University of AL-Shatarh

College of Veterinary Medicine

Molecular and Morphological Identification of Candida species from Urine of Diabetic patients in AL-Shatrah District,Thi-Qar Province south of Iraq

A Thesis

Submitted to the Council of Veterinary Medicine College/University of Al-Shatrah, in partial fulfillment of the requirements for a Master's Degree (M.Sc.) in Veterinary Sciences (Microbiology).

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2025 AD **1447 AH**

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

((ٱلَّذِينَ ءَامَنُواْ وَكَانُواْ يَتَّقُونَ لهُمُ ٱلْبُشْرَىٰ
فِي ٱلْحَيَوٰةِ ٱلدُّنْيَا وَفِي ٱلْأَخِرَةِۚ لَا تَبْدِيلَ
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صدق الله العلي العظيم

سورة يونس: آية63, 64

"Certification"

I certify that Thesis entitled **(Molecular and Morphological Identification of Candida species from Urine of Diabetic patients in AL-Shatrah District, south of Iraq)** was prepared under my supervision at the Department of Microbiology/ College Veterinary Medicine / University of AL-Shatrah as a partial fulfillment of the requirements for the Degree of Master of Science in Microbiology.

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Date: / / 2025

The recommendation of the head of the department, in view of the available recommendation, I forward this thesis for debate by the examination committee.

Signature

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Date: / / 2025

Dedication

To the owner of the greatest honor, the venerable prophet, our prophet Mohammed the messenger of Allah and Imam Hussian (Allah pray and peace upon him and his family), and I request his intercession in the doomsday, to his chaste sinless household, and his kind companions.

To the persons who supplicated Allah for me well and gave me the encouragement, motivation and road drawing....My Lovely Family

To the source of sciences and knowledge ....My Professors

To my angels who always be there for me, with there loves, kindliness and support....My Father ,My Mother and My Hasband

To my sweetheart and my motivation....My Daughter and My Son

To all who helped me in this research....

Farah2025

Acknowledgement

I would like to begin by expressing my deepest gratitude to my supervisor, Assist Prof Dr. Dhurgham Ali ALhasan whose continuous support, valuable guidance, and insightful feedback have been essential throughout every stage of this work. His patience, encouragement, and expertise helped me overcome numerous challenges and shaped this project into its final form. I am truly grateful for the time and effort He dedicated to guiding me and for believing in my potential from the very beginning.

I am also sincerely thankful to all the individuals and colleagues who contributed, directly or indirectly, to the completion of this work. the President of Al-Shatra University, Professor Dr. Basim Abdul-Hussein Jarallah ,the dean of the College Assist Prof Dr. Nabeel Mahdi , Assist Prof Dr. Hekmat Kazem , Assist Prof Dr. Mohammed Abdul- Alabass and the dean of the College of Applied Medical science Assist Prof Dr Mohammed Halol . Also I would also like to extend my sincere thanks to Mr. Hisham Jabbar Flayyih.

I wish to express my appreciate to all academic and technical staff of department of microbiology in college of veterinary medicine / university of Shatra, especially head of department Dr. Nothaila Rasheed Hameed.

To my friends, thank you for being a constant source of motivation, and encouragement

Most importantly, I wish to express my heartfelt appreciation to my family. To my parents, thank you for your unconditional love, endless patience, and unwavering belief in me. Your sacrifices, prayers, and continuous encouragement have been the foundation upon which I built this journey. To my siblings and loved ones, your support gave me strength when I needed it most.

This achievement would not have been possible without each and every one of you. I am forever grateful..

Summary

Candiduria often occurs when one or more predisposing factors are present such as diabetes mellitus (DM).The most common causative agents being Candida albicans, C. parapsilosis, C. glabrata, C. tropicalis, C. guilliermondii, and C. dubliniensis. This study was conducted to detect candiduria in urine specimens of diabetic and none-diabetic patients as well as healthy individuals as a negative control who visiting General hospital and clinics of Al-Shatrah District, Thi-Qar Governorate. The specimens were collected and cultured on Sabouraud dextrose agar (SDA) supplemented with azthromycin aseptically. Identification of Candida species was reported depending on the morphological and molecular methods. All identified species and strains were submitted to the antifungal sensitivity test.

The current research showed three species of Candida were identified: Candida albicans, C. glabrata, and C.krusei. The first species produced white – creamy, green, and pale colonies on SDA, Candida chromagar medium (CCM), and TTC agar, respectively. While C. glabrata revealed…Brownish white ,creamy…… and C.krusei appeared violet on the mentioned media, respectively. PCR using internal transcript spacer regions particularly ITS 1 and ITS 4 confirmed the identification and sequencing these gene regions demonstrated various strains belonged to these identified Candida species.

Among (200) urine specimens of the diabetic patients, …41.. appeared positive results while …14.. positive ones of 25 specimens obtained from none-diabetic patients. Obviously, no positive results were detected in specimens of the healthy individuals.The identified strains revealed different values of the antifungal sensitivity test. Only 3 strains and 9 strains of C. albicans were sensitive to disc of amphotericin B to nystatin, respectively compared with

Clotrimazole, Fluconazole,Itraconazole, and Ketoconazole while all strains of C.krusei were reported resistant. Concerning, 1, 3, and 14 strains of C.glabrata were sensitive to amphotericin B, intraconazole, and nystatin, respectively

Chapter One

Introduction

1. Chapter One

1.1. Introduction

Candidiasis is the most prevalent systemic and opportunistic mycosis, with the most common causative agents being Candida albicans, C. parapsilosis, C. glabrata, C. tropicalis, C. guilliermondii, and C. dubliniensis (Jawet). Several species of the yeast genus Candida can cause candidiasis, as they are part of the normal flora found on the skin, mucous membranes, and in the gastrointestinal tract (Reference). These Candida species colonize the mucosal surfaces of all humans shortly after birth, and the risk of endogenous opportunistic infection is always present (Reference).

The genus Candida is highly diverse and causes various types of candidiasis, including cutaneous, mucocutaneous, and systemic infections. Among the commensal yeasts in human mycoflora, C. albicans is the primary pathogenic species associated with urinary tract infection (UTI) candidiasis (Gharaghani et al., 2022). Candida species are the most common organisms found in the fungal UTIs (Reference). These infections are primarily observed in hospitalized patients, while they are less frequent in community settings (Reference).

In most cases, candiduria results from colonization or contamination rather than a true infection (Reference). It is crucial to differentiate between simple colonization and an actual Candida urinary tract infection (UTI). Candiduria often occurs when one or more predisposing factors are present, with diabetes being the most significant (Reference). Diabetes mellitus (DM) is a major contributor to the development of candiduria in affected individuals (Reference). The condition created by DM allows Candida species to colonize target tissues effectively (Reference).

The Aim of the Study

The current study aims to identify Candida species including strains as the causative agents of urinary tract infections (UTIs) in diabetic patients in the Al-Shatrah District, Thi-Qar Governorate, in southern Iraq. This aim was pursued through the following objectives:

1. Collection of urine samples from diabetic patients in hospitals and clinics within the Al-Shatrah District to determine which strain has a higher frequency in this area.

2. Isolation of Candida species from these samples, with identification based on their morphological and molecular characteristics.

3. Conduct antifungal sensitivity tests on drugs used in hospitals and clinics to treat diabetic patients' UTIs, comparing them with pure forms of other antifungal agents.

Chapter Two

Literature Review

2. The Literatures Review

2.1 Candidiasis

Numerous microorganisms coexist in the human body as commensals and typically do no harm to their host. A common fungus in the skin, genitourinary tract, gastrointestinal tract, and oral cavity of the majority of healthy people is Candida albicans (Pang et al.,2025)

The fungus Candida contains pathogenic fungi that cause the common fungal infection known as candidiasis. As a commensal fungus that is primarily found in the skin, gastrointestinal system, and genital tract, Candida is an essential component of the human normal microbiota (Shiyadeh et al., 2024).

Candidiasis can cause symptoms like pruritus, pain, and discharge, or it can appear as superficial lesions on the skin or mucosal surfaces like the mouth and vulvovaginal regions. When candidiasis gets worse, it can lead to systemic infections, where the fungus invades tissues andenters the bloodstream, affecting organ function. The species involved and their virulence factors, determine how severe these infections are. The lack of effective treatment options is made worse by virulence factors, which include biofilm formation and enzyme production. These factors frequently contribute to the development of resistance mechanisms (Moraes and Ferreira-Pereira , 2019:Fan et al.,2022;Ghannoum .,1999).

Candida is receiving more and more attention as a result of its pathogenicity and the development of drug resistance. The development of genomics technologies has provided effective instruments for examining the pathogenic processes and drug resistance traits of Candida (Huang et al.,2025).These infections are especially prevalent in immunocompromised patients, including those receiving chemotherapy, organ transplant recipients, and those infected with HIV (Bhattacharya et al., 2020; Fisher et al., 2022). Approximately 60% of

deaths worldwide are caused by invasive candidiasis, which affects over 1.5 million people annually (Denning 2024). Human invasive infections have been known to be caused by Candida species; nearly all of these infections were brought on by Candida .(C albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida krusei, and Candida auris. Invasive candidiasis (IC) is thecollective term for serious diseases brought on by Candida species. Because of the clinical state of the patients-who are critically ill, hospitalized, immunocompromised, and in the latter stages of life-IC is linked to a high death rate, especially in cases of candidemia(Rodríguez-Cerdeira et al.,2025).

Candida spp appearance as a pathogen is primarily opportunistic. Advances in medicine have contributed to an increase in these opportunities. One cause of Candida infections is when antibiotic treatments upset the microbiome's equilibrium.These treatments can affect important commensal bacteria that are essential to the balanced microbiome as well as the infectious bacteria that cause disease (Dongari-Bagtzoglou et al. 2009), permitting the development of Candida infections. Secondly, immunological suppression is a trigger. Due in large part to the growing number of immunocompromised patients brought on by treatments like cancer chemotherapy, infection rates have skyrocketed over the last 20 years , organ transplants, steroid treatment (Huang et al.,2025). .The usage of indwelling catheters is a persist as sources of infection due to the growth of biofilms on their surfaces(Singh et al. 2024).

Candiduria though historically regarded as a colonization, the presence of Candida spp. in urine becomes more significant in specific clinical situations, such as in critically ill patients, immunocompromised people, or people with chronic illnesses, because of the potential for active infection and complications (Castellano-Sánchez et al.,2025).Patients with risk factors like chronic illness, recent invasive procedures, intensive care unit (ICU) admission, and exposure to broad-spectrum antibiotics are particularly susceptible to these infections in

nosocomial settings.(Genceli et al.,2025). A condition known as candiduria occurs when urine contains Candida species. Hospitalized patients frequently have candiduria, particularly those with diabetes mellitus and those undergoing medical treatment, particularly those utilizing urine catheters. Despite being a normal component of the body's flora, some species of Candida have the ability to opportunistically cause UTIS (Garini et al.,2025).

When the host's immune system is weakened, Candida species that are normally present in the vulvovaginal flora may cause opportunistic infections. There is a symbiotic relationship between the vaginal microbiota and Candida species; asymptomatic colonization is commonand can persist for years. Pregnant women may have 30% vaginal tract Candida colonization, compared to 20% in young, asymptomatic women (Mucci et al., 2017; Ghaddar et al., 2020). Additionally, Candida sp. Horm contraception is one of the risk factors that influences the growth of Candida sp., which can alter the normal balance of vaginal flora. In hormonal contraception, the estrogen hormone can raise the amount of glycogen in vaginal epithelial cells, which promotes the growth of candida. Urine testing can be used to identify this condition, which may lead to vulvovaginal candidiasis (Cahyanti& Qurrohman.,2025) Candiduria may result from retrograde transmission through the urethra or hematogenous spread after candidemia (Odabasi and Mert .,2020).

The most common infectious agent among the Candida species that cause urogenital candidiasis is Candida albicans. Nonetheless, non-albicans Candida (NAC) species like C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei have been reported more frequently all over the world (Nagi et al.,2025).

2.1.1 Role of candida in disease

2.1.1.1 Candidiasis

A number of infections, ranging from skin or mucosal infections to more serious conditions like invasive candidiasis, are referred to as "candidasis." These conditions include urinary tract infections, bloodstream infections (candidemia), endocarditis, central nervous system infections, and chronic disseminated candidiasis (Fernández et al., 2023). Candida is found all over the world, but it is more common in immunocompromised individuals with severe diseases like HIV and cancer. Candida is one of the most common pathogens associated with infections acquired in hospitals. People who have recently had surgery, received a transplant,or are in intensive care units (ICUs) are especially at risk (Robbins and Cowen.,2025).

2.1.1.2 Superficial and local infections

Fungal infections can manifest as more severe disseminated systemic infections or as superficial cutaneous or mucosal diseases with signs of local invasion. Fortunately, fungal organism exposure usually does not result in infection when the host's immune system is intact (Telles et al., 2017).

2.1.1.3 Systemic infection

Systemic infection via the heart can spread to the brain, eyes, lungs, joints, liver, and reproductive organs, among other organs.Bloodstream fungi can cause endocarditis, which is inflammation of the heart valves, meningitis, which is inflammation of the brain, and fungemia,of the fungal infection affects how the disease progresses (Rasheed,2025).Fungal infections, such as those caused by Candida albicans, have become major causes of morbidity and death in people with weakened immune systems (Cancer chemotherapy, AIDS, organ or bone marrow transplantation (Peters et al., 2010).

In recent years, other species of Candida have become more prevalent and isolated, despite Candida albicans being the most common cause of candidemia. Preventive measures include treating infected areas, maintaining clean, dry skin free of open wounds, using antibiotics sparingly, maintaining a healthy lifestyle with a balanced diet, and practicing good hygiene (Yapar, 2014).

2.2 Genus of Candida

The genus Candida is highly diverse; the majority of its species can grow filamentously (pseudohyphae, pseudo mycelium) or as yeasts (blastospores). Due to their ability to form actual hyphae, or mycelium, Candida albicans and Candida dubliniensis are the only two species believed to be polymorphic. Candida belong to the fungal domain and are sexual, diploid, eukaryotic yeasts (Lim et al., 2012). For a considerable amount of time, humans and Candida species have coexisted as opportunistic pathogens that colonize healthy people's skin and mucosal surface(Mahalingam et al., 2022). Since it has no sexual development at all, the genus Candida,which has about 150 species,is categorized as a deuteromycete. From a medical standpoint, seven of them are recognized as the most important species that cause disease: C. glabrata, C. guilliermondii, C. krusei, C. kyfer, C.albicans, C. tropicalis, and C. parapsilosis (Rudenko et al., 2021).

2.2.1 History of Candida

Hippocrates recorded oral candidiasis, which is caused by Candida species, for the first time in 377 BCE. He called it "aphthae" or thrush, a term thought to be derived from an Anglo-Saxon or Scandinavian word. In his fourth-century BC work Epidemics, Hippocrates documented his findings regarding oral candidiasis (Rippon J,1988)

In the second century AD, Galen also mentions oral Candida infections, calling them "aphthas albus." Galen saw sick children with oral candidiasis, also known

as thrush. In addition, Pepys' diary from 1665 mentions this disease. At the same time, however, there was a great deal ofconfusion about the cause of candidiasis. It was unclear whether an infectious agent, the host, or a combination of the two were the main causes of the infection. The theory that an infectious agent causes oral thrush was backed by researchers like Rosen von Rosenstein (1771) and Underwood 1784). The etiological agent for the invasive form of thrush described by Rosen von Rosenstein was not identified (Tajane et al.,2025).

The first instance of esophageal candidiasis in a newborn was documented by Vernon in 1835, indicating that the infection was contracted during birth canal transit. When a typhoid patient developed oral thrush in 1839, Langenbeck discovered a fungal agent that he called a"Cryptogamic Plant." But instead of thrush, he blamed it for causing fever (Vila et al.,2020)

In 1853, Rubin suggested the term "Albicans," which means "melt-in," to describe the fungus that causes Thrush disease. Gruby had previously identified this species as a member of the genus Sporotrichum in 1842 (Martin ,et al .2004) . Along with other names like Mycotorula, Torulopsis, and Negligent for this underappreciated yeast. In 1839, a typhoid patient had Candida albicans isolated from them. In 1912, Catellani documented a case of bronchopulmonary candidiasis involving yeasts other than Monilia albicans (Calderone, 2002).

Candida Spp.	**Researcher discovery and the year**	**Clinical manifestation**
C. albicans	In 1839	a typhoid patient
C. auris	Satoh et al. (2009)	Infected ear canal
C. ciferrii	Gunsilius et al. (2001)	Bloodstream infection
C. crures		
C. dubliniensis	Sullivan et al. (1995)	Oral candidiasis
C. guilliermondii	Castellani (1912)	Bloodstream infection
C. glabrata	Anderson (1917)	Bloodstream infection
C. haemulonii	Lavarde et al. (1984)	Bloodstream infection in a renal

		failure patient
C. kefyr	Castellani (1914)	Infection of the ear, gastrointestinal tract, and vagina
C. krusi	Castellani (1914)	Bloodstream infection
C. lusitaniae	Pappagianis et al. (1979)	Bloodstream infection
C. lipolytica	Rex et al. (1995)	Bloodstream infection
C. parapsilosis	Ashford (1928)	Diarrhea
C. stellatodia		
C. tropicalis	Castellani (1910)	Bloodstream infection
Candida viswanathii	Randhawa and Chowdhary (1959)	Fatal case of meningitis

2.2.2 Scientific classification of candida

The Latin name "candid," which describes its white hue, was the source of the scientific term "Candida (Raju and Rajappa, 2011).

The kingdom's classification Updates on fungi are ongoing, and recent research has often included information from DNA sequences. Though it is still challenging in some groups, mycologists are increasingly collecting historical taxa and neotyping or epitypifying them using new material (Tedersoo et al., 2018). After making the first microscopic observations of yeast cells in 1680, Anton van Leeuwenhoek categorized them into the following groups: phylum Ascomycota, class Saccharomycetes, order Saccharomycetales, family Saccharomycetaceae, genus Candida, and kingdom Fungi (Silva and Pires de Oliveira 2017).

Taxonomy by(Prakash, 2016)

- Kingdom:_ Fungi
- Phylum:_ Ascomycota
- Sub phylum:_ Ascotina.
- Class: _Ascomycetes

- Order:_ Saccharomycetales
- Family:_ Saccharomycetaceae
- Genus:_ Candida

There are two types of Candida species: pathogenic and non-pathogenic. Pathogenic Candida refers to Candida species that can infect humans and cause illness. Candida glabrata, C. albicans, C. krusei, C. tropicalis, C. auris, and C. parapsilosis are the most common pathogenic (Pappas et al., 2016b).

Non-pathogenic Candida is a genus that cannot infect or cause disease in humans. Although infections are frequently associated with Candida species, other strains of the fungus are considered non-pathogenic and pose no risk to human health. Only three non-albicans Candida and C. orthopsilosis, are commonly found on healthy skin and have the potential to become pathogens (Kühbacher et al., 2017).One well-known characteristic of Candida species is their heterotrophic nutritional mode, which increases their pathogenicity and ability to cause candidiasis while also facilitating their development and multiplication in a range of host habitats.(Mayer et al., 2013).

2.2.3 Epidemiology of candida

Regional data is crucial for assessing the shift and figuring out the situation at the national level because the prevalence rates of candiduria and UTIs caused by Candida spp. vary by geographic location(Alam et al.,2023) .Despite the fact that epidemiological research on Candida is still growing globally, there is a dearth of data in Arab nations because standard clinical laboratories do not provide sensitive, accurate, and specific diagnoses (Ghazi et al., 2019). Worldwide, including in the Arabian Peninsula, Candida infections are a significant contributor to both invasive and non-invasive mycosis. For characteristics candidemia and candiduria, there are current epidemiological and trends, clinical manifestations, species distribution and resistance for antifungal

susceptibility (Ahmad et al.,2025). Candida species can cause a group of diseases known as disseminated candidiasis, which includes endocarditis, vaginitis, meningitis, endophthalmitis, and chronic fatigue syndrome .Candida infections have become more commonplace worldwide, and in certain patient populations, their fatality rates have risen to over 70% (Singh et al., 2020). Depending on the underlying conditions, the crude mortality rates for individuals with invasive candidiasis or candidemia vary "between" 40% and 60% (Wu et al.,2017).

Of all cases of candidiasis, approximately half are caused by Candida albicans, with the remaining cases being caused by non-albicans Candida species. There is serious concern about infections caused by C. tropicalis, C. glabrata, C. parapsilosis, C. krusei, C. dubliniensis, and C. auris. It is nowthought that some of these non-Albican species of Candida are emergent opportunistic pathogens (Caceres et al., 2019; Talapko et al., 2021).

2.2.4 Pathogenicity of Candida species

In Candida species, pathogenesis is a complicated process involving a number of different pathways and mechanisms. The host and the microbe are both involved in this intricate and multidimensional mechanism (Negri et al., 2010; Kadosh, 2019; Mba and Nweze, 2020). The rising prevalence of diseases brought on by commensal yeasts like Candida spp., which appear to be harmless, is currently one of the biggest mysteries in microbiology. The disease must first adhere to the host cells before invading the tissue in order to manifest. Adaptation is thecapacity to quickly respond to stressors, absorb nutrients, or multiply in different environments. As Candida species form complex intracellular networks known as biofilms, they become more prone to illness and more resistant to antifungal medications (Ciurea et al., 2020).

The most common fungal pathogen found in clinical settings is Candida spp. Numerous virulence factors and fitness attributes, including the capacity to

evade host defenses through morphological transitions between yeast and hyphal forms, the formation of biofilms on hosttissue, multiple transcriptional circuits, and morphological and phenotypic switching, all contribute to Candida pathogenicity. cytolytic proteins system, metabolic flexibility, genomic plasticity, pH fluctuation tolerance, and thigmotropism tissue-damaging extracellularhydrolytic enzymes. strong system for acquiring nutrients, invasions and adhesion, and phagocytosis escape. avoidance of the immune system and cooperative co-aggregation with the local microbiota. Some of the main pathogenic determinants of Candida species include theircapacity to effectively respond to a variety of stressors and their resistance to antifungal drugs (Mba and Nweze, 2020 ; Talapko et al.,2021).

2.2.5 Virulence factor of Candida species

The term "virulence," which comes from the Latin "virulent" (virus = poison and virulent = poisonous), describes the capacity of pathogenic microorganisms to infiltrate the host and acquire its nutrients by eluding the host's defenses and proliferating to inflict harm, all while expressing virulence factors (Robles et al., 2013).

Candida species can create infections and elude host immune responses because of their wide range of virulence factors. The virulence factors of Candida albicans have been thoroughly studied, and numerous investigations have been carried out to clarify the virulence mechanisms of Candida non-albicans . Shape alterations, biofilm formation, and the generation of hydrolytic enzymes are common virulence factors among the Candida genus (Singh et al.,2021)

Table( ).Comparing distinct traits and important virulence factors between various species of Candida (Gaffar et al.,2025).

Species	Unique Characteristics	Key Virulence Factors
C. albicans	In infections that spread widely, white cells are more virulent, while in infections that affect the skin, opaque cells are more virulent.	• Yeast–hyphal transition • Adhesins (Als3, Hwp1) • Biofilm formation • White-to-opaque switching • Saps (1–10)
C. auris	Exists in the forms of yeast, filamentous, or pseudohyphal cells; reduced neutrophil phagocytosis in contrast to Candida albicans	• Adhesins • Biofilm formation • Salt tolerance • Sapas (1–7) • Thermotolerance
C. tropicalis	Greater ability than Candida albicans to form biofilms and stronger candidalysin	• White-to-opaque switching • Sapts (1–4) • Adhesins • Biofilm formation • Candidalysin
C. glabrata	Survival and persistence within macrophages; absence of hyphal growth	• Biofilm formation • Yapsins (1–11)
C.	Exists in the cell morphologies	• Sapps (1–3)

parapsilosis	of yeast or pseudohyphae.	• Biofilm formation • Lipases (Lip1 and Lip2)

2.2.5.1 Dimorphism and Phenotypic switching

In response to environmental stimuli, pathogenic fungi that can switch between hyphal and yeast morphology are referred to as dimorphic. Certain fungi produce conidia while living as filamentous hyphae in saprobic environments. The body temperature of 37°C causes the conidia to transform into yeast cells when they are inhaled by mammals or traumatically inoculated. This change encourages immune evasion and the spread of fungus (Ramírez et al.,2025).

Yeast cells spread to other regions via a phagocyte-dependent mechanism, with neutrophils and macrophages acting as dissemination vehicles. By directing growth in response to contact with a solid surface, the hypha, on the other hand, invades and damages host tissue-a process known as thigmotropism (Giusiano, 2021).Despite the fact that temperature is the primary stimulus that affects the phase transition, other factors like the availability of pH, CO2, exogenous cysteine, estradiol, nitrogen sources, lipoxygenases, cyclooxygenases, and quorum-sensing molecules also favor these changes (Ramírez et al.,2025).

Phenotypic switching occurs very frequently in the colonies of the majority of Candida species.Longer, bean-shaped opaque phase cells can develop from round, ovoid, white phase cells in Candida albicans. specifically expressed in opaque cells. There is less information available regarding the phenotypic changes of non-C. albicans, which revealed that dark brown colonies were prevalent among the vaginal isolates of women with VVC. Light brown, white, dark brown, very dark brown, or uneven colonies of wrinkles can result from "core switching," which has been observed in C. glabrata. One of the virulence

characteristics that is changed by phenotypic flipping is the production of hyphae. The specific environmental conditions of each anatomical location influence every single virulence factor that mediates the pathogenicity of Candida. Consequently, changing a woman's natural environment in the lower genital tract may increase her risk of getting a Candida infection. (Deorukhkar and Roushani, 2017).

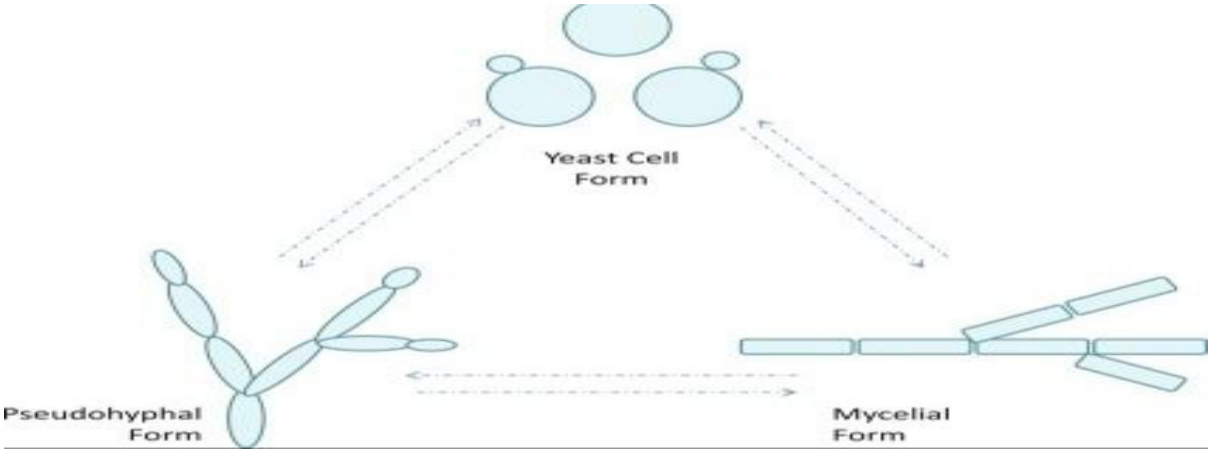


Figure (-): Morphological transformations in Candida albicans (Sharma et al.,2023)

2.2.5.2 Adhesion

The initial stage of Candida spp. colonization of healthy individuals' mucosal surfaces or any other tissue is adhesion (Nikou et al.,2019). The primary process of forming a biofilm structure is adhesion, which enables the formation of mature biofilm on both types of surfaces (Czechowicz et al ., 2022). Studies have demonstrated that specific Candida species can adhere to these specific cells. Furthermore, some Candida species can stick to the surface of medical devices, which often promotes infections associated with the devices, like VVC in women who use intrauterine devices(IUDs) for contraception. First, adhesions-specialized surface proteins-help Candida cells stick to biotic and abiotic surfaces. This process is aided by the physicochemical properties of the cell surface. Either hydrophobic interactions or host ligands, such as serum proteins and extracellular matrix components of the host tissues like collagen,

fibronectin, laminin, vitronectin, and enacting, cause adhesions to stick to abiotic surfaces (Hoyer et al., 2008). Adhesins like Hwp1 (hyphal wall protein 1) and Als3 (agglutinin-like succession) play a crucial role in C. albicans adhesion to the host cells because they are essentially communicated during hyphae formation (Talapko et al., 2021). Proteins, fibrinogens, collagen, laminin, vitronectin, and fibronectins are among the ligands that C. albicans' adhesins can recognize and bind to them (Czechowicz et al., 2022).

2.2.5.3 Biofilm formation

Microbial biofilms have been shown to provide resistance to antimicrobial drugs and to protect against the host's immune system. The gastrointestinal, respiratory, cardiovascular, and urinary organ systems are among the human organ systems on which biofilms can grow. The yeast Candida albicans can cause a variety of illnesses, from bothersome thrush to serious and potentially fatal candidiasis (Johnson et al.,2025).

Microbial communities known as biofilms are ordered, structured communities of cells that stick to solid surfaces or are found at liquid-air interfaces. The cells firmly attach to one another and embed themselves in a template of extracellular materials that the cells produce (Mahto et al., 2022; Sauer et al.,2022). Invasive hyphal forms and yeast, which are tiny, oval cells, make up Candida biofilms (McKloud et al., 2021). Hexosamine, phosphorus, uronic acid, proteins, carbohydrates, and nucleic acids make up the matrix of Candida biofilms (Pereira et al.,2021). Candida biofilms have structural integrity because of their extracellular polysaccharide matrix, which supports adhesion to surfaces and other biopolymers (Macias-Paz et al.,2023) .Immune system defense, cell dispersion regulation, and azole, polyene, and echinocandin resistance are all facilitated by this matrix (Borgeat et al.,2021).

Fungi colonies sach as Candida albicans encased in a thick layer of protective extracellular matrix form a scaffold. It consists of several stages, starting with

the adhesion and growth of yeast cells on surfaces, followed by the transition from yeast to hyphal, which enhances the biofilm's overall structural stability, and the subsequent production of extracellular matrix by component cells. As a result, a strong three-dimensional biofilm structure develops, ultimately opening the door for dispersion (Wijaya et al.,2023) Candida species can adhere to surfaces thanks to the formation of biofilms. This helps spread the fungus by releasing non-adherent fungal cells into the bloodstream, which helps the fungus spread into organs and form new biofilms (Wijaya et al.,2023). Additionally, biofilms' large extracellular matrix protects against antifungal agents and the host immune system. In response to these stimuli, the cells that make up the biofilm strengthen their defenses (Pierce et al.,2017).

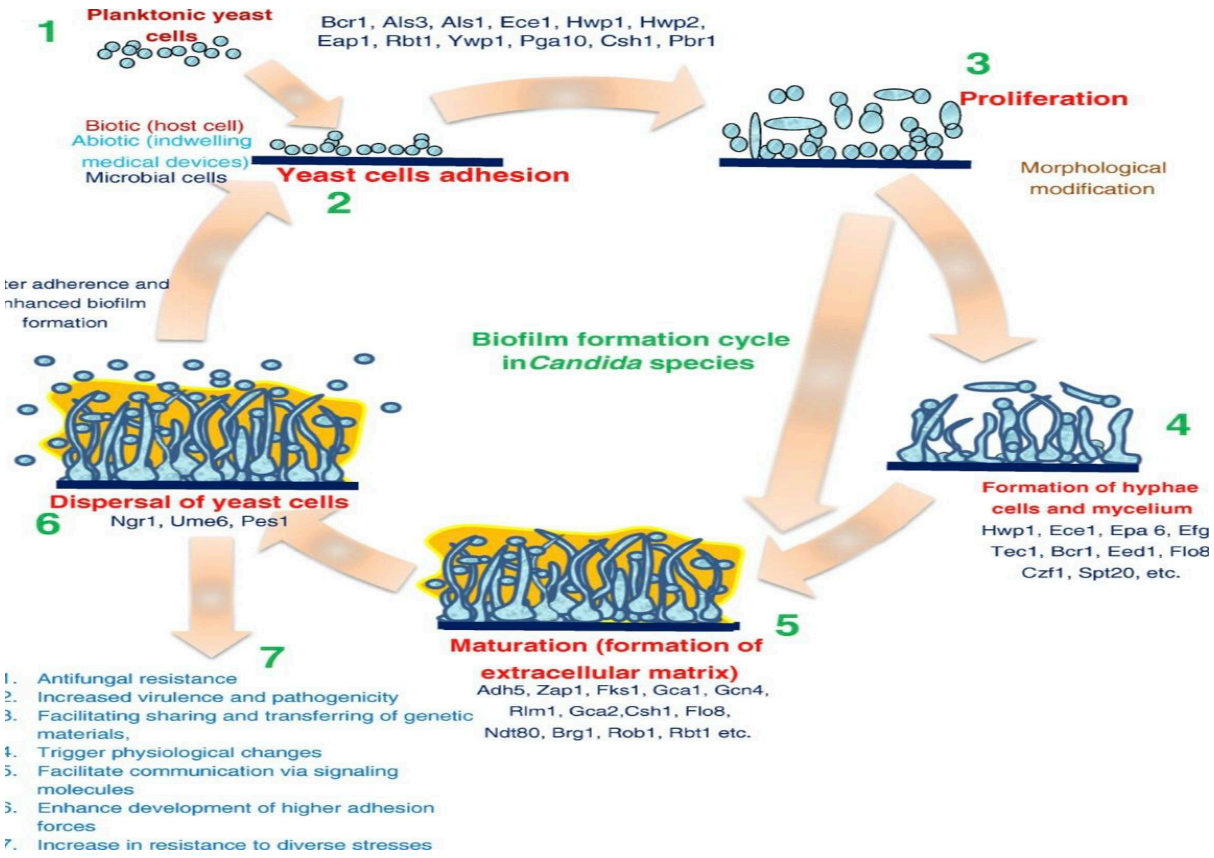


Figure ( ): Biofilm formation in Candida spp (Mba and Nweze,2020)

2.2.5.4 Hydrolytic Enzyme

Candida species have the ability to release extracellular hydrolytic enzymes after adhering to the surfaces of host cells. In addition to facilitating nutrient uptake, the release of hydrolytic enzymes during infection enhances an organism's capacity for adhesion, invasion, and the destruction of host immune factors (Silva et al., 2011; Mba and Nweze, 2020). For example, secreted aspartyl proteinases are exoenzymes. enzymes necessary for colonization and organ attack, including lipases, esterase, phospholipases, hemolysins , and proteinases .

Secreted aspartyl proteinases (SAPs): A family of 10 SAP genes (SAP1-SAP10) encode secreted aspartic proteases (Saps), which are essential to Candida's virulence because they adhere to epithelial host tissue and break down host tissue proteins like hemoglobin, keratin, collagen, laminin, albumin, fibronectin, and mucin (Kadry et al.,2018).

Hemolysins: are chemicals substance that hydrolyze red blood cells(RBCs) to liberate iron, which is necessary for this fungal process's growth and metabolism (63) (Chopra et al., 2015; Cain and Smith, 2021). Patients with poorly managed diabetes may be at risk for candidiasis due to the positive correlation between glucose concentration and hemolysin production in Candida, which is linked to elevated blood and salivary glucose levels (Williams and Lewis, 2011; Wu et al., 2020).

Esterase and lipase: It is unclear how Candida's production of extracellular lipase and esterase contributes to pathogenic processes (Williams and Lewis, 2011). Esterases and lipases are both capable of hydrolyzing the ester bonds found in glycerides (Monteiro et al., 2021). Extracellular lipases have a variety of functions, such as breaking down lipids for nutrients, colonizing host cells and tissues, influencing immune cells to trigger non-specific inflammatory

processes, and lysing rival microorganisms for self-defense (Karkowska-Kuleta et al., 2022).

Phospholipases: are a different class of extracellular enzymes that initiate inflammatory processes, adhere to host tissues, digest lipids for nutrient acquisition, and work in concert with other enzymes (Faris, 2016; El-Houssaini et al.,2019; Aparna et al.,2023).

Candidalysin is one important virulence factor for C. albicans infections. Hyphae release candidalysin into an invasion pocket during mucosal infection, where it dose-dependently activates the immune system and damages cells (Lee et al.,2025)

2.2.5.5 Invasion and dissemination

Two main mechanisms of Candida species invasion are induced endocytosis and active penetration. In order to break down intestinal lining cells and their tight junctions, active penetration-a crucial step in intestinal invasion-requires the release of hydrolytic enzymes, most notably candidalysin. Agglutinin-like sequence proteins play a key role in induced endocytosis, a process that occurs when fungal and host surface proteins interact to enable endocytosis in interactions with stratified epithelial and endothelial cells (Lim et al.,2021).

2.2.5.6 Immune evasion

Several tactics are used by Candida albicans to avoid detection by the immune system. By hiding cell-wall components, it can evade the host immune system's detection in response to metabolic cues like low oxygen and iron levels and high lactate levels, which lowers the expression of K-glucan (Tripathi et al., 2020). Proteases, lipases, and phospholipases are among the enzymes it secretes to neutralize immune proteins, including antibodies, as well as enzymes like lysozymes 65 and defensins (Oliver et al.,2019). By neutralizing pH, hyphal transitions (Westman et al.,2018) and rupturing phagocytes through

intra-endosomal growth, Candida albicans can also evade phagocytosis. Moreover, it causes pyroptosis, a type of cell death brought on by an inflammasome (Oliver et al.,2019).

2.2.5.5 Mycotoxin

Mycotoxins are poisonous secondary metabolites that are made by fungus-related organisms and can kill or seriously harm humans and other animals. (Nji et al., 2022). According to (Nji et al., (2022) and(Tshabalala et al., 2016) , aflatoxins, fumonisins, patulin, ochratoxin A, deoxynivalenol, zearalenone, and gliotoxin are among the most prevalent mycotoxins.

2.2.6 Medically important Candida species

Box ( )The six Candida species that are most clinically significant (Lass-Flörl et al.,2024)

Candida species	Featers
Candida albicans	• 90% of people are colonized • Can impact almost every organ the most prevalent clinical manifestation is candidemia. • Reduced involvement in outbreaks and otomycosis. • The most significant species in human medicine • Endogenous sources account for the vast majority of infections. • Infrequently, resistance occurs.
Nakaseomyces glabratus **(previously known as *Candida glabrata*)**	• Healthy microbial flora colonizer • Widely found in the soil and water of the environment • Significant morbidity and mortality (40-60%) are caused by infection in the second most important species in the USA and northwest Europe. • For this species, the source of infection is

	typically endogenous; however, some studies have found horizontal transfer. • Quickly develops echinocandin resistance, and azole resistance is common.
***Pichia kudriavzevii* (previously known as *Candida krusei*)**	• Temporary presence of mucosal membranes in healthy people. • Widely found in nature (fruits and vegetables) • Impacts both transplant recipients and those with hematological malignancies. • Fluconazole resistance that develops quickly, along with multidrug resistance (echinocandins and azoles) • Isolation rates are lowest in Indonesia, South Korea, and Thailand, and highest in North America and Europe (Czech Republic, 7.6%).
Candida tropicalis	• Found on the mucosa, skin, and nails. • Widely found in the environment, including the Amazon forest, soil, and water. • Fluconazole resistance, primarily in India, with Turkey, Spain, and Algeria following. • Patients with cancer are at risk in the northern hemisphere; this species is the most significant in India and Pakistan and the second most important in Latin America. • Transferring horizontally in hospitals • strong producer of biofilms
Candida auris	• 10% of those colonized by skin colonizers go on to develop invasive infections. • Either the skin or the nearby contaminated healthcare environment is the endogenous source of the infection. • In connection with significant outbreaks in the medical field • quickly emerging as a significant pathogen that causes invasive infections, especially in nations

	with low and middle incomes . • Resistance patterns by clade, with clade I being more resilient than clades III and IV (invasive infections are rarely linked to clades II and V).
***Candida parapsilosis* species complex**	• Colonization affects 10% of adults. • Most infections are exogenous. • impacts both newborns and the elderly. • In connection with inadequate hand hygiene and total parenteral nutrition. • powerful in vivo biofilm-producing. • Propensity for clonal outbreaks and increased fluconazole resistance. • frequently found in southern Africa and southern Europe.

2.2.7 Renaming the species of Candida

Many species of Candida have had their names changed recently. Table 2 lists these Candida species' previous and current names. Recent data about molecular-based epidemiology, diagnostics, and taxonomy inform this new nomenclature. The clinical significance of these species has not changed as a result of these taxonomic shifts, though. From mild mucocutaneous candidiasis to serious disseminated diseases, they are still linked to a variety of infections. Furthermore, the increasing prevalence of antifungal resistance in these species poses serious difficulties for clinical practice, making treatment approaches and diagnosis (Kidd SE et al.,2023)

TABLE( ): Nomenclature changes in clinically Candida spp. (Tajane et al.,2025)technologies in

NO.	Prior name	Recent name
1	*Candida guilliermondii*	*Meyerozyma guilliermondii*
2	*Candida glabrata*	*Nakaseomyces glabrata*

3	*Candida colliculosa*	*Torulaspora delbrueckii*
4	*Candida famata*	*Debaryomyces hansenii*
5	*Candida fabianii*	*Cyberlindnera fabianii*
6	*Candida catenulata*	*Diutina catenulata*
7	*Candida bracarensis*	*Nakaseomyces bracarensis*
8	*Candida kefyr, Candida pseudotropicalis*	*Kluyveromyces marxianus*
9	*Candida krusei*	*Pichia kudriavzevii*
10	*Candida rugosa*	*Diutina rugosa*
11	*Candida pseudorugosa*	*Diutina pseudorugosa*
12	*Candida pelliculosa, Pichia anomala*	*Wickerhamomyces anomalus*
13	*Candida pararugosa*	*Diutina pararugosa*
14	*Candida norvegensis*	*Pichia norvegensis*
15	*Candida neorugosa*	*Diutina neorugosa*
16	*Candida nivariensis*	*Nakaseomyces nivariensis*
17	*Candida lusitaniae*	*Clavispora lusitaniae*
18	*Candida lipolytica*	*Yarrowia lipolytica*

2.3 Laboratory diagnosis of candida

In order to identify the phenotypic and genetic traits of a Candida infection, laboratory diagnostic techniques employ both traditional and molecular approaches (Musinguzi et al.,2022). To diagnose and identify Candida species, conventional and biochemical methods are frequently employed. Since antifungal susceptibility patterns differ among these species, precise microscopic analysis and culture on Sabouraud's dextrose have long been essential diagnostic tools for fungal infections (Fortún et al., 2012;Bakhshiet et al., 2016 ; Song et al., 2020). In addition to improving the mortality rates from

these infections, a proper diagnosis will result in treatment that is suitable for the patient. For treatment to be effective, it is crucial to correctly identify Candida species and determine the susceptibility profile to the primary antifungal agents of medical interest in vitro. Therefore, in order to guarantee accurate diagnosis, appropriate treatment, and an enhanced quality of life for critically ill patients, it is imperative that clinicians and laboratory technicians adopt a standard of conduct (Dias., 2020).

2.3.1 Microscopic diagnosis

Microscopy can be used in the lab to diagnose Candida infections. There are many false positives and negatives, and the sensitivity of microscopy ranges from 60% to 70%. Gram staining of budding yeast cells allows colonies to be confirmed on culture. It is possible to confirm presence of Candida albicans using a germ tube test. Candida species can be distinguished using chromogenic agar medium while maintaining sensitivity (Sobel and Akins, 2015)

2.3.2 Macroscopic diagnosis

The purported colonies of Candida spp. on SDA are smooth and have a cream color at 30 °C. Following seven more days of incubation, the colonies developed into wrinkly, creamy, white colonies that smelled like yeast. Over the course of one to two days, C. albicans colonies rapidly form in the yeast extract agar, but they have a fermented smell and a soft, creamy white color (Al-oebady, 2015). According to (Grabe et al., 2015), men with suspected UTIs, women with unusual and pregnant women, symptoms should all have a urine culture.

Other differential media that enable the identification of specific Candida species based on colony appearance and color after primary culture have emerged in recent years. Pagano-Levin agar or commercially available chromogenic agars, such as CHRO Magar C. albicans ID, Fluroplate, or

Candichrom albicans, are examples of such media. The advantage of these is that they allow for the determination of the presence of multiple Candida spp. in a single infection, which can be crucial when choosing subsequent treatment options. (Sharma et al., 2021).

2.3.2.1 Chromogenic agar test

All of the Candida isolates were put into chrom agar Candida medium, which is meant to help differentiate between different species of Candida by looking at the colors and shapes of their colonies. For C. species, particularly C. albicans, chromogenic agar is a more useful and modern method. It contains chromogenic chemicals and enzymatic substrates. Using CHROM agar for species distinction would be beneficial for quick and easy differentiation (Collee et al., 1996). Verification of Chrome Agar-identified Candida species: A green colony indicating the formation of a germ tube was formed by Candida albicans, a pink colony by Candida krusei, a dark blue colony by Candida tropicalis, and a cream-to-white smooth colony by Candida glabrata (Tsega and Mekonnen, 2019).

2.3.3 MALDI-TOF MS

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was technique first used to identify fungi in the early 2000s, but it was only applied to a small number of species (Hun-Yun Chen et al., 2005). The successful identification of bacteria using MALDI-TOF MS in clinical laboratories sparked interest in using the technique for fungal pathogens. Due to the reliability of MALDI-TOF MS, which has been shown in numerous studies, it is now frequently used in medical settings for yeasts and is being used more and more for molds (Hendrickx et al., 2017).

2.3.5 Molecular diagnosis of Candida species

In a variety of medical specialties, such as virology, mycology, parasitology, and bacteriology, recent developments in molecular techniques have transformed the direct detection of microbial DNA in blood samples, expediting the diagnostic procedure and characterizing microorganisms. Numerous techniques have been created, and some of them have been sold (Opota et al., 2015; Ginn et al., 2017; Sinha et al., 2018; Cendejas-Bueno, 2019).

The development of molecular technology has made it possible to quickly identify and detect fungi in a variety of fields, including science and medicine. The internal transcribed spacer (ITS) region, a highly conserved nucleotide sequence among fungal species, was one of several fungal genome targets that were examined. To diagnose Candida isolates as soon as possible, down to the species level (Imran, 2015).

These methods are costly, but there is no denying their increased speed and accuracy. For molecular diagnosis and species identification, molecular methods like polymerase chain reaction (PCR), multilocus enzyme electrophoresis (MLEE), fragment length polymorphism (RFLP), electrophoretic karyotyping (EK), random amplified polymorphic DNA (RAPD), multilocus sequence typing (MLST), and real-time polymerase chain reaction (real-time PCR) are appropriate used for identifying and diagnosing Candida species (Bakhshi et al., 2016; Dadar et al., 2018; Aslani et al., 2018).

PCR and MALDI-TOF MS are two costly methods for diagnosing candida species and figuring out their relative severity (Yazdanparast et al., 2015). Phylogenetic analysis is required to better understand the evolution of the genus Candida and pinpoint evolutionary pathways for specific gene families (Diezmann et al., 2004).

Universal fungal primers were utilized in a straightforward DNA extraction and PCR amplification procedure to amplify rDNA from a set of reference strains and clinical isolates.Automated dye was used to sequence the entire internal transcribed spacer (ITS) barcoding sequence region in order to determine the genotypes of Candida (Pryce et al., 2003).

Since the internal transcribed spacer (ITS) non-coding regions are easily amplified using universal primers that are compatible with the majority of fungal species, they have emerged as significant molecular targets for taxonomy and identification (Al-Shuhaib et al.,2019). Fungal species and strains are better identified by the ITSI and ITS4 domains than by the small subunit (18s), large subunit (28s), and 5.8s regions (Pryce et al., 2003 ; Paul et al.,2020).

2.3.4 Ultrasonography

Typically, computed tomography is the preferred imaging test for certain patients with Candida UTIs, while ultrasound is the first imaging study of choice. Prostate and epididymis evaluation, as well as ball detection, can be done with imaging studies. Fungal balls can be diagnosed by excretory urography or retrograde pyelonephritis, renal or perinephric abscesses, obstruction at any level in the urinary tract, or fungal pyelography. Using imaging features that show a low-density filling defect, calcified areas, and gas bubbles all at the same time, upper urinary tract fungus balls Candida bezoars) can cause obstruction. When antifungal treatment is not working for a patient, imaging tests are also recommended to assess potential complicating factors. Urinary retention should be assessed using the postvoid residual test in patients who have persistent candiduria (Odabasi& Mert 2020).

2.4 Invasive candidiasis risk factors

(Pappas et al.,2018;Bassetti et al.,2022;kulberg et al.,2016)

When the following medical procedures are carried out, the proliferation of Candida species in the skin or mucosal flora, with multiple body sites of culture-based colonization, may be a risk factor.

1. Mechanical ventilation
2. Utilizing broad-spectrum antibiotics
3. Extended hospitalization in an intensive care unit or acute care treatment center

Indwelling prosthetic devices, such as external ventricular drains, ventriculoperitoneal shunts, or left ventricular assist devices

1. Gastrointestinal perforation.
2. Indwelling prosthetic devices, such as external ventricular drains, ventriculoperitoneal shunts, or left ventricular assist devices.
3. Gastrointestinal surgery.
4. Extensive burns.
5. Instrumentation for the urinary tract.
6. Use of intravenous drug .
7. Parenteral nutrition in whole.
8. Peritoneal dialysis or Haemodialysis.
9. Mucitis brought on by chemotherapy.
10. Intravascular indwelling catheters.
11. Necrotizing or acute pancreatitis.

The following are instances of acquired or intrinsic immunosuppression.

1. Diabetes mellitus.

2. Invasive candidiasis susceptibility due to genetic differences in immune-related genes.

3. Age extremes (especially for tiny, defenseless newborns).

4. Disease caused by graft-versus-host.

5. Prolonged and severe neutropenia.

6. Immunosuppressive therapy, such as corticosteroid use.

7. Infections with viruses like COVID-19.

8. Haematopoietic stem cell transplantation and solid organ transplantation recipients.

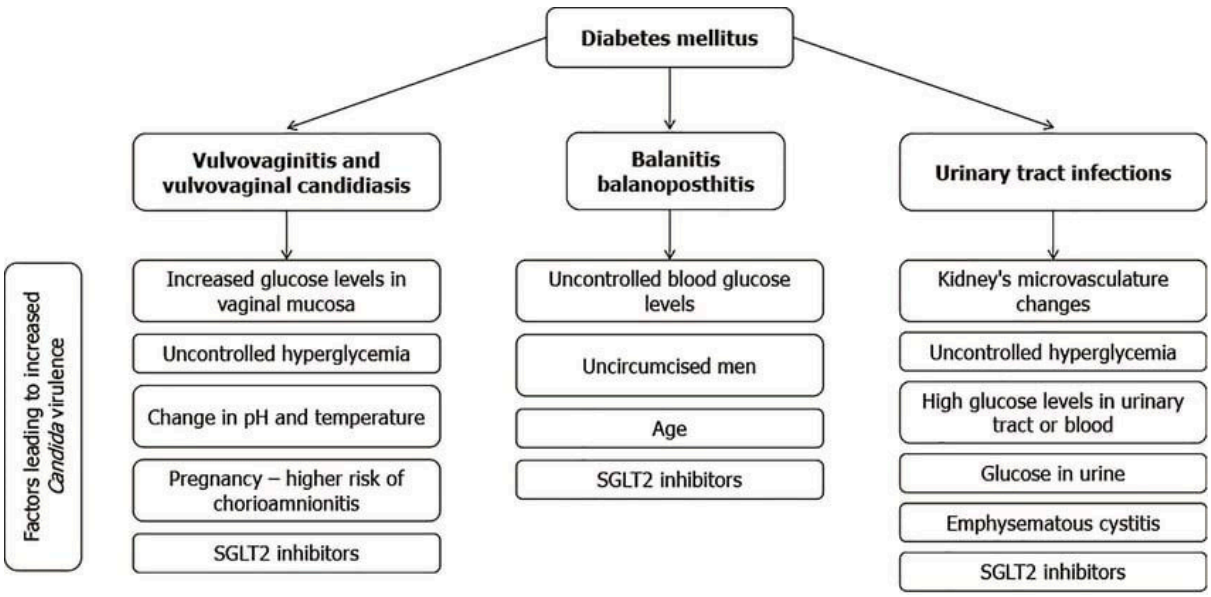


Figure ( ):lists the main causes of urogenital candidiasis in people with diabetes mellitus (Talapko et al.,2022). SGLT2 : Sodium glucose cotransporter2

2.4 Human Urinary System

The "urinary tract" (UT) is a crucial part of the human body that serves as a drainage system, eliminating waste and extra fluid from the bloodstream through urine during urination and controlling electrolyte levels(90)(White., 2021)The human body's homeostasis depends heavily on the urinary tract system.The kidneys, ureters, bladder, and urethra are the first among several organs that make up this system, which is also referred to as the renal system (Chromek and Brauner, 2008; Woolf et al., 2019; Brenner, 2022), Figure (-).

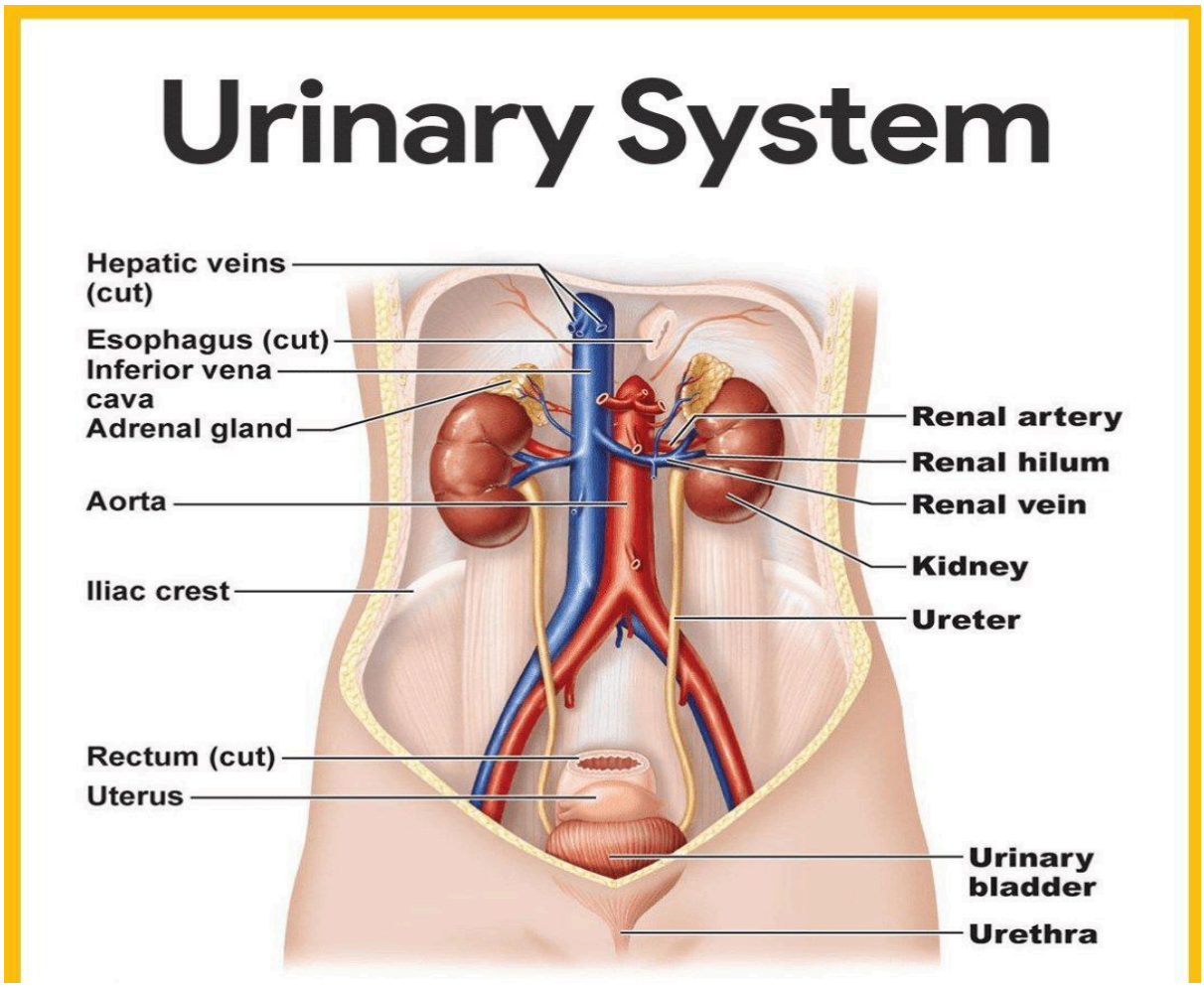


Figure (-):The urinary system Components (Tortora and Derrickson, 2017)

2.4.1 Urinary Tract Infection

Urinary tract infections (UTIs) are a major contributor to the use of health care resources and a chronic and fatal global health issue. According to estimates, UTIs caused 405 million infections and 267,000 fatalities worldwide in 2019. Women are 3.6 times more likely than age-matched men to get a UTI, and 50% of all women are predicted to get at least one UT12,3 in their lifetime. The symptoms, which include pain, urgency, and frequency, all have a detrimental impact on quality of life. The incidence rises with age and exhibits a strong sex bias (Timm et al.,2025). One of the infections that is most frequently diagnosed in both hospital and community settings is urinary tract infection (UTI). A UTI may be caused by bacteria or fungi. Evidence suggests that fungi, particularly Candida species, are now responsible for a higher percentage of UTIs,particularly in critically ill patients, while Escherichia coli, Proteus, and Pseudomonas species are responsible for a declining percentage. A recent study showed that two distinct fungi, type C. albicans and another species of Candida, or E. coli and Enterococcus faecalis, as well as other urinary tract pathogens, could coexist in urine samples (Dias,2020).

UTIs are prevalent throughout the world, and effective treatment is hampered by antimicrobial resistance (AMR). Due to the social-biological nature of AMR, interdisciplinary approaches are necessary for interventions (Onyango,2025). It is possible to isolate other species, including Candida glabrata, Candida parapsilosis, Candida tropicalis, Candida kefir, Candida lusitanae, Candida guilhermondi, and Candida dubliniensis, even though Candida albicans is the most frequently reported species in urine culture . Recently, the urine of a hospitalized patient suffering from candidemia revealed the presence of another species, Candida auris (Dias,2020).

Women of all ages are susceptible to urinary tract infections (UTIs), which are a common health concern. Women's unique lower urinary tract anatomy and its

close proximity to reproductive organs make them more vulnerable to UTIs.Women are more susceptible to UTIs due to a number of life stages and medical conditions, such as pregnancy, postmenopause, diabetes, epilepsy, urinary catheterization, and surgery (Anwar., et al .2025).

2.4.1.1Classification of UTI

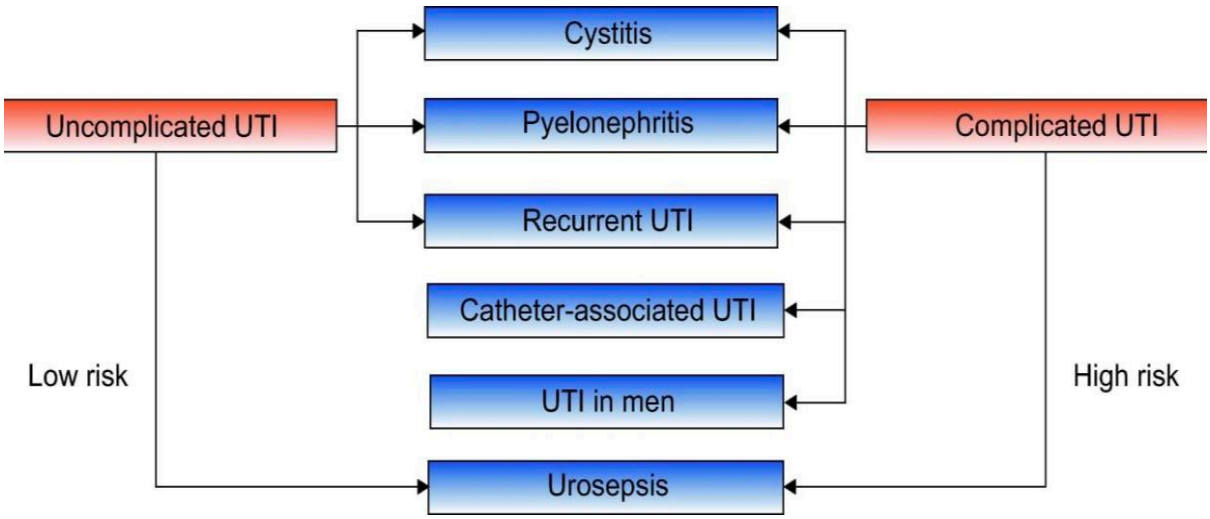
UTIs can be classified as uncomplicated or complicated , When risk factors like age, diabetes, catheterization, patient comorbidities, and spinal cord injury are present (Mancuso et al.,2023).

2.4.1.1.1 Complicated UTI

Urinary tract infections that are linked to conditions that impact the urinary tract or host defense, such as urinary tract obstruction, urinary retention brought on by neurological conditions, immunosuppression, renal transplantation, renal failure, pregnancy, finding foreign bodies, and other drainage devices or indwelling catheters, are referred to as complicated UTIs. Complications of complex urinary tract infections include kidney stones, congenital malformations, and anatomical abnormalities of the urinary system. Kidney and pelvic infections,foreign bodies, and indwelling catheters or other drainage devices, are referred to as complicated UTIs. Complications of complex urinary tract infections include bladder irritation, kidney stones, congenital malformations, and anatomical abnormalities of the urinary system. Kidney and pelvic infections, and in more severe cases, bladder dysfunction, renal dysplasia, increased renal pelvis dilatation, and renal failure are among the symptoms (Banday et al., 2009; Kranz et al., 2017; El et al., 2021; Lombel et al, 2022).

2.4.1.1.2 Uncomplicated UTI

A urinary tract infection is considered uncomplicated if there are no functional renal failure or anatomical or functional abnormalities in the urinary tract. These infections can be nonfunctional and present with complicated symptoms, including flank pain and fever, when they occur in the upper urinary tract. There will be difficulty urinating and no other symptoms if the infection is in the lower urinary tract. The two types of these infections are categorized as upper urinary tract infections (pyelonephritis) and lower urinary tract infections (cystitis). Sexual activity ,prior UTIs, and female gender are among the numerous risk factors linked to cystitis, vaginal infection, Genetic susceptibility, diabetes and obesity (Schliemann et al., 2010; Flores-Mireles et al., 2015; Richa et al., 2016; Kranz et al., 2017).



Figure(-):Complicated and uncomplicated UTI (Kranz et al.,2024).

2.5 Diabetes Miletus

Diabetes is a condition that develops when your body is unable to properly produce, convert, or use glucose from food as fuel. The body produces glucose, a type of sugar, during food digestion. People who have diabetes have high blood glucose levels, which can harm all bodily systems if they are not treated (Diabetes, 2016)..The definition of diabetes mellitus varies de pending on one's viewpoint, and it is not a single disorder. Medically speaking, it refers to a group of metabolic disorders brought on by partial or complete insulin insufficiency and linked to hyperglycemia. Chronic hyperglycemia exposure can cause peripheral nerve, kidney, or retinal microvascular problems. Because they take too long to appear, these characteristics of diabetes cannot be used to define the condition Because they are often present in the pre-diabetic state, the so-called macrovascular complications of diabetes-myocardial infarction, stroke, and peripheral arterial disease-occur more frequently. In order to highlight the clinical issues that the majority of patients face, it has been proposed that diabetes be defined as "premature atherosclerosis with associated hyper-glycaemia.(Egan, & Dinneen, 2019).Diabetes is a major global health concern, with a high disease burden that has significantly increased in most developed and developing nations in recent decades. 537 million adults worldwide have diabetes, according to estimates from the International Diabetes Federation. If effective preventative measures are not taken, this number is expected to rise to 693 million or more by 2045. The Global Action Fund for Fungal Infections, on the other hand, estimates that over 300 million people of all ages experience a fungal infection each year, which can have a major seriously impact their health (Talapko et al., 2022). which also covers urogenital infections brought on by Candida-related yeasts. Given how common these two often coexisting clinical conditions are throughout the world, it is not surprising that genitourinary candidiasis is currently common in primary care as well as

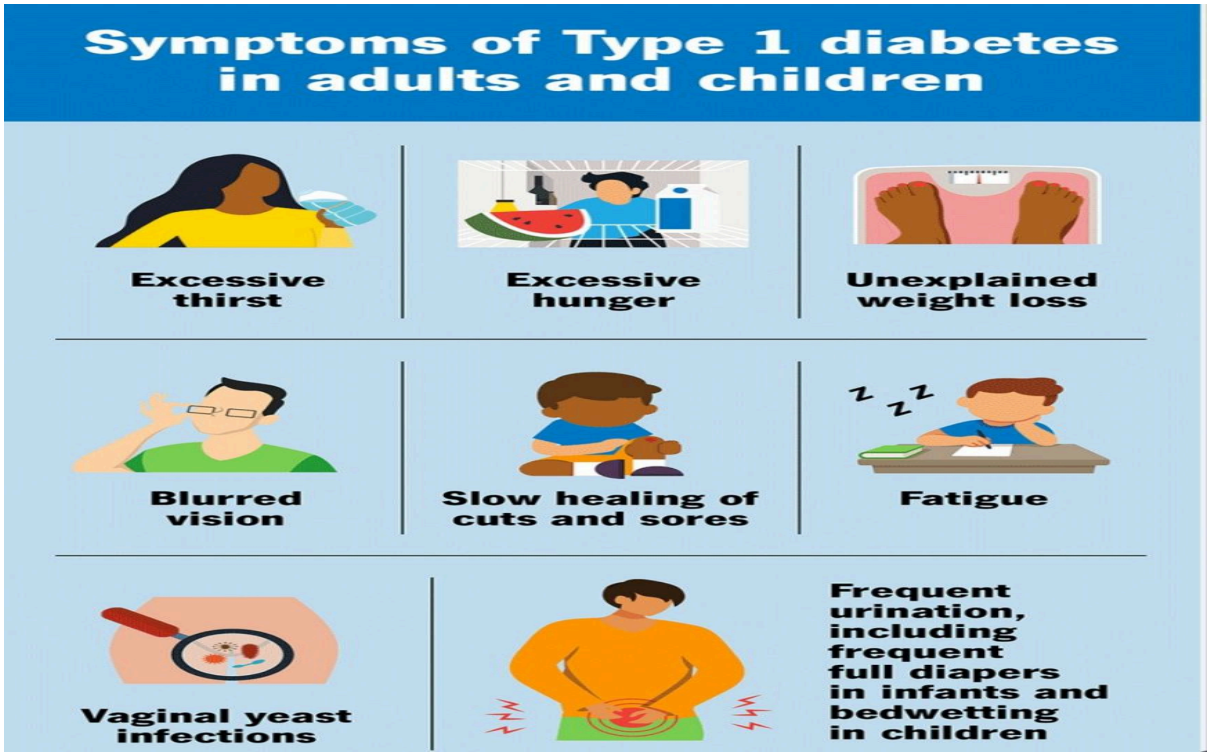
secondary and tertiary care settings among diabetic patients (Nyirjesy and Sobel., 2013). Fasting blood glucose levels of up to 5.6 mmol/L are considered normal in the quotidian clinical approach. A crucial criterion for the diagnosis of diabetes mellitus is when these values are greater than 7 mmol/L; prediabetes is indicated by values between 5.6 and 6.9 mmol/L. As a result, two glucose readings must always be taken: one on an empty stomach, and the second, one to two hours after eating. Two hours after a meal, blood glucose levels should be below 7.8 mmol/L; if they are still above 11 mmol/L, we can be fairly certain that we have diabetes mellitus. We consider prediabetes or glucose intolerance if these values fall between 7.8 and 11 mmol/L (Talapko et al., 2022)

2.5.1Diabetic history

Medical professionals in ancient Egypt had been aware of diabetes mellitus for almost 3,500 years, based on its symptoms but not its etiology. The 1550 BC Ebers papyrus, named for Egyptologist Geary Ebers, was discovered in a grave in the southern Egyptian region of Thebes in 1862. A polyuric syndrome, most likely diabetes, is one of the illnesses described in the papyrus. Several treatments for this syndrome were proposed by the Egyptians, including a mixture of earth, wheat, and bowes (Ahmed.,2002). Ancient Indian Verdic medical texts provided detailed descriptions of two types of diabetes-like conditions: congenital and late-onset. Indians also observed the connection between diabetes and diet, sedentary lifestyle, obesity, and heredity (Algaonker .,1972).

2.5.2 Classification of Diabetes Miletus

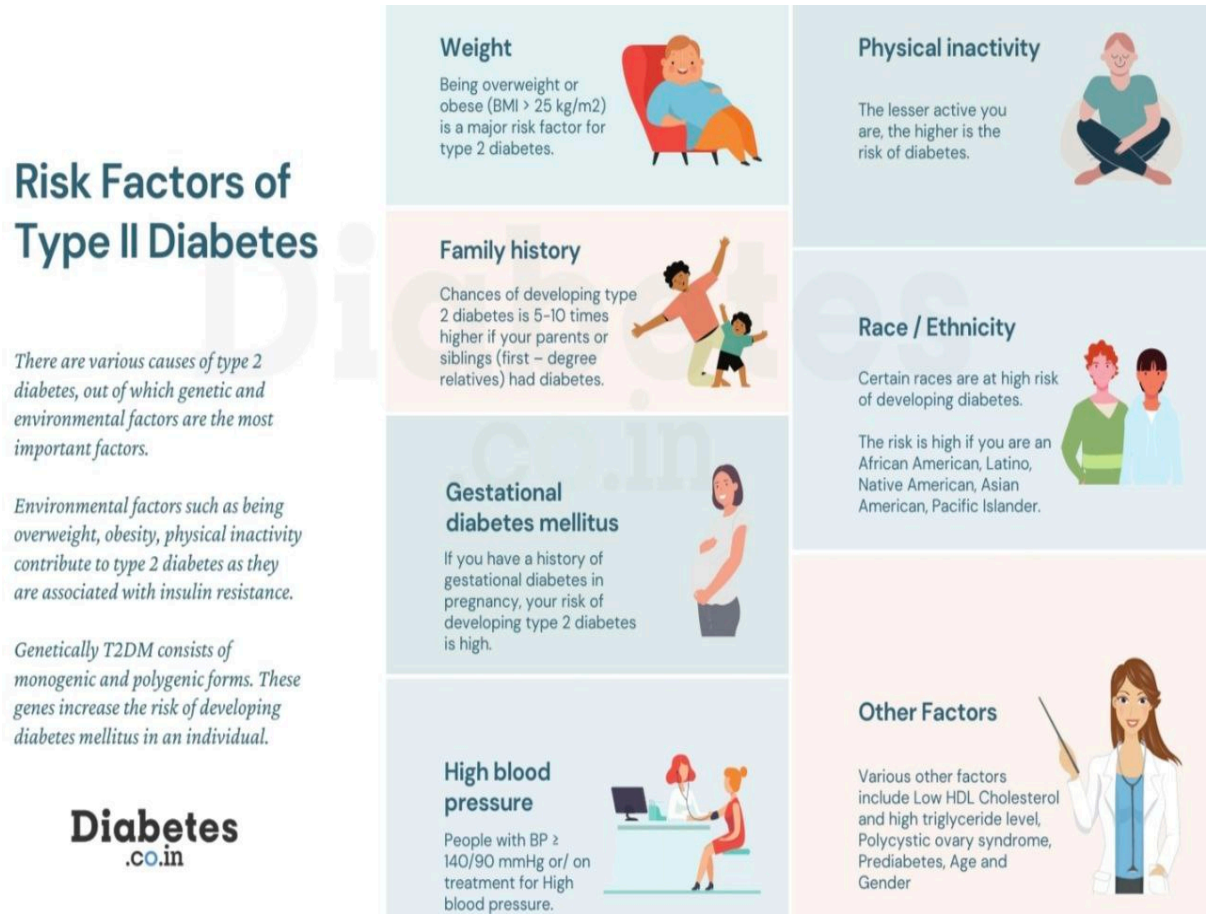
Type 1 diabetes:Pancreatic B-cells are destroyed in type 1 diabetes, typically by inflammatory processes caused by autoimmune diseases. Serum autoimmune markers include zinc transporter ZnT8, insulin, tyrosine phospha tases IA-2 and IA-2B, islet cell autoantibodies, and autoantibodies to glutamic acid decarboxylase (GAD). The progression rate of this damaging process can vary, but it usually results in complete insulin insufficiency with undetectable plasma C-peptide levels (Egan, & Dinneen, 2019).



Figure(-):T1DM (https://share.google/LdykinAwpTMGavdDe)

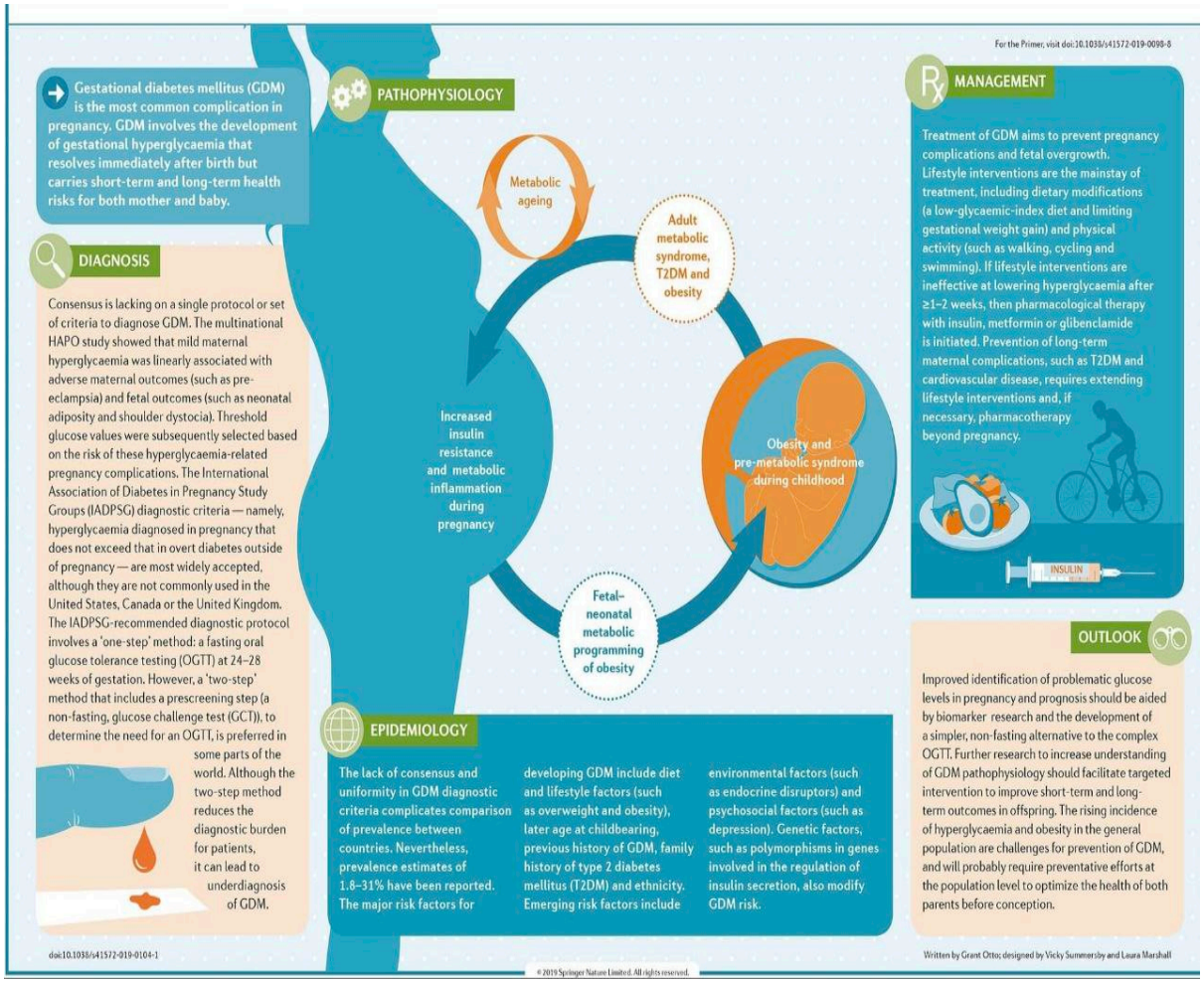
Type 2 diabetes: Type 2 diabetes accounts for 90-95% of all cases of diabetes and is the most common type. Insulin action and stashing diseases are the two main characteristics that set it apart. When diagnosed with this type of diabetes, these individuals do not receive insulin treatment to survive, and they continue to do so throughout their lives. The causes of this kind of diabetes are conceivably numerous. The specific causes are unknown, but there is no

evidence of autoimmune destruction of B-cells. Most people with type 2 diabetes are overweight, but not all of them are; excess weight can lead to insulin resistance. Age, rotundity, and a lack of physical activity all raise the risk of type 2 diabetes (Patial et al., 2025)



Figure(-):Risk factors of T2D(https://share.google/xZYUAxo1VrOOjBNUp)

Gestational diabetes: The definition of gestational diabetes is carbohydrate intolerance. excluding women who most likely have overt pre-gestational diabetes, and having an onset or initial recognition during pregnancy. It is a frequent pregnancy-related medical issue that is linked to a higher risk o f negative outcomes. Despite the fact that the condition usually goes away after giving birth, women who are affected should be evaluated for postpartum diabetes and informed that they have a higher chance of developing type 2 diabetes in the future (Egan, & Dinneen, 2019).



Figure(-):GDM (McIntyre et al., (2019).

2.5.4 Role of Diabetes in accurance of Candida

Candidauria, or the presence of Candida yeasts in the urine, is a sign that the urinary tract has been infected or colonized by Candida species. Patients with diabetes may be at risk for this condition because of a weakened immune system(Falahati et al., 2016). Additionally, patients with diabetes (67%) and those without immunocompromised conditions (60%) also had high mortality rates. In diabetic patients, glucose and fructose are the primary pathophysiologic and nutritionally significant sugars; however, Candida sp. growth is also significantly influenced by other simple carbon sources. assessed the rate of C. albicans growth in the presence of varying glucose and fructose concentrations in order to gain a better understanding of the nutrient acquisition strategy and its

potential connection to diabetic patients'hyperglycemic state. The frequent yeast infections that occur in patients with uncontrolled diabetes may be related to the authors' finding that the growth of Candida albicans is directly correlated with glucose concentration (Rodrigues et al., 2019).It is noteworthy that fructose exhibited the ability to inhibit Candida albicans. Accordingly, foods high in fructose may help stop candidiasis from developing. The outcome of oral Candida sp. biofilms is significant, particularly for patients who wear prostheses (Man et al.,2017).

In actuality, other carbon-based substances like lactose, sucrose, and maltose lessen the effectiveness of antifungal medications while increasing the density of the fungal population. The effects of glucose on Candida sp. susceptibility to antifungal agents in diabetic mice were investigated in a recent studAccording to that study, amphotericin B (AmB) and voriconazole (Vez) have the biggest declines in antifungal medication efficacy. Through hydrogen bonding, glucose showed a greater affinity for binding to Vez , reducing the antifungal agents' susceptibility during chemotherapy .Furthermore, it was verified that Amß and Vez both displayed two hydrogen bonds that stabilized the glucose and three significant hydrogen bonds, respectively.y (Rodrigues et al., 2019).

The same study's in vivo findings suggested that the physiologically significant elevated blood glucose levels in DM mice may interact with the available selective agents during antifungal therapy, reducing glucose activity through complex formation. Complexes of Vcz-glucose and AmB-glucose appear to be less effective than their pure counterparts. Therefore, if we are to control infectious diseases, it is crucial that we choose the right medications for DM patients.(128)(Mandal et al., 2014).Culture of C. glabrata in a glucose-enriched medium reduces its sensitivity to fluconazole in there study(Rodrigues et al.,2017)

It examined how glucose affected the transcriptome of Candida albicans in order to modify carbon assimilatory pathways during pathogenesis. Significant effects of blood glucose levels on C. albicans gene regulation were revealed by the increased resistance to oxidative and cationic stressors as well as resistance to miconazole. While Vcz and AmB became less effective, anidulafungin showed no discernible susceptibility level (Rodaki et al., 2009).

2.6 Antifungal susceptibility

2.6.1 History of Antifungal

Fungal infections can affect the skin, nails, mouth, lungs, and blood, and antifungal agents are medications used to treat them . When topical antiseptics like phenol and iodine were first used to treat superficial fungal infections in the late 19th century, the history of antifungal agents began. however, stated that these agents had a narrow range of activity and were not very effective. A new era of antimicrobial therapy was ushered in by Fleming's 1928 discovery of penicillin, but it was quickly discovered that penicillin and other antibiotics lacked antifungal activity. Thus, in the middle of the 20th century, the hunt for particular antifungal agents started . Amphotericin B, which was isolated from a soil bacterium in 1955, was one of the first discoveries in antifungal therapy.

2.6.2 Type of Antifungal

Currently, there are four types of medications used to treat Candida. Flucytosine, azoles, echinocandins, and polyenes (amphotericin B). In this study, two categories are used (Moghimi et al., 2022).

2.6.2.1 Polyenes group

The Streptomyces spp. bacteria are the producers of a broad class of antibiotics known as polyenes.Their efficiency stems from their interaction with sterols found in fungal cell membranes, which increases membrane permeability and

allows ions and cell enzymes to leak out, ultimately leading to cell death (Ghannoum and Rice , 1999)

Amphotericin B: It is the most potent treatment for systemic fungal infections and one of the most significant compounds in the polyene group (Jawetz 2007). Streptomyces, an aerobic bacterium, was used to isolate amphotericin A and B. nodosus, which was separated from a soil sample in Venezuela's Orinoco River Valley. When amphotericin B binds to the ergosterol found in the fungal cell membrane, it alters the permeability of the membrane, allowing ions to leak out and ultimately leading to cell death. It has been suggested that this antibiotic works through oxidative reactions in the membrane as a second mechanism. (Schmidt, 2019). Amphotericin B is a broad-spectrum antibiotic that works well against a variety of molds and yeasts(Drew ,2010).(114)

Nystatin : The discovery of this antibiotic in 1950 made it the first to be used to treat fungal infections. When this antibiotic was first discovered and then it was later called Nystatin , it was known as "Fungicidin" and is produced by the bacteria Streptomyces noursei (Sobel, ,2015) . Nystatin is a topical medication that can be applied topically as a cream, ointment, or suppository to treat superficial and vaginal infectious diseases (Shahid et al.,2010). Its inability to be absorbed from the gastrointestinal tract means that it cannot be used to treat systemic fungal diseases (Tuite and Lacey, 2013).

2.6.2.2 Azoles group

In the clinic, azole antifungals were a major class of antifungals that were used extensively thirty years ago. Currently, there are roughly 40 azole-based drugs, and based on their structural types, they can be categorized into four groups:

1-First generation: Imidazole such as clotrimazole and miconazole.

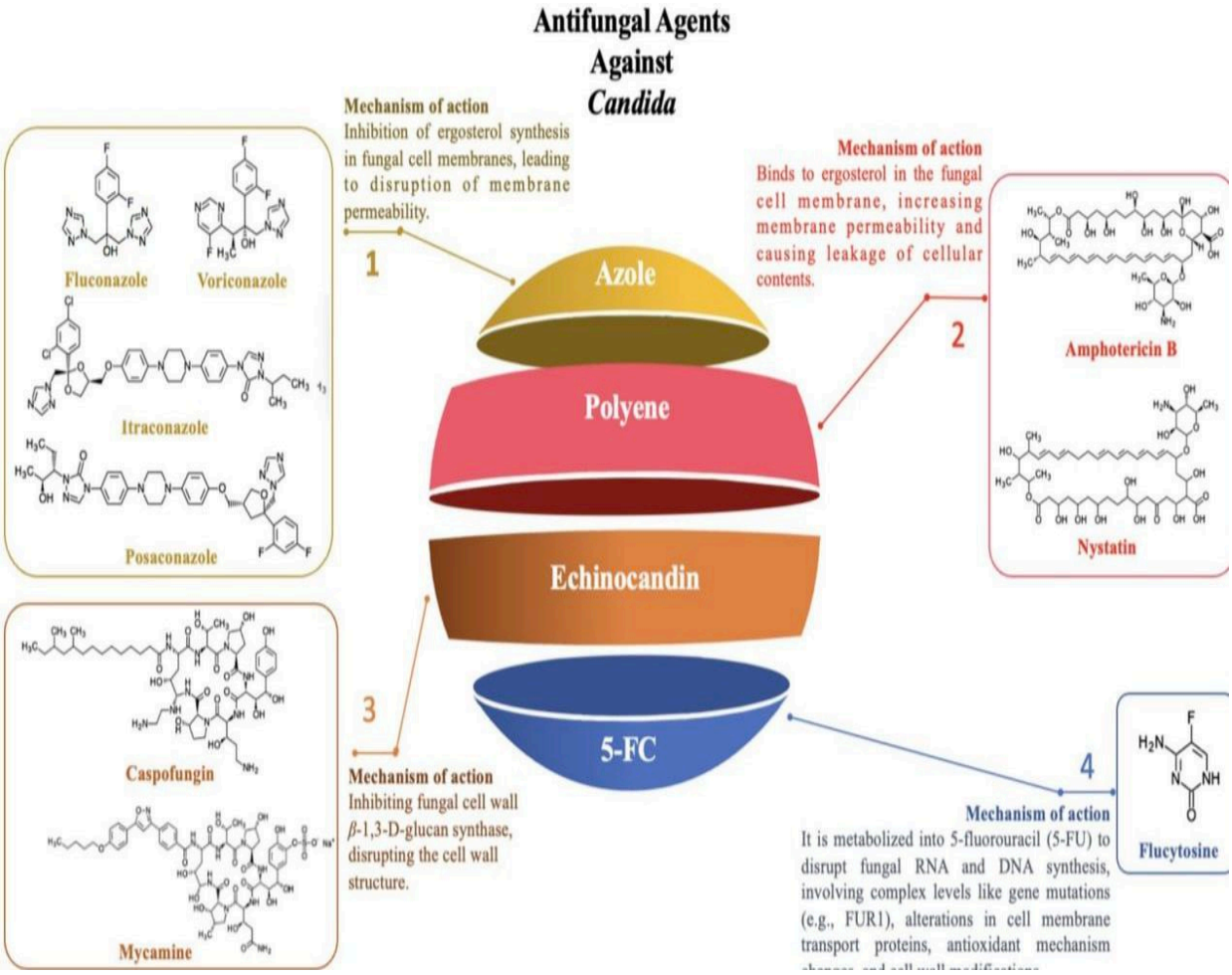
2-Second generation: Triazole including itraconazole and fluconazole.

3-Third generation: Medications include voriconazole, isavuconazole, posaconazole and albaconazole.

4-Fourth generation: VT-1598, quilseconazole, and oteseconazole are examples of side chains that have been added to tetrazol (Tsubamoto, 2017:Teixeira, 2022)

2.6.3 The antifungal mechanism

In order to treat candidiasis, antifungals-drugs belonging to various classes that target distinct biological processes-can either inhibit (fungistatic) or eradicate (fungicidal) the growth of this harmful yeast. Among these biological processes is the synthesis of the cell wall and membrane (Bhattacharya et al., 2020).



Figure(-):Antifungal Agents Against Candida (Huang et al., 2025).

2.6.3.1 Inhibitors of the Protein Biosynthesis

Additionally important are antifungals that interfere with the synthesis of nucleic acids. First developed in the 1950s, they are among the oldest classes of antifungal drugs. 5. Fucytosine (SFC) is an antifungal that prevents nucleic acid synthesis. 5FC can enter the susceptible cells thanks to the cytosine permease enzyme (Bhattacharya and colleagues, 2020 The conversion of 5FC to 5-Fluorouridine triphosphate (5FUTP) yields 5-Fluorouracil (5FU). 5FUTP, as opposed to uridine triphosphate (UTP), is absorbed into the fungal RNA, which impacts protein translation. Alternatively, 5-Fuorodeoxyuridine Monophosphate (SFdUMP) can be produced from SFU, which inhibits the essential enzyme thymidylate synthase from performing its DNA synthesis function (Vermes et al.,2000) .

2.6.3.2 Antifungals that Affect the Biosynthesis of Ergosterol

Ergosterol is the main sterol present in fungal cell membranes, including the mitochondrial and plasma membranes. Sterols in yeast cells help to maintain the fluidity of lipid membranes and support a variety of vital processes, including vesicle formation and protein sorting,cytoskeleton organization, endocytosis, and mating (Caspeta et al.,2014). Sterols and sphingolipids combine to form lipid rafts in the cell membrane. These lipid rafts contain a variety of physiologically important proteins that are involved in signaling, mating, nutrition transport, and stress response (Andres and Alexandro,2020)

The production of ergosterol is catalyzed by a group of 25 different enzymes. Since ergosterol is a lipid that is necessary for plants and fungi but absent from humans, this biosynthesis pathway is an ideal target for medications (Kodedova and Sychrova,2015).

2.6.3.3 Inhibitors of the Biosynthesis of Cell Walls

Along with ergosterol, antifungals also target the biosynthesis of cell walls in Candida species. The main defense against osmotic stress is the fungal cell wall, which is its rigid outer layer. The enzymes involved in cell wall biosynthesis are important targets for antifungals because mammalian cells lack cell walls(Popolo et al.,2001). Echinocandins are antifungal drugs that attack the cell wall (Munro,2010).

2.6.4 The antifungal resistance mechanism

Antimicrobial resistance (AMR) is a serious global health concern (Collaborators, 2022). When compared to antibacterial medications, antifungal resistance is a significant concern in clinical practice because antifungal agents are much less readily available (Denning, 2022). This is because fungi are eukaryotic organisms that resemble mammalian cells in many ways, It making challenging to identify appropriate targets for antifungal drugs (Sanglard, 2016; Gow et al., 2017).. Frequent use of antifungal agents and risk factors have increased along with the number of patients with infectious diseases, and empirical administration or overuse of selective therapies are contributing to the rise in antifungal resistance (Mendes et al.,2017). Over time, Candida species develop virulence factors and resistance mechanisms to the medications and antibiotics that are used, making them resistant to antifungals. Frequent use of medications and antifungals causes the body's immunity to deteriorate and the risk of developing a yeast infection to rise. By stimulating the host's immune system, antifungals can help eradicate a Candida infection (Zheng et al., 2005). Fungi have systems that expel the antifungal outside of the cell and can form biofilms that block the antifungal from reaching the target site, which is one of the reasons they develop resistance to antifungals. A genetic mutation brought on by the use of antifungals may also result in the emergence of resistant strains. As the number of individuals with immunodeficiency has increased, so

too have these resistant strains, which pose a significant global concern. (Saxena et al., 2007).

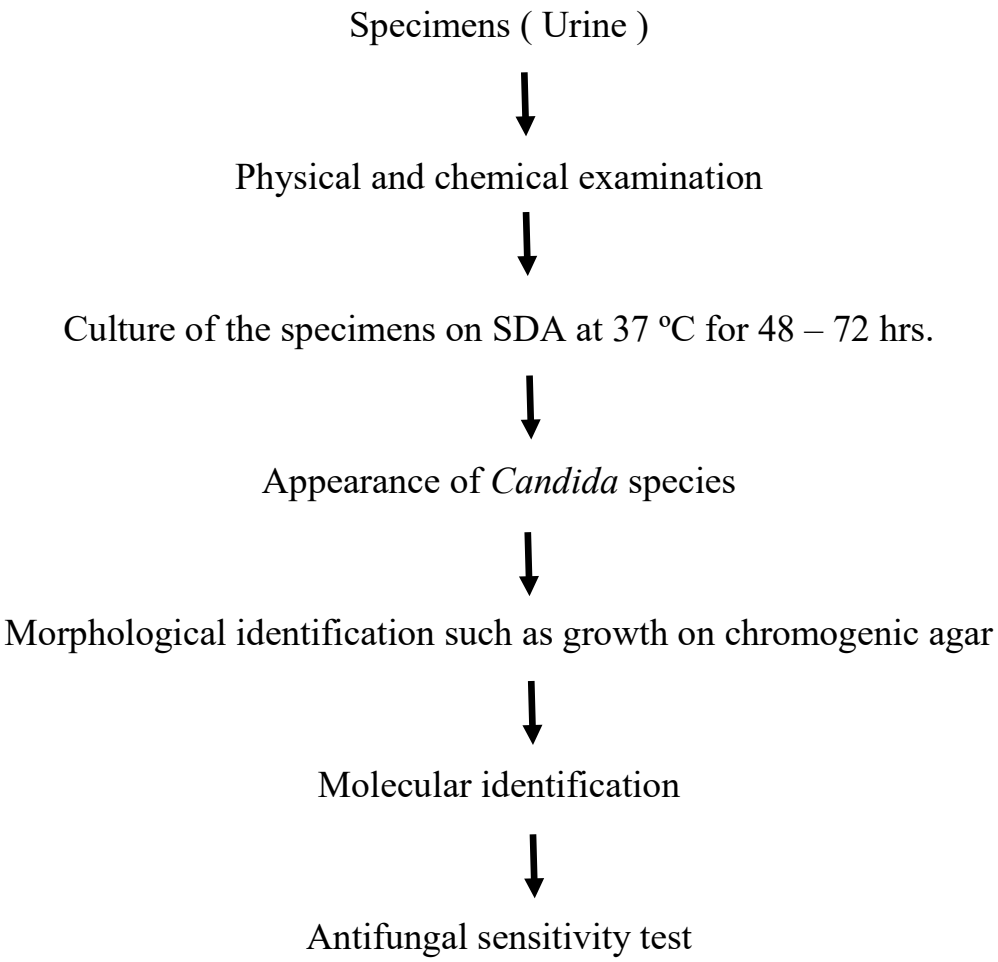
Chapter Three

Materials and Methods

3- Materials and Methods

3.Materials and Methods

Fig. (3-…): Diagram summarizes the plan of this study.



3.1 Study Area

The spatial boundaries of the study area are mainly represented by the city of Shatrah, the center of Shatrah district, which is affiliated with Dhi Qar Governorate. The district is administratively bordered by Maysan Governorate to the east and Al-Nasr district to the west, and it is bordered to the north by Al-Rifai district and then Al-Nasiriyah district to the south. It is located between latitudes (31.24 - (31.28) north and longitudes (46.8 - 46.12) east. The area of the study area is 20.7 km2, equivalent to 5.3% of the district's area of 384 km2 (Latif,2023).Thi-Qar provience,s climates are described as hot in summer and

humid in winter for an administrative purpose . The province of Thi-Qar is of great historical, social,and tourist importance (Hyndman and Jennifr,2007).



MAP OF IRAQ

https://en.m.wikipedia.org/wiki/Geography_of_Iraq



Map of Thi Qar Provance



Map of Al-Shatrah Diatract

3.1.1. Collection of Specimens

The specimens were urine collected from diabetic patients in General Hospital and clinics of AL-Shatrah District located in Thi-Qar Governorate,south of Iraq. These specimens were aseptically collected using sterile plastic cub tubes and cultured on Sabouraud dextrose agar (SDA) supplemented with azithromycin. Additionally, other parameters of the urine were also examined such as color, odor, turbidity, and pH value. Also, urine of non-diabetic patients infected with urinary tract infection (UTI) was obtained as well as specimens of healthy individuals were collected as a negative control, while specimens of non-diabetic patients represented a positive control. Collection of the specimens was conducted from August 2024 to January 2025. The total number of specimens was (250) And shown in (Table, 3-1).

Table (3-1): Total number of specimens used in this study.

No. of Specimens			
Healthy individuals	**Patients with UTIN**	**Patients with DMO**	**Total**
25	**25**	**200**	**250**

.►Patients with DMO: Patients with diabetes mellitus

3.2. Materials

The instruments, devices, other substances, and agents were shown in (Table 3.2, Table 3.3, Table 3.4, Table 3.5, Table 3.6, Table 3.7, Table 3.8, Table 3.9, and Table 3-10).

Table (3.2): A list of the instruments and equipment used in the present study.

NO	Instruments and devices	Manufacture
1	Biological Safety Cabinet	Iraq
2	Autoclave	Labtech
3	Vortex Mixer	Germany Industrial Corp Taiwan
4	Light Microscope with Camera	Olympus CX33 /Japan
5	Incubator	Memmert
6	Sensitive Balance	Sartorium Germany
7	Benzene Burner	Naugra / India
8	Colony accounter	Colony star Germany
9	Oven	Memmert
10	Refrigerator	
11	PH/MV/ISE/Temperature Meter	Adw Romania
12	Distillar	Boeco Germany
13	Hot plate	a LFA
14	Centrifuge	Biomerieux / France
15	Phone Camera	Redmi 14 pro plus
16	Water bath	Memmert /Germany

17	Timer	Himedia (India)
18	Microwave	China
19	Micropipette (1-10, 10-100 & 100-1000 nl)	Dragon / China
20	Ipendorf- centrifuge	15000g/1 min / china
21	UV Gel documentation	Cleaver (UK)
22	Gel Electrophoresis system	Cleaver (UK)
23	Thermal Cycler (PCR)	Multigene Potimax / Genmany
24	MacFarland solution	BioMerieux France/
25	Cotton Swabs	Himedia /India
26	Slides	Himedia /India
27	Disposable Syringe	Superestar /India
28	Cover slides	Himedia /India
29	Petri Dishes	Concord /Lebanon
30	Tips	China
31	Rack tip	Afco /Jordan
34	Filter Paper	china
37	Plain tubes	ALS /China
38	Disposable Loop	Himedia/India
39	Ice box	Tank /Egypt
40	Zipper bag	Iran

Table (3.3): Culture media used in this study.

NO	Culture ,Media	Symbols	
1	Sabouraud Dextrose Agar	SDA	TMMEDIA/India
2	Malt Extract Agar	MEA	Himedia /India
3	Brain Heart Infusion Agar	BHIA	Himedia /India
4	Michrom™ Candida Differential Agar	CCA	Accumix/India
5	Muller Hinton Agar	MHA	Accumix/India
6	Potato Dextrose Agar	PDA	Accumix/India
7	Sabouraud Dextrose Broth	SDB	Himedia /India
8	Brain Heart Infusion Broth	BHIB	Oxoid /England
9	Christensen's urea agar		Himedia/India
10	Corn Meal Agar	CMA	Himedia/India

Table (3.4): Materials used in this study.

NO	Chemicals	Company/	Country
1	Green Safe View	BioBasic	Canada
2	DNA Marker ladder 2000-100bp	iNtRON	Korea
3	Glycerol	Biolife	Italy
4	TBE buffer	iNtRON	Korea
5	Free nuclease water buffer	Bioneer	Canada
6	Nuclease free water	Promega	USA
7	Agarose	Bioneer	Canada
8	Loading buffer	Geneaid	Taiwan

9	Oligo Primers	Macrogen	South Korea
10	Urea suplement		
11	Azithromycin		
12	Lactophenol Blue dye		
13	Tween 80	Oxide	Germany
14	NaCl	CD- H	India
15	2,3,5- triphenyl tetrazolium chloride (TTC)		
16	Peptone		
17	Glucose		Iraq
18	Agar Agar	Accumix	India
19	Normal saline (0.9)	Choueifat	Lebanon
20	Liquid Nitrogen	Medite	USA

Table (3.5): Antifungal agents used in this study.

In accordance with CLSI, 2019, the Antifungal discs employ in the case study revealed in table (3.5) and this study serves as the basis for the global values of antibiotics .

NO	Antifungal	Symbol	Concentration	Company
1	Itraconazole	IT	10	Himedia /India
2	Ketoconazole	KT	10	Himedia /India
3	Clotrimazole	CC	10	Himedia /India

4	Nystatin	NY	100	Himedia /India
5	Fluconazole	FLC	25	Himedia /India
6	Amphotericin B	AP	100	Himedia /India

( ):Drugs given to the patients at Hospital (SGH)

NO	antifungal	Sympol	Concenration	origen	Company
1	Nystatin	Ny	500000IU	Baghdad\Iraq	Wadi AL-Rafidain
2	Fluconazole	Flc	150 mg	Arbil\Iraq	Awamedica

Table (3.6): The commercial Kits used in this study.

NO	Kits	Origin
1	gSYNC™ DNA Extraction Kit	Geneaid /Taiwan
2	Universal PH strips	China
3	GoTaq ® Green PCR master Mix	Promega /USA

Table (3.7): Fungi /yeast gSYNC™ DNA Extraction Kit.

NO	Material	Company	Country
1	PBS buffer	Geneaid	Taiwan
2	proteinase K		
3	GSB buffer		
4	absolute ethanol		

5	W1 buffer		
6	wash buffer		
7	Elution Buffer		

Table (3.8): The primers used annealing temperature and the products size for ITS gene..

Product size(bp)	Annealing Temperature	Primer Name	Oligonucleotide sequence (5′-3′)	Referance
500-800 bp	58	ITS 1	F-TCCGTAGGTGAACCTGCGG	Zhao , et al.,2001;Yin et al., 2017
		ITS 4	R-TCCTCCGCTTATTGATATGC	

Table (3.9): PCR reaction compounds used in this study.

NO	Reagents	Volume µl
1	Green Master Mix 2X (Promega): Taq DNA polymerase, dNTPs, MgCl2 and reaction buffer	13 µl
2	Primer forward	1 µl
3	Primer reverse	1 µl
4	DNA template (30-100ng)	3-4 µl
5	Free water	6-7 µl
	Total	25 µl

Table (3.10): PCR protocol used in this study.

Step		Temp.	Time	Cycle
Initial Denaturation		95	5.00 min	1 time
1	Denaturation	95	0.30 sec.	35 cycle
2	Annealing	56	0.30 sec.	
3	Extension	72	0.45 min	
Final Extension		72	10.00 min	1 time

3.2. Methods

3.2.1. Sterilization Methods

Table (3.11): Types of sterilization methods used in this study

Types of sterilization	Sterilized materials	Conditions
Autoclaving	Media and related equipments such as flasks and tubes	121˚C, 15 pounds/inch2 for 15 minutes
Millipore filter paper syringes	All substances which destroyed in high temperature degrees	0.22 µM
Absolute ethanol	►Tip of Loop,L-shape (100%) ►Hands ,Incubator and surface (70%)	Concentration100% with Burner and 70%
Burner	Loop ,L-shape And Cotton swab	
Oven		180 C^{o}
Clorophorm		

3.2.2. Preparation of Reagents and Solutions

3.2.2.1. McFarland (0.5) Turbidity Standard (Jain *et al*., 2016; Vaknin *et al*., 2022)

1. The 0.5 McFarland was prepared by mixing 0.5 ml of a 1.175% (w/v) barium chloride dehydrate ($BaCl_2$.2H2O) solution with 99.5 ml of 1 % (v/v) H_2SO_4.
2. The 0.5 McFarland standard tubes were sealed with parafilm to prevent evaporation and stored for up to 6 months in the dark at room temperature (22- 25°C), for checking the accuracy of the density prepared 0.5 McFarland standard by using aspectrophotometer, the absorbance of the wavelength of 625 nm should be between 0.08 and 0.1.
3. The 0.5 McFarland standards can be compared visually to a suspension of bacteria in sterile saline or nutrient broth.

3.2.2.2.Preparation of Culture Media

Except SDA with NaCl and TTC agar, all the media used in this work were prepared in the laboratory based on the manufacturer's instructions. Each prepared medium was then labeled and stored at 4°C in the refrigerator.

3.2.2.2.1. Sabouraud Dextrose Agar

The SDA was used for cultivation and isolation of *Candida* species and prepared by dissolving (65 gm) of the medium powder in a liter of distilled water. This medium was autoclaved. After cooling, azithromycin (250mg/L) was added to the medium to prevent bacterial growth. Aseptically, pour this medium into sterilized Petri dishes and store until use.

3.2.2.2.2. Malt Extract Agar

The MEA was prepared as SDA except amount which was dissolving (50gm) in a liter of distilled water. This medium was for sub-culture of *Candida* species.

3.2.2.2.3. Chromogenic Candida Agar

The CCA was prepared dissolving (45.9 gm) in a liter of sterilized distilled water that the liter was autoclaved. After cooling, the amount of the medium powder was added into the water until dissolving the powder completely. Then pouring in Petri dishes. The medium was used for cub-culture and differentiation of *Candida* species depending on the pigmented colonies.

3.2.2.2.4. Brain Heart Infusion Agar

The BHIA was prepared by dissolving (49 gm) powder in a liter of distilled water. This medium was distributed in the screw tubes, and autoclaved. After sterilization, the tubes were placed in a slant position and left until solidified. This medium was used for sub-culturing of *Candida* species.

3.2.2.2.5. Muller-Hinton Agar

The MHA was prepared by dissolving (38 gm) medium powder in a liter of distilled water, heated to dissolve the contents, and autoclaved. After cooling, the medium was poured in Petri dishes for performing antifungal sensitivity test.

3.2.2.2.6. Brain Heart Infusion Broth

The BHIB was prepared by dissolving (37 gm) medium powder in a liter of distilled water, distributed in tubes, and autoclaved. This medium was used to activate *Candida* species. For preservation of yeasts, amount of glycerol was added into BHIB for getting 80% concentration of the medium.

3.2.2.2.7. Sabouraud Dextrose Broth

The SDB was prepared by dissolving (65 gm) of the medium powder in a liter of distilled water. This medium was poured in tubes, autoclaved, and store until use.

3.2.2.2.8. Urea Medium Broth

The UMB was prepared by dissolving (24 gm) of medium powder a liter of distilled water, and distributed in screw glass tubes in which each tube contained (4.5 ml) of this medium. The sterilization was performed using autoclave. Aseptically, 0.5 of sterilized (Millipore filtrated) urea was added into tube. This medium was used for testing ability of *Candida* species to utilize urea.

2.2.2.2.9. Sabouraud Dextrose Agar with 6.5% NaCl (Alvares et al., 2009)

1. Dissolving SDA amount in 6.2 gm in 100 ml distal water .
2. Mixing this amount with NaCl for obtaining **6.5%** of the salt.
3. pH value was adjusted at: 5.5.
4. Preparing Petri dishes contained this medium.
5. This medium was used to differentiate between *Candida albicans* and none-albicnas species.

2.2.2.2.10.TTC agar (Velegraki and Logotheti, 1998)

The TTC agar was prepared using the following components:

1. 2,3,5- triphenyl tetrazolium chloride (TTC)…….....1 gm.
2. Peptone…….....10 gm.
3. Glucose…….....10 gm.
4. Distilled water……1000 ml.

After dissolving the components, pH value was adjusted at 7 and Petri dishes of the medium were prepared. This medium was used to differentiate between germ tube *Candida* species such as *C. albicans* and *C.dubliniensis*.

The TTC stock solution was prepared by dissolving (1 gm) of TTC in 10 ml of distilled water, sterilized using Millipore filter paper syringe, and and stored at 4° C. One ml of TTC stock solution was added to the basal medium to give a final concentration of 100 µg/ml.

3.3.Isolation of *Candida* species (Gharanfoli,A 2019)

1. All urine specimens were inoculated on Petri dishes of SDA supplemented with azithromycin.
2. The dishes were incubated at 37 ºC for 28-72 hrs.
3. After completing incubation period, yeast growth was preserved until applying other tests.

3.4. Morphological Identification (Reference)

1. Sub-culture of *Candida* species were done on SDA, MEA, BHIA, and CCA at 37 ºC for 28-72 hrs.
2. The identification was recorded depending on the color and shape of colonies.
3. Regarding to the identification, table (3-12) shows parameters in which *Candida* species can be differentiated depending on the CCA medium.

Table (3.12): Parameters of CCA medium to differentiate *Candida* species.

Candida species	Color of colonies
Candida albicans	Green
Candida dubliensis	Green
Candida glabrata	Creamy to purple

Candida krusei	Pink
Candida parapsilosis	Creamy to pale pink
Candida tropicalis	Blue

3.4.1. Germ Tube Test (Dimeski *et al*., 2014)

1. Full-loop of *Candida* species was cultured in a plastic tube contained 0.5 ml of egg albumin and incubated at 37 ºC for 2-3 hrs.
2. After completing incubation period, a drop of the incubated tube was placed on a clean slide, covered with a lid.
3. The slide was examined under-microscope (40 X) for detecting germ tube.

3.4.2. Urea Test (Alhasan 2015)

1. Activation of *Candida* species was done.
2. Full-loop of *Candida* species was inoculated in a tube contained sterile urea and its medium as shown in (3.2.2.2.7.).
3. All tubes were incubated at 37 ºC for 28-72 hrs.
4. If color of medium changes into pink or red, it indicates positive result while remaining yellow color, it indicates negative manifestation.

2.4.3.Growth at 45 °C (Abood, 2014).)

1. This test was used to differentiate between *C. albicans* and none-C. *albicans* species.
2. *Candid* species was grown on SDA at 45°C for 48 -72 hrs.
3. If growth appears, it indicates species of *C. albicans*.

2.4.4. Growth in SDA with NaCl (6.5 %)

1. Activation of *C. albicans*.
2. Preparation of SDA with NaCl (6.5 %).

3. Inoculation of *C. albicans* on a Petri dish contained this medium.
4. All dishes were incubated at 37°C for 48 -72 hrs.
5. If growth appears, it indicates *C. albicans*.

2.4.5.TTC agar (Velegraki and Logotheti, 1998)

1. Activation and growth of *Candida* species on BHIA.
2. Amount of yeast growth was streaked on Petri dishes contained TTC agar.
3. All dishes were incubated at 37° C for 24 hrs.
4. If growth reveals pale pink to white colonies, it indicates *C.albicans* while red to maroon growth reports *C. dubliniensis.*

3.5. Preservation of *Candida* species

3.5.1.Short Time Preservation (Modern Reference)

The yeast isolates were stored on slant agar in screw- capped test tubes for a short period from one to three weeks and stored at 4C° (Abood, 2014).

3.5.2.Long Time Preservation (Abood, 2014; Casagrande Pierantoni *et al*., 2021)

The yeast isolates were maintained at low temperatures for an extended length period in a medium containing (20-15%) glycerol. The medium was prepared by adding (2 ml) of glycerol to (8 ml) of SDB; then it was dispensed into a small- screw capped tubes and sterilized by autoclave. Once cooled, the medium was inoculated with one pure isolated colony and incubated at 37C° for 18 hrs. The tubes were stored in deep freeze at -20 °C.

3.6. Molecular Identification of *Candida* species

3.6.1. DNA Extraction

The fungal DNA was extracted depending on instruction of Geneaid (Taiwan) kit and as following steps:

1. Activate Candida growth on SDA at 37 ºC for 24 hrs.
2. Using liquid nitrogen, grind 20-50 mg of fungi or yeasts in a ceramic mortar. Then, add 200-500 µL of PBS buffer and pour the mixture into a 1.5 ml Eppendorf tube.
3. Incubate for 10 minutes at 60°C, stirring every 3 minutes, after adding 20 µl of proteinase K.
4. To each tube, add 200 µl of GSB buffer, and use a vortex mixer to mix. After 10 minutes of incubation at 60°C, each tube was mixed every three minutes. The necessary elution buffer (100 µl per sample) should now be heated to 60°C in preparation for step (8), DNA elution.
5. Immediately shake the lysate cell wall vigorously to mix in 200 µl of absolute ethanol.
6. Transfer all of the mixture, including any precipitate, to the spin column, which was put in a 2 ml collection tube. A centrifuge was then run for one minute at 14,000 rpm. After disposing of the filtrate, put the spin column in a fresh 2-milliliter bag.
7. After adding 400 µl of W1 buffer to the spin column and centrifuging it for one minute at 14,000 rpm, the flow-through was disposed of and used again.
8. Each column was filled with 600 µl of wash buffer, centrifuged for 1 minute at 14,000 rpm, the flow through was disposed of, and the column was put into a fresh 2 ml collection tube. The membrane was then dried by centrifuging again for 1 minute.
9. The purified DNA was eluted by centrifuging the spin column at 14000 rpm for 30 seconds after placing it in a fresh 1.5 ml tube and adding 75-100 µl of elution buffer to the center of the column matrix. The column

was left to stand for at least three minutes to allow the elution buffer to be fully absorbed.

10. Finally, agarose gel electrophoresis was prepared as:

- ✓ 20 ml of 1 X TBE (Tris Boric EDTA) buffer and 0.30 g of agarose were combined in a beaker, and the mixture was heated to the boiling point in a microwave oven to create a 1.5% gel.
- ✓ Put one milliliter of Green Safe View Dye (APM) in a beaker. The comb was carefully fixed at one end, the solution was allowed to cool to between 50 and 60 degrees Celsius, and it was then poured into the gel chamber and allowed to solidify for 20 to 30 minutes at room temperature.
- ✓ After carefully removing the comb, the gel tray was put inside an electrophoresis chamber with 1 X TBE buffer, making it about 1-2 mm above the gel's surface.
- ✓ Horizontal agarose gel electrophoresis was used to test the PCR reactions. As previously described, a 1.5% gel was made. 3 µl of the PCR product into a pocket or insertion cavity.
- ✓ Volume 3ul of the length standard was used to estimate the size of the PCR products, and one pocket per row was used for the length standard (100 bp DNA ladder). The gel was put on a UV screen after the electrophoresis run was finished, which was conducted at 70V and 85 mA.
- ✓ Pouring the agarose in electrophoresis device.

3.6.2. Evaluation of DNA Concentration

The extracted DNA was evaluated using a Nano-drop spectrophotometer, which measures the concentration of DNA (ng/µl) and checked the purity of DNA by reading the absorbance at (260/280 nm).

3.6.3. Polymerase Chain Reaction (PCR)

The PCR process was carried out depending on contents of and protocol (Tables: 3.7, 3.8, 3.9. and 3.10). ITS1 and ITS4 primers were used in this study and protocol that was shown in (Table, 3.10). The mixture of all compounds were reported in (Table,3.9) for obtaining PCR reaction process. After completing process, genes were submitted into agarose electorphoresis and then visualized using ultraviolet device.

3.6.4. Sequencing and Alignment of Genes

1. Obtaining amplified PCR products using two primers (ITS 2 and ITS 4).
2. Using Macrogen program, the genetic sequences were examined.
3. All nucleotide sequences were aligned using BioEdit software, which includes a graphic view tool to illustrate the genetic variations (such as mutations) among nucleotides sequences compared with the standard strain sequences of Candida isolates. Mega x software was also used to construct a phylogenetic tree (The neighbour-Joining (NJ) algorithm, which has been classified. The nucleotide sequences were analyzed and processed using NCBI-Blast Alignment identification http://www.ncbi.nlm.nih.gov/BLAST /Blast.cgi).

3.7. Antifungal Susceptibility Test

3.7.1. Disc Diffusion Test

1. Activate *Candida* species using BHIA at 37 ºC for 24 hrs.
2. Preparation of MHA Petri dishes.
3. Using sterile cotton swab, inoculation of each dish with suspension (1.5 x 10^{8} cell per ml) *Candida* species and spreading on the dish completely.
4. Antifungal discs (Amphotercin B ,Clotrimazole, Fluconazole, Itraconazole , Ketoconazole and Nystatin) were placed on the dishes.

5. All dishes were incubated at 37 ºC for 24-72 hrs until inhibitory zones (IZs) appeared which they were examined using mm.
6. The values of MICs were compared with CSL information.

3.7.2.Minimum Inhibitory Concentrations

1. Activate *Candida* species using BHIA at 37 ºC for 24 hrs.
2. Preparation of SDB tubes.
3. Preparation of *Candida* suspension (1.5 x 10^{8} cell per ml) compared with McFarland standard (0. 5).
4. Groups of the tubes were numbered from 1- 11 in which each tube contained 9 ml of the sterilized medium.
5. Preparation of stock concentration of antifungal agents.
6. Serial concentrations of each antifungal agent were prepared suing those numbered tubes. Additionally, two control tubes were done in which the negative control presented tube contained SDB only while positive one contained SDB and antifungal agents only.
7. Except control tubes, all tubes were inoculated with 100 µL of Candida suspension separately.
8. All tubes were incubated at 37 ºC for 24-72 hrs.
9. After completing period, the tubes were compared with control ones.
10. If the tube has turbidity, it does not indicate presence of inhibitory activity due to concentration of antifungal agents.
11. The values of MICs were compared with CSL information.

3.8. Statistical Analysis

The current data were statistically analysis using statistical software program, SPSS, (Statistical Package of Social Science version 26), based in using both descriptive and, non-parametric, Descriptive Chi-Square, and independent sample t test at p. value < 0.05.

3.9. Biosafety and Hazard Material Disposing

Every contaminated item, including swabs and petri dishes, was autoclaved and burned to ensure biosafety. Every bench was cleaned with 70% alcohol both before and after work, per Bergen and Shelhamer (1996).

3.10.Athesis strategy

Using search engines like Google Scholar, PubMed, and Science Direct, a thorough literature search was carried out in order to prepare this thesis, which examines the historical perspective of various medical aspects of Candida. Medical Subject Headings terms such as "history of Candida," "taxonomy of Candida," "Candidiasis," "virulence/pathogenicity of Candida," "laboratory diagnosis of Candidiasis," and "treatment of Candidiasis" were used in the search. These search engines yielded pertinent English-language reference articles. This article was prepared by compiling the data collected from these research manuscripts.

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Chapter Four

Results

4. Chapter Four: Results

4.1.Distribution of candidurea depending on specimen sources.

The present research showed that the diabetic patients infected with candidurea had a high value of the infection (Table, 4-1) compared with non-diabetic ones and healthy individuals.

Table (4-1): Distribution of candidurea depending on the specimen sources.

No. of Specimens	Healthy individuals N(%)	None-diabetic patients(NDM) N(%)	Diabetic patients (DM) N(%)
Positive results	0 (0%)	14(56%)	41 (20.5 %)
Negative results	25 (100%)	11 (44%)	159 (79.5%)
Total	25 (100%)	25 (100%)	200(100%)

4.2. Distribution of *Candida* species in diabetic patients depending on ages of patients

The diabetic patients distribution according to age of patient and Candida species(C. albicans, C. krusei and C. glabrata) to foure age groups. the age group (5-15), (16-25),(26-35) ,(36-45),(46-55),(56-65) ,(66-75),and (76-85). The study recorded most of were in the Fourth age group in C. glabrata species . The results recorded a significant difference at p. value < (0.122)and CalX2= (14.23) more than TabX2= (14.06) at df=7, as shown in Table (4-2)

Table (4-2): Distribution of *Candida* species in diabetic patients depending on the ages of patients.

Ages of patients (year)	No. of *Candida* species							
	C. albicans		*C. krusei*		*C. glabrata*		Totals	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
5-15	3	21.42	0	0 %	0	0%	3	7
16-25	0	0%	1	50%	2	8%	3	7

26-35	3	21.42 %	0	0%	3	12%	6	14
36-45	2	14.30%	0	0%	9	36%	11	27
46-55	3	21.42%	0	0%	7	28%	10	24
56-65	1	7.14%	1	50%	4	16%	6	15
66-75	2	14.30%	0	0 %	0	0	2	5
76-85	0	0%	0	0 %	0	0	0	0 %
Totals	14	%34	2	% 5	25	% 61	41	% 100
CalX2= 14.23			TabX2= 14.06		df= 7		p. value 0.122	

4.3. Distribution of *Candida* species in diabetic patients depending on sex of patients

Female patients with diabetes had a larger percentage of *Candida* species than men in which Table (4-3) contained the distribution. Statistically, there was a significant difference between males and females with diabetes at p. value <0.05 and CalX2= 10.44 more than TabX2= 9.48 at df =4 .

Table (4-3): Distribution of *Candida* species in diabetic patients depending on the sex of patients.

Sex groups	No. of *Candida* species							
	C. albicans		*C. krusei*		*C. glabrata*		Total	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Male	1	7%	0	0%	5	20%	6	15%
Female	13	93%	2	100%	20	80%	35	%85
Total	14	34%	2	5%	25	61%	41	%100
CalX2= 10.44			TabX2= 9.48		df= 4		p. value 0.046	

4.4. Distribution of *Candida* species in diabetic patients depending locations of patients.

Candida species were distributed in the diabetic patients according to locations of the patients that live. Rural had a larger percentage of *Candida*

species than those who live in the city Rural had a larger percentage of the species than those who live in the city. (Table, 4-4).Statistically, there was a significant difference between two groups at p. value <0.05 and CalX2= 16.06 more than TabX2= 15.50 at df=8, as shown in Table (4-4).

Table (4-4): Distribution of *Candida* species in diabetic patients depending the locations of patients.

Candida species	No. of species					
	City		Rural		Total of species	
	NO.	%	No.	%	No.	%
C. albicans	6	46%	8	29 %	14	34%
C.krusei	0	0 %	2	7 %	2	5%
C. glabrata	7	54%	18	64 %	25	61%
Total	13	32%	28	68%	41	100%
CalX2= 16.06	TabX2= 15.50		df= 8		p. value 0.032	

4.5. Distribution of *Candida* species in diabetic patients depending Date of collection specimen

The following table shows the total number of diabetic patients' samples (positive and negative) distributed according to the sequence of months during the study period. We noticed that the highest percentage was during the month of December, while the lowest percentage appeared during the month of January.

Table (4-5): Distribution of *Candida* species in diabetic patients depending the locations of patients.

Month / Results	Negative	Positive				Number of sample in
		C. albicans No(%)	*C. krusei* No(%)	*C. glabrata* No(%)	Total N(%)	
August	21(81%)	1(4 %)	0(0%)	4(15%)	5(19%)	26 (13%)
September	29(83%)	2(5.7%)	1(2.8%)	3(8.5)%	6(17%)	35(17.5%)
October	38(88%)	2(5%)	0(0%)	3(7%)	5(12%)	43(21.5%)
November	20(74%)	4(15%)	0(0%)	3(11%)	7(26%)	27(13.5%)
December	25(64%)	2(5%)	1(3%)	11(28%)	14(36%)	39(19.5%)
January	26(87%)	3(10%)	0(0%)	1(3%)	4(13%)	30(15%)
Totals	159(79.5	14(7%)	2(1%)	25(12.5%)	41(20.5%)	200

4.6. Distribution of *Candida* species in diabetic patients depending on type of therapy

Non-insulin dependent diabetic patients appeared a high value of *Candida* species compared with insulin dependent patients. Table (4-6) showed statistical non- significant difference between two groups at p. value <0.05 and CalX2= 8.05 less than TabX2= 5.99 at df=2.

Table (4-6): Distribution of *Candida* species in diabetic patients depending on type of therapy in which the patients treated.

Candida species	No. of species					
	Insulin therapy		**Non-insulin therapy**		**Total of species**	
	No.	**%**	**No.**	**%**	**No.**	**%**
C. albicans	9	60%	5	19%	14	34%
C. krusei	0	0 %	2	8%	2	5%
C.glabrata	6	40%	19	73%	25	61%
Total	15	37%	26	63%	41	100%
CalX2= 8.15	TabX2= 5.99		df= 2		p. value 0.124	

4.7. Distribution of *Candida* species depending on the presence of diabetes mellitus and comorbidities in diabetic patients

Diabetic patients with comorbitites appeared remarkable evidence compared with non-diabetic patients infected with UTI and healthy individuals as in Table, (4-7).

Table (4-7):Distribution of *Candida* species depending on the presence of diabetes mellitus and comorbidities in diabetic patients

Candida species	No. of species									
	Healthy individuals (Negative control)		Patients with UTIN (Positive control)		Patients with DMO		Patients with DMC		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
C. albicans	0	0 %	0	0 %	4	40%	10	32.2 %	14	345
C. krusei	0	0 %	0	0 %	0	0 %	2	6.5%	2	5%
C. glabrata	0	0 %	0	0 %	6	60 %	19	61.3%	25	61%
Total	0	0 %	0	0 %	10	24%	31	76%	41	100%
CalX2= 7.84			TabX2= 11.07		df= 5		p. value 0.469			

4.7. Isolation and Identification

4.7.1. Isolation and Diagnosis of Candida Species

and Candida Species Diagnosis According to the specimen culture results on SDA, out of 250 urine specimens, 59 isolates were obtained; the total percentage of Candida isolates from both patients and the control group was (56%). With a rate of (20.5)%, the Candida species isolated from patients werefound in 41 urine samples from 200 diabetic patients with meletis. Regarding the 25 UTI patients in the control group, the candida isolated from 14 urine samples and all 25 healthy control samples came back negative.Note

,every sample cultured should be have 25 colony or more than to be positive sample .

4.7.2. Identification of *Candida spp.*

Through microscopic analysis and chromogenic agar characteristics, Candida species were identified. Candida albicans 14 (%), Candida krusei 2 (%), and Candida glabrata 25 (%) were the most common ones found in diabetic patients. The study found that the species of Candida isolated differed significantly (p. value <), as illustrates.

4.3.4. Morphological Characteristics of *Candida* Growth

4.3.4.1. Culturing on Sabouraud Dextrose Agar

As seen in Figure(4-1), the morphological traits of Candida colonies on Sabouraud dextrose agar were round, soft, smooth, wrinkle-prone, white to creamy, and had a distinct yeast odor.

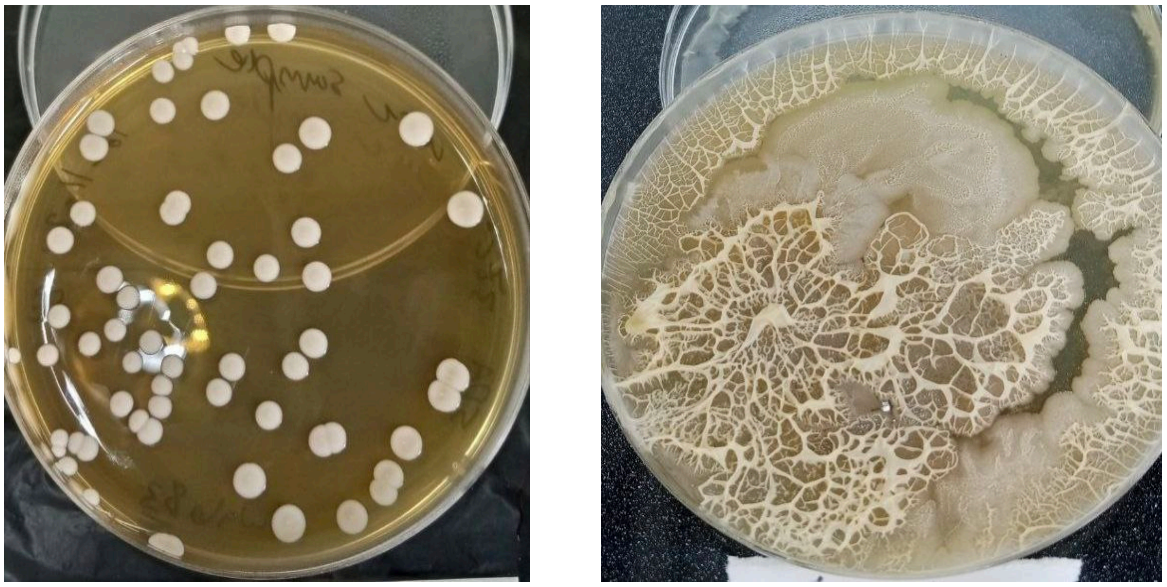


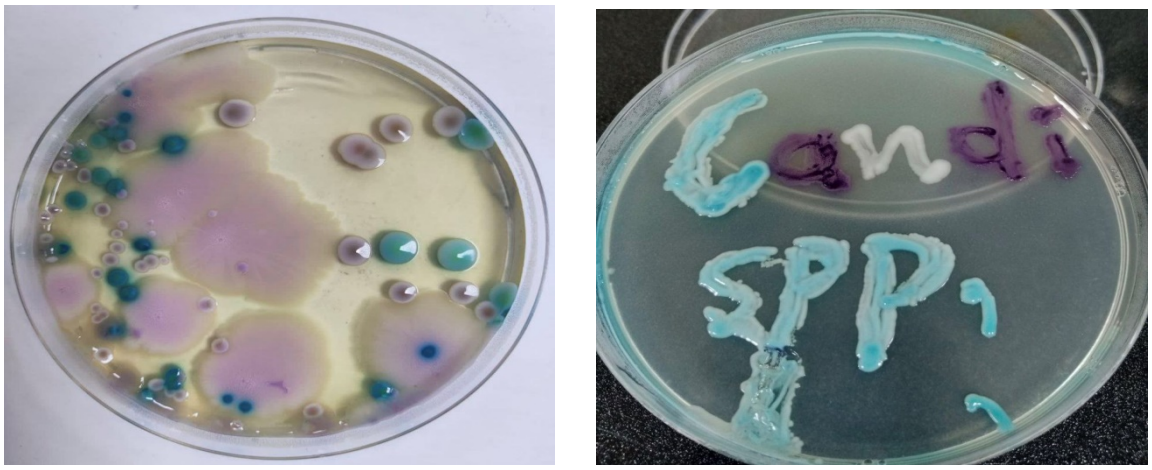
Figure (4-1): Colonies of Candida species on SDA medium at 37° C for 72 hours

4.3.5. Phenotype Identification of Candida Species

In order to identify phenotypes, chromogenic agar and germ tubes were used as follows:

4.3.5.1. Candida Chromogenic Agar (CCA)

Subculturing the isolates of Candida species grown on SDA media on chromogenic agar medium allowed for their recognition as well. The various isolates of Candida displayed a range of colors in their colonies. After 48 hours of incubation, it can be recognized by the color changes that correspond to the growth of the Candida species. Candida glabrata is indicated by greenish colonies of Candida albicans, blue colonies of Candida tropicalis, pink colonies of Candida Krusei, and purple colonies, as per company protocol Figure(4-2), Appendix( ).



Figaure(4-2) :Candida species on tow media from Chromogenic agar

4.3.5.2. Germ Tube Test

The formation of germ tubes distinguishes Candida albicans from other yeast species.However, under a microscope, nine C. albicans isolates tested positive for germ tube formation, which is a long, tube-like projection that extends from the yeast cell. These tubular extensions are a stage in the development of true hyphae.

4.3.5.3. Test of Temperature Test (Growth at 45º C)

This test was used to distinguish between different species of Candida at 45°C for a 24- to 48-hour incubation period. Candida tropicalis showed no growth, C. albicans appeared to be growing strongly, C. glabrata moderately, and C. krusei weakly in the current study Figure(4-3).

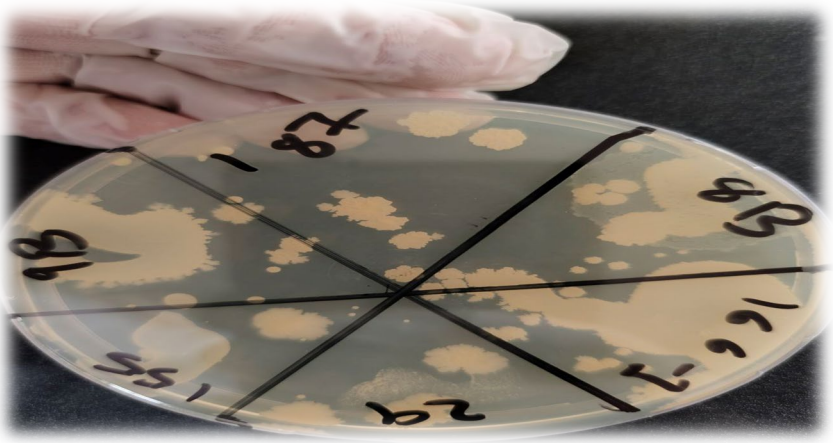
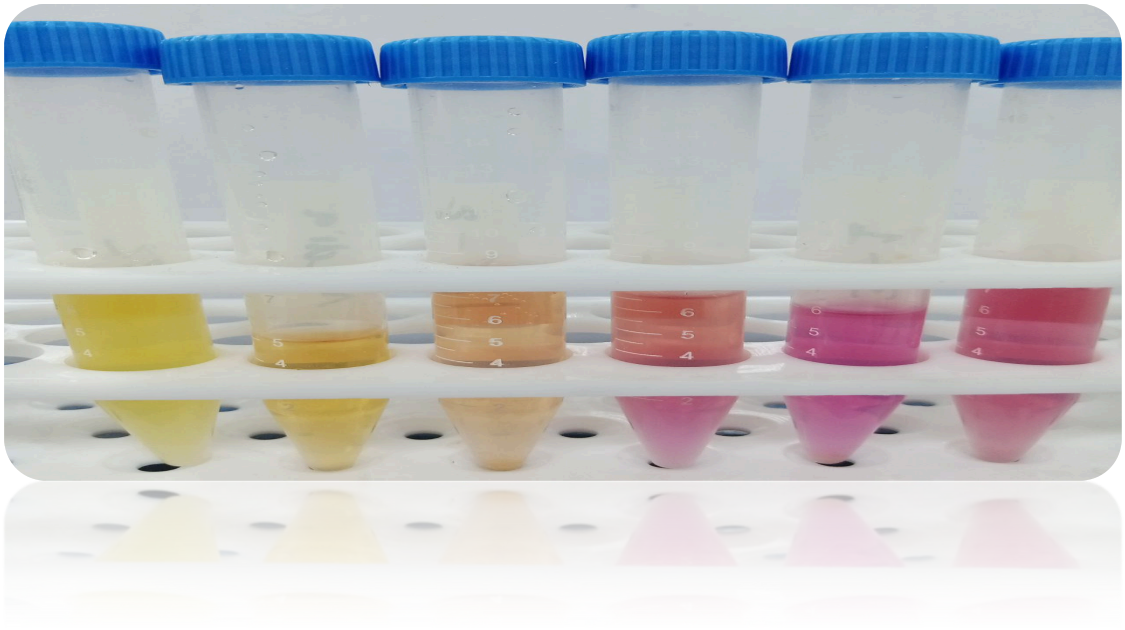


Figure (4-3): Growth of Candida spp. at 45º C.

4.3.5.4.Urease Test

Urea was prepared and sterilized using Millipore filter (0.22 µM) syringe and tubes containing urea broth medium were also prepared and autoclaved. Each tube contained 4.5 ml of the broth and 0.5 of the urea was added to the tube. All tubes were inoculated with 100µL of a suspension had active Candida species and the tubes were incubated at 37 ºC for 72 hrs. If color of the yellow broth changed into pink, it indicates positive result compared with negative control tube (Alhasan, 2015).



Figure(4-4):Candida strains changing color of media in Urease test

4.6 Molecular identification of Candida species isolated from Urine of Diabetic patients and UTI patients and another Healthy

Figure () shows the molecular analyses of three Candida species diagnosis by CHROM AGAR media isolated from Urine of Diabetic patients and UTI patientssigns were sequenced using two primers ITS1 and ITS4 .The target region (ITS) of the collected ITS1 and ITS4 universal primers were used to amplify DNA, and the results showed that DNA from all species of Candida were identified and to detect all strain . 53bands with the same molecular size between 500 and 900 bp were visible in the gel picture.

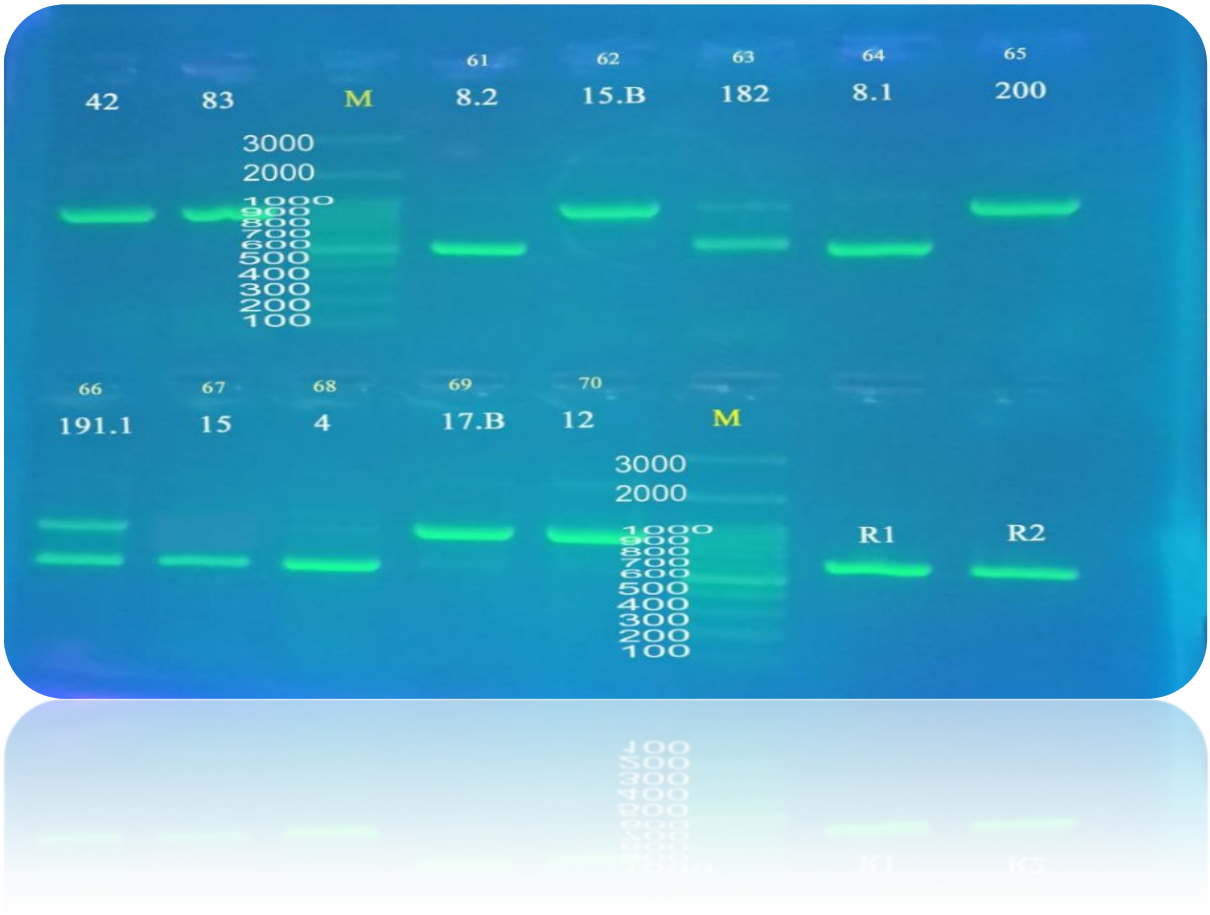


Figure (4:5): Agarose gel separation 1.5% of PCR results for the ITS1-ITS4 internal transcribed spacer regions M: DNA marker (100-2000); Lane 42 ,83, 15B, 182, 200, 191.1, 15, 17B, 12 : C. glabrata (662bp); Lane4, 4B, 35 : C. albicans (451bp); Lane 8.1, 8.2,28 : C. krusei (461bp)

The PCR product for Candida species isolated from Patiants signs in urine, were sent to Macrogen Company in south Korea for sequencing, were blasted in NCBI against standard strains of Candida spp., to identify the genetic variation compare with NCBI fungi. The sequencing results found that all isolated species using ITS1 and ITS4 primer pairs were confirmed to be Candida species with a similarity percentage 100% .

Table (4-8): Identified strains of *Candida albicans* isolated from urine of diabetic patients

No of isolate	NCBI Identification					Accession Number (our study)	
	NCBI BLAST identity	strain	NCBI accession number	Query cover (%)	Identity (%)	Accession Number	Size (bp)
CADM4	*C. albicans*	1405	KY101918.1	100	100	PV942615	451 bp
CADM6	*C. albicans*	1405	KY101918.1	100	100	PV942616	451 bp
CADM29	*C. albicans*	M350B	KP675583.1	100	100	PV942617	451 bp
CADM30F1	*C.albicans*	M350B	KP675583.1	100	100	PV942618	451 bp
CADM31F1	*C.albicans*	M350B	KP675583.1	100	100	PV942619	451 bp
CADM35	*C.albicans*	M350B	KP675583.1	100	100	PV942620	451 bp
CADM76	*C. albicans*	M151B	PQ895580.1	100	100	PV942621	451 bp
CADM113	*C. albicans*	1405	KY101918.1	100	100	PV942622	451 bp
CADM118	*C.albicans*	1504	PP851333.1	100	99.78	PV942623	451 bp
CADM125	*C.albicans*	1504	PP851333.1	100	99.78	PV942624	451 bp
CADM148	*C.albicans*	1405	KY101918.1	100	100	PV983990	451 bp
CADM150	*C.albicans*	1504	PP851333.1	100	99.78	PV942625	451 bp
CADM198F1	*C.albicans*	1504	PP851333.1	100	100	PV942626	451 bp

Table (4-9): Identified strains of Candida glabrata isoleted from urine of diabetic patients

No of isolate	NCBI Identification					Accession Number (our study)	
	NCBI BLAST identity	strain	NCBI accession number	Query cover (%)	Identity (%)	Accession Number	Size (bp)
CGDM 12	*C. glabrata*	14225	MN699325.1	100	100	PV942637	662 bp
CGDM32	*C .glabrata*	14225	MN699325.1	100	100	PV942638	662 bp
CGDM42	*C . glabrata*	H246B	KP674929.1	100	100	PV942639	662 bp
CGDM57	*C. glabrata*	P87	KM065474.1	100	99.09	PV942650	662 bp
CGDM83	*C. glabrata*	H246B	KP674929.1	100	100	PV942640	662 bp
CGDM84	*C. glabrata*	0346	PP851215.1	100	100	PV942641	662 bp
CGDM85	*C. glabrata*	P87	ON016575.1	100	100	PV942642	662 bp
CGDM21F2	*C. glabrata*	H246B	KP674929.1	100	100	PX057260	662 bp
CGDM110	*C.glabrata*	156	KM065474.1	100	98.34	PV942643	662 bp
CGDM130	*C. glabrata*	DMic 113886	JN008127.1	100	100	PV942645	662 bp
CGDM136	*C. glabrata*	DMic 113886	JN008127.1	100	100	PV942646	662 bp
CGDM146	*C. glabrata*	H246B	KP674929.1	100	100	PV942647	662 bp
CGDM151	*C. glabrata*	P87	KM065474.1	100	99.09	PV942648	662 bp
CGDM153	*C. glabrata*	P87	KM065474.1	100	99.09	PV942649	662 bp
CGDM165	*C. glabrata*	P11	ON016549.1	100	98.94	PV942651	662 bp
CGDM166F2	*C. glabrata*	P11	ON016549.1	100	98.94	PV942652	662 bp
CGDM115	*C. glabrata*	156	ON016575.1	100	98.34	PV942644	662 bp
CGDM173	*C. glabrata*	H246B	KP674929.1	100	100	PV942653	662 bp
CGDM178	*C. glabrata*	0346	PP851215.1	100	100	PV942654	662 bp

CGDM191F2	*C. glabrata*	0346	PP851215.1	100	100	PV942655	662 bp
CGDM193F1	*C. glabrata*	0346	PP851215.1	100	100	PV942657	662 bp
CGDM193F2	*C. glabrata*	DR-8	MN371898.1	100	100	PV983991	662 bp
CGDM182	*C. glabrata*	DR-8	MN371898.1	100	100	PV983992	662 bp
CGDM194	*C. glabrata*	DMic 113886	JN008127.1	100	100	PV94265	662 bp
CGDM198F2	*C. glabrata*	DMic 113886	JN008127.1	100	100	PV942658	662 bp
CGDM200	*C.glabrata*	DMic 113886	JN008127.1	100	100	PV942659	662 bp
CGDM203F1	*C. glabrata*	DMic 113886	JN008127.1	100	100	PV942660	662 bp
CGDM203F2	*C. glabrata*	m11b	>KP675167.1	100	100	PV983993	662 bp

Table (4-10): Identified strains of Pichia kudriavzevii isoleted from urine of diabetic patients

No of isolate	NCBI Identification					Accession Number (our study)	
	NCBI BLAST identity	strain	NCBI accession number	Query cover (%)	Identity (%)	Accession Number	Size (bp)
CKDM8	Picha kudravzevii	AUMC 7770	JQ425391.1	100	99.78	PV942665	461 bp
CKDM28	Picha kudravzevii	AUMC 7770	JQ425391.1	100	99.78	PV942666	461 bp

Table (4-11): Identified strains of Candida isoleted from urine of Just UTI patients as a control group

No of isolate	NCBI Identification					Accession Number (our study)	
	NCBI BLAST identity	strain	NCBI accession number	Query cover (%)	Identity (%)	Accession Number	Size (bp)
CGUT2B	***C. glabrata***	H246B	KP674929.1	100	99.22	PV942662	662 bp
CGUT14B	***C.glabrata***	H246B	KP674929.1	100	99.78	PV942661	662 bp
CGUT15B	***C.glabrata***	H246B	KP674929.1	100	99.22	PV942664	662 bp
CGUT17B	***C.glabrata***	H246B	KP674929.1	100	99.78	PV942663	662 bp
CAUT4B	***C.albicans***	M162B	KP675340.1	100	100	PV942627	662 bp
CAUT5B	***C. albicans***	**M162B**	**KP675340.1**	**100**	**100**	PV942628	662 bp
CAUT6B	***C. albicans***	UOA/HCPF	KC253975.1	100	99.58	PV942629	662 bp
CAUT10B	***C.albicans***	0316	PP851199.1	**100**	**100**	PV942630	662 bp
CAUT12-B1	***C.albicans***	1504	PP851333.1	**100**	**100**	PV942631	662 bp
CAUT12-B2	***C. albicans***	strain M350B	KP675583.1	**100**	**100**	PV942632	513 bp
CAUT13B	***C.albicans***	H286A	KP674989.1	100	99.62	PV942633	662 bp
CAUT19B	***C.albicans***	H286A	KP674989.1	100	99.62	PV942634	662 bp
CAUT24B	***C.albicans***	1469	PP851321.1	**100**	**100**	PV942635	662 bp
CAUT25B	***C.albicans***	1469	PP851321.1	**100**	**100**	PV942636	662 bp

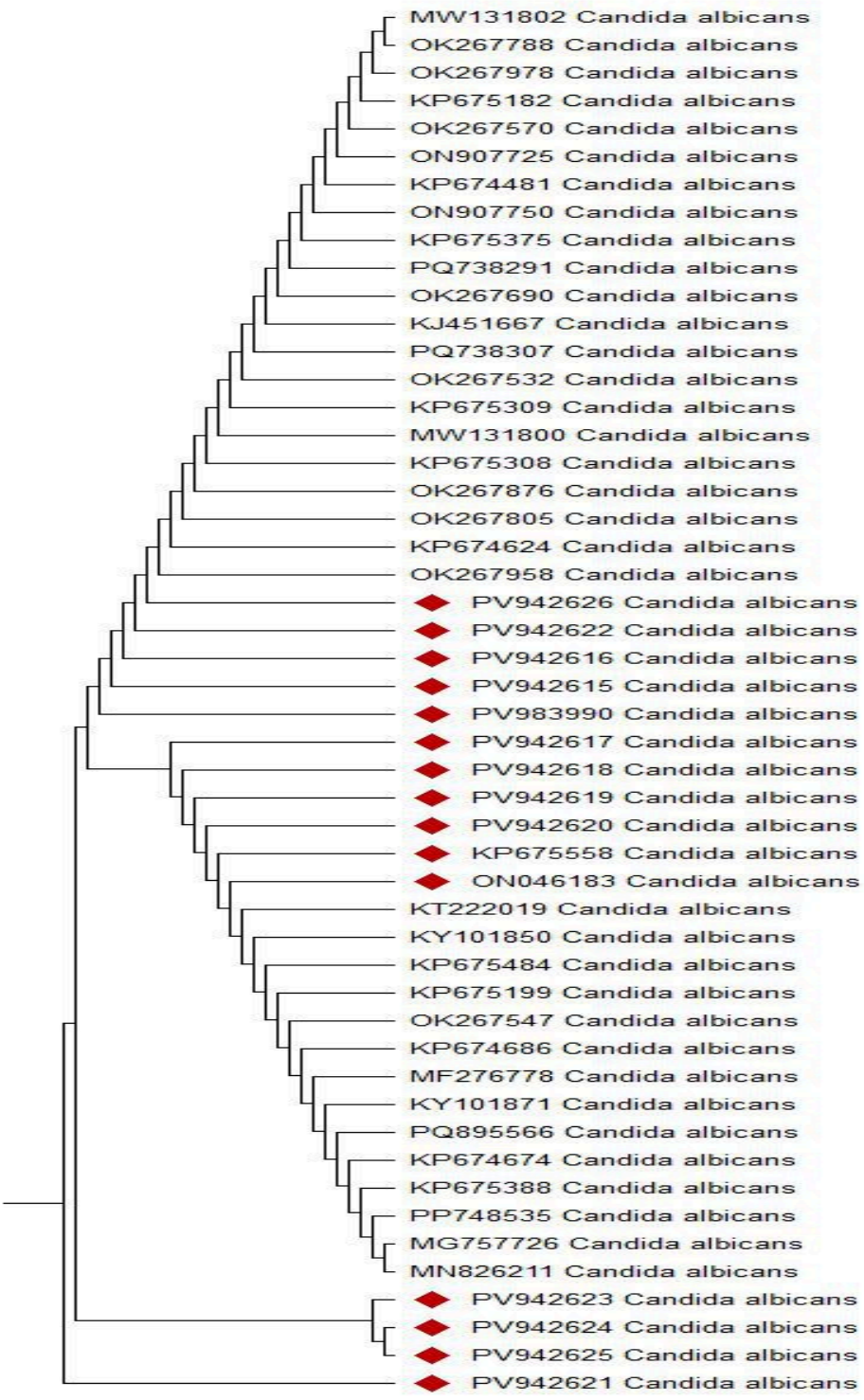


Figure.(4-6): Identified strains of *Candida albicans* isolated from urine of diabetic patients infected with UTI.

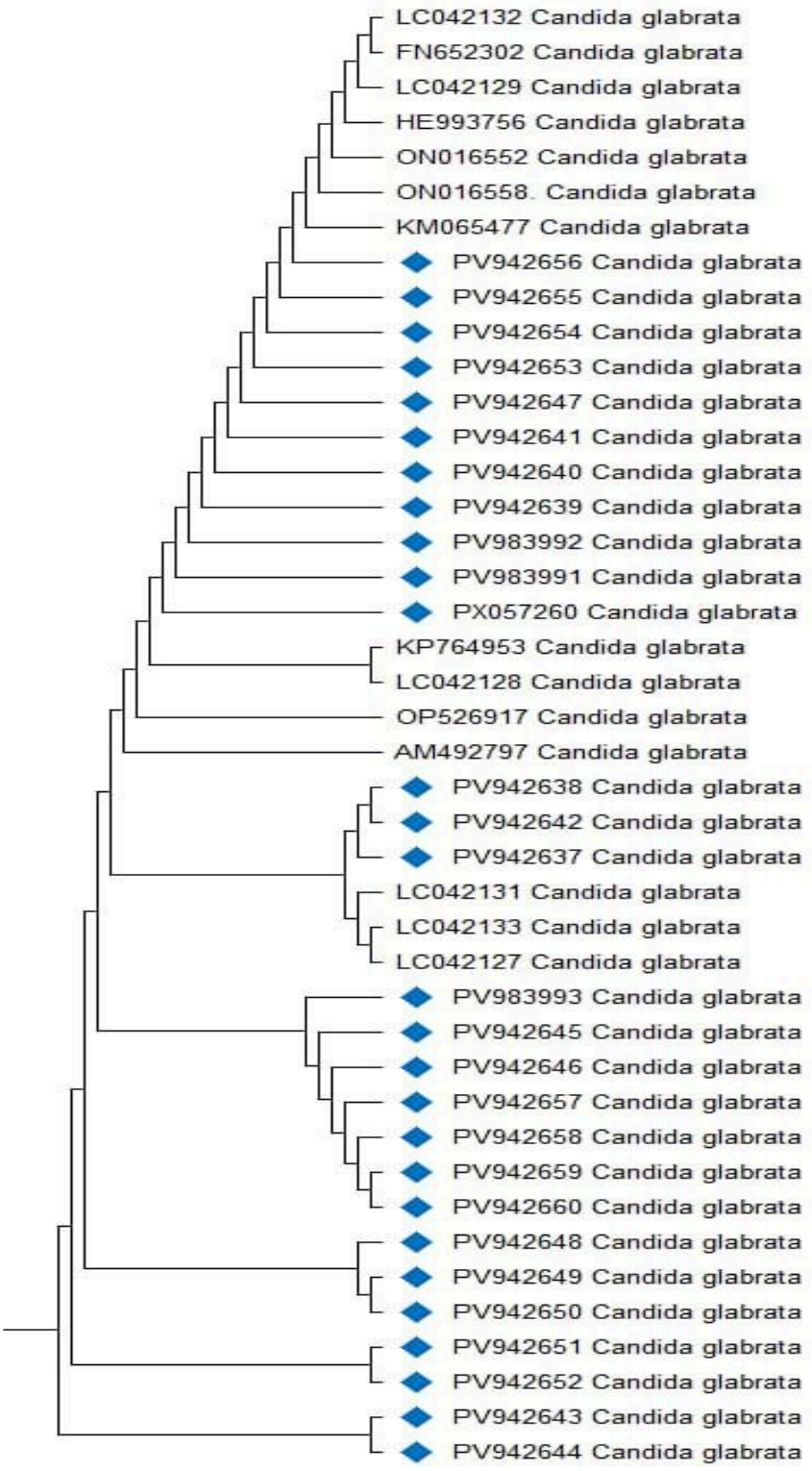


Fig.(4-7): Identified strains of *Candida glabrata* isolaated from urine of diabetic patients infected with UTI.

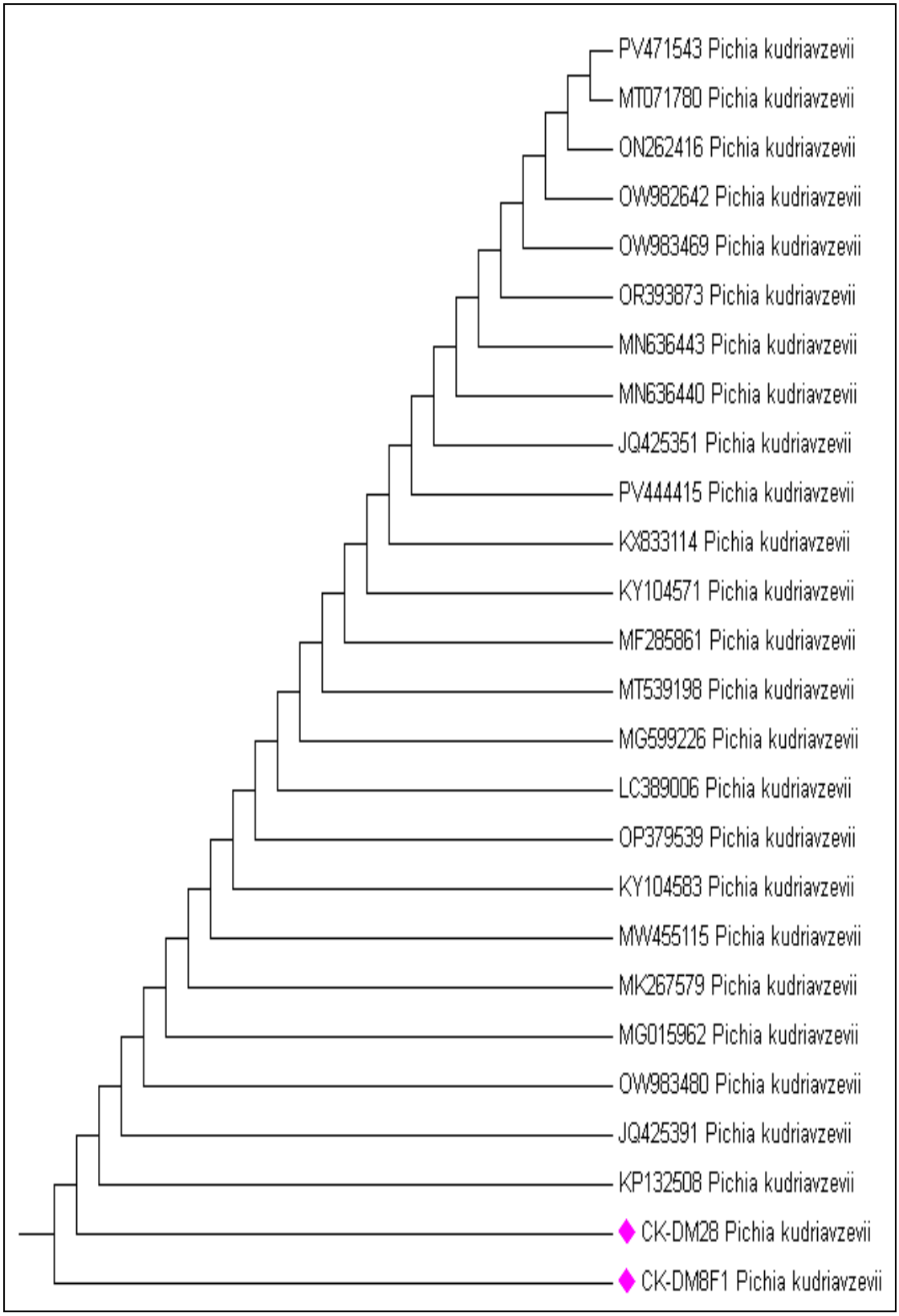


Fig.(4-8): Identified strains of *Candida krusei* isolated from urine of diabetic patients infected with UTI.

4.10. Antifungal sensitivity test

Table (-): Ratios and number of Candida strains isolated from diabetic patients against antifungal agents.

Candida Spp.	Pattern	AP	CC	FLC	IT	KT	NS
C. albicans strians	S	3 (21.43%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (64.29 %)
	I	7 (50%)	1 (7.14%)	0 (0%)	2 (14.29%)	1 (7.14%)	1 (7.14%)
	R	4 (28.57%)	13 (92.86%)	14 (100%)	12 (85.71%)	13 (92.86%)	4 (28.57%)
C. krusei strian	S	0 (0 %)	0 (0%)	0 (0 %)	0 (0%)	0 (0 %)	0 (0 %)
	I	0 (0%)	2 (100%)	0 (0%)	2 (100%)	2 (100%)	0 (0%)
	R	2 (100%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)
C. glabrata strians	S	1 (3.57%)	0 (0%)	0 (0%)	3 (11 %)	0 (0%)	14 (50.0 %)
	I	13 (46.43%)	2 (7%)	1 (3.6%)	2 (7%)	3 (10.71%)	0 (0%)
	R	14 (50.0 %)	26 (93%)	27 (96.4%)	23 (82 %)	25 (89.29%)	14 (50.0%)
C. albicans strians		$CalX^2$ = 22.18		$Tabx^2$ = 11.07	DF= 5	P. value= 0.001	
C. krusei strian		$CalX^2$ = 28.16		$Tabx^2$ = 11.07	DF= 5	P. value = 0.002	
C. glabrata strians		$CalX^2$ = 31.21		$Tabx^2$ = 11.07	DF= 5	P. value = 0.000	

Chapter Five

Discussion

5.Chapter Five: Discussion

5.1 Discussion

5.1 Clinical Study

The lack of laboratory criteria makes it difficult to distinguish candiduria from a frank UTI. It is also known that Candida is a commensal of the urogenital tract. Making a conclusive diagnosis of Candida is made more difficult by the presence of this organism in the urine sample. Another problem with UTI is that candiduria alone could be the only sign of invasive candidiasis, which could have dangerous consequences (especially in patients with weakened immune systems).According to studies done globally, the prevalence of candiduria in people with type 2 diabetes mellitus varies from 2.27% to 30%, with significantly higher rates in women (Talapko et al.,2022).

Significant differences were observed in the patients' clinical presentations and demographic profiles throughout this study, including age, sex, diabetes treatment, chronic illnesses, residency, use of antibiotics, etc. It has been noted that the prevalence of diabetes patients with UTI

5.2.Distribution of Candida species in Diabetic Patients

5.2.1 Distribution of Candida spp. According to Age

This age difference indicates a significant risk factor for diabetes mellitus and UTIs. The age groups of 36 to 45 (27%)and 46 to 55(24%) had the greatest number of infection by Candida positive cases, according to this study.Since Diabetic patiants in these age groups engage in more sexual activity, it is reasonable to assume that they would have a higher incidence and a maternal history, which

explains the higher prevalence reported among the younger population. Similarity findings showed that UTIs are the most prevalent infection in older people (Girma and Aemiro, 2022).and The findings of this study do not align with those in Iraq's Kurdistan Region (Mohammed et al.,2022)and this study agreement with (Gharanfoli et al.,2019; Uzoh et al.,2016)and dis agree with (Okonkwo and Umeanaeto, 2020) (Sampson and George 2020). which stated that the oldest women made up the smallest percentage. Therefore, it might be because women tend to miss their periods and they are past childbearing age. In women, aging lessens the effects of estrogen and may result in fewer infections. Due to their low levels of estrogen and corticosteroids, infrequent use of contraception to avoid pregnancy, and infrequent use of medications-all of which make them resistant to Candida infections-they may also have an increased vaginal immunity (Fidel Jr et al.,2000; Nelson et al.,2013).

In study of (Sampson and George, 2020; Okonkwo and Umeanaeto, 2020), who stated that candiduria occurs more frequently. Candida infections are most likely to occur as a result of increased reproductive activity, poor personal hygiene, drug abuse, and contraceptive use. These factors, along with an increase in estrogen, create an environment that is conducive to Candida growth.

5.2.2 Effect of Sex on frequency of candida isolation

Our goal was to determine the prevalence of UTIs among DM patients and investigate the variety of uropathogens by comparing the data with those of non-diabetic individuals who also had UTIs and other healthy individuals. Poorly managed diabetes was linked to a higher risk of UTIs, according to both our research and a study conducted in Sudan. This demonstrates the significance of

glycemic control in the management of diabetes and its impact on the risk of UTIs(Nath et al.,2021).

The study's findings showed that female diabetic patients had a noticeably greater prevalence of candiduria than did their male counterparts.Candida albicans accounted for 93% of the cases, followed by Candida glabrata at 80% and Candida krusei at 100%. Females made up 85% of the total cases. Conversely, only 15% of the cases of candiduria were found in males with diabetes. The distribution of Candida species among these was as follows: 20% had Candida glabrata, 7% had Candida albicans, and 0% had any evidence of Candida krusei.

5.2.2.1The frequency of Candida isolation in women with diabetes

The study's findings demonstrated that the percentage of isolates that were not Candida albicans was typically higher than that of Candida albicans. It was found that the most frequently isolated species from urine among diabetic women was Candida glabrata.Nonetheless, there is still debate about the fundamental causes of the higher prevalence of C. glabrata infections in diabetic patients. Although non-diabetic women with urinary tract infections had higher levels of Candida albicans, diabetic patients in this study had a higher prevalence of Candida glabrata than C. albicans. The results presented here are in contrast to those of (Savetha et al. (2021b), who discovered that C. albicans species were more prevalent in both diabetic and non-diabetic groups.

According to the results, the most common species of Candida was C. glabrata, and the incidence of candida among women with diabetes mellitus was 85% and that agreement (Samy, R. and Aljaza, D. (2023).

Causes of elevated *Candida glabrata* levels in diabetic patients' urine immune system compromise. Patients with diabetes are more vulnerable to fungal infections, such as those brought on by *Candida glabrata*, because diabetes can impair immunity. elevated blood glucose levels An environment that is favorable to the growth of Candida species, including Candida glabrata, can be produced by elevated glucose levels in tissues and urine. disorders of the urinary tract. Patients with diabetes are more susceptible to urinary tract infections, including those brought on by *Candida glabrata* , because they may have conditions like urinary stasis or use indwelling catheters (Roetzer., 2011)and (Chen et al.,2018).

5.2.3 Lactation of diabetic patients that infected by candiduria

The study results showed that the percentage of microbial isolates recorded among residents of rural areas reached 68% (28 isolates), which is a percentage higher than that recorded among residents of urban areas, which reached 32% (13 isolates).

The study's findings indicated that, in contrast to city dwellers, the majority of hospital visitors came from rural and surrounding areas. In addition to the nature of common occupations like agriculture and animal husbandry, which increase the likelihood of exposure to pathogens ,this is attributed to a number of factors, chief among them the environmental conditions that are prevalent in these areas and are conducive to the growth of microbes. One of the main causes of delayed diagnosis and treatment in rural areas is the low quality of healthcare services available there, which compels patients to seek care in urban hospitals and health centers.

On the other hand, it is observed that a significant percentage of urban dwellers favor private clinics and hospitals, which could limit the study's generalizability

and impact its findings due to the possibility of bias in the representation of various population groups.

5.2.4 Data of date specimen collected

This study aimed to investigate the prevalence of Candida species in the urine of diabetic patients over a six-month period. A total of 200 urine samples were collected; among these, 41 samples (20.5%) tested positive for Candida species.

Monthly Distribution A notable variation in the prevalence of Candida was observed across different months:

The highest positivity rate was recorded in December, with 14 out of 39 samples (35.9%) testing positive. This spike may be associated with seasonal factors such as colder weather, increased antibiotic use, and potential immune suppression during the winter months.

November followed with a positivity rate of 25.9% (7/27 samples). Similar environmental and behavioral factors may have contributed to this elevated rate.

On the other hand, the lowest prevalence was observed in January, where only 4 out of 30 samples (13.3%) were positive. This decrease could be due to variations in patient lifestyle, improved glycemic control following the holiday season, or reduced exposure to risk factors.Other months showed moderate positivity rates:August: 5/26 samples (19.2%),September: 6/35 samples (17.1%) and October: 5/43 samples (11.6%)

Interpretation and Possible Explanations

The findings suggest that diabetic patients are at a significant risk of developing Candida urinary tract infections (Candiduria). The fluctuating positivity rates across months may be influenced by several factors:

Seasonal changes affecting immunity, hydration status, and personal hygiene.

Use of antibiotics or corticosteroids, which may disrupt normal microbial flora.

Poor glycemic control, which promotes fungal growth in the urinary tract.

Additionally, Candida colonization in diabetic patients may occur asymptomatically, and the clinical significance of positive cultures should be evaluated carefully.

5.2.5 Distribution of Candida species depending of diabetic meletis therapy

Association Between Candiduria and Type of Diabetes Treatment .A total of 200 urine samples were collected from diabetic patients to investigate the occurrence of candiduria and its possible association with the type of diabetes treatment. Out of these, 41 samples (20.5%) tested positive for Candida species.

When stratified by treatment type:26 positive cases (63%) were among patients not receiving insulin therapy,while only 15 cases (37%) were from patients on insulin therapy.

This distribution suggests a higher prevalence of candiduria among non-insulin-treated diabetic patients compared to those who receive insulin. This finding may imply a potential link between the type of glycemic control method and the risk of fungal urinary tract colonization or infection.

Non-insulin-treated patients may have poorer glycemic control, especially if relying solely on oral hypoglycemics or lifestyle modifications, leading to glycosuria and a favorable environment for Candida growth.

Insulin therapy may be associated with tighter blood glucose regulation, potentially reducing the risk of candiduria.However, further investigation is needed to determine whether the difference is statistically significant, and to control for

confounding factors such as age, duration of diabetes, antibiotic use, immune status, and comorbidities.

5.2.6 The frequency of Candida depended comorbidities with diabetes meletis

According to the study's findings, just 10 microbial isolates, or 24% of the total, were obtained from patients with diabetes, whereas 31 isolates, or 76% of the total, were obtained from patients with diabetes who also had chronic illnesses. The two control groups-those who had UTIs but did not have any chronic illnesses and those who were healthy but had chronic illnesses-did not have any microbial isolates because their respective percentages were zero percent.

The study's findings demonstrated that people with diabetes and other chronic conditions are more likely to get candiduria than people with diabetes alone. This is mostly because chronic diseases weaken the immune system, which increases vulnerability to fungal infections like Candida by impairing the effectiveness of the immune response. Furthermore, managing these patients' medical conditions is made more difficult by the comorbidity and therapeutic overlap brought on by multiple comorbidities, which also raises the risk of complications. This patient group frequently has polypharmacy, which can result in adverse drug reactions and interactions that impair immunity and raise the risk of infection.

5.3. The effect of media on frequency of *Candida* isolation

The data showed that SDA medium is essential for fungal dominance; this could be because of the media component's effect and the assimilation of dissolved materials in such media, as well as the fact that many fungi can grow in a wide temperature range (10-45°C). It is regarded as a temperature range (10-45°C). It is

regarded as a primary medium for fungal isolation as a result(14) (Scognamiglio et al., 2010)..

Candida isolates were found to be impacted by the type of medium used in this study; the best media for Candida growth from the urine of diabetic patients who tested positive for Candida were SDA 83% and MEA 70%. These findings are in conflict with(12)(Pashley et al. (2012), that yeast isolates in this study were found to be influenced by the type of medium, with MEA 56%, SDA 54%, and MSA 8% being the best medium for yeast growth and positive for Candida. Alsodisagree with Jabar & Ajaza (2022), who discovered that the best growth media were MEA 44% and SDA 35%, respectively.

For a wide variety of organisms, numerous studies have demonstrated that the MEA agar recipe offers exceptional morphological growth characteristics across a wide range of features (Abdullah et al., 2018).

Depending on the researcher, different materials, components, or pH values of each medium, as well as different culture conditions, may result in differences in the ratios of Candida isolates between media. SDA has long been suggested by doctors as the culture medium for fungi. Although inoculating across multiple media may be desirable, this is not always possible due to sample sizes that are insufficient for each medium and related expenses. Particularly in minor ulcers, consecutive inoculation may have resulted in a decreased fungal load in samples,which could account for this decline in positive.

In order to isolate and diagnose Candida, the primary medium used was Sabouraud Dextrose Agar (SDA). The facultative fungi.

The results suggest that the media's impact on the isolates may differ depending on the type of fungus-some fungi grow on SDA but not on MEA, and vice versa-as

well as the type of food material included in each medium, including temperature and PH.

5.3.1 Growth at 37 C

Growing the isolated species at 37°C produced favorable results during the study. After the isolates were cultivated on SDA medium, their growth was tracked every day at this temperature. It was discovered that every species that was tested could thrive at this temperature.The phenotypic features of developing colonies, such as color, colony shape, height above the medium's surface, and texture, are in line with the conclusions drawn by Al-Eidami (2012).

5.3.2 Chrome identification of Candida spp isolated from Urine of Diabetic and UTI Patients

Chromatogenic agar medium is used to differentiate Candida species. Considering the color that results from the isolates' interaction with the medium (Musa et al., 2020). Because it is selective and allows direct speciation based on color production, the CHROMagar Candida culture medium was selected over the widely used Sabouraud dextrose agar (Benyounes et al., 2025)

The chromogenic mixture, a substrate that contains the enzymes of each type of yeast, allows this test to be highly accurate in diagnosing the various species that belong to this genus. For instance, type C. albicans produces an enzyme called BN-Acetyl galactos aminidase that acts on its own substrate in the diagnostic medium

(Jabra et al., 2001). Using the information from the chromogen diagnostic medium, which causes colonies to form different colors depending on the type of Candida, type C. albicans showed up on chrome agar medium in a light green color that set it apart from the other species But C. krusei appears purple because it secretes its own enzymes that interact with the basic substance that is impacted by these

enzymes, giving it a purple appearance. Chromagar is used to isolate and diagnose Candida species from oral samples by observing the color and appearance of the colony in the medium. (Tintelnot et al., 2000).

5.3.3 Germ tube test

The germ tube test is a quick and simple method for differentiating Candida albicans and Candida dubliniensis from other species (Hilmioglu et al., 2007; Matare et al., 2017). and this result disagree with (Othman et al., 2018) All Candida species tested negative for the germ tub test, according to the study's findings, with the exception of C. albicans, which tested positive And agree with (Yakasiri & Siddabathuni, 2020)that appear both C.albicans and C.dubliniesis were positive for germ tube test.

The formation of germ tubes in all study isolates within three hours of incubation was thought to be a key characteristic that sets Candida albicans apart from other species (JO et al., 2005; Deorukhkar et al., 2012).

The majority of Calbicans isolates produce germ tubes, whereas other species are unable to do so. The germ tube looked like a long tube that extended from yeast cells and lacked constriction at the location where it attached to the mother yeast cell (S C Deorukhkar & Roushani, 2018), and the outcome is consistent with (Abdulla & Mustafa, 2020).

5.3.4.Urease test for strains of Candida

The enzyme urease, which is constitutively expressed, hydrolyzes urea to produce ammonia and carbon dioxide.

$(NH_2)_2$ co $+H_2o \longrightarrow CO_2 + 2NH_3$

2% urea and phenol red, a pH indicator, are present in urease test media. Ammonia production raises the pH, which causes the color to shift from yellow (pH 6.8) to bright pink (pH 8.2). The highly buffered medium known as urea broth (Stuart's urea broth) needs a lot of ammonia to raise the pH above 8.0, which causes a color shift. This medium offers every essential nutrient for which it is unique (Brink, B. 2010).

According to our current study, this test yields inconsistent results when used on the same species; although some samples were from the same species, others had negative results. The same is true for strains that belong to the same genus; we found that strains that should be genetically similar produced different results.and appear degrees in color yellow ,Salmon and pink.

These variances show the potential for influencing elements, such as minute genetic alterations, testing circumstances, or variations in target gene expression, to impact test accuracy and this result dis agree with (Seeliger, H. P. (1956) and (29)(Mahon et al., 2022) and (Al-Khafaji (2017) and Al-Hamdani (2020) where researchers wrote that Candida species was negative for this test

5.3.5. Candida Grows at 45°C.

Temperature typically has a significant influence on the activity and efficacy of various microorganisms due to its effects on the rate of growth, metabolic

functions, enzymatic activities, and physiological traits of numerous yeasts (3)(Precht, 2013).

According to this study, high temperatures (45°C) were applied to both Candida albicans and non-C. albicans species, and the number of colonies formed enabled the classification of Candida growth as vigorous, moderate, or weak. Candida albicans showed rapid growth, whereas the other species ranged from weak to moderate , These results are consistent with research carried out by (Casagrande Pierantoni et al., 2021) and (Mahon and Lehman., 2022)

5.3.6 Nacl + Salt

When isolated Candida strains from diabetic patients were tested, the majority of species showed that they could grow in the given environment. Some strains of Candida albicans and Candida glabrata, however, that were isolated from various individuals displayed uneven growth, growing in some samples but not in others. Strain-specific variations in salt tolerance are probably the cause of this variability. This type of testing is useful for determining how well Candida species tolerate environmental stressors, especially salt-induced osmotic stress.

Additionally, it sheds light on Candida's adaptability and phenotypic diversity, which advances our knowledge of how the organism behaves in various ecological and therapeutic contexts.

5.4. Resistance of Antifungal Drugs

Techniques for testing for antifungal susceptibility are available to determine antifungal resistance and the best treatment plan for a specific fungus. Clinical microbiology relies on these methods to understand the local and global spread of antifungal resistance and to decide on the best course of action for a fungal

infection. According to the study's findings, all forms of Candida were resistant to fluconazole. This information was in conflict with(Mohamedi et al. (2015).

To identify the most effective treatment for a particular fungus and to identify antifungal resistance, antifungal susceptibility testing techniques are available. These techniques are used in clinical microbiology to determine the best treatment for a fungal infection and to understand the prevalence of antifungal resistance locally and globally (Alastruey-Izquierdo et al., 2015).

To identify the appropriate treatment for a particular fungal pathogen and to assess antifungal resistance, antifungal susceptibility testing techniques are available (Alastruey-Izquierdo et al.,2015). The development of multidrug resistance (MDR) in fungal pathogens, which restricts the supply of antifungal drugs, is a significant concern when treating invasive Candida infections (Peyclit et al.,2021). Antifungal resistance has emerged as a result of long-term use in the treatment of Candida infections (Kadry et al.,2018).

The disc diffusion method was used in the current study to determine the in-vitro susceptibility of strains of Candida. The results indicated that all isolated Candida species were resistant to sex specific antifungal agents. According to our findings, amphotericin B( ) and nystatin( ) were the most effective antifungals against Candida species. Resistance to fluconazole( ), clotrimazole ( ), and itraconazole ( ) and Ketoconazole was most prevalent. These results are consistent with those of

(Sheneef et al.,2017; Ahmed et al.,2018; Samy and Aljaza, 2023; Al-Qaysi and AL-Rubaie,2023)

The disc diffusion or kerby power method was used in our study to determine the in-vitro susceptibility for yeast strains Candida, and the most effective antifungals Nystatin ( ) and Amphtricin B ( )

The best antifungal medications in our study for all Candida species that were diagnosed with amphotericin B resistance were nystatin and amphotericin B; these results are consistent with (Ahmed et al., (2018) The yeast isolates exhibited the highest sensitivity to Nystatin ( )and Amphotericin B ( )according to an in vitro sensitivity test.

The disc diffusion method is more effective than other techniques for testing for antifungal drug susceptibility, according to Kumar et al. (2010). The disc potency, inoculum size, temperature, and incubation time all affect the disc diffusion methods' ability to detect antifungal susceptibility.

5.5 Molecular identification of Candida species isolated

The advancement of molecular techniques is very helpful for the rapid identification and detection of fungi in a variety of fields, including science, medicine, and business (Hariharan and Prasannath, 2021). While the phenotypes of Candida species are very similar, their epidemiology, genetic traits, susceptibility to antifungals, and virulence profile are different (Musinguzi et al.,2022). The majority of research revealed that the ITS regions can be sequenced to accurately identify pathogenic yeast(Farahyar et al., 2020), therefore to identify and genotype the yeast species that were isolated here, ITS primers (ITS1 and ITS4) were used(Shirvani and Fattahi, 2021).

According to the ITS sequence, Candida was identified differently than based on morphological characteristics (such as CHROM agar). Given that other fungal species share a common nucleotide sequence and the ITS region sequence is non-coding, this implies Nagla et al. (2019), In this study, ITS primers (ITSI and ITS4) were used to identify and genotype the isolated yeast species.

The results showed that C. glabrata and C. albicans made up the majority of the sample (33%), with C. krusei, Mohammed et al. (2015) concurred with these findings. showed that C. glabrata and C. albicans were the most frequently isolated species, with percentages of 40% and 30%, respectively. This was in contrast to Oufi et al. (2022), which found that C. albicans outnumbers other species.

The sequencing results indicated that 28 isolates (61%) of Candida glabrata, 14 isolates (34%) of Candida albicans, and 2 isolates (5%) of Candida krusei were identified.

5.5.1 Strains of Candida isolated from Diabetic patiants

The results of this study demonstrate that three strains of Candida were found in the urine specimens of diabetic patients and in the candiduria of patients who were dispersed throughout the hospitals and clinics in the studied area. Regarding UTI, however, 90% of UTIs are caused by bacteria, while the remaining percentage are caused by fungi, such as Candida species, and DM is a risk factor that predisposes the infection (Shirvani and Fattahi, 2021) . The findings of this study, which identified multiple strains of Candida species, were consistent with the occurrence of candiduria.

Additionally, the genomes of Candida species undergo genetic mutations, giving rise to new strains and genotypes of these species (Zarei et al., 2022). The different strains of Candida species found in the current study can be explained by

this manifestation. Strains may arise as a result of genetic mutations caused by treatment with different antifungal agents, changes in glucose levels, and other aberrant physiological factors.Additionally, ITS 1 and ITS4 were frequently used in this context to identify Candida species strains (Hadid and Abed, 2023). The

current work's findings appeared to match the concept of the researchers who discovered strains of Candida species.

In this study, urine samples from patients with diabetes mellitus and urinary tract infections (UTIs) yielded a total of 19 different strains from three of Candida spcies. Most of the Strains (n = 13), which included 8 strains of Candida glabrata, 4 strains of Candida albicans, and 1 strain of Candida krusei, were taken from diabetic patients.

By contrast, only 8 strains were taked from UTI patients who did not have diabetes. Of these, Candida albicans was the most common species (n = 7), whereas there was only one strian of Candida glabrata found. There was no evidence of Candida krusei in any samples from UTI patients.

These findings suggest that C. glabrata is more common in diabetics, while C. albicans was more commonly linked to UTI patients who were not diabetics. Additionally, two strains of C. glabrata, designated H246B and 1504, were isolated from both groups of patients with diabetes and those with urinary tract infections. This suggests that there may be strain overlap or that colonization is influenced by common environmental/host factors.

Conclusions and Recommendations

Conclusions

1. Appearance of candiduria in diabetic patients in Al-Shatrah District more than none-diabetic ones.
2. *Candida albicans*, *C. krusei*, and *C. glabrata* are isolated from these patients remarkably.
3. This study identified various strains belonged to these species.
4. Interestingly, majority of the strains were reported resistant to the antifungal drugs.

Future Works

1. Further study in the renal failure patients including diabetic patients.
2. Applying whole genome analysis for identifying these strains.
3. Clinical further study to get why resistance of identified Candida strains appeared.

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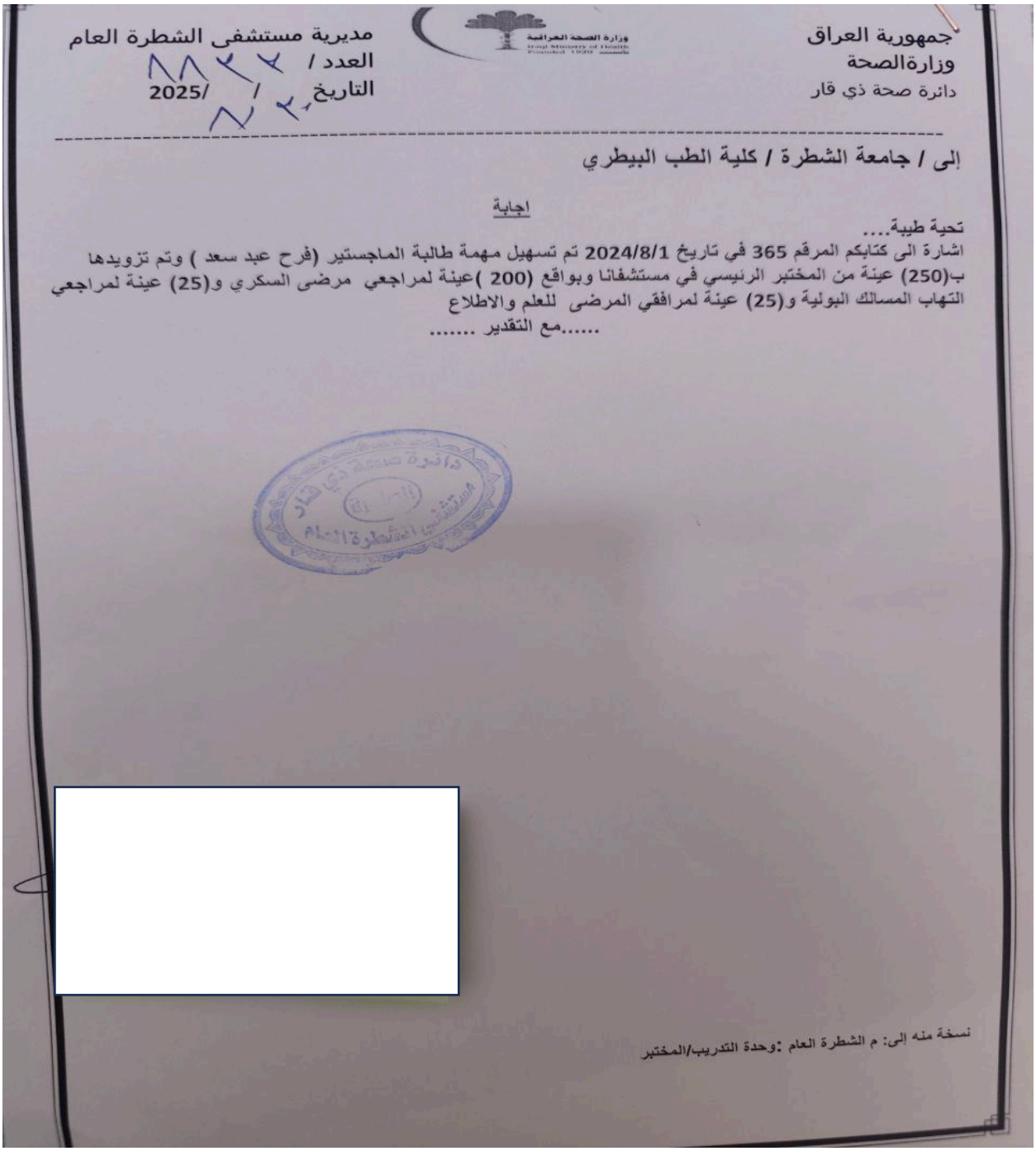
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Appendixes

Appendix 1: collection of specimen



جمهورية العراق
وزارة الصحة
دائرة صحة ذي قار

مديرية مستشفى الشطرة العام
العدد / ٨٨٢٢
التاريخ / ٢٠/ ٨ /2025

إلى / جامعة الشطرة / كلية الطب البيطري

اجابة

تحية طيبة....
اشارة الى كتابكم المرقم 365 في تاريخ 2024/8/1 تم تسهيل مهمة طالبة الماجستير (فرح عبد سعد) وتم تزويدها ب(250) عينة من المختبر الرئيسي في مستشفانا وبواقع (200)عينة لمراجعي مرضى السكري و(25) عينة لمراجعي التهاب المسالك البولية و(25) عينة لمرافقي المرضى للعلم والاطلاع
.....مع التقدير .....

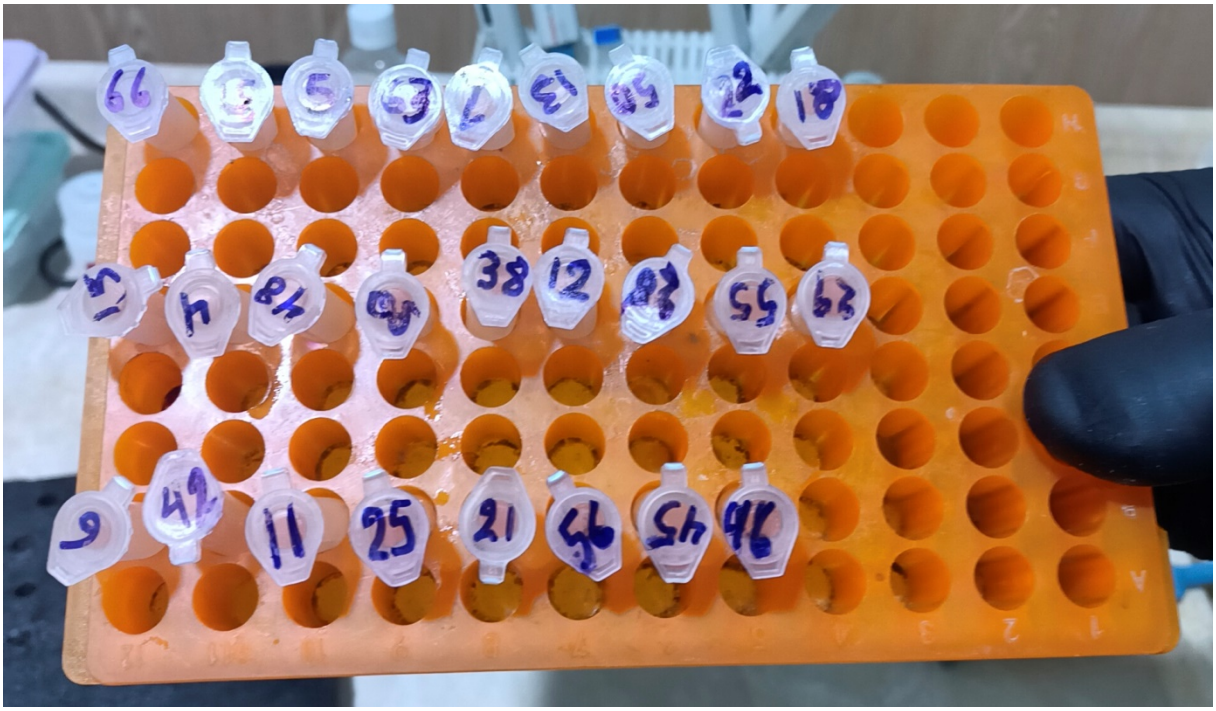
دائرة صحة ذي قار
مستشفى الشطرة العام

نسخة منه إلى: م الشطرة العام :وحدة التدريب/المختبر

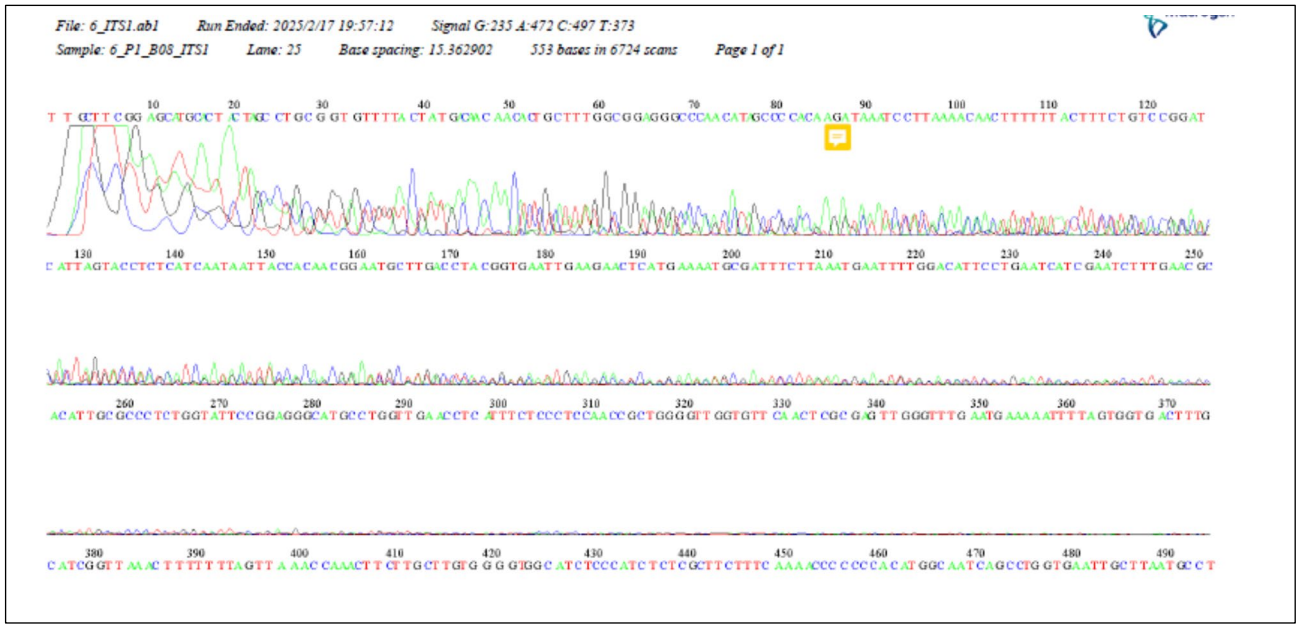
Appendix 2: Questionnaire of patients

No.	Name of patiant	sex	age	Taking conts	Taking antibiotic	Therapy of diabetes meletis	Rate of sugar	comorbidities	Location of patiant	Date of collection samples
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										

Appendix 3: DNA extraction for Sampeles of Candida



Appendix 4 : Analysis sequencing for once of the samples in current study



Appendix 5 : Registration in the Gene bank ITS

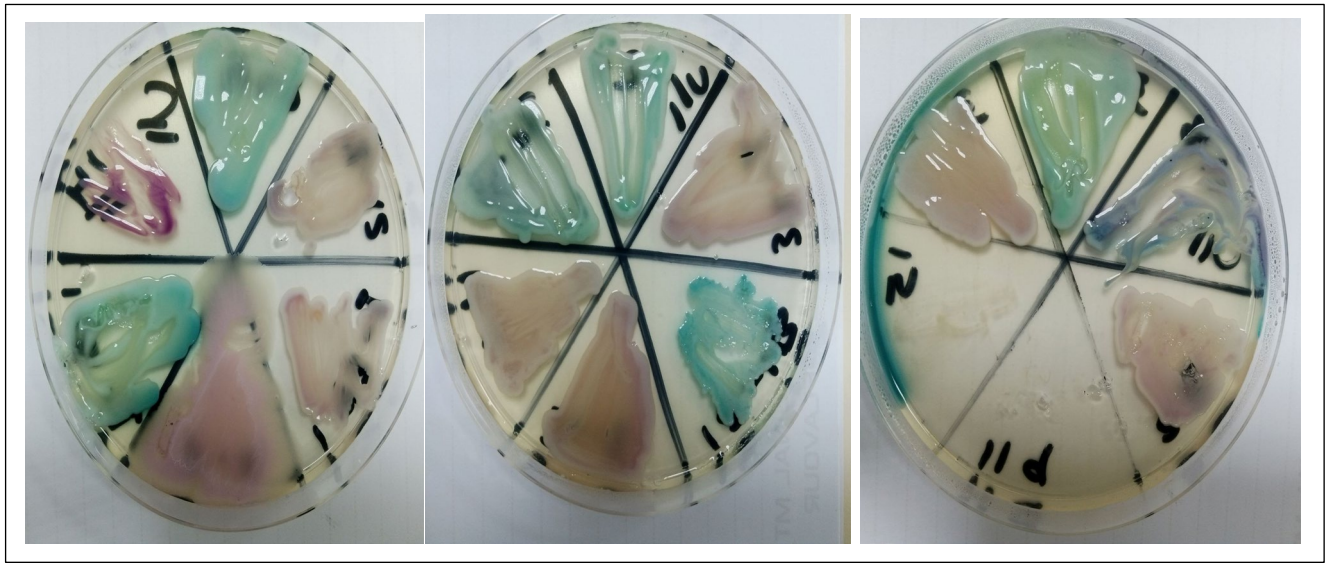
CGDM4

```
LOCUS       CADM4                     451 bp    DNA     linear   PLN 17-JUL-2025
DEFINITION  Candida albicans strain CADM4.
ACCESSION
VERSION
KEYWORDS    .
SOURCE      Candida albicans (Candida stellatoidea)
  ORGANISM  Candida albicans
            Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina;
            Pichiomycetes; Debaryomycetaceae; Candida/Lodderomyces clade;
            Candida.
REFERENCE   1  (bases 1 to 451)
  AUTHORS   Saad,F.A. and Alhasan,D.A.
  TITLE     Molecular and Morphological Identification of Candida species from
            Urine of Diabetic patients in AL-Shatrah District, south of Iraq
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 451)
  AUTHORS   Saad,F.A. and Alhasan,D.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-JUL-2025) Veterinary Medicine college, AL-Shatrah
            Uinversity, Shatrah, Shatrah, Thi-Qar 64001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /organism="Candida albicans"
                     /mol_type="genomic DNA"
                     /strain="CADM4"
                     /db_xref="taxon:5476"
                     /geo_loc_name="Iraq"
                     /collection_date="2025-01-15"
     misc_RNA        <1..>451
                     /note="contains internal transcribed spacer 1, 5.8S
                     ribosomal RNA, internal transcribed spacer 2, and large
                     subunit ribosomal RNA"
BASE COUNT      118 a    106 c    102 g    125 t
ORIGIN
        1 acttgctttg gcggtgggcc cagcctgccg ccagaggtct aaacttacaa ccaatttttt
       61 atcaacttgt cacaccagat tattactaat agtcaaaact ttcaacaacg gatctcttgg
      121 ttctcgcatc gatgaagaac gcagcgaaat gcgatacgta atatgaattg cagatattcg
      181 tgaatcatcg aatctttgaa cgcacattgc gccctctggt attccggagg gcatgcctgt
      241 ttgagcgtcg tttctccctc aaaccgctgg gtttggtgtt gagcaatacg acttgggttt
      301 gcttgaaaga cggtagtggt aaggcgggat cgctttgaca atggcttagg tctaaccaaa
      361 aacattgctt gcggcggtaa cgtccaccac gtatatcttc aaactttgac ctcaaatcag
      421 gtaggactac ccgctgaact taagcatatc a
//
```

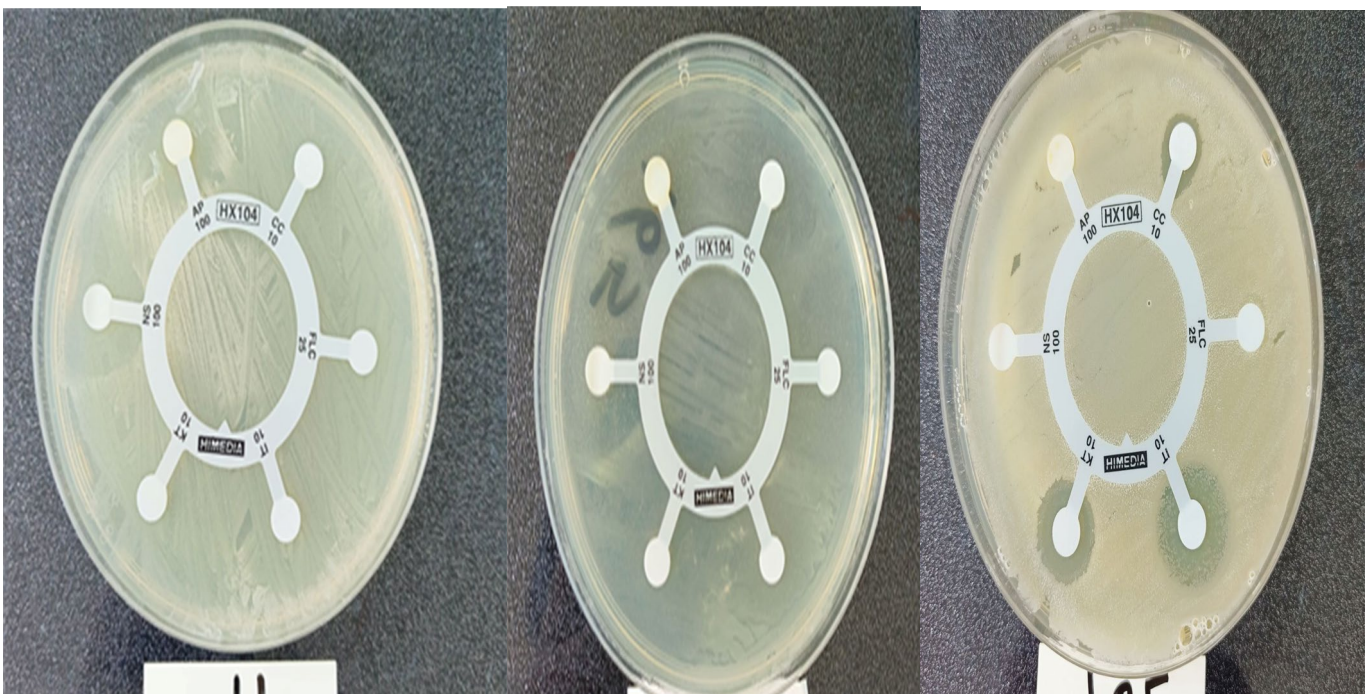
Appendix 6:*Candida* species on Hichromogenic agar



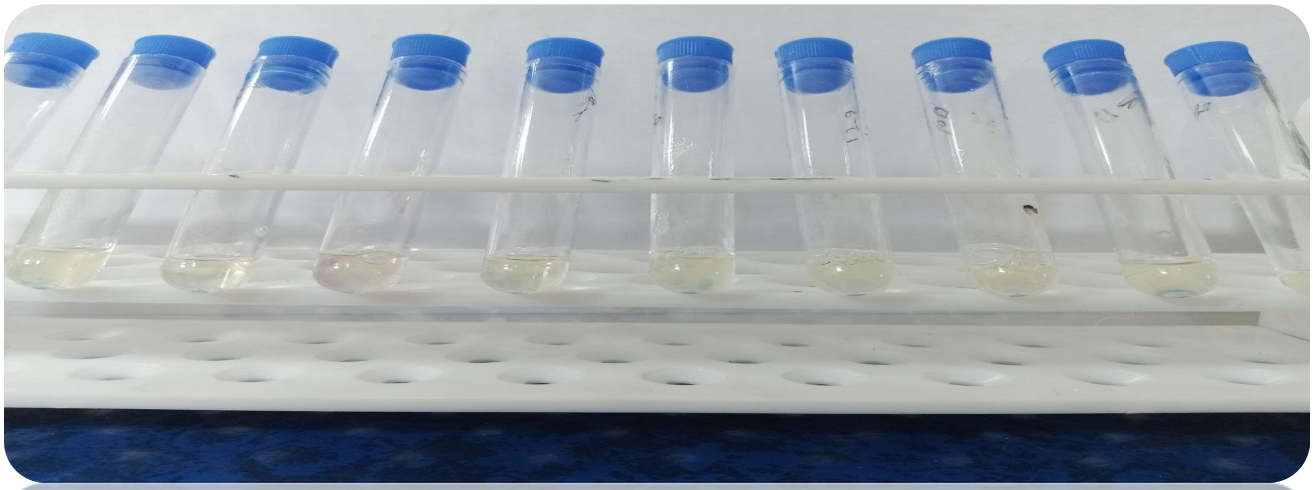
Appendix 7:Candida species on Michrom^{TM} Candida agar



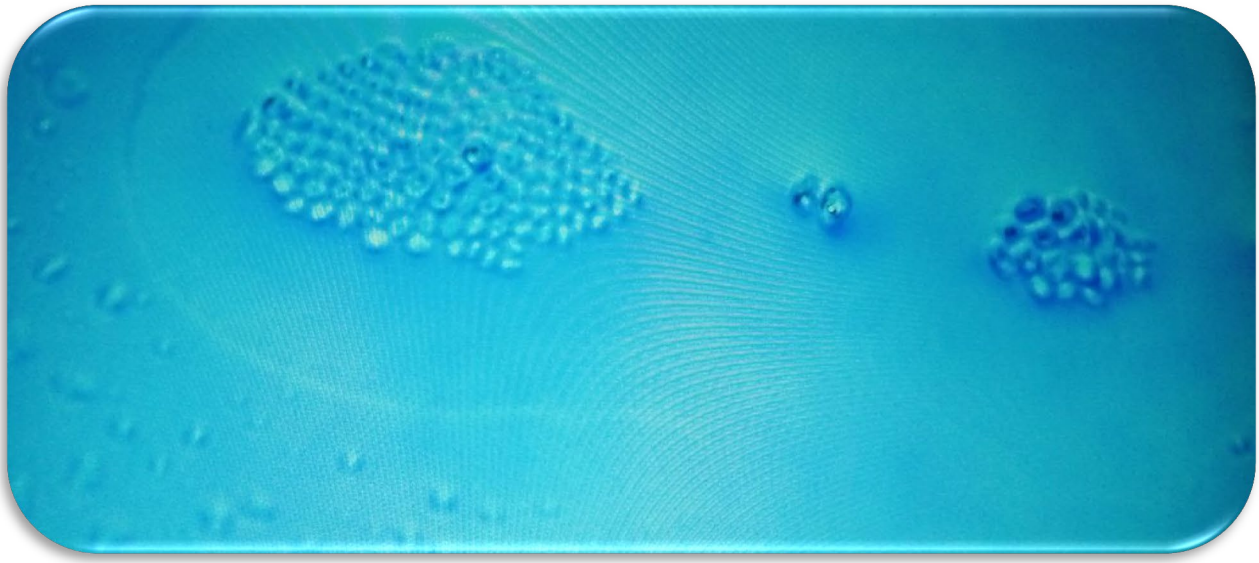
Appendix 8:Antifungal susceptibility test



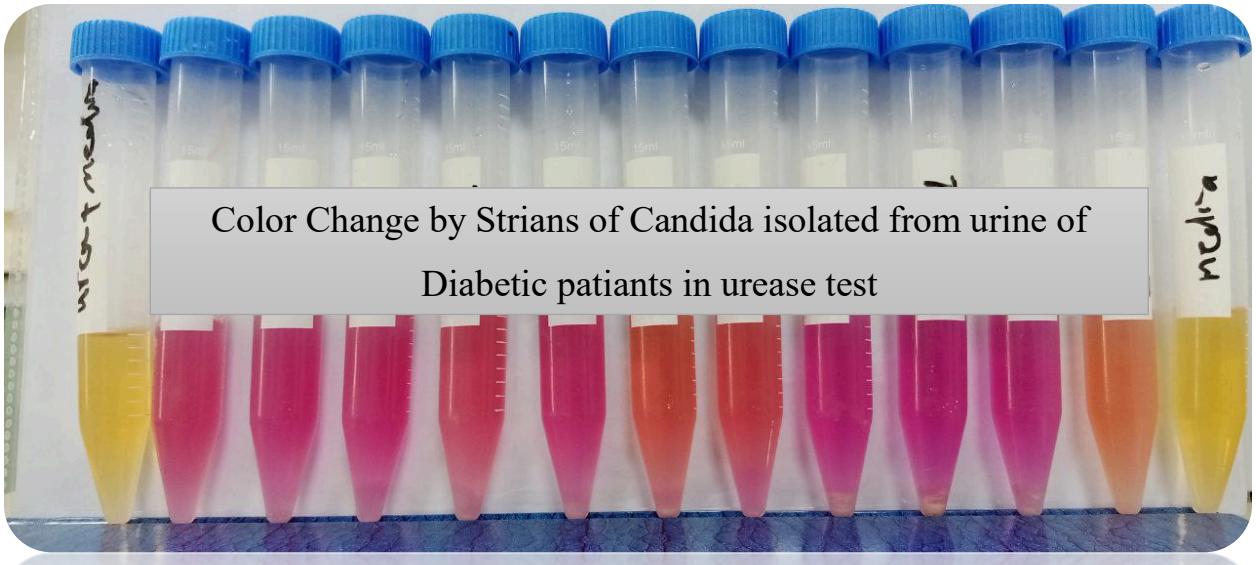
Appendix 9:Germ tube test in Albumin egg



Appendix 10: Microscopic examination of Candida spp. isolates by using Lactophenol blue stain (40 X)



Appendix 11 : Urease test

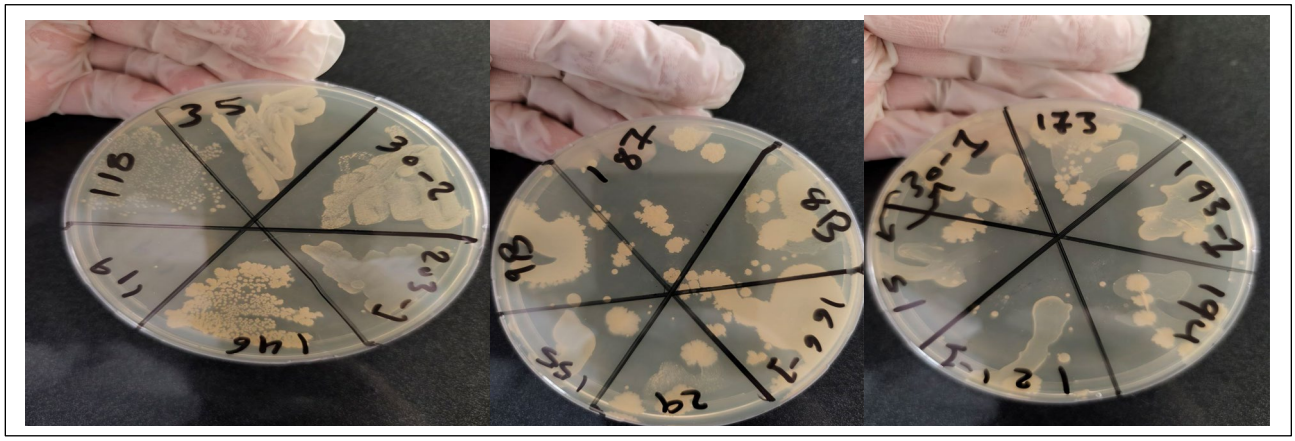


Color Change by Strians of Candida isolated from urine of Diabetic patiants in urease test

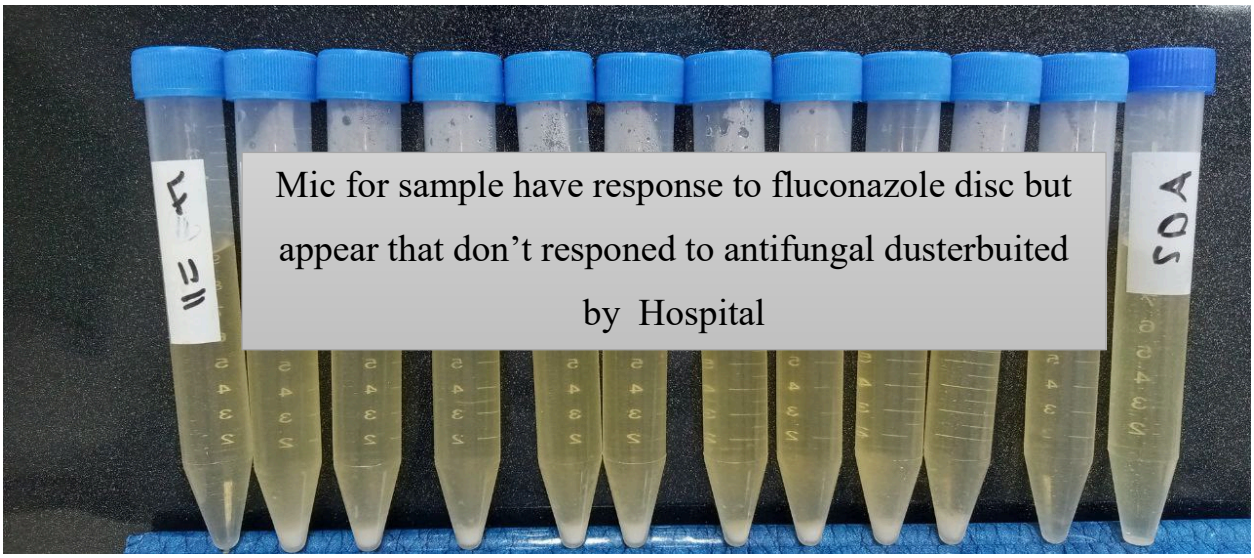
Appendix 12: Nacl + Salt Test



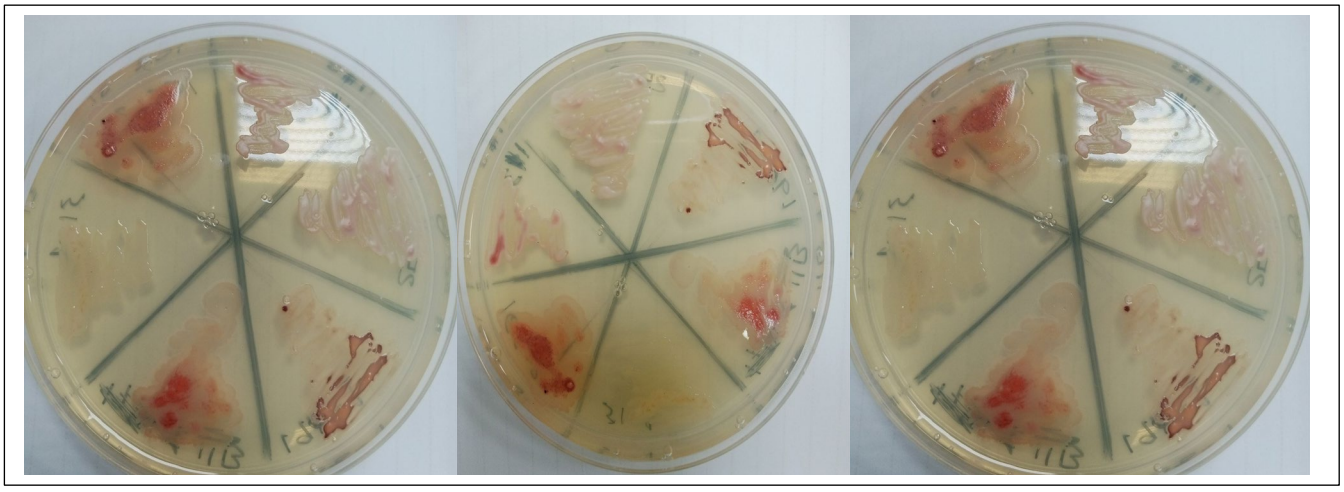
Appendix 13:Growth at 45 C^{o}



Appendix 15:MIC for sampele positive Fluconazole in 37Co



Appendix 15:Growth on TTC agar in 37C^{o}



الخلاصة

تعد إصابة المجاري البولية المتسبب عن خمائر المبيضات واحد من الأمراض التي تظهر إذا ما توفر واحد أو أكثر من العوامل المهيئة ومن بينها داء السكري. أهم أنواع المبيضات التي تسبب تلك الإصابة هي:

Candida albicans, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. guilliermondii*, *C. dubliniensis*.

أجريت هذه الدراسة للتحري عن وجود إصابة المجاري البولية المتسببة عم خمائر المبيضات في عينات إدرار مرضى السكري, والمرضى المصابين بالمجاري البولية غير المصابين بداء السكري فضلا عن عينات مشابهة من الأشخاص الأصحاء كمجموعة سيطرة سالبة. جمعت العينات من المرضى المراجعين لمستشفى قضاء الشطرة العام والعيادات السريرة الخاصة ضمن محافظة ذي قار. جميع تلك العينات زرعت على وسط اكار السابروويد المدعم بالمضاد الحيوي (الازثرومايسين) وبالطرائق المعقمة. أما فيما يتعلق بتشخيص الخمائر فقد اعتمد على الصفات المظهرية سواء العيانية منها أو المجهرية وتم تأكيد التشخيص باستخدام تفاعل البلمرة PCR. بالإضافة إلى ذلك, اجري اختبار الحساسية الدوائية للأنواع والسلالات المعزولة.

إن هذه الدراسة أوضحت وجود ثلاثة أنواع شخصت في عينات الدراسة وهم *Candida albicans*, *C. glabrata*, *C.krusei*. كما ان هذه الأنواع أظهرت مستعمرات بألوان مختلفة حيث نمت خميرة المبيضات الالبكانس باللون الابيض الى الكريمي والأخضر فضلا عن اللون الشاحب على الأوساط الزرعية وبالترتيب SDA, Candida chromagar medium (CCM),TTC agar. تفاعل البلمرة أكد التشخيص ودراسة تتابعات القواعد النتروجينية اظهر سلالات مختلفة تعود إلى تلك الأنواع المشخصة في هذه الدراسة.

من بين مائتين (200) عينة إدرار من مرضى السكري, ظهرت ....موجبة ووجود إصابة بينما ....موجبة من بين (25) عينة إدرار من المرضى المصابين وغير المصابين بالسكري. أما فيما يتعلق بعينات الأصحاء, ظهرت جميعها سالبه. بالإضافة إلى ذلك, ان نتائج الحساسية الدوائية ابدت قيم مختلفة تجاه مضادات الفطريات المستخدمة في هذه الدراسة. ثلاث سلالات من مبيضات الالبكانس متحسسة للامفوترسين بي, في حين أن تأثير النستاتين ثبط تسع سلالات باللمقارنة مع المضادات المختبرة الاخرى. اما فيما يتعل بسلالات المبيضات الكلابراتا, اوضحت هذه الدراسة ان الامفوترسين ثبط سلالة واحدة وثلاث سلالات ثبت

بسبب الانتراكاونازول واربع عشرة ثبطها النستاتين. اللافت للنظر, ان جميع سلالات المبيضات الكروزي مقاومة.

جامعة الشطرة
كلية الطب البيطري

التشخيص الجزئيي والشكلي لاجناس فطر الكانديدا المعزول من بول مرضى السكري في قضاء الشطرة محافظة ذي قار جنوب العراق

رسالة

رسالة ماجستير مقدمة الى مجلس كلية الطب البيطري- جامعة الشطرة وهي جزء من متطلبات الحصول على شهادة الماجستير في اختصاص علوم الطب البيطري (الاحياء المجهرية)

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(بكالوريوس علوم حياة/ كلية العلوم/ جامعة ذي قار (2013)

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2025 ق.م. 1447ه