SHORT COMMUNICATION

# Molecular identification and antifungal susceptibility profile of *Candida* species isolated from patients with vulvovaginitis in Tehran, Iran

**Somayeh Sharifynia, Mehraban Falahati[1], Lame Akhlaghi[1], Alireza Foroumadi[2], Roohollah Fateh[3,4]**

Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, [1]Department of Medical Mycology and Parasitology, Faculty of Medicine, Iran University of Medical Sciences, [2]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, [3]Cellular and Molecular Research Center, [4]Department of Microbiology and Immunology, Faculty of Medicine, Qom University of Medical Sciences, Qom, Iran

**Background:** Rapid and accurate identification and evaluation of antifungal susceptibility pattern of *Candida* isolates are crucial to determine suitable antifungal drugs for the treatment of patients with vulvovaginitis candidiasis. **Materials and Methods:** Vaginal samples were collected from 150 women with suspicious vaginal candidiasis, and then cultured on Sabouraoud's Dextrose Agar with chloramphenicol to isolate *Candida* species. After identification of *Candida* isolates using polymerase chain reaction-restriction fragment length polymorphism technique, antifungal susceptibility testing of four azolic antifungal drugs was carried out using broth microdilution method according to the CLSI M27-A3. **Results:** *Candida* species were isolated from eighty suspected patients (61.79%). The most common pathogen was *Candida albicans* (63.75%). Resistance to fluconazole and ketoconazole was observed in 27.5% and 23.75% of *Candida* isolates, respectively, and only 2% of *Candida* isolates were resistant to miconazole. Interestingly, resistance to fluconazole in *C. albicans* was more than other *Candida* species. **Conclusion:** The results indicated that therapy should be selected according to the antifungal susceptibility tests for the prevention of treatment failure and miconazole therapy can be considered as the best therapeutic choice in the management of vulvovaginitis.

**Key words:** Azolic antifungal drugs, *Candida* species, polymerase chain reaction-restriction fragment length polymorphism, vulvovaginitis candidiasis

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## INTRODUCTION

*Candida* species are the important opportunistic fungi[1] and Candidal vaginitis or vulvovaginal candidiasis (VVC) is one of the most common female problems in the childbearing age, and its prevalence has been increased recently.[2] VVC is considered as recurrent (Recurrent VVC [RVVC]) when at least four episodes occur within 1 year.[3]

Generally, more than 70% of VVC cases are caused by *Candida albicans*, and other patients are infected by nonalbicans species including *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei*.[4] The prevalence of VVC due to nonalbicans species is increasing, whereas these species are often more resistant to the antifungal agents.[5]

The identification of *Candida* isolates and antifungal susceptibility testing are necessary to obtain epidemiological data and avoid therapeutic failure. This study was designed to differentiate *Candida* isolates from patients with vaginitis symptoms referred to obstetrics and gynecology hospitals in Tehran capital of Iran using polymerase chain reaction-restriction fragment length

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**Address for correspondence:** Dr. Roohollah Fateh, Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, Iran.
E-mail: rfateh@muq.ac.ir
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polymorphism (PCR-RFLP) method and determination of the drug susceptibility profile of identified *Candida* species.

## MATERIALS AND METHODS

### Patients and samples

Two vaginal swabs were collected from 150 suspicious patients referred to the several obstetrics and gynecology clinics of Tehran hospitals from February to August 2016. All patients with vulvovaginitis symptoms (irritation, pruritis, soreness, and altered discharge) were enrolled to the study. Consent form was signed by all patients. The patients who taking any antifungal drugs in the past 2 weeks were excluded from the study.

Vaginal swab was subjected to direct examination with 15% KOH and culture on Sabouraud's dextrose agar (Merck, Germany) (Cat no: 1054380500) containing chloramphenicol (50 mg/l).

All isolates were primarily identified by phenotypic methods such as the color of colony on CHROMagar Candida medium (CHROMagar, France) (Cat no: CA220), germ-tube formation in serum and production of chlamydoconidia in corn meal agar with Tween-80 (Merck, Germany).[6]

### DNA extraction and polymerase chain reaction amplification

Genomic DNA was extracted using DNG-Plus kit (SinaClon, Iran) (Cat no: DN8117C). The PCR amplification was carried out in a final volume 25 µl with ITS1 (5′-TCC TCC GCT TAT TGA TAT GC-3′) and ITS4 primers (5′-TCC TCC GCT TAT TGA TAT GC-3′).

### Restriction fragment length polymorphism analysis

The *Msp*I (Fermentas, USA) (Cat no: ER0542) restriction enzyme was used for RFLP assay that described by Mirhendi *et al*.[7] Restriction fragments were separated by 2% agarose gel electrophoresis in TAE buffer for 1 h at 100 V and visualized by ethidium bromide.

### Polymerase chain reaction sequencing

PCR sequencing was used to identify species with similar and indistinguishable RFLP patterns.

### Antifungal susceptibility testing

Susceptibility testing was carried out on the identified isolates using broth microdilution method according to CLSI M27-A3 guideline.[8] Four antifungal drugs were used in this study; fluconazole (Sigma-Aldrich, USA) (Cat no: F8929), ketoconazole (Sigma-Aldrich, USA) (Cat no: K1003), miconazole (Sigma-Aldrich, USA) (Cat no: M1880000), and clotrimazole (Sigma-Aldrich, USA) (Cat no: C6019). The final concentrations for fluconazole were in the range 0.25–128 µg/ml and for other antifungal agents were in the range 0.0313–16 µg/ml. A 100 µl yeast inoculum of 0.5–2.5 × $10^3$ cells/ml in RPMI 1640 medium was added to each well of 96-well microplate. After incubation at 35°C for 48 h, the MIC endpoint was determined as the lowest concentration that resulted in >50% reduction in turbidity as compared to the drug-free control well.[8]

**Table 1: Identification of *Candida* isolates using amplification of the ITS1-ITS4 region and restriction fragment length polymorphism analysis with MspI restriction enzyme**

| Species | VVC, *n* (%) | RVVC, *n* (%) | Total, *n* (%) |
|---|---|---|---|
| *C. albicans* | 28 (59.6) | 23 (69.7) | 51 (63.75) |
| *C. glabrata* | 12 (25.5) | 6 (18.2) | 18 (22.5) |
| *C. tropicalis* | 1 (2.1) | - | 1 (1.25) |
| *C. parapsilosis* | 1 (2.1) | 2 (6.1) | 3 (3.75) |
| *C. krusei* | - | 1 (3) | 1 (1.25) |
| *C. kefyr* | 2 (4.3) | - | 2 (2.5) |
| *C. lusitaniae* | 1 (2.1) | - | 1 (1.25) |
| *C. guilliermondii* | 2 (4.3) | 1 (3) | 3 (3.75) |
| Total (%) | 47 (100) | 33 (100) | 80 (100) |
| 95% CI | 0.48-0.7 | 0.3-0.52 | - |

RVVC = Recurrent volvuvaginal candidiasis; *C. albicans* = *Candida albicans*; *C. glabrata* = *Candida glabrata*; *C. tropicalis* = *Candida tropicalis*; *C. parapsilosis* = *Candida parapsilosis*; *C. krusei* = *Candida krusei*; *C. kefyr* = *Candida kefyr*; *C. lusitaniae* = *Candida lusitaniae*; *C. guilliermondii* = *Candida guilliermondii*; CI = Confidence interval; VVC = Vulvovaginal candidiasis

## RESULTS

Of 150 women with suspected VVC, eighty different *Candida* colonies were isolated from 80 patients (confidence interval 95%: 0.45–0.61). There were 33 (41.25%) cases RVVC and 47 (58.75%) cases non-RVVC.

Table 1 shows the frequency of the clinically important *Candida spp*. isolated from patients with VVC or RVVC. The data clearly showed that *C. albicans* was the most frequently isolated species, followed by *C. glabrata*, *C. parapsilosis*, and *Candida guilliermondii*.

Susceptibility test results for the eighty species showed that resistance to fluconazole (27.5%) and ketoconazole (23.75%) among *C. albicans* strains was frequent. Seventy *Candida* species (87.5%) were sensitive to miconazole and only two species; *C. glabrata* and *C. guilliermondii* were resistant to this drug.

Two isolates of *Candida kefyr* and one isolate of *Candida lusitaniae* were sensitive to all tested antifungal drugs. One species of *C. krusei* isolated from patient with RVVC that this species was resistant to fluconazole and sensitive to ketoconazole, miconazole, and clotrimazole [Table 2].

**Table 2: Sensitivity pattern of *Candida* isolates to several antifungal drugs**

| | Species | Antifungal drugs (μg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fluconazole | | | Ketoconazole | | | Miconazole | | | Clotrimazole | | |
| | | S | DD | R | S | DD | R | S | DD | R | S | DD | R |
| RVVC | *C. albicans* | - | 13 | 10 | 7 | 8 | 8 | 21 | 2 | - | 13 | 8 | 2 |
| | *C. glabrata* | 1 | 2 | 3 | 4 | 1 | 1 | 3 | 2 | 1 | - | 5 | 1 |
| | *C. parapsilosis* | - | 2 | - | 1 | 1 | - | 1 | 1 | - | - | 2 | - |
| | *C. guilliermondii* | 1 | - | - | 1 | - | - | 1 | - | - | 1 | - | - |
| | *C. krusei* | - | - | 1 | 1 | - | - | 1 | - | - | 1 | - | - |
| VVC | *C. albicans* | 26 | - | 2 | 14 | 6 | 8 | 26 | 2 | - | 9 | 15 | 4 |
| | *C. glabrata* | 1 | 7 | 4 | 7 | 4 | 1 | 12 | - | - | 3 | 9 | - |
| | *C. parapsilosis* | - | 1 | - | 1 | - | - | 1 | - | - | 1 | - | - |
| | *C. guilliermondii* | - | 1 | 1 | 1 | - | 1 | - | 1 | 1 | - | 1 | 1 |
| | *C. tropicalis* | - | - | 1 | 1 | - | - | 1 | - | - | - | 1 | - |
| | *C. kefyr* | 2 | - | - | 2 | - | - | 2 | - | - | 2 | - | - |
| | *C. lusitaniae* | 1 | - | - | 1 | - | - | 1 | - | - | 1 | - | - |
| Total, *n* (%) | | 32 (40) | 26 (32.5) | 22 (27.5) | 41 (51.25) | 20 (25) | 19 (23.75) | 70 (87.5) | 8 (10) | 2 (2.5) | 31 (38.8) | 41 (51.2) | 8 (10) |
| 95% CI | | 0.29-0/51 | 0.22-0.42 | 0.18-0.38 | 0.4-0.62 | 0.16-0.34 | 0.19-0.29 | 0.8-0.94 | 0.03-0.17 | 0.0-0.06 | 0.28-0.5 | 0.4-0.62 | 0.03-0.17 |

S = Sensitive; DD = Dose dependent; R = Resistant; CI = Confidence interval; VVC = Vulvovaginal candidiasis; RVVC = Recurrent vulvovaginal candidiasis; *C. albicans* = *Candida albicans*; *C. glabrata* = *Candida glabrata*; *C. parapsilosis* = *Candida parapsilosis*; *C. guilliermondii* = *Candida guilliermondii*; *C. krusei* = *Candida krusei*; *C. tropicalis* = *Candida tropicalis*; *C. kefyr* = *Candida kefyr*; *C. lusitaniae* = *Candida lusitaniae*

## DISCUSSION

VVC is one of the most frequent fungal infections among adult women during their lifetime. The data from this study showed that 33 patient out of 80 cases suffered from RVVC. It may be due to defect in vaginal mucosal immunity of host, antifungal drugs resistance in causative agent of disease, or incomplete treatment of patients.

The main causative agent of VVC is *C. albicans* and is the second main agent of vaginal infections in most countries.[9] In this study, *C. glabrata* was the second most common species (22.5%). The present findings also indicate *C. albicans* was reported as the most common species (63.75%) similar to other studies performed in Iran.[10-12]

The sensitivity pattern of *Candida* isolates to antifungal drugs varies among studies in different regions.[5,13] Al-Abeid *et al*. showed that all *Candida* species were susceptible to nystatin, miconazole, ketoconazole, and fluconazole, and *C. albicans* isolates were more susceptible to azoles than *C. glabrata*.[13] In contrast, in this study, *C. albicans* species were more resistant to fluconazole in comparison with *C. glabrata*. ElFeky *et al*. indicated that only 11.1% of *Candida* isolates were sensitive to fluconazole and did not report resistance to ketoconazole in any species.[5] Whereas, in the present study, 27.5% and 23.75% of tested isolates were resistant to fluconazole and ketoconazole, respectively.

The data indicate that resistance to fluconazole and ketoconazole among tested *Candida* species, especially *C. albicans* is increasing. Therefore, it is recommended that therapy should be selected on the basis of antifungal susceptibility tests for the prevention of treatment failure.

In a study by Salehei *et al*., *C. albicans* isolates were more sensitive to miconazole (49 of 53 isolates) than other antifungal drugs, followed by clotrimazole (41 of 53 isolates).[9] In this study, miconazole was reported as the best antifungal drug with remarkable anticandidal activities. Therefore, miconazole therapy can be considered as a good therapeutic choice in the management of VVC.

## CONCLUSION

This study showed that the main causative agent of VVC and RVVC is *C. albicans* followed by *C. glabrata*, and antifungal susceptibility testing indicated the highest sensitivity of *Candida* species isolated from both infections to azoles was seen against miconazole followed by clotrimazole.

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### Conflicts of interest
There are no conflicts of interest.

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