INTRODUCTION

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide. Many species are harmless commensals of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease. *Candida albicans (C.albicans)* is the most commonly isolated species, and can cause infections (candidiasis or thrush) in humans. Systemic infections of the bloodstream and major organs (candidemia or invasive candidiasis), particularly in immuno-compromised patients, affect over 90,000 people a year in the United States ***(Kourkoumpetis et al., 2011)***.

Overgrowth of several species including *C. albicans* can cause infections ranging from superficial, such as oropharyngeal candidiasis (thrush) or vulvovaginal candidiasis (vaginal candidiasis), to systemic, such as fungemia and invasive candidiasis. Oral candidiasis is common in elderly denture wearers. In debilitated or immunocompromised patients, candidiasis may become a systemic disease producing abscess, thrombo-phlebitis, endocarditis, or infections of the eyes or other organs ***(Chang et al., 2012)***.

Invasive candidiasis (IC) is a major threat to the health of patients in hospitals as well as in the community and its consequent mortality is costly. Several conditions are

associated with the development of candidemia such as the use of antibacterial agents; the presence of a central venous catheter; the use of type 2 histamine receptor blockers (H2); total parenteral nutrition; admission at an intensive care unit (ICU); the use of corticosteroids; surgeries; previous hospitalization; and colonization by *Candida* ***(Lortholary et al., 2017).***

Among *Candida* species, *C. albicans*, which is a normal constituent of the human flora, a commensal of the skin and the gastrointestinal and genitourinary tracts, is responsible for the majority of *Candida* blood stream infections (candidemia). Yet, there is an increasing incidence of infections caused by *Candida glabrata (C.glabrata)* and *Candida rugosa* (*C. rugosa)*, which could be because they are frequently less susceptible to the currently used azole antifungals. Other medically important species include *Candida parapsilosis (C. parapsilosis)*, *Candida tropicalis (C. tropicalis)* and *Candida dubliniensis* (*C. dubliniensis)* ***(Etiz et al., 2015).***

There are many antifungal drugs including those which may interfere with the synthesis of nucleic acids (pyrimidines), microtubules (griseofulvin), synthesis of ergosterol (azoles, allylamines, thiocarbamates, morpholines), cell membrane integrity (polyenes), cell wall synthesis (echinocandins, nicomicinas) and protein synthesis (sodarinas) and other cellular sites.The most common systemic antifungal drugs are amphotericin B and its lipid

preparations, itraconazole, fluconazole, triazoles (voriconazole, posaconazole and ravuconazole) and derivatives of echinocandins (caspofungin, micafungin and anidulafungin) ***(Hazirolan and Yildiran, 2015)***.

Among the causes of high mortality rates that are observed for candidemia is the limited number of systemic antimycotics that are available and the failure of antifungal therapies, which is often due to the emergence of resistance, first against flucytosine and then against fluconazole. Furthermore, multiple studies have shown that cross-resistance may cover other azole class antifungal ***(Dos Santos et al., 2014)***.

Several *candida* speciecs like *Candida krusei* (*C. krusei)* and *C. glabrata* exhibit intrinsic resistance to traditional triazoles like fluconazole and may also demonstrate cross resistance to newer triazoles. Recent studies showed an increase in antifungal resistance of other *candida* species Therefore, species identification and antifungal susceptibility pattern of *Candida* isolates is very important and helps in the selection of appropriate antifungal agents, successful treatment, in antifungal prophylaxis in the immunocompromised host and to prevent the emergence of drug resistance ***(Magill et al., 2014)***.

AIM OF THE WORK

The aim of the present thesis is to determine the local resistance pattern of *Candida* isolates recovered from clinical samples of immunocompromised patients referred to the Central Microbiology Laboratory, Ain Shams University Hospitals to guide treatment planning and to evaluate the performance of VITEK for antifungal susceptibility.

Chapter 1

CANDIDA SPECIES

Candida species (spp.) are eukaryotic opportunistic pathogens that are responsible for the most frequently encountered opportunistic fungal infections. They are the fourth most common cause of hospital-acquired blood stream infections (BSIs) in the United States, and a mortality rate as high as 15- 49% has been seen with these infections ***(Teo et al., 2017)***.

Classification of *Candida:*

Candida reproduce asexually by blastoconidia formation (budding) and sexually by the production of ascospores or basidiospores so classified as Ascomycota, table (1) ***(Tille, 2014)***.

Table (1): Taxonomic classification of *Candida*

Domin	Eukaryota
Kingdom	Fungi
Phylum	Ascomycota
Subphylum	Saccharomycotina
Class	Saccharomycetes
Order	Saccharomycetailes
Family	Saccharomycetaceae
Genus	*Candida*
Candida species	a.*Candida albicans* b.*Non albicans Candida* (NAC)

(Arun Nagendran et al., 2014)

The Genus *Candida* consists of 150-200 species.Few species can cause disease in humans. Although more than 20 different species of *Candida* have been reported as pathogens, more than 90% of invasive infections are attributed to five species, *C.albicans*, *C.glabrata*, *C.parapsilosis*, *C.tropicalis*, and *C.krusei*. Less commonly reported are infections due to *Candida kefyr (C.kefyr)*, *Candida guilliermondii (C.guilliermondii)*, *Candida lusitaniae (C.lusitaniae)*, *Candida stellatoidea (C.stellatoidea)*, and *C.dubliniensis* ***(Pappas et al., 2016)***.

Healthcare facilities in several countries have reported that *Candida auris (C.auris)* as an emerging, multidrug-resistant has been causing severe illness in hospitalized patients. In some patients, this yeast can enter the blood stream and spread throughout the body, causing serious invasive infections. Unfortunately *C.auris* is commonly misidentified as *Candida haemulonii* and other *Candida* species ***(Biswal et al., 2017)***.

Pathogenesis and virulence factors of *Candida* spp:

Candida albicans and other *Candida* spp. are part of the human body's microbiota (i.e, normal flora), but they also have become endemic in most hospitals. Infections may be caused by endogenous yeasts or may be acquired in the hospital. Differentiating among the *Candida* spp. in the clinical laboratory is very important because of the

differences in the virulence of the species and in their susceptibility to antifungal drugs ***(Arendrup, 2013)***.

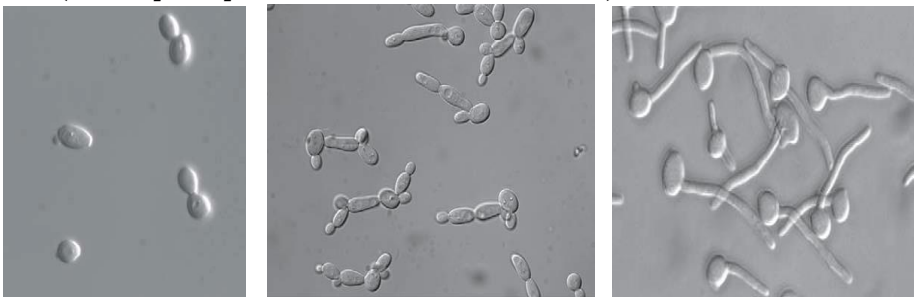
Candida spp. are opportunistic pathogens with different virulent factors that only cause infection when host's immunity is deficient ***(Moran et al., 2012)***. The virulence factors including:

- **Cell wall proteins (CWPs):** One essential but often unrecognized feature of the fungal cell wall is that it is a dynamic structure continuously evolving in response to the environment and during cell cycle ***(Latgé, 2010)***. The *C. albicans* cell wall is composed of three major polysaccharides: β-1,3 glucan, β-1,6 glucan and chitin ***(Nather and Munro, 2008***). Therefore, these proteins have the ability to contribute directly to the virulence of *Candida*. Their role include cell wall assembly, adhesion to host surfaces, immune-modulation, protection of the fungus from host enzymes, permeability toward large macromolecules, promotion of endocytosis by endothelial cells, iron acquisition and coping with oxidative stress ***(Muszewska et al., 2018).***

- **Polymorphism:** The ability of *Candida* species to grow in different forms (budding yeast, pseudohyphae and true hyphae) is associated with the virulence of these pathogens ***(Kabir et al., 2012)***. The hyphal form has been shown to be more invasive and resistant to

phagocytosis than the yeast form. On the other hand the smaller yeast form is believed to represent the form primarily involved in dissemination. While yeast and true hyphae are regularly observed during infection and have distinct functions, the role of pseudohyphae and switching in vivo is rather unclear and chlamydospores have not been observed in patient samples ***(Mayer et al., 2013)***.

This morphological transition corresponds to an adaptive response to environmental changes such as temperature, pH and CO_2 concentration, figure (1) ***(Albuquerque and Casadevall, 2012)***.



(a) Yeast (b)Pseudohyphae (c) Hyphal germ tubes

Figure (1): Different morphological forms of *C. albicans*; (a) yeast budding (b) Pseudohyphae (c) Hyphal germ tubes ***(Kabir et al., 2012).***

- **Metabolic adaptation:** Nutrition is a central and fundamental prerequisite for survival and growth of all living organisms. Metabolic adaptability mediates the effective assimilation of alternative nutrients in dynamic environments. This metabolic flexibility is particularly important for pathogenic fungi during infection of different host niches ***(Fleck et al.,2011)*** Glycolysis,

gluconeogenesis, and starvation responses are all thought to contribute to host colonization and pathogenesis, but their specific contribution may be highly niche-specific and is still only partially understood ***(Mayer et al.,2013)***.

- **Hydrolytic enzymes:** Screated Aspartyl Protease (SAPs), lipase, and phospholipase destroy free and cell-bound proteins that impair fungal colonisation and invasion ***(Asmundsdóttir et al., 2009)***.
- **Adhesins:** It represents a special class of glycosyl phosphatidyl inositol (GPI) anchored cell wall proteins and specifically bind to amino acids and sugars on the surface of other cells or promote adherence to abiotic surfaces ***(Sardi et al., 2013).***The initial event in *Candida* infection is its adherence to host and/or medical device surfaces that mediated by non-specific factors such as hydrophobicity and electrostatic forces and promoted by specific adhesins present on the surface of fungal cells that recognize ligands such as proteins, collagen, fibrinogen and fibronectin ***(Cate et al., 2009)***. Thus, adhesion is an extremely important step in the infection process, and the extent of adhesion is dependent on microbial, host and abiotic surface properties ***(Chaffin, 2008).***
- **Invasins:** Specialized protein expressed on fungal cell wall that mediate binding to host ligands (such as E-cadherin on epithelial cells and N-cadherin on

endothelial cells), thereby triggering engulfment of the fungal cell into the host cell ***(Zakikhany et al., 2007)***.

- **Biofilm formation:** It formed in a sequential process including adherence of yeast cells to the substrate, proliferation of these yeast cells, formation of hyphal cells in the upper part of the biofilm, accumulation of extracellular matrix material and, finally, dispersion of yeast cells from the biofilm complex. Mature biofilms are much more resistant to antimicrobial agents and host immune factors in comparison to planktonic cells ***(Fanning and Mitchell, 2012)***.

Epidemiology of *Candida* spp:

Candida infections are caused by a variety of species. *C. albicans* is the most commonly isolated yeast from infections, accounting for at least 60% to 70% of yeast infections ***(Tille, 2014)***. But with increasing use of azoles there was epidemiological shift from *Candida albicans* to NAC species ***(Jain et al., 2012)***. NAC species are emerging as important opportunistic pathogens where *C.glabrata*, *C.krusei* and *C.tropicalis* have been found to be 32-fold less susceptible to fluconazole than *C. albicans* ***(Agarwal et al., 2011)***.

In Egypt, NAC were predominant (65.6%) where *C.albicans* was the most prevalent species (34.5%) then *C.tropicalis* was the most common isolated NAC species

and the second common isolated species (32.8%), followed by *C.glabrata* (17.3%) and *C. Parapsilosis* (7.5%) ***(Shawky et al., 2017)***.

High-risk groups of developing candidiasis include individuals undergoing blood and Bone marrow transplantation (BMT), solid organ transplantation, or major surgery (especially gastrointestinal [GI] surgery) and those with acquired immunodeficiency syndrome (AIDS), neoplastic disease, immunosuppressive therapy, advanced age, and premature birth ***(Lortholary et al., 2017)***.

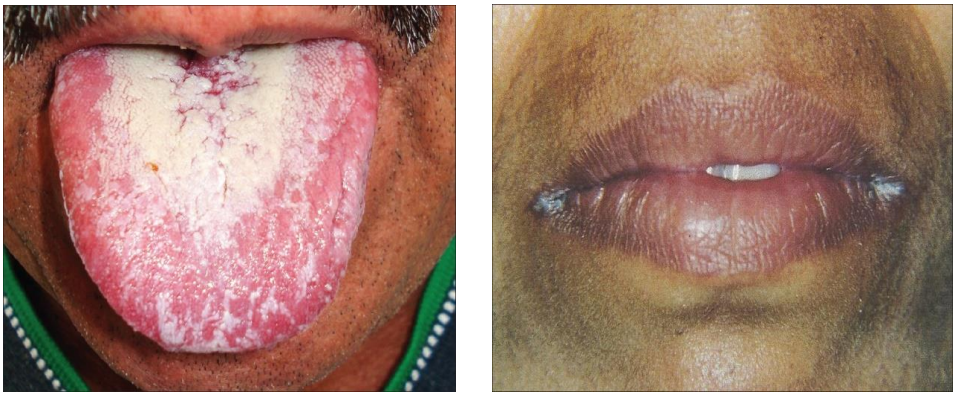
Spectrum of diseases (Candidiasis):

Candida spp. can cause clinically apparent infection of virtually any organ system. Infections range from superficial mucosal and cutaneous candidiasis to wide spread hematogenous dissemination involving target organs such as the liver, spleen, kidney, heart, and brain ***(Moges et al., 2016)***.

1) Mucosal Candidiasis:

Mucosal infections caused by *Candida* spp. (known as"thrush") may be limited to the oropharynx, (figure 2a) or extend to the esophagus and the entire Gastrointestinal tract. In women, the vaginal mucosa is also a common site of infection. These infections are generally seen in individuals with local or generalized immunosuppression. These

infections usually present as white "cottage cheese"–like patches on the mucosal surface. Other presentations include the pseudomembranous type: which reveals a raw bleeding surface when scraped, the erythematous type: flat, red, occasionally sore areas, candidal leukoplakia: non removable white thickening of epithelium caused by *Candida* spp.; and angular cheilitis: sore fissures at the corners of the mouth, (figure 2b), ***(Krishnan, 2012).***



a b

Figure (2): Different lesion of mucosal candidiasis (a.oral thrush and b.angular chelitis), ***(Krishnan, 2012)***

2) Cutaneous Candidiasis:

Candida spp. may cause localized skin infection in areas where the skin surface is occluded and moist (e.g, groin, axillae, toe webs, breast folds). These infections present as a pruritic rash with erythematous vesiculopustular lesions. Onychomycosis and paronychia may occur in the setting of a mixed microbial flora, including *Candida*. The most commonly involved species are *C.albicans, C.parapsilosis, and C.guilliermondii*. Skin lesions may also

appear during the course of hematogenous dissemination. These lesions are of major diagnostic importance; they can be directly biopsied and thus provide an etiologic diagnosis of a systemic process ***(Tille, 2014)***.

3) Chronic Mucocutaneous Candidiasis:

Chronic mucocutaneous candidiasis is a rare condition marked by a deficiency in T-lymphocyte responsiveness to *Candida* spp. These patients suffer from severe unremitting mucocutaneous *Candida* lesions, including extensive nail involvement and vaginitis. The lesions may become quite large with a disfiguring granulomatous appearance ***(Pfaller and Diekema, 2010)***.

4) Systemic Candida Infection:

Candidemia (Blood stream infection) is the commonest invasive fungal infection where *Candida* species are the fourth leading cause of nosocomial blood stream infections (BSIs) in the United States ***(Scorneaux et al., 2017)*** but in developing countries, the exact prevalence is not known due to lack of systematic epidemiological data. The limited studies report a very high incidence of candidemia and unique epidemiology with a different spectrum of *Candida* species ***(Kaur and Chakrabarti, 2017)***. The mortality rate associated with candidemia is high worldwide ***(Da Matta et al., 2017)***.

Although hematogenous candidiasis is most often an endogenous infection arising from the Gastrointestinal or genitourinary tract, it may also result from contamination of an indwelling vascular catheter. Organisms transferred to the hub or lumen of the catheter may form a biofilm within the catheter lumen and subsequently spread into the circulation. Although such infections are no less serious than those arising from an endogenous source, they may be dealt with somewhat more successfully because removal of the catheter essentially removes the nidus of infection. Of course, if the infected catheter resulted in the seeding of distant organs, the consequences and problems in treating the infection would be the same as those arising from an endogenous source ***(Kelly et al., 2015)***.

Urinary tract involvement with *Candida* spp. ranges from asymptomatic bladder colonization to renal abscesses secondary to hematogenous seeding. Bladder colonization with *Candida* spp. is essentially not seen unless a patient requires an indwelling bladder catheter, has diabetes, suffers from urinary obstruction, or has had prior urinary procedures. Benign colonization of the bladder is most common in these settings, but urethritis and/or cystitis may occur. Hematogenous seeding of the kidney may result in renal abscess, papillary necrosis, or "fungus ball" of the ureter or renal pelvis ***(Murray et al., 2016).***

Candida* peritonitis** may be seen in the setting of chronic ambulatory peritoneal dialysis or after gastrointestinal surgery, anastomotic leak, or intestinal perforation. These infections may remain localized to the abdomen, involve adjacent organs, or lead to hematogenous candidiasis. Hematogenous candidiasis may be acute or chronic and usually results in seeding of deep tissues, including the abdominal viscera, heart, eyes, bones and joints, and brain ***(Hasibeder and Halabi, 2014).

Chronic hepatosplenic candidiasis may occur after overt or occult fungemia and presents as an indolent process marked by fever, elevated alkaline phosphatase, and multiple lesions in the liver and spleen ***(Nahum, 2017)***.

Central nervous system candidiasis may occur secondary to hematogenous disease or be associated with neurosurgical procedures and ventriculoperitoneal shunts. Thisprocess may mimic bacterial meningitis, or the course maybe indolent or chronic ***(Murray et al., 2016).***

Cardiac involvement with *Candida* spp. is the result of hematogenous seeding of a prosthetic or damaged heartvalve, the myocardium, or pericardial space. Implantation of heart valves contaminated with *C. parapsilosis* has been reported. The clinical presentation resembles bacterial endocarditis, with fever and a new or changing heart murmur. The vegetations are classically large and friable, and embolic events are more common