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

Somayeh Sharifynia, Mehraban Falahati, Lame Akhlaghi, Alireza Foroumadi, Roohollah Fateh
Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, *Department of Medical Mycology and Parasitology, Faculty of Medicine, Iran University of Medical Sciences, *Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, *Department of Microbiology and Immunology, Faculty of Medicine, Qom University of Medical Sciences, Qom, Iran

INTRODUCTION

Candida species are the important opportunistic fungi 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. VVC is considered as recurrent (Recurrent VVC [RVVC]) when at least four episodes occur within 1 year.

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. The prevalence of VVC due to nonalbicans species is increasing, whereas these species are often more resistant to the antifungal agents.

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

Access this article online

How to cite this article: Sharifynia S, Falahati M, Akhlaghi L, Foroumadi A, Fateh R. Molecular identification and antifungal susceptibility profile of Candida species isolated from patients with vulvovaginitis in Tehran, Iran. J Res Med Sci 2017;22:132.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Address for correspondence: Dr. Roohollah Fateh, Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, Iran.
E-mail: r.fateh@muq.ac.ir
Received: 25-02-2017; Revised: 02-08-2017; Accepted: 13-09-2017
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 were 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 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 × 10³ 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].
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.

The sensitivity pattern of *Candida* isolates to antifungal drugs varies among studies in different regions. Al-Abied et al. showed that all *Candida* species were susceptible to nystatin, miconazole, ketoconazole, and flucanazole, and *C. albicans* isolates were more susceptible to azoles than *C. glabrata*. In contrast, in this study, *C. albicans* species were more resistant to flucanazole in comparison with *C. glabrata*. ElFeky et al. indicated that only 11.1% of *Candida* isolates were sensitive to flucanazole 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 flucanazole and ketoconazole, respectively.

The data indicate that resistance to flucanazole 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 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.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCE

1. Khorvash F, Abbasi S, Yaran M, Abdi F, Ataei B, Fereidooni F, et al. Molecular detection of *Candida* spp. and *Aspergillus fumigatus* in bronchoalveolar lavage fluid of patients with ventilator-associated pneumonia. J Res Med Sci 2014;19:546-50.
2. Matheson A, Mazza D. Recurrent vulvovaginal candidiasis:
A review of guideline recommendations. Aust N Z J Obstet Gynaecol 2017;57:139-45.

3. Diba K, Namaki A, Ayatolah H, Hanifian H. Rapid identification of drug resistant Candida species causing recurrent vulvovaginal candidiasis. Med Mycol J 2012;53:193-8.

4. Ilkit M, Guzel AB. The epidemiology, pathogenesis, and diagnosis of vulvovaginal candidiasis: A mycological perspective. Crit Rev Microbiol 2011;37:250-61.

5. El-Feky DS, Gohar NM, El-Seidi EA, Ezzat MM, Abo-Elew SH. Species identification and antifungal susceptibility pattern of Candida isolates in cases of vulvovaginal candidiasis. Alexandria J Med 2016;52:269-77.

6. Rezaei-Matehkolaee A, Shafiei S, Zarei-Mahmoudabadi A. Isolation, molecular identification, and antifungal susceptibility profiles of vaginal isolates of Candida species. Iran J Microbiol 2016;8:410-7.

7. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important Candida species. Nihon Ishinkin Gakkai Zasshi 2006;47:225-9.

8. Wayne P. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard. CLSI AQ2 Document M27-A3. Vol. 28. Clinical and Laboratory Standards Institute; Pennsylvania, USA: 2008a. p. 6-12.

9. Salehei Z, Seifi Z, Zarei Mahmoudabadi A. Sensitivity of vaginal isolates of Candida to eight antifungal drugs isolated from Ahvaz, Iran. Jundishapur J Microbiol 2012;5:574-7.

10. Diba K, Namaki A, Ayatolah H, Hanifian H. Comparison of biochemical and molecular methods for the identification of Candida species causing vulvovaginal candidiasis and recurring vulvovaginal candidiasis. Iran J Med Microbiol 2014;8:45-50.

11. Hedayati MT, Taheri Z, Galinimoghadam T, Aghili SR, Yazdani Cherati J, Mosayebi E, et al. Isolation of different species of Candida in patients with vulvovaginal candidiasis from Sari, Iran. Jundishapur J Microbiol 2015;8:e15992.

12. Hossein M, Mirhendi SH, Brandão J, Mirdashti R, Rosado L. Comparison of enzymatic method rapid yeast plus system with RFLP-PCR for identification of isolated yeast from vulvovaginal candidiasis. Iran J Basic Med Sci 2011;14:443-50.

13. Al-Abeid HM, Abu-Eleen KH, Elkarmi AZ, Hamad MA. Isolation and characterization of Candida spp. In Jordanian cancer patients: Prevalence, pathogenic determinants, and antifungal sensitivity. Jpn J Infect Dis 2004;57:279-84.