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Vulvovaginal candidiasis clinical signs, and distribution of *Candida* species

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ABSTRACT

Background: Vulvovaginal candidiasis is a global issue of concern due to its association with economic costs, sexually transmitted infections, and ascending genital tract infection. The aim of this study was to determine clinical signs and species distribution of *Candida* species causing vulvovaginal candidiasis.

Methods: A cross-sectional study was conducted from December to June 2021-2024 among 400 women attending Obstetrics and Gynecology outpatient clinics in Sana'a City. The study involved collecting vaginal swabs from each participant and analyzing them in a microbiology laboratory. The swabs were used to identify yeast cells, fungal culturing, and germ tube formation tests. The study also used the VITEK II system to confirm species identification, with the VITEK® 2 YST ID identification card being used to identify *Candida* species.

Results: A study of 400 women found that 91.8% were infected with *Candida albicans*, while 8.2% were infected with other non-*Candida albicans*. The majority were aged 26-35, with secondary education being the most common. Most were married, with 1-3 pregnancies. The most common sign distribution was vaginal discharge with a 99.5% prevalence. Dysuria was the lowest, with a 65.0% prevalence. *Candida* species were most frequently isolated, with *Candida albicans* being the most common. *Candida tropicalis* was found in 1 out of 6 women, while *Candida dubliniensis, Candida famata, Candida glabrata,* and *Candida lusitaniae* were found in 1 out of 6 women.

Conclusions: The high prevalence rate of vulvovaginal candidiasis and observation of a low prevalence rate of *nonalbicans Candida* species in the present study substantiate the importance of conducting continuous epidemiological surveys to measure changes in species distribution from *C. albicans* to *non-albicans Candida* species in Yemen.

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1. Introduction:

Excessive yeast growth in the vagina that causes irritation is referred to as a vaginal yeast infection, also known as vaginal thrush and candidal vulvovaginitis [1,2]. Vaginal itching is the most typical symptom and can get quite bad [1]. Additional signs and symptoms include burning when urinating, redness around the vagina, pain during intercourse, and a thick, white discharge that usually does not smell terrible [1]. Just before a woman's period, symptoms frequently get worse [3]. *Candida* overgrowth is the cause of vaginal yeast infections [1]. Small amounts of this yeast are typically seen in the vagina. *Candida albicans* is the species of yeast that usually causes vaginal yeast infections and is frequently present in the mouth, digestive system, or vagina without eliciting any negative symptoms [1,4]. Although the exact reasons of increased *Candida* growth are unknown, certain risk factors have been found [5]. It is not considered a sexually transmitted infection, though those who engage in sexual activity often may experience it more frequently. Antibiotic use, pregnancy, diabetes, and HIV/AIDS are risk factors. Personal cleanliness, kind of underwear, and tight clothing don't seem to be contributing factors. Testing a sample of vaginal discharge is the method of diagnosis; testing may be advised since the symptoms resemble those of *gonorrhea* and *chlamydia*, two sexually transmitted illnesses [1,6].

One course of treatment is antifungal medicine; this can be used orally with drugs like fluconazole or as a cream like clotrimazole [7]. Wearing loose-fitting garments and cotton underwear is frequently advised as a prophylactic precaution even in the absence of evidence. It's also advised to stay away from scented hygiene products and douching [1, 6]. It has not been discovered that probiotics are helpful for ongoing illnesses [8]. Approximately half of women experience at least two vaginal yeast infections, and 75% of women experience at least one at some point in their lives, also, approximately 5% of people get more than three infections in a year [1,9]. After bacterial vaginosis, it is the second most frequent cause of vaginal irritation [3]. In addition to vulval itching, vulval soreness, and irritation, symptoms of vaginal thrush also include dysuria, which is pain or discomfort during urination, vulval soreness, and irritation, and vaginal discharge, which is typically odorless [10]. Though thick and lumpy, akin to cottage cheese or paper paste, the vaginal discharge linked to yeast infection can also be thin and watery or thick and homogeneous in texture [6]. According to one study, women without a vaginal yeast infection were just as likely as those with one to characterize their discharge as cottage cheese-like [11]. In addition to the thrush symptoms mentioned above, there may also be inflammation in the vagina. Erythema (redness) of the vulva and vagina, vaginal fissuring (cracked skin), edema (swelling from a build-up of fluid), and in more severe cases, satellite lesions (sores in the surrounding area) are all indicators of vulvovaginal inflammation. Although uncommon, this could point to the herpes simplex virus—which causes genital herpes—or another fungal illness [12].

The causes of vulvovaginal candidiasis are frequently unknown, despite the fact that the condition is common. Potential risk factors include sociodemographic traits, the use of oral contraceptives and antibiotics, diabetes mellitus, dietary habits, personal hygiene, sexual activity, and certain immunological deficiencies [13, 14]. The evidence for each of these criteria is contradictory, though. Due to the widespread belief that vaginal candidiasis is a minor illness, it still receives minimal attention in the majority of underdeveloped nations, including Yemen [15]. However, because of its links to rising genital tract infections [19], HIV [18], and sexually transmitted infections [16,17], vulvovaginal candidiasis has been recognized as one of the world's most concerning issues. Although *Candida albicans* is the most frequent cause of vulvovaginal candidiasis, other species of *Candida*, including *C. tropicalis*, *C. glabrata*, and *C. krusei*, are now becoming more common,

especially in women living with HIV [20]. Antifungal agent-resistant yeast infections have been on the rise and are expected to do so in the future. In both *Candida albicans* and *non-albicans* species, resistance to azoles antifungal therapy has increased [21].

The goal of this study was to identify the clinical signs and species distribution of *Candida* species causing vulvovaginal candidiasis in Sana'a, Yemen. There have been a limited number of recent studies discussing candidiases in Yemen, including oral candidiasis [22–24], vaginal candidiasis, and urinary tract candidiasis [25, 26]. As a result, more research is needed to fully understand candidiasis in Yemen.

2. Subjects And Methods

Methods study design and area

A cross-sectional study was conducted from December to June of the years 2021–2024. A study was conducted among women attending obstetrics and gynecology outpatient clinics at different hospitals located in Sana'a City. Laboratory work was performed in the Department of Microbiology and Molecular Biology of the National Center for Public Health Laboratories (NCPHL).

Study population. A total of 400 women who agreed to participate were enrolled in the study. Oral agreement was obtained from all participants after the explanation of the purpose of the study. A questionnaire was used to obtain information on the participants' sociodemographic data and medical history.

Inclusion criteria: Women aged 15–55 who were infected by valvovaginal candidiasis (VVC) were included.

Exclusion criteria: Patients with cervical cancer, women who were currently treated with antifungal in the past 6 months were excluded, females who used vaginal douche within the last 24 hours or spermicidal agents within 72 hours before testing, or females during menstruation at the time of examination.

Specimen collection and examinations: Laboratory examinations were conducted in accordance with standard microbiological practices. Two vaginal swabs were collected from each participant by gynecologists. The vaginal swabs were inoculated into a tube containing approximately 2 ml of sterile physiological saline and were transported immediately to the microbiology laboratory for analysis. At the laboratory, one vaginal swab was used for wet mounting with 10% potassium hydroxide (KOH) to identify the presence of oval to spherical yeast cells with budding. Gram-stained smears for the presence of Gram-positive large oval budding yeast cells with or without pseudohyphae or hyphae. Another swab was used for fungal culturing on Sabouraud's dextrose agar (SDA) containing gentamicin and chloramphenicol (HiMedia, India).

Identification of Candida spp: It was based on the macroscopic appearance of the colonies on SDA as white to creamy, round, soft, and smooth colonies with characteristic yeast odor. **A germ** tube formation test was performed to distinguish between *C. albicans* from other *Candida* spp. As well, isolates were identified based on chlamydospore formation tests on corn meal agar (Oxoid, UK). For confirmation of species identification, isolates were subjected to the VITEK II system. A VITEK®® 2 YST ID identification card (lot 2432153503) was used to identify *Candida* species. All *Candida spp.* isolates were then preserved as frozen stocks in 15% glycerol at -80 °C for further processing.

3. Results

Out of 400 participants, 367 women were infected by *Candida albicans (91.8%),* while 33 women (8.2%) were infected by other *non-Candida albicans*. Table 1 shows data on the socio-demographic characteristics of the study participants. The mean age of the participants was 33.4±8.4 years, ranging from 15 to 55 years old. The majority of the participants fall within the age groups of 26-35, representing 44.5% of the participants with *Candida albicans* and 3.3% with non-*C. albicans*. The most common

educational level is secondary, with 168 cases (42%) for *C. albicans* and 17 cases (4.3%) for other species. The majority of infected women were married, accounting for 356 cases (89%) for *C. albicans* and 32 cases (8%) for other species.

Table 1: Socio-demographic characteristics of studied women suffering from vulvovaginal candidiasis in Sana'a city

Variable	Candidiasis			
	Candida albicans N = 367		Non-*C. albicans* spp N =33	
	No.	%	No.	%
Age groups/years				
15- 25	63	15.8	4	1.0
26- 35	178	44.5	13	3.3
36-45	94	23.5	14	3.5
46-55	32	8.0	2	0.5
Mean ±SD	33.4±8.4			
Educational level				
Illiterate	58	14.5	2	3.3
Primary	56	14.0	6	1.5
Secondary	168	42.0	17	4.3
University	85	21.3	8	2.0
Marital status				
Married	356	89.0	32	8.0
Divorced	2	0.5	0	0.0
Widow	9	2.3	1	0.3
Number of pregnancies				
0	34	8.5	2	0.5
1-3	235	58.8	20	5.0
4-6	81	20.3	10	2.5
>6	17	4.3	1	0.3

Most participants with *Candida albicans* and non-*C. albicans* had 1-3 pregnancies, representing 58.8% and 5% of the participants, respectively. Table 2 shows clinical manifestations: the most common sign distribution was vaginal discharge (99.5%), while the lowest sign distribution was dysuria with the lowest percentage of 65.0%. Additionally, 70.3% of participants reported yeast smell discharge, so we can estimate that 70.3% of the participants had *Candida albicans* infections. Also, participants with *Candida albicans* had a higher percentage of dysuria (65.0%) compared to those with non-*Candida albicans* (35.0%). This suggests that dysuria may be a more common symptom of *Candida albicans* infections.Table 3 shows the frequency of *Candida* species isolated from 400 women of reproductive age with vulvovaginal candidiasis. *Candida albicans* was the most frequently isolated species, found in 367 women (91.8%). *Candida tropicalis* was isolated from 6 women (1.5%). *Candida dubliniensis*, *Candida famata*, *Candida glabrata*, and *Candida lusitaniae* were each isolated from 4 to 6 women (1.0% to 1.5%). Overall, the most frequently isolated *candida* species from females with vulvovaginal candidiasis was *Candida albicans, which* was the most common species isolated.

Table 2: Clinical manifestations among females suffering from vulvovaginal candidiasis in Sana'a city

Clinical manifestations	n = 400 participants			
	Yes	%	No	%
Lower abdominal pain	337	84.3	63	15.8
Pain in vagina	375	93.8	25	6.3
Burning	378	94.5	22	5.5
Pruritus	380	95.0	20	5.0
Vaginal discharge	398	99.5	2	0.5
Yeast smell discharge	281	70.3	119	29.8
Dysuria	260	65.0	140	35.0

Table 3 Frequency of *Candida* spp among reproductive-aged women suffering from vulvovaginal candidiasis in Sana'a city, Yemen

Micro-organism species	Frequency	
	No.	%
Candida albicans	367	91.8
Candida spherica	6	1.5
Candida glabrata	6	1.5
Candida dubliniensis	6	1.5
Candida famata	4	1.0
Candida tropicalis	6	1.5
Candida lusitaniae	5	1.3
Total	400	100%

4. Discussion

According to the current study, vulvovaginal candidiasis is defined as the isolation of *Candida* species in culture from study participants who exhibit abnormalities in their vagina. The three most prevalent causes of vaginitis are trichomoniasis, bacterial vaginosis, and vulvovaginal candidiasis; in Yemen and globally, bacterial vaginosis is the most common cause of vaginal candidiasis [1, 6]. There is little information available about the prevalence of vulvovaginal candidiasis in Yemen. Unfortunately, vulvovaginal candidiasis is not a condition that needs to be reported, and it is frequently diagnosed based just on signs and symptoms, without the assistance of a laboratory. Consequently, Yemen is not well-versed in the range of yeasts linked to the illness and their drug susceptibility profile. Research on the prevalence of vulvovaginal candidiasis differs. With a prevalence of between 17% and 42%, it is the second most frequent infection in the vulvovaginal region in women who have symptoms [13, 14, 28]. The current study's 39.4% infection prevalence rate was within the reported range, however it was greater than the prevalence rates reported by Ahmed *et al.* [17] and Olowe *et al.* [14] in Nigeria and India, but lower than the prevalence rate published by

ERylander *et al*. [27]. Variations in the occurrence and/or recurrence of vulvovaginal candidiasis have been linked to a number of factors, including variations in sociodemographic traits, patient immune status [29], the use of immune suppressive medications and broad spectrum antibiotics [30], and hormonal influences [31]. Age appears to be a significant sociodemographic determinant in the general incidence of vulvovaginal candidiasis. With 44.5% of people having *Candida albicans* and 3.3% having non-*C. albicans* (2nd to 4th decade of life), the age groups 26–35 comprise the majority of research participants. The current outcome and the earlier research were equivalent. According to Sobel *et al*. [32], vulvovaginal candidiasis is uncommon during puberty, which is the first time a person experiences menstruation. However, it becomes more common as one approaches the end of the second decade of life (10–19 years old), peaking in the third and fourth decades of life (30–39 years old) of life. According to the current study, secondary school was the most prevalent educational level for women with vulvovaginits candidiasis—168 cases (42%) of *C. albicans* and 17 cases (4.3%) of other species. The increased rate of infection between secondary levels may be explained by improvements in infection awareness and seeking medical attention. Our results were in conflict with the conclusions drawn by Vadav and Prakash [34], but they were in line with those of Rathod *et al.* [33]. Married women subjects in the current study had greater infection rates (89%) than subjects in other groups. Our outcome agreed with Rathod *et al.'s* findings from India [33]. *Candida albicans* was the most commonly isolated species among the ten species of *Candida* that were isolated in this investigation; it was discovered in 367 women (91.8%). Six women (1.5%) had an individual case of *Candida tropicalis*. *Candida glabrata, Candida lusitaniae, Candida dubliniensis,* and *Candida famata* were isolated from 4–6 women, ranging from 1.0% to 1.5%. The major species found in the current study, *C. albicans,* was also found in previous investigations that were comparable to this one [32, 35]. There has been a growing tendency of non-*albicans Candida* species being recovered, despite the fact that many research on the frequency of various *Candida* species have led to the general consensus that *C. albicans* is the most often isolated species in patients with vulvovaginal candidiasis. This is not the same as the current investigation, where 8.3% of non-*albicans Candida* species were isolated. Conversely, higher recovery rates of non-*albicans Candida* species have been recorded in Belgium [36] and the US [35], respectively. Studies carried out in India [13], Egypt [37], and Iran [38] have also revealed somewhat higher recovery rates of 53.1, 65.0, and 57.5% non-*albicans Candida* species.

We found differences between our study and other previous studies in the recovery rate among non-*albicans Candida* species.Six women (1.5%) in the current investigation had *Candida tropicalis* isolated from them. *Candida glabrata, Candida lusitaniae, Candida dubliniensis,* and *Candida famata* were isolated from 4–6 women, ranging from 1.0% to 1.5%. This is not the same as the earlier work by Trama *et al*. [35], which found a recovery rate of 14.3% for C. *glabrata*, 5.9% for *C. parapsilosis*, and 8.0% for *C. tropicalis*. According to research by Sobel *et al*. [32], Nyirjesy [39], and Sobel *et al*. [40], *C. glabrata* dominated the non-*albicans Candida* species. According to a study by Bauters *et al.* [36], *Candida glabrata* was the most often isolated non-albicans species (16.3%), with *C. parapsilosis* (8.9%), *C. humicola* (1.6%), *C. krusei* (0.8%), and *C. lusitaniae* (0.8%) coming in second and third, respectively. According to Hasanvand *et al*. [41], *Candida albicans* is the most often isolated species, with *Candida glabrata, Candida tropicalis*, and *Candida parapsilosis* following closely behind. Contrary to these observations, the Bitew and Abebaw investigation [42] found that *C. krusei* was the predominant non-*albicans Candidia* species, making up 17.2% of all isolates. The Yemen Ministry of Health currently recommends fluconazole as the first-choice medication for

treating candidiasis, with ketoconazole and miconazole ointment serving as backup antifungal medications, similar to other Middle Eastern and African nations [43, 44].

5. Conclusion

The high prevalence rate of vulvovaginal candidiasis and observation of a low prevalence rate of non-*albicans Candida* species in the present study warrant the importance of conducting continuous epidemiological surveys to measure changes in species distribution from *C. albicans* to non-*albicans Candida* species in Yemen.

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Data Aviliability

The data will be available to anyone upon request from the corresponding author.

A Dispute Of Interest

Regarding this project, there is no conflict of interest.

Author's Contributions

Abeer Abdulmahamood Mohammed Nasher did the fieldwork for this study as part of a PhD in the department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a university. Other authors including prof Hassan al-Shamahy and prof Ahmed Y Al-Jaufy assisted with data analysis, drafting and reviewing the manuscript, and giving final clearance to the study.

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