TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

IDENTIFICATION OF CANDIDA SPECIES ISOLATED FROM CLINICAL SPECIMENS AND THEIR ANTIFUNGAL SUSCEPTIBILITY TESTING

MOHAMMAD ABDEL RAHIM BANI AHMAD

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAM

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MENTOR

Prof. Dr. NEDIM ÇAKIR

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Approval

STATEMENT (DECLARATION)

The data presented in this thesis was obtained in an experiment carried out in the microbiology laboratory/Near East University Hospital. I played a major role in the preparation and execution of the experiment, and the data analysis and interpretation are entirely my own work.

I am aware of and understand the NEAR EAST UNIVERSITY'S policy on plagiarism and I certify that this thesis is my own work I had no unethical behavior in all stages from the planning of the thesis until writing, except where indicated by the references, and the work presented in it has not been submitted in support of another degree or qualification from this or any other university or institute of learning.

Mohammad Abdel Rahim Bani Ahmad

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LIST OF ABBREVIATIONS AND SYMBOLS

%: Percent sign

°C: Celsius

ABC: The ATP-binding cassette

ABCD: Amphotericin B colloidal dispersion

ABLC: Amphotericin B lipid complex

AFST: Antifungal susceptibility testing

AIDS: Acquired immunodeficiency syndrome

AMB: AMPHOTERICIN B

AMI: Antibody-mediated immunity

ATCC: American type culture collection

BCE: Before the Common Era or Before the Current Era

BMD: Broth microdilution

BPs: Break Points

BSIs: Bloodstream infections

CAS: CASPOFUNGIN

CAUTI: Catheter-associated urinary tract infections

CDC: Centers for Disease Control and Prevention

CLABSI: Central-line-associated bloodstream infections

CLSI: The Clinical and Laboratory Standards Institute

CMA: Corn Meal Agar

CMC: Chronic mucocutaneous candidiasis

CMI: Cell-mediated immunity

CNS: The central nervous system

CRBSI: Catheter-related bloodstream infections

CSF: Cerebrospinal fluid

CW: Cell wall

DC: Disseminated candidiasis

DNA: Deoxyribonucleic acid

EC: Esophageal candidiasis

ECDC: The European Center for Disease Control

EPS: Extracellular polymeric substances

ER: Emergency room

ESCMID: The European Society for Clinical Microbiology and Infectious Diseases

et al.: And others

EUCAST: The European Committee for Antimicrobial Susceptibility Testing

FDA: The Food and Drug Administration

FLU: FLUCONAZOLE

GMS: Grocott's Methenamine Silver

HIV: Human Immunodeficiency Virus

hr: hour

ICU: Intensive care unit

IDSA: Infectious Diseases Society of America

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IgM: Immunoglobulin M

ITR: ITRACONAZOLE

IUD: intrauterine device

IV: Intravenous

kg: Kiloigram

KOH: Potassium hydroxide

L-AmB: Liposomal formulation Amphotericin B

L: Liter

lbs: A pound

LPCB: Lactophenol cotton blue

mcg /µg: Microgram

MCZ: MICONAZOLE

mg: Milligram

mg/L: Milligrams per liter

MH-GMB: Mueller–Hinton agar supplemented with 2% glucose and 0.5 μg/mL methylene blue dye

MH: Mueller-Hinton agar

MIC: Minimum inhibitory concentration

mL: Milliliter

mm: Millimeter

n: Number

NAC: Non- albicans Candida

NaCl: Sodium chloride

NCCLS: The National Committee for Clinical Laboratory Standards

NICU: Neonatal intensive care unit

nm: nanometer

No: Number

OPC: Oropharyngeal candidiasis

P-value: Probability value

PAS: Periodic Acid–Schiff

PCR: Polymerase chain reaction

PD: Pharmacodynamics

PDA: Potato Dextrose Agar

pH: Potential for hydrogen

PK: Pharmacokinetics

PLHA: People living with HIV/AIDS

PM: Plasma membrane

PMNL: Polymorphonuclear leucocytes

QC: Quality control

R: Resistant

RPG: RPMI agar with 2% glucose

RPMI: Roswell Park Memorial Institute

RVVC: Recurrent vulvovaginal candidiasis

S: Susceptible

S: Svedberg units

Saps: Secreted aspartyl proteinases

SD: Standard deviation

SDA: Sabouraud Dextrose Agar

SDD: Susceptible dose-dependent

SPSS: Statistical Package for the Social Sciences

TOC: Tween-80, Oxgall, Caffeic Acid

TRM: Tetrazolium Reduction Medium

TRNC: The Turkish Republic of Northern Cyprus

U: Unit

UT: Urinary tract

UTI: Urinary tract infection

VOR: VORICONAZOLE

VVC: Vulvovaginal candidiasis

yrs: Years

µg/mL: Microgram per Milliliter

µm: Micrometer

Thesis Title: Identification of *Candida* Species Isolated from Clinical Specimens and Their Antifungal Susceptibility Testing.

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ABSTRACT

Aim: This study was conducted to determine the distribution pattern of *Candida* species isolated from various clinical specimens and study their antifungal susceptibility profile testing. **Materials and Methods:** A total of 147 samples were included in this study from different clinical specimens. The suspected colonies of *Candida* isolates were primarily identified as yeast cells under the microscope by saline wet mount of preparation and Gram stain. *Candida* isolates were stored in the cryobank® storage system. The stored samples of *Candida* isolates were inoculated on Sabouraud Dextrose Agar (SDA) to get pure colonies. *Candida* species were identified by HiCrome™ *Candida* Differential Agar. Germ tube formation test was conducted for *C. albicans* species only as a confirmatory test. supplemented Mueller-Hinton agar (MH-GMB) was used for antifungal susceptibility testing of *Candida* isolates species. **Results:** In this study out of 147 specimens, 51,7% were females, 48,3% were males, and the majority were seen in the old adults' age group (81,6%). Among specimen types, the urine specimen was dominant with 44,9%. In this study, it was noted that *C. albicans* predominate over *non-albicans Candida* (NAC) species with 55,78% and 44,22%, respectively. In antifungal susceptibility profile testing, *Candida* species demonstrated high resistance to fluconazole (98%) followed by voriconazole (92,5%). Among polyenes and echinocandins, *Candida* isolates showed no resistance to amphotericin B (0,0%) and caspofungin (0,0%). **Conclusion:** This study indicates that high resistance percentages of *Candida* species to azole class antifungal agents. Therefore, this resistance should be taken into consideration in the therapy plan.

Key Words: *Candida* species, Antifungal Susceptibility Test (AFST), CLSI, Disk Diffusion, HiCrome Agar.

Tez Başlığı: Klinik Örneklerden İzole Edilen *Candida* Türlerinin Belirlenmesi ve Antifungal Duyarlılık Testleri.

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Danışmanı: Prof. Dr. Nedim Çakır

Anabilim Dalı: Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji

ÖZET

Amaç: Bu çalışma, çeşitli klinik örneklerden izole edilen *Candida* türlerinin dağılımını belirlemek ve antifungal duyarlılık profillerini incelemek amacıyla yapıldı. **Gereç ve Yöntem:** Bu çalışmaya, farklı klinik örneklerden izole edilen toplam 147 örnek dahil edilmiştir. *Candida* izolatlarından şüphelenilen koloniler, öncelikle mikroskop altında ıslak preparat ve Gram boyası ile tanımlandı. *Candida* izolatları cryobank® stok tüplerinde saklandı. Saklanan *Candida* izolatları saf koloni elde etmek amacıyla Sabouraud Dextrose Agar (SDA) besiyerine ekildi. *Candida* türleri HiCrome™ *Candida* Diferansiyel Agar kullanılarak tanımlandı. Germ tüp testi *C. albicans* türleri için sadece doğrulayıcı bir test olarak gerçekleştirildi. *Candida* türlerinin antifungal duyarlılık testi için %2 Glukoz ve 0,5 µg/mL metilen mavisi eklenmiş Mueller-Hinton agar (MH-GMB) kullanıldı. **Bulgular:** Bu çalışmada kullanılan 147 örneğin %51,7'si kadın, %48,3'ü erkek ve çoğunluğunun yaşlı yetişkin yaş grubunda (%81,6) olduğu görüldü. *C. albicans* %55,78 , *non-albicans Candida* (NAC) türleri ise %44,22 oranlarında tespit edilmiştir. Antifungal duyarlılık testinde, *Candida* türleri flukonazole (%98) ve ardından vorikonazole (%92,5) yüksek direnç gösterdiği tespit edilmiştir. *Candida* izolatlarının polienler ve ekinokandinler grubu antifungaller amfoterisin B (%0,0) ve kaspofungine (%0,0) direnç göstermediği görülmüştür. **Sonuç:** Bu çalışma, *Candida* türlerinin azol sınıfı antifungal ajanlara karşı yüksek direnç oranlarının olduğunu göstermektedir. Bu nedenle, bu direncin tedavi planında dikkate alınması gerektiğini düşünüyoruz.

Anahtar Kelimeler: *Candida* türleri, Antifungal Duyarlılık Testi, CLSI, Disk Difüzyon, HiCrome Agar.

1. INTRODUCTION AND AIM

1.1 Introduction

Candida lives inside our body as normal commensal flora in many places such as the vagina, mouth, throat, esophagus, and gut, even on the skin without causing any diseases. However, *Candida* can cause infection to individuals if the environment in any of those body parts changes in a way that promotes multiply and growth of *Candida* species, or if it enters the bloodstream or internal organs, this infection called "Candidiasis" (CDC, 2019). Through *Candida* species, up to 15 well-defined species of *Candida* can cause infection to the human body. However, more than 90% of invasive candidiasis mainly caused by the most common agents, *C. albicans*, and *non-albicans Candida* (NAC) species *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, and *C. krusei* (Pappas *et al.*, 2015).

Among *Candida* species, each one has specific virulence factors, susceptibility to antifungal drugs, and its spread worldwide, but all species as a whole, serious infections due to these species are generally indicated as invasive candidiasis. According to Clinical Infectious Diseases guidelines of Infectious Diseases Society of America (IDSA), infections with mucosal *Candida* where *Candida* resides as normal flora, are not classically deemed as an invasive disease but are included in IDSA guidelines. Since the last version of these guidelines in 2009, new data related to diagnosis, treatment, and prevention for proven or suspected invasive candidiasis have been available, resulting in noticeable changes in their recommendations for treatment (Pappas *et al.*, 2015)

In immunocompromised patients or critically ill patients who are at risk, *Candida* can cause infection in the mouth and throat is called "Oropharyngeal" or "Thrush" candidiasis (OPC) (Coronado-Castellote & Jimenez-Soriano, 2013). If *Candida* infects the esophagus is called "*Candida* esophagitis" or "Esophageal candidiasis" (EC); one of the most common infections among HIV/AIDS patients (Buchacz *et al.*, 2016). Overgrowth of *Candida* inside the vagina can cause infection is commonly known as "Candidal vaginitis", "Vaginal candidiasis", "Vaginal yeast infection", or

"Vulvovaginal candidiasis" (VVC) (Gonçalves *et al.,* 2016). When *Candida* enters the bloodstream or goes deep into the heart, brain, eyes, kidney, or other parts of the body a serious infection can occur which is commonly termed as "Invasive candidiasis". The term "Candidemia" refers to a *Candida* bloodstream infection which is considered as the most common form of invasive candidiasis (Kullberg & Arendrup, 2015).

Invasive candidiasis is universally recognized in the healthcare community as the main cause of morbidity and mortality (Pappas *et al.,* 2015). *Candida* species are the most common isolated fungal pathogens in the urinary tract (UT) samples (Obručová *et al.,* 2019). As *Candida albicans* and *non-albicans Candida* (NAC) species can cause serious invasive infections, furthermore its variations in susceptibility to antifungal drugs; therefore, swift and correct identification is important to provide immediate and effective treatment (Cameron *et al.,* 2016).

Diagnosing and identification of *Candida* species are considered a great challenge for both microbiologists and clinicians. However, there are many diagnostic methods for diagnosing and identification of *Candida* species divided into two main groups: culture method; which considered the best diagnostic method despite some disadvantages, and Non-culture based diagnostic methods such as PCR, DNA sequencing, and detection of fungal antigens or cell wall components (Sturaro *et al.,* 2018).

Identification of *Candida* species based on traditional methods such as germ tube, unlike other species of *Candida, C. albicans* produces tube-like structure (true hyphae) after incubation in serum within 2 hours at 37°C. But a big concern in this method is that *C. dubliniensis* share this character with *C. albicans,* resulting in misidentification among *Candida* species (Neppelenbroek *et al.,* 2014). A new culture medium HiCrome™ *Candida* Differential Agar (Himedia, Mumbai, India) emerged as a selective and differential medium to identification and isolation of *Candida* species depending on the difference in the color of the colonies resulting from break down chromogenic substrates by the activity of species-specific enzymes (Mehta & Wyawahare, 2016).

HiCrome™ *Candida* differential agar provides results after the inoculation of *Candida* species on it for 24-48 hours at 30-37°C in an aerobic conditions. Then *Candida* species were identified on HiCrome™ *Candida* differential agar media according to the type and the color of the colonies. As per the manufacturer's instructions, *Candida* species will grow as follows; the *C. albicans* grows as light green-green color and smooth colonies, *C. tropicalis* grows as metallic blue to blue color and raised colonies, *C. krusei* grows as purple fuzzy colonies, and *C. glabrata* grows as a cream to white color colonies (Patil *et al.,* 2012). Therefore, the chromogenic agar medium seems to be a very valuable medium for direct and immediate identification of *Candida* species from clinical samples, which provide us with possible treatments (Mathavi *et al.*, 2016).

Recently, antifungal susceptibility testing (AFST) has developed to become unified and now available either from the European Committee for Antimicrobial Susceptibility Testing (EUCAST) or from the Clinical and Laboratory Standards Institute (CLSI; formerly known as the National Committee for Clinical Laboratory Standards [NCCLS]) (the M27-A3, M27-S3, M44-A and M44-S2 documents). EUCAST and CLSI established the standard procedures, the minimum inhibitory concentration (MIC) breakpoints, zone-diameter interpretive breakpoints, and disc diffusion criteria of antifungal susceptibility testing (AFST) (Sanguinetti & Posteraro, 2018). Mostly the methodological differences between EUCAST and CLSI are established in some issues such as medium composition (glucose concentration), incubation time, inoculum size, etc. (Alastruey-Izquierdo *et al.,* 2015).

Antifungal susceptibility testing (AFST) occurs via reference methods such as broth microdilution methods (BMD), commercial methods, and agar-based methods (Cantón *et al.,* 2009). Commercial methods based on agar-based methods (e.g. test strips (E-Test®, bioMérieux; MIC®, Oxoid) and discs saturated with a single antifungal agent) or broth microdilution methods (e.g. Sensititre YeastOne® panel (TREK Diagnostic Systems, Cleveland, USA) and the Vitek 2 system (bioMérieux, Inc.)) (Fothergill, 2012). Commercial methods for antifungal susceptibility testing (AFST) including automated, semi-automated, and manual methods, do not need complex skills and are cost-effective alternative methods. While broth microdilution methods

are consuming too much time and cumbersome for laboratory technicians regardless of their advantages (Alastruey-Izquierdo *et al.*, 2015).

The disk diffusion method is an easily available method to do antifungal susceptibility testing of molds and yeasts. The procedures in this method for testing the susceptibilities of yeasts to antifungal drugs are identical for that used for bacteria (Cantón *et al.*, 2009), including using of Mueller-Hinton agar (MH) supplemented with 2% glucose to enhance yeast growth, and 0,5 mg/L methylene blue dye medium to enhance the zone definition, the pH should be 7,2-7,4 after gelling, The standardized 0.5 McFarland should be used, and plates should incubate at 35 °C for 24 hours or 48 hours for some strains (Alastruey-Izquierdo *et al.*, 2015; Espinel-Ingroff & Cantón, 2007; Fothergill, 2012).

Clinically, most of the invasive fungal infections can be treated by three classes of antifungal drugs: polyenes (e.g., Amphotericin B with its two forms liposomal and lipid formulations), echinocandins (e.g., caspofungin, micafungin), and azoles (e.g., itraconazole, voriconazole, fluconazole, and miconazole) (Fothergill, 2012).

A standardized disk diffusion method for *Candida* spp susceptibility testing has been published by CLSI (M44-A). But till now zone diameter interpretive criteria (breakpoints) are only established for voriconazole and fluconazole (Cantón *et al.*, 2009; Sheehan *et al.*, 2004). Therefore, The clinical significance for combinations of drugs other than fluconazole and voriconazole is unclear (Espinel-Ingroff & Cantón, 2007). However, recent studies compare the correlation between the CLSI (document M44-A) and Neo-Sensitabs tablet assay (Neo-Sensitabs (ROSCO) Diagnostica, Taastrup, Denmark) with five antifungal agents (caspofungin, voriconazole, Amphotericin B, itraconazole, and fluconazole) (Espinel-Ingroff *et al.*, 2007; Vandenbossche *et al.*, 2002)

1.2 Aims and Objectives of Research

1.2.1 Aims

Distribution pattern of *Candida* species isolated from various clinical specimens and study their antifungal susceptibility profile testing.

To accomplish the above-mentioned aims following objectives were set.

1.2.2 Objectives

The study's basic objectives were to investigate:

1. Isolate and identify *Candida* species in collected specimens.
2. Study the distribution of *Candida* species in different clinical specimens.
3. Provide swift and correct diagnosis of *Candida* species.
4. Detect antifungal susceptibility test patterns for *Candida* isolates.

2. GENERAL INFORMATION

2.1 Historical Background

Hippocrates (circa 460–370 BCE), first mentioned oral aphthae or thrush (white patches) in severe debilitation patients in his book "Of the Epidemics" as "mouths affected with aphthous ulceration" in the fourth century BCE (Mayser & Gräser, 2011; McCool, 2010; Refai *et al.*, 2015; Revankar & Sobel, 2012; Segal & Elad, 2010; Vila *et al.*, 2020). In 1665, Pepys Diary reported that "a patient has a rash, a thrush, and a hiccup", perpetuating the idea that oral thrush comes from the host himself. Later, late in the early twentieth century, mycologists approved this conception when Castellani cited previous thrush accounts as "morbid oral mucosa secretions" (McCool, 2010; Refai *et al.*, 2015).

In a textbook on Pediatrics by Rosen Von Rosenstein in 1771 and Underwood in 1784, candidiasis was identified early as a condition of the newborn and infants. Rosen Von Rosenstein reported that the disease was of serious significance and described an invasive form of thrush, even though there was no description of etiology (Mayser & Gräser, 2011; McCool, 2010; Refai *et al.*, 2015; Segal & Elad, 2010; Vila *et al.*, 2020). The first published research on oral candidiasis was in 1786 when a thrush study was conducted by the Royal Society of Medicine in France (Deorukhkar, 2018; Khan & Gyanchandani, 1998; Lynch, 1994; Roux & Linossier, 1890). In 1835, the first case of esophageal candidiasis in newborns was described by Veron and he assumed that newborns acquired the disease while passing across the womb and vagina (Khan & Gyanchandani, 1998; Mayser & Gräser, 2011; Segal & Elad, 2010).

The first description of *Candida* has been published in 1839 by Dr. Bernhard Langenbeck; a university lecturer at the University of Göttingen in Germany, when he found out a fungus in the throat sample from a patient who died as a result of typhoid fever, in spite of he mistook the yeast as the causative agent of typhus and not thrush. The title of his paper was "Finding of fungi on the mucous membrane of the gullet of a typhoid fever corpse" (translated from German) (Knoke & Bernhardt, 2006; Langenbeck, 1839; Mayser & Gräser, 2011; McCool, 2010; Refai *et al.*, 2015; Segal

& Elad, 2010; Vila *et al.*, 2020). Nevertheless, a few years later, Gruby described a fungus as the causative agent of thrush in infants in 1842 in front of the Academy of Sciences of Paris and placed it under the genus "*Sporotrichum*" (Segal & Elad, 2010).

In 1844, similar fungi were found in the sputum and in the lungs of a pneumothorax patient by J.H. Bennett and he criticized Langenbeck's conclusion. Two years later, in 1846, Berg confirmed Langenbeck's observation, they realized that the fungus was, indeed, the causative agent of thrush, then and for the first time the Esophageal candidiasis has been confirmed to be a distinct disease. Berg was the first researcher to sufficiently described the relationship between the thrush and *C. albicans* and he noted that the fungus could be transmitted by feeding bottles and unhealthy conditions in healthy babies when a baby had died as a result of pneumonia and candidal bronchitis (Khan & Gyanchandani, 1998; Lynch, 1994; Mayser & Gräser, 2011; McCool, 2010; Refai *et al.*, 2015; Segal & Elad, 2010; Vila *et al.*, 2020).

Wilkinson, in 1849, described vaginal candidiasis. In 1850, Virchow described the pathogenicity of the fungus in subcutaneous infection. Bonorden in 1851, isolated the fungus from decayed wood and classified it as *Monilia albicans* (Refai *et al.*, 2015; Segal & Elad, 2010). In 1853, Charles Philippe Robin had used *albicans* means "to whiten" to name the causative agent of thrush and he classified it under the genus "*Oidium*" as *Oidium albicans* (Khan & Gyanchandani, 1998; Refai *et al.*, 2015; Segal & Elad, 2010; Vila *et al.*, 2020). The first diagnosis of systemic candidiasis was in 1862 and credited to Zenker (Segal & Elad, 2010). The fungus was described as a yeast and mycelial fungus in 1868 by Hansen and he accepted "*Monilia Candida*" as a correct name. In 1874, Grawitz described the dimorphic nature of the fungus as budding yeast and mycelia forms (Deorukhkar, 2018; Rippon, 1988).

In 1875, Haussman demonstrated the similarity between the causative agent in both oral and vaginal candidiasis, and he also demonstrated transmission of the infection from the vaginal lesion of the mother to the mouth of the baby (Lynch, 1994; Rippon, 1988; Segal & Elad, 2010). Reess, in 1877, classified the fungus under the genus "*Saccharomyces*" as *Saccharomyces albicans* (Refai *et al.*, 2015; Segal & Elad, 2010). In 1887, Plaut found a fungus in rotten wood that can be produced empirical

lesion in the chicken's throat and he concluded that this fungus is the same fungus described by Hansen as the causative agent of human thrush. This was approved in 1890 by Zopf and he classified it as *Monilia albicans* (Deorukhkar, 2018; Khan & Gyanchandani, 1998; Lynch, 1994; Rippon, 1988). Laurent, in 1889, classified the fungus under the genus "*Dematium*" as *Dematium albicans*. In 1897, Johan-Olsen classified the fungus under the genus "*Endomyces*" as *Endomyces albicans* (Refai *et al.,* 2015).

In 1912, Castellani made a comprehensive study on mycosis and he proposed that other yeast species in addition to *Monilia albicans* might be caused candidiasis and he made the first description of other species, currently known as *C. tropicalis*, *C. kefyr*, and *C. guilliermondii*, the name *Monilia* was commonly used in the medical literature according to Castellani's studies (Segal & Elad, 2010; Vincent, 1992). Mello and L.G. Fern (1918) classified the fungus under the genus "*Parasaccharomyces*" as *Parasaccharomyces albicans*. In 1920, Brownlie classified the fungus under the genus "*Blastomyces*" as *Blastomyces albicans* (Refai *et al.,* 2015).

Berkhout, in 1923, after identification of the distinctions between *Monilia* species. Isolated from decaying plants and fruits, and those isolated from medical samples, the *Candida* genus was established to accommodate the latter. The binomial name *Candida albicans* was accepted in 1954 by the Eighth Botanical Congress in Paris as a nomen conservandum. The name, *Candida*, comes from the Latin word "toga *Candida*" which refers to a special whitten robe to Roman Senators. The derived name is possibly referred to the whitish colonies of the *Candida* species on the agar plate or the oral lesions of aphthae or thrush (Mayser & Gräser, 2011; Refai *et al.*, 2015; Segal & Elad, 2010; Vila *et al.*, 2020).

The genus *Candida* includes asporogenous yeasts lying flat with "few hyphae," and falling apart into longer and shorter pieces. Conidia grow by budding on or from the hyphae. Their size is small, and they are colorless (Khan & Gyanchandani, 1998; Neppelenbroek *et al.*, 2014). The fungus; currently known as *Candida albicans*, was isolated from many medical cases, the first time was isolated in 1844 by Bennet from the sputum sample of a tuberculosis patient, then in 1849 by Wilkinson from a vaginal

swab of Vulvovaginal candidiasis (VVC) case, in 1853 by Robin from systemic candidal infection, and in 1861 by Zenker from a brain infection in a debilitated patient (Khan & Gyanchandani, 1998; Rippon, 1988; Segal & Elad, 2010).

In 1870, Parrot described the involvement of *C. albicans* in pulmonary infection and in intestinal disease. Additional pathological entities caused by *Candida* species were identified early in the 20th century, onychomycosis in 1904 by Dubendorfer, dermatitis in 1907 by Jacobi, cystitis in 1910 by Rafin, and chronic mucocutaneous candidiasis (CMC) in 1923 by Forbes, in 1928, Conner described endocarditis, later; in 1943, Suthin noted the relationship between candidiasis and the pathology of the endocrine system (Refai *et al.*, 2015; Segal & Elad, 2010).

Two important medical events in *Candida*'s history are the most interesting. First is the development of broad-spectrum action of antibacterial agents. It has acted as one of the most important predisposing factors for mycotic infections, as it affects the normal flora of the host in favor of the fungi they have no effect on. The second event is the alarming increase in the number of immunosuppressed hosts due to chemotherapy or disease. These two events not only increased the incidence of candidiasis but also led to the appearance of candidiasis manifestations that were previously undocumented. These infections included endophthalmitis, peritonitis, myocarditis, arthritis, myositis, osteomyelitis, meningitis, and others (Khan & Gyanchandani, 1998; Segal & Elad, 2010).

In 1912, Castellani was probably the first to suggest that *Candida* species other than *C. albicans* may be involved in pathological processes while describing the "tea tasters cough". The introduction of antibacterial agents and the increased number of the immunocompromised host may lead to a parallel rise in the overall prevalence of candidiasis and the less pathogenic *non-albicans Candida* (NAC) species. Most particularly. In 1933, Smith and Sano described the first case of candidal meningitis (Segal & Elad, 2010). In 1980, Whelan *et al.* demonstrated the subsequent development of the diploid nature of *C. albicans* and the parasexual genetic system (Deorukhkar, 2018; Whelan *et al.*, 1980). In 1986, Kurtz and his teammates developed a DNA mediated transformation system using a cloned *C. albicans* ADE2 gene. This

acted as an impetus for the rapid progress of the *C. albicans* and *non-albicans Candida* (NAC) species molecular genetic studies. *C. albicans* sequencing is nearly compiled, and new evidence has been obtained for mating in *C. albicans* (Deorukhkar, 2018; Kurtz *et al.*, 1986).

In 1953, Gold *et al.* discovered the polyene Amphotericin B while studying a *Streptococcus nodosus* strain, an aerobic actinomycete obtained from Orinoco River valley Venezuela (Deorukhkar, 2018; Dutcher, 1968). If the 1960s and 1970s are considered the era of Amphotericin B, the 1980s may be considered as the era of ketoconazole, an oral imidazole antifungal agent. The polyene antimicrobial agent nystatin was also introduced in 1990 and fluconazole in 1992 (Deorukhkar, 2018; Maertens, 2004). More efficient drugs with minimal side effects are becoming increasingly demanded (Deorukhkar, 2018).

It was noted in the early 1990s -with the *C. albicans*-specific DNA fingerprinting probe 27A- that some germ tube positive and chlamydospores forming isolates, which were identified as *C. albicans* based on phenotypic characteristics, failed to hybridize efficiently. An in-depth analysis of these organisms was subsequently revealed that they constitute a distinct species clearly distinct from, but closely related to *C. albicans*. *C. dubliniensis* isolates were identified during the intervening four-year period by laboratories from various health-care setups worldwide (Deorukhkar, 2018; Derek J. Sullivan *et al.*, 1999).

After the beginning of the AIDS era, the requirement for accurate and predictive susceptibility testing of fungi became a major issue. Previously, the only significant drug-resistant antifungal experience was evolving azole resistance reported in a number of yeast isolates from patients treated for prolonged periods of chronic mucocutaneous candidiasis (CMC) (Johnson, 2008).

A broth macrodilution method developed by the Clinical and Laboratory Standards Institute (CLSI; formerly known as the National Committee for Clinical Laboratory Standards [NCCLS]) was the first properly optimized and standardized method for yeast. This proved impractical for testing large numbers of isolates and was later modified to allow a microdilution model in microtitre plates, and all methods were

documented in the M27-A3 and M27-S3 documents and these are now a broadly accepted standard (Johnson, 2008).

2.2 The Genus *Candida*: General Characteristics

The *Candida* belongs taxonomically to the kingdom Fungi, subkingdom Dikarya, phylum Ascomycota, subphylum Saccharomycotina, class Saccharomycetes, subclass Saccharomycetidae, order Saccharomycetales, family Saccharomycetaceae, and genus *Candida* (Góralska *et al.*, 2011). The genus *Candida* accommodates a collection of heterogeneous yeast-like fungi (fungi imperfecti), also known as Deuteromycota, which means these fungi producing asexual spores only and their sexual form of reproduction has never been observed. The yeast of the genus *Candida* is oval (3-5µm), cylindrical, or elliptical unicellular or bicellular, with a double-layer cell wall. These yeasts are able to produce true hyphae or pseudohyphae, with some species exceptions like *C. parapsilosis* and *C. glabrata*. *C. parapsilosis* can produce pseudohyphae, but cannot produce true hyphae whereas; *C. glabrata* cannot produce both true hyphae and pseudohyphae (Deorukhkar, 2018; McCullough *et al.*, 1996). Hyphae (singular hypha) have an average diameter of (2-6µm) (Maheshwari, 2016).

Pseudohyphae are formed by budding from yeast cells or hyphae but this bud does not detach itself from the parent cell. At cell-cell junctions, the new growth further elongates and forms filaments with constrictions (Deorukhkar, 2018; McCullough *et al.*, 1996; Silva *et al.*, 2012). Pseudohyphae have no inner cross-walls; known as septa (singular septum). True hyphae are formed from yeast cells, or even as existing hyphae branches. A germ tube formation initiates its development. This germ tube elongates, then branches with defined septa divide the hyphae into separate fungal units (Silva *et al.*, 2012).

Because of its ability to form hyphae and/or pseudohyphae, *C. albicans* and *C. dubliniensis* are considered truly polymeric species of the genus. They may also form a germ tube within 2 hours when inoculated in human serum or other substances at 37°C. For this reason, a positive germ tube test or the Reynolds-Braude Phenomenon is a diagnostic feature of these two species (Deorukhkar *et al.*, 2012; Neppelenbroek *et al.*, 2014; Silva *et al.*, 2012). The genus *Candida* represented by 163 anamorphic

and teleomorphic species in at least 13 genera. In 1998, Meyer *et al.* reclassified the *Candida* species based on their physiological characteristics in 12 groups for identification purposes (C. Kurtzman *et al.*, 2011). Despite the ignorance of the genetic origin of these groups (Deorukhkar, 2018). And with the increase in the number of emerging species about 20 species were identified can cause infection to the human (López-Martínez, 2010).

Many *Candida* species have been implicated in human disease involved *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *C. glabrata*, and *C. lusitaniae*. Among the immunocompromised individuals, *C. dubliniensis* was recognized as an important pathogen. *Torulopsis* has recently been merged within the *Candida* genus; *C. stellatoidea* is included with *C. albicans* and *C. pseudotropicalis* is renamed *C. kefyr* (Lockhart, 2014). A new fluconazole-resistant species, *C. auris*; was first identified in 2009 from the ear discharge of a Japanese patient, in East Asia and had been isolated on five continents in 2018 (Satoh *et al.*, 2009; Spivak & Hanson, 2017). *C. auris* differs in the characteristic of its growth. Because it fails to release daughter cells after budding, it produces large cell aggregates that cannot be disturbed physically. *C. auris* does not produce true hyphae but in vitro rudimentary formation of pseudohyphae could be seen (Borman *et al.*, 2016). The *non-albicans Candida* (NAC) species seem to have a close phenotypic relationship to *C. haemulonii*. *C.auris* has emerged as an essential cause of health-care-associated infections worldwide in recent years (Chowdhary *et al.*, 2017).

Previously, *C. africana* was considered an atypical variant of *C. albicans*. Similar to *C. albicans* and *C. dubliniensis* this species is positive in the germ tube formation but lacks the ability to form chlamydospore (Borman *et al.*, 2013). The list of potentially pathogenic species includes *C. famata* or *C. inconspicua* (Deorukhkar, 2018; Segal & Elad, 2010). Table 2.1 shows the *Candida* species that particularly associated with colonization and disease in humans (Moran *et al.*, 2012).

Table 2.1: *Candida* species of medical importance associated with humans diseases.

***Candida* species**			
C. albicans			
Non-albicans Candida (NAC)			
Commonly encountered *spp.*	Infrequently encountered *spp.*	Rarely encountered *spp*.	
C. glabrata	*C. dubliniensis*	*C. famata*	*C. sake*
C. krusei	*C. guilliermondii*	*C. norvegensis*	*C. haemulonii*
C. parapsilosis	*C. lusitaniae*	*C. inconspicua*	*C. viswanathii*
C. tropicalis	*C. rugosa*	*C. lipolytica*	*C. zeylanoides*
C. kefyr	*C. orthopsilosis*	*C. lambica*	*C. ciferrii*
	C. metapsilosis	*C. nivariensis*	

2.2.1 Reproduction

Candida species reproduce asexually by blastospores formation or by budding and lack a sexual form of reproduction. Typical of Ascomycetes, one bud in each locus results from budding reproduction form, whereas the cells bud repeatedly from one locus in the Basidiomycetes, and therefore, the molecular structure of the cross walls (septa) is transparent and homogenous as in Ascomycetes while tripartite in the Basidiomycetes (Deorukhkar, 2018; Rippon, 1988). Yeast cell produces a protuberance that elongates and gradually separates from the mother cell. In this process, mitosis occurs, where the number of chromosomes remains the same. Figure 2.1 shows the diagrammatic representation of the different budding reproduction stages in *Candida* species (Segal & Elad, 2010).

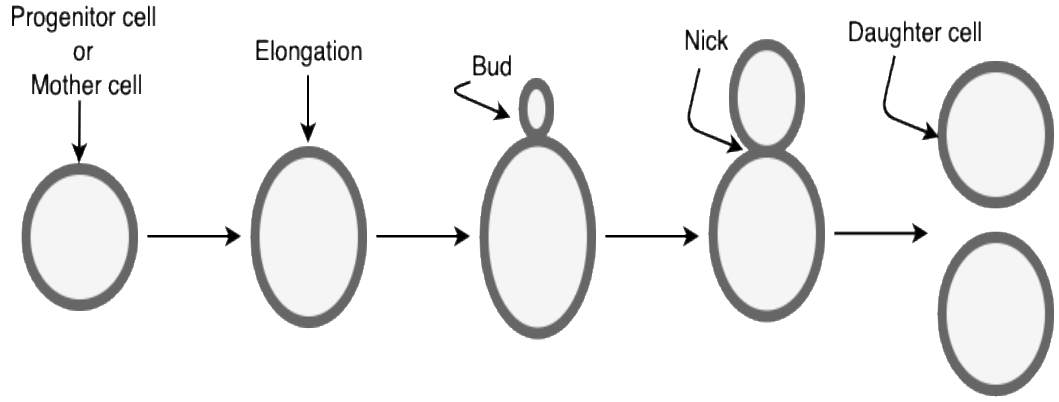

Figure 2.1: Diagrammatic representation of different stages of asexual reproduction by budding in *Candida* species.

2.2.2 Chlamydospores

Chlamydospores are produced in *Candida* species through intercalary or terminal hyphal cells (Deorukhkar, 2018; Rippon, 1988). Chlamydospores are asexual spores that are round, strongly refractile, and resistant (Deorukhkar, 2018; Khan & Gyanchandani, 1998; Rippon, 1988). They are thick-walled and have an average diameter of 8-12µm, larger than other cells. The large size of chlamydospores comes from the storage of reserve nutritional substances (Deorukhkar, 2018; Rippon, 1988). Chlamydospores' thick wall consists of two layers. In nature, the outer layer is a polysaccharide whereas the inner layer is made of lipoprotein. The thick wall shields chlamydospores from harsh environmental conditions (Deorukhkar, 2018; Khan & Gyanchandani, 1998).

Chlamydospore is an amendment of hyphae or a single vegetative cell. Around 60% of isolated *C. albicans* from clinical samples are able to chlamydospores formation on nutritionally deficient media as a result of responding to unfavorable conditions of desiccation and temperature (Deorukhkar, 2018; Rippon, 1988). The formation of Chlamydospore can be stimulated by inoculating *Candida* species isolates on Corn Meal and Rice Starch Agar medium. Corn Meal Agar (CMA) media used to detect the ability to mycelium formation in *Candida* species under ideal conditions. This medium used also to detect the ability to ascospores formation in yeast cells by extending the incubation time up to 30 days (Deorukhkar, 2018; Rippon, 1988).

The Corn Meal Agar (CMA) supplemented with tween 80 (polysorbate) stimulates the chlamydospores formation. Tween-80 allows the development of pseudohyphal, hyphal, and blastoconidial forms of *Candida* species by reducing the surface tension. Chlamydospores are distinguished within a single culture by a large variation in shape and size, and their morphology also helps in the isolate speciation. The staining technique may also be used with 1% lactophenol cotton blue (LPCB) which stains chlamydospores intensely preferentially whereas, pseudohyphae, blastopores, and suspensor cells are either slightly stained or not stained at all (Deorukhkar, 2018; Rippon, 1988). The diagnostic feature of *C. albicans* is the production of chlamydospores. *C. albicans* chlamydospores generally appear independently at the tip or intercalarily. *C. albicans* also form compact blastoconidia clusters along pseudohyphae at regular intervals (Deorukhkar, 2018; Winn *et al.*, 2005).

On nutritionally deficient media, *C. dubliniensis* produces chlamydospores abundantly and readily more than other species as clusters and sometimes as doublets or triplets. chlamydospores formation occurs either on true hyphae or on pseudohyphae (Deorukhkar, 2018; Derek J. Sullivan *et al.*, 1999). True hyphae are short and show hyper branching laterally. In *C. dubliniensis*, chlamydospores are formed abundantly and mostly arrangement in terminal pairs when it inoculates on Tween-80, Oxgall, Caffeic Acid (TOC) Agar within 48 hours at room temperature (Deorukhkar, 2018; Jabra-Rizk *et al.*, 1999). However, a few non-pathogenic *Candida* species can produce chlamydospores as well (Deorukhkar, 2018; Pincus *et al.*, 2007).

2.2.3 Physiology

In *Candida* species, glucose metabolism induces either aerobically (assimilation) through the hexose monophosphate pathway, or anaerobically (fermentation) through the Embden-Meyerhof pathway. Some metabolic pathways such as the Krebs cycle, mitochondrial oxidative phosphorylation, and 80s ribosomal (composed of 60s and 38s subunit) protein synthesis are identical to those in eukaryotic organisms (Braude, 1986; Segal & Elad, 2010). The *Candida* species have a complicated enzymatic apparatus (F. C. Odds, 1988; Segal & Elad, 2010). The functional importance of enzymes such as proteinase and others active in sterol synthesis is important, as they

are specifically active in *Candida* infection pathogenesis. Such enzymes may be used as antimycotic drug targets. Alcohols, fatty acids, acids, and ethanol are catabolic products of metabolism of the *Candida* species (Segal & Elad, 2010).

In the development of morphological characteristics of *Candida* species, the growth temperature plays an important promotor in the formation of chlamydospores and pseudohyphae. In *C. albicans*, chlamydospores formation will be promoted at 25°C while at 37°C or higher (sometimes up to 45°C) promotes the formation of pseudohyphae (Braude, 1986; Segal & Elad, 2010). The capability of growth at 37°C or higher temperature does not occur in all *Candida* species. therefor this ability plays an important pathogenic factor of *Candida* species and helps differentiate between the environmental saprophytes and possible pathogenic strains. With the forming of acid and gas, *Candida* species can ferment different numbers of sugars, particularly glucose, galactose, and maltose. The various species of *Candida* are often can be identified by their pattern of assimilation and fermentation of sugars (Segal & Elad, 2010).

2.3 *Candida* Species of Medical Importance

2.3.1 Commonly Clinically Isolated Species

1. *C. albicans*

Taxonomic synonyms:

Oidium albicans (C.P. Robin., 1853), *Saccharomyces albicans* (Reess.), *Monilia albicans* (Zopf.), *Dematium albicans* (Laurent.), *Syringospora robinni* (Quinquaud.), *Endomyces albicans* (Johan-Olsen.), *Endomyces pinoyi* (Castellani.), *Candida albicans* (Berkhout.) (Lachance *et al.*, 2011; Refai *et al.*, 2015; Segal & Elad, 2010).

C. albicans was described in 1853 by Charles Philippe Robin (Khan & Gyanchandani, 1998; Segal & Elad, 2010). In comparison with other *Candida* species, *C. albicans* consider as the most prevalent pathogenic and rarely isolated from

environmental sources. It is a commensal of the gastrointestinal and genitourinary tract; therefore, most infections are endogenous in origin (Deorukhkar, 2018).

Hasenclever and Mitchell (1961) detected two distinct antigenic groups of *C. albicans*; serotype A and B, based on variations in the mannan component of the cell wall (Auger *et al.*, 1979; Hasenclever & Mitchell, 1961; Segal & Elad, 2010). Clinically, serotype A is more prevalent than serotype B (Deorukhkar, 2018; Meyer *et al.*, 1998). The study of Hasenclever *et al.* (1961) concluded that *C. albicans* serotype A and *C. tropicalis* were antigenically identical and *C. albicans* group B and *C. stellatoidea* were antigenically indistinguishable (Hasenclever *et al.*, 1961; Segal & Elad, 2010).

As *C. stellatoidea* has relatively highly homogenous DNA with *C. albicans* and was considered previously as a sucrose-negative variant of *C. albicans*. Meyer *et al.* (1984) found that there are two different types of sucrose-negative *C. albicans*, identified as *C. stellatoidea*, type I and II (Deorukhkar, 2018; Meyer *et al.*, 1998).

The study of Kwon-Chung *et al.* (1989) shows that *C. stellatoidea* type II was considered as a sucrose-negative mutant of *C. albicans* serotype A, whereas *C. stellatoidea* type I varies from *C. albicans* in many major genetics characteristics; therefore, it is not considered as a mutant of *C. albicans* (Kwon-Chung *et al.*, 1989; Vazquez & Sobel, 2011).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. albicans* colonies are smooth and creamy. but look glistening, waxy, reticulate, smooth, and creamy colonies when incubated for a prolonged time, while in old stock cultures the colonies often look wrinkly and folded (Segal & Elad, 2010). When *C. albicans* grown on SDA supplemented with methylene blue, the colonies fluoresce with yellow color upon exposure to long-wave UV radiation. However, with prolonged storage and repeated subcultures this property could be lost (Deorukhkar, 2018; D. Sullivan & Coleman, 1998). On HiCrome™ *Candida* differential agar, the colonies of *C. albicans* grow with light green color and smooth colonies (F C Odds & Bernaerts, 1994; Patil *et al.*, 2012). On Tetrazolium

Reduction Medium (TRM), the colonies of *C. albicans* grow with pale pink color (Deorukhkar & Saini, 2014).

Microscopic morphology:

On CMA, *C. albicans* forms chlamydospores; clustered blastospores as grape-like shape (true and pseudomycelium) at the end of their hyphae or at their short lateral branches, after incubation at 37ºC for 3 days. While elongated germ tube formation occurs within 2 hours when *C. albicans* incubated in human serum at 37ºC. A prepared smear of *C. albicans* colonies from SDA shows small, oval (4-6 × 6-10µm) yeast-like fungi (Deorukhkar *et al.*, 2012; Neppelenbroek *et al.*, 2014; Segal & Elad, 2010).

Biochemical reactions:

C. albicans can ferment various types of sugar as sucrose, glucose, maltose, galactose, and trehalose with producing acid and gas as final products (Segal & Elad, 2010). It has the ability to assimilating glucose, maltose, galactose, trehalose, inulin, D-mannitol, L-sorbose, and L-arabinose with β-glucosidase production (Deorukhkar, 2018; Hasenclever *et al.*, 1961).

2. *C. tropicalis*

Taxonomic synonyms:

Oidium tropicale (Castellani, 1910.), *Mycotorula japonica* (Yamaguchi.), *Mycotorula dimorpha* (Ciferri and Redaelli.), *Monilia tropicalis* (Castellani and Chalmers.), *Candida tropicalis* (Castellani.) (Lachance *et al.*, 2011; Rippon, 1988; Segal & Elad, 2010).

In comparison with another NAC species, *C. tropicalis* is one of the most commonly isolated from different clinical samples of *Candida* infection (Kothavade *et al.*, 2010). Whereas, it is considered as the most causative agent of nosocomial candidemia (Giri & Kindo, 2012). And as the third or fourth most common of *Candida* species recovered from blood cultures (Kontoyiannis *et al.*, 2001; M. A. Pfaller *et al.*, 1998; Vazquez & Sobel, 2011).

Colony characteristics:

C. tropicalis is similar to *C. albicans* in colony morphology and growth rate when it inoculates on Sabouraud Dextrose Agar (SDA). It produces smooth, cream, and dull glistening colonies. While the colonies look wrinkly when it incubated for 72 hours at 25ºC. In older cultures, prominent mycelia borders are seen (Rippon, 1988; Segal & Elad, 2010).

On HiCrome™ *Candida* differential agar, *C. tropicalis* grows with metallic blue to blue color and raised colonies (F C Odds & Bernaerts, 1994; Patil *et al.*, 2012). On Tetrazolium Reduction Medium (TRM), *C. tropicalis* grows with orange-pink color colonies (Deorukhkar & Saini, 2014).

Microscopic morphology:

On CMA, *C. tropicalis* produces singly blastospores with long branched pseudohyphae, in chain or clusters are often seen at the septa. However, a few strains of *C. tropicalis* are able in initial inoculation to produce true hyphae or chlamydospores. A prepared smear of *C. tropicalis* colonies from SDA shows small, oval (4-8 × 5-10µm) yeast-like fungi (Neppelenbroek *et al.*, 2014; Rippon, 1988; Segal & Elad, 2010).

Biochemical reactions:

C. tropicalis has the ability to ferment various types of sugar as sucrose, glucose, melezitose, maltose, galactose, and trehalose. it can assimilate glucose, maltose, galactose, cellobiose, sucrose, L-sorbose, trehalose, D-xylose, glycerol, ethanol, ribitol, L-arabinose, melezitose, and salicin (Rippon, 1988; Segal & Elad, 2010).

3. *C. glabrata*

Taxonomic synonyms:

Cryptococcus glabratus (Anderson, 1917.), *Torulopsis glabrata* (Lodder and de Vries.), *Candida glabrata* (Yarrow and Meyer.) (Lachance *et al.*, 2011; Rippon, 1988).

In comparison with another NAC species, *C. glabrata* is the only species that has no ability to produce pseudohyphae or hyphae (Paul L. Fidel *et al.*, 1999; Silva *et al.*, 2012).

C. glabrata is one of the most common species isolated from the oral cavity (Segal & Elad, 2010; St Germain & Beauchesne, 1991) or from the vagina of healthy women (F. C. Odds, 1988; Segal & Elad, 2010). The prevalence of *C. glabrata* is increased significantly in human infections (Segal & Elad, 2010). Currently, *C. glabrata* ranks as the second most common causative agent of candidemia after *C. albicans* (Li *et al.*, 2007; Segal & Elad, 2010; Vazquez & Sobel, 2011).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. glabrata* forms glistening, soft, and smooth colonies after incubation at 25ºC for 3 days. No fringes of the mycelia are seen (Vazquez & Sobel, 2011). On HiCrome™ *Candida* differential agar, *C. glabrata* grows as creamy or white color colonies (F C Odds & Bernaerts, 1994; Patil *et al.*, 2012; Vazquez & Sobel, 2011). On Tetrazolium Reduction Medium (TRM), *C. glabrata* grows as pale pink-colored colonies (Rippon, 1988; Segal & Elad, 2010).

Microscopic morphology:

A prepared smear of *C. glabrata* colonies from SDA shows small (2,5 × 4,6µm), oval or round yeast-like fungi, and no true hyphae or pseudohyphae are seen (Rippon, 1988; Segal & Elad, 2010).

Biochemical reactions:

C. glabrata can ferment glucose and trehalose with producing of acid and gas as final products. it has the ability to assimilate glucose, trehalose, and acetate (weak) (Rippon, 1988; Segal & Elad, 2010).

4. *C. krusei*

Taxonomic synonyms:

Saccharomyces krusei, Endomyces krusei, Monilia krusei, Myceloblastanon krusei, Mycotoruloides krusei, Monilia parakrusei, Trichosporon krusei, Candida castellanii (Castellani.), *Mycoderma chevalieri* (Guillierm.), *Mycoderma monosa* (Anderson.), *Mycoderma bordetii* (Kuff.), *Monilia inexpectata* (Mazza.), *Candida lobata* (Bat.), *Candida krusei* (Castellani.) (Rippon, 1988; Segal & Elad, 2010).

In 1914, Castellani suggested the role of *C. krusei* in human infections. *C. krusei* is an optional saprophyte and extensively distributed in the environment. In man, it is considered as a transient commensal (Deorukhkar, 2018; Samaranayake Y & Samaranayake L, 1994).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. krusei* forms dry, dull, and flat colonies. While in old stock cultures the colonies often look wrinkly or smooth and dull with a greenish-yellow color. Mycelium grows densely and spans as a lateral fringe (Rippon, 1988; Segal & Elad, 2010). On HiCrome™ *Candida* differential agar, *C. krusei* grows as purple fuzzy colonies (Patil *et al.*, 2012; Trofa *et al.*, 2008). On Tetrazolium Reduction Medium (TRM), *C. krusei* grows as pink and dry colonies (Rippon, 1988; Segal & Elad, 2010).

Microscopic morphology:

On CMA, *C. krusei* produces long and dense pseudohyphae. The elongated yeast cells have the look of "crossed match sticks" or "tree-like structure". Blastoconidia are always elongated and pseudomycelium grows in vertical branches. Only a few blastoconidia are produced in some strains. A prepared smear of *C. krusei* colonies from SDA shows oval, elongated (2,2-5,6 × 4,3-15,2µm) yeast-like fungi (Deorukhkar, 2018; Samaranayake Y & Samaranayake L, 1994).

Biochemical reactions:

C. krusei is urease positive. it has the ability to ferment only glucose and it can assimilate glucose, glycerol, and ethanol (Rippon, 1988; Segal & Elad, 2010).

5. *C. kefyr*

Taxonomic synonyms:

Monilia pseudotropicalis, Saccharomyces kefyr, Candida pseudotropicalis, MycoCandida pseudotropicalis, Candida kefyr (Castellani.) (Meyer *et al.*, 1998; Rippon, 1988; Segal & Elad, 2010).

In 1914, Castellani was the first one who described *C. kefyr* (formerly known as *C. pseudotropicalis*). *C. kefyr* has been intermittently isolated from the ear, gastrointestinal tract, and vaginal infections. it is often used as a standard control strain for AFST of yeast (Rippon, 1988; Segal & Elad, 2010).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. kefyr* colonies are smooth and creamy. With prolonged incubation, the colonies become dull and smooth with creamy to yellow color. On Tetrazolium Reduction Medium (TRM), the colonies of *C. kefyr* grow with pink color. (Rippon, 1988; Segal & Elad, 2010).

Microscopic morphology:

On CMA, most of *C. kefyr* strains are able to produce pseudohyphae abundantly. The cells, like “logs in a stream”, are elongated and fall apart and lie parallel. Blastoconidia are scanty but become elongated and verticillate when present. A prepared smear of *C. kefyr* colonies from SDA shows oval, small, elongated (2,5-5 × 5-10µm) yeast-like fungi (Rippon, 1988; Segal & Elad, 2010).

Biochemical reactions:

C. kefyr can ferment sucrose, glucose, lactose, galactose, raffinose and inulin. It can assimilate sucrose, glucose, galactose, d-mannitol, salicin, L-arabinose, xylose, cellobiose, ethanol, ribitol, inulin, and glycerol (Rippon, 1988; Segal & Elad, 2010).

6. *C. parapsilosis*

Taxonomic synonyms:

Monilia parapsilosis, Monilia onchophia, MycoCandida parapsilosis (Ashford), *Blastodendrion globosum* (Zach) (Meyer *et al.*, 1998; Rippon, 1988; Segal & Elad, 2010).

In 1928, Ashford was the first one who isolated *C. parapsilosis* in Puerto Rico from the stool sample of a patient suffering from diarrhea. Before 2005, *C. parapsilosis* was divided into 3 groups I, II, and III. Then according to studies that show genetic differences between these groups led to separate them into three distinct species: *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* (Deorukhkar, 2018; Trofa *et al.*, 2008). *C. orthopsilosis* and *C. metapsilosis* are phenotypically similar but genotypically different from *C. parapsilosis*. *Lodderomyces elongisporus* is closely related phenotypically to *C. parapsilosis* and has recently been reported as an infrequent source of bloodstream infections in Asian and Mexican patients (S. Ahmad *et al.*, 2013; Al-Obaid *et al.*, 2018; Tay *et al.*, 2009).

In comparison with other *Candida* species, *C. parapsilosis* is the only species that subsist in a commensal state in the human body. and unlike *C. albicans* and *C. tropicalis*, it is not an obligate human pathogen (Bertini *et al.*, 2013).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. parapsilosis* forms white, creamy, shiny, and smooth or wrinkled colonies. On HiCrome™ *Candida* differential agar, *C. parapsilosis* grows as cream-colored colonies. On Tetrazolium Reduction Medium (TRM), *C. parapsilosis* grows as rose pink color dry colonies (Deorukhkar, 2018; Trofa *et al.*, 2008).

Microscopic morphology:

On CMA, *C. parapsilosis* produces long, thin, and branched pseudohyphae. this feature is related to a specific set of amino acids, such as citrulline. So the variations in cellular and colony morphology of *C. parapsilosis* are linked to this amino acid. It is not able to produce true hyphae like *C. albicans* and *C. tropicalis*. Along pseudohyphae, *C. parapsilosis* produces blastospores singly or in small clusters. the presence of the "giant cells" (large mycelia elements) set as a feature of this species. A prepared smear of *C. parapsilosis* colonies from SDA shows an oval, round, or cylindrical (2,5-4 × 5-9µm) yeast-like fungi (Deorukhkar, 2018; Trofa *et al.*, 2008).

Biochemical reactions:

C. parapsilosis can ferment dextrose only. It can assimilate sucrose, maltose, galactose, dextrose, trehalose, and xylose (Rippon, 1988; Segal & Elad, 2010).

2.3.2 Infrequently Clinically Isolated Species

1. *C. guilliermondii*

Taxonomic synonyms:

Meyerozyma guilliermondii (Wickerham), *Mycotorula guilliermondii* (Castellani), *Monilia arztii* (Ota), *Candida melibiosi* (Lodder & Kreger-van Rij) (C. P. Kurtzman, 2011a; Rippon, 1988; Segal & Elad, 2010).

In 1912, Castellani was the first one who describes *C. guilliermondii*. Previously, *C. guilliermondii* was considered as a rare cause of human candidiasis. However, human infections related to *C. guilliermondii* have been reported from various health-care setups in recent years (Rippon, 1988; Segal & Elad, 2010).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. guilliermondii* forms flat, thin, glossy, and cream to pinkish colonies. With prolonged incubation, the colonies become glistening, smooth, or dull, wrinkled colonies with Yellowish cream to pink color. On Tetrazolium Reduction Medium (TRM), *C. guilliermondii* grows as pink and pasty colonies (Rippon, 1988; Segal & Elad, 2010). On HiCrome™ *Candida* differential agar, *C. guilliermondii* grows as small pink to purple colonies (Nadeem *et al.,* 2010).

Microscopic morphology:

On CMA, *C. guilliermondii* produces sparse or dense, very fine, and short pseudohyphae. Blastoconidia can be seen in clusters or small chains. A prepared smear of *C. guilliermondii* colonies from SDA shows short, ovoid, or small and cylindrical (2-5 × 3-7µm) yeast-like fungi (Rippon, 1988; Segal & Elad, 2010).

Biochemical reactions:

C. guilliermondii can ferment galactose and glucose. It can assimilate sucrose, maltose, galactose, L-sorbose, glycerol, D-glucitol, melezitose, trehalose, D-mannitol, ribitol, and ethanol (Rippon, 1988; Segal & Elad, 2010).

2. *C. dubliniensis*

C. dubliniensis is the novel *Candida* species which was described in 1995 by Sullivan and Coleman. As its name implies, this species was originally identified in Dublin, the capital of the Republic of Ireland, while undertaking an epidemiological study of oral candidiasis in AIDS patients and Irish HIV infected persons (Segal & Elad, 2010; Derek J. Sullivan *et al.*, 1995). This species is reported to be isolated from numerous body sites/samples such as urine, the gastrointestinal tract, vagina, and skin of both non-infected and HIV infected individuals (Sardi *et al.*, 2013).

C. dubliniensis fungemia is reported in patients of end-stage liver disease, hematologic malignancy, and in patients with chemotherapy-induced immunosuppression (Brandt *et al.*, 2000; Deorukhkar, 2018; Meis *et al.*, 1999)

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. dubliniensis* colonies are creamy and white-colored. It grows well at both 30ºC and 37ºC, whereas it shows poor or no growth at 42ºC. Unlike *C. albicans*, *C. dubliniensis* colonies unable to fluoresce when grown on SDA supplemented with methylene blue or when its exposure to long-wave UV radiation. On HiCrome™ *Candida* differential agar, the colonies of *C. dubliniensis* grow with dark green color (Deorukhkar, 2018; Derek J. Sullivan *et al.*, 1995).

Microscopic morphology:

On CMA, *C. dubliniensis* forms abundant chlamydospores usually in clusters or in adjoining pairs along the true hyphae. It is produced solitary blastoconidia or in clusters. *C. dubliniensis* able to produce true hyphae, pseudohyphae, and germ tubes. These features usually diagnostic for *C. albicans* (Rippon, 1988; Segal & Elad, 2010).

Biochemical reactions:

C. dubliniensis can ferment Maltose and glucose (C. P. Kurtzman, 2011a). It assimilates sucrose, galactose, trehalose, maltose, and dextrose. Whereas it does not assimilate Methyl α-D-glucoside, xylose, and lactose as *C. albicans* do (Rippon, 1988; Segal & Elad, 2010).

3. *C. lusitaniae*

Taxonomic synonyms:

Clavispora lusitaniae (Rodrigues de Mirand) (C. P. Kurtzman, 2011a).

C. lusitaniae consider as an emerging *non-albicans Candida* (NAC) species. In 1979, Pappagianis *et al.* were initially described *C. lusitaniae* as a human pathogen that can cause candidiasis (Deorukhkar, 2018; Pappagianis *et al.*, 1979). Holzschu *et al.* were described *C. lusitaniae* as an opportunistic pathogen in humans (Holzschu *et al.*, 1979; Segal & Elad, 2010).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. lusitaniae* colonies are cream to white color at 25ºC. It grows well on media supplement with cycloheximide and at 42ºC (Deorukhkar, 2018; Pappagianis *et al.*, 1979).

Microscopic morphology:

On CMA, *C. lusitaniae* produces plentiful branched pseudohyphae when incubation at 25ºC for 72 hours. Whereas some strains have no ability to produce pseudohyphae. A prepared smear of *C. lusitaniae* colonies from SDA shows ovoid (1,5-6 × 2,5-10µm) yeast-like fungi arranged in pairs or clusters (Deorukhkar, 2018; Pappagianis *et al.*, 1979).

Biochemical reactions:

C. lusitaniae has the ability to ferment cellobiose, galactose, and dextrose. However, sucrose and trehalose can be fermented by a few strains. It assimilates sucrose, galactose, cellobiose, trehalose, maltose, xylose, and dextrose (C. P. Kurtzman, 2011a; Segal & Elad, 2010).

2.3.3 Rarely Clinically Isolated Species

1. *C. famata*

C. Famata is also recognized as *Torulopsis Candida* and *Debaryomyces hansenii*. This *non-albicans Candida* (NAC) species rarely causes infection in humans. *C. Famata* is described for infections with catheter-related bloodstream, peritonitis, acute zonal occult retinopathy, and mediastinitis. *C. Famata* infection mostly occurs in immunocompromised individuals and patients with burning. However, it is considered as a saprophyte commonly found in cheese, dairy, and environmental products (Beyda *et al.*, 2013).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. famata* colonies are white to cream-colored and smooth (Beyda *et al.*, 2013).

Microscopic morphology:

On CMA, *C. famata* produces small blastospores, whereas no pseudohyphae production is seen (Beyda *et al.*, 2013).

Biochemical reactions:

C. famata has the ability to assimilate L-sorbose and L-lactate (Beyda *et al.*, 2013).

2. *C. lipolytica*

C. lipolytica is rarely caused infection in humans. This *non-albicans Candida* (NAC) species considered a saprophyte and commonly found in animals and plants (Deorukhkar, 2018; Krcmery & Barnes, 2002). In 1995, Rex *et al.* described the case of fungemia in a cancer patient treated with fluconazole Intravenously caused by *C. lipolytica* (Deorukhkar, 2018; J H Rex *et al.*, 1995).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. lipolytica* colonies are dry, cerebriform, or creamy, white-colored after incubation for 72 hours at 25ºC (Trabelsi *et al.*, 2015).

Microscopic morphology:

On CMA, *C. lipolytica* produces branched true hyphae. it rarely produces short, branched, and elongated blastospores (Kumar *et al.*, 2013).

Biochemical reactions:

C. lipolytica has the ability to ferment and assimilate dextrose, whereas a few strains are able to assimilate galactose. *C. lipolytica* can produce a urease enzyme (Galán-Sánchez *et al.*, 2014; C. P. Kurtzman, 2011b).

3. *C. lambica*

Previously, *C. lambica* was described as *Mycoderma lambica* and it has similarity with *C. krusei*. It is rarely involved in human infections (Deorukhkar, 2018; Vervaeke *et al.*, 2008).

Colony characteristics:

On Sabouraud Dextrose Agar (SDA), *C. lambica* colonies are smooth, with white to cream-colored. On HiCrome™ *Candida* differential agar, the colonies of *C. lambica* grow flat with mauve-colored. Therefore, HiCrome™ *Candida* differential agar usually

fails to distinguish *C. lambica* colonies from *C. krusei* colonies (Deorukhkar, 2018; Vervaeke *et al.*, 2008).

Microscopic morphology:

On CMA, *C. lambica* produces branched true hyphae. it rarely produces short, elongated, and branched blastospores (Deorukhkar, 2018; Vervaeke *et al.*, 2008).

Biochemical reactions:

The key test to distinguish between *C. lambica* and *C. krusei* is that *C. lambica* has the ability to grow at 45ºC and assimilate xylose (Deorukhkar, 2018; Vervaeke *et al.*, 2008).

2.4 The Pathogenicity of *Candida* Species

There are a number of virulence factors that led to the pathogenicity of the *Candida* species. Virulence factors are all traits needed for the infection to be established and progressed. Those factors could cause damages by directly interact with host cells (Silva *et al.*, 2012).

The significant virulence factors of *Candida* species include adherence or adhesion to host tissues and medical devices, formation biofilms, extracellular hydrolytic enzymes secretion, thigmotropism, polymorphism, and phenotypic switching (Sardi *et al.*, 2013).

- **Adherence**

The most critical step in *Candida*'s colonization and infection is adherence to host tissues. Several cell-signaling cascades control and stimulate this process. Initially, adhesion to the host tissue occurs via non-specific factors such as hydrophobicity and electrostatic forces and is further promoted by specific adhesins on the *Candida* cells surface (Sardi *et al.*, 2013).

Adherence to host tissues plays a crucial role within the pathogenesis of *Candida* infection because it prevents or a minimum of reduces the extent of clearance by the host's defense mechanisms. It also ensures the delivery of enzymes and toxins to the target host cells. *Candida* species can adhere to various host cells including epithelial, endothelial, and phagocytic cells (Calderone & Fonzi, 2001; Deorukhkar, 2018).

- **Biofilm formation**

The *Candida* species can also adhere to the surface of medical devices and form biofilms (Silva *et al.*, 2012). *Candida* species can form biofilm on most medical devices. Biofilms are described as specific and organized surface-associated communities of microbial cells embedded within an extracellular matrix (Deorukhkar, 2018; Kojic & Darouiche, 2004). The adherence of *Candida* cells is directly followed by cell division, multiplication, and biofilms formation. the formed biofilms are resistant to various antifungal drugs; therefore, the surgical removal and replacement of the infected medical devices are included in the treatment plan (Sardi *et al.*, 2013).

Since the precise mechanism of biofilm resistance to antifungal drugs is not completely elucidated. Some hypotheses were suggested as an intrinsic relationship between biofilm and their resistance to antifungal drugs. These hypotheses include growth rate of biofilm cells, presence of physical barriers preventing antimicrobial from exposure to biofilm-embedded cells; these barriers known as a matrix of extracellular polymeric substances (EPS), and presence of phenotypic variants of cells instead of mutants (Persister cells) (Deorukhkar, 2018; Seneviratne *et al.*, 2008).

In vitro, *C. albicans* biofilms show sensitivity to only the echinocandins (caspofungin) and Amphotericin B within three classes of antifungal drugs. Nevertheless, the biofilms formation on medical devices due to Candidal infection is extremely difficult to overcome even with sensitivity to these two antifungal agents. For this reason, despite there are serious implications in case these devices were heart valves, artificial joints, or CNS shunts, the replacement of infected medical devices is necessary (Sardi *et al.*, 2013).

- **Extracellular hydrolytic enzymes**

Candida species produce various exoenzymes that play a crucial role in their pathogenicity by damaging and destroying the cell membranes of host cells. These extracellular hydrolytic enzymes include secreted aspartyl proteinases (Saps), lipases, phospholipase, and haemolysins (Sardi *et al.*, 2013; Silva *et al.*, 2012).

- **Thigmotropism**

The directional hyphal growth shown by *Candida* species on surfaces with specific topologies is known as Thigmotropism. Thigmotropism is one of the major mechanisms that contribute to the *Candida* species virulence. This process helps in the formation of biofilms on various abiotic surfaces such as medical devices and the spread into host tissues. *C. albicans* hyphae Thigmotropism is regulated by extracellular calcium absorption via the calcium channels (Deorukhkar, 2018; Mayer *et al.*, 2013).

- **Phenotypic switching**

Phenotypic switching is defined as the ability of the single strain organisms to switch between different colony phenotypes reversibly and at high frequency (Sardi *et al.*, 2013).

Phenotypic switching is a common technique for adjusting rapidly to different host niches and promoting colonization and infection. This feature also resists neutrophil's Candidacidal behavior. Many strains of *C. albicans* can switch between numerous general phenotypes distinguishable by colony morphology, spontaneously, reversibly, and at high frequencies (Mane *et al.*, 2011).

2.5 The Human Immunity Against *Candida* Species

The development of *Candida* infection in infected individuals includes a combination of well-coordinated events to circumvent the immunity of the host (Deorukhkar, 2017, 2018).

The response of the immune system to *Candida* colonization and infection occurs in many ways initiate with a non-specific (innate immune) response which depends on an acute inflammatory response by various cells like neutrophils or soluble factors like cytokines or complement proteins, followed by a specific (adaptive immune) response which includes the development of specific T cell (cell-mediated immunity) and B cell (humoral immunity) (Deorukhkar & Roushani, 2017; Netea & Maródi, 2010)

Innate immunity by polymorphonuclear leucocytes (PMNL) and macrophages play an important role in the prevention against invasive *Candida* infections, while the dominance of cell-mediated immunity (CMI) is prominent in the defense against mucosal infections. The function of antibody-mediated immunity (AMI), or humoral immunity, remains highly contentious in candidiasis (Deorukhkar, 2017, 2018; P. L. Fidel, 2002).

Cell-mediated immunity (CMI) shows an important role of prevention in immunocompromised patients (HIV or AIDS), individuals on corticosteroid therapy, and transplant recipients who normally have the development of mucosal candidiasis (P. L. Fidel, 2002; Ha *et al.*, 2011).

In humoral immunity, while specific groups of antibodies such as IgG, IgM, or IgA are developed in all clinical forms of candidiasis, with exception of patients who are severely immunocompromised, the protective function of antibody-mediated or humoral immunity is largely unknown (Deorukhkar & Roushani, 2017).

2.6 Epidemiology

Currently, candidiasis is one of the most common mycotic infection, it has the highest effectiveness regarding its intensity and frequency as a complexity infection (López-Martínez, 2010). *Candida* species can cause infection in both immunocompromised patients and immunocompetent individuals. Since it has more severity in immunocompromised patients, candidiasis known as “disease of diseased” (Deorukhkar & Saini, 2014).

Candida species live commensally in various body parts as a normal flora that generally can be isolated from the vagina, skin, throat, and the gastrointestinal tract. *Candida* species are commonly isolated from the oral cavity in both healthy individuals and immunocompromised patients. Colonization of *Candida* species in the newborns' oral cavity is due to many sources such as the maternal birth canal, breastfeeding, pacifiers, and nurses' hands respectively. Oral thrush is commonly prevalent in 5% of newborns and it is noticeable 5 to 8 days after birth (Deorukhkar, 2018; Mooney *et al.*, 1995).

Superficial and mucocutaneous candidiasis may occur in immunocompetent individuals as its highly common infection in humans. Thrush, onychomycosis, cutaneous candidiasis, chronic mucocutaneous candidiasis, and vulvovaginal candidiasis (VVC) are considered as common mucosal infections of superficial and mucocutaneous candidiasis (López-Martínez, 2010).

In healthy individuals, these infections are generally self-limiting and easy to eliminate by local treatment and personal hygiene (Baradkar & Karyakarte, 1999; Deorukhkar, 2018). While in immunocompromised people, particular consideration should be given to mucocutaneous candidiasis, since such infections may become a pathway to systemic spread. Superficial candidiasis is distinctly chronic and recurring, which often signals the beginning of serious types of this fungal infection (López-Martínez, 2010).

Vulvovaginal candidiasis (VVC) is one of the prevalent clinical disorders in 75% of women who had at least one episode of VVC or receive gynecological care. It is also much correlated with obesity, pregnancy, the presence of the intrauterine device (IUD), and the excessive administration of oral contraceptives. Estrogen hormone during pregnancy promotes the invasion and colonization of *Candida* by increasing the glycogen content in vaginal epithelial cells. In diabetic patients, the tissue's high content of glucose increases the colonization of *Candida* as well (Jack D. Sobel, 2014).

Disseminated candidiasis (DC) refers to cases where, in non-adjacent, usually sterile body sites, *Candida* invasion could be seen from cultures or histology results (Eggimann *et al.*, 2003). It is regarded as a destructive disease correlated with elevated morbidity and mortality levels in both immunocompetent individuals and immunosuppressed patients (Lockhart, 2014). it is typically correlated with host defense insufficiencies generally seen in cancer patients undergoing chemotherapy and broad-spectrum antibiotics in the hospital, and ICU (Miceli *et al.*, 2011). It is responsible for 17% of nosocomial infections within European countries (Eggimann *et al.*, 2003).

The infection due to *Candida* species is considered as the second most common cause of catheter-associated urinary tract infections (UTIs) in the USA, while it is the third most common cause of bloodstream infections in the ICU and central-line associated bloodstream infections (CLABSI), and the fourth most common cause of nosocomial bloodstream or health-care-associated infections (Lockhart, 2014; Méan *et al.*, 2008). *Candida* species are responsible for 10% of all infections in the bloodstream and 25% of all urinary tract infections (UTIs) in ICU (Deorukhkar, 2018; Michael A. Pfaller, 1996).

C. albicans and *non-albicans Candida* (NAC) species like *C. glabrata*, *C. tropicalis*, *C. parapsilosis* are responsible together for 95% of clearly discernible *Candida* infections (Deorukhkar, 2018; M. A. Pfaller & Diekema, 2007).

Within *non-albicans Candida* (NAC) species, *C. tropicalis* is the most commonly isolated species from cancer and ICU patients (Deorukhkar & Roushani, 2017). In comparison with *C. albicans* and other *non-albicans Candida* (NAC) species, it has a higher capability of dissemination in neutropenic patients (Silva *et al.*, 2012).

C. glabrata has emerged as an important pathogen, although it was considered as a nonpathogenic saprophyte. It is the second most common species responsible for bloodstream infections in the USA. *C. glabrata* is highly responsible for fungemia in adults more than in neonates and children (Deorukhkar *et al.*, 2014). It occupies the second or third most common associated with different types of candidiasis with the

highest mortality rates as compared with other *non-albicans Candida* (NAC) species (Silva *et al.*, 2012).

C. parapsilosis is occupied as the second most commonly isolated species from sterile body sites of hospitalized patients as compared with other *Candida* species (Silva *et al.*, 2012). It is predominant in several neonatal intensive care units (NICUs) and sometimes correlated with neonatal mortality as compared with other isolated *Candida* species. *C. parapsilosis* is typically spread horizontally by infected external outlets, including as implanted medical devices like catheters or medical fluids, and health-care workers' hands (Deorukhkar, 2018; Trofa *et al.*, 2008).

C. krusei described as a facultative saprophyte (Silva *et al.*, 2012). However, it has appeared more recently as a prominent pathogen with a number of clinical infections usually common in hospitalized patients like fungemia, endocarditis, arthritis, and endophthalmitis (Deorukhkar, 2018; Samaranayake Y & Samaranayake L, 1994).

2.7 The Clinical Diseases Caused by Genus *Candida*

Infections due to *Candida* species have hugely diverse clinical manifestations; tend to range from subacute to acute, and episodic to chronic. The implication may occur in the skin, scalp, nails, fingers, mouth, gastrointestinal tract, throat, bronchi, lungs, and vagina. these infections tend to be more severe systemic as septicemia, endocarditis, and meningitis (Segal & Elad, 2010). Table 2.2 shows the clinical manifestations due to different *Candida* species infections (Deorukhkar, 2018).

Table 2.2: The clinical manifestations of medically important *Candida* species.

***Candida* species**	Clinical Manifestations
C. albicans	Broad-spectrum of clinical symptoms varying from mucocutaneous development to disseminated infections.
C. glabrata	Candidemia, candiduria, disseminated candidiasis.
C. tropicalis	Clinical symptoms varying from candidemia and other disseminated infections in immunocompromised patients.
C. krusei	Candidemia, endophthalmitis, diarrhea in neonates.
C. parapsilosis	Candidemia, medical-device associated infections, infections related to contaminated fluids.
C. kefyr	Disseminated candidiasis.
C. lipolytica	Intravenous catheter-associated candidemia.
C. famata	Candidemia.
C. lusitaniae	Candidemia and other disseminated infections.
C. auris	Broad-spectrum of clinical symptoms in ICU patients.
C. dubliniensis	Oropharyngeal candidiasis in HIV/AIDS patients.

The clinical diseases due to *Candida* species ranging from superficial infections involving skin, scalp, nails, fingers, and mucosal surfaces of different body cavities (superficial candidiasis) to more severe forms of infections involving numerous internal organs such as liver, spleen, heart, kidneys, and brain (systemic candidiasis) to disseminated diseases in addition to allergic diseases. In general, these clinical entities also are known candidiasis can be divided into two large groups superficial candidiasis and deep-seated candidiasis (Segal & Elad, 2010).

Mucocutaneous candidiasis is a special form of superficial infections due to *Candida* species. It includes oropharyngeal candidiasis (OPC), vulvovaginal candidiasis (VVC), balanoposthitis, chronic mucocutaneous candidiasis (CMC), and

gastrointestinal candidiasis (Achkar & Fries, 2010; Akpan & Morgan, 2002; Deorukhkar, 2017, 2018; López-Martínez, 2010; Mane *et al.*, 2010; Segal & Elad, 2010).

Cutaneous candidiasis is another form of superficial candidiasis includes paronychia and onychomycosis candidiasis, diaper dermatitis, and *Candida* granuloma (Jain & Sehgal, 2000; López-Martínez, 2010; Mayser & Gräser, 2011; Segal & Elad, 2010).

Systemic candidiasis is a result of bloodstream infection (candidemia). In systemic candidiasis or deep-seated candidiasis, *Candida* species can reach to any body parts. Therefore several organs may be involved and lead to various infections forms include Candiduria, disseminated candidiasis, candidemia, gastrointestinal candidiasis, candidiasis of the respiratory system, candidiasis of the cardiovascular system, renal and urinary tract candidiasis, central nervous system candidiasis (meningitis), and nosocomial candidiasis (Deorukhkar & Saini, 2016; Fisher, 2011; Henao & Vagner, 2011; Kauffman *et al.*, 2011; Ostrosky-Zeichner *et al.*, 2003; Segal & Elad, 2010). Figure 2.2 shows a schematic diagram of the clinical diseases due to *Candida* species infections (Dabas, 2013; Segal & Elad, 2010).

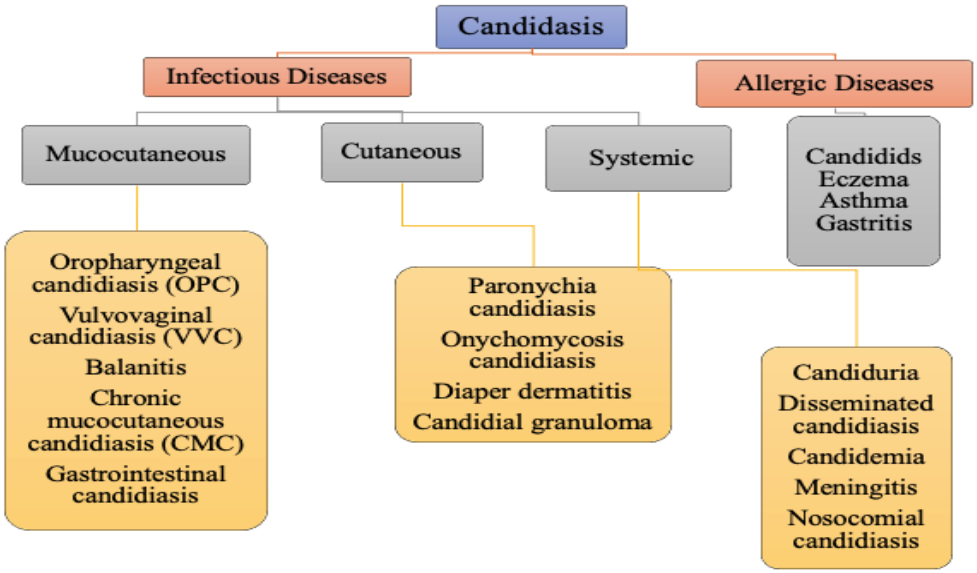

Figure 2.2: Clinical diseases due to *Candida* species infections.

2.8 The Laboratory Diagnostic Techniques of *Candida* Species

Diagnosing and identification *Candida* species are deemed a major obstacle for microbiologists and clinicians alike. Therefore, providing a swift and correct diagnosis of *Candida* species is a major and important requirement to determine the appropriate treatment for *Candida* infection (Deorukhkar & Saini, 2014; Posch *et al.*, 2017; Sturaro *et al.*, 2018).

For effective isolation and accurate identification of the *Candida* species, appropriate specimens should be collected based on infection site, the specimen must be transferred to the laboratory within a period not exceeding 2 hours from collection, Correct and prompt processing of the specimen, and specimen inoculation on proper culture media and incubation at an appropriate temperature (Shivaprakash, 2015). Table 2.3 shows the clinical specimen and the collection Instructions that should be followed (Shivaprakash, 2015).

Table 2.3: The clinical specimens and the methods of collection.

Specimen	Collection Instructions
CSF	o Collection must be done by a physician under aseptic conditions. o Using extreme care in the collection of fluid. Do not contaminate, as any isolate from CSF is considered significant. o Submitting a sufficient volume for tests requested. Do not overfill tubes, as it creates an aerosol upon opening. o 2mL is a suggested volume for fungal culture.
Urine	o The specimen should be taken in the early morning from midstream of urination with an average volume of 5-10mL. o In the case of a urine catheter bag, the specimen must be obtained using aseptic technique and collection must be in a sterile syringe and immediately transfer into a sterile specimen container.

	o Preserved specimens: Store refrigerated (4ºC) or at room temperature. o Preserved specimens, MUST be stored refrigerated (4ºC) Transport to the lab within 12 hours
Blood	o Yellow vacutainer blood tube. o 5 to 10 mL in media containing brain heart infusion broth with 1:10 blood: broth ratio.
BALF	o The specimen should be collected by using fiber optic bronchoscopy and transferred immediately into a sterile container.
Sputum	o The specimen should be taken in the early morning from before having breakfast and after using mouth rinse and brush. o 5-10mL in a sterile container.
pus	o The specimen must be obtained using aseptic technique and collection must be in a sterile syringe from an undrained abscess and immediately transfer into a sterile specimen container syringe and needle. o Pus collected from abscess opened with a scalpel blade.
Stool	o Stool specimens are not processed for fungal culture.

For fungal culture or examination, swabs are not recommended for specimens from other body locations such as the mouth, vagina, cervix, nasopharynx, and ear canal where it is not practicable to extract them through certain means; swabs are collected and approved for laboratory diagnosis. All specimens submitted to the mycology laboratory must be specifically labeled with the name of the patient, sex, age, sample reference number, sample type, department name, date and time of collection, antimicrobial therapy along with a brief related clinical situation and the name of the physician in attendance (Shivaprakash, 2015).

In the case of superficial and mucocutaneous candidiasis laboratory diagnosis is comparatively straightforward and requires predominantly clear microscopic examination of fungal elements (budding yeasts, pseudohyphae, or true hyphae) in

clinical specimens accompanied by species isolation and identification (Deorukhkar & Saini, 2014).

2.8.1 Direct Microscopic Examination

The direct microscopic examination is an appropriate diagnostic technique for liquid and mucoid clinical specimens. It is easy to be done by either 10% of potassium hydroxide (KOH) wet mount preparation or using various staining techniques. The direct microscopic examination is primarily dependent on the type of clinical specimens (Shivaprakash, 2015).

10% (KOH) wet mount of preparation is useful for the demonstration of yeast cells and other fungal elements. In the case of the specimens were hard or slimy like skin, nails scraping, and mucosal secretions, specimens should be cleaned by (KOH) before slide preparation because it increases the demonstration of fungal elements by removing protein residues in the specimens. Wet mount of preparation done by adding a drop of a sterile saline solution (0,85% NaCl) on a clean slide, put the specimen on it and mix it well then covered with slide coverslips. Under the microscope, explore them at different magnifications, at high and low brightness (Deorukhkar & Saini, 2014).

For stained smears, the yeasts and pseudohyphae are best illustrated for solid specimens such as biopsies or macerated tissue. Various staining methods that can be employed involves gram stain, Periodic Acid–Schiff (PAS) stain, and Grocott's Methenamine Silver (GMS) stain (Deorukhkar, 2018).

Gram stain is particularly effective for examining sputum, lung aspirates, gastric washing fluid, pus, urine, and vaginal secretions for demonstrating the details of budding cells and mycelia elements (Shivaprakash, 2015). Yeasts are oval, small (4-6µm), thin-walled, and Gram-positive cells. It can be clearly distinguished in Gram-stained smears due to their large size (Deorukhkar & Saini, 2014; Segal & Elad, 2010).

Periodic Acid–Schiff (PAS) is a useful staining technique for macerated tissue or biopsies where yeast cells and the pseudohyphae appear with a red-purple color (Byadarahally Raju & Rajappa, 2011). In Grocott's Methenamine Silver (GMS) staining technique, yeast cells appear brown-darkish colored (Ma *et al.,* 2013).

The accuracy and the immediately correct diagnosis of candidiasis are playing an important role for suitable treatment (Teles & Seixas, 2015). Wherefore, Abbott in 1995 concluded that the gram staining technique is the most accurate method for VVC diagnosis. whereas, (KOH) wet mount of preparation tends to be mildly accurate due to a high percentage of false-positive and false-negative results (Abbott, 1995; Deorukhkar, 2018).

2.8.2 Isolation of Candida Species

Culture

Sabouraud Dextrose Agar (SDA) is not represented as a selective and differential medium (Deorukhkar, 2018; Samaranayake *et al.*, 1987). SDA is the most commonly used medium for primary isolation of *Candida* species. As a non-fastidious organism, the *Candida* species grows readily on most laboratory media used to isolate the fungus. It induces *Candida* to grow and suppresses the growth of several but not all of the bacteria because of its low pH (Deorukhkar & Saini, 2014; F. C. Odds, 1991).

SDA becomes a selective medium when it supplemented with antibiotics such as tetracycline and gentamicin to prevent any bacterial growth and cycloheximide to eliminate contamination of the saprotrophic fungi (Deorukhkar, 2018; F. C. Odds, 1991). Since there are some *Candida* species such as *C. krusei*, *C. tropicalis*, and *C. parapsilosis* considered as saprotrophic fungus, they tend to be sensitive to cycloheximide. Therefore SDA free of antibiotics or supplemented with antibiotics is being used to tackle this issue (Deorukhkar & Saini, 2014; López-Martínez, 2010).

The inoculated SDA medium could be incubated at room temperature and/or 37ºC with an average of 24-48 hours. the colonies of *Candida* species appear creamy, smooth, pasty, and convex and on prolonged incubation become wrinkled (Deorukhkar & Saini, 2014). While some *Candida* species like *C. glabrata* may need up to 72 hours (Segal & Elad, 2010).

Blood cultures are associated with several significant disadvantages, though considered the current "gold standard" technique for the diagnosis of invasive candidiasis. The technique that is routinely used to cultivate blood is relatively insensitive and can take several days to be positive (Deorukhkar, 2018; Reiss & Morrison, 1993). In addition to approximately 50% of patients with documented invasive candidiasis, blood cultures are negative (Perfect, 2013).

In 1995. Sullivan *et al.* proposed that Potato Dextrose Agar (PDA) is the culture medium of choice for *Candida* species isolated from various clinical samples. it is especially helpful for the differentiation from the same clinical specimen between colonies of different yeast species (Deorukhkar, 2018; D. J. Sullivan *et al.*, 1996).

2.8.3 Identification of *Candida* Species

There are a variety of techniques that can be utilized for identifying *Candida* species from various clinical samples. These include conventional phenotypic methods, such as germ tube and chlamydospores formation, the ability to ferment carbohydrates, and the assimilation of nitrogen and carbon; rapid commercial systems, such as API 20C Aux system, API yeast identification system, and Vitek 2 ID-YST system; and recently molecular techniques such as Multiplex-PCR, and Real-time PCR (Neppelenbroek *et al.*, 2014; SOUZA *et al.*, 2015). The comparison between a variety of techniques that can be utilized for identifying *Candida* species from various clinical samples is shown represented in table 2.4 (Neppelenbroek *et al.*, 2014).

Table 2.4: The comparison between a variety of techniques for identifying *Candida* species.

Identification Techniques	Time required (in hr.)	Cost	Accuracy
conventional methods			
○ Germ tube formation.	2	Low	High
○ Chlamydospore formation.	24-48	Low	Low
○ Carbohydrate fermentation	24-48	Low	Low
○ Carbohydrate assimilation.	24-48	Low	Low
Commercial systems			
○ Vitek 2 ID-YST system.	15	High	High
○ Vitek YBC system.	22-24	Moderate	Moderate
○ API 20C Aux system.	24-72	High	High
○ API *Candida* system.	18-24	High	High
○ API yeast identification system.	24	High	Low
○ ID 32C system.	24-72	Low	High
Chromogenic media-based commercial systems			
○ HiCrome™ *Candida* system.	24-48	Low	Moderate
○ *Candida* ID system.	48	Low	Moderate
○ Auxacolor system.	24-72	Moderate	High
Molecular biology-based techniques			
○ Multiplex-PCR	<2	High	High
○ Nested PCR	4-6	High	High
○ Real-time PCR	4-5	High	High
Non-invasive diagnostic methods			
○ (1,3)-β-d-glucan	1	High	Moderate
○ Galactomannan	8-24	High	Moderate

Conventional Phenotypic Methods.

Germ tube formation.

Germ tube formation or the Reynolds-Braude Phenomenon, as a distinct feature; is the basic method to distinguish between *Candida* species. Since it described in 1960 by Taschdjian (SOUZA *et al.*, 2015)., germ tube becomes the easiest and quick method to discriminate *C. albicans* and *C. dubliniensis* from other *Candida* species. In *C. albicans* and *C. dubliniensis*, the germ tube test shows the ability of these two species to produce short, slender, tube-like structures within 2 hours when they inoculated in human serum at 37ºC (Deorukhkar *et al.*, 2012; Deorukhkar & Saini, 2014).

Various factors can influence the germ tube formation such as the presence of other *Candida* species, bacterial contamination, nature of the medium, inoculum concentration, and incubation temperature (Neppelenbroek *et al.*, 2014). A new specific monoclonal antibody for *C. albicans* germ tube was detected in 2000 by Marot-Lebland *et al.* helps in differentiation between *C. albicans* and *C. dubliniensis* (Deorukhkar, 2018; Marot-Leblond *et al.*, 2000).

Chlamydospore formation.

chlamydospores formation consider as a feature of *C. albicans*, *C. dubliniensis*, and some strains of *C. tropicalis*. this feature is restrictive and requires prolonged (up to several days) incubation in comparison with the germ tube test (Deorukhkar, 2018; Pincus *et al.*, 2007).

C. dubliniensis produces chlamydospores as clusters and sometimes as doublets or triplets. Whereas in *C. albicans*, chlamydospores look single at the ends of true hyphae or on pseudohyphae (Neppelenbroek *et al.*, 2014).

Chromogenic media-based commercial systems.

The identification of *Candida* isolates is used in different chromogenic media. These media have important advantages, such as fast identification, increased isolation efficiency, and separation of mixed infection in clinical samples. Some of these media

may be inoculated directly with clinical specimens while others may require pathogen isolation before inoculation. Compared with conventional media such as SDA, chromogenic media often more expensive (Deorukhkar, 2018).

HiCrome™ *Candida* system.

HiCrome™ *Candida* Differential Agar (Himedia, Mumbai, India) is a selective and differential medium to identification and isolation of *Candida* species according to the variety of colonies' growth colors as a result of the break down chromogenic substrates by the activity of species-specific enzymes (Mehta & Wyawahare, 2016).

As per the manufacturer's instructions, after the inoculation of *Candida* species on HiCrome™ agar for 24-48 hours at 30-37°C in aerobic condition *Candida* species will grow as follows; the *C. albicans* grows as light green to green color and smooth colonies, *C. tropicalis* grows as metallic blue to blue color and raised colonies, *C. krusei* grows as purple color and fuzzy colonies, and *C. glabrata* grows as cream and white color colonies (Patil *et al.*, 2012).

Whereas, in comparison with *C. albicans*, *C. dubliniensis* produces dark green colored colonies. However, due to phenotypic switching, this feature tends to be lost after repeated subcultures and on storage at -70ºC (Neppelenbroek *et al.*, 2014).

In 2005, Sahand *et al.* proposed a new technique to distinguish between *C. albicans* and *C. dubliniensis*. This technique uses Chromogenic *Candida* agar supplemented with Pal's agar. As *C. albicans* grows in light green and smooth colonies, *C. dubliniensis* grows in a bluish-green color and rough colonies (Deorukhkar, 2018; Sahand *et al.*, 2005). In 2003, Horvath *et al.* proposed the CHROMagar medium may be used to identified *C. glabrata* with high accuracy (Deorukhkar, 2018; Horvath *et al.*, 2003). It grows as a dark violet-colored colonies (Deorukhkar, 2018; Hospenthal *et al.*, 2006).

2.9 Antifungal Susceptibility Testing of *Candida* Species

Lately, antifungal susceptibility testing (AFST) has been established to become standardized and is now accessible either from the Clinical and Laboratory Standards Institute (CLSI) (formerly known as the National Committee for Clinical Laboratory Standards (NCCLS)) (M27-A3, M27-S3, M44-A, and M44-S2) or from the European Antimicrobial Susceptibility Research Committee (EUCAST). CLSI and EUCAST have individually developed standard protocols, minimum inhibitory concentration (MIC) breakpoints, interpretive zone-diameter breakpoints, and disc diffusion criteria of AFST (Sanguinetti & Posteraro, 2018).

In 1985, the CLSI established an Antifungal Susceptibility Testing Subcommittee. M27A is the first document that was published in 1997 as a "Reference Method of the Antifungal Susceptibility Test for Broth Dilution: Approved Standard". This document categorized reference strains for some antifungals with ranges of Minimal Inhibitory Concentrations (MIC) and Break Points (BPs) and their activity against both *Candida* and *Cryptococcus*. EUCAST is a joint standing committee of the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), the European Center for Disease Control (ECDC), and the European National Breakpoint Committees (www.eucast.org). In 1997, the EUCAST subcommittee formed an antifungal susceptibility testing (AFST-EUCAST). Susceptibility test standard for yeasts (including *cryptococcus*) was published in 2008. In 2012, this standard was updated. In 2008, a further standard for molds was released. In certain matters, such as medium composition (glucose concentration), incubation time, and inoculum concentration, the methodological discrepancies between EUCAST and CLSI are mostly established (Alastruey-Izquierdo *et al.*, 2015).

Agar based methods are suitable, cost-effective, and easy to perform in the presence of large numbers of isolates in comparison with microdilution broth methods. in addition to its similarity to the procedures of the antibacterial susceptibility test that used routinely in the microbiology laboratory (Deorukhkar, 2018; Rex *et al.*, 1993). The disc diffusion method provides both a qualitative category of interpretation (susceptible (S), susceptible dose dependent (SDD), or resistant (R)) and quantitative

results (inhibition zone). An approved Guideline (M44-A) of CLSI offers the inhibition zone, the value of which can be compared with the MIC value that is given in the broth dilution methods. It is an appropriate method for antifungal susceptibility testing of water-soluble agents such as flucytosine, fluconazole, and voriconazole; and for the determination of the activity of echinocandins against yeast isolates that produce easy-to-read, sharp inhibition zones. This method provides enhanced resistance detection of Amphotericin B (Deorukhkar, 2018; Sheehan *et al.*, 2004).

Selection a suitable growth medium to employee for antifungal susceptibility testing of *Candida* seems to be a critical challenge with disc diffusion method, therefore, CLSI advises the use of (Mueller–Hinton agar) supplemented with 2% glucose and 0,5 μg/mL methylene blue dye (MH-GMB) (Espinel-Ingroff & Cantón, 2007; Sheehan *et al.*, 2004).

2.10 Antifungal Drugs: Mechanism of Action and Drug Resistance

Clinically, three classes of antifungal drugs can be used to treat most Candidiasis infections: polyenes such as Amphotericin B and its two forms of liposomal and lipid formulations, echinocandins such as caspofungin and micafungin, and triazoles such as itraconazole, voriconazole, and fluconazole (Fothergill, 2012).

Most types of drugs commonly in use against fungal infections achievement specifically novel targets for fungi. For example, ergosterol; which is a typical fungal cell sterol, is targeted by most of the antifungal agents. The sterol component of fungal cell membranes is either targeted by blocking the enzymes that are important for their synthesis or by directly depleting ergosterol from the plasma membrane (PM). Whereas, many other antifungal agents that currently in use, are directed against singular components of the fungal cell wall (CW) such as mannan, glucan, and chitin (Prasad *et al.*, 2016).

The resistance of *Candida* species for antifungal drugs is occurring through multiple mechanisms such as efflux of the drug by multi-drug transporters (efflux pumps), changes in concentration of cell components, and via mutations that cause some changes in the target enzymes (Vazquez *et al.*, 2013). Table 2.5 shows

mechanisms of action and multiple resistance mechanisms of antifungal agents (Prasad *et al.*, 2016; Srinivasan *et al.*, 2014).

In 1994, Law *et al.* described the isolation of fluconazole-resistant *C. albicans* from the oral cavities of patients infected with HIV (Berkow & Lockhart, 2017). Revankar *et al.* (1996), Laguna *et al.* (1997), and Maenza *et al.* (1997); reported the isolation of high fluconazole-resistant *C. albicans* from OPC patients infected with HIV (Canuto & Rodero, 2002). In 1995, Goff *et al.* described the isolation of 17 fluconazole-resistant *C. albicans* out of 139 isolated from HIV non-infected patients. In 1996, Sobel *et al.* described the first case due to an azole-resistant *C. albicans* infection from recurrent vulvovaginal candidiasis (RVVC) in HIV non-infected woman (Deorukhkar, 2018; Repentigny *et al.*, 2004).

In 1988, Powderly *et al.* described Amphotericin B-resistance in 30% of *C. tropicalis* strains isolated from blood cultures. It turns out that in *C. tropicalis* strains resistance is gained while receiving Amphotericin B therapy (Deorukhkar, 2018; Powderly *et al.*, 1988).

Three different studies of Hernandez *et al.* (2004), Hakki *et al.* (2006) and Laverdière *et al.* (2006), respectively; concluded that the responsibility of *C. albicans* and *Non-albicans Candida* (NAC) species resistance to echinocandins is till now yet to be clearly understood (Hakki *et al.*, 2006; Hernandez *et al.*, 2004; Laverdière *et al.*, 2006)

Table 2.5: Antifungal agents: Mechanisms of action and resistance mechanisms.

Antifungals Drug Class	Main Activity	Mechanisms of Action	Resistance Mechanisms
Polyenes Amphotericin B Nystatin	Fungicidal	Change membrane function by binding of ergosterol of the target cells leading to pores formation.	Modification of the gene responsible for encoding ergosterol biosynthetic pathway enzymes or glucan synthesis. Significant decrease in the concentration of ergosterol (ablating binding of drug targets).
Azoles Fluconazole Itraconazole Ketoconazole Posaconazole Voriconazole Miconazole	Fungistatic	Blocking the key enzyme, lanosterol 14a-demethylase (*Erg11p*), results in affects ergosterol biosynthesis pathway.	Overexpression or mutations of *Erg11p* gene, resulting in reducing the drug effects. Reducing efflux of the drug via ABC transporters, decreasing drug uptaking, and accumulation.
Echinocandins Caspofungin Micafungin Anidulafungin	Fungistatic or fungicidal	Binding to β(1,3)-D-glucan synthase *Fks1p* or *Fks2p*, leading to blocking the synthesis of the cell wall component (ß-(1,3)-D-glucan)	Mutation in the *FKS1* and *FKS2* genes (binding units).

2.11 Treatment of *Candida* Species Infections

As the antifungal drugs have variety in their cost, safety, indications, side effects, pharmacokinetics (PK)/pharmacodynamics (PD) domains, action mechanism, and administration mode; many factors can impact candidiasis outcomes such as the severity of the infection, species that caused the infection, type of the antifungal drug used in the therapy, and the treatment plan (Michael A. Pfaller, 2012).

Thus, the choice of an antifungal agent for an individual should be suited to the clinical conditions, the history of antifungal drug usage, *Candida* species susceptibility patterns, and the specific objectives of the therapy plan (Deorukhkar & Saini, 2015; López-Martínez, 2010).

In 2008, Rüping *et al.* concluded that Lofty goals of the antifungal therapy revolve around prophylaxis, initial therapy whether empiric, preemptive or targeted or salvage therapy for those immunocompromised patients or who are at high-risk (critically ill patients). The implementation of urgent diagnosis and early treatment of these patients has a significant effect on mortality rates but accurate diagnostic tests are missing. This problem has led to different treatment strategies being employed in parallel (Rüping *et al.*, 2008).

Studies on prophylactic antifungal therapy are sorely missing. However, in 1987, Slotman and Burchard and in 1999, Savino *et al.* confirmed that no effect or only moderate of prophylaxis therapy on the occurrence of infections due to *Candida* species in ICU patients (Méan *et al.*, 2008). In complete contrast, in 1999, Eggiman *et al.* confirmed the intra-abdominal candidiasis elimination through prophylaxis therapy with fluconazole (Deorukhkar, 2018; Eggimann *et al.*, 1999). Later, in 2001 Pelz *et al.* and in 2002, Garbino *et al.* suggested that the initiation of prophylactic therapy may benefit critically ill patients (Méan *et al.*, 2008).

Preemptive administration of IV fluconazole therapy has been shown in the study of Piarroux *et al.* (2004) to prevent the development of proven candidiasis in surgical

ICU patients compared with a historical control group of patients (Piarroux *et al.*, 2004). This modern and developing antifungal treatment strategy has been proven to reduce candidemia amplitude but has no effect on the mortality rate (Tsuruta *et al.*, 2007).

The polyene antifungal drugs; nystatin is isolated from *Streptomyces noursei* species. It was discovered in 1950 by Brown and Hazen. The clinical use of nystatin is limited as a topical antifungal therapeutic option for both OPC and VVC. In 1955, Gold *et al.* discovered Amphotericin B isolated from *Streptomyces noursei*. It is considered as the only antifungal polyene systemically administrable for the treatment of disseminated *Candida* infections (Denning & Hope, 2010). Amphotericin B deoxycholate was used as a typical therapeutic option for systemic mycoses for several decades. Unfortunately, this antifungal agent also poorly tolerated and correlated with side effects such as nephrotoxicity and disruptions of electrolytes (Méan *et al.*, 2008).

A range of reformulated versions of Amphotericin B have been developed to overcome toxicity associated with this agent. The best example of the reformulated versions is the lipid formulation of Amphotericin B. Liposomal formulation (L-AmB; Ambisome), Amphotericin B lipid complex (ABLC), and Amphotericin B colloidal dispersion (ABCD-Amphocil) are currently available usable (Frank C. Odds *et al.*, 2003).

There are two subclasses of the main azole class , the imidazoles (ketoconazole, miconazole and clotrimazole) and the triazoles (fluconazole, itraconazole, voriconazole and posaconazole) (Andriole, 2000; Deorukhkar, 2018).

Ketoconazole was the first bioavailable imidazole for oral administration. It was synthesized in 1976, marketed in 1981 by Janssen Pharmaceutica, it is approved by the Food and Drug Administration (FDA) to use for the treatment of systemic mycoses in 1981 (Maertens, 2004; Terrell, 1999). In certain cases of fluconazole-resistant OPC in AIDS patients, Laguna *et al.* in1997 reported that ketoconazole dosages of 400 to 800 mg/kg as effective (Laguna *et al.*, 1997).

Miconazole was synthesized in 1967 and marketed in 1971 by Janssen Pharmaceutica. It was the first azole available for parenteral administration but its use has been limited due to toxicity associated with IV administration. It has proved to be an effective topical antifungal drug. Miconazole has suboptimal effectiveness and is also responsible for significant local and systemic toxicity. So nowadays it is rarely used (Maertens, 2004).

Fluconazole is a triazole of the first-generation, and one of the world's most given antibiotics (Proia, 2006). It was discovered in 1981 and is active in oral and parenteral administration (Andriole, 2000; Deorukhkar, 2018). Relative to other azoles, the bioavailability of this antifungal agent is very high. This drug has relatively higher water solubility and weak plasma protein sensitivity. Fluconazole is widely used for prophylaxis and treatment DC and superficial candidiasis (Kathiravan *et al.*, 2012). In 1994, Rex *et al.* confirmed that fluconazole to be as successful in the treatment of candidemia in neutropenic patients as and tolerated better than Amphotericin B deoxycholate (Deorukhkar, 2018; John H. Rex *et al.*, 1994). In several recent research, the prophylaxis of fluconazole has provided a significant contribution towards *Candida albicans* and *non-albicans Candida* (NAC) species such as *C. albicans* and *C. dubliniensis* that are innately susceptible to fluconazole but it may also develop secondary resistance (Krcmery & Barnes, 2002).

In 1986, Janssen Pharmaceutica developed itraconazole as an orally active triazole derivative with wide-spectrum antifungal action (Andriole, 2000; Deorukhkar, 2018; Terrell, 1999). It has a significant effect against *C. albicans* and can be used for the treatment of OPC and VVC induced by fluconazole-resistant strains, given that there is no laboratory proof of cross-resistance (Denning & Hope, 2010). Itraconazole has enhanced activity against *C. glabrata* although it lacks reliable activity against *C. krusei* like fluconazole (Ha *et al.*, 2011).

Voriconazole and fluconazole are structurally alike (Canuto & Rodero, 2002; Deorukhkar & Saini, 2015). Pfizer Pharmaceutical has developed Voriconazole, the 2nd generation synthetic triazole, as part of a program designed to enhance the potency and spectrum of fluconazole activity (Maertens, 2004). Voriconazole has low molecular weight and is authorized for first-line treatment of OPC. Voriconazole has a significant effect against all *Candida* species including fluconazole-resistant or less susceptible species such as *C. glabrata* and *C. krusei* (Kathiravan *et al.*, 2012).

Echinocandins originally derived from fermentation broths of various fungi includes *Aspergillus rugulovalvus*, *Aspergillus aculeatus*, *Zalerion arboricola*, and *Papularia sphaerosperma* (Denning, 2002). Echinocandins are a recent contribution toward species *Candida* to the antifungal armamentarium (Kathiravan *et al.*, 2012). All formulations of echinocandin are available IV administration only (Denning, 2003).

Echinocandins have fungicidal or fungistatic action (both in vitro and in vivo) against most *Candida* species irrespective of their resistance or susceptibility to azole or Amphotericin B (Denning, 2003; Giri & Kindo, 2012). The use of echinocandins has greatly increased in many hospitals in recent years. Echinocandins (especially caspofungin) are the drug of choice for the treatment of disseminated candidiasis In critically ill patients who are at risk, clinically dysfunctional, or newly infected or colonized with a *Candida* species believed to have decreased azole susceptibility (Giri & Kindo, 2012).

The first echinocandin to be approved for the treatment of DC was caspofungin. It is used effectively for OPC treatment as well. Caspofungin has a significant effect and slightly less toxic than Amphotericin B, therefore, it's recommended as the first line of treatment for candidemia and DC (Ha *et al.*, 2011; Méan *et al.*, 2008). In 2002, Villanueva *et al.* observed that caspofungin was as successful for treating OPC in HIV-infected patients as Amphotericin B deoxycholate or fluconazole (Villanueva *et al.*,

2002). During the same year, Kuhn *et al.* investigated the impact of caspofungin on the formation of biofilms of *Candida* species. Treatment with caspofungin led to phenotypic modification and destruction of biofilm (Kuhn *et al.*, 2002).

3. MATERIALS AND METHODS

3.1 Design of Study

The study was conducted in the MICROBIOLOGY LABORATORY at the NEAR EAST UNIVERSITY HOSPITAL in the Turkish Republic of Northern Cyprus (TRNC). A total of 147 samples for the study was executed for two intervals (February 2014-February 2016) and (June 2019- December 2019) from different clinical specimens of hospitalized patients from various hospital departments.

The study included *Candida* species isolated from different clinical specimens and the repeated isolates from the same clinical specimen of the same patient were excluded. The patient's demographic features (age, sex, sample date, sample type, department, isolated *Candida* species, Antifungal susceptibility criteria "R", "SDD", "S") were recorded and analyzed.

3.2 Specimens Collection

During the study intervals, a total of 147 clinical specimens were received in the microbiology laboratory. These specimens were categorized by gender, age groups (child, young adults, old adults), sample type (blood, urine, sputum, etc.), and departments where patients were hospitalized (cardiology, anesthesia, chest diseases, and allergy, etc.).

The suspected colonies of *Candida* isolates were identified as yeast cells by saline wet mount using a drop of normal saline was placed in the center of a clean glass slide. A small inoculum of the colony was placed into normal saline and mixed well. Coverslip was mounted to the preparation and examined under the high power objective (40X) of light microscope for budding yeast cells without capsule, as shown in Figure 3.1. Gram stain smear of the suspected colonies of *Candida* was prepared on a clean glass slide. The stained smear was observed under oil immersion (100X) of a

light microscope. *Candida* species appear as Gram-positive budding yeast cells (4-8µm) with or without pseudohyphae, as shown in Figure 3.2.

Candida isolated from all specimens that received during the study target intervals and have a positive growth on blood agar were stored in the cryobank® storage system at -70ºC.

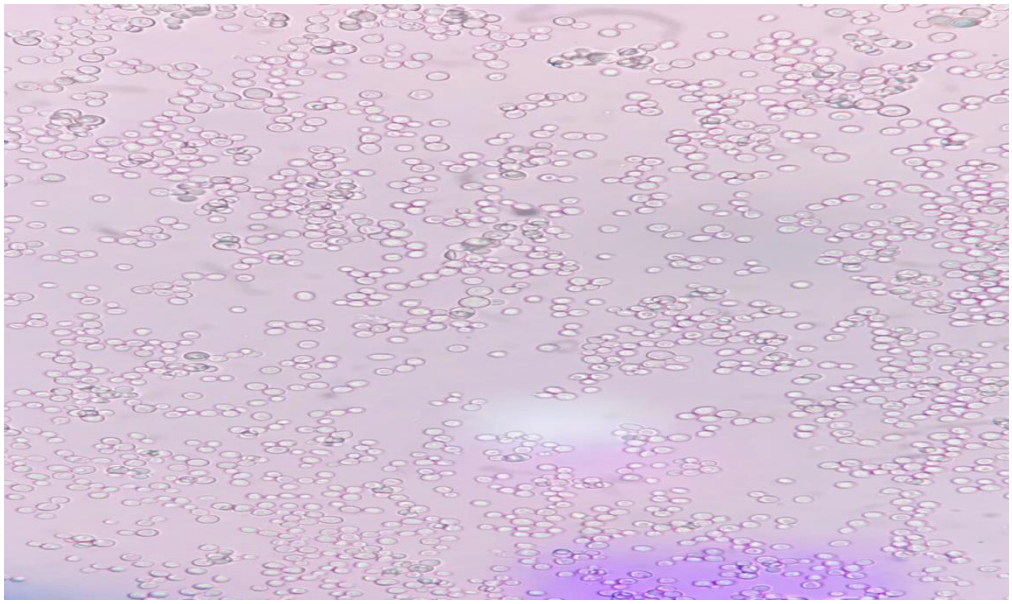

Figure 3.1: Saline wet mount preparation (40X) -Budding yeast of *Candida*.

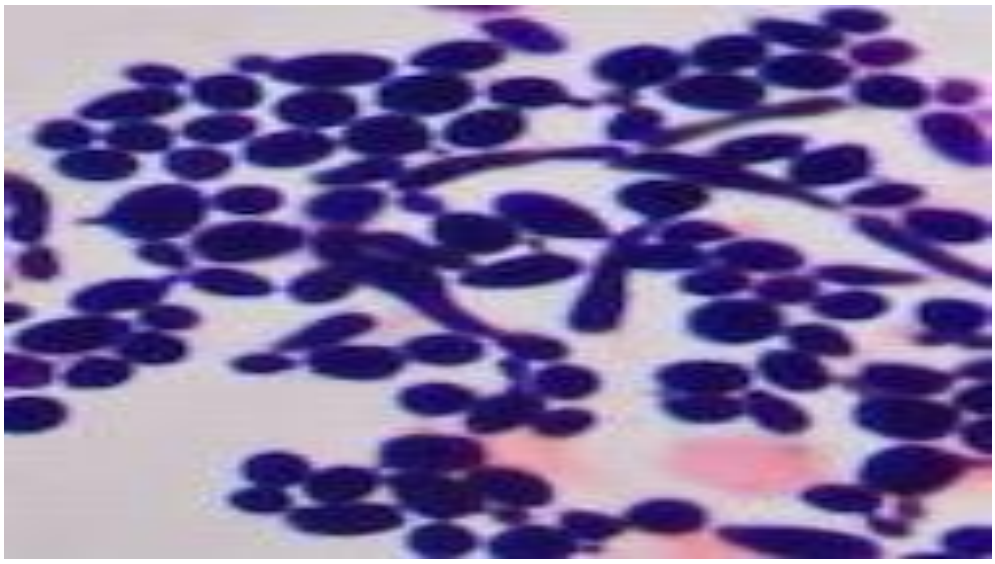

Figure 3.2: Gram stain (100X) -Budding yeast of *Candida*.

3.3 Specimens Culturing

The stored samples of *Candida* isolates were inoculated on Sabouraud Dextrose Agar (SDA) (Merck KGaA, Darmstadt, Germany) to get pure colonies. SDA was prepared as per the manufacturer's directions as follows:

i. Suspend 65,0 grams in 1000 ml purified/distilled water.
ii. Heat to boiling to dissolve the medium completely.
iii. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.
iv. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Candida colonies were obtained after a 48 hours incubation period at 37°C. Colonies are smooth and creamy, as shown in Figure 3.3. These colonies were used for various identification methods of *Candida* species (HiCrome™ *Candida* Differential Agar and germ tube test) and later for antifungal susceptibility testing.

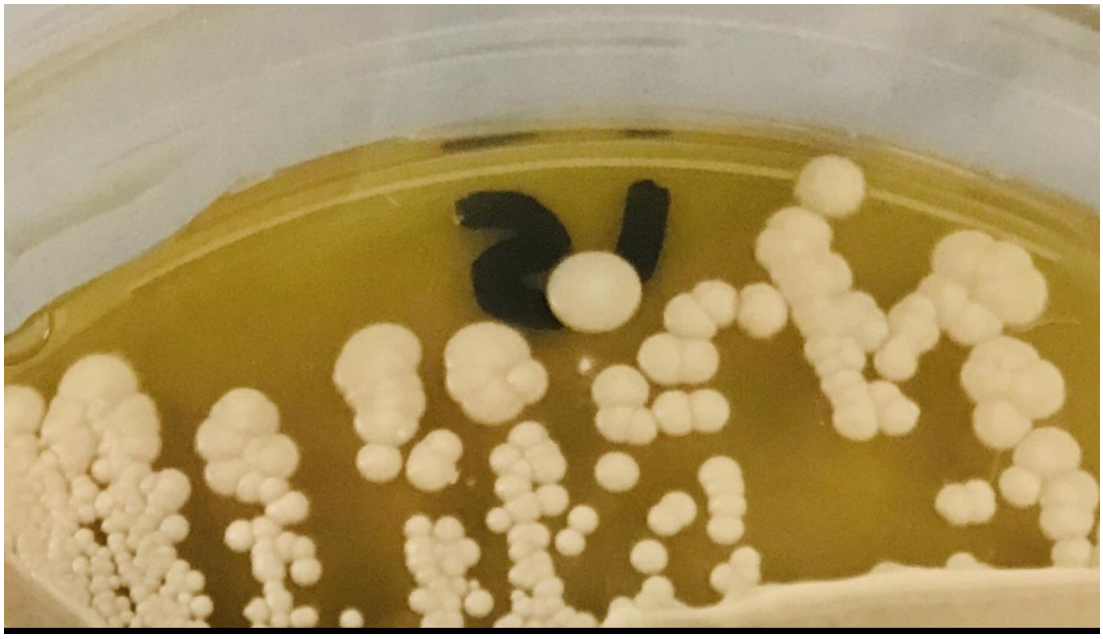

Figure 3.3: Smooth creamy colonies of *Candida* growing on Sabouraud Dextrose Agar (SDA) after 48 hours of incubation at 37°C.

3.4 Species Identification

The isolated *Candida* species were identified by following conventional mycological techniques:

3.4.1 HiCrome™ *Candida* Differential Agar

The HiCrome™ *Candida* Differential Agar (Himedia, Mumbai, India) is Selective and differential medium for rapid isolation and identification of *Candida* species based on the colonies' color difference arising from the breakdown of chromogenic substrates by species-specific enzyme action.

The plates were prepared according to the manufacturer's instructions as follows:

i. Suspend 42,72 grams in 1000 ml purified/distilled water.
ii. Heat to boiling to dissolve the medium completely.
iii. DO NOT AUTOCLAVE.
iv. Cool to 45-50°C. Mix well and pour into sterile Petri plates.
v. Prepared media was kept at 4ºC and used within three days.
vi. Isolates to be identified in chromogenic media were subcultured twice on SDA before inoculation.
vii. A single *Candida* isolate colony was inoculated, and the plate was incubated for 48 to 72 hours at 37ºC.
viii. The results were construed based on color, colonies' morphology, and the presence of halo around them.

Differentiation of *Candida* species on the basis of colony color and morphology on HiCrome™ *Candida* Differential Agar is shown in Figure 3.4.

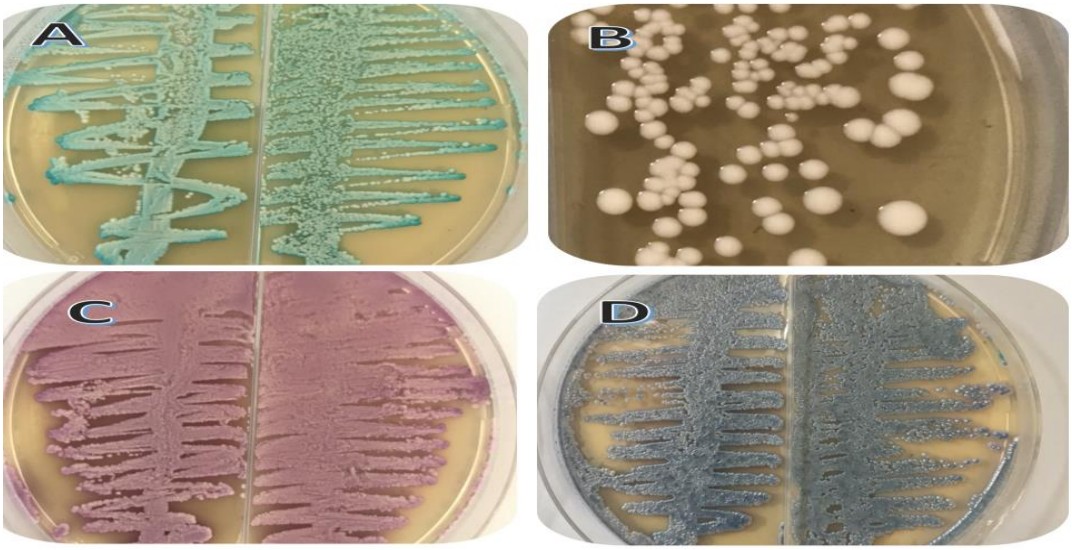

Figure 3.4: Colony color of *Candida* species HiCrome™ *Candida* Differential Agar *C. albicans* (A), *C. glabrata* (B), *C. krusei* (C), and *C. tropicalis* (D).

Table 3.1 shows the color of different *Candida* species on HiCrome™ *Candida* Differential Agar.

Table 3.1: The color of different *Candida* species on HiCrome™ *Candida* Differential Agar.

***Candida* species**	**Colony Color**
Candida albicans	Light green to green
Candida glabrata	Cream to white
Candida krusei	Purple, fuzzy
Candida tropicalis	Metallic blue to blue

3.4.2 Germ Tube Formation Test

Unlike other *Candida* species, *C. albicans* produces a tube-like structure (true hyphae) after incubation in human serum within 2 hours at 37°C. *Candida* species are identified using traditional methods; germ tube formation. But a significant problem in this approach is that *C. dubliniensis* shares this ability with *C. albicans*, contributing

to misidentification of *Candida* species (Neppelenbroek *et al.*, 2014). In this study, this test was performed to confirmatory identification of *C. albicans*.

The procedure for germ tube formation test was as follows:

i. 0.5 mL of human serum was dispensed separately into test tubes.
ii. The colony was taken with an inoculation needle and then inoculated in human serum-containing test tubes to obtain a turbid suspension.
iii. The test tubes were incubated for 2 hours at 37°C.
iv. Placed 2 drops of the suspension from the test tube on a clean microscopic slide, put coverslip onto the suspension drops, and examined under magnification of 40X for the presence of a germ tube.

Germ tube appears as elongated daughter cells which originated from the oval mother yeast cell without shrinkage at their origin (Deorukhkar *et al.*, 2012). Germ tubes formation in *C. albicans* is shown in Figure 3.5.

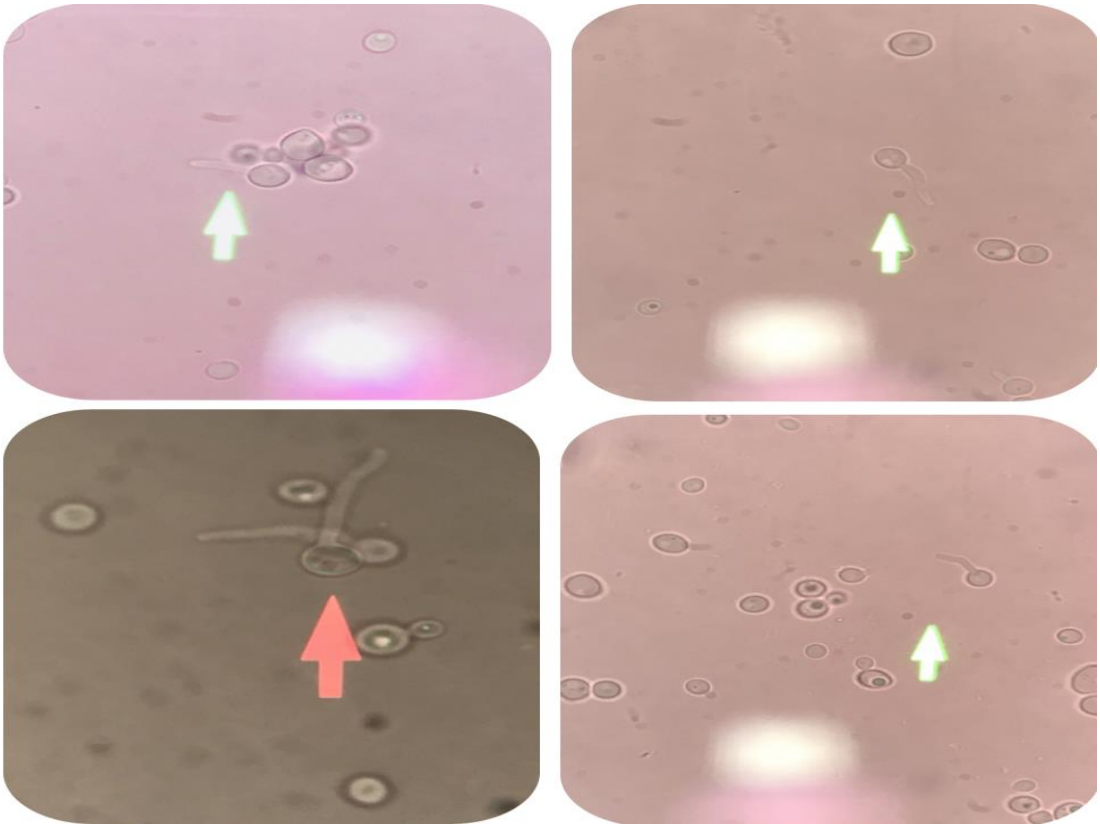

Figure 3.5: Germ tubes formation in *C. albicans* (Indicated by the arrow).

3.5 Antifungal Susceptibility Testing

- **Supplemented Mueller-Hinton Agar (MH-GMB)**

In this study, the disc diffusion method has been performed for the antifungal susceptibility test. The disk diffusion method for yeasts is utilized the same medium that used for bacteria (Mueller-Hinton agar) supplemented with 2% glucose and 0,5 µg/mL methylene blue dye with a pH range 7,2 to7,4. The presence of glucose provides an appropriate growth for the yeasts whereas the presence of methylene blue dye enhances the zone edge definition (Fothergill, 2012).

Mueller-Hinton agar supplemented with 2% glucose and 0,5 µg/mL methylene blue dye (MH-GMB) was prepared as follows (Espinel-Ingroff & Cantón, 2007).

i. Add 100 µL of methylene blue dye to 1000 mL of Mueller-Hinton agar.
ii. Add 20 g of glucose to 1000 mL of Mueller-Hinton agar.
iii. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.
iv. Cool to 45-50°C. Mix well and pour into sterile Petri plates with approximately 4 mm depth.
v. Stored at refrigerator temperature 2 to 8°C and used within 7 days of preparation.

- **Antifungal Discs**

In this study, the antifungal agents used in the disc diffusion method are shown in table 3.2.

Table 3.2: Antifungal discs (Bioanalyse, Ankara, Turkey)

Antifungal Agent	Catalog No.	Symbol	mcG
CASPOFUNGIN	ASD00915	CAS	5
AMPHOTERICIN B	ASD00450	AMB	100 U
MICONAZOLE	ASD05630	MCZ	10

ITRACONAZOLE	ASD04670	ITR	10
FLUCONAZOLE	ASD03930	FLU	25
VORICONAZOLE	ASD09500	VOR	1

Storage and Usage Instructions:

As the manufacturer's instructions, place the discs at -20 to +8 ° C after receipt. The expiry date is only valid for unopened blister packs which are kept under appropriate conditions. If a cartridge is open, it is best to store it for no more than 7 days. Allow room temperature to come in containers before opening to prevent condensation as this can reduce the antimicrobial agent's potency. If opened, the discs should be placed in the container given or any appropriate opaque airtight desiccated container within the dispenser to protect the discs from moisture. Containers will be placed in the refrigerator inside the dispenser and allowed to reach room temperature before opening to avoid condensation from the formation. Return unused discs to the refrigerator once the discs have been applied. First, use the oldest discs. Discard discs that have expired.

- **Preparation of Inoculum:**
  i. From the primary isolation medium, five colonies showing similar morphology are either taken by direct colony suspension method and suspended by using a flamed loop or by using a sterile cotton swab, the yeast cells are picked up and suspended in 4-5 ml sterile saline solution (0,85% NaCl). Mix the cell suspension using a vortex mixer for 15 seconds.
  ii. Once the turbidity is visible, the suspension density is adjusted by the use of a spectrophotometer. The turbidity needs to be balanced to 0,08 to 0,10 at an absorbance of 625 nm for the 0,5 McFarland standard. It needs to be used about an hour after the standard suspension has been prepared.

- **Inoculation into Agar Plates**

Inoculation has been done according to the Kirby-Bauer method as follows:

i. Mix the prepared yeast suspension well with a sterile cotton swab and excess fluid of the swab is removed by slightly pressing and rotating the swab inside the tube, above the fluid level.
ii. Streak the entire agar surface of an MH-GMB plate three times, turning the plate 60° between streaking to obtain even inoculation.
iii. For preventing the excessive moisture of the medium, allow Petri dishes to dry for 3 to 5 minutes, maximum of 15 minutes, at room temperature.

- **Application of Antifungal discs to Inoculated Agar Plates and Incubation.**
  i. The cartridge is opened under the flame and then discs are discharged from the cartridge onto a clean Petri dish with the help of a flamed and cooled forceps.
  ii. The discs have to be distributed into the agar plates no closer than 24 mm from the center to the center and 12 mm away from the edge of the agar plate within 15 minutes.
  iii. Do not move the disc once it has touch into contact with the surface of the agar.
  iv. Place the plates in the inverted position in an incubator set at 35°C (± 2°C) in an aerobic environment within 15 minutes of the discs application.
  v. Incubate all plates for 20 to 24 hours.
- **Reading Inhibition Zones and Interpretation of Results**
  i. Examine the plates after overnight incubation (20-24 hours). If the plate has been streaked satisfactorily and the inoculum is correct, the resulting inhibition zones around the disk are being uniformly clear circular, with a semi confluent growth field.
  ii. Hold the plate just a few centimeters above a black, non-reflective background lit with reflective light.

iii. Measure the diameter of the zone to the nearest full millimeter at the point at which growth is significantly reduced. (It is necessary to ignore the presence of pinpoint microcolonies at the edge of the zone or large colonies within an inhibition zone).

iv. If there is insufficient growth after 24 hours of incubation, re-incubate the plates and read them at 48 hours of initial incubation time.

The CLSI has provides zone diameter interpretive criteria for fluconazole and voriconazole only. However, various studies reported the interpretive criteria of the inhibition zone diameters of many other antifungal agents and are generally identical to MIC interpretative criteria defined in the CLSI documents. Table 3.5 shows zone interpretative criteria (breakpoints) for used six antifungal agents against four *Candida* species (Cantón et al., 2009; Carvalhinho *et al.*, 2012; De la Cruz-Claure *et al.*, 2019; Jabeen *et al.*, 2016; Moges *et al.*, 2016; Oz & Gokbolat, 2018; M. A. Pfaller *et al.*, 2011; Michael A. Pfaller, 2008; Sheehan *et al.*, 2004).

3.6 Statistical Analysis

Qualitative and quantitative data values along with the percentage and mean ± standard deviation (SD) is represented as frequency. The Chi-square test is tested as appropriate on the association between two or more variables. Pictorial explanations of the major results of the study were rendered using an appropriate statistical graph. A $P<0,05$ was deemed significant. All statistical analyses were done using statistical packages SPSS version 25,0 (SPSS Inc. Chicago, IL, USA).

Table 3.3: Zone-diameter (mm) limits for four quality-control strains.

Q.C Strains		Fluconazole 25µg[a,g]	Voriconazole 1µg[a,g]	Itraconazole 10µg[b]	Amphotericin B 100 U[c,e]	Caspofungin 5µg[d,h]	Miconazole 10 µg[e,f]
C. albicans ATCC 90028	S	≥ 19	≥ 17	≥ 20	≥ 19	≥ 18	≥ 20[a]
	SDD	16-18	14-16	12-19	15-18	15-16	12-19[a]
	R	≤ 15	≤ 13	≤ 11	≤ 14	≤ 14	≤ 11[a]
C. krusei ATCC 6258	S	≥ 19	≥ 17	≥ 16	≥ 18	≥ 18	≥ 11
	SDD	16-18	14-16	11-15	12-17	15-17	9-10
	R	≤ 15	≤ 13	≤ 10	≤ 11	≤ 14	≤ 8
C. glabrata ATCC 2001	S	≥ 19	≥ 17	≥ 12	≥ 16	≥ 20	≥ 20
	SDD	15-18	14-16	10-11	11-15	18-19	12-19
	R	≤ 14	≤ 13	≤ 9	≤ 10	≤ 17	≤ 11
C. tropicalis ATCC 750	S	≥ 19	≥ 17	≥ 15	≥ 17	≥ 17	≥ 20
	SDD	16-18	14-16	11-14	12-16	15-16	12-19
	R	≤ 15	≤ 13	≤ 10	≤ 11	≤ 14	≤ 11

S: Susceptible; SDD: Susceptible Dose Dependent; R: Resistant.

a: Sheehan *et al.*, 2004; **b**: Michael A. Pfaller, 2008; **c**: Jabeen *et al.*, 2016; **d**: Cantón et al., 2009; **e**: Moges *et al.*, 2016; **f**: Cantón et al., 2009; **g**: Oz & Gokbolat, 2018; **h**: M. A. Pfaller *et al.*, 2011.

4. RESULTS AND OBSERVATIONS

4.1 Specimens Distribution

In this study, out of 147, 51,7% (n=76) were females and 48,3% (n=71) were males. The mean and median age of the study group was 65,63 ± 22,92 and 71,00 (1,00-99,00). According to the age groups of participant patients, the distribution of their ages was as shown in table 4.1. The specimens were collected from different body sites the majority were urine specimens (44,9%) (n=66), as shown in Table 4.2. The specimens were obtained in the microbiology laboratory from various hospital departments. Table 4.3 shows the distribution of the specimens within different hospital departments.

Table 4.1: The distribution of patients within age groups (Horng, W. B *et al.*, 2001).

Age Groups (yrs.)	Frequency	Percent	Valid Percent	Cumulative Percent
Babies (≤ 2)	1	0,7	0,7	0,7
Child (3-15)	3	2,0	2,0	2,7
Young Adults (16-30)	15	10,2	10,2	12,9
Middle-aged Adults (31-44)	8	5,4	5,4	18,4
Old Adults (≥ 45)	120	81,6	81,6	100,0
Total	147	100,0	100,0	

Table 4.2: The distribution of the specimens from different body sites.

Sample Type	Frequency	Percent	Valid Percent	Cumulative Percent
Urine	66	44,9	44,9	44,9
Blood	7	4,8	4,8	49,7
CSF	1	0,7	0,7	50,3
Sputum	28	19,0	19,0	69,4

Catheter	8	5,4	5,4	74,8
Aspiration	22	15,0	15,0	89,8
Vaginal Swab	12	8,2	8,2	98,0
Abscess Wound	3	2,0	2,0	100,0
Total	147	100,0	100,0	

Table 4.3: The distribution of the specimens from different hospital departments.

Department	Frequency	Percent	Valid Percent	Cumulative Percent
Chest Diseases and Allergy	30	20,4	20,4	20,4
Brain Surgery	3	2,0	2,0	22,4
Anesthesia	31	21,1	21,1	43,5
Laboratory	1	0,7	0,7	44,2
Oncology	1	0,7	0,7	44,9
Urology	3	2,0	2,0	46,9
ICU	1	0,7	0,7	47,6
Gastroenterology	1	0,7	0,7	48,3
Internal Medicine	6	4,1	4,1	52,4
Gynecology and Obstetrics	17	11,6	11,6	63,9
Neurosurgery	6	4,1	4,1	68,0
Geriatrics	6	4,1	4,1	72,1
Cardiology	32	21,8	21,8	93,9
Infectious Diseases	4	2,7	2,7	96,6
ER	3	2,0	2,0	98,6
Child Health and Diseases	2	1,4	1,4	100,0
Total	147	100,0	100,0	

4.2 Species Distribution

In this study, it was noted that *C. albicans* predominate over NAC species. As shown in figure 4.1, the isolation rate of *C. albicans* and NAC species was 55,78% (n=82) and 44,22% (n=65) respectively.

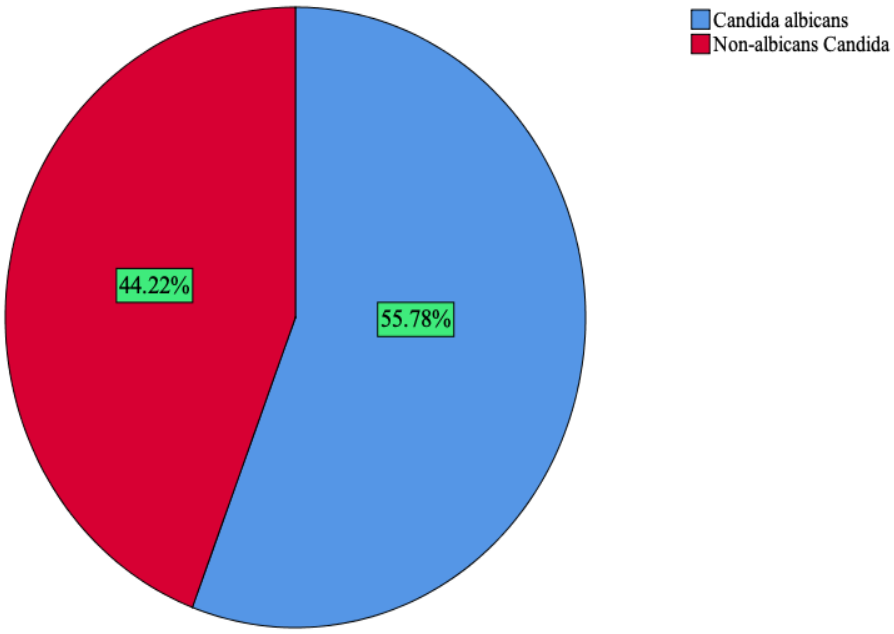

Figure 4.1: Distribution of *C. albicans* and *non-albicans Candida* (NAC) species.

Figure 4.2 shows, the distribution of *C. albicans* and NAC species isolated from different clinical specimens. *C. albicans* was the predominant isolates in some clinical specimens while NAC species were the predominant isolates in other clinical specimens.

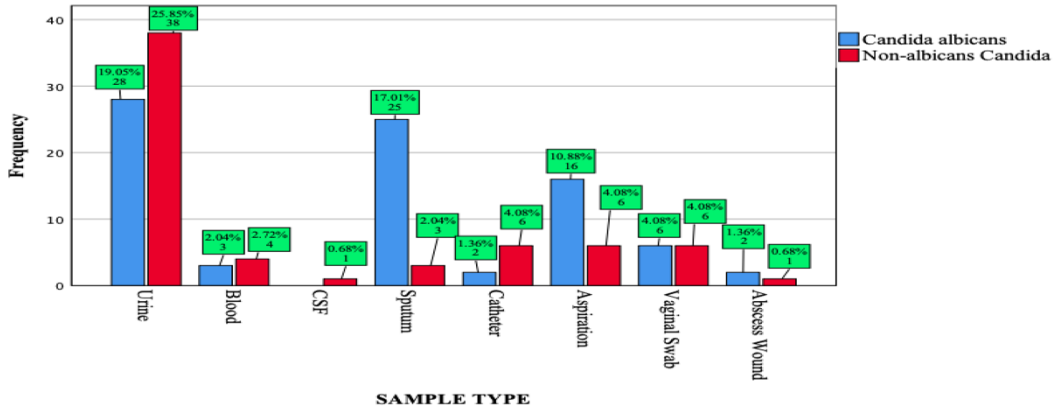

Figure 4.2: Distribution of *C. albicans* and *non-albicans Candida* (NAC) species isolated from various clinical specimens.

The species wise distribution of *Candida* isolates is shown in figure 4.3. The predominant species isolated in this study was *C. albicans* 55,78% (n=82).

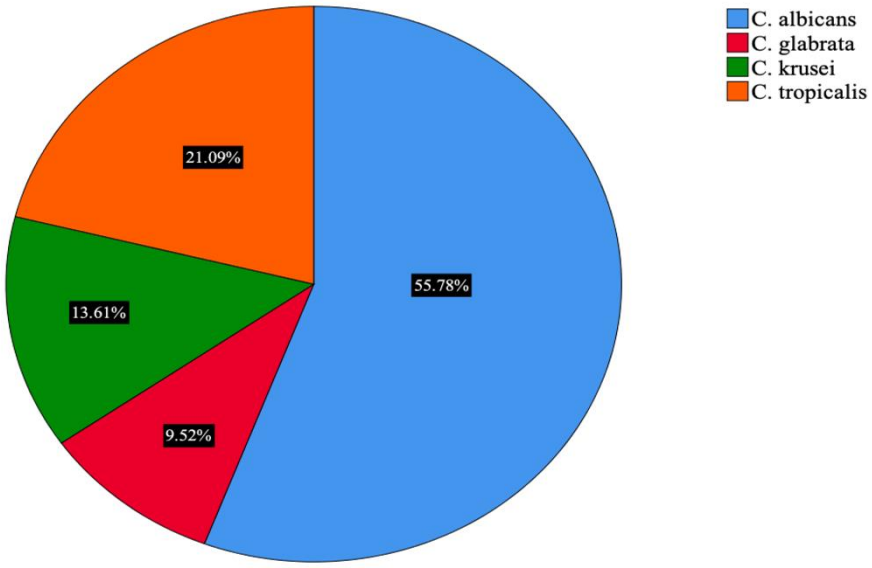

Figure 4.3: Species distribution of *Candida* isolates.

4.3 Antifungal Susceptibility Testing Pattern

In this study, the antifungal susceptibility profile of *Candida* species was screened by the disc diffusion method using MH-GMB.

As shown in Table 4.4, among the azole class of antifungal agents, *Candida* species demonstrated high resistance to fluconazole (98%) (n=144) followed by voriconazole (92,5%) (n=136). Itraconazole resistance was seen in 83,7% (n=123) of *Candida* isolates. Only 18,37% (n=27) of *Candida* isolates showed resistance to miconazole. Among polyenes, *Candida* isolates showed no resistance to Amphotericin B (0,0%) (n=0). Among echinocandins, *Candida* isolates showed no resistance to caspofungin (0,0%) (n=0). (Chi-square test P-value <0,05), whereas no P-value for caspofungin because it is constant. Therefore, no statistics were computed for it.

Table 4.4: Antifungal susceptibility profile of *Candida* isolates by disc diffusion method.

	Total no. of isolates tested	Susceptible isolates n (%)	SDD isolates n (%)	Resistant isolates n (%)
Fluconazole 25µg	147	3 (2)	0 (0)	144 (98)
Voriconazole 1µg	147	11 (7,50)	0 (0)	136 (92,50)
Itraconazole 10µg	147	10 (6,80)	14 (9,50)	123 (83,70)
Amphotericin B 100 U	147	144 (98)	3 (2)	0 (0)
Caspofungin 5µg	147	147 (100)	0 (0)	0 (0)
Miconazole 10 µg	147	99 (67,35)	21 (14,28)	27 (18,37)

As shown in tables 4.5 and 4.6, among azoles, *Candida albicans* demonstrated significantly high resistance to fluconazole and voriconazole compared to NAC species (Chi-square test, P-value <0,05). Fluconazole resistance was observed in 100% (n=82), 95,4% (n=62) of *Candida albicans*, and NAC species, respectively. voriconazole resistance was seen in 98,8% (n=81), 84,6% (n=55) of *Candida albicans*, and NAC species, respectively.

NAC species demonstrated significantly high resistance to itraconazole and miconazole compared to *Candida albicans* (Chi-square test, P-value <0,05). itraconazole resistance was seen in 86,2% (n=56), 81,7% (n=67) of NAC species and *Candida albicans*, respectively. miconazole resistance was observed in 38,5% (n=25), 2,4% (n=2) of NAC species and *Candida albicans*, respectively.

Among polyenes and echinocandins, both (*Candida albicans* and NAC species) have no resistance to Amphotericin B (0%) and caspofungin (0%).

Table 4.5: Antifungal susceptibility profile of *Candida albicans* by disc diffusion method.

	No. of isolates	Susceptible isolates n (%)	SDD isolates n (%)	Resistant isolates n (%)
Fluconazole 25µg	82	0 (0)	0 (0)	82 (100)
Voriconazole 1µg	82	1 (1,2)	0 (0)	81 (98,8)
Itraconazole 10µg	82	1 (1,2)	14 (17,1)	67 (81,7)
Amphotericin B 100 U	82	82 (100)	0 (0)	0 (0)
Caspofungin 5µg	82	82 (100)	0 (0)	0 (0)
Miconazole 10 µg	82	73 (89)	7 (8,5)	2 (2,4)

Table 4.6: Antifungal susceptibility profile of *non-albicans Candida* (NAC) species by disc diffusion method.

	No. of isolates	Susceptible isolates n (%)	SDD isolates n (%)	Resistant isolates n (%)
Fluconazole 25µg	65	3 (4,6)	0 (0)	62 (95,4)
Voriconazole 1µg	65	10 (15,4)	0 (0)	55 (84,6)
Itraconazole 10µg	65	9 (13,8)	0 (0)	56 (86,2)
Amphotericin B 100 U	65	62 (95,4)	3 (4,6)	0 (0)
Caspofungin 5µg	65	65 (100)	0 (0)	0 (0)
Miconazole 10 µg	65	26 (40)	14 (21,5)	25 (38,5)

Table 4.7 shows the antifungal susceptibility profile among *non-albicans Candida* (NAC) species. Among azoles, fluconazole resistance was high in *C. tropicalis* and *C. glabrata*, 93,5% (n=29) and 92,9% (n=13), respectively. Voriconazole resistance was high in *C. tropicalis*, *C. glabrata*, and *C. krusei*, 100% (n=31), 85,7% (n=12), and 60% (n=12), respectively. Itraconazole resistance was high in *C. tropicalis*, *C. glabrata*, and *C. krusei*, 96,8% (n=30), 85,7% (n=12), and 70% (n=14), respectively. Miconazole resistance was high in *C. tropicalis*, *C. krusei*, and *C. glabrata*, 54,8% (n=17), 30% (n=6), and 14,3% (n=2), respectively. Among polyenes and

echinocandins, NAC species have no resistance to Amphotericin B (0%) and caspofungin (0%).

Table 4.7: Antifungal susceptibility profile among *non-albicans Candida* (NAC) species by disc diffusion method.

		Fluconazole 25µg	Voriconazole 1µg	Itraconazole 10µg	Amphotericin B 100 U	Caspofungin 5µg	Miconazole 10 µg
C. glabrata (n=14)	S n (%)	1 (7,1)	2 (14,3)	2 (14,3)	14 (100)	14 (100)	11 (78,6)
	SDD n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7,1)
	R n (%)	13 (92,9)	12 (85,7)	12 (85,7)	0 (0)	0 (0)	2 (14,3)
C. krusei (n=20)	S n (%)	NT	8 (40)	6 (30)	18 (90)	20 (100)	14 (70)
	SDD n (%)		0 (0)	0 (0)	2 (10)	0 (0)	0 (0)
	R n (%)		12 (60)	14 (70)	0 (0)	0 (0)	6 (30)
C. tropicalis (n=31)	S n (%)	2 (6,5)	0 (0)	1 (3,2)	30 (96,8)	31 (100)	1 (3,2)
	SDD n (%)	0 (0)	0 (0)	0 (0)	1 (3,2)	0 (0)	13 (41,9)
	R n (%)	29 (93,5)	31 (100)	30 (96,8)	0 (0)	0 (0)	17 (54,8)

S: Susceptible; SDD: Susceptible Dose Dependent; R: Resistant.
NT: Not tested. *C. krusei* has been identified as a potential multidrug-resistant (MDR) fungal pathogen because of its intrinsic resistance to fluconazole (M. A. Pfaller et al., 2008).

5. DISCUSSION

Infections of *Candida* species have intensified and become more complicated to manage owing to the rise of immunogenic diseases, misuse of broad-spectrum antibiotics, increasing usage of immunosuppressive drugs, malnutrition, endocrine diseases, extensive use of medical devices, aging and an increase in the number of patients (Silva *et al.*, 2017). In addition, in the last two decades, the whole evolution of infectious diseases has undergone significant changes (Deorukhkar *et al.*, 2014). Fungi that were formerly considered nonpathogenic or less virulent are now identified as a significant cause of morbidity and mortality in patients who are immunocompromised and critically ill (Deorukhkar & Saini, 2014).

C. albicans and *non-albicans Candida* (NAC) species were closely correlated with many opportunistic fungal infections (Martins *et al.*, 2015). In past centuries, 92–95% of all *Candida* infection cases have been caused by the five most common agents, *C. albicans*, and *non-albicans Candida* (NAC) species *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis* (Berkow & Lockhart, 2017; Pappas *et al.*, 2015).

Many studies around the world noted an increase in the prevalence of *Candida* infections. In Europe, *Candida* species are recognized as the 6th to 10th causative agent of nosocomial bloodstream infections (BSI). In the USA, *Candida* species are reported as 2nd causative agent of catheter-associated urinary tract infections (CAUTI), as 3^{ed} causative agent of bloodstream infections (BSIs) in the ICU patients and of catheter-related bloodstream infections (CRBSI), and as 4th causative agent of health-care-associated bloodstream infections (Méan *et al.*, 2008).

Most *Candida* isolates in this study were from urine samples. Bukhary (2008) noted that *Candida* species responsible for 10% to 15% of nosocomial urinary tract infections. *Candida* species isolation in urine samples generally puts a clinician and microbiologist in a conundrum about whether the candiduria symbolizes just *Candida* colonization or infection of the lower or upper urinary tract, as well as ascending pyelonephritis and sepsis infections of the renal *Candida* (Bukhary, 2008). In this study, 44,9% (n= 66 out of 147) of the collected specimens were urine samples.

In a recent study, the percentage of *C. albicans* isolated from the urine sample was (23,7%), whereas 76,3% of isolated *Candida* species have belonged to NAC species (Deorukhkar, 2018). As per Fisher's study in 2011, >50% of *Candida* isolates from the urine sample belong to NAC species (Fisher, 2011). In this study, 57,6% and 42,4% were belonged to NAC species and *C. albicans*, respectively.

Candidiasis infections caused by *C. tropicalis* have significantly increased on a global scale, proclaiming this organism as an emerging pathogenic yeast (Kothavade *et al.*, 2010). Among NAC, *C. tropicalis* was the predominant species in various recent studies. Pahwa *et al.* (2014) reported this observation with (38,7%) of NAC isolates were *C. tropicalis* (Pahwa *et al.*, 2014). In this study, the percentage of *C. tropicalis* isolates was 47,7% (n=31 out of 65).

In this study, *C. tropicalis* was the predominant (50%) isolate among collected urine samples. This observation is consistent with previous studies, Paul *et al.* (2004) noted the predominance of *C. tropicalis* among candiduria cases (Paul *et al.*, 2004). And Negri *et al.* (2012) described *C. tropicalis* as 1st or 2nd of NAC species most frequently isolated from candiduria (Negri *et al.*, 2012).

In this study, the rate of isolation of *C. glabrata* among urine samples was 18,4%. Sobel *et al.* (2000) and Deorukhkar (2018) reported a similar percentage (20%) of *C. glabrata* among UTI in their researches (Deorukhkar, 2018; J. D. Sobel *et al.*, 2000). Whereas de Freitas *et al.* (2014) reported *C. glabrata* in 61.3% of nosocomial candiduria (de Freitas *et al.*, 2014).

In the present study, the distribution of Candida species from urine specimens was as follows: *C. albicans* (42,4%), *C. tropicalis* (28,8%), *C. krusei* (18,2%), and *C. glabrata* (10,6%).

Paul et al. (2004) showed that *C. albicans* (19,1%), *C. tropicalis* (42,8%), and *C. glabrata* (19,1%), whereas *C. krusei* not tested in their study. Kobayashi *et al*. (2004) reported that *C. albicans* (35,6%), *C. tropicalis* (22,2%), *C. krusei* (6,7%), and *C. glabrata* (8,9%). Ozhak-Baysan *et al*. (2012) documented that *C. albicans* (44%), *C. tropicalis* (20%), *C. krusei* (6%), and *C. glabrata* (18%). A Zarei *et al.* (2012) noted

that *C. albicans* (53,3%), *C. tropicalis* (3,7%), *C. krusei* (2,2%), and *C. glabrata* (24,4%). Yashavanth *et al.* (2013) reported that *C. albicans* (30,3%), *C. tropicalis* (45,4%), *C. krusei* (15%), and *C. glabrata* (9,1%). Yazdani *et al.* (2016) documented that *C. albicans* (44%), *C. tropicalis* (11%), *C. krusei* (8%), and *C. glabrata* (26%). Deorukhkar (2018) reported that *C. albicans* (23,7%), *C. tropicalis* (24,2%), *C. krusei* (19,6%), and *C. glabrata* (20%). (Deorukhkar, 2018; Kobayashi *et al.*, 2004; Ozhak-Baysan *et al.*, 2012; Paul *et al.*, 2004; R. *et al.*, 2013; Zarei-Mahmoudabadi *et al.*, 2012).

Oropharyngeal candidiasis (OPC) is an opportunistic mucosal infection triggered by *Candida* species being overgrown (Akpan & Morgan, 2002). Although OPC is the most common infection of the fungi in people living with HIV/AIDS (PLHA) (Repentigny *et al.*, 2004). It is not correlated with mortality, however, it leads greatly to morbidity and induces persistent pain or harassment during swallowing which may reduce food intake in immunocompromised and elderly patients (Li *et al.*, 2007).

The predominant isolates of sputum specimens were *C. albicans* and *C. glabrata*. However, *C. albicans* is still seen as a significant source of OPC infection (Jeddy *et al.*, 2011).

In this study, *C. albicans* was predominant isolates from sputum specimens (89,3%) and *C. tropicalis* (10,7%), whereas *C. glabrata* and *C. krusei* are not seen in sputum specimens.

Kliemann *et al.* (2008) noted that *C. albicans* (96,2%), *C. tropicalis* (2,5%), and *C. glabrata* (0,6%), whereas *C. krusei* not tested in their study. Luque *et al.* (2009) reported that *C. albicans* (60,7%), *C. tropicalis* (4,5%), *C. krusei* (5,6%), and *C. glabrata* (5,6%). Baradkar and Kumar (2009) documented that *C. albicans* (78%), *C. tropicalis* (12%), and *C. glabrata* (4%), whereas *C. krusei* not tested in their study. Mane *et al.* (2010) noted that *C. albicans* (74,4%), *C. tropicalis* (7,1%), *C. krusei* (3,2%), and *C. glabrata* (3,9%). Agwu *et al.* (2012) documented that *C. albicans* (87%), *C. tropicalis* (3,6%), and *C. glabrata* (3,6%), whereas *C. krusei* not tested in their study. Mulu *et al.* (2013) noted that *C. albicans* (46%), *C. tropicalis* (7%), *C. krusei* (10%), and *C. glabrata* (25%). Deorukhkar (2018) reported that *C. albicans*

(26,7%), *C. tropicalis* (11,5%), *C. krusei* (5,7%), and *C. glabrata* (26%). (Agwu *et al.*, 2012; Baradkar & Karyakarte, 1999; Deorukhkar, 2018; Kliemann *et al.*, 2008; Luque *et al.*, 2009; Mane *et al.*, 2010; Mulu *et al.*, 2013).

Candida is one of the most common causative agents of vaginal infection (Dan *et al.*, 2002). Since vulvovaginal candidiasis (VVC) is not a reportable infection and has recently been excluded from the list of sexually transmitted diseases, the information about the prevalence and epidemiology of this infection is limited. VVC is an exceedingly common infection of the mucosal *Candida* in women of childbearing age (Jindal *et al.*, 2007).

According to Mohanty (2007) and Jindal (2007) studies, *C. glabrata* being the most common causative agent of VVC, whereas Deorukhkar (2018) reported predominant of *C. tropicalis* (34,1%) followed by *C. glabrata* (21,1%) (Deorukhkar, 2018; Jindal *et al.*, 2007; Mohanty *et al.*, 2007).

In this study, the predominance was for *C. albicans* (50%), *C. tropicalis* (8,3%) *C. krusei* (33,3%) , and *C. glabrata* (8,3%).

Mohanty *et al.* (2007) noted that *C. albicans* (35,1%), *C. tropicalis* (10,8%), *C. krusei* (2,7%), and *C. glabrata* (50,4%). Ahmad and Khan (2009) noted that *C. albicans* (46,9%), *C. tropicalis* (2,8%), *C. krusei* (1,4%), and *C. glabrata* (36,7%). Mahmoudi Rad *et al.* (2011) noted that *C. albicans* (67%), *C. tropicalis* (6,8%), *C. krusei* (5,8%), and *C. glabrata* (18,3%). Guzel *et al.* (2011) reported that *C. albicans* (50,4%), *C. tropicalis* (3,6%), *C. krusei* (3,8%), and *C. glabrata* (35%). Babin *et al.* (2013) reported that *C. albicans* (35,5%), *C. tropicalis* (26,4%), *C. krusei* (15,7%), and *C. glabrata* (20,6%). Vijaya *et al.* (2014) reported that *C. albicans* (66%), *C. tropicalis* (26,4%), *C. krusei* (3,8%), and *C. glabrata* (1,9%). Alfouzan *et al.* (2015) reported that *C. albicans* (73,9%), *C. tropicalis* (0,96%), *C. krusei* (0,96%), and *C. glabrata* (19,8%). Deorukhkar (2018) reported that *C. albicans* (18,2%), *C. tropicalis* (34,1%), *C. krusei* (12,3%), and *C. glabrata* (21,1%). (A. Ahmad & Khan, 2009; Alfouzan *et al.*, 2015; Babin *et al.*, 2013; Deorukhkar, 2018; Guzel *et al.*, 2011; Mahmoudi Rad *et al.*, 2011; Mohanty *et al.*, 2007; Vijaya *et al.*, 2014).

Bloodstream infection (BSI) due to NAC species was significantly higher than *C. albicans*. Similar findings have been reported recently from the USA, Germany, France, China, and India (Falagas *et al.*, 2010). Dagi *et al.* (2016) noted that bloodstream infection (BSI) due to *Candida* species is referred to *C. albicans* and *non-albicans Candida* (NAC) species (47,5% and 52,5%), respectively (Dagi *et al.*, 2016).

In this study, the distribution of *Candida* isolates from blood specimens was 57,1% and 42,9% for NAC species and *C. albicans*, respectively. *C. glabrata* (42,9%) was the predominant among NAC species followed by *C. krusei* (14,3%).

The resistance of *Candida* species to antifungal agents possibly has serious implications for the management of infections (Sanglard & Odds, 2002). This resistance is due to a combination of factors related to the host, an antifungal agent, or the causative agent itself (John H. Rex *et al.*, 2001). There is no or minimal transmission of resistant isolates from patient to patient as candidiasis is not a contagious infection. Unlike bacterial infections, resistance cannot be transmitted through plasmids in the *Candida* species. In *Candida* isolate, therefore, resistance often occurs during exposure to an antifungal drug (Arendrup, 2013).

The antifungal susceptibility testing is useful for both *C. albicans* and NAC species according to the guidelines published by the Infectious Diseases Society of America (IDSA). With *C. albicans* the susceptibility testing is necessary in patients with progressive candidemia or other types of disseminated candidiasis while in the case of NAC species susceptibility testing is extremely significant in patients who have received a prophylactic or therapeutic dose of any drug of azole class antifungal drugs (Pappas *et al.*, 2015).

Clinical and Laboratory Standards Institute (CLSI) has approved M27-A3 for macrobroth and microbroth dilution and M44-A for disc diffusion susceptibility testing of yeast and yeast-like fungi (J. H. Rex *et al.*, 2008; Sheehan *et al.*, 2004). Since the CLSI reference broth microdilution method is technically complicated and time-consuming, many resource-limited hospital laboratories now commonly adopt alternative methods such as disc diffusion and E-testing (John H. Rex *et al.*, 2001).

In this study, the disc diffusion method using Mueller–Hinton agar supplemented with 2% glucose and 0,5 μg/mL methylene blue dye (MH-GMB) was applied to test the antifungal susceptibility profile of *Candida* isolates. Meis *et al.* (2002) developed the Agar Disc Diffusion Method, and Barry *et al.* (2002) further improved it (M. A. Pfaller *et al.*, 2004). Compared to the CLSI standardized form of microbroth dilution, the disc diffusion method is convenient and economical. The disc diffusion method and the routine Kirby-Bauer method for bacterial susceptibility testing are similar; therefore it is easily used in routine laboratories that have a heavy daily workload. (John H. Rex *et al.*, 2001).

Several researchers have used the antifungal susceptibility of *Candida* species using diverse media such as YNBG, antibiotic medium 3, RPMI agar with 2% glucose (RPG), Casitone, and unsupplemented Mueller Hinton agar (M. A. Pfaller *et al.*, 2004). In 2002, Barry *et al.* were first used this medium to test the *Candida* species for antifungal susceptibility. They recognized this medium being superior to the RPG medium (Barry *et al.*, 2002). In 2004, CLSI adopted MH-GMB as the medium of choice for *Candida* species disc diffusion testing (Sheehan *et al.*, 2004).

MH-GMB is cheap, readily applicable, and easily prepared compared to other media. This promotes the growth of all *Candida* species, which are medically significant. In MH-GMB agar, glucose in the medium provides an appropriate growth for *Candida* species whereas the presence of methylene blue dye enhances the zone edge definition and improves visualization of the zone diameters (Fothergill, 2012; John H. Rex *et al.*, 2001).

In this study, the trailing phenomenon (partial growth inhibition over an extended spectrum of antifungal concentrations) was less common on MH-GMB agar along the zone margin. Additionally, on MH-GMB agar there was the limited frequency of macrocolonies in the middle of the clear zone. Based on previous studies, of the many agar media available for *Candida* species to test for antifungal susceptibility, supplemented Mueller-Hinton agar is a good choice to apply antifungal susceptibility testing of *Candida* species.

Candida species resistance to fluconazole is of concern since it is one of the most commonly used first-line antifungal agents for the treatment and prophylaxis of all forms of *Candida* infection. Fluconazole is available as a tablet, orally suspended, and IV formulation (Kathiravan *et al.*, 2012).

Oxman *et al.* (2010) and Deorukhkar (2018) reported the resistance to fluconazole was seen in 34,6% of *Candida* isolates. Resistance to fluconazole was also observed in species considered innately susceptible to fluconazole such as *C. albicans* and *C. tropicalis* (Deorukhkar, 2018; Oxman *et al.*, 2010). Fluconazole resistance was reported in various studies as well. It was seen in 37,5% of *Candida* isolates in Gupta *et al.* (2001) study, 11,7% of *Candida* isolates in Xess *et al.* (2007) study, 36% of *Candida* isolates in Kothari *et al.* (2009) study, in 30,8% of *Candida* isolates in Giri *et al.* (2013) study, in 5,4% of *Candida* isolates in Kiraz and Oz (2011) study, in 2,5% of *Candida* isolates in Fleck *et al.* (2007) study, and in 1,9% of *Candida* isolates in yang *et al.* (2006).

In 2010, Oxman *et al.* warned that simple species identification might not be enough to predict fluconazole susceptibility patterns of *Candida* species (Oxman *et al.*, 2010). In 2002, Krcmery and Barnes concluded that more than 20% of *Candida* species develop resistance during fluconazole treatment and prophylaxis (Krcmery & Barnes, 2002). Vanden Bossche *et al.* (1992) and Sanglard *et al.* (1999) cautioned that both intrinsic and acquired resistance are being to be high especially in areas where fluconazole usage is high (Bossche *et al.*, 1992; Sanglard *et al.*, 1999).

In this study, fluconazole resistance was seen in 93,2% of *Candida* isolates. It was seen in 100%, 95,4% of *Candida albicans*, and NAC species, respectively. Among NAC species, fluconazole resistance was high in *C. tropicalis* (93,5%) isolates and 92,9% of *C. glabrata* isolates.

In this study, voriconazole resistance was seen in 92,5% of *Candida* isolates. Among NAC species, the voriconazole resistance was highest in *C. tropicalis* (100%). Whereas, Deorukhkar (2018) reported 5,3% of *Candida* isolates and 7,4% in *C. tropicalis* among NAC species were resistant to voriconazole (Deorukhkar, 2018). Negri *et al.* (2012) reported the rates of voriconazole resistance in *C. tropicalis* isolates

from Latin America, Europe, and North America were 1,7%, 3,6%, and 2%, respectively (Negri *et al.*, 2012).

Compared with azoles, resistant to polyenes (Amphotericin B) and echinocandins (caspofungin) is quite rare. It is almost always the result of either a decrease in the quantity of ergosterol in the plasma membrane or a change in the target lipid resulting in a decrease in the binding of Amphotericin B to fungal cells (Dick *et al.*, 1980).

In using of disc diffusion method, the zone of inhibition for azole class antifungal agents is not completely clear due to the presence of microcolonies and fuzzier zone resulting in increasing the chances of misinterpretation of results. while the zone of inhibition for polyene (amphotericin B) and echinocandin (caspofungin) classes is clear with sharp margins (Greenfield, 1992).

The condition where mixed cultures are not known (in the case of double *C. albicans* and *C. glabrata* infections) is one of the major disadvantages of the disc diffusion method, the susceptibility of the more susceptible isolate is typically reported as the presence of microcolonies in the inhibition zone should be ignored particularly for azole class of antifungal agents (Greenfield, 1992; Sheehan *et al.*, 2004).

6. CONCLUSION

This study documents that the identification of *Candida* species, which previously seldom conducted in clinical microbiological services for *Candida* species, has become essential for "species-directed therapy". In the distribution of Candida species, the majority was for C. albicans over other NAC species. Consequently, their isolation from clinical specimens can no longer be disregarded as nonpathogens nor can it be simply ignored as contaminants. Furthermore, their susceptibility to antifungal drugs varies; therefore, prompt and correct identification is important to ensure immediate and effective treatment.

Our present study indicates that the majority of isolates were from old adult patients. Moreover, urine specimens followed by sputum specimens were dominant over the other specimens from various body sites.

The present study provides important information about high resistance percentages of *Candida* species to some antifungal agents such as fluconazole, voriconazole, and itraconazole, arranged respectively. Hence, the role of isolating, identifying, and evaluating therapies for *Candida* species has now become a feature of clinical microbiology infrastructure.

The results of this study propose that the resistance in *Candida* species to some azole class antifungal agents should be taken into consideration.

REFERENCES

Abbott, J. (1995). Clinical and Microscopic Diagnosis of Vaginal Yeast Infection: A Prospective Analysis. *Annals of Emergency Medicine*, *25*(5), 587–591. https://doi.org/10.1016/S0196-0644(95)70168-0

Achkar, J. M., & Fries, B. C. (2010). *Candida* Infections of the Genitourinary Tract. *Clinical Microbiology Reviews*, *23*(2), 253. https://doi.org/10.1128/CMR.00076-09

Agwu, E., Ihongbe, J. C., McManus, B. A., Moran, G. P., Coleman, D. C., & Sullivan, D. J. (2012). Distribution of yeast species associated with oral lesions in HIV-infected patients in Southwest Uganda. *Medical Mycology*, *50*(3), 276–280. https://doi.org/10.3109/13693786.2011.604862

Ahmad, A., & Khan, A. U. (2009). Prevalence of *Candida* species and potential risk factors for vulvovaginal candidiasis in Aligarh, India. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *144*(1), 68–71. https://doi.org/10.1016/j.ejogrb.2008.12.020

Ahmad, S., Khan, Z. U., Johny, M., Ashour, N. M., Al-Tourah, W. H., Joseph, L., & Chandy, R. (2013). Isolation of Lodderomyces elongisporus from the Catheter Tip of a Fungemia Patient in the Middle East. *Case Reports in Medicine*, *2013*, 1–5. https://doi.org/10.1155/2013/560406

Akpan, A., & Morgan, R. (2002). Oral candidiasis. *Postgraduate Medical Journal*, *78*(922), 455–459. https://doi.org/10.1136/pmj.78.922.455

Alastruey-Izquierdo, A., Melhem, M. S. C., Bonfietti, L. X., & Rodriguez-Tudela, J. L. (2015). Susceptibility Test For Fungi: Clinical And Laboratorial Correlations In Medical Mycology. *Revista Do Instituto de Medicina Tropical de São Paulo*, *57*(suppl 19), 57–64. https://doi.org/10.1590/S0036-46652015000700011

Alfouzan, W., Dhar, R., Ashkanani, H., Gupta, M., Rachel, C., & Khan, Z. U. (2015). Species spectrum and antifungal susceptibility profile of vaginal isolates

of *Candida* in Kuwait. *Journal de Mycologie Médicale*, *25*(1), 23–28. https://doi.org/10.1016/j.mycmed.2014.10.021

Al-Obaid, K., Ahmad, S., Joseph, L., & Khan, Z. (2018). Lodderomyces elongisporus: A bloodstream pathogen of greater clinical significance. *New Microbes and New Infections*, *26*, 20–24. https://doi.org/10.1016/j.nmni.2018.07.004

Andriole, V. T. (2000). Current and future antifungal therapy: New targets for antifungal therapy. *International Journal of Antimicrobial Agents*, *16*(3), 317–321. https://doi.org/10.1016/S0924-8579(00)00258-2

Arendrup, M. C. (2013). *Candida* and candidaemia. Susceptibility and epidemiology. *Danish Medical Journal*, *60*(11), B4698.

Auger, P., Dumas, C., & Joly, J. (1979). A Study of 666 Strains of *Candida albicans*: Correlation between Serotype and Susceptibility to 5-Fluorocytosine. *The Journal of Infectious Diseases*, *139*(5), 590–594. https://doi.org/10.1093/infdis/139.5.590

Babin, D., Kotigadde, S., Rao, P. S., Rao, T. V., & Babin, M. D. (2013). Clinico-mycological profile of vaginal candidiasis in a tertiary care hospital in Kerala. *Int J Res Biol Sci*, *3*(1), 55–59.

Baradkar, V. P., & Karyakarte, R. P. (1999). Isolation and characterization of *Candida* species in acquired immunodeficiency syndrome. *Indian Journal of Medical Microbiology*, *17*(1), 42.

Barry, A. L., Pfaller, M. A., Rennie, R. P., Fuchs, P. C., & Brown, S. D. (2002). Precision and Accuracy of Fluconazole Susceptibility Testing by Broth Microdilution, Etest, and Disk Diffusion Methods. *Antimicrobial Agents and Chemotherapy*, *46*(6), 1781–1784. https://doi.org/10.1128/AAC.46.6.1781-1784.2002

Berkow, E. L., & Lockhart, S. R. (2017). Fluconazole resistance in *Candida* species: A current perspective. *Infection and Drug Resistance*, *10*, 237–245. https://doi.org/10.2147/IDR.S118892

Bertini, A., De Bernardis, F., Hensgens, L. A. M., Sandini, S., Senesi, S., & Tavanti, A. (2013). Comparison of *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* adhesive properties and pathogenicity. *International Journal of Medical Microbiology*, *303*(2), 98–103. https://doi.org/10.1016/j.ijmm.2012.12.006

Beyda, N. D., Chuang, S. H., Alam, M. J., Shah, D. N., Ng, T. M., McCaskey, L., & Garey, K. W. (2013). Treatment of *Candida famata* bloodstream infections: Case series and review of the literature. *Journal of Antimicrobial Chemotherapy*, *68*(2), 438–443. https://doi.org/10.1093/jac/dks388

Borman, A. M., Szekely, A., & Johnson, E. M. (2016). Comparative Pathogenicity of United Kingdom Isolates of the Emerging Pathogen *Candida auris* and Other Key Pathogenic Candida Species. *MSphere*, *1*(4). https://doi.org/10.1128/mSphere.00189-16

Borman, A. M., Szekely, A., Linton, C. J., Palmer, M. D., Brown, P., & Johnson, E. M. (2013). Epidemiology, Antifungal Susceptibility, and Pathogenicity of *Candida africana* Isolates from the United Kingdom. *Journal of Clinical Microbiology*, *51*(3), 967–972. https://doi.org/10.1128/JCM.02816-12

Bossche, H. vanden, Marichal, P., Odds, F. C., Jeune, L. L., & Coene, M. C. (1992). Characterization of an azole-resistant *Candida glabrata* isolate. *Antimicrobial Agents and Chemotherapy*, *36*(12), 2602–2610. https://doi.org/10.1128/AAC.36.12.2602

Brandt, M. E., Harrison, L. H., Pass, M., Sofair, A. N., Huie, S., Li, R. K., Morrison, C. J., Warnock, D. W., & Hajjeh, R. A. (2000). *Candida dubliniensis* fungemia: The first four cases in North America. *Emerging Infectious Diseases*, *6*(1), 46–49.

Braude, A. I. (1986). *Infectious diseases and medical microbiology*.

Buchacz, K., Lau, B., Jing, Y., Bosch, R., Abraham, A. G., Gill, M. J., Silverberg, M. J., Goedert, J. J., Sterling, T. R., Althoff, K. N., Martin, J. N., Burkholder, G., Gandhi, N., Samji, H., Patel, P., Rachlis, A., Thorne, J. E., Napravnik, S., Henry, K., … Brooks, J. T. (2016). Incidence of AIDS-Defining Opportunistic Infections in a Multicohort Analysis of HIV-infected Persons in the United States and Canada, 2000–2010. *Journal of Infectious Diseases*, *214*(6), 862–872. https://doi.org/10.1093/infdis/jiw085

Bukhary, Z. A. (2008). Candiduria: A Review of Clinical Significance and Management. *Saudi Journal of Kidney Diseases and Transplantation*, *19*(3), 350.

Byadarahally Raju, S., & Rajappa, S. (2011). Isolation and identification of *Candida* from the oral cavity. *International Scholarly Research Network (ISRN) Dentistry*, *2011*.

Calderone, R. A., & Fonzi, W. A. (2001). Virulence factors of *Candida albicans*. *Trends in Microbiology*, *9*(7), 327–335. https://doi.org/10.1016/S0966-842X(01)02094-7

Cameron, S. J. S., Bolt, F., Perdones-Montero, A., Rickards, T., Hardiman, K., Abdolrasouli, A., Burke, A., Bodai, Z., Karancsi, T., Simon, D., Schaffer, R., Rebec, M., Balog, J., & Takáts, Z. (2016). Rapid Evaporative Ionisation Mass Spectrometry (REIMS) Provides Accurate Direct from Culture Species Identification within the Genus *Candida*. *Scientific Reports*, *6*(1), 36788. https://doi.org/10.1038/srep36788

Cantón, E., Espinel-Ingroff, A., & Pemán, J. (2009). Trends in antifungal susceptibility testing using CLSI reference and commercial methods. *Expert Review of Anti-Infective Therapy*, *7*(1), 107–119. https://doi.org/10.1586/14787210.7.1.107

Canuto, M. M., & Rodero, F. G. (2002). Antifungal drug resistance to azoles and polyenes. *The Lancet Infectious Diseases*, *2*(9), 550–563. https://doi.org/10.1016/S1473-3099(02)00371-7

Carvalhinho, S., Costa, A. M., Coelho, A. C., Martins, E., & Sampaio, A. (2012). Susceptibilities of *Candida albicans* Mouth Isolates to Antifungal Agents, Essentials Oils and Mouth Rinses. *Mycopathologia*, *174*(1), 69–76. https://doi.org/10.1007/s11046-012-9520-4

CDC. (2019). *Centers for Disease Control and Prevention. (2019).Candidiasis | Types of Diseses | Fungal Diseases | CDC*. https://www.cdc.gov/fungal/diseases/candidiasis/index.html

Chowdhary, A., Sharma, C., & Meis, J. F. (2017). *Candida auris*: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *PLoS Pathogens*, *13*(5). https://doi.org/10.1371/journal.ppat.1006290

Coronado-Castellote, L., & Jimenez-Soriano, Y. (2013). Clinical and microbiological diagnosis of oral candidiasis. *Journal of Clinical and Experimental Dentistry*, e279-86. https://doi.org/10.4317/jced.51242

Dabas, P. S. (2013). An approach to etiology, diagnosis and management of different types of candidiasis. *Journal of Yeast and Fungal Research*, *4*, 63–74. https://doi.org/10.5897/JYFR2013.0113

Dagi, H. T., Findik, D., Senkeles, C., & Arslan, U. (2016). Identification and antifungal susceptibility of *Candida* species isolated from bloodstream infections in Konya, Turkey. *Annals of Clinical Microbiology and Antimicrobials*, *15*(1), 36. https://doi.org/10.1186/s12941-016-0153-1

Dan, M., Poch, F., & Levin, D. (2002). High rate of vaginal infections caused by *non-C. albicans Candida* species among asymptomatic women. *Medical Mycology*, *40*(4), 383–386. https://doi.org/10.1080/mmy.40.4.383.386

de Freitas, A. R., Baeza, L. C., Faria, M. G. I., Dalben Dota, K. F., Godoy Martínez, P., & Svidzinski, T. I. E. (2014). Yeasts isolated from nosocomial urinary

infections: Antifungal susceptibility and biofilm production. *Revista Iberoamericana de Micología*, *31*(2), 104–108. https://doi.org/10.1016/j.riam.2013.06.004

De la Cruz-Claure, M. L., Cèspedes-Llave, A. A., Ulloa, M. T., Benito-Lama, M., Domínguez-Álvarez, E., & Bastida, A. (2019). Inhibition–Disruption of *Candida glabrata* Biofilms: Symmetrical Selenoesters as Potential Anti-Biofilm Agents. *Microorganisms*, *7*(12), 664. https://doi.org/10.3390/microorganisms7120664

Denning, D. W. (2002). Echinocandins: A new class of antifungal. *Journal of Antimicrobial Chemotherapy*, *49*(6), 889–891. https://doi.org/10.1093/jac/dkf045

Denning, D. W. (2003). Echinocandin antifungal drugs. *The Lancet*, *362*(9390), 1142–1151. https://doi.org/10.1016/S0140-6736(03)14472-8

Denning, D. W., & Hope, W. W. (2010). Therapy for fungal diseases: Opportunities and priorities. *Trends in Microbiology*, *18*(5), 195–204. https://doi.org/10.1016/j.tim.2010.02.004

Deorukhkar, S. C. (2017). *Immunity to Candida Infection: An Overview*. https://doi.org/10.15406/moji.2017.05.00144

Deorukhkar, S. C. (2018). *"Biotyping, Molecular Characterization And Antifungal Susceptibility Of Candida Species Isolated From Various Clinical Specimens In A Rural Tertiary Care Hospital Of Western Maharashtra".* [Pravara Institute Of Medical Sciences (Deemed University)]. https://shodhganga.inflibnet.ac.in/handle/10603/220826

Deorukhkar, S. C., & Roushani, S. (2017). Virulence Traits Contributing to Pathogenicity of *Candida* Species. *Journal of Microbiology & Experimentation*, *5*(5), Article 5. https://www.medcrave.org/index.php/JMEN/article/view/13631

Deorukhkar, S. C., & Saini, S. (2014). Laboratory approach for diagnosis of candidiasis through ages. *International Journal of Current Microbiology and Applied Sciences (IJCMAS)*, *3*, 206–218.

Deorukhkar, S. C., & Saini, S. (2015). Candidiasis: Past, present and future. *Int J Infect Trop Dis*, *2*, 12–24.

Deorukhkar, S. C., & Saini, S. (2016). Why *Candida* Species have Emerged as Important Nosocomial Pathogens? *International Journal of Current Microbiology and Applied Sciences*, *5*(1), 533–545. https://doi.org/10.20546/ijcmas.2016.501.054

Deorukhkar, S. C., Saini, S., & Jadhav, P. A. (2012). Evaluation of different media for germ tube production of *Candida albicans* and *Candida dubliniensis*. *International Journal of Biomedical and Advance Research*, *3*(9), 704–707. https://doi.org/10.7439/ijbar.v3i9.732

Deorukhkar, S. C., Saini, S., & Mathew, S. (2014). *Non-albicans Candida* Infection: An Emerging Threat. *Interdisciplinary Perspectives on Infectious Diseases*, *2014*, 1–7. https://doi.org/10.1155/2014/615958

Dick, J. D., Merz, W. G., & Saral, R. (1980). Incidence of polyene-resistant yeasts recovered from clinical specimens. *Antimicrobial Agents and Chemotherapy*, *18*(1), 158–163. https://doi.org/10.1128/AAC.18.1.158

Dutcher, J. D. (1968). The Discovery and Development of Amphotericin B. *Diseases of the Chest*, *54*, 296–298. https://doi.org/10.1378/chest.54.Supplement_1.296

Eggimann, P., Francioli, P., Bille, J., Schneider, R., Wu, M.-M., Chapuis, G., Chiolero, R., Pannatier, A., Schilling, J., Geroulanos, S., Glauser, M. P., & Calandra, T. (1999). Fluconazole prophylaxis prevents intra-abdominal candidiasis in high-risk surgical patients. *Critical Care Medicine*, *27*(6), 1066–1072.

Eggimann, P., Garbino, J., & Pittet, D. (2003). Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. *The Lancet Infectious Diseases*, *3*(11), 685–702. https://doi.org/10.1016/S1473-3099(03)00801-6

Espinel-Ingroff, A., & Cantón, E. (Eds.). (2007). Antifungal Susceptibility Testing of Yeasts. In *Antimicrobial Susceptibility Testing Protocols* (5th ed, pp. 174–207). http://www.crcpress.com

Espinel-Ingroff, A., Canton, E., Gibbs, D., & Wang, A. (2007). Correlation of Neo-Sensitabs Tablet Diffusion Assay Results on Three Different Agar Media with CLSI Broth Microdilution M27-A2 and Disk Diffusion M44-A Results for Testing Susceptibilities of *Candida* spp. And *Cryptococcus neoformans* to Amphotericin B, Caspofungin, Fluconazole, Itraconazole, and Voriconazole. *Journal of Clinical Microbiology*, *45*(3), 858–864. https://doi.org/10.1128/JCM.01900-06

Falagas, M. E., Roussos, N., & Vardakas, K. Z. (2010). Relative frequency of *albicans* and the various *non-albicans Candida* spp among candidemia isolates from inpatients in various parts of the world: A systematic review. *International Journal of Infectious Diseases*, *14*(11), e954–e966. https://doi.org/10.1016/j.ijid.2010.04.006

Fidel, P. L. (2002). Immunity to *Candida*. *Oral Diseases*, *8*(s2), 69–75. https://doi.org/10.1034/j.1601-0825.2002.00015.x

Fidel, Paul L., Vazquez, J. A., & Sobel, J. D. (1999). *Candida glabrata*: Review of Epidemiology, Pathogenesis, and Clinical Disease with Comparison to C. albicans. *Clinical Microbiology Reviews*, *12*(1), 80. https://doi.org/10.1128/CMR.12.1.80

Fisher, J. F. (2011). *Candida* Urinary Tract Infections—Epidemiology, Pathogenesis, Diagnosis, and Treatment: Executive Summary. *Clinical Infectious Diseases*, *52*(suppl_6), S429–S432. https://doi.org/10.1093/cid/cir108

Fothergill, A. W. (2012). Antifungal Susceptibility Testing: Clinical Laboratory and Standards Institute (CLSI) Methods. In G. S. Hall (Ed.), *Interactions of Yeasts, Moulds, and Antifungal Agents* (pp. 65–74). Humana Press. https://doi.org/10.1007/978-1-59745-134-5_2

Galán-Sánchez, F., García-Agudo, L., García-Martos, P., & Rodríguez-Iglesias, M. (2014). *Candida galli* as a Cause of Tinea Unguium—Molecular Characterization of a Rare Clinical Fungal Entity. *Mycopathologia*, *178*(3), 303–306. https://doi.org/10.1007/s11046-014-9789-6

Giri, S., & Kindo, A. J. (2012). A review of *Candida* species causing blood stream infection. *Indian Journal of Medical Microbiology*, *30*(3), 270. https://doi.org/10.4103/0255-0857.99484

Gonçalves, B., Ferreira, C., Alves, C. T., Henriques, M., Azeredo, J., & Silva, S. (2016). Vulvovaginal candidiasis: Epidemiology, microbiology and risk factors. *Critical Reviews in Microbiology*, *42*(6), 905–927. https://doi.org/10.3109/1040841X.2015.1091805

Góralska, K., Dynowska, M., Barańska, G., Troska, P., & Tenderenda, M. (2011). Taxonomic characteristic of yeast-like fungi and yeasts isolated from respiratory system and digestive tract of human. *Medical Mycology/Mikologia*, *18*(4).

Greenfield, R. A. (1992). Host defense system interactions with *Candida*. *Journal of Medical and Veterinary Mycology*, *30*(2), 89–104. https://doi.org/10.1080/02681219280000141

Guzel, A. B., Ilkit, M., Akar, T., Burgut, R., & Demir, S. C. (2011). Evaluation of risk factors in patients with vulvovaginal candidiasis and the value of chromID *Candida* agar versus CHROMagar *Candida* for recovery and presumptive identification of vaginal yeast species. *Medical Mycology*, *49*(1), 16–25. https://doi.org/10.3109/13693786.2010.497972

Ha, J. F., Italiano, C. M., Heath, C. H., Shih, S., Rea, S., & Wood, F. M. (2011). Candidemia and invasive candidiasis: A review of the literature for the burns surgeon. *Burns*, *37*(2), 181–195. https://doi.org/10.1016/j.burns.2010.01.005

Hakki, M., Staab, J. F., & Marr, K. A. (2006). Emergence of a *Candida krusei* Isolate with Reduced Susceptibility to Caspofungin during Therapy. *Antimicrobial*

Agents and Chemotherapy, *50*(7), 2522–2524. https://doi.org/10.1128/AAC.00148-06

Hasenclever, H. F., & Mitchell, W. O. (1961). Antigenic studies of Candida: I. Observation of two antigenic groups in *Candida albicans*. *Journal of Bacteriology*, *82*(4), 570–573.

Hasenclever, H. F., Mitchell, W. O., & Loewe, J. (1961). Antigenic studiesof *Candida*: II. Antigenic relation of *Candida albicans* group A and group B to *Candida stellatoidea* and *Candida tropicalis*. *Journal of Bacteriology*, *82*(4), 574.

Henao, N. A., & Vagner, B. (2011). Infections of the central nervous system by *Candida*. *Journal of Infectious Diseases and Immunity*, *3*, 79–84.

Hernandez, S., López-Ribot, J. L., Najvar, L. K., McCarthy, D. I., Bocanegra, R., & Graybill, J. R. (2004). Caspofungin Resistance in *Candida albicans*: Correlating Clinical Outcome with Laboratory Susceptibility Testing of Three Isogenic Isolates Serially Obtained from a Patient with Progressive *Candida* Esophagitis. *Antimicrobial Agents and Chemotherapy*, *48*(4), 1382–1383. https://doi.org/10.1128/AAC.48.4.1382-1383.2004

Holzschu, D. L., Presley, H. L., Miranda, M., & Phaff, H. J. (1979). Identification of *Candida lusitaniae* as an opportunistic yeast in humans. *Journal of Clinical Microbiology*, *10*(2), 202.

Horng, W. B., Lee, C. P., & Chen, C. W. (2001). Classification of age groups based on facial features. 4(3), 183-192.

Horvath, L. L., Hospenthal, D. R., Murray, C. K., & Dooley, D. P. (2003). Direct Isolation of *Candida* spp. From Blood Cultures on the Chromogenic Medium CHROMagar Candida. *Journal of Clinical Microbiology*, *41*(6), 2629–2632. https://doi.org/10.1128/JCM.41.6.2629-2632.2003

Hospenthal, D. R., Beckius, M. L., Floyd, K. L., Horvath, L. L., & Murray, C. K. (2006). Presumptive identification of *Candida* species other than *C. albicans* , *C. krusei* , and *C. tropicalis* with the chromogenic medium CHROMagar *Candida*.

Annals of Clinical Microbiology and Antimicrobials, *5*(1), 1–5. https://doi.org/10.1186/1476-0711-5-1

Jabeen, K., Kumar, H., Farooqi, J., Mehboob, R., Brandt, M. E., & Zafar, A. (2016). Agreement of Direct Antifungal Susceptibility Testing from Positive Blood Culture Bottles with the Conventional Method for *Candida* Species. *Journal of Clinical Microbiology*, *54*(2), 343–348. https://doi.org/10.1128/JCM.02432-15

Jabra-Rizk, M. A., Baqui, A. a. M. A., Kelley, J. I., Falkler, W. A., Merz, W. G., & Meiller, T. F. (1999). Identification of *Candida dubliniensisin* a Prospective Study of Patients in the United States. *Journal of Clinical Microbiology*, *37*(2), 321–326. https://doi.org/10.1128/JCM.37.2.321-326.1999

Jain, S., & Sehgal, V. N. (2000). Onychomycosis: An epidemio-etiologic perspective. *International Journal of Dermatology*, *39*(2), 100–103. https://doi.org/10.1046/j.1365-4362.2000.00808.x

Jeddy, N., Ranganathan, K., Devi, U., & Joshua, E. (2011). A study of antifungal drug sensitivity of *Candida* isolated from human immunodeficiency virus infected patients in Chennai, South India. *Journal of Oral and Maxillofacial Pathology : JOMFP*, *15*(2), 182–186. https://doi.org/10.4103/0973-029X.84490

Jindal, N., Gill, P., & Aggarwal, A. (2007). An epidemiological study of vulvovaginal candidiasis in women of childbearing age. *Indian Journal of Medical Microbiology*, *25*(2), 175. https://doi.org/10.4103/0255-0857.32736

Johnson, E. M. (2008). Issues in antifungal susceptibility testing. *Journal of Antimicrobial Chemotherapy*, *61*(suppl_1), i13–i18. https://doi.org/10.1093/jac/dkm427

Kathiravan, M. K., Salake, A. B., Chothe, A. S., Dudhe, P. B., Watode, R. P., Mukta, M. S., & Gadhwe, S. (2012). The biology and chemistry of antifungal agents: A review. *Bioorganic & Medicinal Chemistry*, *20*(19), 5678–5698. https://doi.org/10.1016/j.bmc.2012.04.045

Kauffman, C. A., Fisher, J. F., Sobel, J. D., & Newman, C. A. (2011). *Candida* Urinary Tract Infections—Diagnosis. *Clinical Infectious Diseases*, *52*(suppl_6), S452–S456. https://doi.org/10.1093/cid/cir111

Khan, Z. K., & Gyanchandani, A. (1998). Candidiasis: A review. *PINSA*, *64*, 1–34.

Kliemann, D. A., Pasqualotto, A. C., Falavigna, M., Giaretta, T., & Severo, L. C. (2008). *Candida* esophagitis: Species distribution and risk factors for infection. *Revista Do Instituto de Medicina Tropical de São Paulo*, *50*(5), 261–263. https://doi.org/10.1590/S0036-46652008000500002

Knoke, M., & Bernhardt, H. (2006). The first description of an oesophageal candidosis by Bernhard von Langenbeck in 1839. *Mycoses*, *49*(4), 283–287. https://doi.org/10.1111/j.1439-0507.2006.01237.x

Kobayashi, C. C. B. A., de Fátima Lisboa Fernandes, O., Miranda, K. C., de Sousa, E. D., & do Rosário Rodrigues Silva, M. (2004). Candiduria in Hospital Patients: A Study Prospective. *Mycopathologia*, *158*(1), 49–52. https://doi.org/10.1023/B:MYCO.0000038436.51918.d9

Kojic, E. M., & Darouiche, R. O. (2004). *Candida* Infections of Medical Devices. *Clinical Microbiology Reviews*, *17*(2), 255. https://doi.org/10.1128/CMR.17.2.255-267.2004

Kontoyiannis, D. P., Vaziri, I., Hanna, H. A., Boktour, M., Thornby, J., Hachem, R., Bodey, G. P., & Raad, I. I. (2001). Risk Factors for *Candida tropicalis* Fungemia in Patients with Cancer. *Clinical Infectious Diseases*, *33*(10), 1676–1681. https://doi.org/10.1086/323812

Kothavade, R. J., Kura, M. M., Valand, A. G., & Panthaki, M. H. (2010). *Candida tropicalis*: Its prevalence, pathogenicity and increasing resistance to fluconazole. *Journal of Medical Microbiology*, *59*(8), 873–880. https://doi.org/10.1099/jmm.0.013227-0

Krcmery, V., & Barnes, A. J. (2002). *Non-albicans Candida* spp. causing fungaemia: Pathogenicity and antifungal resistance. *Journal of Hospital Infection*, *50*(4), 243–260. https://doi.org/10.1053/jhin.2001.1151

Kuhn, D. M., George, T., Chandra, J., Mukherjee, P. K., & Ghannoum, M. A. (2002). Antifungal Susceptibility of *Candida* Biofilms: Unique Efficacy of Amphotericin B Lipid Formulations and Echinocandins. *Antimicrobial Agents and Chemotherapy*, *46*(6), 1773–1780. https://doi.org/10.1128/AAC.46.6.1773-1780.2002

Kullberg, B. J., & Arendrup, M. C. (2015). Invasive Candidiasis. *New England Journal of Medicine*, *373*(15), 1445–1456. https://doi.org/10.1056/NEJMra1315399

Kumar, S., Vyas, A., Kumar, M., & Mehra, S. (2013). Application of chromagar *Candida* for identification of clinically important *Candida* species and their antifungal susceptibility pattern. *Int J Biol Med Res*, *4*(4), 3600–3606.

Kurtz, M. B., Cortelyou, M. W., & Kirsch, D. R. (1986). Integrative transformation of *Candida albicans*, using a cloned *Candida* ADE2 gene. *Molecular and Cellular Biology*, *6*(1), 142–149. https://doi.org/10.1128/MCB.6.1.142

Kurtzman, C., Fell, J. W., & Boekhout, T. (2011). *The Yeasts: A Taxonomic Study*. Elsevier.

Kurtzman, C. P. (2011a). Meyerozyma Kurtzman & M. Suzuki (2010). In *The Yeasts: A Taxonomic Study* (pp. 621–624). Elsevier. https://doi.org/10.1016/B978-0-444-52149-1.00047-1

Kurtzman, C. P. (2011b). Yarrowia van der Walt & von Arx (1980). In *The Yeasts: A Taxonomic Study* (pp. 927–929). Elsevier. https://doi.org/10.1016/B978-0-444-52149-1.00082-3

Kwon-Chung, K. J., Riggsby, W. S., Uphoff, R. A., Hicks, J. B., Whelan, W. L., Reiss, E., Magee, B. B., & Wickes, B. L. (1989). Genetic differences between type I and type II *Candida stellatoidea*. *Infection and Immunity*, *57*(2), 527.

Lachance, M.-A., Boekhout, T., Scorzetti, G., Fell, J. W., & Kurtzman, C. P. (2011). *Candida* Berkhout (1923). In *The Yeasts: A Taxonomic Study* (5th ed., Vol. 2, pp. 987–1278). Elsevier. https://doi.org/10.1016/B978-0-444-52149-1.00090-2

Laguna, F., Rodríguez-Tudela, J. L., Martínez-Suárez, J. V., Polo, R., Valencia, E., Díaz-Guerra, T. M., Dronda, F., & Pulido, F. (1997). Patterns of Fluconazole Susceptibility in Isolates from Human Immunodeficiency Virus-Infected Patients with Oropharyngeal Candidiasis Due to *Candida albicans*. *Clinical Infectious Diseases*, *24*(2), 124–130. https://doi.org/10.1093/clinids/24.2.124

Langenbeck, B. (1839). Auffindung von Pilzen auf der Schleimhaut der Speiserohre einer Typhusleiche. *Neue Notizen Aus Dem Gebiete Der Natur-Und Heilkunde*, *12*, 145–147.

Laverdière, M., Lalonde, R. G., Baril, J.-G., Sheppard, D. C., Park, S., & Perlin, D. S. (2006). Progressive loss of echinocandin activity following prolonged use for treatment of *Candida albicans* oesophagitis. *Journal of Antimicrobial Chemotherapy*, *57*(4), 705–708. https://doi.org/10.1093/jac/dkl022

Li, L., Redding, S., & Dongari-Bagtzoglou, A. (2007). *Candida glabrata*, an Emerging Oral Opportunistic Pathogen. *Journal of Dental Research*, *86*(3), 204–215. https://doi.org/10.1177/154405910708600304

Lockhart, S. R. (2014). Current Epidemiology of *Candida* Infection. *Clinical Microbiology Newsletter*, *36*(17), 131–136. https://doi.org/10.1016/j.clinmicnews.2014.08.001

López-Martínez, R. (2010). Candidosis, a new challenge. *Clinics in Dermatology*, *28*(2), 178–184. https://doi.org/10.1016/j.clindermatol.2009.12.014

Luque, A. G., Biasoli, M. S., Tosello, M. E., Binolfi, A., Lupo, S., & Magaró, H. M. (2009). Oral yeast carriage in HIV-infected and non-infected populations in Rosario, Argentina. *Mycoses*, *52*(1), 53–59. https://doi.org/10.1111/j.1439-0507.2008.01542.x

Lynch, D. P. (1994). Oral candidiasis: History, classification, and clinical presentation. *Oral Surgery, Oral Medicine, Oral Pathology*, *78*(2), 189–193. https://doi.org/10.1016/0030-4220(94)90146-5

Ma, L., Xu, R., Shi, J., Zhou, W., Xu, G., Jiang, G., Li, G., & Chen, Z. (2013). Identification of fungi in fungal ball sinusitis: Comparison between MUC5B immunohistochemical and Grocott methenamine silver staining. *Acta Oto-Laryngologica*, *133*(11), 1181–1187. https://doi.org/10.3109/00016489.2013.814156

Maertens, J. A. (2004). History of the development of azole derivatives. *Clinical Microbiology and Infection*, *10*, 1–10. https://doi.org/10.1111/j.1470-9465.2004.00841.x

Maheshwari, R. (2016). *Fungi: Experimental Methods In Biology*, *Second Edition* (2nd ed., Vol. 28). CRC Press.

Mahmoudi Rad, M., Zafarghandi, S., Abbasabadi, B., & Tavallaee, M. (2011). The epidemiology of *Candida* species associated with vulvovaginal candidiasis in an Iranian patient population. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *155*(2), 199–203. https://doi.org/10.1016/j.ejogrb.2010.11.022

Mane, A., Panchvalli, S., Bembalkar, S., & Risbud, A. (2010). Species distribution & antifungal susceptibility of oral *Candida* colonising or infecting HIV infected individuals. *Indian Journal of Medical Research, 131*(6), 836–839.

Mane, A., Pawale, C., Gaikwad, S., Bembalkar, S., & Risbud, A. (2011). Adherence to buccal epithelial cells, enzymatic and hemolytic activities of *Candida* isolates from HIV-infected individuals. *Medical Mycology*, *49*(5), 548–551. https://doi.org/10.3109/13693786.2010.540044

Marot-Leblond, A., Grimaud, L., Nail, S., Bouterige, S., Apaire-Marchais, V., Sullivan, D. J., & Robert, R. (2000). New Monoclonal Antibody Specific for

Candida albicans Germ Tube. *Journal of Clinical Microbiology*, *38*(1), 61–67. https://doi.org/10.1128/JCM.38.1.61-67.2000

Martins, N., Barros, L., Henriques, M., Silva, S., & Ferreira, I. C. F. R. (2015). Activity of phenolic compounds from plant origin against *Candida* species. *Industrial Crops and Products*, *74*, 648–670. https://doi.org/10.1016/j.indcrop.2015.05.067

Mathavi, S., Sasikala, G., Kavitha, A., & Priyadarsini, R. I. (2016). CHROMagar as a primary isolation medium for rapid identification of *Candida* and its role in mixed *Candida* infection in sputum samples. *Indian Journal of Microbiology Research*, *3*(2), 141. https://doi.org/10.5958/2394-5478.2016.00033.9

Mayer, F. L., Wilson, D., & Hube, B. (2013). *Candida albicans* pathogenicity mechanisms. *Virulence*, *4*(2), 119–128. https://doi.org/10.4161/viru.22913

Mayser, P. P. M., & Gräser, Y. (2011). Superficial Fungal Infections. In A. D. Irvine, P. H. Hoeger, & A. C. Yan (Eds.), *Harper's Textbook of Pediatric Dermatology* (p. 62.1-62.34). Wiley-Blackwell. https://doi.org/10.1002/9781444345384.ch62

McCool, L. (2010). *The Discovery and Naming of Candida albicans*. 2.

McCullough, M. J., Ross, B. C., & Reade, P. C. (1996). *Candida albicans*: A review of its history, taxonomy, epidemiology, virulence attributes, and methods of strain differentiation. *International Journal of Oral and Maxillofacial Surgery*, *25*(2), 136–144. https://doi.org/10.1016/S0901-5027(96)80060-9

Méan, M., Marchetti, O., & Calandra, T. (2008). Bench-to-bedside review: *Candida* infections in the intensive care unit. *Critical Care*, *12*(1), 204. https://doi.org/10.1186/cc6212

Mehta, R., & Wyawahare, A. S. (2016). *Evaluation of Hicrome Candida Differential Agar for Species Identification of Candida Isolates from Various Clinical Samples*. *International Journal of Contemporary Medical Research 3*(4), 4, 1219-1222.

Meis, J. F. G. M., Ruhnke, M., De Pauw, B. E., Odds, F. C., Siegert, W., & Verweij, P. E. (1999). *Candida dubliniensis* Candidemia in Patients with Chemotherapy-Induced Neutropenia and Bone Marrow Transplantation. *Emerging Infectious Diseases*, *5*(1), 150–153. https://doi.org/10.3201/eid0501.990119

Meyer, S. A., Payne, R. W., & Yarrow, D. (1998). *Candida* Berkhout. In C. P. Kurtzman & J. W. Fell (Eds.), *The yeasts: A taxonomic study* (pp. 454–573). Elsevier, Amsterdam. https://repository.rothamsted.ac.uk/item/87x5q/candida-berkhout

Miceli, M. H., Díaz, J. A., & Lee, S. A. (2011). Emerging opportunistic yeast infections. *The Lancet Infectious Diseases*, *11*(2), 142–151. https://doi.org/10.1016/S1473-3099(10)70218-8

Moges, B., Bitew, A., & Shewaamare, A. (2016). Spectrum and the in vitro antifungal susceptibility pattern of yeast isolates in Ethiopian HIV patients with oropharyngeal candidiasis. *International Journal of Microbiology*, *2016*.

Mohanty, S., Xess, I., Hasan, F., Kapil, A., Mittal, S., & Tolosa, J. E. (2007). Prevalence & susceptibility to fluconazole of *Candida* species causing vulvovaginitis. *Indian Journal of Medical Research*, *126*(3), 216.

Mooney, M. A., Thomas, I., & Sirois, D. (1995). Oral Candidosis. *International Journal of Dermatology*, *34*(11), 759–765. https://doi.org/10.1111/j.1365-4362.1995.tb04392.x

Moran, G., Coleman, D., & Sullivan, D. (2012). An Introduction to the Medically Important *Candida* Species. *Candida and Candidiasis, Second Edition*, 11–25.

Mulu, A., Kassu, A., Anagaw, B., Moges, B., Gelaw, A., Alemayehu, M., Belyhun, Y., Biadglegne, F., Hurissa, Z., Moges, F., & Isogai, E. (2013). Frequent detection of 'azole' resistant *Candida* species among late presenting AIDS patients in northwest Ethiopia. *BMC Infectious Diseases*, *13*(1), 82. https://doi.org/10.1186/1471-2334-13-82

Nadeem, S. G., Hakim, S. T., & Kazmi, S. U. (2010). Use of CHROMagar Candida for the presumptive identification of *Candida* species directly from clinical specimens in resource-limited settings. *Libyan Journal of Medicine*, *5*(1), 2144. https://doi.org/10.3402/ljm.v5i0.2144

Negri, M., Silva, S., Henriques, M., & Oliveira, R. (2012). Insights into *Candida tropicalis* nosocomial infections and virulence factors. *European Journal of Clinical Microbiology & Infectious Diseases*, *31*(7), 1399–1412. https://doi.org/10.1007/s10096-011-1455-z

Neppelenbroek, K., Seó, R., Urban, V., Silva, S., Dovigo, L., Jorge, J., & Campanha, N. (2014). Identification of *Candida* species in the clinical laboratory: A review of conventional, commercial, and molecular techniques. *Oral Diseases*, *20*(4), 329–344. https://doi.org/10.1111/odi.12123

Netea, M. G., & Maródi, L. (2010). Innate immune mechanisms for recognition and uptake of *Candida* species. *Trends in Immunology*, *31*(9), 346–353. https://doi.org/10.1016/j.it.2010.06.007

Obručová, H., Kotásková, I., Tihelková, R., Holá, V., Růžička, F., & Freiberger, T. (2019). Fluorescent Capillary Electrophoresis Is Superior to Culture in Detecting *Candida* Species from Samples of Urinary Catheters and Ureteral Stents with Mono- or Polyfungal Biofilm Growth. *Journal of Clinical Microbiology*, *57*(4), e01861-18, /jcm/57/4/JCM.01861-18.atom. https://doi.org/10.1128/JCM.01861-18

Odds, F. C. (1988). Candida and candidosis: A review and bibliography. 2nd edition. *Candida and Candidosis: A Review and Bibliography. 2nd Edition.* https://www.cabdirect.org/cabdirect/abstract/19892057780

Odds, F. C. (1991). Sabouraud('s) agar. *Journal of Medical and Veterinary Mycology: Bi-Monthly Publication of the International Society for Human and Animal Mycology*, *29*(6), 355–359. https://doi.org/10.1080/02681219180000581

Odds, F C, & Bernaerts, R. (1994). CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *Journal of Clinical Microbiology*, *32*(8), 1923–1929. https://doi.org/10.1128/JCM.32.8.1923-1929.1994

Odds, Frank C., Brown, A. J. P., & Gow, N. A. R. (2003). Antifungal agents: Mechanisms of action. *Trends in Microbiology*, *11*(6), 272–279. https://doi.org/10.1016/S0966-842X(03)00117-3

Ostrosky-Zeichner, L., Rex, J. H., Pappas, P. G., Hamill, R. J., Larsen, R. A., Horowitz, H. W., Powderly, W. G., Hyslop, N., Kauffman, C. A., Cleary, J., Mangino, J. E., & Lee, J. (2003). Antifungal Susceptibility Survey of 2,000 Bloodstream *Candida* Isolates in the United States. *Antimicrobial Agents and Chemotherapy*, *47*(10), 3149. https://doi.org/10.1128/AAC.47.10.3149-3154.2003

Oxman, D. A., Chow, J. K., Frendl, G., Hadley, S., Hershkovitz, S., Ireland, P., McDermott, L. A., Tsai, K., Marty, F. M., Kontoyiannis, D. P., & Golan, Y. (2010). Candidaemia associated with decreased in vitro fluconazole susceptibility: Is *Candida* speciation predictive of the susceptibility pattern? *Journal of Antimicrobial Chemotherapy*, *65*(7), 1460–1465. https://doi.org/10.1093/jac/dkq136

Oz, Y., & Gokbolat, E. (2018). Evaluation of direct antifungal susceptibility testing methods of *Candida* spp. From positive blood culture bottles. *Journal of Clinical Laboratory Analysis*, *32*(3), e22297. https://doi.org/10.1002/jcla.22297

Ozhak-Baysan, B., Ogunc, D., Colak, D., Ongut, G., Donmez, L., Vural, T., & Gunseren, F. (2012). Distribution and antifungal susceptibility of *Candida* species causing nosocomial candiduria. *Medical Mycology*, *50*(5), 529–532. https://doi.org/10.3109/13693786.2011.618996

Pahwa, N., Kumar, R., Nirkhiwale, S., & Bandi, A. (2014). Species distribution and drug susceptibility of *Candida* in clinical isolates from a tertiary care centre at

Indore. *Indian Journal of Medical Microbiology*, *32*(1), 44. https://doi.org/10.4103/0255-0857.124300

Pappagianis, D., Collins, M. S., Hector, R., & Remington, J. (1979). Development of Resistance to Amphotericin B in *Candida lusitaniae* Infecting a Human. *Antimicrobial Agents and Chemotherapy*, *16*(2), 123. https://doi.org/10.1128/AAC.16.2.123

Pappas, P. G., Kauffman, C. A., Andes, D. R., Clancy, C. J., Marr, K. A., Ostrosky-Zeichner, L., Reboli, A. C., Schuster, M. G., Vazquez, J. A., Walsh, T. J., Zaoutis, T. E., & Sobel, J. D. (2015). Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*, civ933. https://doi.org/10.1093/cid/civ933

Patil, AshaB., Nadagir, ShobhaD., Shepur, T. A., Gadadavar, S., Mythri, B. A., & Shettar, ShyamalaK. (2012). Evaluation of HiCrome differential agar for speciation of *Candida*. *Journal of Academy of Medical Sciences*, *2*(3), 101. https://doi.org/10.4103/2249-4855.132950

Paul, N., Mathai, E., Abraham, O. C., & Mathai, D. (2004). Emerging Microbiological Trends in Candiduria. *Clinical Infectious Diseases*, *39*(11), 1743–1744. https://doi.org/10.1086/426083

Perfect, J. R. (2013). Fungal diagnosis: How do we do it and can we do better? *Current Medical Research and Opinion*, *29*(sup4), 3–11. https://doi.org/10.1185/03007995.2012.761134

Pfaller, M. A., Boyken, L., Messer, S. A., Hollis, R. J., & Diekema, D. J. (2004). Stability of Mueller-Hinton Agar Supplemented with Glucose and Methylene Blue for Disk Diffusion Testing of Fluconazole and Voriconazole. *Journal of Clinical Microbiology*, *42*(3), 1288–1289. https://doi.org/10.1128/JCM.42.3.1288-1289.2004

Pfaller, M. A., & Diekema, D. J. (2007). Epidemiology of Invasive Candidiasis: A Persistent Public Health Problem. *Clinical Microbiology Reviews*, *20*(1), 133–163. https://doi.org/10.1128/CMR.00029-06

Pfaller, M. A., Diekema, D. J., Andes, D., Arendrup, M. C., Brown, S. D., Lockhart, S. R., Motyl, M., & Perlin, D. S. (2011). Clinical breakpoints for the echinocandins and *Candida* revisited: Integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. *Drug Resistance Updates*, *14*(3), 164–176. https://doi.org/10.1016/j.drup.2011.01.004

Pfaller, M. A., Diekema, D. J., Gibbs, D. L., Newell, V. A., Nagy, E., Dobiasova, S., ... & Global Antifungal Surveillance Group. (2008). Candida krusei, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. Journal of clinical microbiology, 46(2), 515-521.

Pfaller, M. A., Jones, R. N., Doern, G. V., Sader, H. S., Hollis, R. J., Messer, S. A., & Group, for T. S. P. (1998). International Surveillance of Bloodstream Infections Due to *Candida* Species: Frequency of Occurrence and Antifungal Susceptibilities of Isolates Collected in 1997 in the United States, Canada, and South America for the SENTRY Program. *Journal of Clinical Microbiology*, *36*(7), 1886. https://doi.org/10.1128/JCM.36.7.1886-1889.1998

Pfaller, Michael A. (1996). Nosocomial Candidiasis: Emerging Species, Reservoirs, and Modes of Transmission. *Clinical Infectious Diseases*, *22*(Supplement_2), S89–S94. https://doi.org/10.1093/clinids/22.Supplement_2.S89

Pfaller, Michael A. (2008). New developments in the antifungal susceptibility testing of *Candida*. *Current Fungal Infection Reports*, *2*(3), 125–133. https://doi.org/10.1007/s12281-008-0019-x

Pfaller, Michael A. (2012). Antifungal Drug Resistance: Mechanisms, Epidemiology, and Consequences for Treatment. *The American Journal of*

Medicine, *125*(1, Supplement), S3–S13. https://doi.org/10.1016/j.amjmed.2011.11.001

Piarroux, R., Grenouillet, F., Balvay, P., Tran, V., Blasco, G., Millon, L., & Boillot, A. (2004). Assessment of preemptive treatment to prevent severe candidiasis in critically ill surgical patients(*). *Critical Care Medicine*, *32*(12), 2443–2449. https://doi.org/10.1097/01.CCM.0000147726.62304.7F

Pincus, D. H., Orenga, S., & Chatellier, S. (2007). Yeast identification – past, present, and future methods. *Medical Mycology*, *45*(2), 97–121. https://doi.org/10.1080/13693780601059936

Posch, W., Heimdörfer, D., Wilflingseder, D., & Lass-Flörl, C. (2017). Invasive candidiasis: Future directions in non-culture based diagnosis. *Expert Review of Anti-Infective Therapy*, *15*(9), 829–838. https://doi.org/10.1080/14787210.2017.1370373

Powderly, W. G., Kobayashi, G. S., Herzig, G. P., & Medoff, G. (1988). Amphotericin B-resistant yeast infection in severely immunocompromised patients. *The American Journal of Medicine*, *84*(5), 826–832. https://doi.org/10.1016/0002-9343(88)90059-9

Prasad, R., Shah, A. H., & Rawal, M. K. (2016). Antifungals: Mechanism of action and drug resistance. In *Yeast Membrane Transport* (pp. 327–349). Springer.

Proia, L. A. (2006). New antifungal therapies. *Clinical Microbiology Newsletter*, *28*(22), 169–173. https://doi.org/10.1016/j.clinmicnews.2006.11.001

R., Y., M.P., S., U.A., B., R., R., & K.B., A. (2013). Candiduria: Prevalence and Trends in Antifungal Susceptibility in A Tertiary Care Hospital of Mangalore. *Journal of Clinical and Diagnostic Research : JCDR*, *7*(11), 2459–2461. https://doi.org/10.7860/JCDR/2013/6298.3578

Refai, M., El-Enbaawy, M., & Hassan, A. (2015). *Monograph On Candida albicans*. https://scholar.cu.edu.eg/?q=hanem/files/candida_albicans.pdf

Reiss, E., & Morrison, C. J. (1993). Nonculture methods for diagnosis of disseminated candidiasis. *Clinical Microbiology Reviews*, *6*(4), 311. https://doi.org/10.1128/CMR.6.4.311

Repentigny, L. de, Lewandowski, D., & Jolicoeur, P. (2004). Immunopathogenesis of Oropharyngeal Candidiasis in Human Immunodeficiency Virus Infection. *Clinical Microbiology Reviews*, *17*(4), 729–759. https://doi.org/10.1128/CMR.17.4.729-759.2004

Revankar, S. G., & Sobel, J. D. (2012). Mucosal Candidiasis. *Candida and Candidiasis, Second Edition*, 419–427. https://doi.org/10.1128/9781555817176.ch26

Rex, J. H., Alexander, B. D., Andes, D., Arthington-Skaggs, B., Brown, S. D., & Chaturveli, V. (2008). Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard—Third edition (M27-A3). *Clinical and Laboratory Standards Institute (CLSI), Wayne, PA*, *28*(14).

Rex, J H, Pfaller, M. A., Barry, A. L., Nelson, P. W., & Webb, C. D. (1995). Antifungal susceptibility testing of isolates from a randomized, multicenter trial of fluconazole versus amphotericin B as treatment of nonneutropenic patients with candidemia. NIAID Mycoses Study Group and the Candidemia Study Group. *Antimicrobial Agents and Chemotherapy*, *39*(1), 40. https://doi.org/10.1128/AAC.39.1.40

Rex, John H., Bennett, J. E., Sugar, A. M., Pappas, P. G., van der Horst, C. M., Edwards, J. E., Washburn, R. G., Scheld, W. M., Karchmer, A. W., Dine, A. P., Levenstein, M. J., & Webb, C. D. (1994). A Randomized Trial Comparing Fluconazole with Amphotericin B for the Treatment of Candidemia in Patients without Neutropenia. *New England Journal of Medicine*, *331*(20), 1325–1330. https://doi.org/10.1056/NEJM199411173312001

Rex, John H., Pfaller, M. A., Walsh, T. J., Chaturvedi, V., Espinel-Ingroff, A., Ghannoum, M. A., Gosey, L. L., Odds, F. C., Rinaldi, M. G., Sheehan, D. J., & Warnock, D. W. (2001). Antifungal Susceptibility Testing: Practical Aspects and

Current Challenges. *Clinical Microbiology Reviews*, *14*(4), 643–658. https://doi.org/10.1128/CMR.14.4.643-658.2001

Rippon, J. W. (1988). Pathogenic fungi and the pathogenic actinomycetes. *Medical Mycology*, 226–229.

Roux, G., & Linossier, G. (1890). *Recherches morphologiques sur le champignon du muguet...*

Rüping, M. J. G. T., Vehreschild, J. J., & Cornely, O. A. (2008). Patients at High Risk of Invasive Fungal Infections. *Drugs*, *68*(14), 1941–1962. https://doi.org/10.2165/00003495-200868140-00002

Sahand, I. H., Moragues, M. D., Eraso, E., Villar-Vidal, M., Quindós, G., & Pontón, J. (2005). Supplementation of CHROMagar *Candida* Medium with Pal's Medium for Rapid Identification of *Candida dubliniensis*. *Journal of Clinical Microbiology*, *43*(11), 5768–5770. https://doi.org/10.1128/JCM.43.11.5768-5770.2005

Samaranayake, L. P., MacFarlane, T. W., & Williamson, M. I. (1987). Comparison of Sabouraud dextrose and Pagano-Levin agar media for detection and isolation of yeasts from oral samples. *Journal of Clinical Microbiology*, *25*(1), 162.

Samaranayake Y, Y. H., & Samaranayake L, L. P. (1994). *Candida krusei*: Biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen. *Journal of Medical Microbiology*, *41*(5), 295–310. https://doi.org/10.1099/00222615-41-5-295

Sanglard, D., Ischer, F., Calabrese, D., Majcherczyk, P. A., & Bille, J. (1999). The ATP Binding Cassette Transporter GeneCgCDR1 from *Candida glabrata* Is Involved in the Resistance of Clinical Isolates to Azole Antifungal Agents. *Antimicrobial Agents and Chemotherapy*, *43*(11), 2753–2765. https://doi.org/10.1128/AAC.43.11.2753

Sanglard, D., & Odds, F. C. (2002). Resistance of *Candida* species to antifungal agents: Molecular mechanisms and clinical consequences. *The Lancet Infectious Diseases*, *2*(2), 73–85. https://doi.org/10.1016/S1473-3099(02)00181-0

Sanguinetti, M., & Posteraro, B. (2018). Susceptibility Testing of Fungi to Antifungal Drugs. *Journal of Fungi*, *4*(3), 110. https://doi.org/10.3390/jof4030110

Sardi, J. C. O., Scorzoni, L., Bernardi, T., Fusco-Almeida, A. M., & Mendes Giannini, M. J. S. (2013). *Candida* species: Current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *Journal of Medical Microbiology*, *62*(1), 10–24. https://doi.org/10.1099/jmm.0.045054-0

Satoh, K., Makimura, K., Hasumi, Y., Nishiyama, Y., Uchida, K., & Yamaguchi, H. (2009). *Candida auris* sp. Nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. *Microbiology and Immunology*, *53*(1), 41–44. https://doi.org/10.1111/j.1348-0421.2008.00083.x

Segal, E., & Elad, D. (2010). Candidiasis. In B. W. J. Mahy, V. ter Meulen, S. P. Borriello, P. R. Murray, G. Funke, S. H. E. Kaufmann, M. W. Steward, W. G. Merz, R. J. Hay, F. E. G. Cox, D. Wakelin, S. H. Gillespie, & D. D. Despommier (Eds.), *Topley & Wilson's Microbiology and Microbial Infections* (p. taw0157). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470688618.taw0157

Seneviratne, C., Jin, L., & Samaranayake, L. (2008). Biofilm lifestyle of *Candida*: A mini review: *Oral Diseases*, *14*(7), 582–590. https://doi.org/10.1111/j.1601-0825.2007.01424.x

Sheehan, D. J., Brown, S. D., Pfaller, M. A., Warnock, D. W., Rex, J. H., Chaturvedi, V., Espinel-Ingroff, A., Ghannoum, M. A., Moore, L. S., & Odds, F. C. (2004). Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline (M44-A). *Pennsylvania, USA: National Committee for Clinical Laboratory Standards M27-A2*, *24*(15).

Shivaprakash, M. R. (2015). *Standard Operating Procedures* [PhD Thesis]. Indian Council of Medical Research.

Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D. W., & Azeredo, J. (2012). *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: Biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiology Reviews*, *36*(2), 288–305. https://doi.org/10.1111/j.1574-6976.2011.00278.x

Silva, S., Rodrigues, C. F., Araújo, D., Rodrigues, M. E., & Henriques, M. (2017). *Candida* Species Biofilms' Antifungal Resistance. *Journal of Fungi*, *3*(1), 8. https://doi.org/10.3390/jof3010008

Sobel, J. D., Kauffman, C. A., McKinsey, D., Zervos, M., Vazquez, J. A., Karchmer, A. W., Lee, J., Thomas, C., Panzer, H., & Dismukes, W. E. (2000). Candiduria: A Randomized, Double-Blind Study of Treatment with Fluconazole and Placebo. *Clinical Infectious Diseases*, *30*(1), 19–24. https://doi.org/10.1086/313580

Sobel, Jack D. (2014). Genital candidiasis. *Medicine*, *42*(7), 364–368. https://doi.org/10.1016/j.mpmed.2014.04.006

Souza, M. N., Ortiz, S. O., Mello, M. M., Oliveira, F. De M., Severo, L. C., & Goebel, C. S. (2015). Comparison Between Four Usual Methods Of Identification Of *Candida* Species. *Revista Do Instituto de Medicina Tropical de São Paulo*, *57*(4), 281–287. https://doi.org/10.1590/S0036-46652015000400002

Spivak, E. S., & Hanson, K. E. (2017). *Candida auris*: An Emerging Fungal Pathogen. *Journal of Clinical Microbiology*, *56*(2), e01588-17. https://doi.org/10.1128/JCM.01588-17

Srinivasan, A., Lopez-Ribot, J. L., & Ramasubramanian, A. K. (2014). Overcoming antifungal resistance. *Drug Discovery Today: Technologies*, *11*, 65–71. https://doi.org/10.1016/j.ddtec.2014.02.005

St Germain, G., & Beauchesne, D. (1991). Evaluation of the MicroScan Rapid Yeast Identification panel. *Journal of Clinical Microbiology*, *29*(10), 2296.

Sturaro, L. L., Gonoi, T., Busso-Lopes, A. F., Tararam, C. A., Levy, C. E., Lyra, L., Trabasso, P., Schreiber, A. Z., Kamei, K., & Moretti, M. L. (2018). Visible DNA Microarray System as an Adjunctive Molecular Test in Identification of Pathogenic Fungi Directly from a Blood Culture Bottle. *Journal of Clinical Microbiology*, *56*(5), e01908-17, /jcm/56/5/e01908-17.atom. https://doi.org/10.1128/JCM.01908-17

Sullivan, D., & Coleman, D. (1998). *Candida dubliniensis*: Characteristics and Identification. *Journal of Clinical Microbiology*, *36*(2), 329–334. https://doi.org/10.1128/JCM.36.2.329-334.1998

Sullivan, D. J., Henman, M. C., Moran, G. P., O'Neill, L. C., Bennett, D. E., Shanley, D. B., & Coleman, D. C. (1996). Molecular genetic approaches to identification, epidemiology and taxonomy of *non-albicans Candida* species. *Journal of Medical Microbiology*, *44*(6), 399–408. https://doi.org/10.1099/00222615-44-6-399

Sullivan, Derek J., Moran, G., Donnelly, S., Gee, S., Pinjon, E., McCartan, B., Shanley, D. B., & Coleman, D. C. (1999). *Candida dubliniensis*: An update. *Revista Iberoamericana de Micología*, *16*, 72–76.

Sullivan, Derek J., Westerneng, T. J., Haynes, K. A., Bennett, D. E., & Coleman, D. C. (1995). *Candida dubliniensis* sp. nov.: Phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. *Microbiology*, *141*(7), 1507–1521. https://doi.org/10.1099/13500872-141-7-1507

Tay, S. T., Na, S. L., & Chong, J. (2009). Molecular differentiation and antifungal susceptibilities of *Candida parapsilosis* isolated from patients with bloodstream infections. *Journal of Medical Microbiology*, *58*(2), 185–191. https://doi.org/10.1099/jmm.0.004242-0

Teles, F., & Seixas, J. (2015). The future of novel diagnostics in medical mycology. *Journal of Medical Microbiology*, *64*(4), 315–322. https://doi.org/10.1099/jmm.0.082297-0

Terrell, C. L. (1999). Antifungal Agents. Part II. The Azoles. *Mayo Clinic Proceedings*, *74*(1), 78–100. https://doi.org/10.4065/74.1.78

Trabelsi, H., Chtara, K., Khemakhem, N., Néji, S., Cheikhrouhou, F., Sellami, H., Guidara, R., Makni, F., Bouaziz, M., & Ayadi, A. (2015). Fungemia Caused by Yarrowia lipolytica. *Mycopathologia*, *179*(5), 437–445. https://doi.org/10.1007/s11046-015-9859-4

Trofa, D., Gácser, A., & Nosanchuk, J. D. (2008). *Candida parapsilosis*, an Emerging Fungal Pathogen. *Clinical Microbiology Reviews*, *21*(4), 606. https://doi.org/10.1128/CMR.00013-08

Tsuruta, R., Mizuno, H., Kaneko, T., Oda, Y., Kaneda, K., Fujita, M., Inoue, T., Kasaoka, S., & Maekawa, T. (2007). Preemptive Therapy in Nonneutropenic Patients with *Candida* Infection Using the Japanese Guidelines. *Annals of Pharmacotherapy*, *41*(7–8), 1137–1143. https://doi.org/10.1345/aph.1K010

Vandenbossche, I., Vaneechoutte, M., Vandevenne, M., De Baere, T., & Verschraegen, G. (2002). Susceptibility Testing of Fluconazole by the NCCLS Broth Macrodilution Method, E-Test, and Disk Diffusion for Application in the Routine Laboratory. *Journal of Clinical Microbiology*, *40*(3), 918–921. https://doi.org/10.1128/JCM.40.3.918-921.2002

Vazquez, J. A., Miceli, M. H., & Alangaden, G. (2013). Invasive fungal infections in transplant recipients. *Therapeutic Advances in Infectious Disease*, *1*(3), 85–105. https://doi.org/10.1177/2049936113491936

Vazquez, J. A., & Sobel, J. D. (2011). Candidiasis. In C. A. Kauffman, P. G. Pappas, J. D. Sobel, & W. E. Dismukes (Eds.), *Essentials of Clinical Mycology* (pp. 167–206). Springer New York. https://doi.org/10.1007/978-1-4419-6640-7_11

Vervaeke, S., Vandamme, K., Boone, E., De Laere, E., Swinne, D., & Surmont, I. (2008). A case of *Candida lambica* fungemia misidentified as *Candida krusei* in

an intravenous drug abuser. *Medical Mycology*, *46*(8), 853–856. https://doi.org/10.1080/13693780802342552

Vijaya, D., Dhanalakshmi, T. A., & Kulkarni, S. (2014). Changing Trends of Vulvovaginal Candidiasis. *Journal of Laboratory Physicians*, *6*(1), 28–30. https://doi.org/10.4103/0974-2727.129087

Vila, T., Sultan, A. S., Montelongo-Jauregui, D., & Jabra-Rizk, M. A. (2020). Oral Candidiasis: A Disease of Opportunity. *Journal of Fungi*, *6*(1), 15. https://doi.org/10.3390/jof6010015

Villanueva, A., Gotuzzo, E., Arathoon, E. G., Noriega, L. M., Kartsonis, N. A., Lupinacci, R. J., Smietana, J. M., DiNubile, M. J., & Sable, C. A. (2002). A randomized double-blind study of caspofungin versus fluconazole for the treatment of esophageal candidiasis. *The American Journal of Medicine*, *113*(4), 294–299. https://doi.org/10.1016/S0002-9343(02)01191-9

Vincent, S. (1992). Origin of the Names of Species of *Candida*. *Medical Mycology 1*, 1–2.

Whelan, W. L., Partridge, R. M., & Magee, P. T. (1980). Heterozygosity and segregation in *Candida albicans*. *Molecular and General Genetics MGG*, *180*(1), 107–113. https://doi.org/10.1007/BF00267358

Winn, W. C., Allen, S. D., Allen, S., Janda, W. M., Koneman, E. W., Schreckenberger, P. C., Procop, G. W., & Woods, G. L. (2005). *Koneman's Color Atlas and Textbook of Diagnostic Microbiology* (6th Revised edition edition). Lippincott Williams and Wilkins.

Zarei-Mahmoudabadi, A., Zarrin, M., Ghanatir, F., & Vazirianzadeh, B. (2012). Candiduria in hospitalized patients in teaching hospitals of Ahvaz. *Iranian Journal of Microbiology*, *4*(4), 198–203.

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Masters Thesis	
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Courses and Certificate

Name	Name of the Institution where take place	year
Medical Lab Technician Certificate	The Ministry of Health, Jordan.	2011
Medical Lab Technician Trainee Certificate	King Hussein Medical Center, Jordan	2011
9th National and 2nd International Congress of Hydatidology Certificate (Attendance)	DESAM Institute, Near East University, Nicosia	2018
Mathematical Modeling in Health Certificate (Attendance)	DESAM Institute, Near East University, Nicosia	2019
Parasitology Academic Course: Essential and Application	Turkish Microbiology Society, TMC-KKTC Microbiology Platform, Nicosia	2020

Foreign Languages	Reading comprehension	Speaking*	Writing*
English	Excellent	Excellent	Excellent

Foreign Language Examination Grade								
YDS	ÜDS	IELTS	TOEFL IBT	TOEFL PBT	TOEFL CBT	FCE	CAE	CPE
•			•	•				

Computer Knowledge

Program	Use proficiency
SPSS	Excellent
Python Programming Language	Excellent
Common Computer Programs and Skills	Excellent