology of different *Candida* species on SDA: a – *C. albicans*, b – *C. glabrata*, c – *C. krusei* and d – *C. tropicalis*.

includes *C. tropicalis*, *Candida lusitaniae* and *Candida kefyr*, while the third includes the rest of *Candida* species. Fifty-seven (90.5%) of the *Candida* isolates in this study were correctly assigned into 3 groups by CAN2 agar.

4.5. API 20C AUX

Fifty-five (87.3%) of the 63 *Candida* isolates were correctly identified to the species level by API 20C AUX.

4.6. PCR-RFLP

Discrete bands of 510–870 bp were obtained from amplification of the ITS-1 and ITS-2 regions of all isolated *Candida* species using the universal primers; IST-1 and IST-4. However, as previously published,[17] only bands of *C. glabrata* (871 bp) and *C. guilliermondii* (610 bp) could be distinguished from bands of other species which were difficult to be differentiated from each other (510–535 bp) (Fig. 2). Thus, while PCR was sufficient for the identification of *C. glabrata* and *C. guilliermondii*, the RFLP technique using the restriction enzyme *Msp* I was used for the identification of additional four *Candida* species: *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* (Fig. 3a and b). There is no recognition site for this enzyme within ITS region of *C. parapsilosis*; therefore, the same band (520 bp) was obtained before and after digestion with *Msp I* enzyme. Repeated testing of the isolates was found to yield consistent results.

4.7. Antifungal susceptibility testing (Table 4)

The most effective antifungal agent used in this study was amphotericin B, where 62 isolates (98.4%)-all except for one *C. parapsilosis* isolate-were sensitive to it, followed by ketoconazole (54 sensitive isolates, 85.7%), voriconazole (52 sensitive isolates, 82.5%), fluconazole (49 sensitive isolates, 77.8%), clotrimazole (39 sensitive isolates, 61.9%) and finally miconazole (32 sensitive isolates, 50.8%). No resistance was detected for ketoconazole, clotrimazole and miconazole, while 7 isolates (11.1%) were resistant to fluconazole, 5 isolates (7.9%) were resistant to voriconazole and 1 isolate (1.6%) was resistant to amphotericin B. Susceptible dose dependent (SDD) isolates were detected for miconazole (31 isolates, 49.2%), clotrimazole (24 isolates, 38.1%), ketoconazole (9 isolates, 14.3%), fluconazole (7 isolates, 11.1%) and voriconazole (6 isolates, 9.5%).

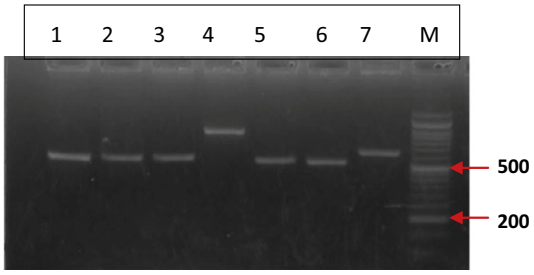

Figure 2 PCR products before digestion with restriction enzyme *MSP I*. Lanes (1, 2): *C. tropicalis* (band at 524), Lane (3): *C. albicans* (band at 535), Lane (4): *C. glabrata* (band at 871), Lane (5): *C. parapsilosis* (band at 520), Lane (6): *C. krusei* (band at 510), Lane (7): *C. guilliermondii* (band at 608) and Lane (M): 50 bp DNA ladder.

5. Discussion

The current local study was designed to identify the spectrum of *Candida* species associated with VVC in a group of 125 patients presenting with vaginal discharge and to assess their antifungal susceptibility pattern. Rate of prevalence of VVC was 50.4% among the studied group. Microscopic examination was not adequate for the diagnosis of VVC in the current study, with a poor sensitivity (26%) but excellent specificity (95.16%). Therefore, as previously reported, a negative smear result does not rule out the presence of disease.[24]

Precise identification of *Candida* at the species level is essential because of emergence of new pathogen species and because of the different antifungal susceptibility profiles.[7,25,26] PCR-PFLP was used in the current study as the gold standard method for *Candida* species identification.[13,14] *C. albicans* was the most common isolated species (60.3%), whereas the overall prevalence of non-*albicans* species was 39.7%. Earlier report from Egypt[27] has recorded higher rate of *C. albicans* in VVC (86.6%), while rates of 59%, 65.95% and 73.9% were reported from Saudi Arabia,[28] Yemen[29] and Kuwait,[30] respectively. Worldwide, rates of the isolation of *C. albicans* in cases of VVC ranged between 47% and 89% in studies from Nicaragua[31], Australia,[32,33] Turkey,[34] Iran,[35] Nigeria[36,37] and India.[38] In these studies, there was an increasing rate of non-albicans species in the more recent studies even in the same country, which possibly could be attributed to a wide spread resistance, inappropriate use of antifungal medications, long term treatments and the use of over-the-counter antimycotics.[39–41] Similar to the current study, *C. glabrata* was the

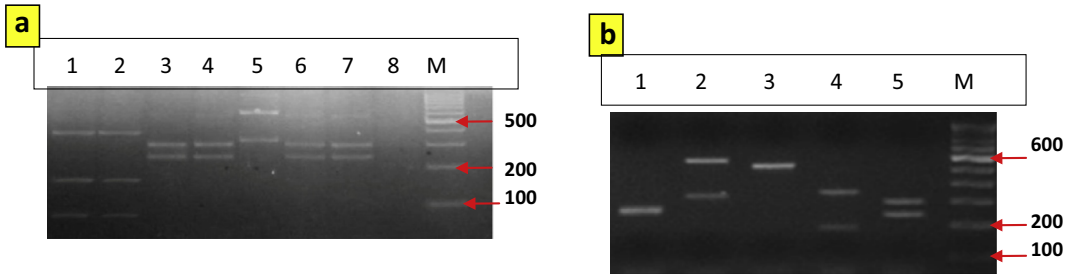

Figure 3 PCR-RFLP products of Candida species using *MSP I* enzyme. (a): Lanes (1, 2): *C. guilliermondii* (3 bands at 80, 150, 350 bp), Lanes (3, 4, 6): *C. albicans* (2 bands at 238, 297 bp), Lane (5): *C. glabrata* (2 bands at 550, 314 bp), Lane (7): positive control (*C. albicans* reference strain ATCC 10231), Lane (8): negative control and Lane (M): 100 bp DNA ladder (Qiagen). (b): Lane (1): *C. krusei* (2 bands at 261, 249 bp), Lane (2): *C. glabrata* (2 bands at 550, 314 bp), Lane (3): *C. parapsilosis* showing 1 band at 520 bp, Lane (4): *C. tropicalis* (2 bands at 180, 340 bp), Lane (5): *C. albicans* (2 bands at 238, 297 bp) and Lane (M): 100 bp DNA ladder.

Table 4 Antifungal susceptibility profile of the different isolated *Candida* species.

	Amphotericin B (%)			Fluconazole (%)			Voriconazole (%)			Ketoconazole (%)			Miconazole (%)			Clotrimazole (%)		
	S	DD	R	S	DD	R	S	DD	R	S	DD	R	S	DD	R	S	DD	R
C. albicans	100	0	0	89.5	0	10.5	89.5	0	10.5	92.1	7.9	0	71.1	28.9	0	78.9	21.1	0
C. glabrata	100	0	0	50	50	0	50	50	0	50	50	0	37.5	62.5	0	12.5	87.5	0
C. tropicalis	100	0	0	100	0	0	75	25	0	100	0	0	50	50	0	50	50	0
C. krusei	100	0	0	20	40	40	100	0	0	100	0	0	0	100	0	80	20	0
C. parapsilosis	80	0	20	60	20	20	60	20	20	60	40	0	0	100	0	0	100	0
C. guilliermondii	100	0	0	100	0	0	100	0	0	100	0	0	0	100	0	66.7	33.3	0
Total	98.4	0	1.6	77.8	11.1	11.1	82.5	9.5	7.9	85.7	14.3	0	50.8	49.2	0	61.9	38.1	0

S = sensitive, DD = susceptible dose dependent, R = resistant.

second most common isolate in cases of VVC in studies from Saudi Arabia (31%),[28] Turkey (34.5%),[34] and Australia (20%).[33] Studies have reported rates of *C. tropicalis* isolation in VVC ranged from 4% to 26.4%[27,31,38] while rates of *C. Krusei* ranged from 3% to 15.7%.[28,31,34,38] Reported rates of isolation of other species were 0.6% for *C. parapsilosis*[28]; 0.6% and 3.6% for *C. kefyr*[28,34] while rates of isolation of *C. dubliniensis* ranged from 0.17% to 29.52%.[2,38]

Although it has been reported that colonies of different yeast species on SDA cannot easily be distinguished from each other,[42] however, in the current study, colony morphology on SDA was found to be helpful in the identification of *Candida* species.

Traditionally, the preliminary identification of *C. albicans* was made through the use of the GTT.[43] However, this test gives also positive results in case of *C. dubliniensis*.[44] Consistent with our GTT results, some studies[45,46] reported similar sensitivity rates but with 100% specificity. Other studies[47] reported lower sensitivity and specificity rates (79.3% and 69.2%, respectively), or higher rates ranging from 92 to 98.8% and 99 to 100%, respectively.[23,27,48,49] Limitations of this test include misinterpretation of elongated blastoconidia as germ tube, absence of germ tube production in some strains of *C. albicans*, and health hazards of handling pooled sera.[10,49,50]

Morphological media as Rice agar-Tween 80 were used for the differentiation of *Candida* species on the basis of mycelial characteristics, size and shape of pseudohyphae and the arrangement of blastoconidia along pseudohyphae.[51] In the current study, 71.4% of *Candida* species were correctly identified by Rice Tween-80 Agar. However its sensitivity for *C. guilliermondii*, *C. krusei* and *C. parapsilosis* was very poor (0%, 20% and 20%, respectively). Contrary to our results, other investigators[52,53] reported that morphology on morphologic media was sufficient to make a final identification.

Chromogenic culture media are very useful for the diagnosis of *Candida*[54] but their main limitation is the low discrimination power among *Candida* species.[55] In this study, 90.5% of *Candida* isolates were correctly assigned into the three groups of yeasts identified by CAN2 agar. However, this medium was actually useful for the identification of *C. albicans* with excellent sensitivity and specificity, while it showed lower sensitivity but excellent specificity for *C. tropicalis* (neither *C. kefyr* nor *C. lusitaniae* were isolated in this study). The other *Candida* species cannot be differentiated from each other by this medium. Our results were in accordance with other studies.[56,57]

The most convenient and popular methods for *Candida* species identification consist of commercially available strips for carbohydrate assimilation and/or enzyme detection.[56] In the present work, 87.3% of the *Candida* isolates were correctly identified to the species level by API 20C AUX. Other studies have reported almost similar results.[58–61]

The standard phenotypic methods used to identify clinical isolates of *Candida* species are time-consuming and not appropriate for rapid, accurate and reliable identification.[7,62] In addition, these techniques rely on phenotypic expression that makes them potentially unreliable due to documented phenotypic switching of *Candida* species.[3,63,64] Consequently,

genotype-based approaches may be preferable for *Candida* species identification.[16,18,65] Currently, PCR-RFLP is the most commonly used method for identification of *Candida* species.[25] Other molecular methods that have been developed for rapid diagnosis of Candida species include random amplified polymorphic DNA (RAPD), DNA sequence analysis and real-time PCR.[66–68] However, these methods are expensive and need skilled workers.[18].

As previously reported,[16,17,62] the PCR-RFLP assay used in this study enables the identification of six medically important *Candida* species which represent up to 95% of *Candida* infection.[17] In addition, some investigators[16,69,70] have reported that, *C. kefyr* and *C. famata* (which were not isolated in the current study) can also be identified using the same protocol used in this study by the size of their PCR products. However, additional enzymes are still needed for the differentiation of *C. albicans* and *C. dubliniensis* as both species have similar RFLP profile when using *Msp* I.[17,71]

Several studies have used the PCR-RFLP method for the identification of *Candida* species using the same single restriction enzyme (*Msp* I) used in the current study[3,7,18,69,70,72] or a different restriction enzyme (*Hae III*).[73] However, the later enzyme had a lower discrimination power.[73] Other studies have used combination of *Msp I* and *Bln I* enzymes which enabled the additional differentiation between *C. albicans* and *C. dubliniensis*,[71,74] while others[16,62] reported that the combination of *Hae III*, *Dde I* and *Bfa I* restriction enzymes enabled the additional identification of *C. stellatoidea*.

In the current study, in-vitro susceptibility was performed by the disk diffusion method. Several studies have similarly reported that amphotericin B was the most effective drug against vaginal *Candida* isolates.[6,38,75,76] Although some studies have reported that ketoconazole was the most effective azole,[27,75,77] as the case in our study, however, since July 2013, the U.S. Food and Drug Administration (FDA) cleared that ketoconazole oral tablets can cause severe liver injuries, adrenal gland problems and harmful drug interactions with other medications and should not be a first-line treatment for any fungal infection. However, the topical formulations of the drug have not been associated with such side effects.[78]

In accordance with our results, similar rates of fluconazole resistance were reported in studies from Egypt[27] and Taiwan.[79] Higher resistance rates were reported in studies from Brazil (32%)[6] and India (16% for *C. albicans*)[38]; while no fluconazole resistance or a very low level of resistance (0.6%) among vaginal *C. albicans* isolates was reported in other studies from Australia and Kuwait, respectively.[30,80] It has been noted that no resistance to fluconazole was reported among *Candida* vaginal isolates in earlier studies conducted in several countries such as US, Italy, Brazil and England.[81–87] As *C. dubliniensis* more easily develops fluconazole resistance than *C. albicans*,[88] the high azole resistance rate detected for *C. albicans* in this study might be due to undifferentiating *C. albicans* and *C. dubliniensis*. In addition, some investigators[6] have reported a high resistance rate for fluconazole (32%) by disk diffusion method, while no resistance to the drug was detected by the standard microdilution method.

Although *C. krusei* is intrinsically resistant to fluconazole,[5] only 40% of the isolates in this study were found to be resistant and *Candida* species exhibited a considerable azole cross-resistance as previously reported.[89–94] Therefore, the azole resistance rate reported in this study warrants further investigation to reassess the usefulness of fluconazole as the most common drug used for the treatment of VVC.

6. Conclusion

C. albicans was the predominant isolated species (60.3%) while the most common non-albicans species was *C. glabrata* (12.7%). API 20C AUX was an accurate phenotypic method for *Candida* species identification, while Chrom ID Candida agar was an effective method for presumptive identification of *C. albicans*. The PCR-RFLP analysis was relatively simple to perform, rapid and highly valuable; however, its direct use on clinical samples has to be evaluated in further studies for more rapid diagnosis. *Candida* antifungal susceptibility testing is recommended to avoid treatment failures.

Limitations of the study include the lack of differentiation of *C. albicans* and the closely related species *C. dubliniensis* and the use of PCR-RFLP for the identification of clinical *Candida* isolates rather than using it directly on clinical specimens.

Conflict of interest

The authors declare that they have no conflict of interest.

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"*All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.*"

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