International Journal of Research in Medical Sciences
Erbaş G et al. Int J Res Med Sci. 2017 Dec;5(12):5117-5120
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20175429

Original Research Article

Antifungal susceptibilities and identification of *Candida* species by using maldi-tof microbial identification system from cervicovaginal samples

Göksel Erbaş*, Uğur Parin, Şükrü Kirkan, Süheyla Türkyilmaz, Serap Savaşan, Hafize Tuğba Yüksel, Sinem Öztürk

Department of Microbiology, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydın, Turkey

Received: 22 September 2017
Accepted: 31 October 2017

***Correspondence:**
Dr. Göksel ERBAŞ,
E-mail: gerbas@adu.edu.tr

ABSTRACT

Background: Among the vaginal diseases seen in women, candidiasis is in the first place. This disease, which is caused by *Candida* species, can sometimes persist very stubbornly. The aim of this study was to determine *Candida* species isolated from vaginal specimens by using VITEK MS (MALDI-TOF Microbial Identification System) rapid identification system and to evaluate their susceptibility to some antifungals.
Methods: In this study, 220 cervicovaginal swab were used. Isolates were identified by VITEK MS rapid identification system. After identification, antifungal susceptibility testing was performed using the M-44 A2 guideline of The Clinical and Laboratory Standards Institute (CLSI).
Results: Total 16.3% (36) of Candida spp. positivity was determined from 220 cervicovaginal samples, and 25 (69.4%) *C. glabrata*, 6 (16.7%*) C. albicans*, 3 (8.3%) *C. kefyr* and 2 (5.6%) *C. krusei* were obtained with Vitek MS. All identified *C. albicans* strains were found to be completely resistant to all antifungals used except nystatin agent, *C. krusei* strains were found to be resistant to flucytosine but sensitive to all other antifungals, *C. glabrata* and *C. kefyr* strains were susceptible to all antifungals within the antifungals used in this study.
Conclusions: It is concluded that it is necessary to distinguish *Candida* species in order to apply a correct treatment. And species selection is very important for the selection of antifungal to be used. Nystatin is recommended if no laboratory tests are to be performed for the diagnosis of Vaginal Candidiasis.

Keywords: Antifungal, Candida, Vaginal Candidiasis, Vitek MS

INTRODUCTION

Candida is a normal commensal organism in the vagina and cause vaginal yeast infection. The most common name of vaginal fungal infections is Vaginal Candidiasis (VC). Vaginal candidiasis is a common fungal infection that affects healthy women of all ages. There are many types of fungi that cause vaginal yeast infections. Among these fungi, the most common is *Candida albicans*, but there is increased awareness of the role of yeasts other than *C. albicans* such as *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *Candida parapsilosis*.[1] In recent studies, non-albicans Candida (NAC) species such as *C. tropicalis*, *C. glabrata* and *C. krusei* and *C. parapsilosis* have been frequently isolated.[2] This data, especially caused by non-albicans species in VC infections, may be due to many factors such as the use of over-the-counter antifungal, increase of high-risk patient populations and geographical differences.

The responses of *Candida* species to antifungals vary. Therefore, determining the species distribution of *Candida* spp. is of great importance in the selection of antifungals. There are several ways to identify *Candida* species. Among these ways Rapid identification systems have been used in recent years. Identification of yeast

isolates is important in order to adjust the antifungal treatment. If complete identification is not made, random antifungal use begins. The random and frequent use of antifungals for treatment and prophylaxis has led to the development of resistance in some fungal species. And also, the distribution of *Candida* species can vary depending on the geographic area where the study was conducted.[3] An increasing number of *Candida* spp. clinical isolates are resistant to antifungal agents routinely used for the treatment of VC. Because of these, it is necessary to identify *Candida* to species level as many non albicans *Candida* have decreased susceptibility to antifungal agents.

In this study, it was aimed to determine *Candida* species isolated from vaginal specimens by VITEK MS rapid identification system and to determine their susceptibility to some antifungals.

METHODS

Samples

In this study, 220 cervicovaginal swab samples taken by specialist doctor during routine smear scanning were used in Aydin/Turkey. Specimens taken from the ectocervix and endocervical duct with sterile swab were brought to the laboratory under cold chain.

Isolation and identification of Candida species

All samples were cultured to 4% Sabouraud Dextrose Agar (SDA) (Merck 1.05438, Biopharma, Turkey) and were incubated separately at 25-37 °C. The growths were checked on a daily basis and after 72 hours, the samples without yeast were excluded from the study. From the colonies that grown in SDA in 2-3 days, in paste stiffness, 0.5-1 mm in diameter, white or cream colored, uniformly bounded and that have distinctive yeast scent were subjected to gram staining.

In Gram staining, Gram positive, oval or prolonged budding yeast cells, individual, sometimes double, triple blastospor clusters and pseudohyphogenic yeast cells isolated as Candida spp. Under gram staining evaluation, microorganisms that were predefined as Candida spp. were conveyed to SDA and pure cultures were obtained.[4-5] Pure isolates were identified by VITEK MS (bioMérieux, France) rapid identification system.

For rapid identification, Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based system (VITEK MS-VMS) with the commercial database version 2.0 is used. Identification by VMS was carried out using a portion of one colony from SDA culture that was applied directly onto the VMS disposable target slide (single deposit) by loop and lysed with 0.5µl of 25% formic acid (bioMérieux, France). After drying completely at room temperature (1-2 min), 1µl of ready-to-use α-cyano-4-hydroxycinnamic acid matrix (bioMérieux-MALDI matrix solution) was applied to the spot and the latter allowed to dry completely (1 min). Species identification was performed on the VMS, version 2.0.[6]

Antifungal suspectibilty tests

Antifungal suspectibilty testing was performed for Candida spp. isolates, using the M-44 A2 guideline of CLSI.[7] The antifungal discs used included ketoconazole (10 µg), fluconazole (10 µg), miconazole (10 µg), Voriconazole (1 µg), nystatin (100 IU) and flucytosine (1 µg) (BD BBL, United Kingdom).

RESULTS

In the present study, total of 220 vaginal swab samples were examined and we speciated *Candida* isolates using VITEK MS rapid identification system. Antifungal susceptibility test was performed after the complete identification of *Candida* spp. When the isolation rates were evaluated, 16.3% (36) of *Candida* spp. positivity was determined. According to the results of identification test with Vitek MS, 25 (69.4%) *C. glabrata*, 6 (16.7%) *C. albicans*, 3 (8.3%) *C. kefyr* and 2 (5.6%) *C. krusei* were obtained (Table 1).

Table 1: Vitek MS identification results.

Species	Number	Percentage
C. albicans	6	16.7
C. glabrata	25	69.4
C. kefyr	3	8.3
C. krusei	2	5.6
Total	36	100

In this study, the antifungal discs used included ketoconazole (10µg), fluconazole (10µg), miconazole (10µg), Voriconazole (1µg), nystatin (100IU) and flucytosine (1µg).

Determination of the antifungal susceptibility test results, all identified *C. albicans* strains were found to be completely resistant to all antifungals used except nystatin (100 IU) agent. *C. krusei* strains were found to be resistant to flucytosine (1µg) but sensitive to all other antifungals. *C. glabrata* and *C. kefyr* strains were susceptible to all antifungals (Table 2).

DISCUSSION

Vaginal candidiasis (VC) is a fungal infection. *Candida* spp. normally is found in the mouth, vagina, digestive system and in normal flora of the skin. However, it is multiplied in such a way as to cause an infection in case of damaged or reduced immune responses due to opportunistic nature. Vaginal candidiasis is a condition that can sometimes be a problem even for healthy women. In candidiasis, the species distribution and antifungal susceptibility of *Candida* isolates varies

between countries, regions, and institutions. Because of this reason, accurate identification of *Candida* isolates from samples is vital for the establishment of etiological diagnosis, selection of appropriate antifungal therapy.[8]

Table 2: Antifungal susceptibility results of Candida species.

Species	Antifungals[a]																	
	KCA			FCN			MCL			VOR			NY			FY		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Candida albicans (n=6)	-	-	6	-	-	6	-	-	6	-	-	6	6	-	-	-	-	6
Candida glabrata (n=25)	25	-	-	25	-	-	25	-	-	25	-	-	25	-	-	25	-	-
Candida kefyr (n=3)	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-
Candida krusei (n=2)	2	-	-	2	-	-	2	-	-	2	-	-	2	-	-	-	-	2

a: KCA-Ketoconazole (10µg), FCN-Fluconazole (10µg), MCL-Miconazole (10µg), VOR-Voriconazole (1µg), NY-Nystatine (100U), FY-Flucytosine (1µg)

In our study 16.3% (36) of *Candida* spp. positive samples were determined in asymptomatic patients. A total of four species of *Candida* spp. were isolated from 220 women. The percentage obtained is 13.7% in agreement with the literature.[9] *Candida* spp. in healthy women belongs to normal genital microflora up to 30%.[10] *C. glabrata* frequency was the highest (69.4%) of the four *Candida* species. Isolation rate of *C. glabrata* are similar to those of Oriel et al and Consolaro et al.[11,12]

It is normal for asymptomatic women to have a high isolation rate of non-albicans yeast. This is explained by the fact that the symptoms of non-albicans are much less than the symptoms of albicans.[13]

One study shows that 70% to 90% of all VC cases of *Candida albicans* are formed and that the nonalbicans species have emerged lately.[14] In recent years, remarkable increases have been observed in infections, particularly those caused by *non-albicans Candida* species.[3] This increase, especially caused by non-albicans species in VC infections, may be due to many factors such as the use of over-the-counter antifungal and the increase of high-risk patient populations. *Candida glabrata* is the first order among non-albicans species in VC disease. *Candida glabrata* can cause VC in 14% of women who are immunosuppressed.[14-16]

In our study, isolation rate of non-Candida albicans spp. was higher (83.3%) compared to *Candida albicans* (16.7%). *C. glabrata* (69.4%) was most common species, followed by *C. albicans* (16.7%), *C. kefyr* (8.3%), *and C. krusei* (5.6%). Okungbowa et al, reported that *C. glabrata* (33.7%) was the most dominant species in their study from genitourinary tract specimens.[17] Besides, *C. albicans* is reported as the most dominant species in many studies.[10,16,18,19] This difference in outcome is thought to be due to the fact that the incidence of *Candida* species varies according to the characteristics of the study group and the geographical region.[20] Because the variation of *Candida* species differs according to the geographical situation, it is thought that such studies should be repeated in different regions.

In fungal infections, antifungal therapy is usually started empirically in routine practice. As a result, the development of resistance is encountered. When the studies done in different regions are examined, various results are encountered. Richter et al, in their study found that the most common fungal agent in vaginal candidiasis is *C. albicans*, and secondly, *C. glabrata* and they found no *Candida* species to be resistant to nystatin same as our results.[21] Also Fan and Lui, reported that all the *Candida* spp. they obtained in their studies were sensitive to nystatin.[22]

In the present study supports the findings of Richter et al and Fan and Lui that all *Candida* species that have been isolated from patients are susceptible to nystatin.[21,22] In addition to this, the identification of *Candida albicans* or non-albicans was very important for the selection of antifungals to be used. Because, while all identified *C. albicans* strains were found to be completely resistant to all antifungals used except nystatin (100IU) agent, *C. glabrata* and *C. kefyr* strains were susceptible to all antifungals. Besides, *C. krusei* strains were found to be resistant to flucytosine (1µg) but sensitive to all other antifungals.

CONCLUSION

As a result, the use of advanced identification system to distinguish in albicans and non-albicans is very important. And, short identification duration with Vitek MS was also quite impressive.

When antifungal susceptibility results are evaluated, it has been observed that species selection is very important in the selection of antifungal to be used and Nystatin is recommended if no laboratory tests are to be performed.

ACKNOWLEDGEMENTS

Authors would like to thank KETEM (Cancer Early Diagnosis Screening and Training Center/AYDIN) staff for helping to retrieve the samples used in the research.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by Adnan Menderes University Faculty of Medicine Ethical Board of Innovative Clinical Research committee (2014/245-13

REFERENCES

1. Holland J, Young ML, Lee O, C-A Chen S. Vulvovaginal carriage of yeasts other than Candida albicans. Sex Transm Infect. 2003;79(3):249-50.
2. Rangaraj S, Uyen B, Ajit PL, Cookson BT. Rapid identification of commonly encountered Candida species directly from blood culture bottles. J Clin Microbial. 2003;41(12):5660-64.
3. Warnock DW. Trends in the epidemiology of invasive fungal infections. Nihon Ishinkin Gakkai Zasshi. 2007;48(1):10-12.
4. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology. 9th edn. Baltimore, Md, USA: Wiliams and Wilkins;1994.
5. Sonmez M, Erbas G. Isolation and Identification of Candida spp. from Mastitis Cattle Milk and Determination of Antifungal Susceptibilities. Inter J Veterinary Sci. 2017;6(2):104-07.
6. Duran-Valle MT, Sanz-Rodriguez N, Munoz-Paraiso C, Almagro- Molto M, Gomez-Garces JL. Identification of clinical yeasts by Vitek MS system compared with API ID 32 C. Med Mycol. 2014;52:342-49.
7. Clinical Laboratory Standards Institute (CLSI). Method for Antifungal disk diffusion Susceptibility testing of Yeasts: Approved Guidelines, second edition. CLSI document M44-A2 (ISBN 1-56238-703-0). Clinical Laboratory Standard Institute, Wayne: Pennsylvania;2009.
8. Shokohi T, Soteh MB H, Pouri ZS, Hedayati MT, Mayahi S. Identification of Candida species using PCR-RFLP in cancer patients in Iran. Ind J Medic Microbiol. 2009;28:147-51.
9. Colombo AL, Guimarães T, Camargo LF, Richtmann R, de Queiroz-Telles F, Salles MJ, et al. Brazilian guidelines for the management of candidiasis–a joint meeting report of three medical societies: Sociedade Brasileira de Infectologia, Sociedade Paulista de Infectologia and Sociedade Brasileira de Medicina Tropical. Braz J Infec Dis. 2013;17(3):283-312.
10. Beikert FC, Le MT, Koeninger A, Technau K, Clad A. Recurrent vulvovaginal candidosis: focus on the vulva. Mycoses. 2011;54:807-10.
11. Oriel JD, Partidge BM, Denny MJ, Coleman JC. Genital infections. Br Med J. 1972;4:761-64.
12. Consolaro ME, Albertoni TA, Yoshida CS, Mazucheli J, Peralta RM, Svidzinski TI. Correlation of Candida species and symptoms among patients with vulvovaginal candidiasis in Maringá, Paraná, Brazil. Rev Iberoam Micol. 2004;21(4):202-5.
13. Spinillo A, Capuzzo E, Egbe TD, Baltaro F, Nicola S, Piazzi G. Torulopsis glabrata vaginitis. Obstet Gynecol. 1995;85:993-98.
14. Paulitsch A, Weger W, Ginter-Hanselmayer G, Marth E, Buzina W. A 5-year (2000–2004) epidemiological survey of Candida and non-Candida yeast species causing vulvovaginal candidiasis in Graz, Austria. Mycoses. 2006;49:471-75.
15. Ozcan SK, Budak F, Yucesoy G, Susever S, Willke A. Prevalence, susceptibility profile and proteinase production of yeasts causing vulvovaginitis in Turkish women. APMIS. 2006;114:139-45.
16. Vermitsky JP, Self MJ, Chadwick SG, Trama JP, Adelson ME, Mordechai E, et al. Survey of vaginal-flora Candida species isolates from women of different age groups by use of species-specific PCR detection. J Clinic Micro. 2008;46(4):1501-3.
17. Okungbowa FI, Isikhuemen O, Dede APO. The distribution frequency of Candida species in the genitourinary tract among symptomatic individuals in Nigerian cities. Revista iberoamericana de micología. 2003;20(2):60-63.
18. St-Germain G, Laverdiere M, Pelletier R, Bourgault AM, Libman M, Lemieux C, Noel G. Prevalence and Antifungal Susceptibility of 442Candida Isolates from Blood and Other Normally Sterile Sites: Results of a 2-Year (1996 to 1998) Multicenter Surveillance Study in Quebec, Canada. J Clinic Microbiol. 2001;39(3):949-53.
19. Dias LB, Melhem MSC, Szeszs MW, Filho JM, Hahn RC. Vulvovaginal candidiasis in Mato Grosso, Brazil: pregnancy status, causative species and drugs tests. Braz J Microbiol. 2011;42:1300-07.
20. Aquino VR, Lunardi LW, Goldani LZ, Barth AL. Prevalence, susceptibility profile for fluconazole and risk factors for candidemia in a tertiary care hospital in southern Brazil. Braz J Infect Dis. 2005;9(5):411-18.
21. Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal susceptibilities of Candida species causing vulvovaginitis and epidemiology of recurrent cases. J Clin Microbiol. 2005;43:2155-62.
22. Fan SR, Lui XP. In vitro fluconazole and nystatin susceptibility and clinical outcome in complicated vulvovaginal candidosis. Mycoses. 2010;54:501-05.

Cite this article as: Erbaş G, Parin U, Kirkan S, Türkyilmaz S, Savaşan S, Yüksel HT, et al. Antifungal susceptibilities and identification of Candida species by using maldi-tof microbial identification system from cervicovaginal samples. Int J Res Med Sci 2017;5:5117-20.