NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

A RETROSPECTIVE STUDY ON CLINICAL ISOLATES OF CANDIDA SPECIES FROM HOSPITALIZED PATIENTS IN NEAR EAST UNIVERSITY HOSPITAL

MSc. THESIS

ADA STELLA ODOGWU

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THESIS SUPERVISOR
ASSIST. PROF. DR. ESREF CELIK

Nicosia
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APPROVAL

We certify that we have read the thesis submitted by **ODOGWU ADA STELLA** titled **"A RETROSPECTIVE STUDY ON CLINICAL ISOLATES OF CANDIDA SPECIES FROM HOSPITALIZED PATIENTS IN NEAR EAST UNIVERSITY**" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

Examining Committee	Name-surname	Signature
Head of the Committee:	Prof. Dr. Nedim Cakir	…..…..……
Committee Member:	Assist. Prof. Dr. Esref Celik	…..…..……
Committee Member:	Assist. Prof Dr. Ayse Seyer	……

Approved by the Institute of Graduate Studies

…../…../20…

Prof. Dr. Kemal Hüsnü Can Başer

Head of the Institute

DECLARATION

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

ODOGWU ADA STELLA

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ACKNOWLEDGEMENT

My utmost thanks, goes to God almighty, who has strengthened me throughout this period of my studies, it has not been totally easy but he has made me a success. I am grateful for his guidance and provision during my research work.

My deepest gratitude goes to my thesis supervisor Associate Professor DR. Esref Celik who has taken my worries to be hers during this whole time, for her continuous advice and support, for being constantly available when I needed her despite the hour or day, I truly appreciate your efforts Ma.

I also would like to offer my thanks to Dr. Emrah Guler who took out his time to explain to me in details when I needed it, I am thankful for him always putting me through my laboratory work

I wouldn't have been able to survive this whole experience if I didn't have guidance and help from colleagues, for that I am thankful to Chris who always helped me out when I needed it, I am grateful to God for blessing me with the best Father and Uncle who always provided for me and made sure I never lacked so as to excel in my studies. Thank you all for seeing me to be worthy of assistance, I will never disappoint you all, thank you.

ODOGWU ADA STELLA

Abstract

Odogwu Ada Stella. A Retrospective study on the clinical isolates of *Candida* species from hospitalized patients in Near East University Hospital, Institute of Graduate Studies, Medical Microbiology and Clinical Microbiology Program, Master Thesis, Nicosia, 2022

Candida species are eukaryotic fungi in the human micro flora that are prevalent in the hospitals and have various species that are emerging as major public health issues. The aim of this study was to evaluate the growth of *Candida* species in hospitalized, outpatients and determine the susceptibility and resistance pattern to the anti-fungal drugs. Lastly, to provide necessary treatment and prevention guidelines for *Candida* infections. To accomplish this, details of patients both male and female from the age range (0-95) years from various departments in the hospital who were also in-patients were taken from the electronic microbiology database in the laboratory, 202 samples between "15th September 2020- 28thFebruary 2022 were taken for the purpose of this study. The ICU department had the highest percentage rate of isolates (70.8%), followed by the cardiology department which was (8.4%), the urology, dermatology, ear nose throat, orthopedic and traumatology (0.5%). *Candida albicans* was the most prevalent species is (68.8%), *Candida parapsilosis* as a slowly emerging species, was the second most described species(11.4%), *Candida lusitaniae* was only isolated once therefore, having the lowest percentage (0.5%). Patients with yeast culture sample reported the most number of isolates (n=117, 57.9%).The antifungal drugs that were analyzed were Amphotericin B (93.1%) sensitive and (6.9%) resistant, Flucytosine (94.6%) sensitive (5.4%) resistant and voriconazole (88.1%) sensitive (11.9%) resistant

Keywords; Candida infections, nosocomial infections, antifungal resistance, flucytosine, Amphotericin B

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ÖZ

Odogwu Ada Stella. Yakın Doğu Üniversitesi, Lisansüstü Eğitim Enstitüsü, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programı, Yüksek Lisans Tezi, Lefkoşa, 2022

Candida türleri, insan mikro florasında bulunan, hastanelerde yaygın, önemli halk sağlığı sorunları yaratan ökaryotikmantarlardır . Çalışmamızını amacı, hastanede yatan veya ayaktan tedavi gören hastalarda üreyen *Candida* türlerini değerlendirmek ve antifungal ilaçlara duyarlılık ve direnç paternini belirlemektir. *Candida* enfeksiyonları için gerekli tedavi ve korunma kılavuzlarını sağlamaktır. Bu amaçla, hastanenin çeşitli bölümlerinde yatan ve ayakta tedavi gören (0-95) yaş aralığındaki kadın ve erkek 202 hastanın bilgileri, "15 Eylül 2020- 28 Şubat 2022 tarihleri arasında laboratuvardaki elektronik mikrobiyoloji veri tabanından alındı. İzolat oranı en yüksek (%70,8) Yoğun Bakım bölümü olurken, bunu %8,4 ile kardiyoloji , üroloji, dermatoloji, kulak burun boğaz, ortopedi ve travmatoloji bölümü (%0,5) izledi. *Candida albicans* en yaygın tür (%68.8), yavaş ortaya çıkan bir tür olarak *Candida parapsilosis* ikinci en çok tanımlanan tür (%11.4), *Candida lusitaniae* yalnızca bir kez izole edildi ve bu nedenle en düşük yüzdeye (%0.5) sahipti. Mantar kültürü örneği olan hastalar en fazla izolat sayısı 117 (%57,9) bildirirken, hastanede yatış durumu ve cinsiyet karşılaştırıldığında herhangi bir ilişki/anlamlılık bulunmadı (p=0,212).Analiz edilen antifungal ilaçlar, Amfoterisin B'ye(%93.1) duyarlı ve (%6.9) dirençli, Flusitosine duyarlı (%94.6) (%5.4) dirençli ve vorikonazole (%88.1) duyarlı (%11.9) dirençli idi.

Anahtar:Kelimeler; *Candida* enfeksiyonları,hastane enfeksiyonları, antifungal direnç, flusitozin, Amfoterisin B

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List of Abbreviations

% - Percentage

Ml - Mililiter

IL - interleukin

CMC- Chronic mucocutaneous candidiasis

MIC- Minimal inhibitory concentration

HIES- Hyper IgE syndrome

SDA- Sabouraud dextrose agar

EMB- Eosine methylene blue

ICU- Intensive care unit

CLR- C-type Lectin receptors

IFN- Interferon receptors

ERG- Ets related gene

BSI- Blood stream infection

CFU- Colony forming unit

RNA- Ribonucleic acid

PCR-Polymerase chain reaction

CDR (1) (2) – Candida drug resistant (1) (2)

ABC- ATP-binding cassette

MFS- Major Facilitator superfamily

NCAC- Non *Candida albicans* Candida

STAT- Signal transducer and activator of transcription

CLSI- Clinical and laboratory standards institute

NNIS- National nosocomial infection surveillance

AFST- Antifungal susceptibility testing

MDRI- Multi drug resistance

RVVC- Recurrent idiopathic vulvovaginal candidiasis

EUCAST- European committee on antibiotic susceptibility testing

ESCMID- European society of clinical microbiology and infectious diseases

ECDC- European center for disease control

ENBC- European National Breakpoint committee

SECTION ONE

Introduction

1.0 The fungal kingdom

A fungus is a eukaryote that takes in nutrients directly through its cell walls (Carris *et al*., 2012), they mostly reproduce sexually/asexually by spores and have a web-like, tubular, filamentous, branched body called the hyphae which can either be septate or non-septate. Some fungi require energy from plants to live and they are known as biotrophs, the saprophytic fungi (largest group of fungi) depend on dead decaying microbial matters for their nutrients while the necrotrophc fungi kills the host plant and uses its nutrient. There are structural components of the fungal cell wall which are indispensable (chitin, glucans and glycoproteins) (Lowman *et al*., 2011). They are very important in sustaining the fauna by their break down of dead organic materials and provide minerals, energy back into the environment (Hawksworth,2001).There have been an indefinite report of new species of fungi at the frequency of 1,200 annually, although previously an estimate of 100,000 species were identified (Blackwell 2011, Kirk *et al*. 2008). A modest number of fungal species that are identified are known to be about 1. 5 million (Hawksworth, 2001), this conclusion was as a result of series of research and studies done by Hawksworth. Fungi have been known to grow in or on invertebrate and vertebrate animals causing diseases, some of these fungi causes an attack on animals/plants that have physiological effect, *Entomophthora musae*, is a model guide that is often observed forming a ring of white spores discharged around the body of a parasitized fly on panes of glass (Bromenshenk *et al*., 2010).

In humans, a series of diseases are caused by fungi. Dermatophytes cause recurrent diseases, they inhabit the hair, skin, finger, toenails. This type of fungi affects the epidermis and causes infections such as tinea that are unattractive and treatment is burdensome as they cause cosmetologic problem, but is hardly serious. In immune-competent people, certain fungal species are the resident micro flora but causes diseases/infections in immunocompromised individuals. A group of fungi exists which causes diseases by inhalation of spores which affect the lungs thereby in some cases causing systemic mycoses. These fungi include *Coccidioides immitis* (coccidioidomycosis, commonly known as valley fever), and *Histoplasma capsulatum* (histoplasmosis) (Carris *et al*., 2012). Opportunistic mycoses are normally not as a

result of humans, plants and animals but as a result of a compromised immune system and underlying medical condition, and in immune competent or immune compromised individuals can cause infections when consumed or placed in wounds. Among these opportunistic mycoses, *Aspergillus fumigatus*, appears to be the predominant specie, settled in pre-existing cavities as a result of tuberculosis.it leads to the production of tiny airborne spores that are constantly inhaled thereby causing it to grow systematically in some individuals bringing about aspergillosis most especially in immune compromised individuals. *Pneumocystis carinii* is the fungus responsible for causing symptoms similar to pneumonia in immune compromised individuals. It is the most opportunistic infection in AIDS patients and is not grown in-vitro.

Candida is the most opportunistic infections worldwide in immune compromised individuals and also causes yeast infections in mucosal surface of healthy people. (Pfaller *et al*., 2012; Richardson & Varnock, 2012).

CHAPTER TWO
Literature Review

2. General information

Candida are yeasts (sometimes molds in the cases of *C.albicans* and *C. dubliniensis*) that exists naturally in the normal human flora such as skin (skin folding), genitourinary and gastrointestinal area and can be identified as colonizing organisms in sputum and the urine of patients with no exception to surgical patients with catheter (Achkar JM& Fries BC, 2010). Approximately, 150 species of *Candida* has been reported. *C. albican*s, *C. krusei*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* (Shoham & Levitz, 2005) are recognized to be the predominant species causing diseases. *Candida albicans* is a major source of superficial, systemic and opportunistic infections causing oral thrush and vaginal candidiasis. Nosocomial infections are present in patients who are admitted into a hospital. Devices such as unsterile catheters, hands of medical personnel, hospital equipment, syringes, are known to be associated with nosocomial infections in hospitalized patients. Every hospitalized patient is at risk of acquiring this infection, susceptible patients are those in the intensive care unit (ICU), COVID ward, organ transplant patients, neonates, pregnant women (Allegranzi, 2011). With an increase in the prevalence of these infections there is prolonged hospital stay, chances of long term disability, antifungal resistance, patients tend to be immunocompromised. There are various fungal parasites which acts as opportunistic pathogens for this nosocomial infection, they can cause both endogenous and exogenous source of infection, *Candida* infections arise from the patient's micro biota, *Candida albicans* is still recognized as the principal fungus causing nosocomial infections (Guinea, 2014).

2.1 *Candida* species

There are more than 150 known species of *Candida* but few are recognized as major disease causing organisms which pose a risk to public health.

2.1.0 *Candida dubliniensis*

It was reported initially in the mid 90's, there is close relation between *Candida dubliniensis* and *Candida albicans* which can be seen phenotypically but however, their epidemiological data shows their differences in the ways and manner they cause diseases and respective mode of drug use, this fungus has been linked to be a cause of oral thrush in individuals infected with HIV, nevertheless, it has also been clinically diagnosed in patients who are not carriers of HIV, a certain amount can also be discovered in the genital region of some females with vaginitis (faggi E*et al*., 2005, Miron D*et al*., 2005). Most isolate of *Candida dubliniensis* show susceptibility to fluconazole (MIC range, 0.125 to 1.0 g/ml) this can also be seen in some commercial antifungal drugs such as ketoconazole, itraconazole and amphotericin (lofler *et al*., 1997).*C.dublinensis* can be differentiated from *C. albicans* using the PCR.

2.1.1 *Candida krusei*

Candida krusei causes diseases most especially in immune compromised individuals with urinary tract infections it is an unusual cause of vaginitis (Hepburn *et al*., 2003, Singh *et al*., 2002). *C. krusei* infections have increased in the previous years as a result of its permanent resistance to fluconazole which is a commonly used antifungal for immunocompromised patients. When identifying *C. krusei* as an isolate of *Candida inconspicua*, one should be very careful as they are similar in their forms. These two species are most easily differentiated by the production of pseudohyphae by *C. krusei* (Kurtzman *et al*., 2000). This isolate is well recorded in humans who are known to be resistant to fluconazole, however, it is recently reported in systematic infections in patients administered caspofungin (Pelletier *et al*., 2005). This fungus is a cause of vaginitis in relatively aged women, recurrent introduction to antifungals causes a shift in the micro flora of the vagina (Abi-Said et al., 1997, Wingard JR 1992) (Singh S et al., 2002).

2.1.2 *Candida lusitaniae*

When compared with the other species of Candida, this fungus is important due to its rare sensitivity to antifungal drugs. (Wingward JR 1995, Blinkhorn, 1989, Fromtling

et al., 1993). It has the ability to acquire a swift resistance to amphotericin B, certain strains have developed resistance permanently making it important to detect amphotericin B resistance pattern at the initial phase of treatment of infections caused by this *Candida* specie (Merz WG 1984).

It was reported initially by a group of scientists as a frequent colonizer of the gastrointestinal area of animals that are warm blooded (Baker et al., 1984), this specie is a resident flora of the digestive system, respiratory system of patients who are hospitalized with infections of the urinary tract making no exception. Reports have been made noting its recovery from both the vagina and skin of a (Silverman *et al*., 2001).

2.1.3 *Candida kefyr*

This specie was initially discovered in 1931 and was hardly isolated clinically, in immune compromised individuals it was found to cause diseases from time to time (Hazen KC, 1995). Since then the organism has been reassigned various times and has become known as a pathogen (Corpus *et al*., 2004), irrespective of the insignificant number of articles written concerning *Candida kefyr*, an approximately ten numbers of clinical studies and reports have supported this specie's ability to cause harm to patients (Corpus et al., 2004), it causes various diseases and also infects the blood of individuals, however it is comparatively an infrequent cause of these infections. In immune competent patients, it can be identified as a source of candidemia, Candiduria and vaginitis (Abu-Elteen *et al*., 1997, Listemann *et al*., 1998).

Reports of its ability to be resistant has been described to be as a result of combination with amphotericin B therapy (Pfaller MA *et al*., 2004) and forming resistance to common antifungals (Pfaller *et al*., 2006)

2.1.4 *Candida glabrata*

In the previous years, C. glabrata has been known to be a resident of the micro flora which means its inability to cause diseases to humans (Haley LD, 1961, Stenderup A&Pederson G T, 1962), nevertheless, due to the extensive use of antifungals and combination therapies, it has been reported to play a major part in the increase of invasive and mucosal candidiasis (Hitchcock C A et al., 1993,Knoke M et al., 1997,

Komshian S V et al., 1989, Pfaller M A, 1996, Schwab U et al., 1997, Vanden-Bossche H et al., 1992, Willocks L et al., 1991, Wingard J R et al., 1993). According to previous studies, it is in most cases the second or third most common cause of candida diseases which depends on the part of the body infected, in immune compromised individuals, it causes a serious infection especially those suffering from diabetes mellitus (Geiger A M et al., 1995, Sinnott J T, 1987, Sobel J D, 1988, Wingard J R, 1995). As a result of compromised immune system in hospitalized patients, there is high mortality rate.

2.1.5 *Candida parapsilosis*

This specie of *Candida* is responsible for various diseases in humans (Trofa, D et al., 2008), it is the most predominant specie among the many fungus species that cause infections in the hands of humans (Silva S et al., 2012), compared to other *Candida* species, and it causes a series of diseases in immune compromised individuals as well as patients undergoing surgical procedures (Trofa, D et al., 2008). Studies have shown that approximately 8-15% of infections in hospitalized patient is mostly caused by *C. parapsilosis* (Silva S et al., 2012). It is difficult to treat because of its complicated mechanisms (Papadimitriou-Olivgeris, M et al., 2018). A study was conducted byDögen, et al., in Turkey which indicated that this *Candida* specie is found on the surface of household equipment (Dögen, A et al., 2017).

2.1.6 *Candida tropicalis*

There is a hospital acquired transmission of *C.tropicalis* which occurs in new born as a result of cross contamination. It is reported to be among the major causes of candidemia in cancer patients(Kontoyiannis et al., 2001; Leung et al., 2002; Goldani & M´ario, 2003; Weinberger et al., 2005; Vigouroux et al., 2006; Nucci & Colombo, 2007). It is prevalent in sea water, people in marine environment, it is harder to eradicate than *C.albicans* because it is in the tropics. Ingestion of food high in antimicrobial properties such as ginger help in eradication of this fungus.

2.2 Types of candidiasis

2.2.1 Invasive candidiasis

Unlike candida of the mouth, throat, it is considered an important infection that causes candidemia, systemic diseases, and also affects the eye, candidemia is a common hospital infection in hospitalized patients. The risk factors associated with the growth of invasive candidiasis are trauma, recent abdominal surgery, dialysis, broad spectrum antibiotic therapy, central venous catheters, organ tumors (Masur *et al*., 1977; Kullberg & Arendrup, 2015). A study in the US which took place in a surgical ICU noted that prior surgery, acute renal failure, parenteral nutrition, and central venous catheters were impartially related with increased risk for developing candidemia. While in Spain, a study center reported that self-reliant risk factors for the growth of candidemia were sepsis, prior surgery, parenteral nutrition, and *Candida* colonization at different regions. All patients with candidemia should be given adequate special attention including antifungal combination therapy and removal of infected catheters as that is a major source of infection (Evans, 2010). Candidemia can be gotten endogenously from the host's micro flora as it is a member of the human micro flora or it can be acquired exogenously from the surrounding environment such as blood transfusion, surgery, and catheterization, it can also be acquired as a result of unprotected blood vessels which after acute burns (Pfaller M A*et al*., 2014; Pfaller M A & Diekema, 2007). Brazil has noted an increase in the prevalence of candidemia (Almirante B*et al*., 2005), country of Spain (Almirante B*et al*., 2005) and in the U. S. (Pfaller M A*et al*., 2014).

2.2.2 Vaginal candidiasis

This is predominant in the female reproductive system, studies have shown that at least 75% of sexually active women worldwide have experienced vaginitis ((Schroppei K*et al*., 1994; Lisiak M*et al*., 2003). The most common causative *Candida* specie is *Candida albicans* ((Simoes J A*et al*., 1998; Da Rosa M I and Rimel, 2004; Ken H, 1991; Ferre J, 2000), and essential details are; hormonal variation associated with an increase in vaginal pH promotes these infections, patients exposed to broad spectrum antibiotic therapy, in the cases of patients with diabetes, the use of oral contraceptives is also an infection booster. Clinical symptoms of vaginitis are present in women when there is a high count of fungal growth which is more or identical to 105 CFU/ml of

vaginal fluid (Carlson P*et al*., 2000). Dyspareunia is a recognized symptom (Barousse M M*et al*., 2005).

In men, balanitis which commonly appears after sexual contact presents a rash followed by small pustules with purulent discharge, more or less abundant, although this infection is defined, it can sometimes progress to the groin or perianal region, the crucial factors here are abuse of antibiotic therapies, patients with diabetes and vaginal secretions of the sexual partner (Mayer *etal*., 2013), it occurs in uncircumcised males but is not restricted to them only.

2.2.3 Candiduria

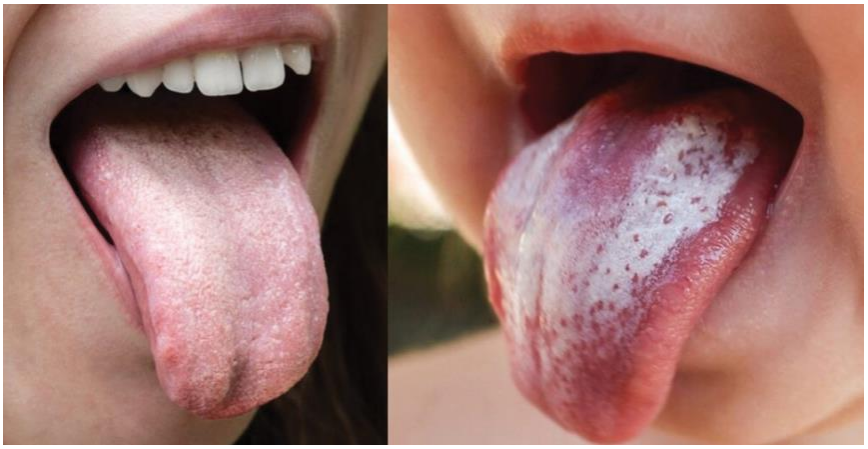
This is the ability of fungi to exist in urine causing urinary tract infections, it can be as a result of bladder colonization due to the presence of venous catheters, primary or disseminated mycosis. There are a number of fungal organisms that are present in urine, however, *Candida* species have become prevalent nosocomial infections (Lundstrom et al., 2001). Diabetes mellitus, indwelling catheter, abuse of antifungal drugs, surgery, immunosuppressive therapy, chronic renal failure in addition with so many others are known risk factors associated with Candiduria, it is important to consider that the older the age, the more chances of cases of Candiduria in a patient

2.2.4 Candidiasis of the mouth, throat

This is the more common type of mucocutaneous candidiasis, it is also known as thrush characterized by the presence of small spots or white adherent painless patches in the mouth, it usually extends from the lining of the mouth to the cheeks and is most commonly observed in infants, it is a source of sore throat when it progresses to the roof of the mouth, gums or tonsils and finally to the back of the throat. This infection is caused by broad spectrum antibiotics, chemotherapy, dry mouth, immune deficiencies and can be treated by regular mouth washing/rinsing, use of antifungal drugs (Dangi et al., 2010; Akpan & Morgan 2010). Oropharyngeal candidiasis is common among invalids suffering from HIV, neonates, immunocompromised host, geriatrics in the community, and diabetic patients.

Figure1

Two sets of oral cavities, on the left is a normal oral cavity while on the right is an oral thrush.



(Lynch, 1994)

Other classes of candidiasis are; intrauterine candidiasis which occurs during pregnancy, anal candidiasis characterized by intense itching and burning around the anus, and nail candidiasis (*candida* is not considered a normal flora of the nails)

2.3 Virulence traits of Candida species

There are various virulence factors which are responsible for the infectious process in this pathogenic yeast, these several virulence traits include adherence to (epithelial cells, endothelial cells, extracellular cells, C3b), hydrolytic enzymes (phospholipases, secreted aspartyl proteinase), phenotypic switching, cell wall components (glucan, glycoproteins, chitin).

2.4 Epidemiology

Several *Candida* species are known to cause systemic and superficial diseases in immune compromised patients which at most times affect mucosal surfaces and epidermal layers of the skin (Hasan F*et al*., 2009). Candidiasis makes up a large proportion of infections that people suffering from AIDS undergo (Fidel P L JR, 2006;

Hasan F*et al*., 2009). Invasive candidiasis is caused by the specie *Albicans* (Horn *et al*., 2009) leading to a high mortality rate and as a result causes a rise in economic and medical importance, this poses as a huge public health challenge (Almirante B*et al*., 2005; Lai C C*et al*., 2012).

Non *Candida albicans* have been reported to have an increased rate of infectivity despite *C.albicans* being the most rampant cause of invasive fungal infection. A study in 2019 produced *C. glabrata* as the specie isolated alongside other non albicans species with majority of isolates being that of *C. albicans* (Janaina *et al*., 2013). Epidemiological changes arise as a result of broad spectrum antifungal use, low immunity level, being a geriatrics patient (Horn D L*et al*., 2009). Investigations in European countries further proved that *C. albicans* was the major cause of as the rate at which other species were produced were significantly lower, (Tortorano A M*et al*., 2006), epidemiological changes in other parts of America have been reported.

There have been several species recognized in immune competent individuals. The species *C. dubliniensis* in addition to *C. albicans* were found to be closely associated (Sullivan D J*et al*., 2004). There is an extensive number of *C. dubliniensis* reported from patients infected with HIV and AIDS and also from oral cavities (Tintelnot K*et al*., 2000; Lasker B A*et al*., 2001). Following the initial report as stated earlier, (Khan *et al*., 2012), succeeding studies have described this specie to be endemic as it affects humans, (Loreto E S*et al*., 2010; Z. Khan S*et al*., 2012) and non- humans creating an avenue for inter-host transmission (Nunn*et al*., 2007).

C. parapsilosis appeared as an important hospital acquired disease causing fungus which presents itself clinically in a series of ways such as fungemia (Canto'n E*et al*., 2011), it causes infections in new born, however in contrast, it affects patients of all age in Latin America (Almirante B*et al*., 2005; Nucci M*et al*., 2010). Pires R H*et al*. (2011a).

There is a level of concern as regard the increase in the rate at which antifungals are becoming resistant and also in the number of infections caused by species other than *C. albicans* (Pereira G H*et al*., 2010). Due to the significance in increase in anti-fungal resistance, cautious steps have been taken to expand anti-fungal compounds that have been developed since the 90s (J,C,O Sardi et al., 2013), due to increased availability of the anti-fungals, the immune system has become prone to selection resulting in anti-

fungal resistance. Medical practitioners preserve the right of administering these drugs while waiting for diagnosis which causes an increase in exposure (Rodrı´guez-Tudela J L*et al*., 2007).

2.5 *Candida* biofilm

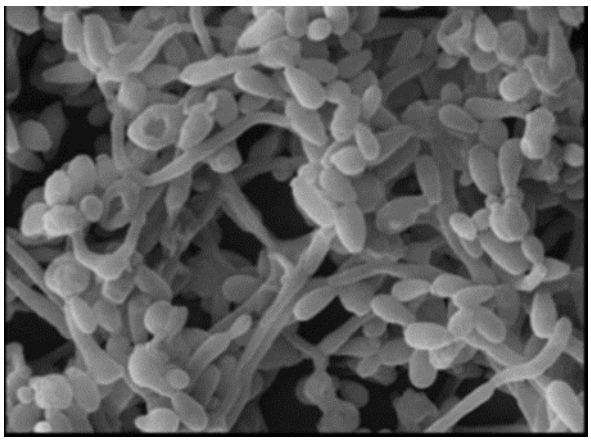
The ability of fungi to form biofilm helps in its ability to cause diseases (Martinez L R& Fries B C, 2010). Studies have shown that a vast number of diseases caused by *C. albicans* is as a result of biofilm formation (Ramage G& Lo´pez-Ribot J L, 2005). Silva S*et al*. (2009) reported that other non *albicans* species can also form biofilm. There are corroborations with past studies that have shown that these fungi are almost non recognizable when they exist by themselves causing them to exist in a group of other organisms (Soll D R, 2008). Villar-Vidal M*et al*. (2011), in comparison with *C. dubliniensis, C. albicans* has a higher number of biofilm formation. The ability of *Candida* species to adhere to materials and host cell is the initial step in biofilm formation. Adhesion of these *Candida* species to materials is interrupted by forces and also specific adhesins (Ramage G & Lo´pez-Ribot J L, 2005; Chaffin W L*etal*., 1998; Chandra J*et al*., 2005).

C. parapsilosis biofilms are made up of a large proteinous and carbohydrate content in comparison to *C. glabrata* and *C. tropicalis* (Silva S*et al*., 2009, 2011b; Baillie G S& Douglas L J, 1999). Biofilm formation by *C. albicans* has a subset of yeast which is considered to be extremely resistant once adhesion has taken place and is self-reliant of the up regulation of efflux pumps and cell membrane composition (LaFleur M D*et al*., 2006).

Connection of fungi in a biofilm matrix is for their protection and improving a relationship where every fungi benefits from the next causing them to adapt in an unstable environment (Davey & O'toole, 2000). Formation of Biofilms sustain fungi's resistivity to antifungals and its ability to cause diseases while also escaping the host immune system, and at the same time being under duress from other organisms. As a result of all these, it is important to note that formation of biofilm requires a lot of stages to treat (Ozkan S*et al*., 2005).

Figure 2

A microscopic representation of C.albicans biofilm



(J.C.O Sardi *et al*., 2013)

Mortality rate is high in diseases caused by *Candida* species associated with Catheter (Viudes A*et al*., 2002; Finkel J S & Mitchell A P, 2011), antifungal therapies such as combination therapy or monotherapy cost a fortune yearly in America (Finkel J S& Mitchell A P, 2011). Similar to bacterial biofilm formation, candida biofilms also exhibit resistance which makes it difficult to treat except it is as a result of surgical procedure (Finkel J S& Mitchell A P, 2011) Catheter acquired infections are considered serious and common, therefore many investigations have been focused on its biofilm formation (Finkel J S & Mitchell A P, 2011). Although, biofilms can also be formed on several other devices (Nett J E et al., 2010)

Invasive infections is common as a result of biofilm formation which makes it possible for Candida to grow alongside other bacterial species. (Klotz S A*et al*., 2007a; Harriott M M& Noverr M C, 2011). For all we know, biofilms are yet to be recognized in the gastrointestinal tract (Harriott M M& Noverr M C, 2011).

2.6 Antifungal susceptibility Testing

Fungal infections are known to cause diseases and infections in immune compromised patients in recent years leading to an increase in mortality and as a result of broad spectrum antifungal therapy, resistance to antifungal drugs have increased. There are three classes of antifungals that are used clinically in treating fungal infections; azoles

which include but are not limited to (posaconazole, and isovuconazole); (anidulafungin);polyenes (amphotericin B) (Anna *et al*.,2015). The antifungal drugs that were tested on in this study were Flucytosine, voriconazole, amphotericin B. The use of recently developed antifungals have been helpful to clinicians with the now use of combination therapy which works faster and better in serious cases.

Antifungal susceptibility methods are useful in detecting antifungal resistance and discovering the best method in treating a specific fungus. According to studies previously done on antifungal susceptibility testing, there are various reference testing methods that are used which are; Broth micro dilution method, agar-based methods, (Ana *et al*., 2015).

Micro dilution methods are the reference techniques for antifungal susceptibility testing, irrespective of its benefits, these methods of testing for antifungal sensitivity or resistivity is quite complicated and takes a lot of man power, energy and time. Some of the machines readily available do not require this much energy and time, and are easy to handle and help to manage cost. (Anna *et al*., 2015).

2.6.1 Broth dilution

This is a method which is used to test for susceptibility of an organism to a drug, it is most common for bacteria and widely used in the US, but is also used for antifungal testing. (Giagiani *et al*., 1978), it generally involves the use of a micro titer plates filled with a certain quantity of broth, antifungals or antibiotics are added as well to the micro titer plate and inoculum to be tested on as well (Berkow *et al*., 2020).

The micro dilution method makes use of two recognized guidelines for clinical testing; the CLSI and methods established by (EUCAST) (Arendrup et al., 2017). The CLSI and EUCAST use the same approach but a notable difference in the concentration of their glucose is seen, same standard is used in the assessment of the finishing point of both guidelines. There is also similarity on the basis of growing their clinical breakpoint which leads to the outcome of comparable results in their antifungal sensitivity and resistivity (Berkow *et al*., 2020). But they have specific differences which are discussed in the table below:

TABLE 2.6.1 *the differences between Clinical and laboratory standards institute (CLSI) and European committee on antimicrobial susceptibility testing (EUCAST) methods of broth micro dilution*

(Berkow *et al*., 2020)

Guidelines	**CLSI M27 A4**	**EUCAST E.DEF 7.3.1**
RPMI(Rosewell park memorial institute medium) glucose content	It has a 0.2% glucose content	There is a difference as compared to CLSI with a 2% glucose
Composition of antifungal drugs	A stock was prepared of concentration at least 1,280 g/ml or 10 times the highest concentration to be tested, whichever is greater	it is advisable to prepare stock of concentration which is preferably 200 X times higher than the recognized excessive concentration which is to be tested in the plate
Composition of an organism	The yeast should be subcultured atleast twice	There is no accuracy on the quantity of subculture
Microdilution plate	Micro titer Plates with U-shaped wells are recognized	Unlike CLSI, Tissue-treated plates with flat-bottomed wells are used in this instance
Inoculum size	Yeasts, 0.5 $X10^3$ to 2.5 $X10^3$ cells/ml; filamentous fungi (non-Dermatophytes), $0.4X10^4$ to $5X10^4$ cells/ml; filamentous fungi (Dermatophytes), $1X10^3$ to $3X10^3$ cells/ml	Yeasts, $1X10^5$ to $5X10^5$ cells/ml; filamentous fungi, $2X10^5$ to $5X10^5$ cells/ml
Assessment method	Optical	Spectrophotometric

Amphotericin B reading	A complete decrease in growth, or the first optically clear well	There is a ≥90% decrease in growth
Miscellaneous		Recommends against the use of low-evaporation lids

2.7 EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

The organization was selected in this study because it has strict breakpoints in an ability to control the rising rate of antifungal resistance. There are separate organizations which work closely with EUCAST such as the ECDC, ENBC and ESCMID. The AFST-EUCAST which is considered a sub-committee of the EUCAST came into existence 25 years ago, and few years later came up with a basis on which yeasts were tested on (Arendrup *et al*., 2012). Another standard for mold was published in 2008 (Berkow *et al*., 2020). Differences between CLSI and EUCAST criteria are found in (Table 1). Nevertheless, there is similarity between both guidelines (Chryssanthou E *et al*., 2006; Pfaller MA *et al*., 2007).

2.8. Mechanism of Resistance against Antifungal agents

There are three main mechanisms of antifungal resistance; (Claudia Spampinato and Darío Leonardi 2013) which all act differently. Studies are available which give more details on antifungal resistance against the different antifungal classes (Z. A. Kanafani 2008, P.Vandeputte *et al*., 2012, D.S Perlin 2009, J.Peman *et al*., 2009).

2.8.1 Azole resistance

In decade past, azoles have been used to a large extent, they are widely used in treating severe fungal infections and are also readily available for prevention of further infections, azoles are known to be safe hence, their wide usage (J F G M Meis and P E Verweii, 2001, Hoffman et al., 2000, D.M.Livermore, 2004). Eventually, reports of

high percentage of patient resistance to azole was described (S.W Redding *et al*., 2003). Patients suffering from HIV infected with oral thrush are known to exhibit resistance to azoles (D.J Skiest *et al*., 2007). Although, patients infected with other forms of diseases such as vaginitis show less resistivity (M.Ribeiro *et al*., 2005).

2.8.1.1. Reduced Drug Intracellular Accumulation

An accountable process needed in reducing concentration of drugs here are based on up regulation of efflux pumps (R. D. Cannon *et al*., 2009). These transporters are different based on their specificity and origin of their vitality needed to withdraw the drug (R. D. Cannon *et al*., 2009). The Cdr machine has the ability to release all antifungals belonging to the azole class and are a member of the ABC transporters. They are hidden by *Candida* drug resistance 1 and 2 genes in *C. albicans* (R. D. Cannon *et al*., 2009). There is the availability of a second pump which is not a primary transporter and is particular for fluconazole. (R. D. Cannon et al., 2009).*C. glabrata* (R. Torelli *et al*., 2008, D Sanglard *et al*., 1999. J.E. Bennett *et al*., 2004), *C. dubliniensis* (CdCDR1, CdCDR2) (G. P Moran et al., 1998), *C. krusei* (ABC1 and 2) (E. Lamping *et al*., 2009, S. K. Katiyar & T. D. Edlind, 2001), *C.tropicalis* (CDR1-homologue) isolates (P. Vandeputte et al., 2012, J.-P. Vermitsky & T. D. Edlind, 2004, J P. Vermitsky *et al*., 2006, H.F. Tsai et al., 2006).

2.8.1.2 Drug processivity

The aim of azole antifungals is the lanosterol 14-α-demethylase is the target for azoles and a situation where this is changed leads to a reduction in the association between enzymes and azoles (Favre B et al., 1999).

2.8.1.3. Prevention of the Drug Effect

There are processes which help this effect; initial process has to do with upregulation which leads to an increase intracellular of the target protein (Claudia Spampinato and Darío Leonardi 2013). Regardless of the fact that the latter process is uncommon, it has been noted in isolates of *C. albicans* (C.M. Martel *et al*., 2010)

2.8.1.4. Echinocandin Resistance

Antifungal drugs belonging to the class “Echinocandin” are approved by medical practitioners as the go to drugs for severe candida diseases. Nevertheless, patients infected with *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* have been described to have an increasing rate of resistance to echinocandins (S. Hernandez *et al*., 2004, M Krogh-Madsen *et al*., 2006. M Hakki *et al*., 2006, T Pasquale *et al*., 2008, B Alexander *et al*., 2013, M A Pfaller *et al*., 2012). From 2001 to 2010, *C. glabrata* developed in resistance drastically (B. Alexander *et al*., 2013). Furthermore, isolates of *C. glabrata* have developed a dual resistance to azoles and echinocandins (B. Alexander *et al*., 2013

Secondary resistance is as a result of drug processivity which is explained below

2.8.1.5. Drug Processivity

The FKS1 or FKS2 genes are responsible for resistance in this class of antifungals whichoccurs as a result of the change, addition or removal of the nucleotide base(J. N Kahn et al., 2007, S. Park et al., 2005, S. V. Balashov *et al*., 2006) that hides the β-glucan synthase complex (S. V. Balashov *et al*., 2006). Changes in the nucleotide base in FKS1 only affected the rate of reaction of enzyme saturation (G. Garcia-Effron *et al*., 2009).

2.8.1.6. Polyene Resistance

Irrespective of its more than 30 years of clinical usage, amphotericin B has established a very minimum resistance. This class of antifungal is well known for its level of toxicity and side effects, making it difficult to be prescribed as a prophylactic which has become a major issue in its usage commercially (R Laniado-Laborın & M N Cabrales-Vargas 2009, D. Ellis, 2002). For candida to be resistant to polyenes, it depends on the specie. The species *C. glabrata* and *C. krusei*most of the time exhibit sensitivity to amphotericin B. as a result of this, a directive from the infectious diseases society of America states that Amphotericin B should be administered more than usual

to patients infected *C. glabrata* and *C. krusei*(J.H. Rex *et al*., 2000). An outstanding proportion of isolates of *C. glabrata* and *C. kruseiC. lusitaniae* and *C. guilliermondii*, have been noted to indicate resistivity to amphotericin B (D. P. Kontoyiannis and R. E. Lewis, 2002, (P. G. Pappas et al., 2004).

Resistance to polyenes is as a result of the mechanism mentioned below

2.8.1.7. Counteraction of the Drug Effect

Secondary resistance in this class of antifungals is likely because of a decrease or lack thereof ergosterol. It is reported that *Candida* species resistant to polyenes have a reduction in their ergosterol in comparison to *Candida* species sensitive to polyenes (Claudia Spampinato and Darío Leonardi 2013). These shortages can be as a result of the deprivation of the ability to cause a change in the nucleotide base in the ERG3 or ERG6 genes (A. Espinel-Ingroff, 2008, P. Vandeputte *et al*., 2007, S. L. Kelly *et al*., 1997).

2.8.1.8. Flucytosine Resistance

Flucytosine has a chief resistance level which is quite low. And a latter resistance which is dependent on disruption of various enzyme pathways (Claudia Spampinato and Darío Leonardi 2013).

2.8.1.9. Reduced Drug Intracellular Accumulation

A removal, addition or change of the nucleotide base in the FCY2 gene affects uptake of the drug (J. Peman *et al*., 2009, A. Espinel-Ingroff, 2008)

2.8.2. Prevention of the Drug Effect

Resistance to Flucytosine following exposure to previous diseases can also stem from an alteration in its nucleotide base in the FCY1 gene which helps in coding for the cytosine deaminase (Claudia Spampinato and Darío Leonardi 2013).Various changes in the nucleotide base have been detected in some *Candida* species (J.Peman, 2009, A. Espinel-Ingroff, 2008, F. Chapeland-Leclerc et al., 2005, P. Vandeputte *et al*., 2011)

2.9. Aim

This investigation was aimed at evaluating the growth of *Candida* species in hospitalized patients, out-patients and also to determine the susceptibility and resistance pattern to the antifungal drugs used in this study which were (amphotericin B, Flucytosine, Voriconazole) and to provide necessary treatment and prevention guidelines for *Candida* infections.

SECTION THREE

Materials and methods

3.1 Study Group

This investigation was done in Near East University Hospital. For this retrospective study, 202 samples of *Candida* species were obtained from various hospitalized patients (in/out patients) from 15th September 2020- 28th February 2022, samples from various departments of the hospital were involved in this study. EMB and Blood agar were the medium used but in the case of specific *Candida* species identification, (SDA) was used. These different media were all without cycloheximide.

3.2 Tools and Equipment

- Petri-dish
- Inoculating pin
- Test-tubes
- Test-tube rack
- Vitek 2 compact system (Biomerieeux)
- Automatic pipette (Gilson Pipetman.Dk60063, Biyomedikal 2179. Made in France)
- Autoclave (model OT40L. Miive Steam Art. Biyomedikal 2189)
- BD BACTEC 9120 machine
- Incubator (Heraeus Thermo Scientific. Biyomedikal 2184)
- Microscope Slides
- Electronic microscope
- Blood culture bottle BD BACTEC (becton, Dickinson and company,

3.2.1 Kits and Chemicals:

- Blood agar– Becton, Dickinson and company. France.
- EMB agar Becton, Dickinson and company, France
- SDA -Becton, Dickinson and company, France

3.2.2 VITEK 2 Compact machine (BIOMERIEUX)

The VITEK 2 compact instrument provides efficacy in patient outcomes through reliable microbial identification (ID) and antibiotic/anti-fungal susceptibility testing (AFST), it also helps laboratory capabilities with reduced hands on time and rapid reporting abilities (Sanders et al., 2001).

Figure 3

An image of a VITEK2 COMPACT machine from the company "BIOMERIEUX"

From Near East University laboratory

3.2.3 BD BACTEC 9120 machine

BD 9000 system offers a unique, reliable, safe, shorter service, shorter protocol and higher recovery rate than any other blood culture system

Figure 4

An automated BD BACTEC 9120 machine

From Near East University laboratory

Figure 5

A BACTEC 9120 station showing the blood culture bottle

From Near East University laboratory

3.3. Inclusion criteria

Data of Patients aged 0-95 were entered in this investigation indicating that there was no restriction to the age limits. Samples such as yeast culture, urine, aspirate, vaginal swab, abscesses/wound scrapings, liquid, sputum, catheter tip, blood and others known to be colonizers for *Candida* species were taken into consideration

3.4. Exclusion criteria

For the purpose of this study, there were cases of repeated cultures where isolates that were not identified as *Candida* were discarded.

3.5. Data collection

Each of the patients were assessed based on age, gender, hospital department, sample type, in/out patient at the time of sample collection and resistance as well as sensitivity to antifungal drugs were recorded.

Once the samples arrived in the lab, Such as blood, stool, urine, aspirate culture with labels detailing the patient's name, barcode number, date of sample collection, type of sample, details were written in a record book (starting from the number 1) as well as the electronic microbiology laboratory database. All culturing was done in Blood and EMB agar with exception to a situation where the clinicians asked directly for a yeast

culture or if *Candida* species were detected on Blood or EMB, then we had to culture and sub culture on SDA (Sabouraud dextrose agar)

Culturing of the samples took place in a special cabinet which was already sterilized and allows no form of contamination or cross contamination, in the special cabinet we find the inoculating loop/pin, flame.

3.6. Urine samples;

The inoculating loop was sterilized until it was red hot and after cooling down for 2-3 seconds, a sample was taken and streaked on the agar plates, Blood and EMB respectively. The cultured plate was incubated in an incubating machine for 24-48hrs at 35ºC, after 24 hours it was observed for microbial growth and a series of biochemical tests and Gram staining were performed until a pure culture was attained, if *Candida* was detected on either of the agars, it was sub-cultured on SDA until a pure culture was gotten.

3.6.1 Aspirate samples;

In this particular test, a slide was needed to perform a Gram staining test. It was labelled also with the patients details

A sample was taken with a swab and spread in a section of the blood agar, then a sterilized loop was used to streak further on the agar plate and this procedure was repeated for the EMB agar also, and it was put into the incubator alongside the smeared slides at 35^0C for 24-48hours, as previously done with the urine samples, it was observed for growth and a biochemical and Gram staining test were also performed before finally taking it for identification in the VITEK machine.

3.6.2 Blood culture;

Nurses took 2 culture bottles from each patient, one bottle from 1 patient's arm after 15 minutes interval, a second bottle from the other arm. For adult patients 8-10ml of blood was used for each bottle, and for pediatric patients 1-3ml of blood was used. Once the bottle got to the lab the patients details such as barcode number, date of sample collection, time, which department it came from into the electronic

microbiology database was inputted, the BACTEC machine was opened to scan the barcode on the blood culture bottle and the barcode on the BACTEC machine and the machine automatically gave indication on where to put the bottle. BACTEC machine indicate if the blood culture has bacteria or fungi (*Candida*) in the blood. If the test result is negative, it gives a negative result after 7 days, if there is presence of candidemia, the machine gives a positive sign after 1 day, if the infection is very severe the machine gives a result in few hours, therefore, the greater the infection rate, the faster the machine gives a positive sign.

A positive sign indicates that the bottle be taken out and a drop of blood was taken with a sterile syringe and cultured on blood agar and EMB agar plate, once Candida was detected after 24-48hrs of incubation at 35ºC, it was sub-cultured on SDA and after a pure culture was obtained the organism was identified through colony morphology, Gram staining and observation under the microscope where the presence of yeast was observed, 2-3 colonies were mixed in a saline solution (in a test-tube) and put in the VITEK 2 machine for species identification.

3.6.3. Anti-fungal susceptibility testing

Two test tubes are needed for this testing, one test tube was for identification, the other for anti-fungal susceptibility testing. 2-3 colonies of the sample was taken and mixed with the saline solution, McFarland method was used in this study, with the range 1.80-2.20 for *Candida* species, while 2.8µl (for Gram positive and *Candida* species) was transferred from the first test tube into the second one. It was taken to the VITEK 2 machine to determine its resistance or sensitivity and results were taken the next day. For the purpose of this study I was more interested in 3 anti-fungal drugs (Amphotericin B, Flucytosine and voriconazole).

3.7. Gram staining:

It helps to classify fungi into Gram positive and Gram negative, the first step in Gram staining is to;

- ✓ Get a clean side, using an inoculating loop, the sample is smeared on the slide, and heat fixed by passing through a flame gently 1-3 times. Make sure to not apply too much heat as that can possibly kill the organism
- ✓ Crystal violet is applied to the slide and after 1 minute is gently rinsed off under running water for 2-3 seconds.
- ✓ A dropper is used to flood the slide with Gram's iodine and allowed to sit for 1 minute, and rinsed off under running water.
- ✓ The slide is rinsed with alcohol (decolorizing agent) for about 5-10 seconds, followed immediately with a rinsing under running water
- ✓ Safranin (counter stain) is applied and allowed to sit for 1 minute, then washed off under a running water, and blot dry. The Gram negative cells will retain the color of the Safranin (pink/red), Gram positive cells appear purple/blue.
- ✓ Examine the slide under oil immersion using an electronic microscope, an amplification of X40 is recommended for candida species (Anne Marie Helmenstine, 2019)

3.8. Biochemical tests

There are series of biochemical tests which are performed to help in identification of candida species as well as other microorganisms, the VITEK 2 compact instrument helps to do this in a shorter amount of time with improved accuracy.

3.9. Statistical data analysis

The Pearson chi square test and Fisher's exact test were the testing methods for the results in this study, with an SPSS Demo version 22for all statistical analysis. A P value of 0.005 was considered significant.

SECTION FOUR

Results

The results from this retrospective study shows that data were gotten from 202 patients with *Candida* isolates in Near East University Hospital from the period 15th September 2020- 28th February 2022. There were patients of different age groups and gender involved in this study from various departments of the hospitals who were either in-patients or out-patients.

The distribution of the male and female gender involved in this study and their P-value is detailed in Table 4.1

Table 4.1: *Distribution of the gender base of Candida isolates*

Gender	**Number**	**%**	**P-Value**
Male	116	57.4	
Female	86	42.6	
Total	202	100	0.553

Overall, there was a significant difference between the inpatients and outpatients in this study, this is further explained in the table below

Table 4.2: *Distribution of in-patients and out-patients*

Patients	**Number**	**%**	**P-Value**
Inpatients	194	96	
Outpatients	8	4	
Total	202	100	0.037

The distribution of the various *albicans* and non *albicans Candida* species has been outlined in Table 4.3

Table 4.3: *Distribution of the various Candida species isolates*

Candida species	**Number**	**%**
Candida albicans	139	68.8
Candida parapsilosis	23	11.4
Candida tropicalis	14	6.9
Candida glabrata	13	6.4
Candida krusei	5	2.5
Candida famata	4	2.0
Candida kefyr	3	1.5
Candida lusitaniae	1	0.5
Total	202	100

Table 4.4 gives a more detailed explanation of the *Candida* strains (*albicans and non-albicans*) involved in this study and their sensitivity and resistivity patterns in all patients involved

Table 4.4; *Distribution of the susceptibility and resistance pattern of Candida species in all patients*

***Candida* species**	**Number**	**%**	**Amphotericin B**	**Flucytosine**	**Voriconazole**
Candida albicans	120	59	Sensitive	Sensitive	Sensitive
Candida albicans (1)	2	1	Resistant	Sensitive	Resistant
Candida albicans (2)	1	0.5	Resistant	Resistant	Sensitive
Candida albicans (3)	7	3.5	Sensitive	Sensitive	Resistant
Candida albicans (4)	6	3	Resistant	Sensitive	Sensitive

Candida albicans (5)	3	1.5	Sensitive	Resistant	Sensitive
Candida tropicalis	12	6	Sensitive	Sensitive	Sensitive
Candida tropicalis (1)	1	0.5	Sensitive	Resistant	Sensitive
Candida tropicalis (2)	1	0.5	Sensitive	Sensitive	Resistant
Candida glabrata	10	5	Sensitive	Sensitive	Sensitive
Candida glabrata (1)	2	1	Sensitive	Sensitive	Resistant
Candida glabrata (2)	1	0.5	Sensitive	Sensitive	
Candida famata	4	2	Resistant	Sensitive	Sensitive
Candida parapsilosis	12	6	Sensitive	Sensitive	Sensitive
Candida parapsilosis (1)	10	5	Sensitive	Sensitive	Resistant
Candida parapsilosis (2)	1	0.5	Resistant	Sensitive	Resistant
Candida krusei	5	2.5	Sensitive	Resistant	Sensitive
Candida lusitaniae	1	0.5	Sensitive	Sensitive	Sensitive
Candida kefyr	3	1.5	Sensitive	Sensitive	Sensitive
TOTAL	202	100			

Isolates were observed to be isolated mostly from yeast culture (n=117, 57.9%), with a more detailed observation outlined in Table 4.5

Table 4.5: *Distribution of the different clinical samples involved in this study*

Samples	**Number**	**%**
Yeast culture	117	57.9
Sputum	31	15.3
Aspiration	23	11.4
Urine	15	7.4
Abscesses/wounds	6	3
Catheter culture	4	2
Blood	3	1.5
Vaginal/urethral discharge	3	1.5
Total	202	100

Clinical samples were collected from patients in different hospital departments with the majority of *Candida* infections isolated from ICU department, more details are outlined in the table below

Table 4.6: *Distribution of Candida species from the various departments in the hospital*

Department	**Number**	**%**
ICU	146	72
Cardiology	17	8.4
Chest disease and Allergy	10	5
Pediatrics and Diseases	4	2

Infectious diseases	4	2
Internal medicine	3	1.5
Obstetrics and Gynecology	3	1.5
General surgery	3	1.5
Neurology	2	1
Geriatrics	2	1
Oncology	2	1
Neurosurgery	2	1
Urology	1	0.5
0rthopedic and Traumatology	1	0.5
Ear Nose and Throat	1	0.5
Dermatology	1	0.5
Total	202	100

The *Candida* species involved in this study were tested on three different antifungals and exhibited various rates of sensitivity and resistance as detailed below

Table 4.7: *Distribution of the sensitivity-resistance percentages of antifungals in Candida species*

Amphotericin B	**Number**	**%**	**Valid (%)**	**Cumulative (%)**
Sensitive	188	93.1	93.1	93.1
Resistant	14	6.9	6.3	100
Total	202	100	100	

Flucytosine	Number	%	Valid (%)	Cumulative (%)
Sensitive	191	94.6	94.6	94.6
Resistant	11	5.4	5.4	100
Total	202	100	100	

Voriconazole	Number	%	Valid (%)	Cumulative (%)
Sensitive	178	88.1	88.1	88.1
Resistant	24	11.9	11.9	100
Total	202	100	100	

SECTION FIVE

Discussion

Candida is an endogenous and exogenous fungi that has over 150 species but with few species causing nosocomial infections, the different *Candida* species leads to fungal infection causing mycoses. Endogenous *Candida* is found on the human micro flora where it colonizes the mucosal surfaces, gastrointestinal tract, skin etc., and exogenous *Candida* can be acquired through environmental factors; colonization of hospital equipment, colonization of intravenous catheter or through the hands of professional health workers. Candidiasis affects various parts of the human body; there is the presence of superficial candidiasis, invasive/systematic candidiasis and candidiasis of the mouth, oropharyngeal candidiasis. Many of these infections have developed resistance to the anti-fungal drugs readily available, which can be as a result of broad spectrum anti-fungal use, weakened immune system, underlying diseases. *Candida albicans* is the most prevalent strain and it exhibits polymorphism which makes it able to adapt to the environmental conditions and evade host's immune system thereby causing severe diseases. The susceptibility patterns of *Candida* species were investigated using the EUCAST guidelines.

Candida species were reported to be more prevalent in the male patients than females with a mean age of (67.53±15.46) and (68.94±18.14) respectively, no correlation was found between the above mentioned mean ages, with a P- value of 0.553

In this study, as described in Table 4.2, there were more number of isolates reported in in-patients than was seen in out-patients, this is further proving that *Candida* is a prevalent nosocomial infection most especially among hospitalized patients, it explains that the hospital is an environment when not properly sanitized can become a huge source of infection and diseases where it should rather be a safe zone from all these infections. Patients most times come into the hospital to get treated for a cut in the leg and as a result of cross contamination and hospital acquired infections, when admitted can develop a more acute infection due to the presence of nosocomial infections, these findings support a publication from (F. Galle et al., 2006), who stated that the 115 hospitals participating in the national nosocomial infection surveillance experienced a spike in hospital acquired infections between 1980-1990 (Beck-sague *et al*., 1980-1990).

Few species are regularly isolated from the hands of Health care workers such as *C. parapsilosis* and *C. albicans* as stated by Verduyn Lunel FM et al., 1999; Pfaller MA et al., 1998; Strausbaugh L J et al., 1994, this aligns with our present study as described in Table 4.3 which shows *Candida albicans* to be the prevalent species succeeded by *C. parapsilosis* 11.4% which is slowly emerging as a public health issue. And in the same light also giving more details why the ICU department which houses a large number of anesthesiologists had the highest number of isolates from the various departments in the hospital, given that this particular department requires the regular use of hands of medical staff. For the purpose of this investigation, *C. tropicalis* is the third isolated organism, according to Mayhall, 2004 which demonstrates that "*Candida albicans* has more prevalence in hospitals in comparison to *C. tropicalis*" (Mayhall CG, 2004).

Candida albicans to this very day still remains the predominant species in the *candida* family, and also reported to be the most isolated species as a result of this investigation with a 68.8%, there are more cases of *Candida albicans* in this study as compared to other species possibly due to the use of immunosuppressant drugs which can lower the immunity level of patients thereby making them prone to this pathogen which are resident flora of the GIT, genitourinary tract, to name a few. These findings are consistent with the reports of Fridkin SK &Jarvis WR 1996; Beck-sague C *et al*., 1993 who discovered a large number of hospital acquired infections describes *Candida* species as the causative fungi. And also Fridkin *et al*., 1996 who again discovered that the species causing a widespread of diseases is *C. albicans*. (Emori TG 1993, Fridkin SK *et al*., 1996) also stated that NNIS in 1990-1992 described *C. albicans* as being among the top ranks of disease causing organisms isolated from systemic regions which harbors severe infections. This further proves that for over 20 decades, *Candida albicans* is the predominant species causing most nosocomial diseases particularly in immunocompromised patients.

Several publications have described candidemia to be a significant reason of nosocomial diseases leading to loss of lives (Wisplinghoff H *et al*., 2004; Fraser V J*et al*., 2018) reported candidemia as the top ranked cause of hospital acquired infections seen among the Americans with a death rate of 57%. Nevertheless, my study produced a low outcome of candidemia with only a 1.5%.

Table 4.4 explains the various *Candida* species isolated in this study and their susceptibility and resistance patterns in all patients involved in this study. It shows *Candida albicans* to exhibit sensitivity and resistivity in different patients, *C. albicans* (1, 2, 3, 4, 5) explicitly shows a case where a patient with an isolate of *C. albicans* is sensitive to all anti-fungal drugs used in this study, and also a case where another patient *C. albicans* (1) with the same species shows resistance to one of the drugs and sensitivity to the rest of the anti-fungal, this can be attributed to the fact that the patient might have developed a resistance mechanism to that particular anti-fungal while the other patient has not, it can also be due to the age of the patient and the fact that this particular patient might be immunocompromised. For the non-*albicans* species in this study with similar outcomes, it implies that these species are increasing in pathogenicity. *Candida glabrata* (2) showed no form of activity to voriconazole which is explained as an inconclusive result, possibly due to the fact that the anti-fungal didn't provide a clear sensitive or resistance result.

Candida krusei was the species that was the most resistant to Fluconazole and this is most probably due to the recent increase in the use of immunosuppressive drugs which has led to alternatively an increase in *C. krusei* infections, Jay Shankar Singh Yadav et al., 2012 explains why this may be so as *C. krusei* obtained a permanent resistance to a number of triazole anti-fungal drugs especially fluconazole which is a main drug in anti-fungal therapy, therefore this poses as a major health concern.

There were more yeast cultures isolated from patients in this study, from both male and female patients but most especially females, the high rate of the number of this yeast culture in patients indicate that a lot of consumption of anti-fungal, birth control pills, sugar and alcohol have taken place, as well as an alteration in the pH value of the vagina in some of the female patients. Abscesses/wound had six samples with (3%), Twenty-three (11.4%) aspirate samples, thirty-one (15.3%) sputum cultures, 15 (7.4%) samples were urine, 3 (1.5%) blood sample, 4 (2%) catheter culture which causes blood stream infections in hospitalized patients, and 3(1.5%) were vaginal/urethral discharge.

Navarro EE *et al*., 1997 who stated that "*Candida* species isolated from patients in the ICU have been described to be a significant reason for the spread of UTIs". As noted in this study, age of participants range from 0-95, with most of the patients being of

the older age, this can contribute to the presence of Candiduria and is supported by sobel JD, 1999; Lundstrom T and Sobel J, 2001; Sobel JD *et al*., 1999; Kauffman CA et al., 2000; Nucci M, 2000, Who reported that "*Candida* colonization, which include prolonged stay in the hospital, broad spectrum anti fungal use" to name a few are known risk factors for Candiduria. And the older the patient, the more viable they are for infections, most especially nosocomial infections.

In alignment to the findings in this study which reported cases of Candidemia in the ICU department, a study carried out in France reported that patients are exposed to conditions of acquiring Blood stream infections when stationed in the ICU, in comparison to when they are in other hospital departments (Brun-Buisson *et al*., 1996).

There is a vast difference between the numbers of isolates in the ICU department 72% as compared to other departments in table 4.6 This is due to the fact that patients who are in the ICU have an already existing condition which makes them susceptible to infections, as a result of the severity of the procedures in this department, it is very common for patients to develop invasive candidiasis, and also cross contamination has a high possibility of occurring in this department. The cardiology department produced the second most number of isolates 8.4% with the least number of isolates from the urology, dermatology, while ear nose throat, orthopedic and traumatology had the least number of isolates with (0.5%)

High levels of sensitivity were reported from this investigation as compared to its resistivity, which does not neglect the fact that resistance to antifungals yet again were observed. Voriconazole emerged to exhibit the highest resistivity among the antifungal drugs tested on the fungi which is in alignment with a publication by (Oxman D A*et al*., 2010; Lortholary O*et al*., 2011; Fothergill A W*et al*., 2014) that described that NAC have intrinsic resistance to azoles, this offers an explanation to why azoles in this study (Voriconazole) appear to be more resistant to *Candida* species, and also as stated by (Siikala E*et al*., 2010; Rautemaa R & Ramage G, 2011), prophylactic administration of low dose of azole derivatives such as voriconazole, fluconazole for an extended period to prevent manifestation of diseases in immunocompromised can lead to the development of high level resistivity.

Resistance to Amphotericin B (polyenes) is unusual in isolates of *Candida albicans* which can be due to a change in sterols, (Kanafani Z A & Perfect J R, 2008) is a report that is consistent with the findings of my study which clearly signifies that Amphotericin B is a sensitive antifungal with a 93.1% when tested on all the *Candida* species

Pfaller MA *et al*., 2000; Diekema DJ *et al*., 2002; Pfaller MA *et al*., 2001 educates us that blood isolates of *Candida albicans* happen to be sensitive to amphotericin B and fluconazole which makes them important in prevention of candidemia and treatment purposes. And also, Diekema DJ *et al*., 2002; Berrouane YF *et al*., 1999; Collin B *et al*., 1999, reported that the constant administration of fluconazole clinically allows for the opportunity to monitor this fungi's ability to be susceptible in order to put a stop to the distribution of resistance species.

Flucytosine (5-FC) produced the highest level of sensitivity as a result of this study with a 94.6%. Tassel D & Madoff M A, 1968); Francis P & Walsh, T J, 1992) collaborates with the finding in this study by stating that 5-FC monotherapy in cases of systematic or disseminated candidiasis has proven to be successful among every age group. Nevertheless, Monotherapy with 5FC is restricted because of the constant resistivity (A. Vermes *et al*., 2000) letting us know that resistance is not rare in 5-FC, which is why A. Vermes *et al*., 2000 suggests that monotherapy with 5-FC is not recommended. Although, as a result of this study, monotherapy with 5-FC produced such level of sensitivity in Table 4.6. Medoff, G (1971) and Montgomerie *et al*., 1975 described that in combining amphotericin B and 5-FCin clinical therapies indicates synergism and also, Thaler et al., 1988 reported the partnership of amphotericin B and 5-FC in treating hepatosplenic and diseases caused by *Candida* species. Moreover, previous studies have reported that this mode of treatment is effective for candida meningitis patients (Smego *et al*., 1984).

SECTION SIX

Conclusion

Conclusion

Finally, Candida albicans is still a prevalent fungal infection and it can be seen that Candiduria growth in most cases is as a result of broad spectrum antifungal, low doses of antifungals over a period of time is not the best approach to curbing resistance pattern and Flucytosine is a more responsive drug in treating candidiasis with Amphotericin B following close which shows clearly that a merger of Amphotericin B and 5-FC should be prioritized.

Recommendation

The hands of health workers should be frequently sanitized as well as hospital equipment at intervals to prevent cross contamination

For patients with Candiduria, it could be believed that it is as a result of antifungal therapy and for patients with infections to be treated adequately because there are very few antifungal drugs with clinical success, AFST has to be largely and readily available than it is presently.

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ADA STELLA ODOGWU

Sakir 9 apartment, Gonyeli, Nicosia, Northern Cyprus .

+905428582904

Stellaodogwu98@gmail.com

A dedicated individual with a high degree of moral worth looking for creative ways to be involved in the public health sector. To grow company and personal goals by attaining challenging positions and providing leadership within the profession, thus carefully showing the passion I have for improving the quality of life as well as human care in the best possible ways

Experience

August 2013- August 2014

LABORATORY ASSISTANT, DANGOTE SUGAR REFINERY FACTORY

- ★ assisted the full time lab technician in running tests in the laboratory
- ★ assisted in daily activities in the office (printing, photocopying, coffee runs)
- ★ Teamed up with 2+ interns in arranging files and documents for the department head

JUNE 2017- AUGUST 2017

LABORATORY ASSISTANT, ST WILLIAMS SPECIALIST HEART CLINIC

- ★ Assisted the lab technician in running tests in the laboratory, which included blood samples, urine, sugar level tests, HIV, malaria, typhoid, and filling of reports which made his work easier
- ★ From time to time assisted the cardiologist in EKG tests, working as his assistant alongside a nurse resulted in efficacy
- ★ worked alongside the pharmacist 2/3 times every week resulting in fast results

JANUARY 2019-OCTOBER 2019

Production Assistant, Osun state investment company

- ★ Teamed up with other professionals in the production of tabled water which involved start to finish production processes.
- ★ Assisted 3 other staff members in fixing labels on the plastics manually hereby increasing productivity by 40%
- ★ Alongside one intern and sometimes 2, we were involved in positioning of the plastics for automatic packaging by the machine
- ★ Assisted quality control unit in running tests when the need arose
- ★ assisted The quality control officer and department supervisor in testing for water quality

OCTOBER-DECEMBER 2020

Interim assistant administrator, Thierry technologies

- assisted in calling truck drivers at least 5-8 times a day depending on how busy the day was
- I was in charge of preparing the invoice at least once during my stay in the company
- I prepared documents for various companies we (the company) were working for, such as IHS and Huawei
- I was assigned to be in charge of a cluster region which housed up to 5 cars and trucks coming in and out on a daily basis

- Took down details of truck drivers and waybills details of every site they went to dispense diesel in my jurisdiction

MAY 2021 (2 weeks)
Laboratory intern, Near East University microbiology laboratory

- I observed the culturing processes of organisms in the lab.
- I assisted in viewing organisms under the microscope when I was needed

Education

22ND FEB- 21ST JUNE, 2021-2022
MSc: Medical Microbiology and clinical microbiology, NEAR EAST UNIVERSITY TRNC

Having completed my coursework, thesis and seminar , my CGPA is at 3.71 (high honors).

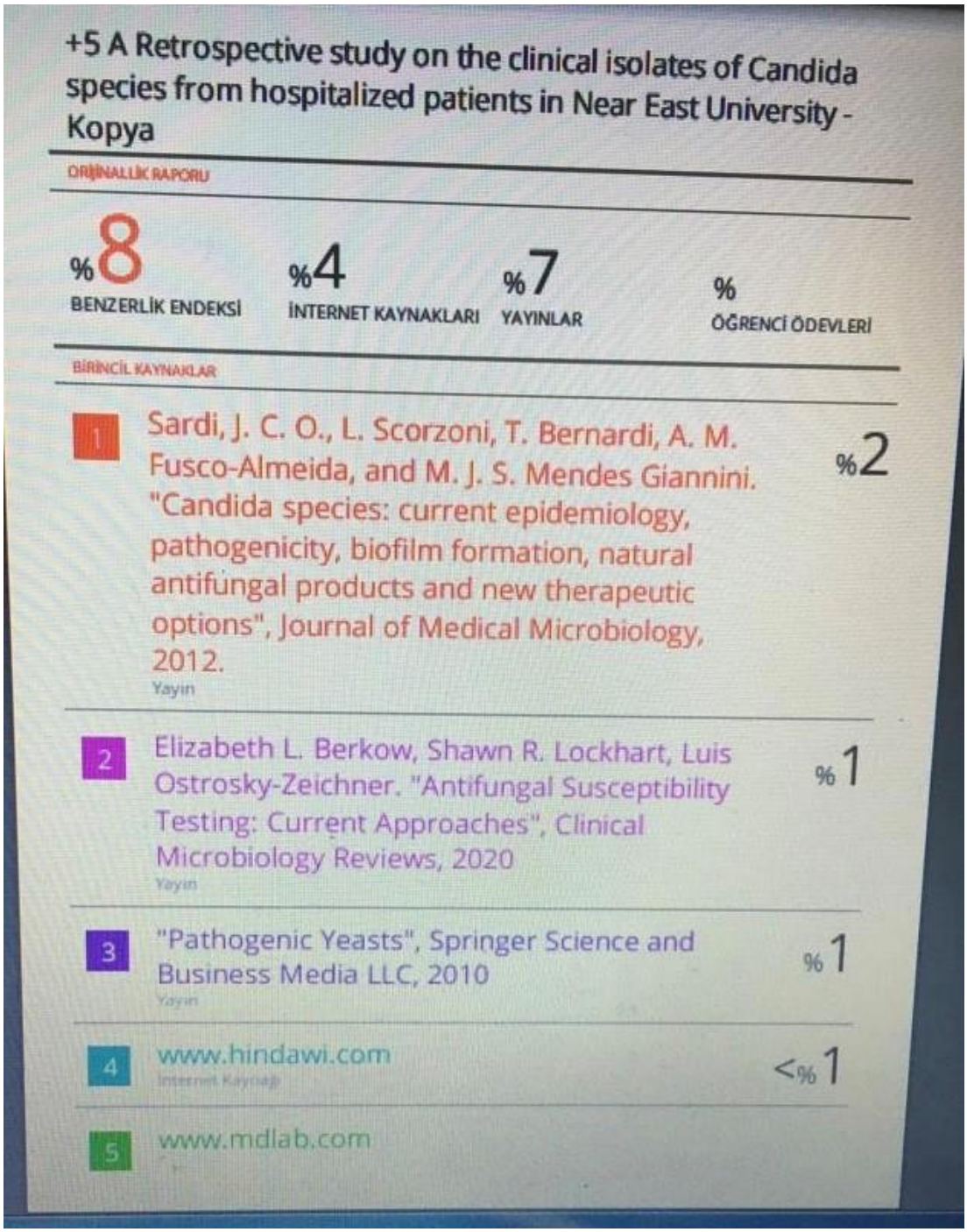
1ST SEPTEMBER - 26TH JUNE, 2014-2018
B.SC. MICROBIOLOGY, BOWEN UNIVERSITY IWO, OSUN STATE
Graduated with a 3.05 which is considered a 2:2

Skills

• Basic microbiology skills • Highly customer centric • Fast learner and Critical intense thinker	• Inquisitive and very ready to gain knowledge • Team Spirited and Maintenance of cordial work relationship • Excellent communication abilities

Activities

I am passionate about giving back to the less privileged in the society and administering free health care in any way I can, I volunteered in a desensitizing activity in the year 2019 alongside my committee members which gained me a membership position in NDLEA (national drugs, law and enforcement agency)



+5 A Retrospective study on the clinical isolates of Candida species from hospitalized patients in Near East University - Kopya

ORİJİNALLİK RAPORU

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BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	Sardi, J. C. O., L. Scorzoni, T. Bernardi, A. M. Fusco-Almeida, and M. J. S. Mendes Giannini. "Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options", Journal of Medical Microbiology, 2012. Yayın	%2
2	Elizabeth L. Berkow, Shawn R. Lockhart, Luis Ostrosky-Zeichner. "Antifungal Susceptibility Testing: Current Approaches", Clinical Microbiology Reviews, 2020 Yayın	%1
3	"Pathogenic Yeasts", Springer Science and Business Media LLC, 2010 Yayın	%1
4	www.hindawi.com İnternet Kaynağı	<%1
5	www.mdlab.com	