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

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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 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

polymorphism (PCR-RFLP) method and determination of the drug susceptibility profile of identified C. albicans 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 GAT TGA TAT GC-3').

Restriction fragment length polymorphism analysis
The MspI (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 × 10^5 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]

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].
The sensitivity pattern of *Candida* isolates to antifungal drugs varies among studies in different regions. 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*. 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. 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). In this study, miconazole was reported as the best antifungal drug with remarkable antifungal 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.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCE**

2. Matheson A, Mazza D. Recurrent vulvovaginal candidiasis:

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**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. 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.

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