Original Research Article

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

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Received: 22 September 2017
Accepted: 31 October 2017

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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*. In recent studies, non-albicans Candida (NAC) species such as *C. tropicalis*, *C. glabrata* and *C. krusei* and *C. parapsilosis* have been frequently isolated. 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. 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 blastospore 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. 

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 susceptibility tests

Antifungal susceptibility 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 fluocytosine (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>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>6</td>
<td>16.7</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>25</td>
<td>69.4</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>3</td>
<td>8.3</td>
</tr>
<tr>
<td>C. krusei</td>
<td>2</td>
<td>5.6</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>KCA</th>
<th>FCN</th>
<th>MCL</th>
<th>VOR</th>
<th>NY</th>
<th>FY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=6)</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>25</td>
<td>-</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(n=25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida kefyr</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida krusei</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(n=2)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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 (83.5%) 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 fluconazole (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
