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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.

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

3.5. Antifungal susceptibility testing

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

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