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Isolation, Identification and Antifungal Susceptibility Testing of *Candida* species isolated from patients in Misrata City

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Abstract: Fungal infections caused by *Candida* species have become increasingly common, particularly among hospitalized and immunocompromised patients. These opportunistic pathogens can cause a wide range of infections, from superficial mucosal infections to life-threatening systemic conditions. study was conducted to isolate and identify of *Candida* spp. from clinical samples, including urine, sputum, and vaginal swabs, and to test their susceptibility to five different antifungal against the isolated species and analyzed antifungal resistance patterns using disk diffusion method. A total of 80 samples were collected from hospitals in Misrata. 50 *Candida* isolates were obtained was culture on Sabouraud Dextrose Agar (SDA)and incubated at 37°C for 24-48h . Additionally the advanced Phoenix M50 system was used for accurate identification. revealed the isolation and identification of three *Candida* species from clinical samples: *C. albicans, C. tropicalis*, and *C. sake*. Among them, *C. albicans* was the most common species. The gender distribution analysis in this study showed a higher prevalence of fungal infections among females, accounting for 74% of the cases. showed variability in drug efficacy, with CLO 50 (clotrimazol) being the most effective antifungal agent the isolated *Candida* species, making it the preferred choice for treating fungal infections. On the other hand, ITC 50 (Itraconazole) was the least effective against all *Candida* isolates, , the results indicated that *C. albicans* was highly sensitive to most antifungal agents, except for ITC 50.

Keywords: Fungal infections ,*Candida* spp, pathogens, drug efficacy, antifungal agents

Introduction

The genus *Candida* belongs to the group of imperfect fungi within the division Deuteromycotina and the class Blastomycetes. However, some strains within this genus have been found to produce ascospores, leading to their reclassification under the division of sac fungi (Ascomycotina) within the class Hemiascomycetes (Muhammed ,2021) . Fungal infections, particularly those caused by *Candida* species, are a significant cause of urinary tract infections (UTIs) (Dias , 2020). *Candida albicans* is the most common pathogen responsible for fungal infections and is often found in healthy individuals. It coexists with other *Candida* species such as *C.dubliniensis*, *C. krusei*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* (Jabar and Aljaz , 2022). Opportunistic *Candida* infections are associated with several factors, including physiological (age children and the elderly), pregnancy, endocrine 2 diseases such as diabetes (Jayalakshmi *et al*.,2014), indwelling urinary catheters, and immunosuppressive therapy (Zarei-Mahmoudabadi *et al*., 2012). Additional factors include long-term antibiotic use, chemotherapy, surgical manipulation of the urinary tract, and extended stays in intensive care units (Jamil *et al*., 2016). These risk factors have become more prevalent due to intensive cancer treatment regimens and complications from abdominal, cardiac, and thoracic surgeries, organ transplantation, and burns (Rashwan *et al*., 2010). *Candida* becomes an opportunistic pathogen when certain virulence factors are present. These factors include the ability to adhere to epithelial cell surfaces, produce lipid-degrading enzymes, and form germ tubes (Hussein ,2024). Additionally, *Candida* produces aspartate proteinases (SAPS), which are extracellular proteins that play a key role in its pathogenesis. These proteins facilitate attachment and tissue invasion (Alenzi , 2016), biofilm formation, and metabolic adaptation (Cirurea *et al*., 2018). Other virulence factors include phenotypic switching, allowing *Candida* to transition between unicellular budding forms (blastospores) and pseudo- or true hyphae, enabling survival under conditions conducive to disease development (Singh *et al*., 2020).*Candida* infections in the urogenital tract manifest as vulvar candidiasis, balanitis in males, and vulvovaginal candidiasis in females. Infections can also occur in the oral

cavity, gastrointestinal tract, and urinary tract. For instance, candiduria is often asymptomatic, with yeasts incidentally observed during routine urine analysis or culture (Kuffman *et al*., 2011).The increasing diversity of *Candida* species and their varying resistance to antifungal agents necessitate rapid diagnosis to control these infections and prevent their spread. Non-selective and repeated antifungal use has led to the development of resistant strains, modifications in permeability barriers, 3 and changes in drug targets (Tasneem *et al*., 2017). Drug resistance is a major cause of treatment failure (Mahajan *et al*., 2015). Research problem Because of the increased infections caused by different *Candida* species over the past decade and the observed changes in the species causing candidiasis and empirical antifungal therapy. There is an increase in the *Candida* species being more prevalent with varying susceptibility to antifungals Accurate diagnosis of these infections and determination of antifungal susceptibility are essential to improve treatment management and reduce rates of epidemiological resistance.

Importance of research

Candida species and their varying resistance to antifungal agents necessitate rapid diagnosis to control these infections and prevent their spread. The non-selective and repeated use of antifungals has led to the development of resistant strains, modifications to the permeability barrier, and changes in drug targets. Drug resistance is a major cause of treatment failure**.** Therefore, monitoring fungal infections through accurate species identification and antifungal testing is crucial due to the rising resistance**.** This study provides valuable data on the diversity of *Candida* species in healthcare settings in Misrata, supporting the development of effective prevention andtreatment strategies.

objectives Study

This study aims to**:**

1- isolate and identify different types of *Candida* from Urine, Sputum and Vaginal swabs samples.

2- Evaluate their sensitivity to the five antifungals agents.

A study was conducted by Alenzi ,(2016) in Saudi Arabia, aiming to examine *Candida* species in urine samples from patients with urinary tract infections (UTIs) associated with obstructive uropathy.The results using the API system showed that *Candida albicans* was the most common species. Kim *et al*., (2016) conducted a study in Korea on *Candida* spp. The study analyzed 2,508 *Candida* isolates from various clinical specimens . The aim was to determine the isolation frequency and characteristics by specimen type, gender. The findings revealed that the most frequently isolated species were *Candida albicans* (48.56%). In a study conducted by Mahmoudabadi *et al*.,(2015) in Iran the prevalence of candiduria and fungal urinary tract infections was investigated among patients.Ten microliters of uncentrifuged samples were cultured on CHROM agar *Candida* plates and incubated at 37°C for 24-48 hours aerobically. *Candida* species were identified based on colony morphology on CHROM agar and germ tube production. Was the results the 744 patients, 49.5% were female and 50.5% were male.. The most common species identified were *C. albicans* (53.3%). In a study conducted by Singla *et al*.,(2102) in India, the researchers investigated the presence of *Candida* species in urine samples and the associated therapeutic challenges. This study emphasizes the importance of monitoring *Candida* colonization in ICU patients and identifying appropriate treatment strategies, particularly given the increasing resistance to antifungal agents like Fluconazole .

Materials and Methods

Sample Collection:

This corss –sectiona study was conducted from July 2024 to December 2024. A total of 80 samples were collected, including:49 Urine,11 Sputum 15 and 20 Vaginal swabs samples. Samples were collected from several healthcare centers in Misrata inclusion:

Misurata Medical Center, Alamal Laboratory, Misurata Central Laboratory, Almarga aboratory, Aldaka Laboratory, Alhakma Laboratory, Ibn Sine Laboratory, Alsalam Laboratory.

After collection, the samples cultured in Sabouraud's Dextrose then incubated at 35-37°Cfor within 2-3 days. Also, the samples were frozen at -80°C until reactivation.

Isolation of *Candida* spp.

Obtained *Candida* isolates were sub cultured on Sabouraud's Dextrose Agar plates, and were incubated at 37°C. The growth was monitored by examining the plates at 24, 48, and 72 hour .The culture plates were observed for the following:

(Appearance of colonies Color of colonies Morphology of colonies(. These observations were recorded for further identification and characterization of the isolates.

Identification *of candida*

1- Macroscopic identification through cultured plates.

The colonies of *Candida* spp. were studied for their morphological characteristics.

2- Microscopic Identification of *Candida* spp.

The *Candida* spp. isolates were identified microscopically using the Gram stain method (Jamil *et al* , 2016) . Gram Stain is a microscopic technique used to differentiate and classify bacteria and fungi based on the structure of their cell wall and presence of invasion **(**pseudohyphae or hyphae). This stain was used to divide organisms into two main groups:

●Gram-positive.

●Gram-negative.

3- Biochemical Tests

The Catalase Test The colonies grown on the SDA medium were taken at 48hours of age and spread on a glass slide. then, were added 1-2drop of 3% hydrogen peroxide(H_2O_2),and the result were recorded.(Muhammed ,2021)

4- Germ Tube Test for Identification of *Candida albicans*

Few colonies of *Candida* cultures were inoculated into 0.5ml of human serum in a test tube and incubated at 37°C for 2-4 hour. After incubation a loop-full of the culture was placed on a glass slide overlaid with a cover-slip, and examined microscopically for the presence or absence of germ tubes. The germ tubes were observed as long, tube like projections extending from the yeast cells with no constriction or septa at the point of attachment to the yeast cells. The formation of germ tubes is indicative of *Candida albicans* (Othman *et al*., 2018).

5- Phoenix M50 System

In this study, *Candida* samples collected from hospitals in Misrata were analyzed at the Misrata Central Laboratory using the Phoenix M50 system to identify the fungal species responsible for infections (Masoud and Elmajbery ,2021).

6- Antifungal Susceptibility Test

All the *Candida* isolates were tested for antifungal susceptibility (table1) using the Kirby-Bauer method (disc diffusion) on Mueller-Hinton agar supplemented with 2% glucose. Commercially available antifungal discs were used, and the zones of inhibition were measured after 24–48 hour of incubation at 37°C (Singh *et al*., 2020). Antibiotic Antifungal were selected according to guidelines recommended by the Clinical and Leboratory standred (CLSI,2019)(Jabar and Aljaza ,2022).The *Candida* spp. suspension was prepared and inoculated onto the surface of a plate media. *Candida* spp. that was grown for 24 to 48 hour at 37°C were suspended in sterile 0.85% saline, then adjusted to McFarland standard (0.5). Performance Standards for Antifungal Susceptibility Testing of Yeasts according to CLSI M44. Sterile antifungal discs were placed on the surface of each agar plate using a sterile pair of forceps. The plates were then incubated aerobically at 37°C for 24–48 hour. The diameter of the inhibition zone was measured after 24–48 hour of incubation (Othman *et al*., 2018).

Table 1. antifungal used for *Candida* isolates.

Antifungal discs	Symbol	Concentration(µ/m1)
Voriconazole	**VO**	(1 µg)
Fluconazole	**FLu**	(25 µg)
Itraconazole	**ITC**	(50µg)
Ketoconazole	**KCA**	(10µg)
clotrimazol	**CLO**	(50 µg)

Results

Distribution of Clinical Samples

The clinical samples were classified into three main types sputum, vaginal swabs, and urine. The distribution percentage of negative (-ve) and positive (+ve) isolates for each sample type was calculated. Additionally, the overall percentage and frequency of the total number of samples tested were also presented as table (2) and fig(1).

Table 2. Distribution of Clinical Samples

Clinic samples	Number of samples tested		Number of positive isolated + ve		Number of positive isolated - ve		Total
	Frequency	%	Frequency	%	Frequency	%	
Sputum	11	13.8	7	63.6	4	36.4	80
Vaginal swab	20	25	14	70	6	30	
urine	49	61.2	29	59.2	20	40.8	100
total	80	100.0	50	62.5%	30	37.5	

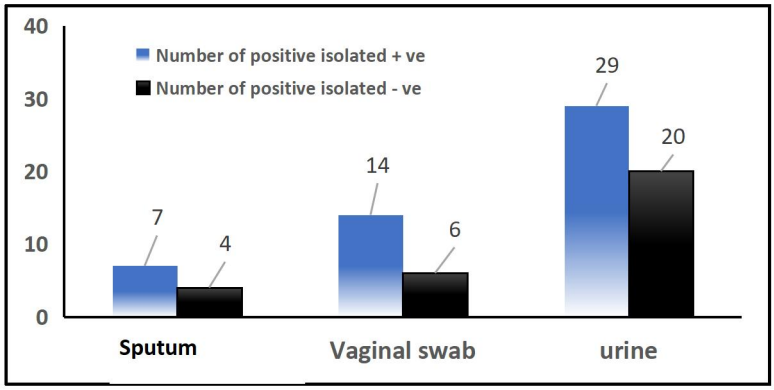

Fig.1 Frequency of clinical samples.

A practical study was conducted on a sample of 80 individuals. Among these, 11 sputum samples were collected, representing 13.8% of the total sample size. Of these ,7 confirmed for fungal infection, constituting 63.6%, while 4 were negative for *Candida* spp. ,representing 36.4%. Additionally, 20 vaginal swab samples were collected, accounting for 25% of the total sample. Out of these , 14 confirmed for fungal infection, representing 70%, and 6 were negative for Candida spp, representing 30%.And 49 urine samples were collected, making up 61.2% of the total sample. Of these , 29 confirmed for fungal infection, 21 constituting 59.2%, and 20 were negative for Candida spp, representing 30%.And 49 urine samples were collected, making up 61.2% of the total sample. Of these , 29 confirmed for fungal infection, 21 constituting 59.2%, and 20 were negative for Candida species, representing 40.8%.In summary, the total number of samples positive for fungal infection was 50, representing 62.5% of the overall sample size. Conversely, 30 samples were negative for fungal infection, accounting for 37.5% of the total sample size.

Macroscopic Identification through Cultured plates

The study confirmed the traceability of 80 isolates to *Candida* spp. The results showed that 50 samples of *Candida* spp. were successfully isolated. The identification was achieved by examining cultural, microscopical characteristics, and performing biochemical tests, as detailed below. The results showed that the colonies of Candida spp. grown on SDA medium appeared white to cream- colored, smooth in texture, and round in shape . These colonies exhibited distinctive phenotypic traits, being glossy, which demonstrated the suitability of the medium for optimal cultivation.

Microscopic Identification of *Candida* spp.

All species exhibited oval to round cells, varying in size and budding was clearly in cells (Fig.2). Gram stain tests were performed on the isolated *Candida* spp. to determine their basic microbial characteristics.

Fig2. *Candida* spp. cells stained

The results showed that all species were Gram-positive, as they retained the crystal violet stain, giving them a dark violet color under microscopic examination.

The Gram stain

Gram stain tests were performed on the isolated *Candida* spp. to determine their basic microbial characteristics. The results showed that all species were Gram-positive, as they retained the crystal violet stain, giving them a dark violet color under microscopic examination as shown in (Figure 3). The cells ranged in size from small to medium. The cells appeared in small clusters or scattered, while in some cells they were organized into chain-like formations.

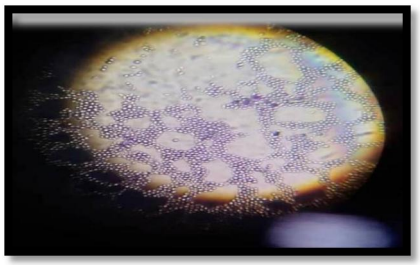

Fig 3 . *C.albicans* cells stained with Gram stain (40x Magnification).

Biochemical Tests
Catalase Test
The results of the Catalase test showed that all isolates were capable of producing the catalase enzyme, with a 100% production rate. This indicates that each isolate has enzymatic activity that enables it to break down hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2
Germ Tube Test for identifying *Candida albicans*
The results of the germ tube test showed that 30 out of 50 synthetic *Candida* isolates (60%) were able to form germ tubes, indicating the presence of Candida albicans. Meanwhile, 20 samples (40%) showed negative results, suggesting the possibility of other species(C. tropicalis c. sake.). These results confirm that germ tube formation is specific to *C. albicans*, as the germ tube forms around the yeast cell in the presence of serum, which plays a crucial role in penetrating epithelial cells and accessing the bloodstream (Fig 4).

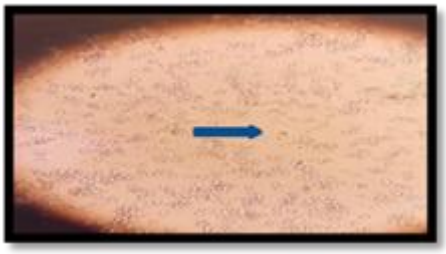

Fig4. The Germ Tube indicated by an arro for the type *C.albicans* (100x magnification).

Using the Phoenix M50 system
In this study 12 *Candida* samples collected from hospitals in Misrata were analyzed using the Phoenix M50 system to identify the fungal species responsible for infections. These species are listed in the following table (3) illustrates the fungal species names of *Candida* and the sample types collected in this study using the Phoenix.

Table 3. fungal species names of *Candida*

Species Name	Sample Type	Frequency Count
C. albicans	Vginal swab	4
C. tropicalis	Urin –Sputum	5
C. sake	Urin	3

The samples were obtained from different sources, including vaginal swabs urine and sputum samples, to identify the species responsible for the infections.

Classification of infected samples by gender
Infected Samples by Gender The total sample size consists of 50 individuals. Males account for 13 individuals, representing 26% of the total sample, while females account for 37 individuals, representing 74% of the total sample. The results indicate that females constitute the majority of the sample, making up approximately three-quarters of the sample as table (4).

Table4. Gender Distribution of *Candida* Isolates.

Gender	Frequency	Percent	
Male	13	26%	
Female	37	74%	
Total		50	100%

The total sample size consists of 50 individuals. Males account for 13 individuals, representing 26% of the total sample, while females account for 37 individuals, representing 74% of the total sample. The results indicate that females constitute the majority of the sample, making up approximately three-quarters of the sample.

Determination of *Candida* spp.

In Clinical Sample the data from table (5) clearly indicate the presence of *Candida albicans* in saliva, vaginal, and urine samples. The highest prevalence was observed in urine samples, where *Candida albicans* was detected in 18 out of 29 urine samples, representing 62.1% of the total urine samples. This was followed by vaginal swap samples, with 9 out of 14 samples (64.3%) testing positive for *Candida albicans*. The lowest prevalence was found in sputum samples, with only 3 out of 7 samples (42.9%) showing the presence of *Candida albicans*. Data from the table show that other Candida was detected in 11 out of 29 urine samples (37.9% of the total), 5 out of 14 vaginal swab samples (35.7% of the total), and 4 out of 7 sputum samples (57.1%) of the total.

Table(5).Determination of *Candida* spp. in Clinical Sample Type

Isolated species	sputum samples		Vaginal samples		urine samples		Total
	Frequency	%	Frequency	%	Frequency	%	
c. albicans	3	42.9	9	64.3	18	62.1	50
Non-*c.albicans*	4	57.1	5	35.7	11	37.9	
Total	7	100	14	100	29	100	100

To study the differences Chi-Square .A statistically significant difference was observed. in the occurrence of *Candida albicans* among the saliva, vaginal, and urine samples. The p-value(0.05) for the Chi-Square test was found to be less than 0.001, which is highly statistically significant. This indicates strong evidence that the observed distribution of *Candida albicans* varies significantly from the expected distribution, confirming a substantial difference in the frequency of *Candida albicans* among the saliva, vaginal, and urine samples Specifically.

Antifungal Susceptibility Testing

Antifungal susceptibility of *Candida* spp. was evaluated Using the Disk diffusion method The results were shown in figure (5).

Fig 5. Antifungal susceptibility testing of *Candida* spp. Isolates.

Based on the data presented in table (5), it is evident that ITC 50 was the least effective antifungal agent compared to the others tested against the three *Candida* species (*C. albicans*, *C. tropicalis*, *C. sake*), as all isolates showed resistance with no effective response. The remaining antifungal agents varied in their activity, where CLO 50 demonstrated the highest inhibitory effect, followed by FLU 25 which exhibited the lowest activity, while KCA 10 and VO 1 showed moderate effectiveness. Among the 27 tested species, *C. albicans* was the most affected, showing high sensitivity to most antifungal agents except ITC 50 to which it was resistant, indicating that it can be effectively treated with antifungals such as

KCA 10 and CLO 50. In contrast, *C. tropicalis* and C. sake showed lower responses compared to *C. albicans*, suggesting that these species require more specific antifungal agents such as CLO 50 to achieve high effectiveness. Regarding the statistical analysis, the sensitivity test of the three *Candida* species to VO1 recorded a Chi-square value of 3.000 with a p-value of 0.223, which is greater than the usual significance level (0.05), therefore the null hypothesis could not be rejected and no significant association was observed between the *Candida* species and their sensitivity to VO1. Similarly, the sensitivity test to FLU 25 showed a Chi-square value of 6.000 with a p-value of 0.199, which is also higher than 0.05, indicating insufficient statistical evidence to confirm an association between the *Candida* species and their sensitivity to FLU 25.

Table (5):Effect of some antifungal against *candida* spp

Species of *Candida*	Types of antifungal used in the test				
	KCA 10	CLO 50	Flu 25	VO1	ITC 50
C. sake	R	S	S	R	R
C .tropicals	R	S	R	R	R
C. albicans	S	S	S	S	R

Discussion

Distribution of Clinical Samples

The results of this study showed the isolation of 50 samples from urine, sputum, and vaginal swabs, which were analyzed to determine the presence *of Candida* spp. The results are consistent with the findings of Hussein (2024), that used the same types of samples. This agreement enhances the credibility of the current study's results and suggests that the diversity of sample types can be an important factor in understanding the spread of *Candida* infections across the urinary tract, respiratory system, and genital system. This study differs from some previous studies in terms of sample types. A study by Jamil *et al*., (2016) showed that urine samples are primarily associated with *Candida* infections in the urinary tract or genital system. However ,The study added a new dimension by isolating *Candida* from diverse clinical samples such as urine, sputum, and vaginal swabs, reflecting the diversity of infection and enhancing the validity of our findings. clinical samples from multiple diverse sites.

Most of researches studied the prevalence of different species such as *Candida albicans* and *Candida tropicalis* in urine, and did not focus on evaluating the antifungal susceptibility of the species, This gap in previous researches contribute to a lack of information regarding the effectiveness of antifungal treatments for different types of infections, The study addressed by evaluating the resistance of *Candida* species to locally used antifungal agents in hospitals in Misrata. This contributes to improvd local treatment strategies based on species identification and their drug susceptibility. While the study by Masoud and Elmajbery,(2021) provided valuable data on the oral fungal microbiome in diabetic and non-diabetic patients in Benghazi, Libya, The 30 study adds a new dimension by focusing on clinical samples from multiple diverse sites such as urine, sputum, and vaginal swabs, enhancing our understanding of *Candida* species distribution across various clinical settings. Furthermore .

Macroscopic Identification through Cultured plates

This study revealed that most samples were isolated using the traditional Sabouraud Dextrose Agar (SDA), which is the same method used in many previous studies. For example, the study by Masoud and Elmajbery, (2021) also employed this culture medium to isolate fungal species from diabetic. Through the examination of cultural, microscopic, and biochemical characteristics, colonies grown on SDA medium displayed a smooth, white to cream-colored appearance and a round shape .According to Hussein (2024), *Candida* species demonstrate specific phenotypic traits when cultured on this medium. These observations are consistent with the findings of Singh *et al*.,(2013), which reported that colonies exhibited a glossy, cream-colored, smooth, and circular appearance.

The Gram stain

species exhibited positive result when subjected to Gram staining, as the cells displayed an oval to spherical, oval to elongated, or cylindrical form resembling yeast like fungi dark violet of *Candida*. This finding was in line with the findings of Khadka,(2017) . And study Jamil *et al*.,(2016) The

Gram stain test is a crucial step that complements other tests.

Germ Tube Test

All isolates not formed a germ tube. The result found that only *C. albicans* can make germ tubes. Similar findings were reported by Jamil *et al*., (2016) .Only *C. albicans* can produce the germ tube in this experiment. The results indicate that the Germ Tube test is an effective and rapid tool for identifying *Candida albicans*, the species most closely associated with pathogenicity.

Catalase test

All *candida* isolates produced catalase enzyme by 100% rate highlights the importance of this enzyme in the survival and life cycle of *candida*, the Catalase is one of the key protective enzymes that help microorganisms including *candida*, withstand oxidative stress caused by the hydrogen peroxide within cells. This characteristic provides *candida* with resistance to host defense mechanisms ,including the production of free radicals and peroxides a part of the immune response . *Candida* species with higher catalase production may be more efficient at colonizing and causing infection ,emphasizing the enzymes role in pathogen survival under stress conditions. These results align with Muhammed *et al*.,(2021) have demonstrated the role of catalase in protecting *candida* from oxidative stress.

Classification of infected samples by gender

Samples from 50 patients with *Candida* infection were analyzed to determine gender ratios of infection. The results showed that the infection rate women was higher than among men, with infection rates distributed as follows: The incidence among women was 74%, which reflects women's exposure to greater risk factors for Candida infection, such as vaginal infection or the influence of hormones .The infection rate among men was lower, reaching 26%. This is often associated with a urinary tract infection or prolonged use of antibiotics . These results are consistent with Singh *et al*.,(2012), who reported a higher rate of urinary 32 colonization by fungi in females in intensive care units. Physiological and hormonal factors may contribute to this increased fungal colonization in females, highlighting the need for close monitoring of this population that have shown higher rates of infection among women compared to men. The importance of directing health awareness programs is highlighted towards prevention, especially among women, focusing on risk factors such as chronic diseases and uncontrolled. Results showed that the percentage of *C.albicans* isolation represented the larg percentage of the species isolated from the various clinical samples mentioned above, and this agrees with (Al-Obady, 2017), as five species belonging to the genus *Candida* were isolated from the oral cavity, vagina, and urine, and was *C.albicans* is the most prevalent and also agrees with study (Hussain, 2011). isolated five species belonging to the genus *Candida* from the or oral cavity *C.albicans* at the forefront, followed by the follow *C.tropicalis*, *C. parapsilosis*, *C. sake* .

Using the Phoenix M50 system

This study align with several previous studies that focused on fungal species identification and the use of advanced diagnostic techniques such as the Phoenix system as study (Masoud and Elmajbery, 2021), the Phoenix 100 apparatus was used, while this study employed the Phoenix M50.This similarity in diagnostic tools enhances the reliability of the findings from both studies and underscores the value of the Phoenix system in providing accurate and rapid fungal species.

Antifungal Susceptibility Testing

Regarding the antimicrobial susceptibility testing used in this study, which included CLO 50, ITC 50, FLUO 25, KCA 10, and VO 1,this study showed that CLO 50 was the most effective against all tested *Candida* species. This aligns with the results of several previous studies, such as the study by Ali *et al*., (2024), which demonstrated high efficacy of certain antifungal agents against *C. albicans* and other species. Singh *et al*., (2020) also indicated the effectiveness of Voriconazole, which is comparable to CLO 50 in some 33 contexts. this study suggests that CLO 50 is the most effective option, which is consistent with the findings of Elmanama *et al*., (2020), where other studies showed that CLO 50 or similar drugs like Amphotericin B were effective in treating *Candida* infections in general. In this study, the results showed that ITC 50 was the least effective on all tested species, which aligns with previous studies such as the one by Ali *et al*., (2024), which demonstrated resistance of several species to Fluconazole and Itraconazole. This indicates that there are challenges in using ITC 50 with certain species like C. albicans and *C. krusei,* as pointed out by Singh *et al*. (2013). In this study the effectiveness of FLU 25 was moderate and varied, showing potent activity against *C. tropicalis* but less effective against *C. albicans*. This aligns with the results Othman *et al*., (2018), found that some species, like *C.albicans*,exhibited resistance to certain antifungals like Fluconazole. This suggests that FLU 25 may not be the best option for all species. Furthermore, these findings are consistent with the study by Ali *et al*.,(2024), which showed resistance to Fluconazole in several species but greater effectiveness against other species such as *C. tropicalis* and *C. sake*.

Conclusion

The results of this study confirm that *Candida albicans* is the most prevalent *Candida* species, particularly in urine and vaginal samples, with urine identified as the most favorable environment for *Candida* proliferation. The study also found that females have higher infection rates compared to males. Furthermore, antifungal susceptibility testing revealed variability in drug effectiveness, with FLUO 25 being the most effective agent against the isolated species. This study highlights the importance of using advanced diagnostic techniques such as the *Phoenix M50* system to improve isolation accuracy and detect antifungal resistance patterns. It also emphasizes the need for pre-treatment antifungal sensitivity testing to ensure optimal therapy, especially in light of increasing resistance in some species to conventional antifungal agents.

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