Rafidain Journal of Science
https://rsci.uomosul.edu.iq
Vol. 35, No.1, pp. 29-38, 2026 (March)

Isolation and Identification of Candida Species Causing Vulvovaginal Candidiasis Using the VITEK 2 System in Duhok City/ Iraq

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p-ISSN: 1608-9391
e -ISSN: 2664-2786

Article information

Received: 20/4/2025
Revised: 14/6/2025
Accepted: 24/6/2025

DOI: 10.33899/rsci.v35i1.61976

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ABSTRACT

Vulvovaginal candidiasis (VVC) is a prevalent gynecological disease characterized by vaginal wall inflammation that is caused by Candida species. VVC impacts almost three-quarters of all women throughout their reproductive years. This study was aimed to assess the incidence and species distribution of Candida in Duhok women presenting with vaginal Candidiasis. Using selective media Sabouraud dextrose agar (SDA), germ tube test (GTT), chlamydospore formation, and CHROMagar Candida. A total of 200 samples of high vaginal infection were collected from patients in Azadi teaching hospital/Duhok city. A total of 71 samples Candida species, 114 with normal flora, 14 with bacterial infection, and 1 with parasite infection. These isolates were subjected to GTT, chlamydospore formation, and inoculation on CHROMagar Candida.

Candida albicans was the main species isolated (67.6%), followed by C. glabrata (23.9%), C. krusei (7.0%), and C. tropicalis (1.4%) based on colony color. Following this, species confirmation and antifungal susceptibility testing are performed using the VITEK 2 system.

Regarding antifungal sensitivity, micafungin and voriconazole have proven to be the most effective, exhibiting the lowest MIC values. These are followed by caspofungin, fluconazole, amphotericin B, and flucytosine, indicating their relative efficacy in treating Candida infections. This comprehensive approach ensures accurate identification and optimal treatment strategies for Candida species.

Keywords: Vaginal candidiasis, chlamydospores formation, CHROMagar, Vitek 2 system.

INTRODUCTION

Vulvovaginal candidiasis is the most common type of vaginitis, second only to bacterial vaginosis. The infection arises from the multiplication of yeasts in the mucosal lining of the female vaginal canal. Yeast infections are primarily attributed to *Candida* species, impacting 70-75% of women at least once during their lives, especially young women of reproductive age. Approximately 50% of adult women may experience a recurrence, whereas 5-8% will report 4 or more episodes. In 20% of healthy asymptomatic women, *Candida* species may be found in the lower vaginal flora (Sobel, 2007). Approximately 85-95% of yeast strains isolated from the vagina are *C. albicans*; however, the prevalence of non-albicans Candida (NAC) appears to be increasing (San *et al.,* 2023).

The primary NAC is *C. glabrata*; however, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* have been sporadically identified as causal agents of vulvovaginal candidiasis (VVC) (Singh *et al.,* 2003).

Vaginal infections caused by NAC are clinically indistinguishable from those caused by *C. albicans*. Moreover, most NAC species exhibit resistance to azole antifungal treatment, making the infections they cause often difficult to control. Consequently, NAC species *C. glabrata* and *C. krusei* are the primary organisms in persons with recurrent vulvovaginal candidiasis (RVVC) (San *et al.,* 2023). *Candida* species are opportunistic infections that can cause diseases under unexpected circumstances and rarely impact healthy individuals (Macias-Paz *et al.,* 2023). Approximately 20% of asymptomatic healthy women devoid of abnormal vaginal discharge may possess *Candida* species in their lower genital tracts. A variety of predisposing factors promote the progression from asymptomatic colonization to symptomatic vaginitis, including the following: Some individuals may have a genetic susceptibility to *Candida* colonization or vaginitis. Pregnant women demonstrate a greater incidence of vaginal colonization than non-pregnant women. Vaginal colonization by *Candida* is more common in women with diabetes than in those without diabetes.

The use of broad-spectrum antibiotics predisposes patients to vulvovaginal candidiasis by eliminating beneficial bacterial flora, hence promoting *Candida* overgrowth in the vagina. The use of oral contraceptives with high amounts of estrogen. Immunocompromised states arising from reasons like HIV/AIDS, corticosteroid treatment, organ transplantation, and cancer therapy (Sobel, 2016).

The implementation of antifungal susceptibility tests is crucial due to the emergence of antifungal resistance in certain pathogen strains and the possible involvement of several infections. Therefore, it is crucial to identify the most appropriate drug for particular organs. This study seeks to assess the incidence and species distribution of Candida in women with vaginal candidiasis in Duhok, investigate key predisposing factors related to its development, and identify antifungal-resistant strains.

MATERIALS AND METHODS

Samples collection

This investigation involved the collection of 200 high vaginal swab samples from married female patients suspected of having vaginitis. The samples were obtained at Azadi Teaching Hospital in Duhok throughout a three-month period, from September to November. The study participants ranged in age from 18 to 70 years, offering a thorough representation of the adult married population susceptible to vaginitis. The collection method employed a sterile speculum to reach the upper vaginal area, enabling the acquisition of accurate and uncontaminated samples (Khan *et al.,* 2019). This method was chosen to provide reliability and accuracy in sample collection, while enhancing diagnostic precision for identifying putative causal agents of vulvovaginal infections. The collected swabs were later examined in the laboratory to isolate *Candida* species; the primary fungal pathogens associated with VVC. Stringent aseptic techniques were utilized throughout sample management and culturing to maintain integrity and avert contamination, so ensuring that the results accurately reflected the presence of *Candida* and other microorganisms within the research population (Nenadić and Miloš, 2015).

Direct microscopy

Direct microscopy was employed as an initial diagnostic method for all collected samples to promptly identify fungal elements. Each vaginal swab was examined using a 10% potassium hydroxide (KOH) preparation on a sterile glass slide. The KOH solution aids in the elimination of epithelial cells and other debris, hence enhancing the visibility of fungal structures under the microscope. This method enabled the direct identification of yeast cells, pseudo-hyphae, and mycelia, which are unequivocal indicators of *Candida* infections. Although direct microscopy is a swift and cost-effective technique, it has limitations in sensitivity and specificity, particularly in differentiating *C. albicans* from NAC species (Sobel, 2007).

Cultural media

Sabouraud Dixtrose Agar (SDA) was utilized as the primary culture medium for the culture-based identification of *Candida* species in all vaginal swab samples. All collected swabs were immediately inoculated onto SDA plates and incubated at 37°C for 24 to 48 hours to promote optimal fungal growth. Samples exhibiting colony morphology characteristic of *Candida* were classified as positive and subsequently sub-cultured onto CHROMagar Candida, a chromogenic differential medium designed to facilitate the identification of several *Candida* species based on colony color and morphology. CHROMagar Candida provided a rapid and effective technique for distinguishing among commonly seen species, such as *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis*. The two-step culture process, which includes initial screening with SDA followed by species distinction with CHROMagar Candida, enhanced the accuracy and effectiveness of *Candida* identification, especially in recognizing mixed infections that are difficult to detect (Nadeem *et al.,* 2010).

Germ tube test

The GTT was utilized for all culture-positive samples as a quick and economical technique to distinguish *C. albicans* from NAC species (Guzel *et al.,* 2011; Hoppe and Frey, 1999). This assay relies on the ability of *C. albicans* to produce germ tubes, which are tubular extensions that emerge from yeast cells when cultivated with human serum. This study entailed inoculating a little sample from each positive colony into sterile serum and culturing it at 37°C for 2 to 4 hours (Cárdenes-Perera *et al.,* 2004; Hoppe and Frey, 1999). Following incubation, a drop of the serum-yeast suspension was placed on a glass plate and examined microscopically for the presence of germ tubes. The formation of these unbranched filamentous extensions is considered a definitive indicator of *C. albicans*. NAC species do not produce genuine germ tubes in these conditions, allowing for a simple and effective first differentiation among species.

Chlamydospores formation

After doing the GTT on all *Candida*-positive samples, Corn Meal Agar (CMA) was utilized to confirm the identify of *C. albicans* by observing chlamydospore formation. The test was performed on all isolates demonstrating germ tube formation by streaking them onto CMA plates, followed by incubation at 25°C for 24 to 48 hours. Subsequent to incubation, the cultures were subjected to microscopic examination to identify the presence of chlamydospores, which are large, spherical, thick-walled structures that confirm the isolates as *C. albicans*. This technique enhanced the accuracy of species identification by validating unique morphological traits (Rashak *et al.,* 2024).

Identification by Vitek 2 system

The VITEK 2 Compact system (bioMérieux, France) was employed for supplementary verification and precise identification of all *Candida* isolates obtained in this study. This automated method enables species-level identification based on the organism's metabolic activity, employing the YST ID card designed exclusively for yeast identification. Subsequent to initial identification using CHROMagar, GTT, and chlamydospore assessment, all isolates were analyzed utilizing the VITEK 2 system. Each pure colony was suspended in a 0.45% saline solution to attain the requisite turbidity (about 1.8-2.2 McFarland standard) and thereafter inserted into the machine using the YST ID card. The system analyzed the biological responses for several hours and generated accurate species identifications. The outcomes from the VITEK 2 system were fully consistent with the initial identification performed using CHROMagar: *C. albicans* green colonies, *C. glabrata* white colonies,

C. krusei white to pink colonies, and *C. tropicalis* blue colonies. This extensive concordance between chromogenic medium and automated biochemical identification confirmed the reliability of both methods (Pincus, 2010).

Antifungal susceptibility by VITEK 2 system

Subsequent to the identification step, antifungal susceptibility testing was performed for each *Candida* species using the VITEK 2 system. This automated method provided accurate and rapid results, enabling the evaluation of minimum inhibitory concentrations (MICs) for different antifungal agents. The susceptibility profile for micafungin, voriconazole, caspofungin, fluconazole, amphotericin B, and flucytosine was established by assessing each isolate individually. This technique was crucial for evaluating the effectiveness of antifungal medications and detecting potential resistance patterns, thereby enabling informed clinical decision-making and targeted therapy (Wiegand *et al.,* 2008).

RESULTS AND DISCUSSION

Identification of *Candida* species

To detect the presence of fungal components, the first microscopic examination of high vaginal swab samples was performed using wet mount preparation. This direct examination method provided a quick and easy diagnostic tool for detecting fungal infections before culture results were obtained.

The microscopic image Fig. (1) clearly displays characteristic oval budding yeast cells, which supports the initial diagnosis of vulvovaginal candidiasis. After that all samples cultured on SDA agar to isolate and identify *Candida* species. Creamy to white colonies detected which are characteristic of *Candida* species as in Fig. (2).

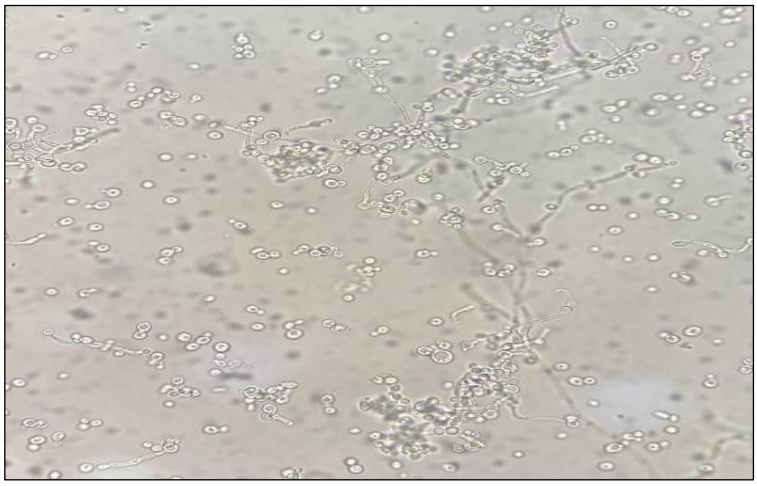

Fig. 1: Yeast cells and pseudo-hyphae under a light microscope.

Fig. 4: Germ tube of *C. albicans*.

Fig. 2: Colony of *Candida tropicalis* on SDA agar.

Following initial culture on SDA, isolates were sub-cultured on CHROMagar *Candida* to help identify *Candida* species based on colony color. The medium provided for easy differentiation: *C. albicans* appeared as green colonies, *C. tropicalis* as blue, *C. krusei* as white to pink, and *C. glabrata* as white. Fig. (3) clearly shows the variation in colony form.

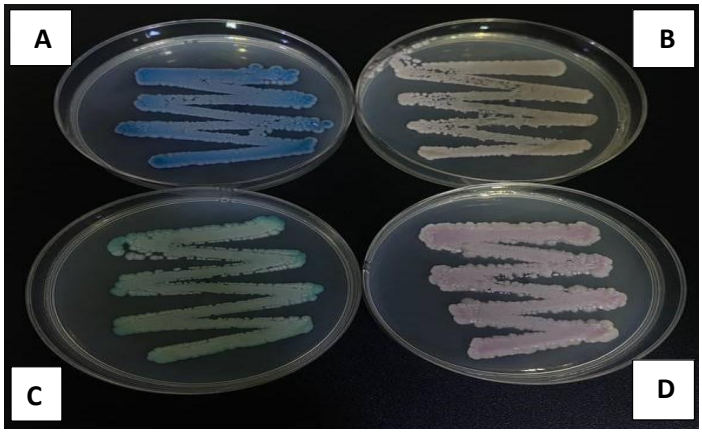

Fig. 3: Colony color of *Candida* species on CHROMagar A. (*C. Tropicalis*, Blue), B. (*C. glabrata*, White), C. (*C. albicans*, Green), D. (*C. krusei*, White to Pink).

After identification on CHROMagar Candida, all green colonies suspected to be *C. albicans* were examined using the germ tube method. The results showed that all samples had germ tubes, confirming that they were *C. albicans*. The germ tubes appeared as tiny, tube-like extensions of yeast cells. This is clearly demonstrated in Fig. (4). For additional confirmation, the same green colonies suspected of being *C. albicans* were cultured on corn meal agar. All of the examined isolates produced chlamydospores, which are thick-walled, spherical spores that *C. albicans* generally forms. The presence of chlamydospores supported the identification of the isolates as *C. albicans* as in Fig. (5).

Fig. 5: Chlamydospores of *C. albicans.*

Following identification, all isolates that showed distinct colony colors on CHROMagar Candida were confirmed using the VITEK 2 Compact system to ensure precise species identification.

The green colonies *C. albicans*, blue *C. tropicalis*, white to pink *C. krusei*, and white *C. glabrata*, were confirmed to be the same species detected by CHROMagar Candida. The VITEK 2 results were completely consistent with the chromogenic media, confirming the accuracy and reliability of both methods.

After species confirmation, each *Candida* isolate underwent antifungal sensitivity testing with the VITEK 2 Compact system. The test measured each species' susceptibility to routinely used antifungal drugs. This method was critical for finding resistant strains, notably in non-albicans Candida. The results showed varied sensitivity patterns across species. (Tables 1 and 4) show detailed antifungal susceptibility profiles for *C. albicans*, *C. krusei*, *C. tropicalis*, and *C. glabrata*.

Table 1: Antifungal susceptibility for *C. albicans*.

Antifungal	MIC	Interpretation
Voriconazole	< = 0.12	S
Fluconazole	< = 0.5	S
Caspofungin	< = 0.12	S
Micafungin	< = 0.06	S
Amphotericin B	1	S
Flucytosine	< = 1	S

Table 2: Antifungal susceptibility for *C. krusei*.

Antifungal	MIC	Interpretation
Voriconazole	< = 0.12	S
Fluconazole		
Caspofungin	< = 0.12	S
Micafungin	< = 0.06	S
Amphotericin B	< = 0.5	S
Flucytosine	8	R

Table 3: Antifungal susceptibility for *C. tropicalis*.

Antifungal	MIC	Interpretation
Voriconazole	< = 0.12	S
Fluconazole	2	S
Caspofungin	< = 0.12	S
Micafungin	< = 0.06	S
Amphotericin B	< = 0.5	S
Flucytosine	< = 1	S

Table 4: Antifungal susceptibility for *C. glabrata*.

Antifungal	MIC	Interpretation
Voriconazole	< = 0.12	S
Fluconazole		
Caspofungin	< = 0.12	S
Micafungin	< = 0.06	S
Amphotericin B	1	S
Flucytosine	< = 1	S

The association study of multiple parameters associated with vaginal candidiasis is presented in (Table 5). The correlation coefficient and p-value for each variable elucidate their associations with vaginal candidiasis. Furthermore, 200 clinical samples were analyzed for VVC, employing wet mount preparation, germ tube testing, and VITEK machine analysis for fungus identification (Table 6).

The correlation study demonstrates that diabetes and the presence of discharge or odor show substantial correlations ($p < 0.05$) with high correlation coefficients, indicating a robust association with vaginal candidiasis. Itching exhibits the strongest correlation coefficient (26.702) and a significant p-value (0.000), indicating that is a pivotal symptom associated with vaginal candidiasis.

Conversely, characteristics including pregnancy and lower abdominal pain exhibit weaker correlations and elevated p-values, signifying less meaningful relationships with vaginal candidiasis.

These findings highlight the significance of comprehending the numerous factors that lead to vaginal candidiasis, which can inform clinical evaluations and improve patient education.

Table 5: Correlation analysis of various factors related to vaginal candidiasis.

No.	Variables	p-value	correlation coefficient
1	Pregnancy	0.578	1.096
2	Diabetics	0.016	8.329
3	Discharge/smell	0.012	8.784
4	Pain in the lower abdomen	0.841	0.346
5	Itching	0.000	26.702

Table 6: Distribution of *Candida species* isolated from patient with suspected vulvovaginal candidiasis.

Candida sp.	No. of cases	Percentage %
Candida species	71	35.5
Candida albicans	48	67.6%
Candida glabrata	17	23.9%
Candida krusei	5	7.0 %
Candida tropicalis	1	1.4 %
Non- *Candida* growth	129	64.5 %

The findings of this study reveal that *C. albicans* is the predominant species linked to VVC, representing around 67.6% of cases, consistent with global evidence that underscores its increased virulence, biofilm formation ability, and adaptation to the vaginal environment. This result is consistent with the finding of (Narges *et al.,* 2021) who also reported C. *albicans* as the dominant species in vaginal candidiasis. NAC species, such as *C. glabrata* (23.9%), *C. krusei* (7.0), and *C. tropicalis* (1.4%), acquiring clinical significance due to their inherent resistance to antifungal drugs such azoles. The distribution of non-albicans species in this study contrasts with those documented by (Anh *et al.,* 2021), which exhibited a distinct prevalence pattern. In contrast to our findings, their results indicated a greater or lesser prevalence of specific non-albicans species, suggesting regional or clinical variability.

Diagnostic methods encompassed wet mount preparation, which, although effective for rapid screening, exhibits lower sensitivity compared to advanced techniques; the GTT, which is specific and expedient for identifying *C. albicans* but constrained in species differentiation; and the VITEK machine, which offered definitive identification and uncovered the presence and diversity of NAC species that conventional methods might overlook. Consistent with the current work (Mishra *et al.,* 2017) utilized a blend of conventional and automated techniques for the identification of Candida

species. *C. albicans* infections typically respond favorably to first-line antifungals such as fluconazole; however, the emergence of NAC species like *C. glabrata* and *C. krusei*, which exhibit diminished azole susceptibility, highlights the necessity of performing antifungal susceptibility testing to guarantee effective treatment.

The correlation analysis indicates that diabetes and the presence of discharge or odor are highly associated with vaginal candidiasis, with itching showing a particularly strong correlation. This suggests that patients displaying these symptoms may necessitate more comprehensive assessment and tailored treatment strategies. The detection of a solitary instance of *C. tropicalis* necessitates surveillance for uncommon species, as they may signify developing resistance trends or epidemiological changes. (Toprak, 2022) indicates a definitive association between diabetes and an elevated chance of acquiring vaginal candidiasis. The compromised immune response and heightened glucose levels in diabetic individuals foster an environment conducive to the proliferation of *Candida*.

The heightened incidence of NAC species aligns with global trends concerning antifungal resistance and extended azole usage; however, discrepancies in prevalence between studies may be affected by geographic location, patient demographics, or previous antifungal treatment. Additional investigation is required to evaluate antifungal resistance trends among various *Candida* species and to analyze host-related factors, such immunosuppression, diabetes, or hormonal fluctuations, that may affect species dispersion, thereby informing preventative and treatment approaches.

CONCLUSIONS

A total of 200 high vaginal swab samples were collected from patients at Azadi Teaching Hospital. The present study underscores the importance of *C. albicans* as the principal pathogen in vulvovaginal candidiasis, while simultaneously recognizing the rising prevalence of non-albicans species. Precise species identification, together with antifungal susceptibility testing, is essential to enhance treatment efficacy and mitigate the dissemination of resistant strains. Ongoing monitoring and sophisticated diagnostic techniques are crucial for improving patient outcomes and guiding public health initiatives. Furthermore, understanding the correlation between various risk factors and the prevalence of VVC will inform targeted prevention strategies and clinical management, underscoring the need for continued research into antifungal resistance patterns and the essential host factors affecting species distribution.

ACKNOWLEDGEMENTS

I wish to convey my profound appreciation to my supervisor for their unwavering support, direction, and encouragement during this study. I express my gratitude to the Biology Department/College of Science for furnishing the academic environment and resources essential for the completion of this study. Finally, I am profoundly grateful to my parents for their steadfast love, patience, and support.

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عزل وتشخيص انواع المبيضات من المصابين بالتهابات مهبلية باستخدام نظام فايتيك 2 في مدينة دهوك/ عراق

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الملخص

داء المبيضات الفرجي المهبلي هو مرض نسائي شائع يتميز بالتهاب جدار المهبل الناتج عن انواع من المبيضات. يصيب هذا المرض ما يقارب ثلاثة ارباع النساء طوال سنوات الانجاب. هدفت هذا الدراسة الي تقييم معدل انتشار المبيضات وتوزيعها لدى نساء دهوك المصابات بداء المبيضات المهبلي. شخصت الخمائر التي تم جمعها على اوساط انتقائية شملت الزرع على وسط SDA واختبار تكوين الانبوب الجرثومي GTT وتكوين السبورات الكلاميدية واختبار CHROMagar Candida. جمعت 200 عينة من اصابات المهبل عالية الكثافة من مريضات في مستشفى ازادي التعليمي/ مدينة دهوك· كان من بين العينات 71 عينة شخصت على انها انواع من المبيضات، منها 114 عينة تحتوي على فلورا طبيعية، و14 عينة اصابة بكتيرية، وعينة واحدة اصابة طفيلية. خضعت هذه العزلات لاختبار تكوين الانبوب الجرثومي، وتكوين كلاميدوسبور، وزراعتها على وسطCHROMagar Candida. كانت *C. albicans* هي النوع الرئيسي(67.6%) ، تليها *C. glabrata* (23.9%) و *C. Krusei* (7.0%)، و*C. Tropicalis* (1.4%)، وذلك استنادًا إلى لون المستعمرات .بعد ذلك يجري تأكيد النوع واختبار حساسية مضادات الفطريات باسخدام نظام VITEK 2.

وفيما يتعلق بالحساسية لمضادات الفطريات، ثبت أن Micafungin وVoriconazole هما الأكثر فعالية، حيث أظهرا أقل قيم للتركيز المثبط الأدنى (MIC). تلاهما كلٌّ من caspofungin وfluconazole وamphotericin B وflucytosine، مما يعكس فعاليتها النسبية في علاج عدوى المبيضات. ويضمن هذا النهج الشامل تحديدًا دقيقًا ووضع استراتيجيات علاجية مثلى لأنواع المبيضات.

الكلمات الدالة: داء المبيضات المهبلي، تكوين السبور الكلاميدي، وسط CHROMagar Candida، نظام .VITEK