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Determining the frequency of *Candida* species in women with candidal vaginal infection

Mahin Tafazoli, Mahboobe Gholami¹, Zahra Mohebbi-Dehnavi², Fatemeh Shaghghi³, Zahra Kamali¹

Department of Midwifery, Evidence-Based Care Research Center, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran, ¹Department of Nursing and Midwifery, Neyshabur University of Medical Sciences, Neyshabur, Iran, ²PhD Candidate in Reproductive Health, Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Isfahan University of Medical Sciences, Isfahan, Iran, ³PhD Candidate in Reproductive Health, Department of Midwifery, Faculty of Medicine, Social Development and Health Promotion Research Center, Gonabad University of Medical Sciences, Gonabad, Iran

Address for correspondence:

Zahra Kamali,
School of Nursing and Midwifery, Neyshabur University of Medical Sciences, Neyshabur, Iran.
E-mail: kamaliz@num.ac.ir

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Abstract:

BACKGROUND: Candidiasis vaginitis is an opportunistic and common mucosal infection. Although *Candida albicans* is the most common isolated species, other species of *Candida* such as *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* have also increased significantly in recent years. The aim of this study was to identify *Candida* species in women with candidal vaginal infection in order to promote health in women.

MATERIALS AND METHODS: This cross-sectional descriptive study was performed on nonpregnant women of childbearing age who referred to health centers in Neyshabur in 2018. At the beginning of the study, 163 people entered the study and the culture results were positive in 68 of them. *In vitro*, culture was performed in subdextrose agar medium containing chloramphenicol and gentamicin. Species identification was performed using *Candida* specific culture medium (Saburo dextrose agar and chloramphenicol). After culture, 15% of the residual wet slide with potash (KOH) was prepared and observed with a lens. Finally, the data were analyzed with SPSS software version 24.

RESULTS: The participants in the study were women with a mean age of 35.55 ± 5.47 and the average number of deliveries was 1.38 ± 1.15 . In this study, the prevalence of *C. albicans* was 59.7%, *C. tropicalis* was 14.8%, *C. krusei* was 15%, and *C. glabrata* was 7.61% and also *krusei* and *tropicalis* were 3% simultaneously. Therefore, *C. albicans* was the most common species isolated from clinical specimens.

CONCLUSION: In this study, the most common species after *albicans* were *C. tropicalis* and *cruciferous*. Considering the role of *C. glabrata* in the development of resistance to antifungal drugs, the lower prevalence of this species in the present study can be associated with improved treatment outcomes in patients.

Keywords:

Candidal infection, *Candida* species, health promotion, vaginal infection, women

Introduction

Candidal infection is the second most common vaginal infection^[1] which is associated with significant economic costs and physical, mental, and psychological complications. This

disease is an opportunistic and common mucosal infection that is found in the genitals of 30%–80% of asymptomatic women or women with mild symptoms.^[1] About 75% of women develop candidiasis vulvovaginitis at least once, and 45% of women develop two or more attacks

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each year. Some also have chronic and recurrent infections.^[2]

Vaginal candidiasis is a common infection of the female genital tract that is accompanied by yellow or milky discharge, cheesy with inflammation, redness and severe itching, and sometimes sores or pustules.^[3] Synonymous names for candidiasis include candidiasis, moniliasis. Today, this infection is the fourth most common cause of nosocomial infections.^[4] Candidiasis has been seen and reported in various forms in all cities of Iran depending on the severity and weakness of the immune system.^[5] *Candida* species cause candidiasis in 20–25 cases.^[6] Factors that predispose to vaginal candidiasis include: antibiotic therapy, pregnancy, uncontrolled diabetes mellitus, use of oral contraceptives (especially high-dose formulations), suppression of the immune system, and wearing tight underwear. The most common symptom of candidal vulvovaginitis is itching and then vaginal burning.^[1-6] The most important pathogens in candidiasis are *Candida albicans*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida pseudotropicalis*, *Candida stellatoideae*, and *Candida C.* The most common cause of this disease is *C. albicans*, which is a natural resident of the gastrointestinal tract, oral mucosa, and vagina and is often acquired at birth when passing through the vagina. Other species of *Candida* are sometimes part of the natural flora of the skin and mucous membranes, and some are found in nature, soil, and various materials that have a more limited pathogenicity overall.^[7] In recent years, the number of organisms resistant to antifungal drugs has increased and caused problems in treatment. Approximately 10% of patients with candidal vaginitis do not respond to initial treatment.^[6-9] More than 50 years after the discovery of candidal systemic infections, the main drug in treatment is still amphotericin B, although this may change with the introduction of new azoles such as voriconazole. The main problem in the treatment with amphotericin B is the toxicity of this drug, the high cost of lipid formulations has overshadowed its practical use. Some recommend the use of fluconazole in high doses in many cases for candidal infections, which are not only resistant to fluconazole but also due to repeated and long-term therapeutic use of fluconazole and its prophylactic use (for example in acquired immunodeficiency syndrome patients), even *C. albicans* may become resistant to it.^[1] Triazole group drugs such as fluconazole and terconazole and imidazoles including clotrimazole, miconazole, itraconazole, voriconazole, ketoconazole, and their polyunsaturated drugs such as nystatin are generally used to treat candidiasis vulvovaginal infections. The study of Nazeri *et al.* showed that among the patients with candidal vulvovaginitis identified, 13 (12.3%) had recurrent candidal vulvovaginitis. *C. albicans* and then *C. glabrata* had the highest frequency.^[10]

The study of Khorsand *et al.* showed that 77.7% of *C. albicans*, 9% of *Candida parapsilosis*, 7% of *C. glabrata*, 4% of *Candida tropicalis*, and 1% of *Candida kefir* were isolated.^[11]

Candidal vulvovaginitis, like other superficial fungal infections, is treated with azole antifungal drugs, but overuse of azoles may cause colonization of resistant species such as *C. glabrata* and *Candida krusei*. Among the reasons for recurrence and chronicity of candidal vaginal infection is lack of proper treatment and increase in nonalbicans species and inherent or acquired resistance of some species to azole drugs^[12-14]. Therefore, accurate identification of *Candida* species is necessary to treat this infection. Therefore, considering the prevalence of candidal vulvovaginitis and the importance of knowing the different species in treatment, a study was conducted to identify *Candida* species in patients referred to health centers in Neyshabur in 2018.

Materials and Methods

Study design and setting

This is a cross-sectional descriptive study that was performed on all nonpregnant women of childbearing age and referred to Neyshabur Health Centers in 2018.

Study participants and sampling

At the beginning of the study, 163 people entered the study, and the results of culture were reported to be positive in 72 people. Four people were excluded from the study due to dissatisfaction with the continuation of cooperation. Using the results of the study of Janani(2011) *et al.* and with the formula of ratios, the sample size with power 80%, coefficient 95%, and calculation of loss 20% using SPSS software (IBM Company, Armonk, NY, USA), 72 people were calculated.^[12] Inclusion criteria were literacy, consent to participate in the study, age range 15–49 years, marriage, no recurrent candidiasis vulvovaginitis, obtaining at least a score of 3 out of 18 of the symptoms, and criteria of vaginal candidiasis.^[9-12]

Data collection tool and technique

The questionnaires used were demographic questionnaire (7 questions) and fertility questionnaire (10 questions), questionnaire to diagnose the signs and symptoms of candidiasis. The questionnaire of personal characteristics and fertility was prepared according to the objectives of research and study of the latest related sources and articles and in consultation with supervisors and consultants. Then, content validity was used to determine the validity. In this way, this form was provided to seven midwifery faculty members and after making the necessary corrections, it was used in the research. Candidiasis of the vagina is an infection in which the patient's total score of clinical signs and symptoms (symptoms such as discharge, itching, burning, vulvar irritation, vaginal irritation,

and pain during intercourse) is diagnosed based on the severity of the symptoms of candidiasis. This questionnaire was prepared based on the study of similar studies and authoritative sources in this field. Content validity was used to determine validity. In this way, this form was provided to seven members of the midwifery faculty and after making the necessary corrections, it was used in the research. Its reliability was determined by the equivalence reliability method (agreement of evaluators). Thus, this form was completed separately for 10 of the simultaneous research units by the researcher and the women's consultant, then the correlation coefficient between the calculated results and its reliability was confirmed with $r = 0.90$. The minimum score is 3 out of 18. Each of the above items is given a score between 3 and 1, which is a total score of 18. A patient who scores at least 3 is clinically infected.

By observing ethical points and obtaining written consent from the patient, first, the entry and exit conditions were examined. Patients were selected as the sample if they obtained at least a score of 3 according to the symptoms of vaginal candidiasis. Then, the demographic and midwifery profile questionnaire was completed by the researcher for the patients.

A sample of vaginal discharge was taken from the posterior vaginal cold socket by a swab and sent to the laboratory for culture in Saburo dextrose agar medium and direct test. In the laboratory, culture was performed in Sabour dextrose agar medium containing chloramphenicol and gentamicin made by Conda company. After the growth of *Candida* colonies, the species were identified using the specific culture medium of *Candida* made by HiMedia Company. Immediately after receiving the samples, in the laboratory using swap, the sample was cultured on saber and dextrose agar and chloramphenicol (SC) medium and kept in an incubator at 37°C for 48 h. After culture, a 15% wet direct sludge sample with potash (KOH) was prepared and observed with $\times 40$ lens. At this stage, if the sample of false mycelium and *Candida* blastoconides were positive, it could be seen. After determining the results of initial culture on SC medium, some colonies were passed in a table on HiMedia chromium agar differential medium to determine *Candida* species and kept in an incubator at 37°C for 24–48 h. After the necessary time, according to the guide table of the culture medium and according to the color change created in the culture medium, the desired candidate species were determined. Based on the results of *in vitro* culture, various types of vaginal infections (*C. albicans*, *glabrata*, *tropicalis*, and other species) of the vagina were identified.

Ethical considerations

At the beginning of the study, written consent was obtained from all participants to participate in the study.

All research units were assured that their information would be confidential and that participating in this study would not harm them. At first, the code of ethics (IR.MUMS.NURSE.REC.1397.044) was obtained from the Vice Chancellor for Research of Mashhad University of Medical Sciences to enter the research environment.

After data collection and coding, analysis was performed with SPSS software version 24 and using descriptive and analytical statistical tests. All statistical tests were performed with 95% confidence interval and 0.05% alpha coefficient. In this study, $P < 0.05$ was considered significant.

Results

During the sampling period, 163 patients who presented with symptoms of candidal vaginal infection were tested directly and cultured vaginal secretions, which were positive in 68 patients. The age range of participants in this study was 15–49 years and their mean age was 35.55 ± 5.47 years. Other demographic characteristics, individual and family characteristics, and fertility of research units are listed in Tables 1 and 2.

In this study, *C. albicans* was the most common species isolated from clinical specimens. Also in this study, out of 68 patients, in 66 patients, one species of *Candida* and several species of *Candida* in two patients were isolated simultaneously [Table 3].

Discussion

The prevalence of vaginitis infection in women is high and other species of *Candida* such as *glabrata*, *cruzei*, *parapsilosis*, and *C. tropicalis* have been increasing in recent years. As a result, the aim of this study was to identify *Candida* species in women with candidal vaginal infections referred to health centers in Neyshabur. In this study, the prevalence of *C. albicans* was 59.7%, *C. tropicalis* was 14.8%, *C. krusei* was 15%, and *C. glabrata* was 7.61% and also *krusei* and *tropicalis* were 3% simultaneously. According to the research findings, like most studies, *C. albicans* is the most common cause of candidal infection in the present study. The results of this study are in line with other studies in this field.^[15-17] In the study of Mahmoodirad *et al.* (2011), *C. albicans* had the highest frequency and *Candida cruzei* and *C. tropicalis* had the lowest frequency.^[18] This study was in line with the present study in terms of the most abundant species. In the study of Buskemi *et al.*, (2005) about 80% of the species isolated from patients were related to *C. albicans*, 14.3% were related to *C. glabrata*, 5.9% were related to *C. parapsilosis*, and 8.5% were related to *C. tropicalis*.^[15] While in the present study, *C. albicans* species are less

Table 1: Demographic and midwifery characteristics of research units

Variable	n (%)
Female education level	
Elementary and literacy at the level of reading and writing	37 (54.5)
Middle school	15 (22.6)
Diploma	13 (18.05)
Above the diploma	3 (4.4)
Female occupation	
Housewife	3 (93.95)
Working at home	2 (3.02)
Working outdoors	2 (3.02)
Student	0
Spouse's education level	
Elementary and literacy at the level of reading and writing	16 (23.5)
Middle school diploma	27 (39.7)
Above the diploma	14 (20.65)
Above the diploma	11 (16.15)
Spouse job	
Manual worker	9 (12.25)
Government job	22 (32.35)
Freelance	33 (48.55)
Other items	4 (6.85)
Family income level	
Less than enough	50 (73.55)
Enough	17 (25.0)
More than enough	1 (1.45)
Irregular bleeding between periods	
Yes	5 (7.3)
No	63 (92.7)
Type of contraception	
Condom	13 (19.1)
Withdrawal	44 (64.7)
Without prevention	11 (16.2)
Duration of infection	
Less than a week	4 (5.85)
1 week-1 month	35 (51.5)
More than a month	29 (42.65)
Dysmenorrhea	
Lack of dysmenorrhea	5 (7.35)
Mild dysmenorrhea	27 (39.7)
Medium dysmenorrhea	16 (47.1)
Severe dysmenorrhea	2 (5.9)
Dyspareunia	
Lack of dyspareunia	4 (5.85)
Mild dyspareunia	35 (51.5)
Medium dyspareunia	29 (42.65)
Time of onset of dyspareunia	
Beginning of intercourse	60 (88.25)
End of intercourse	8 (11.75)
Nonvaginal intercourse	
Yes	3 (4.4)
No	65 (95.65)

Table 1: Contd...

Variable	n (%)
Type of delivery	
NVD	52 (76.45)
Cesarean section	16 (23.55)

NVS=Normal vaginal delivery

Table 2: Mean and standard deviation of demographic variables of research units

Variable	Mean±SD
Female age (years)	35.5±55.47
Spouse age (years)	36.6±29.46
Number of deliveries	1.1±38.15
BMI	26.2±02.88
Number of intercourses during the last month	1±7.02
Duration of menstrual bleeding (days)	6.1±2.8
Number of days elapsed since the first day of menstruation	13.5±2.4
Menstrual intervals	26.8±4.3

BMI=Body mass index, SD=Standard deviation

Table 3: Frequency of studied units by candidate species

Variable	n (%)
Candidate species	
<i>Candida albicans</i>	40 (59.6)
<i>Candida tropicalis</i>	10 (14.8)
<i>Candida krusei</i>	11 (15.0)
<i>Candida tropicalis</i> and <i>krusei</i>	2 (3.0)
Species <i>glabrata</i>	5 (7.61)

than Buskemi study and other species are more common. Grigoriou *et al.* attributed the greater prevalence of *C. albicans* to better adhesion to vaginal mucus and believe that although *Candida* is still more common, other species are increasing. Some nonalbicans candida species are growing. In their study, the reason for the increase in nonalbicans species was the excessive use ofazole drugs.^[16] In a study by Richter *et al.*, the reason for the increase in nonalbicans species was the excessive use ofazole drugs.^[19] In Mahmoudi Rad's study, which was performed to investigate the epidemiology of *Candida* species causing vulvovaginitis in Iran, 67% of cases of *C. albicans*, then *C. glabrata* and *C. tropicalis* were reported to be the cause of this infection.^[18] Co-infection with albicans and glabrata was also predominant in infections involving several species simultaneously. In this study, simultaneous infection was observed in *C. tropicalis* and *C. krusei* species. In some studies, *C. glabrata* is second in prevalence^[20-22] and is the most common species after *C. albicans*.^[23] However in the present study, this species is in the last category and is less common than other species. Considering the role of *C. glabrata* in the development of resistance to antifungal drugs, the lower prevalence of this species in the present study can be associated with improved treatment outcomes in

Contd...

patients. In this study, the most common species after *C. albicans* were *C. tropicalis* and *C. krusei*.

In this study, one species of *Candida* was isolated from 65 patients (54%) and two species were isolated from two patients (6%). In Richter's study 4.8% of patients,^[19] in Fun's study 0.02% of patients,^[20] in Abolten's study 0.12% of patients,^[21] and in Kunzelman's study 5% of patients^[23] had more than one type of *Candida* species. The results of these studies were less than the results of our study. In addition, in the Wheeler study, in 13%^[24,25] of patients, more than one type of *Candida* was identified as the causative agent, which was higher than the results of our study.

One of the strengths of this study was determining the type of candidal infection based on the results of laboratory culture (*C. albicans*, *glabrata*, *tropicalis*, and other species) of the vagina. Furthermore, the gold standard method for diagnosing candidal infection and culturing vaginal secretions in a specialized laboratory of parasitology and mycology under the supervision of an associate professor of mycology was used.

Limitation and recommendation

One of the limitations of this study is the lack of diagnostic methods in the diagnosis of other *Candida* species such as *glabrata*. Another weakness of this study is that it was performed on women who were not pregnant during the study. If this study is performed on pregnant women, the results of the study can be used to identify the causes of preterm delivery and premature rupture of the amniotic sac. Therefore, it is recommended that this study be performed to compare pregnant women and women in the general population.

Conclusion

The most common *Candida* species in our study were *albicans*, *tropicalis*, and *krusei*. In all studies, despite the increase in other species, *C. albicans* is still the most common cause of candidal vulvovaginitis. The results of the present study are in line with other studies and show a relative increase in nonalbicans species.

Acknowledgment and ethical moral code

This article is taken from a research project approved by Mashhad University of Medical Sciences with ethics code IR.MUMS.NURSE.REC.1397.044. The researchers hereby express their gratitude and appreciation to the Vice-Chancellor for Research of Mashhad University of Medical Sciences, Health Centers, and Research Units.

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Conflicts of interest

There are no conflicts of interest.

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