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**ISOLATION AND MOLECULAR IDENTIFICATION OF CANDIDA
SPECIES FROM URINARY TRACT INFECTION IN HAWIJA
CITY**

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ISOLATION AND MOLECULAR IDENTIFICATION OF CANDIDA SPECIES
FROM URINARY TRACT INFECTION IN HAWIJA CITY

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ABSTRACT

ISOLATION AND MOLECULAR IDENTIFICATION OF CANDIDA SPECIES FROM URINARY TRACT INFECTION IN HAWIJA CITY

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Master of Science in Biology

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The study investigated the isolation and identification of *Candida spp.* from the urinary tract of different ages and genders in Hawija. The study included separating and identifying the species from the urinary tract using culture media and PCR technique, as well as identifying the species that spread the infection on a large scale, determining the age and gender most susceptible to *Candida*, and studying the relationship between candiduria and other diseases such as diabetes. The total sample was 125 samples, 23 samples appeared (18.4%) positive patients for *Candida* infection, while 81.6% (102) were negative. 23 of *Candida* infections were due to the *Candida albicans* group. In terms of gender, urinary tract infections are most often observed in older and pregnant females than in men. Referring to the variables of sugar, cumulative sugar, and age in candidiasis indicated that there were significant differences between the group of patients when compared with the control group, where the percentage of candidiasis infection with an average means of 50 years. The means of diabetes mellitus and cumulative glucose were higher in patients with *Candida*. Women were more likely to contract candidiasis than men. Where 23 results showed that *C. albicans* in sample No. 1 with a percentage of 99.60%, the second sample by 97.62%, and the third sample with 99.41%. The fourth with 95.81%, the fifth with 99.61%, the sixth with 87.55%, the seventh with 99.40%, the eighth with 99.83%, the ninth with 99.21%, and the tenth with 91.58 %. The results indicated that the percentage ranged between 95-100. The percentage of the spread of *C. albicans* in the targeted area in abundance, which amounts to 40% of the samples that were positive for the infection in the culture tests,

while the remaining results indicated that 60% of the *Candida* spread less according to the results of the studies.

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Keywords: *Candida* spp, Urinary tract, Chromagar *Candida*, PCR assay



ÖZET

HAWIJA ŞEHRİNDE İDRAR YOLU ENFEKSİYONUNDAN CANDIDA TÜRLERİNİN İZOLASYONU VE MOLEKÜLER TANIMLANMASI

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Bu çalışma ile Hawija'da farklı yaş ve cinsiyetlerin idrar yollarından *Candida spp* izolasyonunu ve tanımlanmasını araştırıldı. Çalışma, kültür ortamı ve PCR tekniği kullanılarak üriner sistemden türlerin ayrılması ve tanımlanmasının yanı sıra enfeksiyonu geniş çapta yayan türlerin belirlenmesi, *Candida*'ya en duyarlı yaş ve cinsiyetin belirlenmesi ve candidüri arasındaki ilişkinin araştırılmasını içeriyordu. Toplam örnek 125 idi, 23 örnekte (%18.4) *Candida* enfeksiyonu için pozitif hasta görüldü, %81.6'sı (102) negatifti. Cinsiyet açısından bakıldığında, idrar yolu enfeksiyonları erkeklere göre yaşlı ve hamile kadınlarda daha fazla görülmektedir. Diyabet, kümülatif diyabet ve yaş değişkenlerine atıfta bulunulan kandidiyazis hastalarında, yaş ortalaması 50 olan kandidiyazis enfeksiyonu yüzdesinin kontrol grubu ile karşılaştırıldığında hasta grupları arasında önemli farklılıklar olduğunu göstermektedir. *Candida*'lı hastalarda diabetes mellitus ve kümülatif glukoz ortalamaları daha yüksekti. Kadınların kandidiyaza yakalanma olasılığı erkeklerden daha yüksekti. Burada sonuçlar *Candida albicans*'ın %99,60'lık ile 1 No'lu numunede, ikinci numunede %97,62'lik ile ve %99,41'lik ile üçüncü numunede olduğunu göstermiştir. Dördüncüsü %95,81, beşincisi %99,61, altıncısı %87,55, yedincisi %99,40, sekizincisi %99,83, dokuzuncusu yüzde %99,21 ve onuncu %91,58 ile numunede olduğunu göstermiştir. Sonuçlar, yüzde 95-100 arasında olduğunu gösterdi. *C. albicans*'ın hedeflenen alanda bol miktarda yayılma yüzdesi, kültür testlerinde enfeksiyon için pozitif olan örneklerin

%40'ına tekabül ederken, kalan sonuçlar çalışmaların sonuçlarına göre Candida'ların %60'ının daha az yayıldığı tespit edilmiştir.

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Anahtar Kelimeler: Candida spp, İdrar yolu, Chromagar Candida, PCR testi



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LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees Celsius
g	Gram
L	Liter
mg	Milligram
min	minutes
mL	Milliliters
mM	Millimoles
ng	Nanogram
μL	Microliter

LIST OF ABBREVIATIONS

ALS	Agglutinin-like sequence
BLAST	Basic in-situ search tool for nucleotide sequencing
CFU	Colony-forming unit
DSPS	Delayed sleep phase syndrome
NACA	Non-albicans Candida
NASBA	Nucleic acid sequence-based amplification
NCBI	National Center for Biotechnology Sequencing Information
PCR	Polymeric chain reaction
PIFs	Prolactin inhibiting factors
RCTs	Recent randomized monitoring trials
RPL	Recurrent pregnant loss
RSA	Recurrent spontaneous abortion
SNP	Single nucleotide polymorphism
WHO	World health organization
UTI	Urinary tract infections

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1. INTRODUCTION

For both nosocomial and community-acquired infections, urinary tract infections (UTIs) are among the most frequently diagnosed illnesses (Sardi *et al.* 2013). The causes of UTI are fungus and bacteria. According to certain data, the percentage of UTIs caused by *Klebsiella pneumoniae*, fungus, and *Streptococcus agalactiae* has increased while the percentage of *Proteus* species, *Eschericia coli*, and *Pseudomonas* species has decreased. In recent decades, the frequency of UTIs brought on by fungi, particularly *Candida* species (candiduria), has grown by two to three times (Behzadi *et al.* 2015, Bongomin *et al.* 2017). There are asymptomatic and symptomatic variants of candiduria. The majority of people who excrete *Candida* in their urine don't exhibit any symptoms. On the other hand, individuals with pyelonephritis, renal candidiasis, cystitis, epididymorchitis, and prostatitis experience symptomatic candiduria (Fisher *et al.* 2011). It's possible for deep fungi infections to cause candiduria. Studies demonstrate that most situations associated with a reported development of *Candida* in urine cultures are temporary and unrelated to systemic infection (Rivett *et al.* 1986, Fisher *et al.* 2011). However, candiduria develops in 10% of blood infections brought on by *Candida* (Odds and Davidson 2000). *Candida* infections of the urinary tract and kidneys can produce discomfort, hematuria, dysuria, micturating, and pyuria as frequent symptoms (Rivett *et al.* 1986). Rarely do these illnesses happen without any risk factors. In this regard, a number of significant risk factors for these infections include organ transplantation, diabetes, a protracted hospital stay, antibiotic usage, recurrent bacterial infections, aging, and catheter use. The use of a catheter, particularly in patients referred to intensive care units, is the most frequent risk factor for candiduria (Kauffman *et al.* 2000). In this regard, a research looking into UTI found that *Candida* species caused UTI in 26.5% of persons using catheters (Vaezi *et al.* 2017). As a result of organisms adhering to and colonizing catheter surfaces, they might adhere to the bladder's mucosa and irritate it (Almirante *et al.* 2006). *Candida albicans*, a member of the *Candida* genus, is the pathogen that has been identified from the majority of clinical samples (Rudramurthy *et al.* 2017). However, *Candida glabrata* was identified as the dominating species in a global surveillance investigation (Cortese *et al.* 2018). Other

Candida species isolated from UTIs include *Candida krusei*, *Candida tropicalis*, *Candida guilliermondii*, and *Candida parapsilosis* (Sobel *et al.* 2011, Kalantar *et al.* 2015). The epidemiology, risk factors, and species distribution of candiduria have all been studied in a few studies with limited sample sizes. It appears that the difficulty to separate the colonization of infections makes the definition of candiduria challenging (Fisher *et al.* 2011). Repeat urine cultures and imaging investigations that identify infection foci are efficient methods for establishing a differential diagnosis in individuals with candiduria (Maharjan *et al.* 2018). Because *Candida* strains differ in their antifungal susceptibility patterns, it is necessary to distinguish between species even if candiduria patients have a low death rate (Bono and Reygaert 2021). For instance, fluconazole medication can cause *C. glabrata* related UTI to develop. The correct therapy and preventing the condition from becoming chronic depend on identifying the microbial agent that caused the infection. On the other hand, some research indicates that managing UTIs by eliminating risk factors is more beneficial than treating them (Fisher *et al.* 2011). In light of this, the current study's objectives were to identify the prevalence rate of candiduria and ascertain the contribution of predisposing variables to the development of this infection. ITS4 and ITS5 primers were used to amplify DNA. Following the reference approach, phenolchloroform and isoamyl alcohol were used to isolate genomic DNA (Behzadi and Behzadi 2011). Using general fungal ITS5 and ITS4 primers, which amplify areas with 650 bp fragment length, PCR was carried out, encoding ITS4 and ITS5 (ITS5: 5'GGAAGTAAAAGTCGTAACAAGG-3', ITS4:5'-TCCTCCGCTTATTGATATGC-3'). Using the ITS4 primer and the Applied Biosystems 3730 XL Bioneer (Korea), all PCR-amplified products were sequenced. Through local blast, a sequence search was carried out using a molecular database kept by the NCBI (Marchler-Bauer *et al.* 2002).

2. LITERATURE REVIEW

2.1 The Urinary Tract

The kidneys, ureters, bladder, and urethra make up the urinary system, commonly referred to as the urinary tract or renal system. The urinary system's functions include removing waste from the body, controlling blood volume and pressure, electrolyte and metabolite levels, and blood pH. The body's drainage system for eventually eliminating urine is the urinary tract. The renal artery and renal vein provide the kidneys considerable blood supply and drainage, respectively. Nephrons are functional subunits that make up each kidney. Wastes (in the form of urine) leave the kidney through the ureters, tubes made of smooth muscle fibers that carry urine to the urinary bladder, where it is stored after additional processing and blood filtering. The urinary system is made up of two kidneys, a bladder, two ureters, and a urethra.

Kidneys, two organs that are about the size of a fist and resemble beans. Its just below ribs on either side of spine. To remove toxins and keep fluid balance, kidneys filter 120 to 150 24 h/L quarts of blood each day. This method results in a daily pee production of 1 to 2 L quarts (McKechnie 1981).

Ureters, transfer urine from the kidneys to the bladder via very thin muscle fibers (Hanna *et al.* 1976).

Before being ejected through the urethra, the urine produced by the kidney's nephrons is carried to the urinary bladder for storage. The bladder of the urinary system is a sac that stores urine. When filled with urine, it extends into the belly from its location in the extraperitoneal area of the pelvis, which is behind the pubic bones. The body and apex, which are located above the ureteric orifices, make up the upper portion of the bladder. The fundus, trigone, and neck make up the lower portion of the bladder. In healthy people, the bladder can hold roughly 500 mL (Sellers *et al.* 2018). Two pairs of muscles

in the urinary system function as a sphincter to block the urethra and hold urine in the bladder between journeys to the bathroom.

Up until your brain transmits instructions to pee, the internal sphincter muscles of the bladder neck and urethra remain closed.

The muscles of the external sphincter surround the internal sphincter and apply additional pressure to maintain the urethra's closure. To prevent urine leakage, you can intentionally contract the pelvic floor muscles and external sphincter (D'Ancona *et al.* 2019).

2.2 Fungi

Fungal infections are a major source of morbidity and mortality globally and are regarded as a severe health issue, especially in those with immune system impairments (Vallabhaneni *et al.* 2016). Infections caused by fungi have significantly increased during the past two decades. Increasing numbers of people with weakened immune systems, have been linked to this high prevalence (Ortega *et al.* 2010, Junqueira *et al.* 2012, Li *et al.* 2015, Terças *et al.* 2017).

Candidiasis is the most prevalent fungus related illness impacting people worldwide (Lewis *et al.* 2012, Kwamin *et al.* 2013, Ferreira *et al.* 2013). Cutaneous candidiasis, onychomycosis, mucosal candidiasis, and systemic candidiasis are a few of the several kinds of candidiasis (Kim and Sudbery 2011). A crucial truth is that both individuals with weakened immune systems and healthy individuals can contract candidiasis (Raman *et al.* 2013). Another illness caused by *Candida* spp. is called candidemia, which is the most important and common nosocomial fungal infection and has a significant death rate (up to 49%) in patients with weakened immune systems. Depending on the patient's health, age, and location, there are different associations between *Candida* and bloodstream infections. Although *Candida* species are only the primary cause of sepsis or septic shock in less than 5% of cases, candidemia is such a

significant infection that it is linked with these conditions in 10% to 40% of cases (Sardi *et al.* 2013).

Numerous species from the genus *Candida* have been found in human samples, and over half of these species have been linked to severe illnesses. The bulk of fungal infection isolates still come from the most common species, *C. albicans* (Li *et al.* 2015, Terças *et al.* 2017). However, there are now much more instances of other *Candida* species. These species are *C. parapsilosis*, *C. famata*, *C. guilliermondii*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. metapsilosis*, *C. orthopsilosis*, and *C. lusitaniae* (Li *et al.* 2015, Kaur *et al.* 2016).

When it comes to the availability of osmolarity, nutrients, pH, temperature change, and the amount of accessible oxygen, *C. albicans* is a species that exhibits a great degree of adaptability. The genus *Candida* poses a severe threat to human health because to this trait, the high antifungal resistance of the species, their pathogenic characteristics, and their propensity to create biofilms with other species. Because of this, *Candida* species are very adaptive and have a variety of survival techniques that might influence their proliferation and alter their susceptibility profiles (Paramythiotou *et al.* 2014).

Furthermore, it might take a while to pinpoint a specific *Candida* species, which could prevent the adoption of precise medicines. For instance, several species of *Candida* cannot be distinguished by microbiological testing utilizing certain growth medium. Frequently, it takes several days to learn about a *Candida* species' antifungal susceptibility (Clancy and Nguyen 2013). Although there are newer molecular methods, including fluorescence in situ hybridization, commercially available analysis tools cannot tell *C. glabrata* from *C. albicans*, *C. parapsilosis*, or *C. krusei* when quickly detecting yeast (PNA-FISH). These statistics are important since inadequate antifungal medication is delayed and is linked to an increased death rate (Bassetti *et al.* 2014).

2.3 Epidemiology and Mechanisms of Resistance

Nearly 1.4 million people die from invasive fungal infections each year, making them a significant contributor to morbidity and mortality worldwide (Brown *et al.* 2012). Fungi make up the bulk of eukaryotes in the human gut microbiome, which invade the human host at a number of different body regions. When immune systems are suppressed or natural barriers are broken, the majority of these organisms have the capacity to act as opportunistic pathogens (Huseyin *et al.* 2017). Bloodstream fungal infections, which are predominantly caused by yeasts belonging to the genus *Candida*, are associated with significant death rates (45-75%), and provide a major risk to those with weakened immune systems, such as those with AIDS and cancer, those who have undergone organ transplants, and premature infants. As the prevalence of fungal infections has grown, so has the usage of antifungal drugs for both treatment and prevention. Due to the scarcity of antifungal medication classes, there are sadly very few therapy choices for invasive fungal infections. Azole antifungals, fungistatic drugs that target the formation of membrane sterol, have been the main prophylaxis/therapy for *Candida* infections, with *C. albicans* being the predominate infecting species, for a long time (e.g., fluconazole). The extensive usage of echinocandin antifungal medications is a result of epidemiological changes in infecting organisms toward non-albicans *Candida* species that are either naturally azole resistant (like *C. krusei*) or rapidly develop resistance (like *C. glabrata*). *C. albicans* is the most common bloodstream infection in therapeutic settings. However, *C. glabrata* infections have been on the rise for a number of years and are now the second most frequent *Candida* bloodstream infection in North America, accounting for 18–25% of all *Candida* isolates. It serves as the main bloodstream fungal pathogen in various situations, such as those involving patients with hematological malignancies (Farmakiotis *et al.* 2014).

2.4 Urinary Tract of Candidiasis

Candida infections of the urinary system are the most common nosocomial fungi in the globe. The most frequent cause of nosocomial fungal urinary tract infections is *C. albicans*, but the distribution of *Candida* species is changing quickly. Likewise, the rise

in urinary tract candidiasis has prompted the emergence of *Candida* species that are resistant to antifungal medications. They take a close look at the uropathogenesis of *C. albicans* and the three most prevalent *Candida* species that cause urinary tract candidiasis and their global distribution (Behzadi *et al.* 2015).

C. albicans and unrelated organisms in a wide variety of healthy individuals, *Candida* (NACA) species are regarded as significant components of the microbial normal flora in the vagina, alimentary canal, and oral cavity. Furthermore, in premenopausal and healthy females, they proliferate on the outside of the urethral orifice. Immune deficiencies may be the root of an imbalance between NACA yeasts, *C. albicans*, and the other host normal flora. In this state, the host may develop candidal UTIs as a result of the commensal yeasts of *Candida* changing into opportunistic pathogenic bacteria (Fisher *et al.* 2011).

Candiduria, *C. albicans* and NACA species can be found in urine in both asymptomatic and symptomatic UTIs (Voltan *et al.* 2014, Behzadi *et al.* 2015).

2.5 Urinary Tract Infection

Typically, a simple UTI simply affects the bladder. Germs invading the bladder mucosal membrane result in cystitis, an inflammatory reaction. The primary cause of UTIs is enteric coliforms, which commonly exist in the periurethral vaginal introitus. These germs go up the urethra and into the bladder to create the UTI. A UTI is frequently brought on by sexual activity because it encourages bacterial invasion of the bladder. Regular urination and bladder emptying reduces the risk of a UTI in most people (Maharjan *et al.* 2018).

The perfect environment for bacterial development is urine. It is less suited for bacterial development when there are organic acids, a pH lower than 5, and significant quantities of urea. It is also known that frequent urination and large urine volumes reduce the incidence of UTI (Bono and Reygaert 2021).

2.6 Candidiasis Epidemiology

Under physiological settings, the genus *Candida* exists as saprophytes, populating the mucosal surfaces and external genitalia of both males and females, although they are abundantly present close to the urethral meatus of healthy women of reproductive age (Fisher *et al.* 2011, Sardi *et al.* 2013). In <1% of cases, measurable levels of *Candida* species can be identified in urine samples from healthy persons who have been voided. However, they account for 10% or more in tertiary-care hospitals and specialty institutions and 5% of all positive urine culture findings in primary care settings (Fisher *et al.* 2011). A typical clinical finding is the presence of a *Candida* species in the urine, or candiduria. Up to 90% of patients with *Candida* UTIs reportedly required a urinary catheter and were hospitalized (Fisher *et al.* 2011, Yashavanth *et al.* 2013, Padawer *et al.* 2015, Datta *et al.* 2018). Especially if the patient exhibits no symptoms, candiduria in adult individuals may commonly indicate colonization or contamination of the samples (Jacobs *et al.* 2018). Doctors should not dismiss this clinical finding quickly as the presence of yeasts in the urine may very well be connected to their function in the genesis of UTIs or it may be an indicator of or an underlying illness in the genitourinary system (e.g. vulvovaginal candidiasis). Additionally, candiduria may be a sign of disseminated candidaemia, which has a mortality rate of 30–40% (Pongrácz *et al.* 2015, Şular *et al.* 2018). A *candida* UTI is primarily distinguished by $10^5 < \text{CFU/mL}$ of yeasts discovered in urine, which often matches with the patient's symptoms, as opposed to candiduria, which is defined as 10^4 - 10^5 CFU/mL of yeasts found in urine (Fisher *et al.* 2011). *Candida* species can reach the upper urinary tract through the circulation, but they can also climb to the urinary tract through a colonization focus that is either close to or right at the urethra (retrograde infection pathway) (antegrade infection route). The female sex, diabetes mellitus, extremes of prolonged hospitalization, age, admission, recent use of broad-spectrum antibiotics or immunosuppressants, intensive care unit (ICU), dysfunction of the bladder and urinary stasis, UTI, transplantation, nephrolithiasis, congenital or structural abnormalities of nephrolithiasis, catheterization, and concurrent bacteriuria or bacterial UTIs are risk factors for *Candida* urinary tract and candiduria (Sobel *et al.* 2011). It is known that HIV-positive women are more likely to develop vaginal colonization of *Candida* species, frequently ones that are not *C.*

albicans. Additionally, numerous studies have shown that HIV-positive patients have higher levels of *Candida* species colonization on nearly all mucosal surfaces, which combined with higher levels of manifested *Candida* infections, particularly those of the genitourinary tract, will be seen as a result of the patient's general immunosuppression (Ribeiro *et al.* 2020). The fact that yeasts typically have poor adherence to bladder mucosa and no tropism regarding the tissues of the urinary system further emphasizes the significance of the aforementioned underlying factors in the development of candiduria. Given the wide regional variation in the epidemiology of *Candida* UTIs and candiduria, it is critical to review local data in order to assess changes over time and consider how the national situation stacks up against global statistics.

2.7 Candidiasis

A superficial infection of the mouth, vagina, or skin that results in white or red patches, itching, irritation, or both is the most prevalent kind of candidiasis (Rafiq 2021).

The esophagus and other internal organs may get seriously infected in people with weaker immune systems (Talapko *et al.* 2021).

Under a microscope, a sample of the contaminated material is analyzed before being submitted to a lab for cultivation. Antifungal treatments can be ingested or given topically to the afflicted region, although intravenous drugs are needed for severe infections (Lombardi and Ouanounou 2020).

Candida is usually found on the skin and intestines, and for women, in the genital area. *Candida* usually causes no problems when present in these areas, but the fungus sometimes causes a skin infection or an infection in the mouth (infects the mucous membrane) or an infection in the vagina. Even those with strong immune systems are susceptible to contracting this fungus, but those with diabetes, cancer, AIDS, and pregnant women are more likely to do so or for a longer period of time. People with AIDS frequently have candidiasis especially oral and esophageal candidiasis. Due to the

fact that antibiotics eradicate the normally occurring bacteria in a person's body that compete with *Candida*, and *Candida* can spread uncontrolled in persons who use antibiotics (Rowan-Nash *et al.* 2019).

2.8 Adhesion and Invasion of Candidiasis

There are currently no comprehensive studies of *Candida* species in the gut environment that just focus on commensalism. Additionally, nothing is known about the function of adhesins during *Candida* colonization of the gastrointestinal system (Witchley *et al.* 2019). The present understanding of adhesins, their function in different niches that *Candida* species occupy, and their possible function during intestinal colonization will be covered in this subsection. The agglutinin-like sequence (ALS) gene family contains the *C. albicans* adhesins that have been the subject of the most research (Hoyer 2001). Which code for a collection of sticky GPI-anchored proteins. Previous research has shown that these proteins are located on the surface of fungus cell walls (Hoyer *et al.* 1999). A structure mostly made of mannans, glucans (β -1,6 and β -1,3) and chitin (Gow *et al.* 2017). There are eight members of the ALS gene family (ALS9 and ALS1 - ALS7), with ALS1 and ALS3 receiving the most research. Different ALS family members are expressed during *C. albicans* yeast and hyphal morphologies (Almeida *et al.* 2008). Hyphae are the principal source of expression for ALS3 and the non-ALS adhesin Hyphal Wall Protein 1 (Almeida *et al.* 2008). In fact, it's possible that the distinctive array of adhesins is necessary for adaptation to the various niches filled by *Candida* species. Early research has revealed that *C. albicans* is more adept at adhering to vaginal epithelial cells, uroepithelial cells, and buccal epithelial cells (Romo and Kumamoto 2020), comparing intestinal epithelial cells and monolayers generated from epithelium to different *Candida* species in vitro (Modrzewska and Kurnatowski 2015). More recent research have started to clarify specific functions, even if these investigations did not explicitly address the function of certain adhesins (Martin *et al.* 2013). The hyphal specific adhesin, ALS3, was demonstrated by Zakikhany *et al.* (2007). to be significantly increased during epithelial infection in vitro and that ablation of ALS3 reduces this adherence. Similar to this, ALS2 deletion reduces adhesion. Importantly, deletion of ALS5, ALS6, or ALS7 increased adhesion, emphasizing the

intricate functions of adhesins. Additionally, The hyphal-specific adhesin HWP1 is significantly expressed during colonization and infection of the oral epithelium, and research utilizing mice with oropharyngeal candidiasis has demonstrated that its deletion decreases pathogenicity. This article provides a summary of their current understanding of adhesins' function in tissue culture systems and animal models. These findings coupled with the knowledge that *C. humilis* gastrointestinal tracts can be colonized by *albicans* very successfully (Shao *et al.* 2019), assert that several adhesins may be necessary for intestinal colonization and that specific adhesins are indeed needed for *C. albicans* to engage successfully with the various environmental niches it encounters.

2.9 Molecular Diagnostic Methods

Tools for the clinical diagnosis of candidiasis have been developed using PCR-based approaches (Kanbe *et al.* 2005). Within 1 hour after the isolation sample, PCR enables the detection of *Candida spp.* high specificity and sensitivity characterize this technique. When primers are used to selectively amplify the internal transcribed spacer (ITS) region of fungal ribosomal DNA, PCR may also be used to confirm the presence of fungus. On the other side, frequent use of PCR during labor would necessitate a substantial capital expenditure for additional equipment, local availability of a 24-hour period, and higher cost per test (Wei *et al.* 2010). An organism may be identified down to the species level with the use of certain oligonucleotide probes, and they can potentially reliably identify mixed fungal infections. These methods consist of PCR and NASBA (Nucleic acid sequence-based amplification). Target sequences in PCR-based approaches may be either species specific, *Candida* genus-specific, or universal panfungal sequences at this time. Molecular tactics currently supplement traditional methods and are only employed in speciality laboratories. Target sequences most frequently use the rRNA gene, which has excellent specificity and sensitivity for *Candida* infections ranging from 78% to 100% (Alexander and Pfaller 2006, Trtkova and Raclavsky 2006). While nested PCR boosts detection sensitivity and specificity, it also amplifies the likelihood of false positive findings. Since NASBA is a particular and sensitive RNA, real-time PCR enables quick detection of the amplicon synthesis.

Six different *Candida* species may be detected using an amplification approach. NASBA is more costly than PCR since it only identifies active yeast cells and doesn't require a heat cycling equipment (Trtkova and Raclavsky 2006).

2.10 *C. albicans*

One may consider *C. albicans* to be a tautology. *Candida* derives from the latin word candidus, which means white. The latin word albicans, which means turning white, is the present participle of albic. It is a tautology since this causes white to become white.

It is frequently abbreviated as *Candida*, candidiasis, or thrush. *C. albicans* has been referred to by more than one hundred different synonyms (Akah 2018). The *Candida* genus contains descriptions of more than 200 species. The first mention of thrush, most likely brought on by *C. albicans*, appears in Hippocrates' book of the Epidemics, which goes back to 400 BCE and describes oral candidiasis, as shown in Table 2.1 (Vila *et al.* 2020).

Table 2.1 Classification of *C. albicans*

CLASSIFICATION	TYPES FUNGI
Kingdom	Fungi
Division	Ascomycota
Class	Saccharomycetes
Order	Saccharomycetales
Family	Saccharomycetaceae
Genus	<i>Candida</i>
Species	<i>C. albicans</i>

2.11 Morphology

Due to phenotypic flipping and the transition from bud to hypha, *C. albicans* displays a diverse spectrum of morphological morphologies. Environmental variables trigger the quick yeast-to-hyphae transformation (filamentation). When it occurs more slowly and spontaneously, phenotypic flipping can result in as many as seven distinct phenotypes in

certain strains. The white to opaque changeover mechanism has been the subject of the most research (an epigenetic process) (Wooten *et al.* 2021). There have also been descriptions of other systems. David R. Soll and colleagues found two systems (the white to opaque switching system and the highfrequency switching system) (Vico *et al.* 2021). Environmental factors including the amount of CO₂, the anaerobic environment, the medium being utilized, and the temperature can frequently but not always affect switching in *C. albicans*. *C. albicans* is a yeast that is between 10 and 12 microns in size. Chlamydo spores, which are spores that can grow on the pseudohyphae, can survive in harsh settings like dry or hot seasons (Bartnicka *et al.* 2019).

2.12 C. albicans and Polymorphism

The mortality is minimal in UTIs caused by *C. albicans* despite the high prevalence of morbidity. However, individuals with AIDS and systemic candidiasis have a significant death rate. The method of fungal colonization and infection is determined by *C. albicans*' morphology. The three types of *C. albicans* include septate truehyphae, which are septate pseudohyphae with loose septa, and single-celled budding yeast, which has an ovoid, spherical shape. Documented data show that yeast cells and genuine hyphae both contributed to UTI candidiasis, and *C. albicans*' pseudohyphal form is thought to be a switch-building variant of the fungus *in vivo*. In the life cycle of *C. albicans*, an apparent morphological evolutionary route may be detected.

The *C. albicans* filamentous form is an invasive morphology of the fungus that is seen in solid tissues, including the kidneys, and is capable of producing a significant number of proteases. These enzymes have an increased capacity for hydrolysis, disruption, and progression inside the host tissues. Contrarily, the yeast form of *C. albicans*, which has a limited capacity for invasion, is an efficient pathogenic morphology for spreading across the host's mucosal membranes and liquid form structures (Mayer *et al.* 2013, Behzadi *et al.* 2015).

2.13 Adhesion and Invasion

C. albicans uses a unique group of proteins termed adhesins in both life modes, including pathogenesis and commensalism, in order to successfully cling to host cells, other inanimate surfaces or *C. albicans* cells. As a result, the first and most crucial need for colonization of commensal or pathogenic strains of *C. albicans* is a solid attachment that can withstand being washed away. In the filamentous form of *C. albicans*, the activity of adhesion is mediated by two sets of protein families, including ALS (ALS9 and ALS1-7) and Hwp1 adhesins. ALS3 is the most important ALS protein for adhesion. Both the ALS and Hwp1 genes produce the aforementioned proteins, respectively (Brunke and Hube 2013, Behzadi *et al.* 2015).

However, in pathogenic strains of *C. albicans* hyphal structure, invasion is a natural process. Invasins are often used to orchestrate two complementary invasion processes in which host cells are invaded. To initiate endocytosis and the trojan horse mechanism, these mechanisms are comprised (hyphal active penetration).

Invasins, specific proteins found on the surfaces of hyphal cells, are the mediators of the triggered endocytosis mechanism. Both inactive and active fungal cells have the ability to attach to host cell ligands, such as endothelium cell N- and epithelial cell E-cadherin. The proteins ALS3 and Ssa1 are found in the most significant invasins. ALS3 is an adhesin-invasin protein that is used in the fungal hyphae of *C. albicans* for attachment and invasion. Additionally, ALS3 and Ssa1 function as invasins in *C. albicans* hyphal structures since they are both members of the heat shock protein 70 (HSP70) family. The early stages of invasion appear to need the triggered endocytotic process (Mayer *et al.* 2013, Wu *et al.* 2013).

2.14 *C. glabrata*

Several clinical symptoms, ranging from mucocutaneous overgrowth to bloodstream infections, are caused by *Candida* species, which are part of the natural microbiota of

the oral cavity, gastrointestinal, and vaginal tracts. *C. glabrata*, hitherto thought to be non-pathogenic, was quickly linked to a wide range of human illnesses. When patients exhibit any degree of immunosuppression, these clinical situations become increasingly frequent and difficult to treat year after year. Both *C. glabrata*'s ability to produce biofilms and its great resistance to conventional antifungal treatments contribute to these challenges. As a result, this review aims to provide an overview of the biology, epidemiology, and pathology of *C. glabrata* as well as outline an approach to the organism's resistance mechanisms based on recent research (Rodrigues *et al.* 2014).

2.15 C. krusei

C. krusei cultures on Sabourauds (SDA) medium were flat, dry, spreading, ground glass appearance, variants dull and no separated, glabrous, while Chromagar showed that pale pink colonies. Microscopic examination large, flat, dry with a delicate feathery periphery. Dalmau Plate Culture on Cornmeal with Tween 80 Agar (This in vitro approach has advantages in that it permits the media conditions to be varied to mimic different host environments. In addition, the concentration of agar can be varied to determine the effects of altering the rigidity of the matrix into which the cells invade, as this provides a better indicator of invasive growth than the ability to form hyphae in a liquid culture. Testing under multiple conditions can be used to identify mutant cells with the strongest defects. Therefore, protocols and media for analyzing invasive growth of *C. albicans* under different conditions will be described that are appropriate for testing a single strain or high-throughput analysis of a collection of mutant *C. albicans* strains) (Nadeem *et al.* 2010).

3. MATERIALS AND METHODS

3.1 Study Design

The current study was conducted on 125 patients suffering from urinary tract infections associated with diabetes for the age group from 25 to 55 in Hawija General Hospital from the period from April 2022 to June 2022, based on the diagnosis of the field supervisor in the sample test, the samples were divided into two groups:

- ❖ The first group 1: 95 patients who suffer from urinary tract infections .
- ❖ The second group 2: 30 people with urinary tract infection with diabetics.

We cultured urine on the medium of the sparoid agar using laboratory dishes. We used cotton, torch, lube, tubes to preserve urine and adhesive tape to prevent air from entering the cultured dishes. The dishes were left inside an incubator after being planted under sterile conditions to prevent contamination. The cultivation was close to a flame of fire to sterilize the atmosphere surrounding the cultivated sample. After cultivation, the sample was left for 48 hours, after waiting for the implantation period and the appearance of positive samples. By melting the media and cooling it to a temperature of 50°C, then under sterile conditions, we poured the agar into dishes and placed it in an opposite way to prevent water from touching the surface when evaporation occurs inside the medium. We took the positive samples and planted them on the prepared medium and waited for the mushroom growth period to know the species.

3.2 Materials

3.2.1 Laboratory instruments

In the Table 3.1, the materials used in the method of work and the origin of the materials used in the study

Table 3.1 The Equipment and instrument are used in this study

NO	EQUIPMENT AND INSTRUMENT	COMPANIES/ORIGIN COUNTRY
1	Incubator Memmart	Germany
2	Sensitive Balance Ohaus	USA
3	Sterile surgical gloves China	China
4	Incubator Memmart	Germany
5	Refrigerator Concord	Lebanon
6	Disposable Petri dishes 9 cm and 6 cm size China 9- Test tube China	China
7	Test tube China	China
8	Slides China	China
9	Hood Lab Tech	Japan
10	Hood Lab Tech	Korea
11	Rack	-
12	water bath	-

3.2.2 Agricultural media

In the Table shown 3.2, the culture media used in the study and the origin of the culture media.

Table 3.2 Agricultural media used in this study

No	Name of agar	The manufacture company	Origin
1	Sabouraud Dextrose Agar Medium (SDA)	Himedia	India
2	Chrom agar candida (cac)	Himedia	india

3.2 Electric Relay

100 bp DNA lader. BM301 Storage: 2-8°C for six months; At -20°C for 2 years Concentration: 0,12 mg/mL Description 100 bp DNA Ladder is a ready-to-load, mixed molecular weight tag containing eight linear double-stranded DNA fragments. The

DNA ladder is suitable for use as molecular weight standards for agarose gel electrophoresis.

The DNA ladder contains 100 bp-1.5 kb of DNA fragments. The 500 bp (100 ng/5 μ L) band doubled its intensity compared to the other reference band action bands. The ready-to-use molecular weight tag highlights the DNA size range from 100 bp to 1.5 kb. All bands of restriction enzyme digest plasmids. Brighter reference range 500 bp, make up 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1,000 bp, 1,500 bp buffer 10 mM Tris-HCl (pH 8.4), 10 mM EDTA, 0.02% bromophenol blue, 5% glycerol notes.

In general, we recommend using 100 bp of DNA ladder per mm of gel lane, or 5 μ L per gel lane. The 100 bp DNA ladder is not intended for accurate quantification of DNA mass but can be used for quantification of molecules by weight of linear double-stranded DNA fragments. Do not use the 100 bp DNA ladder with polyacrylamide gels. For long-term storage, store at -20°C. If stored at -20°C, mix well after thawing.

3.3 Primers

Molecular diagnosis was carried out using PCR technique depending on the heterogeneity in the ITS internal transcribed Spacer region to confirm the diagnosis of *Candida* species in this study, where the fungus DNA was extracted and its target region was amplified using all ITS1 and ITS4 primers as in Table 3.3.

Table 3.3 DNA primers and their nitrogenous base sequences used to confirm the diagnosis of *Candida* spp

NO	PRIMERS	SEQUENCES
1	ITS1	5'-TCCGTAGGTGAACCTGCGG-3'
2	ITS4	5'-TCCTCCGCTTATTGATATGC-3'

ITS1: Forward

ITS4: Reverse

3.4 Sterilization

The used glassware was sterilized in the electric oven at a temperature of 180 °C for a period of 30 minutes, and the culture media was sterilized with an autoclave at a temperature of 120 °C and a pressure of 15 pounds for a period of 15 minutes.

3.5 Laboratory Examinations of Samples

Urine swabs were examined in two ways:

3.5.1 Direct microscopical examination

A 10 min, 1500 rpm. centrifuge was used to separate four mL of urine. The supernatant was removed, one drop was deposited on a clean slide, and the glass was covered before being examined in 40X.

3.5.2 Indirect examination

The swabs taken on SDA solid sabroid medium were grown in plastic dishes, and the cultured dishes were incubated at 37 mm for 24-48 hours. During the incubation period, the characteristics were observed. The formalities of the yeast colonies, including the color, texture and shape of the colony on both sides of the plate, were neglected in which growth did not appear (Ellis 1994).

3.6 Isolation and Purification

Individual colonies were isolated and purified from all samples previously cultured on sabroid medium.

3.7 Identification

3.7.1 Morphological characteristics

The external appearance of the developing colonies on SDA solid sabrooid medium was examined and colony colour, shape, texture, diameter, height and odor (Ellis *et al.* 2007).

3.7.2 Microscopic characteristics

Take a portion of the colony mediated by the vector (loop) and mix with a drop of dye cotton blue lactophenol, then the sample was spread on a sterile glass slide and covered with a cover slide, then it was examined by light microscopy under the force of 10X and 40X to observe pseudohyphal and giant spores. A second smear was taken on another sterile glass slide, stained with chromium dye, fixed on a fire flame and examined for sprouting (Ellis *et al.* 2007) (Figure 3.1).

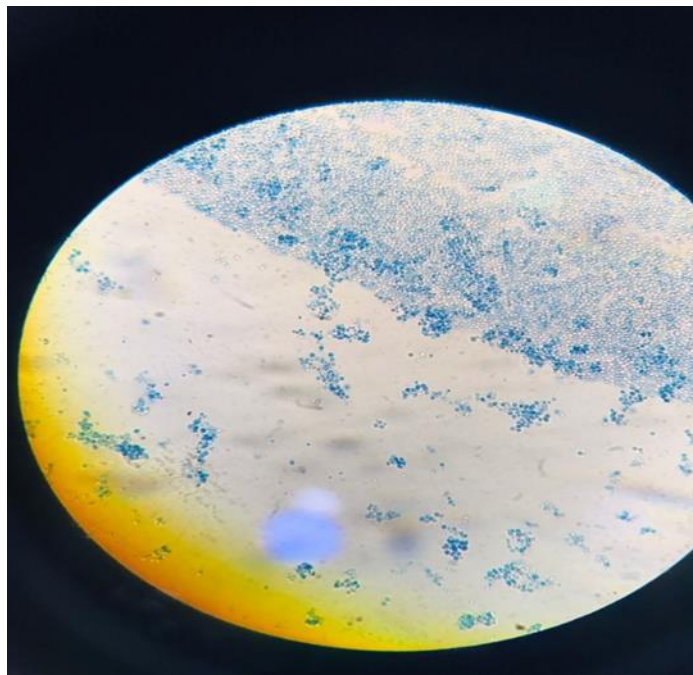


Figure 3.1 *C. albicans* yeast dyed with cotton blue lactophenol dye (40X magnification)

3.7.3 Sabouraud dextrose agar medium (SDA)

Prepared according to manufacturer's instructions, 65 g agar was dissolved in 1000 mL of distilled water, then antibiotic gentamycin (100 mg/L) was added and the medium was sterilized by steam autoclaving at 15 psi and 121 °C for 15 minutes. Cool to 45–50 °C, add 0.05 g/L gentamycin and pour into sterile petri dishes. The medium was used to isolate, culture, and preserve *Candida* isolates (Rey 2009).

3.7.4 Germ tube test

A tiny test tube containing 0.5 mL of human serum was pipetted, and the serum was then infected with a yeast colony using sterile wire loops. A drop of the serum yeast culture was transferred to a glass slide using a pasteur pipette, and the glass slide was then covered with a cover glass after the tube had been in a water bath at 35–37 °C for two–three hours. The 10 X and 40 X objectives were used to evaluate the preparedness. Yeast cells that created germ tubes had hyphal like extensions and didn't constrict at the place of origin (Figure 3.2).

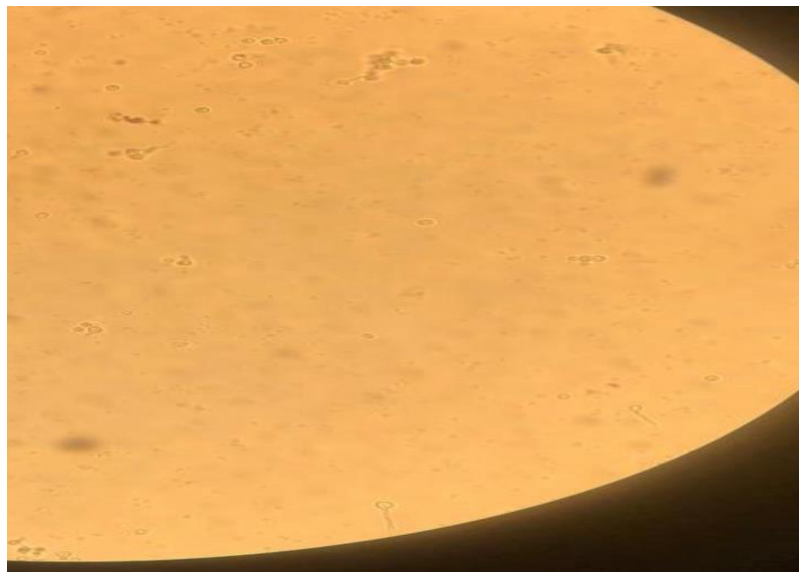


Figure 3.2 Germ tube test

3.7.5 Chrom agar Candida

The results of this study reveal that Chromagar *Candida* can accurately differentiate between *C. albicans*, *C. tropicalis*, and *C. krusei* based on colonial color and shape. Chromagar *Candida* has a 99% specificity and sensitivity for *C. albicans*, a 98% sensitivity for *C. tropicalis*, and a 100% sensitivity for *C. Krusei*.

3.8 Biochemical Tests

3.8.1 Growth test on chromium-agar-Candida medium

The test was carried out by taking a portion of the pure yeast colony by a sterile vector, aged 24 hours, developing on SDA medium and plotting it on chrom agar medium. The dishes were incubated at 37 °C for 24-48 hours. The strains were diagnosed according to the manufacturer's instructions, which can identify *Candida spp.* by the color of the colonies and their outward appearance. *C. albicans* is green and *C. tropicalis* is blue, while *C. glabrata* is light pink to cream, either *C. krusei* pink *C. lusitaniae* dark and pale pink to purple (Horvath *et al.* 2003).

3.8.2 Preservation of fungal isolates

The fungal isolates obtained after their growth were preserved by culturing them on SDA medium containing anti-chloramphenicol at a temperature of 25 °C for 7-14 days in a tilted SDA medium. After the incubation period, they were placed in the refrigerator at a temperature of 4 °C. For a period of two months, taking into account its renewal every two months and in duplicate for each isolate by reactivating it on SDA medium in a petri dish and the process was repeated again (Rey 2009).

3.8.3 Molecular diagnosis PCR

The PCR was performed in a private laboratory (Golden Steps) in Tikrit City to investigate *Candida spp.* The fungus DNA was extracted and the ITS target region was amplified using each of the primers. This diagnosis was made according to the following steps: The DNA was extracted from a pure and active yeast colony using a Chelex100 kit, making a solution of 100 Chelex from BioRad in the United States of America. The extraction was carried out according to the manufacturer's instructions as in the following steps:

1. Transfer about 200 mg of fungal growth colonies to a sterile ceramic container using liquid nitrogen. The fungal colonies were crushed and then transferred to sterile 1.5 mL tubes.
2. After that, 180 μL of universal digestion buffer solution and 20 μL of ProteinaseK enzyme were added to each sample and then mixed well using a homogenizer, then the samples were incubated at a temperature of 56 °C for 30 minutes using a water bath for each tube.
3. Add 100 μL of universal buffer PF solution and mix using a mixer and incubate the tubes at 20 °C for 30 minutes.
4. The samples were placed in a centrifuge at 10,000 rpm for 5 minutes, then the supernatant was transferred to a new 1.5 mL tube.
5. Add 100 μL of universal buffer BD solution and mix well with a mixer.
6. After that, 96% absolute ethanol was added to all samples and mixed well using a mixture.
7. Then the mixture was transferred to special tubes containing a DNA extraction filter equipped with the kit, which were placed in collecting tubes of a capacity of 2 mL, and then placed in a centrifuge at a speed of 12000 rpm for a minute, then the precipitate was disposed of.
8. After that, 500 μL of universal PW solution was added, then the tubes were placed in a centrifuge at 12000 rpm for 1 minute, and then the precipitate was discarded.

9. The 10-EZ containing DNA was placed in sterile 1.5 mL tubes and then The tubes were placed in a centrifuge at 12,000 rpm for two minutes to dry the 10-column membrane EZ was removed from alcohol and then the precipitate was discarded.
10. Adding 50 μ L of TE Buffer solution to dissolve the DNA inside the 10-EZ column filter and then incubated at room temperature for a minute and then all tubes were placed in Centrifuge at 12,000 rpm for 1 minute to collect DNA.
11. Measuring the purity of DNA for all samples in the Nanodrop a spectrophotometer

3.9 Statistical Analysis

The data of the experiment were statistically analyzed using the SPSS statistical analysis program, for the estimator, the averages and the standard error. The differences between the means were compared based on Dunkin's multiple-negotiation test at a probability level ($P \leq 0.05$).

4. RESULTS AND DISCUSSION

Of the total 125 samples, 23 samples appeared (18.4%) positive patients for *Candida* infection, while 102 (81.6%) were negative, 2 (8.6%) patients showed symptoms. 11 (47.8%) of 23 *Candida* infections were due to *C. glabrata* group. UTIs are most often observed in older and pregnant females than in men.

Using the questionnaire applied to the patients and analyzing the medical records, it was possible to verify some factors associated with UTI. In UTI patients, Diabetes were the most frequent disease. Among the variables analyzed for patients with UTI infection or not, we found that there were no statistically significant differences for the only risk factor for recurrent UTI in this population which was the presence of diabetes in patients ≥ 50 years.

4.1 The Patient Compared with Control Group

The results of the study, patients have significantly higher value than control group, in terms of age, RBS and HbA1c, where the percentage of *Candida* infection with an average age of 50 years.

Means of diabetes mellitus and cumulative glucose were higher in patients with *Candida*. Women were more susceptible to infection with candidiasis than men, as the information from patients indicated that the frequent use of antibiotics and consulting a specialist doctor is one of the most important causes of candidiasis in women.

Table 4.1 Mean and SD age, RBS, and HbA1c in this study

Parameter	Result		
	Control group	Patient group	P
Age (Year)	32.481 \pm 9.142	50.3051 \pm 13.643	0.0351
RBS (mg/dL)	83.791 \pm 16.748	124.637 \pm 26.758	0.0047
HbA1c (%)	4.3591 \pm 1.693	6.9514 \pm 2.85901	0.0491

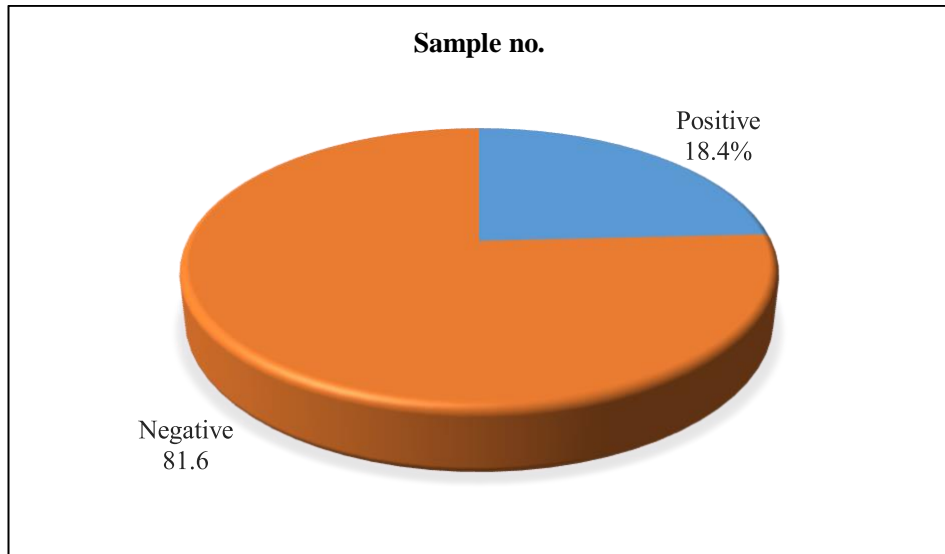


Figure 4.1 It shows the number of positive and negative isolates compared to the total

Macroscopic features: The type *C. albicans* appeared when planted on the media of the saproid dextrose agar in the form of white colonies of cream-colored, convex, with a smooth texture and grow in the incubation period 4-2 days at 37°C (Figure 4.3).



Figure 4.2 Yeast *C. albicans* on the medium of the sabroide dextrose agar, under 37 °C, aged 48 hours

4.2 Diagnosis Using Chromagar Candida Medium

By following the instructions of the aforementioned medium manufacturer and the criteria specified by researchers Odds Bemaerts 1994, 46 isolates were diagnosed to the species level, all of which appeared in light green, indicating that they are *C. albicans* (Figure 4.4). The examination was conducted for the purpose of confirming the diagnosis, as the medium chromium agar is one of the most efficient and reliable media for the diagnosis of *C. albicans*, as well as the possibility of achieving it in a short time. This result agrees with Nadeem *et al.* (2010) and Mohammed *et al.* (2009), the results also matched Manzoor *et al.* (2018).



Figure 4.3 *C. albicans* yeast on the medium chromium agar, under temperature 37°C, age 48 hour

It is also noted from Figure 4.4 that the percentage of *C. albicans* isolate was 43.47% of all urine samples that gave a positive result, and it is consistent with many studies that

showed that *C. albicans* is the most important cause of UTI among other causes. It has been demonstrated by Fisher *et al.* (2011), Helbig *et al.* (2013) and Behzadi *et al.* (2015). The high incidence of *C. albicans* in urine samples may reflect vaginal candidiasis through the rise of yeasts from the urogenital tract to the urogenital tract. It is also noted from the Figure 4.4, that the infection rate is lower than what was recorded by the study of Mohammed *et al.* (2009) in Kirkuk (30.6%) and other countries such as Egypt, Iran, Brazil and Turkey, in which the infection rates reached 34.5%, 48.35%, 42.63% and 44%, respectively (Omar *et al.* 2008, Mahmoudabadi *et al.* 2013, Voltan *et al.* 2014, Özer *et al.* 2016). It also does not agree with the same studies mentioned above by isolating it from other species other than *C. albicans*. Table 4.3 shows the distribution of samples.

Table 4.2 *Candida* positive samples results

CANDIDA OF SPECIES	NUMBER OF SPECIES	%
<i>C. albicans</i>	10	43.47
<i>C. glabrata</i>	11	47.82
<i>C. krusei</i>	2	8.2

4.3 Germ Tube Formation Test

Ten selected isolates of *C. albicans* showed their ability to form a germination tube, which is characterized by its parallel walls and the absence of constriction in the area of contact with the mother cell, as shown in Figure 4.5. The formation of the germ tube is a diagnostic characteristic of the isolated type, as well as being an essential feature in the transformation of yeast from its healthy symbiotic form to the pathogenic (filamentous) form (Torosantucci *et al.* 2004, Fazly *et al.* 2013) through its ability to penetrate the layer of epithelial cells lining the body and tissues and reach the bloodstream as well as its importance in feeding mushrooms (Sudbery *et al.* 2004). Found in the cell wall of the yeast *C. albicans* (Blostein *et al.* 2017) the germination tube, mother cell and bud were distinguished, as well as pseudohyphae.



Figure 4.4 Formation of the germ tube by yeast *C. albicans* in serum after two hours Incubation, at 37°C (400 x strength)

4.4 Isolated Yeast Diagnosis

The colony was examined microscopically after staining with cotton-blue lactophenol dye, and the shape of the cells appeared spherical to oval or single, longitudinal and bud shaped, and this result was identical (Ellis *et al.* 2007). Yeasts after chromium staining are more visible when compared to cotton blue lactophenol dye. The appearance of yeast cells stained blue due to the peptidoglycan layer retaining this dye in the cell wall (Sudbery *et al.* 2004), in which the outer cell wall components contain a polysaccharide in the form of chiting glucans, followed by mannoproteins (Mc-Courtie 1981). In addition, ovarian cells are gram positive (Anderson and Soll 1986) and that the dye lactophenol blue has an important role in observing chlamydia spores and other fungal hyphae (Webb *et al.* 1998), as shown in Figure 4.6, 4.7, and 4.8.

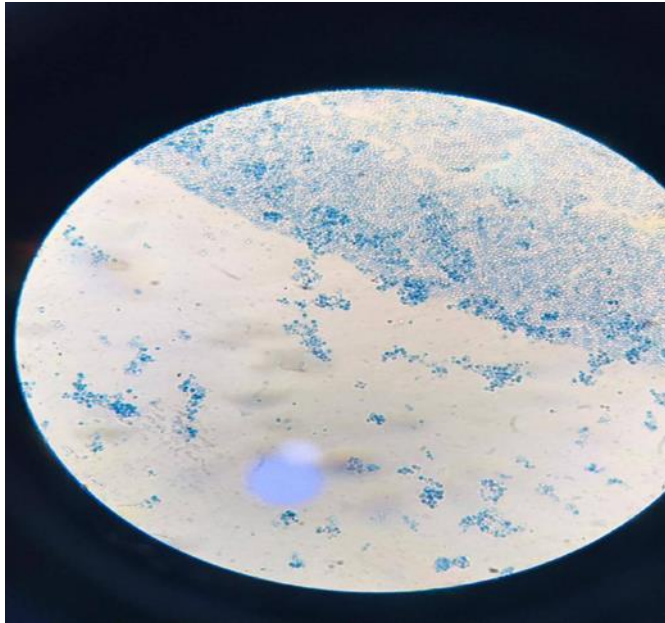


Figure 4.5 Yeast *C. albicans* dyed with cotton blue lactophenol dye (under 40X magnification)



Figure 4.6 It shows the growth of *Candida* species on the medium of *Candida* agar vines

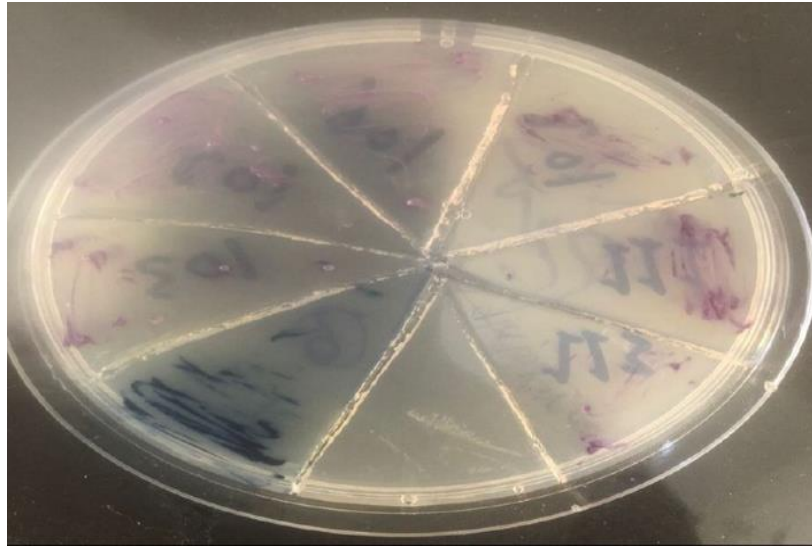


Figure 4.7 The figure shows the growth of samples on chromium agar and the presence of positive infections with fungi that took their own color

A group of samples were cultured with chromium agar and the results showed positive *Candida* infection and took its own color, as shown in Figure 4.9.



Figure 4.8 Samples used in determination of nucleotide sequences of samples based on DNA sequencing technology

4.5 Molecular Diagnosis Using PCR Technique

The test was carried out using PCR technique to confirm the diagnosis of 10 isolates of *Candida* based on the primers (Figure 4.9), due to the fact that the traditional methods adopted in the diagnosis of yeasts, including the species belonging to the genus *Candida spp.*, which are based on the identification of phenotypic criteria are no longer sufficient due to the overlap of these criteria with other species that are classified within other species of the same genus *Candida spp.* In addition to the genetic variation among them, microorganisms farms, even if they belong to a particular group, differ in their growth characteristics and phenotypic characteristics. The difference does not necessarily mean the genetic structure, especially between farms and isolates that belong to the same genus, to the same gender (Mohieldin *et al.* 2013) (Figure 4.10).

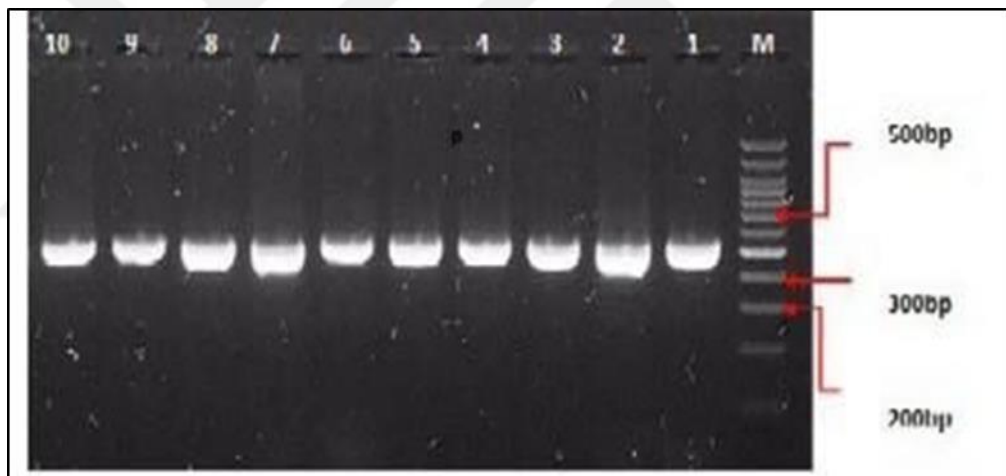


Figure 4.9 Bands of *Candida* obtained by PCR

The results showed that the tested samples belong to the *C. albicans*. That 52.85% of the tested samples were of the type *C. albicans*, and this percentage is not consistent with Al-Aboudi *et al.* (2016), whose results showed that 90% of the tested samples were *C. albicans* noting that the test included samples from the mouth and thigh regions. The reason for the difference in percentages is due to the difference in the geographical area and the environment of the patients, in addition to the culture of the patients, which also differs.

4.6 Electrophoresis Gel Result

The results indicated the presence of DNA in all *C. albicans* isolates as shown in Figure 4.10 and Table 4.3. Thus, the DNA will be extracted according. This result may agree with many studies that have been concerned with isolating *Candida* and who scored the first place with *C. albicans* yeast compared to other types. Among these studies, Al-Hamdani (2020) where the number of isolates reached 49 isolates with a rate of 63.64%, but it does not agree with the study of Vainionpää *et al.* (2019), which did not record any infection of *C. albicans*, followed by *C. tropicalis*, *C. lusitaniae*, *C. krusei*, *C. glabrata*, with a number of isolates. In Beriberi (2015) study, the highest percentage was recorded. *C. albicans* infection with a number of 35 isolates, with a percentage of 92%, followed by *C. tropicalis* with a number of 2 isolates with a percentage of 5.26%, and finally *C. glabrata* with a single isolate with a percentage of 2.64%, many studies have indicated that the main cause of *Candida* infection, whether in the mouth or urinary tract is the yeast *C. albicans*, followed by *C. glabrata* in addition to other types in children and adults (Sahai and Kumar 2018).

Isolation and diagnosis of *Candida* by identifying three species of the genus among the samples, with *C. glabrata* being the dominant species, representing 8.8% of all samples that make up this study. The second most frequent species in the study in general is *C. albicans* with 8% of the samples. A third type was isolated with a lower percentage, *C. krusei*, which was 1.6%.

4.7 DNA Sequence Analysis Results

A skunk was conducted for the selected isolates, which were positive in our study to ensure their conformity in the initial diagnosis in general and their conformity with the diagnosis using PCR technology and using the NCBI BLAST program to find out the similarity between the results of samples in traditional diagnostic methods and compare them with the results of PCR where the program depends on the global gene bank and is the basis in obtaining the nucleotide sequence of the DNA bundle in the isolates diagnosed with UTI in our study. Where the results showed that the percentage of

congruence between the samples in our study with the NCBI is similar with percentages that we will mention sequentially were given in Table 4.4. The results indicated that the percentage ranged between 95-100. The percentage of the spread of *C. albicans* in the targeted area in abundance, which amounts to 40% of the samples that were positive for the infection in the culture tests, while the remaining results indicated that 60% of the *Candida* spread less according to the results of the studies. Follow the gene listed in Table 4.4 and Figure 4.11 Beriberi (2015) indicated the highest infection rate with *C. albicans*, 35 isolates, 92%, followed by *C. tropicalis* with two isolates, 5.26%, and finally *C. glabrata* with one isolate, 2.64%. Several studies have indicated that the main cause of *Candida* infection, both in the mouth and in the groin area, is *C. albicans*, followed by *C. glabrata*, as well as other types of *Candida* in children and adults (Al-Aboudi *et al.* 2016, Al-Hamdani 2020)

Table 4.3 Of the global isolates and their accession numbers in NCBI that were compared with them through the BLAST website, and the percentage of congruence with the isolates under study

Isolation number	Molecular Diagnostics	Similarity ratio %	Accession number
1	<i>C. albicans</i>	99.60	KP675504.1
2	<i>C. albicans</i>	97.62	KP674616.1
3	<i>C. albicans</i>	99.41	MN419364.1
4	<i>C. albicans</i>	95.81	MF443749.1
5	<i>C. albicans</i>	99.61	KP674937.1
6	<i>C. albicans</i>	87.55	JN606277.1
7	<i>C. albicans</i>	99.40	KP675195.1
8	<i>C. albicans</i>	99.83	KP675381.1
9	<i>C. albicans</i>	99.21	MN419374.1
10	<i>C. albicans</i>	91.58	MH534941.1

Sequence ID: **MH534941.1** Length: **501** Number of Matches: **1**

Range 1: 2 to 487 [GenBank](#) [Graphics](#) ▼ Next Match ▲

Score	Expect	Identities	Gaps	Strand
671 bits(363)	0.0	446/487(92%)	2/487(0%)	Plus/Plus
Query 10	TGC- TTATTGCACCACATGTGTTTTCTTTGAAACAAACTTGCTTTGGCGGTGGGCCCAG	68		
Sbjct 2	TGCATTATTGCACCACATGTGTTTTCTTTG-AACAAACTTGCTTTGGCGGTGGGCCCAG	60		
Query 69	CCTGCCGCCAGAGGTCTAAACTTACAACCAATTTTTTATCAACTTGTCACACCAGATTAT	128		
Sbjct 61	CCTGCCGCCAGAGGTCTAAACTTACAACCAATTTTTTATCAACTTGTCACACCAGATTAT	120		
Query 129	TACTAAAAATCCAAACTTTCCACCACCGAACCTTTGGTTCCCCCTCCATTAAAAAACCC	188		
Sbjct 121	TACTTATAATCCAAACTTTCCACCACCGAACCCCTGGTTCCCCCTCCATTAAAAAACCC	180		
Query 189	ACCAAATGGCAAACCTAATAAGAATTGGCAAATTTCTGAATCCACCAAACCTTTAACCC	248		
Sbjct 181	ACCAAATGGCAAACCTAATAAGAATTGGCAAATTTCTGAATCCTCCAATCCTTTAACCC	240		
Query 249	CCCTTGGCCCTCCGGGAATCCCGAAGGCATGGCCGGTTAACCTCCTTTCCTCCTCAA	308		
Sbjct 241	CCCTTGGCCCTCCGGGATTTCCCGAAGGCATGGCTGGTTAACCTCCTTTCCTCCTCAA	300		
Query 309	CCCCCTGGTTGGTGGTTAACCATACACCTGGGTTGGTTGGAAAAAGGGAATGGTAAA	368		
Sbjct 301	CCCCCTGGTTGGGGGTGAACCATACCAATTGGGTTGGCTGGAAAAACGGAATGGTAAA	360		
Query 369	GGCGGATTCCTTTGACCAAGGCCTAAGGCTAAACAAAAACCATGCTTGGGGCGGTACCG	428		
Sbjct 361	GGGGGATCCCTTTACCATGGCTAAGGCCAACCCAAAAACCTTGGTTGGCGCGGGAACCT	420		
Query 429	TCAACCAGTTTATCTTCCAACCTTTGACCTCCAATCCAGGAAGAACACCCCTTAACCTTA	488		
Sbjct 421	CCAACCACTATAACCTCCAACCTTTTACCTCCAATCCAGGAAGAACACCCCTGAACCTTA	480		

Figure 4.10 Matching the nucleotide sequences in the ncbi database to the nitrogenic base sequences of the genes that are being studied

4.8 The locations and Types of Different Mutations

The results of a sequence test for inflated gene exons revealed that the gene regions under investigation had nucleotide variations that took the following forms, as shown in Table 4.5.

Table 4.4 The locations and types of different mutations

ID SEQUENCE	NUCLEOTIDE	LOCATION	MUTATION TYPE	IDENTITY	GAPS
MH534941.1	A→--	115	Deletion	92%	0
MH534941.1	C→T	207	Transversion	92%	0
MH534941.1	T→A	235	Transversion	92%	0
MH534941.1	T→C	326	Transition	92%	0
MH534941.1	A→C	392	Transition	92%	0
MH534941.1	G→T	403	Transversion	92%	0
MH534941.1	A→T	425	Transversion	92%	0
MH534941.1	G→C	431	Transversion	92%	0

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

1. 125 samples were taken and studied, 23 of them were positive and the rest were negative, and the positive samples were distributed; *C. albicans* = 10, *C. glabrata* = 11, and *C. krusei* = 2, and our study on *C. albicans* has been completed because it is the target fungi in our study.
2. The electrophoresis test was performed on the target samples in order to confirm and prepare for DNA extraction, and all the *Candida* results showed that they are indeed positive.
3. The results of the polymerase chain reaction assay indicated that the percentage of similarity with the World Bank for Genes was high and with high diagnostic accuracy, as it was as in Table 4.3.
4. One of the most common reasons contributing to the development of *Candida* infections is the frequent antibiotic or steroid treatment and the lack of follow-up of the effect of the treatments with the specialist doctor, as well as diabetic patients.
5. The percentage of women infected with candidiasis was more in our study, at a rate of 53%, due to the use of treatments that contribute to eliminating the body's resistance and thus be the cause of candidiasis.

5.2 Recommendation

1. Maintain the cleanliness of the affected areas (genitals area).
2. Do not take antibiotics without consulting a specialist.
3. In case of feeling infected with any type of fungi (*C. albicans*) thanks to resorting to early examination using PCR technology and searching for the code (KP675381.1), it will be 99.83 according to the results of our study, moving towards the positive.

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