JOURNAL OF ZANKOY SULAIMANI

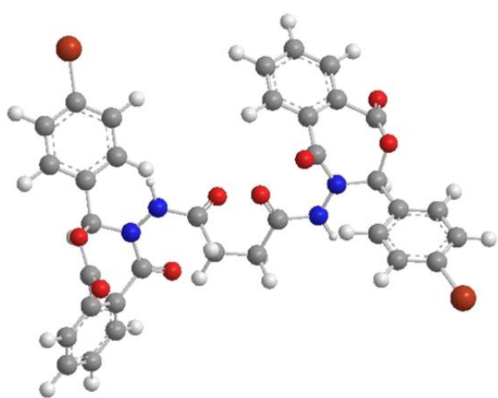
AUTHOR'S COPY

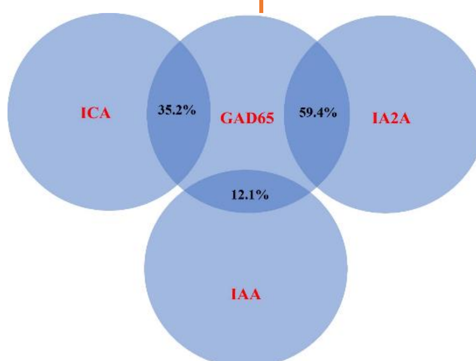
Part -A- (Pure and Applied Sciences)

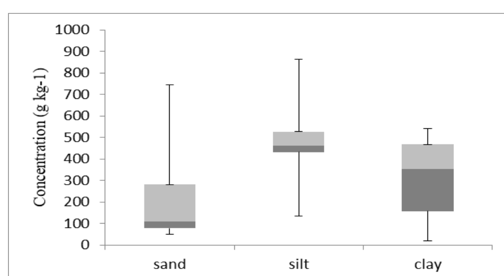
VOLUME 25 ISSUE 2 December 2023

ISSN: 1812-4100

www.jzs.univsul.edu.iq







Journal homepage www.jzs.univsul.edu.iq
Journal of Zankoy Sulaimani
Part-A- (Pure and Applied Sciences)
ISSN:1812-4100

Molecular identification of *Candida spp.* isolated from women with vulvovaginal candidiasis in Koya District, Iraq

Kale Mohammed Ali Mohammed[1]* & Taha Jalal Omar Zrary[1]

1 Department of Medical Microbiology, College of Science and Health, Koya University, Koya, Kurdistan Region, Iraq
*Corresponding email: kale.mohammed@koyauniversity.org

Article info

Original: 04/05/2023
Revised: 01/07/2023
Accepted: 02/07/2023
Published online: 20/12/2023

Keywords:
Vulvovaginal candidiasis, Prevalence, PCR, Candida species, Candida albicans.

Abstract

Vulvovaginal candidiasis (VVC) is the second most common vaginal infection among women of reproductive age. It is an opportunistic mucosal infection. *Candida* species causes VVC, which affects millions of women annually. The objective of this study was to isolate and identify the species of *Candida* from married women who had VVC attending Shahid Doctor Khalid Hospital & Haji Qader Health Centre in Koya, Iraq using traditional and molecular methods. This research was carried out, from December 2021 to May 2022; 150 vaginal swabs from married patients with symptoms of VVC were collected. Swabs were microscopically examined, grown on Sabouraud Dextrose Agar (SDA), and HiCrome™ Candida Differential agar and Germ Tube Test were used to identify Candida isolates. Next, the results were validated using the molecular method called polymerase chain reaction (PCR) to amplify and detect the ITS region in this study. The chitin synthase gene (CHS1) confirmed all *C. albicans* isolates. Out of the 150 vaginal swabs gathered, 84 (56%) cases were positive for vaginal candidiasis. Ages 26-30 were found to have the most significant proportion of positive patients, and Women who were pregnant had a more positive culture than women who were not. Identifying six different species of *Candida* was accomplished through the application of both traditional and molecular techniques in the study; they are *C. albicans* (88.1%), *C. glabrata* (4.8%), *C. kefyr* (2.4%), *C. krusei* (2.4%), *C. parapsilosis* (1.2%), and C. *tropicalis* (1.2%). Finally, we concluded that VVC was shown to be prevalent in Koya city, with the predominant species as *C. albicans* among all other species.

Introduction

Vulvovaginal candidiasis (VVC) is the most common yeast infection affecting the genital area [1]. However, VVC is characterized by *Candida* spp, signs and symptoms of inflammation and the absence of other infectious causes [2,3]. Vaginal Candidiasis is an infection caused by overgrowth of *Candida* species as an opportunistic pathogen affecting the genital tract [4]. The human body has multiple regions, including the vagina, in which *Candida* species are considered a part of the normal microbiota [5]. After Bacterial vaginosis (BV), the second most frequently occurring reason for vaginal infections [6]. Millions of women visit gynecologic offices, clinics, and health centres yearly for vaginal and urinary tract issues. The majority of these women complain of vulvovaginal candidiasis [7]. While the infection is not fatal, it causes high morbidity, discomfort, and pain with a health care cost [8]. At least once in their lifetimes, vulvovaginal candidiasis affects around 75% of women worldwide. Around 5 - 9% of women with VVC will experience recurrent VVC (RVVC) when they have three or more symptoms within twelve months [9]. The probability of being infected with *Candida* is <20% for people who are not pregnant, but it can go up to 30% during pregnancy [10]. Pregnant women have an increased risk of vulvovaginal candidiasis (VVC) due to changes in their immune system, elevated estrogen levels, and improved vaginal glycogen production mechanisms

[11]. Pregnant women are more likely to experience recurrence of VVC, and their response to treatment is reduced [12]. VVC's clinical signs and symptoms include vaginal discharge that resembles cottage cheese, swelling, itching, discomfort, irritation, burning, dyspareunia and painful urination [13]. pregnancy, contraceptives, diabetes mellitus, use of antibiotics, age, and some behavioural factors are risk factors for VVC [2]. In 85-95% of VVC cases, *C. albicans* are responsible, while non-albicans *Candida* species cause the infection in less than 10% of patients—especially *C. glabrata*, or *C. parapsilosis, C. tropicalis,* and *C. krusei* [10]. The distribution of the various *Candida* species found in women with VVC varies significantly depending on the populations and locations included in the studies [14]. Traditional methods to identify *Candida* species involve performing microscopic examination, analyzing colonies' morphological features on chromogenic candida agar and assessing germ tube production. Molecular techniques like Polymerase Chain Reaction (PCR) are employed to study, which is considered the most accurate and sensitive method for detecting *Candida* species [15]. Thus, this study aimed to isolate and identify *Candida* species from married women who exhibit vaginal candidiasis. This was accomplished by utilizing both traditional and molecular techniques.

Materials and methods

The Medical Microbiology Department's Research and Ethical Committee of the College of Science and Health, Koya University, has approved the study. Consent to participate in the study was received from each person who took part in the research.

Sample collection

This research was carried out at Shahid Doctor Khalid Hospital & Haji Qader health centre in Koya, Iraq, from December 2021 to May 2022; 150 vaginal swabs from married patients with symptoms of VVC were collected. The patients' ages were diverse, ranging between 17 to 52 years. Vaginal samples were collected using sterile cotton swabs. From each patient, two vaginal swabs were taken. The swab stick was swiftly returned to its casing. It was then labelled with the patient's name and, assigned a number, then taken to the Koya University Science & Health Research Center (SHRC) laboratory.

Direct examination of samples

The first set of vaginal swab samples was mixed with 10% KOH and examined under the microscope for Candida's presence. The existence of both hyphae and budding yeast cells enabled the candidiasis diagnosis to be established [16].

Samples culturing and isolating Candida species

The second set of vaginal swab samples was placed on Sabouraud Dextrose agar (SDA) that contained 1% chloramphenicol (CHLORWAVE, India) for cultivation [17]. Each agar plate was then put in an incubator set at 37° C and examined for signs of growth every day for 7 days [18]. Gram stain was applied to the colonies from the SDA plate to confirm *Candida* growth [19]. all isolated colonies were inoculated on HiCrome™ Candida Differential agar (HiMedia, India) and incubated at 37º C for 48 hours [20].

Germ tube test

To verify the generation of germ tubes, the colonies on the SDA plate underwent a germ tube test. To conduct a germ tube test, 3-4 colonies are mixed with 500 µl of human serum, incubated at a temperature of 37°C for 2-4 hours, and then looking for the formation of a germ tube under the microscope. Positive germ tube tests confirmed *C. albicans* colonies [19].

Molecular identification of Candida isolates

Molecular techniques identified all isolation. Fresh subcultures of the isolates were used for DNA extraction by the colony PCR, with slight modifications. Briefly, from the 24h culture, 2-3 colonies of each sample were suspended in 50 μL of ddH2O and vortexed to make a homogenized suspension. Then place in the thermocycler for 20minute at 95º C, followed by centrifugation for 2 min at 12,000 rpm. The DNA-containing supernatant was collected and stored at -20 °C for subsequent steps of PCR [21]. The universal primers, forward ITS1-F (5′-TCCGTAGGTGAACCTGCG-3′) and reverse ITS4-R (5′

TCCTCCGCTTATTGATATGC-3′), were utilized to amplify the intergenic spacer regions (ITS) of 5.8 S ribosomal DNA (rDNA) [22]. A volume of 20 µl in total, PCR reactions contained 10 µl of Add Taq Master (2x conc, Addbio, South Korea), 1 µl of each forward primer ITS1-F and 1 µl of reverse primers ITS4-R, the primers were 10 pmol, and 2 µl of genomic DNA, adding 6 µl of nuclease-free water to complete the total volume. The PCR was carried out using the BIO-RAD thermal cycler and was conducted as a single cycle of initial denaturation performed at a temperature of 95 ºC for 5 minutes. This is followed by 35 amplification cycles, consisting of denaturation at 95 ºC for 30 seconds, annealing at 57 ºC for 30 seconds, and extension at 72º C for 40 sec. The procedure was finished with a final extension step that was carried out at 72º C for 5 min.

Confirmation of the Candida albicans by detection of CHS1 gene

For further validation, all the amplicons previously amplified using ITS primers and were 532 bp in size were re-analyzed using a different set of primers CHS1. The chitin synthase gene (CHS1) from *C. albicans*, 122bp in size, can be amplified by conventional PCR [21]. The colony PCR was carried out with the same procedure previously mentioned using forward CHS1-F (5'- CGCCTCTGATGGTGATGAT-3') and reverse CHS1-R (5'-TCCGGTATCACCTGGCTC-3') primers [23]. The PCR program was as follows: The PCR process began with an initial denaturation step at 95º C for 5 min, followed by 40 cycles of denaturation at 95ºC for 30 sec, annealing at 52º C for 30 sec, and extension at 72º C for 30 sec. The PCR was completed with a final extension step at 72º C for 7 min.

Agarose gel electrophoresis

Following PCR amplification, agarose gel electrophoresis was used to verify the existence of amplification. Run 1% agarose gel with ethidium bromide (EtBr) and a 100 bp DNA ladder (Genedirex) in 1X TBE buffer at 80 V for 80 min. After the run, the DNA molecules were visualized and photographed using a UV Gel Imager SynGene 1409.

Sequencing

Sequencing was performed on 20 samples that were amplified using ITS1-F forward primers (10 pmol) (Sanger sequencing/ ABI 3500, Macrogen Genome Center, Republic of Korea). After DNA extraction, PCR was done on 20 samples. A volume of 40 microliters in total, PCR reactions contained 20 µl of Add Taq Master (2x conc, Addbio, South Korea), 2 µl of each forward primer ITS1-F and 2 µl of reverse primers ITS4-R, the primers were 10 pmol and 4 µl of genomic DNA, adding 12 µl of nuclease-free water to complete the total volume. The PCR was carried out using the BIO-RAD thermal cycler and was conducted as a single cycle of initial denaturation performed at a temperature of 95º C for 5 min. This is followed by 35 cycles of amplification, consisting of denaturation at 95º C for 30 sec, annealing at 57º C for 30 sec, and extension at 72º C for 40 sec; the procedure was finished with a final extension step that was carried out at 72º C for 5 min. The samples run on gel electrophoresis to verify the existence of amplification. After that, 20 µl of the PCR product with 15µl of ITS1-F forward primers (10 pmol) were send for sequencing.

Statistical analysis

The data was analyzed using the SPSS software (version 26).

Results

Microscopic Characterization

The results indicated that yeast cells, with or without pseudohyphae, were present in 55 out of 150 specimens (36.7%), whereas 95 samples (63.3%) did not show any yeast cells (Table 1).

Macroscopic Characterization on Sabouraud Dextrose Agar Medium

One hundred fifty swab samples were subjected to a 7-days culture on SDA. Of the 150 swab samples, 84 (56%) showed positive culture. In comparison, 66 samples (44%) showed negative results (Table 2). The colonies that were isolated were examined for the morphological features: The isolated colonies were examined for their size, ranging from small to large. At the same time, their color varied from white to creamy. The colonies displayed a round or curved shape; their texture ranged from smooth and soft to

wrinkled. Additionally, the characteristic odor of yeast was detected. To confirm the candida samples using gram stain to identify the morphology of different spp. Of *Candida* (Figure 1).

The positive cases of candidiasis among the age group (26 -30 years) were represented by 27.4%, and among the age group (36-40 years) was represented by 19%. In comparison, the age group of patients (51 years and older) was the lowest percentage, 1.2% (Table 3).

The positive *Candida* cultures were recorded in pregnant women 22 out of 33(66.7%) than in non-pregnant women 62 out of 117 (53%) (Table 4).

Macroscopic Characterization on CHROM agar media

The chromogenic medium can differentiate *Candida albicans* from non-albicans species through color changes. The positive species obtained from SDA were subjected to subculturing on Chromogenic Candida agar; after incubation for 48 hours, the colonies on CHROM agar exhibited a distinct color. *Candida* species Color on HiCrome agar appeared as follows: *Candida albicans* (light green), *Candida glabrata* (cream to white), *Candida kefyr* (cream to white with a slight purple centre), *Candida krusei* (purple, fuzzy), *Candida parapsilosis* (white to cream), *Candida tropicalis* (blue) (Figure 1 and Table 5).

Germ tube test

This test was applied to 84 *Candida* isolates. The germ tubes appeared in 74 out of 84 (88.1%), while the remaining 10 isolates (11.9%) were negative for germ tubes.

Molecular identification of Candida isolates

The positive cultures were verified through PCR by targeting the ITS region. Agarose gel electrophoresis was employed to analyze all the PCR products, as shown in (Figure 3, Table 5): Six different species of *Candida* were detected among all the isolates; the identified *Candida* species among all isolates were: *C. albicans* (88.1%), *C. glabrata* (4.8%), *C. kefyr* (2.4%), *C. krusei* (2.4%), *C. parapsilosis* (1.2%), and *C. tropicalis* (1.2%). Twenty isolates were sequenced and analyzed using BLAST to determine the species with the closest match, as shown in (Table 6).

Confirmation of the Candida albicans by detection of CHS1 gene

The confirmation of all *C. albicans* isolates was conducted via polymerase chain reaction (PCR) targeting the chitin synthase gene (CHS1); the fragment size was 122 bp (Figure 4).

Table 1: Results of samples treated with KOH.

		Frequency	**Percent (%)**
Samples	Negative	95	63.3 %
	Positive	55	36.7 %
	Total	150	100.0 %

Table 2: Results of cultures on SDA.

		Frequency	**Percent (%)**
Samples	Negative	66	44.0 %
	Positive	84	56.0 %
	Total	150	100.0 %

Table 3: The occurrence of vulvovaginal candidiasis in different age categories.

			Result		Total
			Negative	Positive	
	17-20	Count	4	8	12
Age Group		% within Result	6.1%	9.5%	8.0%
(Years)	21-25	Count	9	15	24
		% within Result	13.6%	17.9%	16.0%
	26-30	Count	6	23	29
		% within Result	9.1%	27.4%	19.3%
	31-35	Count	13	8	21
		% within Result	19.7%	9.5%	14.0%
	36-40	Count	16	16	32
		% within Result	24.2%	19.0%	21.3%
	41-45	Count	7	8	15
		% within Result	10.6%	9.5%	10.0%
	46-50	Count	8	5	13
		% within Result	12.1%	6.0%	8.7%
	51 and above	Count	3	1	4
		% within Result	4.5%	1.2%	2.7%
Total		Count	66	84	150
		% within Result	100.0%	100.0%	100.0%

Table 4: Compares the percentage of isolated *Candida* among pregnant and non-pregnant women.

Samples		%	Result		Total
			Negative	Positive	
	Non-Pregnant	Count	55	62	117
		within pregnant	47	53.0	100.0%
	Pregnant	Count	11	22	33
		within pregnant	33.3	66.7	100.0%
	Total	Count	66	84	150
		within pregnant	44	56	100.0%

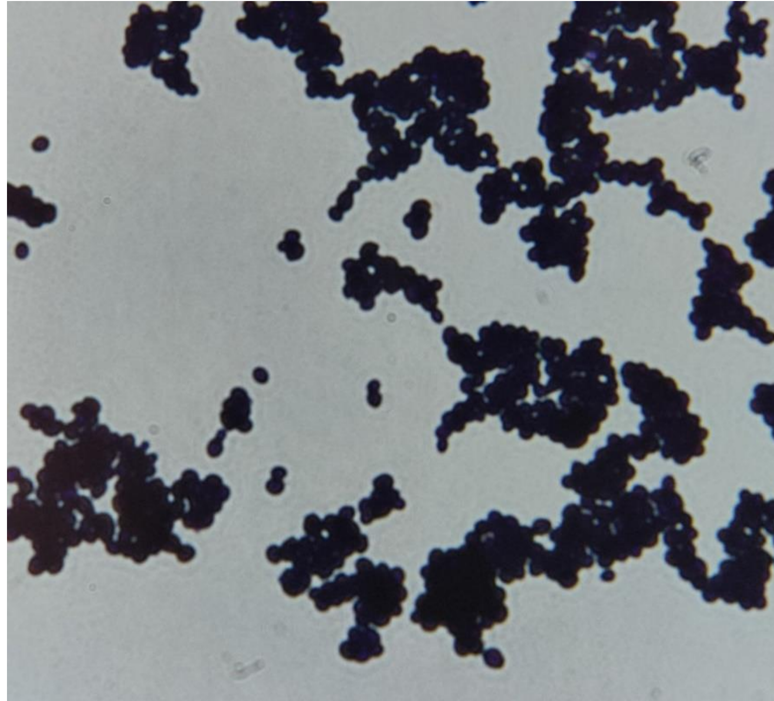


Figure 1: *Candida albican* appearance treated with gram stain by light microscope under (100X).

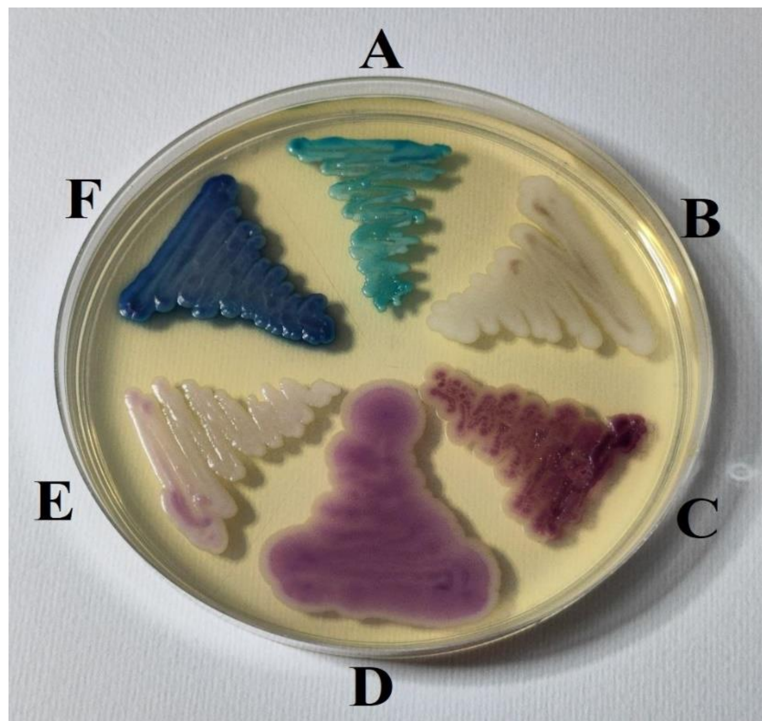


Figure 2: The diverse *Candida* species exhibit distinct colony colors and morphologies when cultured on HiCrome™ Candida Differential Agar, **A**/ *C. albicans*, **B**/ *C. glabrata*, **C**/ *C. kefyr*, **D**/ *C. krusei*, **E**/ *C. parapsilosis*, **F**/ *C. tropicalis.*

Table 5: Properties and distribution of *Candida* species that were separated and identified.

Candida Species	Isolates Number	%	Color of the colony on CHROM agar	PCR with ITS1-ITS4 primer found the size of the fragment (bp)
C. albican	74	88.1	light green	532
C. glabrata	4	4.8	cream to white	874
C. kyfer	2	2.4	cream to white with a slight purple centre	722
C. krusei	2	2.4	purple, fuzzy	500
C. parapsilosis	1	1.2	white to cream	516
C. tropicals	1	1.2	blue	521
Total	84	100.0		

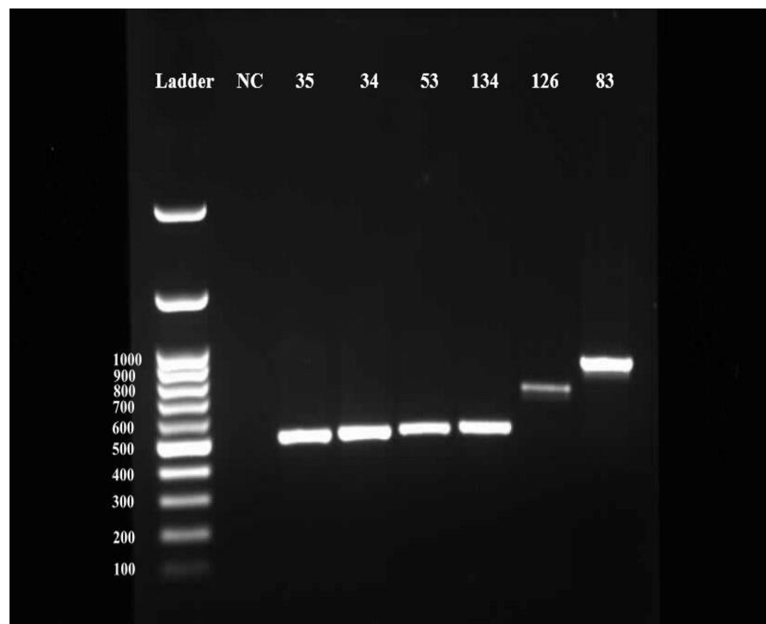


Figure 3: The agarose gel electrophoresis technique was used to visualize the ITS-PCR products of Candida isolates, which showed distinct bands. Lane 1: Ladder I00 bp (Genedirex), Lane 2: negative control (NC), Lane 3: *C. krusei* (35), Lane 4: *C. parapsilosis* (34), Lane 5: *C. tropicalis* (53), Lane 6: *C. albicans* (134), Lane 7: *C. kefyr* (126), Lane 8: *C. glabrata* (83).

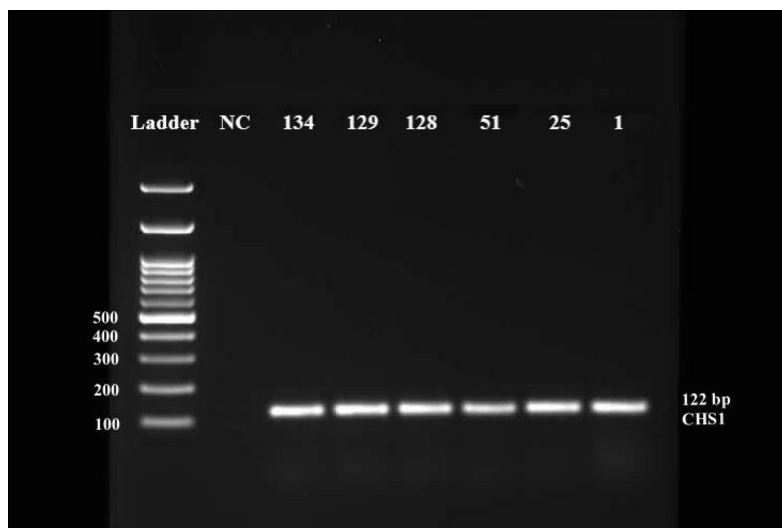


Figure 4: The identification of *C. albicans*, CHS1 primers were used, which produced a 122bp band. Lane 1: Ladder I00 bp (Genedirex), Lane 2: negative control (NC), Lane 3-8: *C. albicans*.

Table 6: Identification of *Candida* species isolated from married women using rDNA sequencing and matching the obtained sequences with the NCBI GenBank database.

Number of isolates	BLAST accession number	Most closely related in Genbank
C-8	MN263159	*C. albicans*
C-14	OW988313	*C. albicans*
C-25	KY575037	*C. albicans*
C-34	AB109234	*C. parapsilosis*
C-35	MN310535	*Pichia kudriavzevii (Candida krusei)*
C-51	KY112740	*C. albicans*
C-53	KX664616	*C. tropicalis*
C-62	KP674930	*C. albicans*
C-83	MK300696	*C. glabrata*
C-90	KM921928	*Kluyveromyces marxianus*
C-94	OM049825	*C. glabrata*
C-98	MF351876	*C. albicans*
C-106	ON875339	*C. glabrata*
C-109	MF276787	*C. albicans*
C-115	KP674762	*Pichia kudriavzevii (Candida krusei)*
C-116	OM523868	*C. glabrata*
C-126	OW985263	*Kluyveromyces marxianus*
C-134	KP675591	*C. albicans*
C-141	MH534916	*C. albicans*
C-147	KP675546	*C. albicans*

Discussion

The *Candida* species is responsible for various diseases, including vulvovaginitis [12]. The overgrowth of yeasts in the vaginal mucosa is the leading cause of VVC, A health issue that impacts millions of women around the globe [24].

In the current research, based on the vaginal swab culture, 56% (84/150) instances of VVC were found, and these findings are similar to those reported by Hussein *et al.* [25], which were 56.8%. Our study observed a prevalence rate that was greater than the rate reported by Babin *et al.* [26] (48.4%) and an analysis done by Ugwa [27], who found a prevalence rate of 84.5% in the North-West region of Nigeria.

Considering age, the age range where a positive VVC was most likely to occur was between 26 to 30 years 23 (27.4%), followed by the 36–40 age group 16 (19%). This report agreed with Al-Aali [28], which showed that the peak age for vaginal infections was between 20 and 40. One possible explanation for this could be increased sexual activity, inadequate personal hygiene practices, contraceptive usage, and drug abuse among this age group. As a result, Women of reproductive age are more prone to developing vaginal candidiasis.

Pregnant and non-pregnant women both showed the following. Among pregnant women, it was revealed that the prevalence of VVC was 66.7%, while non-pregnant women had a majority (53%). This result is higher than the result reported in the study of Toua *et al.* [29]. The findings of the study showed that the prevalence of VVC was among pregnant women (55.4%) than among non-pregnant women (35.4%). The higher rate of positive cases reported by Hynniewta *et al.* [30] was 77.27% among pregnant women. Literature has identified various physiological changes during pregnancy that may Elevate the risk of VVC in pregnant women. Factors contributing to this condition include immune system suppression, elevated levels of reproductive hormones, glycogen accumulation, low vaginal pH, and reduced cell-mediated immunity [31].

In the present study, the culture method and molecular methods were used for identifying *Candida* spp. Our analysis detected different species of *Candida*, among which *C. albicans* was the most commonly found

(88.1%) and *non-albicans Candida* (NAC) (11.9 %) of cases. The result is similar to Abdullah's [32]. The highest *Candida* species found were *C. albicans* (86.6%) and *non-albicans Candida* (NAC)(13.4%) of cases. In a study by Mohammed *et al.* [33], the most common non-albicans species found in the survey was *C. glabrata*, according to the results, with a prevalence of 40%, followed by *C. albicans* (30%). A possible explanation for this could be the development of resistance to antifungal drugs in *Candida* species and the weakened immune system that makes individuals more vulnerable to infection. Another potential factor could be changes in hormone levels [34].
A traditional technique to identify *Candida* species involves culturing the sample on chromogenic agar. As the conventional method relies on culturing the *Candida* species, it cannot differentiate between non-albicans *Candida* species. Conversely, The ITS sequencing technique can differentiate between *Candida* species, including *C. albicans* and non-albicans. Twenty strains were sequenced and then analyzed using BLAST to determine the species with the closest match.

Conclusions

The findings of this study indicated a significant prevalence of VVC (56%) among married women. The study found that VVC was more common among women aged 26-30 years and had a higher frequency in pregnant women; among different *Candida* species, the most significant proportion of the isolated strains (88.1%) were identified as *C. albicans*, making it the most commonly occurring species. Utilizing the ITS sequencing method for molecular identification proved more effective in determining and verifying the precise *Candida* species.

Recommendations

Molecular identification of *Candida* spp. Isolate from different samples taken in different seasons.

Conflict of interest

The authors confirm that they are not affiliated with or involved in any organization or entity with financial interests.

References

1. Martins N, Ferreira ICFR, Barros L, Silva S, Henriques M. (2014). Candidiasis: Predisposing Factors, Prevention, Diagnosis and Alternative Treatment. Mycopathologia. Jun;177(5–6):223–40.
2. Sobel JD. (2007). Vulvovaginal candidosis. Lancet. Jun 9;369(9577):1961–71.
3. Sobel JD, Faro S, Force RW, Foxman B, Ledger WJ, Nyirjesy PR, et al. (1998). Vulvovaginal candidiasis: Epidemiologic, diagnostic, and therapeutic considerations. American Journal of Obstetrics and Gynecology. Feb;178(2):203–11.
4. Altayyar I, Shiha A, Wadedy N. (2016). Prevalence of vaginal candidiasis among pregnant women attending different gynecological clinic at South Libya. European Journal of Experimental Biology. Feb 1;6:25 9.
5. Hussein M, Yassin A, El-Gelany F. (2019). Characterization, Virulence Factors and Antifungal Susceptibility of Vulvovaginal Candida Isolated from Women at Qena, Egypt. Egypt J Microbiol. May;0(0):0–0.
6. Barousse MM, Espinosa T, Dunlap K, Fidel PL. (2005). Vaginal Epithelial Cell Anti-Candida albicans Activity Is Associated with Protection against Symptomatic Vaginal Candidiasis. Infect Immun. Nov;73(11):7765–7.
7. Habibipour R. (2023). Prevalence Rate of Vulvovaginal Candidiasis and Identification of Candida Species in Women in Referred to Hamedan Hospitals 2013 - 2014, West of Iran. Zahedan J Res Med Sci [Internet]. 2016 Mar [cited 2023 Jan 22];18(3). Available from: https://brieflands.com/articles/zjrms 6250.html#abstract
8. Dabas P. (2013). An approach to etiology, diagnosis and management of different types of candidiasis. Available from: https://www.semanticscholar.org/paper/An-approach-to-etiology%2C-diagnosis-and management-Dabas/9a292e14f55b99598e3dabd2523a6baef4b8c19c

9. Sobel JD, Donders G, Degenhardt T, Person K, Curelop S, Ghannoum M, et al. (2022). Efficacy and Safety of Oteseconazole in Recurrent Vulvovaginal Candidiasis. NEJM Evidence [Internet]. Jul 26 [cited 2023 Mar 30];1(8). Available from: https://evidence.nejm.org/doi/10.1056/EVIDoa2100055
10. Aguin TJ, Sobel JD. (2015). Vulvovaginal candidiasis in pregnancy. Curr Infect Dis Rep. Jun;17(6):462.
11. Ghaddar N, Anastasiadis E, Halimeh R, Ghaddar A, Dhar R, AlFouzan W, et al. (2020). Prevalence and antifungal susceptibility of Candida albicans causing vaginal discharge among pregnant women in Lebanon. BMC Infect Dis. Dec;20(1):32.
12. Emam S, Aboelazm A, Morad AW. (2012). Exoenzymes Production and Antibiotic Susceptibility of Candida Species Isolated from Pregnant Women with Vulvovaginitis. J Am Sci. Jan 1;8.
13. Ghaddar N, El Roz A, Ghssein G, Ibrahim JN. (2019). Emergence of Vulvovaginal Candidiasis among Lebanese Pregnant Women: Prevalence, Risk Factors, and Species Distribution. Infectious Diseases in Obstetrics and Gynecology. Jul 28;2019:e5016810.
14. Achkar JM, Fries BC. (2010). *Candida* Infections of the Genitourinary Tract. Clin Microbiol Rev. Apr;23(2):253–73.
15. Oufi Z, Mohammed A, Abdullah S. (2022). Conventional and Molecular Identification, Incidence and Species Distribution of Candida Associated with Vaginal Candidiasis among Women Attending Gynecological Clinic at Duhok Province, Kurdistan - Iraq. Acta microbiologica Bulgarica. Mar 27;38:18–25.
16. Shawaky SM, Shammari MMAA, Sewelliam MS, Ghazal AAER, Amer AN, Shawaky SM, et al. (2022). A study on vaginitis among pregnant and non-pregnant females in Alexandria, Egypt: An unexpectedly high rate of mixed vaginal infection. AIMSMICRO. May 5;8(2):167–77.
17. Matare T, Nziramasanga P, Gwanzura L, Robertson V. (2017). Experimental Germ Tube Induction in Candida albicans: An Evaluation of the Effect of Sodium Bicarbonate on Morphogenesis and Comparison with Pooled Human Serum. Biomed Res Int. 2017 Jun 5:1976273.
18. Al Halteet S, Abdel-Hadi A, Hassan M, Awad M. (2020). Prevalence and Antifungal Susceptibility Profile of Clinically Relevant Candida Species in Postmenopausal Women with Diabetes. Fernandes L, editor. BioMed Research International. 2020 Nov 26:1–9.
19. Krishnasamy L, Krishnakumar S, Santharam P, Saikumar C. (2018). Isolation and identification of Candida Species in Patients with Vulvovaginal Candidiasis. J Pure Appl Microbiol. Dec 30;12(4):2269–73.
20. Kaup S, Sankarankutty J, Balasubrahmanya HV, Kulkarni S, Nirmala M. (2016). Speciation of Candida using HiCrome Candida Differential Agar. IntJCurrMicrobiolAppSci. Jul 15;5(7):267–74.
21. Shekhany KAM. (2021). Isolation and genotyping of Candida albicans involved in vaginal candidiasis among pregnant women in Sulaymaniyah and Erbil cities. Zanco Journal of Medical Sciences (Zanco J Med Sci). Apr 27;25(1):493–502.
22. Fujita SI, Senda Y, Nakaguchi S, Hashimoto T. (2001). Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. J Clin Microbiol. Oct;39(10):3617–22.
23. Jordan JA. (1994). PCR identification of four medically important Candida species by using a single primer pair. J Clin Microbiol. Dec;32(12):2962–7.
24. Zeng X, Zhang Y, Zhang T, Xue Y, Xu H, An R. (2018). Risk Factors of Vulvovaginal Candidiasis among Women of Reproductive Age in Xi'an: A Cross-Sectional Study. Biomed Res Int. Jun 7;2018:9703754.
25. Hussein M, Yassin A, El-Gelany F. Characterization, Virulence Factors and Antifungal Susceptibility of Vulvovaginal Candida Isolated from Women at Qena, Egypt. Egyptian Journal of Microbiology. 2019 May;0(0):0.
26. Babin D, Kotigadde S, Rao P, Rao T. (2013). Clinico-mycological profile of vaginal candidiasis in a tertiary care hospital in Kerala. International Journal of Research in Biological science. Mar 8;3:55–9.
27. Ugwa E. (2015). Vulvovaginal candidiasis in Aminu Kano teaching hospital, North West Nigeria: Hospital based epidemiological study. Ann Med Health Sci Res. Jul 24;5(4):274.
28. Al-Aali KY. (2015). Prevalence of Vaginal Candidiasis among Pregnant Women Attending Al-Hada Military Hospital, Western Region, Taif, Saudi Arabia. May;4(5).
29. Toua V, Djaouda M, Gaké B, Menye DE, Christie EA, Tambe E, et al. (2013). Prevalence of vulvovaginal candidiasis amongst pregnant women in Maroua (Cameroon) and the sensitivity of Candida albicans to extracts of six locally used antifungal plants. Int Res J Microbiol. Mar;4(3):89–97.
30. Hynniewta BC, Wanswett Chyne W, Phanjom P, Donn R. (2019). Prevalence of Vaginal Candidiasis among pregnant women attending Ganesh Das Government Maternity and Child Health hospital, Shillong, Meghalaya, India. IJAM. Dec 23;22(2):1–11.
31. Disha T, Haque F. (2022). Prevalence and Risk Factors of Vulvovaginal Candidosis during Pregnancy: A

Review. Baig AA, editor. Infectious Diseases in Obstetrics and Gynecology. Jul 20;2022:1–14.
32. Abdullah SMZ. (2020). Prevalence of Candida spp. from in women with vulvovaginal infection in Maternity teaching hospital in Erbil, Iraq. Iraq Med J [Internet]. Mar 26 [cited 2022 Jul 17];4(1). Available from: http://localhost/ojs3307/index.php/imj/article/view/776
33. Mohammed A, Ali J, Abdullah S. (2015). Identification of Candida spp. isolated from vaginal swab by phenotypic methods and multiplex PCR in Duhok, Iraq. Int J Res Med Sci. Nov;3211–6.
34. Trama JP, Adelson ME, Raphaelli I, Stemmer SM, Mordechai E. (2005). Detection of Candida species in vaginal samples in a clinical laboratory setting. Infect Dis Obstet Gynecol.13(2):63–7.