e-ISSN: 0974-4614
p-ISSN: 0972-0448

Prevalence of non-albicans *Candida* species isolated from vulvovaginal samples of women and heifers

Nabaa A. Hasan [1]*, Zainab A.A. Al-Haddad [2]

[1, 2] Department of Veterinary Public Health/ Zoonotic Diseases Research Unit, College of Veterinary Medicine, Baghdad University, Baghdad, Iraq.
Emails: naba.ali2204m@covm.uobaghdad.edu.iq [1], zaynab.az99@covm.uobaghdad.edu.iq [2]
ORCIDs: [0009-0009-8646-890X] [1], [0009-0006-0983-5508] [2]
* ***Corresponding author***

Abstract
The prevalence of non-albicans *Candida* (NAC) species is increasing globally, leading to pathological changes in both humans and animals. This study aimed to investigate the prevalence of NAC in women and heifers using traditional methods based on phenotypic characterization and antifungal susceptibility. Three hundred vaginal samples were collected-150 from women and 150 from heifers. The samples were cultured on Sabouraud Dextrose Agar (SDA) and HiChromo Agar and identified using the VITEK-2 system. Out of 300 samples,64 sixty four yeast like growth samples were identified via the VITEK-2, 50 tested positive for candidiasis, with 32 positive samples from women and 18 from heifers. Using HiChrome Agar, only one isolate from a heifer and 12 isolates from women were identified as *Candida albicans*, while the remaining were classified as non-albicans Candida (NAC) species. The VITEK system revealed variability in NAC prevalence between women and heifers. The most prevalent NAC in women was *Candida glabrata* [13 out of 38 (34.2%)], followed by *C. albicans* [11 out of 38 (33.8%)] and *C. tropicalis* [3 out of 38 (7.8%)]. In heifers, the dominant species was *C. tropicalis* [8 out of 26 (30.7%)], followed by *Millerozyma farinosa* [6 out of 26, (23.1%)] and *C. krusei* [5 out of 26 (19.2%)]. The isolation rates of *Candida* species differed between women and heifers, with *C. albicans* and C. *glabrata* being predominant in women, while *C. tropicalis* and *C. krusei* were more common in heifers. The study also identified diverse antifungal susceptibility profiles among the Candida species associated with vulvovaginitis. *C. glabrata exhibited* resistance to fluconazole, micafungin, and caspofungin; *C. tropicalis showed* varied susceptibility to fluconazole; *C. krusei displayed universal* resistance to fluconazole and flucytosine; and unexpected fluconazole resistance was observed in *C. albicans*. This study concluded that NAC is associated with vulvovaginitis in women and was reported here for the first time in heifers.

Keywords: Non-Albicans Candida, Vulvovaginal candidiasis, Women, Heifers

Introduction
The prevalence of fungal infections is increasing worldwide due to various factors facilitating fungi growth in humans and animals (1). Fungal species are normal inhabitants of the intestinal tract, as well as the vaginal and oral cavities of humans and animals. The reproductive system, particularly the vagina, serves as a conducive environment for fungal growth, providing essential growth factors (2). Vulvovaginitis is a common clinical condition characterized by inflammation of the vulva and vaginal mucosa, primarily caused by bacterial and fungal species (3, 4). Vulvovaginal candidiasis (VVC) is typically caused by *Candida* species in the female lower genital tract, predominantly by *Candida albicans* (5). Furthermore, both non-albicans and albicans Women's urogenital infections may be linked to *Candida*. *Candida* species are known to be the leading fungal pathogens responsible for vulvovaginitis in women, with an estimated 75% of women experiencing at least one episode during their lifetime (6, 7). Makanjuola et al. (8) indicate that NAC species account for 10% to 45% of VVC cases. Recent studies have reported an increasing prevalence of non-albicans *Candida* (NAC) species, such as *Candida glabrata*, *C. tropicalis*, and *C. krusei*, often associated with antifungal resistance **(**9, 10). Non-albicans Candida (NAC) infections are frequently associated with several factors, including severe illness, immunosuppression, age, broad-spectrum antibiotics, and the empirical use of antifungal drugs. The clinical symptoms caused by NAC species are often similar, and some NAC species exhibit inherent resistance or may acquire resistance to commonly used antifungal medications (11).
The current study aimed to isolate and identify NAC strains from women and heifers suffering from vulvovaginitis in the vicinity of Baghdad. Most of the female participants in this study dealt with cows,

either in milking or husbandry roles. *Candida species* are opportunistic pathogens that can colonize the udders of cows. Additionally, the misuse of antibiotics for the prevention and treatment of cows has led to an increased prevalence of fungi within animal organ systems. This research contributes to a better understanding of the prevalence and resistance patterns of NAC species among women and heifers in rural areas around Baghdad City, Iraq. Also provides crucial insights for the clinical management of candidiasis in both humans and animals.

Materials and Methods

Sample collection

A total of three hundred vaginal swab samples were collected, consisting of 150 samples from women and 150 from heifers in rural areas around Baghdad city. Informed consent was obtained from all women participating in the study according to the method described by Forney et al. (12). Vaginal swabs from Heifers were collected using sterile cotton swabs as described by Deng et al (13). All participants were instructed to refrain from using any Antifungal medication or douching for at least 2 to 6 day**s** before sample collection. A trained professional conducted the sample collection**.**

Culture and isolation

The three hundred swab samples were inoculated onto Sabouraud's Dextrose Agar (SDA) plates (HiMedia Laboratories LLC, USA.) supplemented with chloramphenicol (0.05 g/L) to inhibit bacterial growth. Plates were incubated at 37°C for 48-72 hours under aerobic conditions to promote the growth of yeast-like fungi. The SDA medium, with its acidic pH and nutrient composition, is specifically chosen to support the development of Candida species (14). Plates were monitored and examined for yeast-like fungal colonies based on morphological characteristics during incubation. Suspicious colonies were repeatedly Subcultured onto fresh SDA plates to obtain pure colonies. The pure yeast colonies were subjected to a series of morphological and biochemical tests for preliminary identification, including microscopic examination of cell morphology and germ tube testing (11, 16).

Germ tube methods

Germ tube methods were performed to distinguish between *C. albicans* and NAC species. A small portion of each pure yeast colony was inoculated into 0.5 mL of human serum and incubated at 37°C for 2-3 hours. Following incubation, the serum suspensions were examined microscopically at 100X magnification for germ tubes, which are characteristic of *Candida albicans* and appear as slender tube-like outgrowths extending from the yeast cells. This germ tube test is a definitive method for identifying *Candida albicans*
(16-18).

Chromogenic media

Another subset of the purified isolates was inoculated onto HiChrome Candida Differential Agar plates (HICHROME, Experts in Chromatography, Canada). *Candida species* can be differentiated based on colony color, facilitating easier identification. The plates were incubated at 37°C for 24 to 48 hours, after which the colonies were examined for specific colors (19, 20).

Biochemical identification using the Vitek 2 compact system

This study utilized the VITEK-2 system for diagnosing non-albicans Candida (NAC), as used before in different organisms (76) which involved several steps (21). The identification card was inoculated with a suspension of pure yeast isolates using an integrated vacuum apparatus. A test tube containing the yeast suspension was placed into a specialized rack (cassette). The filled cassette was manually inserted into the vacuum chamber. Once the vacuum was applied and the air was reintroduced into the chamber, the yeast suspension was forced through a transfer tube into micro-channels, filling all the test wells. The inoculated card was later transported through a mechanism that cut off the transfer tube and sealed it before loading it into the carousel incubator.

Antifungal susceptibility testing

Antifungal susceptibility testing was performed using the VITEK 2 automated system (bioMérieux, France) with AST-YS08 cards. Yeast suspensions were prepared to a 2.0 McFarland standard in 0.45%

saline. The cards containing fluconazole (1-64 µg/mL), voriconazole (0.12-8 µg/mL), amphotericin B (0.25-16 µg/mL), flucytosine (1-64 µg/mL), caspofungin (0.25-4 µg/mL), and micafungin (0.06-4 µg/mL), were automatically filled, sealed, and incubated for up to 24 hours. Results were interpreted according to CLSI guidelines, with *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 used as quality controls (22, 23).

Statistical analysis

Descriptive statistics, including frequency and percentage of *Candida species*, were calculated using t-test analysis (24).

Results and discussion

Isolation and identification of Candida species

A total of three hundred specimens were studied, consisting of 150 women and 150 subjects suspected of having *Candida* vaginitis in rural areas around Baghdad, Iraq. Results indicated that based on phenotypical characterization, sixty-four specimens (21.3%) assessed positive for Vulvovaginitis. Fifty of which were candidiasis (VVC), As shown in Table 1, of the sixty-four positive samples for yeast spp., 38 (59.3%) and 26 (40.7%) were isolated from women and Heifers respectively. Statistically, the isolation of *Candida* species from women was higher than that from heifers, which is expected since women are more frequently exposed to fungal contamination. In contrast, animals may possess immunity against such pathogens (7). Successful isolation and culturing of yeast-like organisms on Sabouraud Dextrose Agar (SDA) were revealed. In the initial culture, after 48 hours of incubation at 37℃, colonies displayed a robust and creamy appearance, consistent with yeast morphology, suggesting the presence of Candida**.** Following three rounds of subculturing, consistent yeast-like growth was noted, indicating the stability and viability of the isolated strain. This consistency across subcultures confirms the isolate's identity and ensures its reliability for further analysis (25). The Germ Tube Test, a reliable method for identifying Candida albicans, yielded 12 positive results from women and only one positive result from heifers. This germ tube confirms the isolate's identity as *C. albicans*, a species known for its significant role in vulvovaginal candidiasis. In contrast, non-albicans *Candida* (NAC) species did not exhibit germ tube formation. These findings suggest that *Candida albicans* may not be the predominant species in vulvovaginitis cases in heifers (Table 1**)**. HiChrome media as advised by M. Faraj (74) was very helpful and consistently confirmed the Germ Tube test results, allowing differentiation between *Candida albicans* and non-albicans species based on colony color. Specifically, 11 samples from women and one from heifers displayed a greenish-blue color indicative of *Candida albicans* (Figure 1).

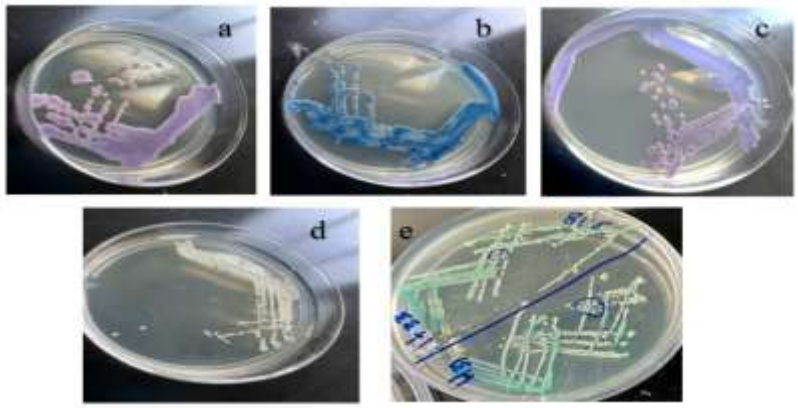

Figure 1: Yeast growth on differential HiChrome Agar plates; (a) Suspected *C. glabrata*; (b) Suspected *C. tropicalis*; (c) Suspected *C. krusei*; (d) Suspected *C. parapsilosis*; (e) Greenish-blue colonies growth on HiChrome media suspected to be *C. albicans*.

In Figure 1 (a), the colonies appear smooth and compact, exhibiting a lavender (purple) to pink hue, which is characteristic of *Candida glabrata,* as noted by Fidel *et al.* (26), M. Risan (75) and Kucukates et al. (27). This species is often differentiated from others by its inability to ferment sugars like glucose and its typical presentation as smaller, non-yeast-forming colonies on chromogenic media, as Silva *et al.* (28) established. In Figure 1 (b), the yeast colonies display a blue-green coloration, indicating *Candida tropicalis*, *whi*ch aligns with Kucukates *et al.* (27). The blue coloration of *Candida tropicalis* is due to the hydrolysis of specific chromogenic substrates by enzymes produced by the organism. This finding is supported by research from Alwaily et al. (29), and Perry (30). In Figure 1(c), the colonies exhibit a distinctive pink-to-mauve color, which is characteristic of *Candida krusei*, aligning with the observations made by Nadeem et al. (31) and Al-Rubaie and Al-Qaysi (32). Figure 1(d) shows white to cream-colored colonies, indicating *Candida parapsilosis*, consistent with the findings of Silva et al. (2012) and Mohammed et al. (2020). The colony morphology on HiChrome agar typically appears smooth and glossy, as supported by Gómez-Molero et al. (2021) in Figure 1. Finally, Figure 1(e) illustrates yeast colonies with a distinctive greenish-blue coloration, characteristic of *Candida albicans*, which is reported by Silva et al. (28), Charles et al. (33), and Jarallah and Al-Haddad (34). Twenty-seven samples from women and twenty-five from heifers displayed various colors, indicating the presence of non-albicans Candida species (Table 1).

Table 1: The primary isolation on SDA, Germ tube test, and HiChrome media for women and heifers' vulvovaginal swabs

Samples	Yeast-like growth on SDA	Germ tube		HiChrome media	
		Positive	Negative	Greenish-blue color (C. albicans)	Other colors (non-albicans *Candida*)
Women	38	12	26	11	27
Cattle	26	1	25	1	25
Total	64	13	51	12	52

These findings highlight the presence of non-albicans Candida species in both human and heifer samples. The use of SDA for primary isolation effectively identified yeast-like organisms, as indicated by Nagano et al. (35), Gupta et al. (36), and De Angelis et al. (37). Furthermore, the Germ Tube Test and HiChrome media provided critical differentiation between *Candida albicans* and non-albicans species, as Nadeem et al. (20135) and Rathore et al. (38) agreed. The higher incidence of yeast-like growth in women (38 samples) compared to heifers (26 samples) suggests a greater prevalence or susceptibility to vulvovaginitis caused by *Candida species* in women, as noted by Lines et al. (39) and Anh et al. (40). This research confirmed that 21.3% of yeast isolation differs from the 51% reported by Anh et al. (40) and from the isolation percentages of *Candida species* established by Brandão et al. (41) and N. Abdulla (73) .Recent observations report found a shift towards non-albicans *Candida species*, highlighting their emerging clinical importance. In line with the literature, increasingly recognized that non-albicans species pose significant pathogenic risks, often associated with antifungal resistance and distinct pathogenicity compared to *Candida albicans*. These results emphasize the necessity of comprehensive diagnostic approaches, including using SDA for primary isolation, the Germ Tube Test for rapid screening, and HiChrome media for differentiating between *Candida albicans* and *non-albicans species*.

Identification of Candida species in women using VITEK 2 system

The VITEK 2 system in this study identified yeast species in 38 samples from women with vulvovaginitis. The results revealed a diverse range of Candida and other yeast species, underscoring the complexity of vulvovaginitis etiology, closely related to findings by Eleutério et al. (42)**.** The most prevalent species identified was *Candida glabrata,* accounting for 13 out of 38 samples (34.21%), (**Table 2)**. Our findings align with Mahmoudi-Rad et al. (43), Who found that *C.albicans* and *C. glabrata* were the most frequently isolated species from the samples and indicated that the most prevalent mixed infection identified in cases of vaginal candidiasis was a combination of *Candida glabrata* and *Candida albicans.*

Makanjuola *et al.* (8**)** found that *Candida glabrata* is the most common cause of non-Albicans vulvovaginal candidiasis (NAC-VVC). In contrast, Sheppard et al. (44), Kalkanci *et al.* (45) and Fornari *et al.* (46) reported that *Candida albicans* were the most prevalent species found in cases of vulvovaginal candidiasis. This study confirms that the second most common species was *Candida albicans*, identified in 11 samples (33.8%). This aligns with previous findings**.** Despite being traditionally considered the leading cause of vulvovaginitis, the comparable prevalence of *Candida glabrata* suggests a shifting trend in pathogen dominance, like earlier studies, other *non-Albicans Candida* species were also identified, including *Candida tropicalis* in 3 samples (7.8%), *Candida Krusei* in 2 samples (5.26%), and *Candida parapsilosis*, *Candida kefir*, and *Candida fermata, each* found in 1 sample (Table 2)**.** These findings are consistent with recent literature indicating an increasing incidence of *non-Albicans Candida* species in vulvovaginitis cases. (44,47,48,49). Additionally, the study identified non-Candida yeasts, such as *Zygosaccharomyces* in three samples (11.4%), *Rhodotorula* in two samples (7.6%), and *Trichosporon spp.* in 1 sample (3.8%). These *non-Candida* yeasts complicate the clinical picture, as they are less commonly associated with vulvovaginitis and may pose unique treatment challenges. This finding expands our understanding of the vaginal mycobiome. Zygosaccharomyces, primarily known for its role in food spoilage, is rarely reported in human clinical samples, as noted by Stratford (50)**.** Its presence in vaginal specimens is particularly unusual and may warrant further investigation into its ecological role in the vaginal environment. While *Rhodotorula* species can cause opportunistic infections in immunocompromised individuals, they are not typically associated with vulvovaginitis, according to Wirth and Goldani (51). *Trichosporon* species are considered rare causative agents of vulvovaginitis, especially among immunocompromised individuals, as Colombo et al. (52) suggested. However, their prevalence in this study (3.8%) aligns with the understanding that they are uncommon pathogens in vaginal infections. The identification of these *non-Candida* yeasts corresponds with recent research indicating that the vaginal mycobiome is more diverse than previously recognized, as shown by Bradford and Ravel (53). This study supports the notion that there is an increased diversity of fungal flora in patients with recurrent vaginal candidiasis, including the presence of uncommon yeast species, which agrees with the unexpected results of this study.

Table 2: Identification of candidiasis in women using VITEK 2

Species	Women (Total No: 38)	
	No.	Percentage (%)
Candida glabrata	**13**	34.21
Candida tropicalis	**3**	7.8
Candida krusei	**2**	5.26
Candida parapsilosis	1	3.8
Candida kefyr	**1**	3.8
Candida famata	**1**	3.8
Candida albicans	**11**	33.8
Zygosaccharomyces	**3**	11.4
Rhodotorula	**2**	7.6
***Trichosporon* spp.**	**1**	3.8

Identification of Candida species in heifers using VITEK 2 system

Table 3 summarizes the VITEK 2 system results regarding the identification of yeast species in twenty-six samples collected from heifers with vulvovaginitis. This is the first report of the isolation of non-albicans Candida (NAC) from heifers, although studies have documented its isolation from cow milk (54). The most prevalent species identified was *Candida tropicalis,* found in eight of the twenty-six samples (30.7%). Most studies, including those by Alfouzan et al. (55) and Rezaei-Matehkolaei et al. (56), have indicated that the most common causes of vulvovaginitis are *C. albicans* and *C. glabrata* of vulvovaginal candidiasis towards non-albicans Candida (NAC) species.

The high incidence of *C. tropicalis* in this study is agreed by Megri et al. (57), who noted its virulence and ability to form biofilms, contributing to infections. The second most identified species was *Millerozyma farinosa*, detected in six samples (23.07%). This species is less frequently associated with clinical infections, as shown by Hong et al. (58), indicating that *Millerozyma farinosa* has been reported in various environments. However, its high occurrence in this study may suggest adaptability and the potential to cause infections under certain conditions, supported by Mollaschi et al. (59).

Table 3: Identification of candidiasis in heifers using VITEK 2

Species	Cattle (Total No: 26)	
	No.	Percentage (%)
Candida tropicalis	8	30.7
Candida krusei	5	19.2
Candida parapsilosis	2	7. 6
Candida utilis	1	2.6
Candida pelliculosa	1	2.6
Candida albicans	1	2.6
Rhodotorula	2	7.6
Millerozyma farinosa	6	23.07

Candida krusei was identified in 5 samples, accounting for 19.2%, which aligns with the findings of Alwaily et al. (29). *C. krusei* was the second most common species identified, following *C. albicans.* Other species detected included *Candida parapsilosis* (2 samples, 7.6%), *Rhodotorula* (2 samples, 7.6%), *Candida utilis* (1 sample, 2.6%), *Candida pelliculosa* (1 sample, 2.6%), and *Candida albicans* (1 sample, 2.6%)**.** These results suggest a shift in the species distribution in vulvovaginitis cases, with *Candida tropicalis and krusei* becoming more prevalent while *Candida albicans* have decreased significantly. This distribution contrasts with global trends, where *Candida albicans* is the dominant species in vulvovaginitis, as noted by Alfouzan et al. and Rezaei-Matehkolaei et al. (55,56). However, it aligns with the findings from Husni et al. (49), N. Mohammed (77) and Soriano et al. (60), who reported an increase in *non-albicans Candida* (NAC) species in regions with high antifungal use or recurrent infections.

Antifungal sensitivity testing of Candida species using the VITEK 2 system

The antifungal sensitivity profiles of various Candida species isolated from vulvovaginitis cases in women and Heifers were assessed using the VITEK 2 system. The results are summarized in Table 4, which indicates susceptibility (S), intermediate susceptibility (I), and resistance (R) to six antifungal agents: fluconazole, voriconazole, amphotericin B, micafungin, caspofungin, and flucytosine. The antifungal sensitivity profile of *Candida glabrata* in this study reveals a concerning pattern of resistance and susceptibility. Notably, all thirteen samples of *C. glabrata* exhibited resistance to fluconazole, micafungin, and caspofungin. This finding is consistent with global trends, as *C. glabrata* was reported for its resistance to fluconazole, due to the widespread use of azoles in clinical settings**.** The resistance to micafungin and caspofungin raises additional concerns, given that these Echinocandins are typically considered first-line treatments for *C. glabrata* infections. This resistance pattern may indicate emerging concerns that require close monitoring, as echinocandin-resistant *C. glabrata* can be particularly difficult to treat. However, all *C. glabrata* isolates were sensitive to voriconazole, amphotericin B, and flucytosine, providing alternative treatment options. Voriconazole's effectiveness aligns with its role as a broader-spectrum azole, often used when fluconazole resistance is present, as Pappas et al. (61) highlighted. Additionally, Sobel (62) Indicates that amphotericin B continues to be an effective agent against most *Candida* species, including *C. glabrata.* The sensitivity of all samples to flucytosine further supports the potential for combination therapy with amphotericin B, particularly in cases of severe or invasive candidiasis, as confirmed by Sigera and Denning (63).

Table 4: Antifungal sensitivity testing for detected species using the VITEK 2 system

Species	Fluconazole			Voriconazole			Amphotericin B			Micafungin			Caspofungin			Flucytosine		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. glabrata	-	-	13	13	-	-	13	-	-	-	-	13	-	-	13	13	-	-
C. tropicalis	8	-	3	11	-	-	11	-	-	11	-	-	11	-	-	11	-	-
C. krusei	-	-	7	7	-	-	7	-	-	7	-	-	7	-	-	-	-	7
C. kefyr	-	-	1	1	-	-	1	-	-	-	-	1	-	-	1	1	-	-
C. parapsilosis	2	-	1	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-
C. utilise	-	-	1	1	-	-	1	-	-	-	-	1	-	-	1	-	1	-
C. albicans	-	-	12	12	-	-	12	-	-	12	-	-	12	-	-	12	-	-

Candida tropicalis exhibited mixed susceptibility profiles. Among the eleven isolates studied, eight were susceptible to fluconazole, while three showed resistance and that agree with . However, all eleven isolates were susceptible to voriconazole, amphotericin B, micafungin, caspofungin, and flucytosine. This susceptibility pattern suggests that voriconazole and echinocandins remain effective treatment options for *Candida tropicalis* infections, consistent with findings from Takakura et al. (64), Pfaller et al. (65), and Kessler et al. (66). *Candida krusei* demonstrated universal resistance to fluconazole and flucytosine, which aligns with its known intrinsic resistance (67). All seven isolates were susceptible to voriconazole, amphotericin B, micafungin, and caspofungin, as previously reported by Pfaller et al. (68), Badiee and Alborzi (69) and K. Othman (72). *Candida kefyr* showed susceptibility to most antifungal agents tested, including voriconazole, amphotericin B, and flucytosine, which agrees with Badiee and Alborzi (69). However, *it exhibi*ted resistance to fluconazole, micafungin, and caspofungin, as noted by Ahmad *et al.* (70). *Candida parapsilosis* displayed sensitivity to fluconazole in two samples, while one isolate was resistant. All three isolates were susceptible to voriconazole, amphotericin B, micafungin, caspofungin, and fluconazole. This finding aligns with the works of Kucukateset al. (27), and Pfaller et al. (65, 68). *Candida utilis* showed resistance to all tested antifungal agents except for voriconazole and amphotericin B, which were sensitive, with flucytosine showing intermediate susceptibility. These results are consistent with Sigera and Denning (63). The high susceptibility of all isolates to voriconazole may be attributed to its more significant in vitro activity against *Candida species* than fluconazole. This is supported by previous research, while the high effectiveness of amphotericin B is likely due to its broad-spectrum activity against fungi, including Candida, which is also well documented (10,15, 41). *Candida albicans* demonstrated complete sensitivity to voriconazole, amphotericin B, micafungin, caspofungin, and flucytosine in 12 samples, although it showed resistance to fluconazole.

Conclusion

This study reports for the first time the isolation and identification of non-albicans Candida (NAC) species from heifer vulvovaginitis and confirms the presence of NAC in women's vaginitis. The research provides significant insights into the prevalence and antifungal susceptibility of Candida species causing vulvovaginitis in both women and heifers. The predominance of NAC species, accounting for 81.3% of isolates, indicates a shift in the etiology of vulvovaginal candidiasis. The species distribution varied between women and heifers, with *C. glabrata* and *C. tropicalis* emerging as the most prevalent species. This diversity emphasizes the need for accurate identification methods in clinical and veterinary settings. The observed antifungal susceptibility profiles, particularly the resistance patterns in *C. glabrata* and the unexpected fluconazole resistance in *C. albicans* highlight the evolving challenge of antifungal resistance. These findings underscore the critical importance of species-specific antifungal susceptibility testing to guide treatment strategies effectively. The presence of less common yeast species, including non-Candida yeasts, complicates the clinical picture and suggests a more complex vaginal mycobiome than previously recognized. This complexity calls for a more nuanced approach to diagnosing and treating vulvovaginitis.

In conclusion, this research highlights the shifting landscape of vulvovaginal candidiasis etiology and the growing importance of NAC species. It emphasizes the need for precise diagnostic methods and tailored antifungal therapies based on species identification and susceptibility testing. These findings have significant implications for humans and animals, potentially leading to more effective management strategies for vulvovaginitis.

Declaration

We, the authors of this manuscript, confirm that neither we nor our relatives nor any business with which we are associated have any personal or business interest. We also confirm that the disclosed information is correct and that no other situation of actual, potential, or apparent conflict of interest is known to us.

Finding

This study received no financial support.

Availability of data and materials

All data from the current study are available in this article.

Authors' contributions

Zainab A.A. Al-Haddad conceived the idea, developed the theory, and performed the computations. She verified the analytical methods. Nabaa Ali Hasan, a graduate student, did all the experimental analysis and discussed the results to contribute to the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Ethical considerations
The authors considered ethical concerns and women's consent before work. This article was originally written without copying from other articles.
Ethical Approval
All subjects involved in this research were treated humanely, adhering to the guidelines outlined by international and national human and animal care and using criteria of ethical standards defined in the 1964 Declaration of Helsinki.

References

1. Pal C, Bengtsson-Palme JB, Kristiansson E, Joakim Larsson DG. Co-occurrence of resistance genes to antibiotics, biocides, and metals reveals novel insights into their co-selection potential. BMC Genomics. 2015;16:964. doi: 10.1186/s12864-015-2153-5
2. Al-Rubaie RM, Al-Qaysi AS. Detection of Some Virulence Factors in Candida albicans Obtained from Different Clinical Specimens of Iraqi Patients. Ibn AL-Haitham J. Pure Appl. Sci.2024; 37: 1-11.
3. Ranđelović G, Mladenović V, Ristić L, Otašević S, Branković S, Mladenović-Antić S, Bogdanović M, Bogdanović D. Microbiological aspects of vulvovaginitis in prepubertal girls. Eur. J. Pediatr. 2012; 171 (8): 1203-1208. doi: 10.1007/s00431-012-1705-9. Epub 2012 Mar 1.
4. Bruins MJ, dos Santos CO, Damoiseaux RAMJ, Ruijs GJHM. Bacterial agents in vulvovaginitis and vaginal discharge: a 10-year retrospective study in the Netherlands. Eur. J. Clin. Microbiol. Infect. Dis. 2021;40: 2123-2128.
5. Alizadeh M, Kolecka A, Boekhout T, Zarrinfar H, Ghanbari Nahzag MA, Badiee P, Rezaei-Matehkolaei A. et al. Identification of Candida species isolated from vulvovaginitis using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Curr Med Mycol. 2017; 3: 21-25.doi: 10.29252/cmm.3.4.21
6. Farr A, Effendy I, Frey Tirri B, Hof H, Mayser P, Petricevic, L.et al. Guideline: Vulvovaginal candidosis (AWMF 015/072, level S2k). Mycoses. 2021; 64:583-602.
7. DOI: 10.1111/myc.13248. Epub 2021 Feb 27
8. Czechowicz P, Nowicka J ,Gościniak G. Virulence Factors of Candida spp. and Host Immune Response Important in the Pathogenesis of Vulvovaginal Candidiasis. Int J Mol Sci, 2022; 23(11): 5895.doi: 10.3390/ijms23115895.
9. Makanjuola O, Bongomin F, Fayemiwo SA. An Update on the Roles of Non-albicans Candida Species in Vulvovaginitis. J Fungi *(Basel),* 2018; 4(4): 121. DOI: 10.3390/jof4040121
10. Taei M, Chadeganipour M, Mohammadi R. An alarming rise of non-albicans Candida species and uncommon yeasts in the clinical samples; a combination of various molecular techniques for identification of etiologic agents. BMC Res Notes, 2019;12(1):779.
11. doi: 10.1186/s13104-019-4811-1.
12. Husni R, Bou Zerdan M, Samaha N, Helou M, Mahfouz Y, Saniour R, Hourani S. et al. Characterization and susceptibility of non-albicans Candida isolated from various clinical specimens in Lebanese hospitals. Front Public Health.2023;11:1115055.doi: 10.3389/fpubh.2023.1115055
13. Deorukhkar SC, Saini S, Mathew S. Non-albicans Candida Infection: An Emerging Threat. Interdiscip Perspect Infect Dis, 2014; 615958. doi: 10.1155/2014/615958
14. Forney LJ,Gajer P, Williams CJ, Schneider GM, Koenig SSK, McCulle SL, Karlebach S, Brotman RM, Davis CC, Ault K , Ravel J . Comparison of Self-Collected and Physician-Collected Vaginal Swabs for Microbiome Analysis. J Clin Microbiol. 2010; 48 (5).https://doi.org/10.1128/jcm.01710-09
15. Deng F, McClure M, Rorie R. *et al.* The vaginal and fecal microbiomes are related to pregnancy status in beef heifers. J Animal Sci. Biotechnol. 2019; 10:92. https://doi.org/10.1186/s40104-019-0401-2
16. Murray, P, Baron E, Jorgensen J, Pfaller M, Yolken R. Manual of Clinical Microbiology: 9th edition. Washington DC, American Society for Microbiology; 2007.
17. Deorukhkar SC, Saini S, Mathew S. Virulence Factors Contributing to Pathogenicity of Candida tropicalis and Its Antifungal Susceptibility Profile. Int J Microbiol. 2014, 456878.doi: 10.1155/2014/456878.
18. Terlecka JA, Du Cros PA, Orla Morrissey, C, Spelman D. Rapid differentiation of Candida albicans from non-albicans species by germ tube test directly from BacTAlert blood culture bottles. Mycoses. 2007; 50(1):48-51.doi: 10.1111/j.1439-0507.2006.01307.x.
19. Marinho SA, Teixeira AB, Santos OS, Cazanova R F, Ferreira CA, Cherubini K, de Oliveira SD. Identification of Candida spp. by phenotypic tests and PCR. Braz. J. Microbiol. 2010;41(2):286–294. doi: 10.1590/S1517-83822010000200004
20. Talaro,K, Chess B. Foundations in Microbiology (ed.) New York. *NY: McGraw-Hill Education* .2015.
21. Nadeem SG, Hakim ST, Kazmi SU. Use of CHROMagar Candida for the presumptive identification of Candida species directly from clinical specimens in resource-limited settings. *Libyan J Med. 2010;* 5. doi: 10.3402/ljm.v5i0.2144
22. Borman AM, Fraser M, Johnson EM. CHROMagarTM Candida Plus: A novel chromogenic agar that permits the rapid identification of Candida auris. Med. Mycol. 2021; 59(3):253-258. doi: 10.1093/mmy/myaa049
23. Sariguzel F, Berk E, Koç AN, Sav H, Aydemir G. Evaluation of CHROMagar Candida, VITEK2 YST and VITEK® MS for identification of Candida strains isolated from blood cultures. Infez Med.2016;24(1):90. PMID: 27031906
24. Pfaller M, Diekema D, Procop G, Rinaldi M. Multicenter comparison of the VITEK 2 antifungal susceptibility test with the CLSI broth microdilution reference method for testing amphotericin B, flucytosine, and voriconazole against Candida spp. J. Clin. Microbiol. 2007; 45(11): 3522-8.doi: 10.1128/JCM.00403-07

25. Fothergill AW. Antifungal Susceptibility Testing: Clinical Laboratory and Standards Institute (CLSI) Methods. *In:* HALL, G. S. (ed.) Interactions of Yeasts, Moulds, and Antifungal Agents: How to Detect Resistance. Totowa, NJ: Humana Press, 2012.
26. Dunn OJ, Clarck VA. Basic statistics: a primer for medical sciences. New Jersey. USA, John Wiley and Sons Inc, 2009.
27. Hadi HS, AlSultany SJ. Isolation and identification Candida species among renal failure Iraqi patients. Drug Invent. Today,2022; 14(6): 812–16.
28. Fidel PL, Vazquez JA, Sobel JD. (1999). Candida glabrata: review of epidemiology, pathogenesis, and clinical disease with comparison to C. albicans. *Clin. Microbiol. Rev.*, 1999;12: 80-96.doi: 10.1128/CMR.12.1.80
29. Kucukates E, Gultekin NN, Alisan Z, Hondur N, Ozturk R. Identification of Candida species and susceptibility testing with Sensititre Yeast One microdilution panel to 9 antifungal agents. Saudi Med J. 2016 2016; 37:750-7.
30. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol. Rev.2012; 36: 288-305.DOI: 10.1111/j.1574-6976.2011.00278.x
31. Alwaily ER, Abood MS, Flaih MH. Identification of Candida Krusei by 18S rRNA Gene and Investigation of SAP1 Gene in Samples Isolated from Female Genital Tract Infection. *International Symposium on Multidisciplinary Studies Innovative Technologies*. 2022; 31.319-5 DOI: 10.1109/ISMSIT56059.2022.9932855
32. Perry JD. A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin. Microbiol. Rev.2017; 30(2):449-479. DOI: 10.1128/CMR.00097-16
33. Habib KA, Najee EN, Abood MS. Identification of Candida species Isolated from Vulvovaginal Candidiasis Patients by Chromgen agar and PCR-RFLP Method. Baghdad Science Journal. 2016; 13: 291-297.DOI: 10.21123/bsj.13.2.291-297
34. Mohammed NA, Muhsen TAA, Risan MH. Isolation and diagnosis of some candida species from some Baghdad city hospitals with PCR technique and evaluation of the effectiveness of some antifungals. Plant Archives. 2020*;* 20: 3895-3900.
35. Pravin Charles MV, Kali A, Joseph NM. Performance of chromogenic media for Candida in rapid presumptive identification of Candida species from clinical materials. Pharmacognosy Res. 2015; 7 Suppl 1): S69-73.DOI: 10.4103/0974-8490.150528
36. Jarallah MA, Al-Haddad ZAA. Isolation and molecular detection of Candida albicans from human and domesticated small animals in Baghdad, Iraq. Biochem. Cell. Arch. 2022; 22, 2085-2092.
37. Nagano Y, Millar BC, Goldsmith CE, Walker JM, Elborn JS, Rendall J, Moore J E. Development of selective media for the isolation of yeasts and filamentous fungi from the sputum of adult patients with cystic fibrosis (CF). Journal of Cystic Fibrosis. 2008; 7 (6): 566-572.DOI: 10.1016/j.jcf.2008.06.007.
38. Gupta V, Abhisheik K, Balasundari S, Devendra NK, Shadab K, Anupama M. Identification of Candida albicans using different culture media and its association in leukoplakia and oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2019*;* 23(1), 28-35.DOI: 10.4103/jomfp.JOMFP_157_18
39. De Angelis G, Menchinelli G, Torelli R, De Carolis E, Posteraro P, Sanguinetti M, Posteraro B. Different detection capabilities by mycological media for Candida isolates from mono- or dual-species cultures. PLoS One.2020; 15, e0226467.
40. Rathore N, Lalwani J, Singh P, Meena M. Speciation of Candida species isolated from cutaneous skin lesions using CHROMagar and conventional methods. Asian J.Med.Sci. 2024; 15, 113-117.DOI**:** https://doi.org/10.3126/ajms.v15i2.59383
41. Lines A, Vardi-Flynn I, Searle C. Recurrent vulvovaginal candidiasis. *bmj*, 2020; 369:m1995. doi: https://doi.org/10.1136/bmj.m1995.
42. Anh DN, Hung DN, Tien TV, Dinh VN, Son VT, Luong NV, et al. Prevalence, species distribution and antifungal susceptibility of Candida albicans causing vaginal discharge among symptomatic non-pregnant women of reproductive age at a tertiary care hospital, Vietnam. BMC Infect. Dis.2021; 21, 523.ID: mdl-34082699
43. Brandão LDS, Boniek D, Resende Stoianoff MA, da Mata FMR, de Azevedo PRM, Fernandes JV, Andrade, V. S. Prevalence and antifungal susceptibility of Candida species among pregnant women attending a school maternity at Natal, Brazil. Lett. Appl. Microbiol.2018; 67(3): 285-291. DOI: 10.1111/lam.13034. Epub 2018 Jul 12.
44. Eleutério J Jr, Campaner AB, de Carvalho NS. Diagnosis and treatment of infectious vaginitis: Proposal for a new algorithm. Front Med (Lausanne). 2023;10: 104007.*2.* DOI: 10.3389/fmed.2023.1040072.
45. Mahmoudi Rad M, Zafarghandi A, Amel Zabihi M, Tavallaee M, Mirdamadi Y. Identification of Candida species associated with vulvovaginal candidiasis by multiplex PCR. Infect Dis Obstet Gynecol, 2012, 872169.DOI: 10.1155/2012/872169
46. Sheppard DC, Locas MC, Restieri C, Laverdiere M. Utility of the germ tube test for direct identification of Candida albicans from positive blood culture bottles. J. Clin. Microbiol.2008; 46: 3508-9.DOI: 10.1128/JCM.01113-08
47. Kalkanci A, Güzel AB, Khalil IIJ, Aydin M, Ilkit M, Kuştimur S. Yeast vaginitis during pregnancy: susceptibility testing of 13 antifungal drugs and boric acid and the detection of four virulence factors .Med. Mycol. 2012; 50:585-593.DOI: 10.3109/13693786.2012.662597
48. Fornari G, Vicente VA, Gomes R R, Muro M D, Pinheiro R L, Ferrari C, Herkert P F, et al. Susceptibility and molecular characterization of Candida species from patients with vulvovaginitis. Braz. J. Microbiol.2016;47(2):373–380. doi: 10.1016/j.bjm.2016.01.005

49. Bitew A, Abebaw Y. Vulvovaginal candidiasis: species distribution of Candida and their antifungal susceptibility pattern. *BMC Womens Health.2018;* 18 (1): 94. doi: 10.1186/s12905-018-0607-z.
50. Waikhom SD, Afeke I, Kwawu GS, Mbroh HK, Osei GY, Louis B, Deku JG, et al. Prevalence of vulvovaginal candidiasis among pregnant women in the Ho municipality, Ghana: species identification and antifungal susceptibility of Candida isolates. *BMC Pregnancy Childbirth.* 2020; 20(1): 266.doi: 10.1186/s12884-020-02963-3.
51. Mushi MF, Olum, R, Bongomin F. Prevalence, antifungal susceptibility and etiology of vulvovaginal candidiasis in sub-Saharan Africa: a systematic review with meta-analysis and meta-regression.Med.Mycol.2022; 60(7);myac037, https://doi.org/10.1093/mmy/myac037
52. Stratford, M. (2006). Food and Beverage Spoilage Yeasts. *In:* QUEROL, A. and FLEET, G. (eds.) *Yeasts in Food and Beverages*. Berlin, Heidelberg: Springer Berlin Heidelberg.
53. Wirth F, Goldani LZ. Epidemiology of Rhodotorula: an emerging pathogen. Interdiscip Perspect Infect Dis. 2012, 465717. DOI: 10.1155/2012/465717. Epub 2012 Oct.
54. Colombo AL, Padovan AC, Chaves G M. Current knowledge of Trichosporon spp. and Trichosporonosis. *Clin. Microbiol. Rev.*, 2011; 24, 682-700 .DOI: 10.1128/CMR.00003-11.
55. Bradford LL, Ravel J. The vaginal mycobiome: A contemporary perspective on fungi in women's health and diseases. Virulence. 2017; 8 (3): 342-351.DOI: 10.1080/21505594.2016.1237332.
56. Dworecka-Kaszak B, Krutkiewicz A, Szopa D, Kleczkowski M, Biegańska M. High prevalence of Candida yeast in milk samples from cows suffering from mastitis in Poland. Scientific World Journal, 2012, 196347. DOI; 10.1100/2012/196347. Epub 2012 Apr 24.
57. Alfouzan W, Dhar R, Ashkanani H, Gupta, M, Rachel C, Khan ZU. Species spectrum and antifungal susceptibility profile of vaginal isolates of Candida in Kuwait. J Mycol Med. 2015; 25: 23-8.DOI: 10.1016/j.mycmed.2014.10.021
58. Rezaei-Matehkolaei A, Shafiei S, Zarei-Mahmoudabadi A. Isolation, molecular identification, and antifungal susceptibility profiles of vaginal isolates of Candida species. Iran J Microbiol. 2016; 8: 410-417.PMID: 28491253
59. Megri Y, Arastehfar A, Boekhout T, Daneshnia F, Hörtnagl C, Sartori B, et al. (2020). Candida tropicalis is the most prevalent yeast species causing candidemia in Algeria: the urgent need for antifungal stewardship and infection control measures. Antimicrob. Resist. Infect. Control. 2020; 9: 50. DOI: 10.1186/s13756-020-00710-z.
60. Hong SI, Suh YS, Kim HO, Bae IG, Shin JH, Cho OH. Successful Treatment of Catheter-Related Blood Stream Infection By Millerozyma farinosa with Micafungin: A Case Report. Infect Chemother. 2018: 50(4):362-366 https://doi.org/10.3947/ic.2018.50.4.362
61. Mollaschi EMG, Iskandar EN, Esposto MC, Prigitano A, Rigano G, Rotola G, Caneva G, Cavanna C. Catheter-related blood stream infection caused by Millerozyma farinosa in an immunocompetent patient: a case report and a brief review of the literature. New Microbiol.2022; 45: 142-147.
62. Soriano A, Honore PM, Puerta-Alcalde P, Garcia-Vidal C, Pagotto A, Gonçalves-Bradley DC, Verweij PE. Invasive candidiasis: current clinical challenges and unmet needs in adult populations. J. Antimicrob. Chemother. 2023; 78(7):1569–1585. DOI: 10.1093/jac/dkad139
63. Pappas PG, Kauffman C A, Andes D, Benjamin DK, Jr, Calandra TF, Edwards JE, Jr., Filler SG, Fisher JF, et al. Clinical Practice Guidelines for the Management Candidiasis: 20 09Update by the Infectious Diseases Society of America. Clin. Infect. Dis. 2009; 48, 503-535. https://doi.org/10.1086/596757
64. Sobel JD. Vulvovaginal candidosis .*Lancet. 2007;* 369, 1961-71.
65. Sigera LSM, Denning DW.Flucytosine and its clinical usage. Ther Adv Infect Dis, 2023; 10, 20499361231161387.
66. Takakura S, Fujihara N, Saito T, Kudo T, Iinuma Y, Ichiyama S, Group Taj IM SS. (2004). National surveillance of species distribution in blood isolates of Candida species in Japan and their susceptibility to six antifungal agents including voriconazole and micafungin. J. Antimicrob. Chemother. 2004; 53: 283-289.
67. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty Years of the SENTRY Antifungal Surveillance Program: Results for Candida Species From 1997-2016. Open Forum Infect Dis,2019; 6(Suppl 1): S79-S94. DOI: 10.1093/ofid/ofy358.
68. Kessler SQS, Lang PM, Dal-Pizzol TS, Montagner F. Resistance profiles to antifungal agents in Candida albicans isolated from human oral cavities: systematic review and meta-analysis. Clin Oral Investig,26 ,2022;26(11):6479–6489. DOI: 10.1007/s00784-022-04716-2
69. Lee Y, Puumala E, Robbins N, Cowen LE.Antifungal Drug Resistance: Molecular Mechanisms in Candida albicans and Beyond. Chem Rev,2021; 121(6):3390–3411. DOI: 10.1021/acs.chemrev.0c00199
70. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Nagy E, Dobiasova S, Rinaldi M, Barton R, Veselov A. Candida krusei, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. J. Clin. Microbiol.,2008; 46(2):515-21. DOI: 10.1128/JCM.01915-07. Epub 2007 Dec 12.
71. Badiee P, Alborzi A. Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five-year study. Iran J Microbiol,2011; 3 (4): 183-188. PMC3330181.
72. Ahmad S, Khan Z, Al-Sweith N, Alfouzan W, Joseph L, Asadzadeh MC. Candida kefyer in Kuwait: Prevalence, antifungal drug susceptibility and genotypic heterogeneity. PLoS one. 2020; 15, e0240426. DOI: 10.1371/journal.pone.024.
73. Alwan, M.J. (2012) 'Isolation and identification of candida species from urogenital tract infections of women in Baghdad City', *The Iraqi Journal of Veterinary Medicine*, 36(0E), pp. 115–119. doi:10.30539/iraqijvm.v36i0e.404.

74. Karwan I. O. Isolation and Identification Candida spp from Urine and Antifungal Susceptibility Test. Iraqi Journal of Science. 2018 Nov. 28 [cited 2024 Dec. 9];59(4B):1981-8. Available from: https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/554
75. Nareen Q.Abdulla The Efficacy of Antifungal Medications and Plant Extracts Against Candida albicans Isolated from Vulvovaginitis Women. (2023). *Iraqi Journal of Science*, *64*(2), 560-572. https://doi.org/10.24996/ijs.2023.64.2.6
76. Muhsen.M. F. , Kamil M. AL-Jobori1 , Evaluation of Vitik2 System for Clinical Identification of Candida species and Their Antifungal Exposure Test.(2020) Iraqi Journal of Biotechnology, April2020, Vol. 19, No. 1, 28-39**.**
77. Mohsen H. Risan, Molecular Identification of Yeast Candida glabrata from Candidemia Patients in Iraq Iraqi Journal of Science, 2016, Vol. 57, No.2A, pp: 808-813
78. 76. Ahmed F. K. Role of Proteus mirabilis DNA in Comparison to Candida albicans DNA in Rats' Joints Infection. (2023). *Iraqi Journal of Science*, *55*(3B), 1170-1182. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/11544
79. Najwan Abbas Mohammed Detection The Prevalence of Adhesins and Extracellular hydrolytic enzymes genes in Candida albicans Biofilm Formation. (2021). *Iraqi Journal of Science*, *58*(2C), 988-1000. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/5931