CHARACTERIZATION OF YEAST ISOLATES AND ASSESSMENT OF ASSOCIATED RISK FACTORS AMONG INPATIENTS AT NAIROBI SOUTH HOSPITAL, KENYA

Akweya Charity Lyavuli

H56/38327/2020

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of Degree of Master of Science in Medical Microbiology, University of Nairobi

December, 2023

Declaration

This research project is my original work and has never been submitted by anyone for the award of any degree award in any University or College.

……*clyavuli*…… 09/12/2023

Signature: Date:

Akweya Charity Lyavuli

H56/38327/2020

Supervisors' Approval

This Research thesis has been submitted for examination with my approval as the assigned Supervisor.

…… 11/12/2023 ……

Signature: Date:

Dr. Florence Mutua

University of Nairobi

Department of Medical Microbiology and Immunology

…… 11/12/23 ……

Signature: Date:

Dr. Winnie Mutai

University of Nairobi

Department of Medical Microbiology and Immunology

Dedication

To my beloved siblings Isaac Stephen, Moses Elias, Dali Edcar, Grace Sabato and Simeon Yhoshwua and to my parents, Mr. Jairus Akweya, and Mrs. Beatrice Aleyo Akweya who sacrificed a lot for me to reach here.

Acknowledgement

First and foremost, I would like to express my gratitude to the almighty God for the strength and perseverance he gave me to accomplish this research. Secondly, I would like to thank Dr. Hassan Adam Mohamed proprietor of Nairobi South Hospital for allowing me to conduct this research in his facility. I am also deeply grateful to my supervisors Dr. Florence Mutua and Dr. Winnie Mutai for their full guidance and advisory to make the research successful. My extended appreciation goes out to Dr. Gloria Susanne Omosa, Dr. Martin Mulinge, Mr. Kenneth, my parents, Mr. Jairus Akweya, and Mrs. Beatrice Aleyo Akweya and my friends, colleagues as well as all my family for their continuous support and motivation throughout the research and without whom I would not have had the forbearance to accomplish this research. My special thanks goes to the Nairobi South Hospital, especially the laboratory Department and Mrs. Ann Mwangi and Mr. Donald Omollo for their material and physical support in the hospital. I would also like to convey my appreciation to the Uandishi Manuscript Writing Programme for their material support in manuscript writing workshops. Finally, I am very thankful to all other people who assisted me at different stages of my research with physical or intellectual support.

Table of Contents

List of Tables

List of Figures

Abbreviations and Acronyms

AFDR - Antifungal Drug Resistance

ART - Anti- Retroviral Therapy

AST- Antifungal Susceptibility Testing

BA - Blood Agar

BMT - Bone Marrow Transplantation

BSI - Blood Stream Infections

C – *Candida* or *Cryptococcus*

℃ -Degrees Celsius

CDC - Centers for Disease Control and Prevention

CI– Confidence Interval

CLED - Cysteine Lactose Electrolytes Deficient

CLSI - Clinical and Laboratory Standards Institute

CRAG - Cryptococcal Antigen

CSF - Cerebrospinal Fluid

CVC(s) - Central Venous Catheters

CYP51A1 – Cytochrome 51A1

DNA – Deoxyribonucleic acid

ERG 11 - Ergosterol 11

GAFFI - Global Action Funds for Fungal Infections

GIT – Gastrointestinal Tract

HIV/AIDS – Human Immunodeficiency Virus/ Acquired Immuno-Deficiency Syndrome

ICU - Intensive Care Unit

ITS - Internal Transcribed Spacer

IV – Intravenous

KEML: Kenya Essential Medicines List 2019

KOH – Potassium Hydroxide

MAC – MacConkey

MALDI-TOF MS - Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

MDR - Multiple Drug Resistance

MFC (s) - Minimal Fungicidal Concentrations

MIC – Minimum Inhibitory Concentration

NCCLS M - National Committee for Clinical Laboratory Standards- Microplate

NSH - Nairobi South Hospital

PCR - Polymerase Chain Reaction

RNA – Ribonucleic Acid

SDA – Sabouraud's Dextrose Agar

T – *Trichosporon*

TAT – Turn Around Time

UON-KNH – University of Nairobi – Kenyatta National Hospital

WHO - World Health Organization

$\chi 2$ – Chi-Square

Operational Definition of Terms

Antifungal Drug Resistance: This is the condition in which an antifungal drug no longer effectively treats a fungal infection (Solomon, 2021).

Anti-Retroviral Therapy: Treatment using a drug combination that inhibits the human immunodeficiency virus (HIV) or other types of retroviruses from multiplying in the human body (Sadeghi *et al.,* 2018).

Antifungal Susceptibility Testing: In clinical microbiology laboratories, there are invitro methods for detecting fungus susceptibility and resistance by determining the quantity of medicine required to inhibit a microorganism to a given degree, known as the MIC, in order to pick the optimum antifungal agent (Kathuria *et al.,* 2015).

Blood Stream Infections: This is an invasive infection of blood with a microorganism (Wall and Lopez-Ribot, 2020).

Catheterization: The placement or use of a catheter or into the trachea, heart vein, or ureter (Solomon, 2021).

Inpatient: An inpatient is a patient who resides in the hospital while receiving medical treatment (Wall and Lopez-Ribot, 2020).

Multiple Drug Resistance: A yeast species is considered to be multiple drug resistant if it is not sensitive to more than one antimicrobial drug class as a result of the accumulation of resistance genes (Arendrup & Patterson, 2017).

Minimal Fungicidal Concentrations: Defined as the lowest antifungal concentration that kills 99.9% of the inoculum, produces no observable growth to be seen, or produces less than three colonies (Kathuria *et al.,* 2015).

Minimum Inhibitory Concentration: The concentrations of an antimicrobial agent that totally inhibit visible fungal growth expressed in (µg/mL) (Kathuria *et al.,* 2015).

Neutropenia: It refers to a condition wherein the neutrophil count of an individual falls below 1500 per microliter of blood, either acutely or chronically (Kathuria *et al.,* 2015).

Yeast Infection: It refers to a medical condition in which the body becomes infected with fungi from the genera *Candida*, *Cryptococcus*, or *Trichosporon* (Kathuria *et al.,* 2015).

Abstract

Background: Yeast infections are neglected silent killers that cause massive morbidity and mortality among infected patients globally. There is growing concern that the population of hospitalized patients likely to be infected by yeast has grown due to increases in various risk factors such as immunosuppressive conditions and therapies, and persistent neutropenia. Treatment failure due to antifungal resistance among critically ill patients may also limit therapeutic options in these patients.

Methodology: The goal of this study was to retrospectively describe the proportion and distribution of yeast isolates recovered from inpatients records at Nairobi South Hospital between October 1st, 2018 and September 30th, 2022, to describe the isolates' antifungal drug sensitivity pattern to amphotericin B, flucytosine, fluconazole, itraconazole and voriconazole, and to assess the associated risk factors.

Study Results: A total of 308 inpatients out of the 2006 examined for microbial culture and sensitivity tested positive for yeast isolates, giving an overall proportion of 15%. Non - *albicans* species of *Candida* were the most common species, 51%, then *Candida albicans,* 38% and other less commonly isolated yeasts of *Cryptococcus*, 10% and *Trichosporon,* 2% species. Overall, 90% of all isolates were resistant to amphotericin B, 70% were resistant to itraconazole, 61% resistant to fluconazole, while 74% and 44% of the yeast isolates were sensitive to flucytosine and voriconazole. Multiple drug resistance (MDR) was present in 48% of the yeast isolates. Yeast isolate recovery was observed in all cases of comorbidities documented.

Conclusion: *Candida albicans* was the most common yeast species isolated. Flucytosine had the highest sensitivity in this study, while amphotericin B and the azoles were less sensitive. All the yeast isolates showed some level of multi-drug resistance. Patients in the intensive care unit and high dependency unit were more likely to have yeast isolates than patients in the general ward. However, there was no link found between the presence of a comorbidity and the risk of yeast infection among inpatients. Information on the yeast profile and antifungal susceptibility patterns is important for effective patient management and should be routinely monitored.

.

Chapter One: Introduction

1.1 Background

Currently, there are about 5 million fungal species with only 100,000 of these species identified, and only 300 have been linked to human infections (Wall and Lopez-Ribot, 2020). Although yeast-related infections and deaths are reported in Africa, but accurate data is lacking (Fausto *et al.,* 2019). According to the World Health Organization (WHO), an estimated 47.6 million persons fall ill with fungal infections in Africa annually, with 1.7 million of these having a severe infection (WHO, 2018). These estimates are generalized and as a result, they are most likely understated, since Africa is a continent with many countries thus the actual prevalence of cases in Africa is unknown. In addition, a rough estimate of the disease burden in Kenya suggests that at any given time, approximately 7% of the population of Kenya is afflicted with yeast infections (Guto *et al.,* 2016). However, the true weight of yeast infections in Kenya is yet to be determined. There is limited research and systematic methods for diagnosis and data collection on yeast infections in the country (Solomon, 2021).

1.2 Purpose of the Study

This research study aimed at providing a retrospective description of the proportion of yeast isolates recovered from inpatients records at Nairobi South Hospital between October 1st, 2018 and September 30th, 2022, to describe the isolates' antifungal drug sensitivity pattern to Amphotericin B, Flucytosine, Fluconazole, Itraconazole and Voriconazole, and to assess the risk factors associated with yeast infection.

1.3 Problem Statement

The exact effect of yeast infections on patients is yet to be recognized in the same way that other pathogenic microorganisms have (Pfavayi *et al.,* 2021). Disease incidence has been

attributed to low immune status, which predisposes to yeast infections (Casadevall, 2018). Recent years have seen diagnostic advances in identification of more yeast species primarily associated with clinical infections. Despite the fact that *Candida albicans* accounts for the vast majority of yeast infections in humans, other yeasts such as *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *Cryptococcus* species, and *Trichosporon* species, have gained clinical interest due to varied sensitivity to antifungals agents (Sadeghi *et al.,* 2018). Further, the management of yeast infections is complicated by the limited number of available antifungal agents and poses a challenge in instances of drug resistant strains which contribute to increased morbidity and mortality. The increasing proportion of diseases, such as HIV and diabetes that suppress the immune system has led to increase in yeast infections (Casadevall, 2018).

Further, the limited number of available antifungals also contribute to the high rates of morbidity and mortality associated with yeast infections (Pfavayi *et al.,* 2021). It is important to monitor changes in risk factors for yeast infections and add to those reported by the World Health Organization (Rajasingham *et al.,* 2019; WHO, 2018). The ministry of health under Kenya List of Essential Medicines and Kenya Pharmacy and Poisons Board, approved several antifungal agents such as echinocandins, polyenes, azoles, and caspofungin, to treat yeast infections (KEML, 2019). Nonetheless, reports from around the world indicate rise of multidrug-resistant yeast infections. However, efforts are being made to identify alternative drug targets and therapeutic strategies (Jallow & Govender, 2021). A yeast species is considered to be multiple drug resistant if it is not sensitive to more than one antimicrobial drug class as a result of the accumulation of resistance genes (Arendrup & Patterson, 2017).

1.4 Justification of the Study

Infections by various types of yeast species pose a threat to the lives of inpatients and the health care system globally (Fausto *et al.,* 2019). However, the true weight of yeast disease infections in Kenya is yet to be determined. To assess the burden and impact of yeast infections among

inpatients, it is necessary to conduct more epidemiological and microbiological research (Guto *et al.,* 2016). Further, many identified risk factors have not been assessed locally as risk factors for yeast infections. There is also inadequate data on some yeast species, such as *Candida auris*, which has been connected to multiple antifungal drug resistance. This study seeks to describe the actual proportion and distribution of yeast species among inpatients at hospitals in Nairobi, Kenya and to describe their antifungal drug sensitivity pattern. This will aid in monitoring the distribution of yeast isolates in hospitalized patients. Besides, the yeast species antifungal sensitivity patterns are crucial in the fight against yeast antifungal drug resistance lastly, surveillance will guide the selection of the most potent antifungal agent for treatment of a particular yeast isolate and may further provide information for local antibiograms.

1.5 Broad Research Question

What are the characteristics of the yeast isolated and the potential risk factors that predispose inpatients at Nairobi South Hospital between 1st October 2018 and 30th September 2022 to yeast infections?

1.5 .1 Research Questions

I. What are the proportion and distribution of yeast isolates recovered from inpatients at Nairobi South Hospital between 1st October 2018 and 30th September 2022?
II. What is the antifungal drug susceptibility pattern of the yeast isolates?
III. What are the potential risk factors that predispose inpatients at Nairobi South Hospital between 1st October 2018 and 30th September 2022 to yeast infections?

1.6 Broad Objective

To retrospectively characterize yeast isolates and assess the potential risk factors that predispose inpatients at Nairobi South Hospital between 1st October 2018 and 30th September 2022 to yeast infections.

1.6.1 Specific Objectives

I. To describe the proportion and distribution of yeast isolates recovered from inpatients at Nairobi South Hospital between 1st October 2018 and 30th September 2022.

II. To characterize the antifungal drug susceptibility pattern of the yeast isolates.

III. To assess the potential risk factors that predispose inpatients at Nairobi South Hospital between 1st October 2018 and 30th September 2022 to yeast infections.

Chapter Two: Literature Review

2.1 Overview

Candida, *Cryptococcus*, and *Trichosporon* yeasts cause mild to potentially fatal infections in both immune-competent and immunocompromised people (Richardson, 2005). To be precise, yeast infections have caused more than 1.6 million deaths annually, with varying degrees of severity (Fausto *et al.,* 2019). Varieties of yeast infections have become more common as a result of global warming and shifting climates (Fausto *et al.,* 2019). In addition, there is an increased frequency of bloodstream infections (BSIs) caused by yeast species, with *Candida albicans* being frequent (Richardson, 2005). This is associated with the growing population of immunosuppressed individuals such as inpatients with HIV/AIDS, organ transplantation, hematological diseases, burns, or malignant tumours (Rahi *et al.,* 2021).

The proportion of yeast infections has been rising in recent years, particularly among hospitalized patients (Richardson, 2005). These opportunistic yeast infections could include *Candida*, *Cryptococcus*, or *Trichosporon (*Rahi *et al.,* 2021). However, surveillance of diseases caused by the yeast has yet to be initiated in most healthcare facilities located in developed and developing nations. Hence, the available data suggesting the intensity of yeast infection globally is quite conservative (Casadevall, 2018). Pathogenic yeast infections are thought to be the primary cause of host loss in immunocompromised inpatients, with approximately 65 percent of cases occurring as coinfections, making them difficult to diagnose in the early stage (Rahi *et al.,* 2021). In addition, most yeast infections remain poorly understood in terms of clinical characteristics and prognostic factors because of limited research studies globally. The available research on yeast has been performed in Europe and the United States. However, data relating to proportion and incidence of yeasts and their susceptibility to agents with antifungal properties are scarce in Asian countries such as China (Guto *et al.,* 2016). A rough estimate of

the disease burden in Kenya suggests that at any given time, approximately 7% of the population of Kenya is afflicted with yeast infections (Guto *et al.,* 2016).

2.2 Yeasts of *Candida* Species

According to Goemaere *et al.,* (2018), invasive yeast infections are becoming more frequent, with *Candida* species responsible for about 80% of these infections (Fontecha *et al.,* 2019). According to Zaoutis *et al.,* (2005), candidiasis is linked to a 49 percent attributable death rate in hospitalized patients. However, in patients who have septic shock and have not received antifungal medication, it can rise to 98 percent (Fontecha *et al.,* 2019). Currently, the vast majority of *Candida* species are divided into four cryptic complexes (Fontecha *et al.,* 2019).

2.2.1 *Candida albicans* complex

The genus *C. albicans* contains three species in the complex: *C. albicans sensu stricto (s.s)*, *C. dubliniensis*, and *C. africana* (Fontecha *et al.,* 2019). These yeasts are common pathogens with a lot of phenotypic similarities. *C. albicans s.s* is the most common yeast strain in this complex (Fontecha *et al.,* 2019). *C. dubliniensis* and *C. africana* are two yeast strains that were previously referred to as *C. albicans* biovarieties (Fontecha *et al.,* 2019). They also share similar morphophysiological characteristics as those of *C. albicans s.s* (Fontecha *et al.,* 2019).

2.2.1.1 *Candida albicans sensu stricto (s.s)*

Candida albicans s.s is a normal flora of the human gut flora, but it can become a fatal pathogenic yeast in immunocompromised patients (Gow and Yadav, 2017). It can cause infections ranging from superficial to systemic, with a 50% mortality rate in all bloodstream cases (Spampinato and Leonardi, 2013a). On Sabouraud's dextrose (SDA) agar, *C. albicans* forms soft, creamy colonies, while on chrome agar, it forms, green colonies. At 37℃, they produce germ tubes and chlamydospores on cornmeal agar. When observed under a microscope, it also produces pseudo-hyphae and true hyphae (Torosian and Mauger, 2019). *C.*

albicans has demonstrated antifungal resistance to the first-line drugs used in treatment. As a result, antifungal susceptibility needs to be determined (Gow and Yadav, 2017).

2.2.1.2 *Candida dubliniensis*

C. dubliniensis was initially identified as the cause of oral candidiasis in the United Kingdom in 1995 when it was recognized as a separate yeast (Torosian and Mauger, 2019). It has been recently discovered opportunistic or convenient yeast having a strong relation to *C. albicans s.s* but differs in epidemiology, pathogenicity, and in vitro Fluconazole resistance. It was recently discovered as a rare cause of invasive illness among patients with cancer undergoing treatment (Fontecha *et al.,* 2019). It is, however, far less common than other *Candida* species (Torosian and Mauger, 2019). *C. dubliniensis* is still being linked to candidiasis affecting the mouth in HIV/AIDS patients. *C. dubliniensis* has been shown in the laboratory to generate both chlamydospores and germ tubes, which play a role in its pathogenicity (Torosian and Mauger, 2019). This unique species, on the other hand, is less resilient in cultures at higher temperatures (Torosian and Mauger, 2019).

2.2.1.3 *Candida africana*

Candida africana is a pathogenic *C. albicans* species complex member. *C. africana*, first isolated in Africa in 1995, was proposed in 2001 as a new species within the *C. albicans* complex (Gharehbolagh *et al.,* 2020). *C. africana* has been isolated from mucocutaneous membranes, urine, oral-nasangeal swabs and aspirates, and blood samples. It has been associated with a variety of human infections, such as candidiasis, pharyngitis, and septicemia (Gharehbolagh *et al.,* 2020). Unlike the other *C. albicans* complex yeasts, *C. africana* cannot form chlamydospores and cannot metabolize glucosamine, N-acetylglucosamine, trehalose, or DL-lactate (Gharehbolagh *et al.,* 2020). However, it still can form germ tubes just like *C. albicans* and *C. dubliniensis* (Gharehbolagh *et al.,* 2020). Besides, molecular research has

revealed a close genetic relationship between *C. albicans* and *C. africana* (Gharehbolagh *et al.,* 2020). Consequently, differentiating *C. africana* from the other members of the *C. albicans* complex using traditional identification methods is difficult (Gharehbolagh *et al.,* 2020).

2.2.2 *Candida glabrata* complex

The *Candida glabrata* species complex consists of *Candida glabrata sensu stricto*, *Candida nivariensis*, and *Candida bracarensis* (Sadeghi *et al.,* 2018). C *glabrata* uses constant host macrophage attack, and biofilm formation, and is not as aggressive in nature to induce strong host immune reactions as an immune evasion mechanism (Sadeghi *et al.,* 2018). Lastly, *C. glabrata s.s* is becoming more common due to its decreased susceptibility to azole drugs (Sadeghi *et al.,* 2018).

2.2.2.1 *Candida glabrata sensu stricto (s.s)*

Invasive candidiasis is caused by *C. glabrata s.s* a common infection. *C. glabrata s.s* infections have the greatest fatality rates among non- *albicans Candida* species (Sadeghi *et al.,* 2018). *C. glabrata* is a commensal that has been isolated from the mucosal surfaces of normal individuals (Sadeghi *et al.,* 2018). However, it has become a growing concern among inpatients as it also causes mucosal infections (Rodrigues and Nosanchuk, 2020). Previous studies have reported *C. glabrata s.s* as the most predominant non- *albicans Candida* species are isolated among patients with impaired immunity such as HIV/AIDS and cancer patients (Sadeghi *et al.,* 2018). It is also implicated in causing 15% of all candidemia (Sadeghi *et al.,* 2018). Today, *C. glabrata*-related candidemia is frequent, and its primary cause has been identified to be the extensive use of immunosuppressants and antifungals (Chakrabarti, 2015). *C. glabrata* produces creamy colonies on Sabouraud's dextrose agar and is light pink in colour on chrom agar (Sadeghi *et al.,* 2018). It does not produce pseudo-hyphae as *C. albicans* does (Nadeem *et al.,* 2010). However, following the discovery of the *C. glabrata* complex, traditional

laboratory methods have shown limitations in detecting the two unusual species (Sadeghi *et al.,* 2018).

2.2.2.2 *Candida bracarensis*

Candida bracarensis is a recently discovered cryptic *C. glabrata* complex species (Sadeghi *et al.,* 2018). *C. bracarensis* is an opportunistic yeast linked to bloodstream infections in immunocompromised patients, including those undergoing bone marrow transplants (CuencaEstrella *et al.,* 2011). The first isolates were obtained from a vaginal exudate of a Portuguese patient and blood cultures of a UK hospital patient (Cuenca-Estrella *et al.,* 2011). The organism can be distinguished from other *C. glabrata* species in the complex by its white colour on chrom agar (Sadeghi *et al.,* 2018). Also, the DNA sequencing of the D1/D2 gene and internal transcribed spacer (ITS) primers can be used to distinguish *C. bracarensis* from other yeasts in the same complex (Cuenca-Estrella *et al.,* 2011).

2.2.2.3 *Candida nivariensis*

Candida nivariensis is another cryptic yeast species that belong to the *C. glabrata* complex (Cartier *et al.,* 2020). It was first described in the year 2005 using a DNA sequencing technique from clinical bronchoalveolar lavage, blood culture, and urine samples collected from three Spanish patients in a single institution in the Canary Islands (Cartier *et al.,* 2020). Despite its rarity, it has been connected to deep-seated infections in people with impaired immune systems (Cartier *et al.,* 2020). In contrast to other *C. glabrata* complex species isolates, Itraconazole, Fluconazole, and Voriconazole are less efficacious against *C. nivariensis* isolates, with much higher Flucytosine MICs being reported (Silva *et al.,* 2009).

2.2.3 *Candida parapsilosis* complex

The *Candida parapsilosis* complex is comprised of three genetically diverse yeast species: *Candida parapsilosis sensu stricto*, *Candida orthopsilosis*, and *Candida metapsilosis (*Silva *et*

al., 2009). In several hospitals in Europe and South America, *C. parapsilosis* is the second-most frequent cause of yeast infection after *C. albicans* (Trofa *et al.,* 2008). Three families of *C. parapsilosis* isolates have been described (I to III) (Adam *et al.,* 2019). Despite this complex of yeasts' genetic similarity, Tavanti *et al.,* were able to divide yeast from *Candida parapsilosis* into three groups based on the great degree of variety they showed (Tavanti *et al.,* 2005, 2007). Group 1 was designated for *C. parapsilosis s.s*, whereas Group 2 is assigned to *C. orthopsilosis*, and the group that *C. metapsilosis* was put in was 3 (Tavanti *et al.,* 2005, 2007). However, due to a proline to alanine replacement in the beta-glucan synthase Fks1 target, the yeasts of *C. parapsilosis* complex show reduced sensitivity to echinocandins (Tavanti *et al.,* 2005, 2007).

2.2.3.1 *Candida parapsilosis sensu stricto (s.s)*

According to Silva *et al.,* (2009) *C. parapsilosis s.s* has the highest proportion of this complex worldwide, and is the most common isolate in hematogenous infections (Silva *et al.,* 2009). *C. parapsilosis* was first isolated from a stool sample in 1928 and was observed to be a non-maltose fermenter by Arford who referred to it as “Manila” due to this property *(*Trofa *et al.,* 2008). Infection rates are especially high in neonates, which could be due to either the neonates' vulnerable skin barrier or the hospital's high microbial population, which would permit horizontal transmission from healthcare practitioners to the neonates (Silva *et al.,* 2009).

C. parapsilosis is found on the skin of humans, mouth, throat, gastrointestinal tract (GIT) and genitals naturally (Richardson, 2005). Due to the widespread use of invasive devices in NICUs and ICUs, including as central venous catheters (CVCs), *C. parapsilosis* has been associated with significant outbreak of candidiasis (Silva *et al.,* 2009). *C. parapsilosis s.s* is distinguished from other yeast strains by the appearance of pink colonies on chrom agar (Sadeghi *et al.,* 2018). Besides, they exhibit aggregates of blastophores in corn meal agar and biofilm formation (Nadeem *et al.,* 2010).

2.2.3.2 *Candida orthopsilosis*

Although *C. orthopsilosis* is related to *C. parapsilosis,* it is seldom associated with infection (Silva *et al.,* 2009). *C. orthopsilosis* isolates have been shown to damage reconstituted human epithelial cells (Silva *et al.,* 2009). As a result, it has recently been identified as a persistent yeast among individuals with septic arthritis associated with systemic lupus erythematosus (SLE), despite treatment (Silva *et al.,* 2009).

2.2.3.3 *Candida metapsilosis*

C. metapsilosis can be rarely isolated from diverse samples of hospitalized patients such as from urine and catheters (Giri and Kindo, 2012). It has the ability to form biofilms on epidermal tissues it attacks (Silva *et al.,* 2009). By being problematic for phagocytes to engulf and destroy, *C. metapsilosis* isolates circumvent the immune system (Silva *et al.,* 2009).

2.2.4 *Candida haemulonii* complex

According to Sadeghi *et al.,* (2018), the proportion of candidiasis caused *C. haemulonii* complex and closely related species is rising. This complex's yeast species include *C. haemulonii* and *C. duobushaemulonii*, *C. auris* and *C. pseudohaemulonii* (Silva *et al.,* 2009). These species frequently develop resistance to Amphotericin B or echinocandins (Sadeghi *et al.,* 2018).

2.2.4.1 *Candida haemulonii sensu stricto (s.s)*

C. haemulonii infections are uncommon and these organisms are challenging to identify without sophisticated diagnostic identification techniques such as MALDI-TOF-MS hence, scarce information is available about the species in this genus (Sadeghi *et al.,* 2018). More often than not, *C. haemulonii s.s* infections are found in diabetic wounds or in those with peripheral vascular disease (Sadeghi *et al.,* 2018).

2.2.4.2 *Candida haemulonii var. vulnera*

The first case of *C. haemulonii var. vulnera* infection was isolated in a mature adult with a liver abscess in Peru (Mutua *et al.,* 2010). *C. haemulonii var. vulnera* has been linked to the development of liver abscess in immunocompromised hosts, under situations such as mucositis and neutropenia that foster the spread of yeast from the gastrointestinal tract (Solomon, 2021). Typically, *C. haemulonii var. vulnera* is resistant to both Amphotericin B and Fluconazole (Spampinato and Leonardi, 2013b).

2.2.4.3 *Candida doubushemolonii*

The yeast *C. duobushaemulonii* is a member of the *C. haemulonii* species complex it causes infection rarely among immune susceptible individuals (Solomon, 2021). *C. duobushaemulonii* is closely related to *C. auris*, hence is often misidentified (Solomon, 2021). Given its tolerance to numerous antifungals, *C. duobushaemulonii* causes more septicemia than was earlier known and creates a grave concern (Wall and Lopez-Ribot, 2020). *C. duobushaemulonii* causes septicemia, severe lower extremities wound infections and vulvovaginal candidiasis (Sadeghi *et al.,* 2018).

2.2.4.4 *Candida auris*

Candida auris multi-fungal drug resistant yeast that has attracted the attention of the public health community (Solomon, 2021). *C. auris* was first isolated from a hospitalized patient in Japan in the year 2009 (Giri and Kindo, 2012). *C. auris* is a leading cause of systemic infection, with a 35.2 percent monthly mortality rate among hospitalized (Sadeghi *et al.,* 2018). In the microbiology lab, *C. auris* is difficult to isolate using common techniques like the VITEK model DL-96 I and II, and it is constantly mistaken for other *Candida* species like *C. haemulonii* or *C. famata* (Dmytruk and Sibirny, 2012).

2.2.4.5 *Candida pseudohaemulonii*

Candida pseudohaemulonii is phylogenetically close to *C. haemulonii* (Sugita *et al.,* 2006). *C. pseudohaemulonii* has been implicated in causing candidemia among immunocompromised patients, and has been recovered from blood samples of such patients though rarely (Sugita *et al.,* 2006). The first identification of this azole and polyene-resistant yeast was done in Thailand in the year 2006 (Sugita *et al.,* 2006).

2.2.5 *Candida tropicalis*

C. tropicalis has been implicated as being the first major cause of *C.* non-*albicans* bloodstream infections among neutropenic inpatients (Zuza-Alves *et al.,* 2017). *C. tropicalis* produces creamy soft colonies on Sabouraud's dextrose agar and has dark blue colonial morphology that diffuses into surrounding agar in Chrom agar following a 48-hour incubation at 35°C (Zuza-Alves *et al.,* 2017). In corn meal agar, *C. tropicalis* similarly produces pseudo-hyphae with blastoconidia (Giri and Kindo, 2012). *C. tropicalis* has been reported to be more prevalent among neutropenic patients. It has the ability to form biofilms, which contributes to its virulent nature (Zuza-Alves *et al.,* 2017). *C. tropicalis* has demonstrated resistance to azoles like Fluconazole (Zuza-Alves *et al.,* 2017).

2.2.6 *Candida lusitaniae*

The gastrointestinal tracts of warm-blooded animals typically contain *Candida lusitaniae* as part of the natural flora (Khan *et al.,* 2019). However, when an immunocompromised state develops in human beings, *C. lusitaniae* can lead to septicemia (Khan *et al.,* 2019). On Chrom agar, *C. lusitaniae* forms pink-grey like, purple colonies, while on corn meal agar, it produces branching pseudo hyphae (Khan *et al.,* 2019). At present, there is an increase in the number of incidences of *Candida lusitaniae* among cancer patients undergoing chemotherapy (Khan *et al.,* 2019). *C. lusitaniae* is resistant to Amphotericin B (Khan *et al.,* 2019).

2.2.7 *Candida krusei*

The infections due to *Candida krusei* are rare though common in patients with haematological conditions (Khan *et al.,* 2019). *C. krusei* occurrence in hospitalized patients is frequently linked to use of prophylactic antifungal medication (Khan *et al.,* 2019). After 48 hours incubation at 35°C, *C. krusei* appears as a white rose on Chrom agar (Khan *et al.,* 2019).

2.3 Yeasts of *Cryptococcus* Species

Cryptococcus var neoformans and *Cryptococcus var gattii* are basidiomycetous yeast that are found in the natural environment (Iyer *et al.,* 2021). They are encapsulated yeasts that cause cryptococcosis, an infectious disease with a widespread distribution (Chayakulkeeree and Perfect, 2007). The *Cryptococcus* yeast is divided into three varieties and five serotypes: *C. neoformans var grubii* (serotype A), *C. neoformans var neoformans* (serotype D), *C. neoformans* (hybrid AD), and *C. gattii* (sero- types B and C) this is based on the antigenic determinants of the capsular polysaccharide (Iyer *et al.,* 2021).

At least most human beings have been exposed to this yeast after inhalation of the arthroconidia of the yeast (Iyer *et al.,* 2021). Individuals in advanced HIV/AIDS states are most vulnerable to cryptococcal infections, however, this yeast of *Cryptococcus var neoformans* rarely causes any infection among immune competent individuals (Chayakulkeeree and Perfect, 2007). The most common clinical outcome of cryptococcosis is subacute or chronic meningocephalitis, meningitis and lung disease (Iyer *et al.,* 2021).

According to the US Centers for Disease Control and Prevention (CDC), globally around one million new cryptococcal meningitis cases occur each year (Chayakulkeeree and Perfect, 2007). Sub-Saharan Africa has the highest burden of cryptococcosis with an estimate of more than 70% of all the new cases reported by CDC (Sadeghi *et al.,* 2018). In low-income nations like Africa, the mortality rate from cryptococcal meningitis is still 70%, compared to 20– 30%

in high-income nations (Rajasingham *et al.,* 2019). This is thought to be due to delay and inadequate provision of rapid sensitive test methods for diagnosing *C. neoformans* (Zuza-Alves *et al.,* 2017). Additionally, the high price and limited choice of first-line antifungal medications have increased mortality (Solomon, 2021). The incapability of low-income nations to effectively monitor and manage treatment toxicity levels, the recurring complications of elevated intracranial pressure, immune reconstitution inflammatory syndrome linked to cryptococcal meningitis, and antiretroviral therapy are all key contributors to mortality (ART) (Rajasingham *et al.,* 2019).

Reports from CDC state that there are approximately 11,900 cases of cryptococcal meningitis among HIV/AIDS patients (Rajasingham *et al.,* 2019). Other disease outcomes include pulmonary disease and involvement of the skin, bone and lymph node (Zuza-Alves *et al.,* 2017). These outcomes are fatal if untreated and also have high morbidity. The annual global disease burden among HIV/AIDS for cryptococcosis is estimated to be 278,000 cases resulting in 181,000 deaths (Rodrigues and Nosanchuk, 2020). However, incidences cryptococcosis disease among children with HIV/AIDS has been reported to be rare in areas where there is a high of the disease burden among adults (Rajasingham *et al.,* 2019). As a result, *C. neoformans* infection is a major global health concern (Zuza-Alves *et al.,* 2017).

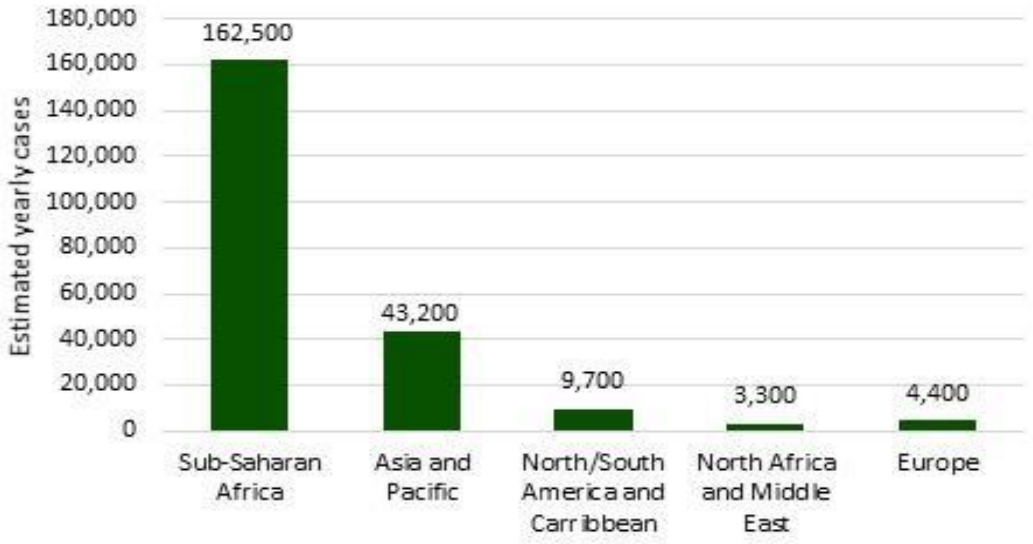

Figure 1: World Burden of Cryptococcal Meningitis Associated with HIV/AIDS (Rajasingham *et al.,* 2019).

2.4 Yeasts of *Trichosporon* Species

Trichosporon is a basidiomycete yeast with septate hyphae, arthroconidia, and pseudo hyphae (Miceli *et al.,* 2011). Yeast of this genus are occasionally isolated in the medical laboratories among immune susceptible patients with eventual fatal infections (Karabay *et al.,* 2006). *Trichosporon* species possess virulence through biofilm formation, lipase and protease production (Karabay *et al.,* 2006). *Trichosporon* was first proposed in 1865 by Hermann Beigel, who noted that it caused hair infections (Karabay *et al.,* 2006). The taxonomy and nomenclature of *Trichosporon* have transformed several times over the years (Karabay *et al.,* 2006). Previously, all *Trichosporon* species were considered to be *Trichosporon beigelii*, besides the strain thought to primarily affect superficial hair and sporadically induce deep infection (Karabay *et al.,* 2006). However, advanced studies have identified remarkable morphological, physiological, and biochemical diversity in *T. beigelii* (Miceli *et al.,* 2011). Hence, a revision of the genus *Trichosporon* was revised into twenty species by (Karabay *et al.,* 2006). The current taxonomy of *Trichosporon*'s 20 yeast species comprises six pathogens:

T. asahii, *T. asteroides*, *T. cutaneum*, *T. inkin*, *T. mucoides*, and *T. ovoides* (Karabay *et al.,* 2006). Yeasts of *Trichosporon* species have become of concern due to an increment in the frequency of infections in immunocompromised inpatients (Gonzalez-Duarte *et al.,* 2015). These *Trichosporon* yeasts have a broad geographical distribution (Karabay *et al.,* 2006). Some of the yeast species of *Trichosporon* are resistant to antifungal drugs in vitro (Karabay *et al.,* 2006). *Trichosporon* species has been reported to be the second after *Candida* species cause of bloodstream yeast infections especially among inpatients with malignant hematological diseases (Miceli *et al.,* 2011). *Trichosporon*osis is frequently found in people who have undergone heart valve surgery, third degree burns, HIV/AIDS, or long-term corticosteroid usage (Adam *et al.,* 2019). Half of all infections in the genus are caused by *T. asahii*, and the death rate for sepsis greater than 70% (Karabay *et al.,* 2006). Furthermore, the cell walls of *Trichosporon* species and encapsulated *C. neoformans* share antigenic similarity, so there is a possibility of antigenic cross-reaction between the two yeast genera, resulting in a positive cryptococcal latex test in patients with disseminated *trichosporon*osis (Rodrigues and Nosanchuk, 2020).

2.5 Risk Factors Associated with Yeast Infections

Among the primary risk factors for the onset of yeast infections are immune suppression regimens for transplant patients, cytotoxic chemotherapy in cancer patients, and broad-spectrum antibiotics use (Richardson, 2005). Traumatic inoculation of yeast into the bloodstream can occur via skin or mucous membrane breaks or wounds (Gonzalez-Duarte *et al.,* 2015). Burns, surgery, and patients in the intensive care unit (ICU) are extremely vulnerable to yeast infections, because their immune systems are compromised (Wudhikarn *et al.,* 2020). People with diabetes, on parenteral feeding, neonates, and the elderly are all predisposed to yeast infection (Richardson, 2005).

Lastly, catheters and other indwelling devices such as urinary catheters and IV ports/lines provide pathogenic yeast with access to the body (Gonzalez-Duarte *et al.,* 2015). Invasive yeast infections are particularly common in patients with chronic neutropenia caused by a hematological malignancy (Richardson, 2005). Risk factors for yeast infections are classified as host-related factors and health-care-related factors, and these risk factors may increase the likelihood of having yeast infections either independently or in combination (Wudhikarn *et al.,* 2020).

2.5.1 Host Related Factors

2.5.1.1 Immunocompromised Conditions

The immune system acts as a natural defense against yeast infections in healthy people (Giri and Kindo, 2012). Hence, yeasts that traverse physical anatomical barriers in humans avoid complement, phagocytes, antibodies, and cell-mediated immunity in order to cause an infection (Giri and Kindo, 2012). However, when these systems are suppressed, the risk of yeast infection increases (Giri and Kindo, 2012). Several diseases cause immunodeficiency, the most well-known of which is HIV/AIDS (Gonzalez-Duarte *et al.,* 2015). This is because HIV infects CD4+ T cells, weakening the entire immune system (Gonzalez-Duarte *et al.,* 2015). As a result, there will be an increase in the incidence of yeast infections such as Cryptococcosis and Candidemia in HIV infected individuals, showcasing the critical role of CD4+ T cells (Gonzalez-Duarte *et al.,* 2015).

2.5.1.2 Prior Colonization with Yeast Infections

Prior colonization with yeast is a risk factor for yeast infections, however, the colonization index has to be higher than 0.5 score for the yeast infection to actualize (Richardson, 2005). Therefore, regular sequential point screening for colonization at multiple sites and patient samples can thus be used to define a hospital's colonization index and determine yeast

proportion (Richardson, 2005). Yeast colonization promotes its growth and increases the risk of infection in patients with kidney disease, those on antihistaminic medications, and the critically ill (Richardson, 2005).

2.5.1.3 Neutropenia

Neutropenia is a haematological disorder that entails a neutrophil count of less than 500 neutrophils per microliter in peripheral blood film (Wudhikarn *et al.,* 2020). Neutropenia may be induced by haematological disease activity, chemotherapy, or an adverse drug reaction (Wudhikarn *et al.,* 2020). The intensity of chemotherapy is determined by a low neutrophil count (Wudhikarn *et al.,* 2020). However, patients with chemotherapy-induced neutropenia are more likely to develop yeast infections, particularly *C. tropicalis* (Wudhikarn *et al.,* 2020). Lastly, a prolonged neutropenic phase of more than seven days promotes seeding of yeasts of *Candida* and other yeasts in vital organs such as liver and spleen that is evident in patients as persistent fever unresponsive to antibiotics, polymyalgias, reduced renal function and lesion formation in the liver and spleen and macronodular skin lesions (Wudhikarn *et al.,* 2020).

2.5.1.4 Hematological Malignancy, Cancer, and Solid Organ Transplantation

Among the most prominent infectious disease-related bring about death in patients with hematological malignancies such as acute leukemia and following allogeneic bone marrow transplantation is systemic yeast infections (Richardson, 2005). This is due to the prolonged neutropenic state that patients undergo as a result of having acute myeloid or acute lymphoid leukemia as well as receiving anti-neoplastic therapy to completely eradicate malignant bone marrow cells (Richardson, 2005). Moreover, patients are given antibiotics during this severe and protracted neutropenic period, rendering them more susceptible to yeast infections (Rahi *et al.,* 2021). The intensity of conditioning regimen for hematological patients has been lowered

over time which has in return decreased the incidence of yeast infections by shortening the duration of neutropenia (Richardson, 2005).

2.5.1.5 Neonates

Transmission of yeast to neonates occurs both vertically from maternal flora and horizontally through contaminated medical devices (Juyal *et al.,* 2014). Yeasts of *C. albicans* and *C. parapsilosis* are the most notorious in causing system infections among these neonates (Richardson, 2005). Although enterobacteria and other microbes colonize newborns' gastrointestinal tracts at delivery or breast-feeding, any disturbance in this process can lead to the colonization of pathogenic yeasts (Juyal *et al.,* 2014).

Numerous risk factors, such as prior antibiotic exposure, the presence of indwelling vascular catheters, and endotracheal intubation, predispose newborns to yeast infections (Gonzalez-Duarte *et al.,* 2015). Additionally, infants in intensive care units typically receive little breast milk, and some of them may require whole parenteral nutrition, which increases the risk of yeast infections (Juyal *et al.,* 2014). Due to their low birth weights, the extremely preterm require catheterization, have longer neonatal intensive care unit stays, and thus are 10% more likely to develop yeast infections (Juyal *et al.,* 2014).

2.5.1.6 Comorbidities and Autoimmune Diseases

Concurrent infections that predispose patients to increased risk of yeast infections of either *Trichosporon*, *Cryptococcus* or *Candida* species include: diabetes mellitus, use of corticosteroid drugs and organ transplantation (Chagas-Neto *et al.,* 2008). Patients with chronic granulomatous disease have a genetic disorder of phagocytic cells such as neutrophils, which serve as the first line of defense against yeast infections (Henriet *et al.,* 2013). Yeasts of *Candida* and *Trichosporon* genera are typically isolated from patients who suffer from chronic granulomatous disease (Chagas-Neto *et al.,* 2008).

In addition, to the common pre-existing comorbidities that predispose patients to *Trichosporon* yeast infection especially *T. asahii* pulmonary diseases, end-stage renal disease and malignant tumours have also been reported (Chagas-Neto *et al.,* 2008). Immunosuppressive therapy without Clinical Activity Score (CAS), disseminated intravascular coagulopathy, acute renal failure, decompensated liver cirrhosis, and lung disease have all been identified as conditions that may elevate the likelihood of cryptococcosis (Chagas-Neto *et al.,* 2008).

2.5.2 Health Care-Related Factors

2.5.2.1 Intravascular Catheters and Implanted Devices

Yeast colonization of the indwelling devices allows for a direct passage into the bloodstream without extensive gastric colonization (Nagao *et al.,* 2014). However, severely sick patients usually need a variety of vascular and other indwelling medical devices, such as triple lumen catheters, for treatment (Nagao *et al.,* 2014). To be specific, at least in 20–80% of most catheterization cases, has resulted in systemic yeast infections (Richardson, 2005). To support this, according to a study conducted in Japan, complete parenteral feeding, is one of the primary risk factors for the development of yeast infections (Nagao *et al.,* 2014). For example, *trichosporon*osis caused by *T. asahii* have been reported among peritoneal dialysis patients (Richardson, 2005).

According to studies, sepsis induced by yeasts isolated from catheters grows more aggressively than other microorganisms, hence the yeasts can be adequately diagnosed within 30 hours (Nagao *et al.,* 2014). Patients with yeast infections brought on by catheters most likely have a larger inoculum, which would account for why they grow more rapidly and why follow up studies show a lower death rate after catheter removal (Richardson, 2005).

2.5.2.2 Broad Spectrum Antibiotic Use

According to several research studies, the proportion of yeast infections has been steadily rising over time, while the number of antimicrobial-resistant bacterial infections has also increased, resulting in significant mortality (Saini *et al*, 2021). The use of inappropriate and excessively high doses of antibiotics, such as third generation cephalosporins for all bacterial infections, which disrupt the normal microbial flora on the skin and mucosal surfaces, as well as better antibiotic regimens, which increase the survival of patients who are susceptible to yeast infections, are likely to blame for this (Richardson, 2005).

Therefore, the quantity and variety of antibiotics administered may have an impact on the risk of yeast infections such candidemia (Pilmis and Monnier, 2020). Studies have revealed that antibiotics with an anti-anaerobic action and those with higher gastrointestinal concentrations have some impact on the gut flora (Pilmis and Monnier, 2020). They aid in the reported accelerated colonization over time seen in intensive care unit patients (Richardson, 2005). More antibiotic action results in a net reduction in the amount of normal gut flora and an increase in the number of hospitalized patients with pathogenic yeast colonization (Pilmis and Monnier, 2020).

2.5.2.3 Parenteral Nutrition

Colonization of the catheter or the infusion ports in parenteral nutrition, confers favourable conditions for yeast growth (Pilmis and Monnier, 2020). According to Pilmis and Monnier, (2020), the utilization of parenteral feeding results in the development of mucosal atrophy and a deterioration of mucosal epithelial barrier function, which may have an impact on gut microbiome and potentiate yeast entry into bloodstream (Richardson, 2005).

2.5.2.4 Length of Intensive Care Unit Stay (≥ 7 Days)

Numerous studies have emphasized the necessity to regulate the length of patients stay in intensive care unit since it is inversely correlated with the incidence of yeast infections (Nagao *et al.,* 2014). Moreover, compared to patients in other ward, intensive care unit a patient have demonstrated greater rates of fatal yeast infections (Richardson, 2005). Therefore, it is a recommended practice to assess the risk related to the length of stay using the number of days prior to the start of the first infection (Richardson, 2005).

2.5.2.5 Surgical Procedures

Research studies have revealed a strong correlation between yeast infection and prior surgery (Richardson, 2005). This finding could be explained by a variety of factors, including gastrointestinal surgery and the impact of excised parts on intestinal flora (Nagao *et al.,* 2014). According to studies, individuals with an increased anastomotic leak, chronic gastrointestinal orifice, or acute necrotizing pancreatitis are more likely to develop septicemia due to yeast (Richardson, 2005).

2.5.2.6 Immunosuppressive Therapy

Certain medications can cause immune suppression (Richardson, 2005). This is done on purpose, such as when immunosuppressive medications are administered to allogenic Hematopoietic stem cell transplantation (HSCT) patients in an effort to avoid graft-versus-host disease and minimize rejection in bone marrow transplant (BMT) recipients (Choe and Crawford, 2019). Due to lymphopenia brought on by these immunosuppressive regimens, the patient is more prone to infections with *Trichosporon*, *Candida* and *Cryptococcus* species (Choe and Crawford, 2019). Neutropenia can also result from myelosuppressive treatment for some patients (Choe and Crawford, 2019). However, leukemia patients undergoing remission

induction treatment are at a higher risk of yeast infection because their neutropenic phase is prolonged for longer than 7 days (Richardson, 2005).

2.6 Mechanism of Antifungal Drug Activity and Resistance of Yeasts

Only a small number of the several antifungal medications that are available for therapeutic use are used to treat yeast infections today (Spampinato and Leonardi, 2013a).

2.6.1 Azoles Activity

The azole class contains the most effective Kenya Essential Medicines List 2019 (KEML)

approved antifungal drugs for treating yeast infections (KEML, 2019). They function by damaging the cell membrane of yeasts and by inhibiting lanosterol 14-α-demethylase activity (Spampinato and Leonardi, 2013b). This enzyme produces ergosterol in yeast cell membranes (Spampinato and Leonardi, 2013b).

Azoles drugs are specific in that they only target the ergosterol of yeast rather than the cholesterol of the host cell due to structural variations between the two (Spampinato and Leonardi, 2013b). Azoles can be applied topically and are effective against sepsis caused by yeasts (Spampinato and Leonardi, 2013b). Azoles are divided into three categories: imidazole, which include clotrimazole and ketoconazole, triazoles, which include Fluconazole, Itraconazole, Voriconazole, and Posaconazole (Spampinato and Leonardi, 2013b).

2.6.1.1 Resistance to Azoles

The fundamental cause of azole resistance is a change in the structure of the enzyme lanosterol 14- α-demethylase, which results in a reduced affinity for binding to the ergosterol 11 (ERG 11) target (Morio *et al.,* 2017), this mutation results in overexpression of efflux pumps, which reduces intracellular azole sequestration (Ganeshkumar *et al.,* 2021). A specific example of this sort of resistance has been demonstrated in *Candida* species, where the ERG 11 target

undergoes point mutation, resulting in an increase in the quantity of CYP51A1 as ERG 11 is overexpressed (Ganeshkumar *et al.,* 2021). Another example is overexpression of various efflux pumps due to changes in yeast's genetic makeup, leading in multiple drug resistance (MDR) (Morio *et al.,* 2017).

Reduced intracellular antifungal drug sequestration is associated with heightened expression of resistance genes (Morio *et al.,* 2017). Multiple drug resistant, along with CDR1 and CDR2 ABC transporters, is usually the main facilitator of resistance genes (Ganeshkumar *et al.,* 2021). Multiple drug resistant in Fluconazole susceptible *C. albicans* is undetectable, whereas it is dramatically enhanced in Fluconazole resistant yea8st species (Ganeshkumar *et al.,* 2021).

2.6.2 Echinocandins Activity

Echinocandins, like caspofungin are lipopeptides that interfere with the formation of the β-1-3-dglucan synthase, which is key in biosynthesis of yeast cell wall (Chakraborty *et al.,* 2020). In order to cure yeast infections, this antifungal class was initially utilized in 2001 to impede the activity of β-1-3-d-glucan synthase (Chakraborty *et al.,* 2020). The catalytic enzyme β-1-3-d-glucan synthase has an FKS genes that is primarily regulated by Rho, a GTP-binding protein (Chakraborty *et al.,* 2020).

2.6.2.1 Resistance to Echinocandins

Resistance is caused by amino acid point mutation in hotspot areas of FKS subunits of β-1-3-dglucan synthase, which reduce the enzyme's sensitivity to medication (Chakraborty *et al.,* 2020).

Drug adaptation occurs as a result of intracellular biological stress networks, which favours the development of resistant FKS strains (Spampinato and Leonardi, 2013b). Evidence-based treatment, prophylaxis, gastrointestinal repositories, and intra-abdominal yeast infections are all clinical aspects that cause echinocandin resistance (Chakraborty *et al.,* 2020). Since FKS 1

and FKS 2 genes are found in yeast such as *C. glabrata,* and *Trichosporon* species the antifungal drug is less responsive to 1,3--D-glucan synthase due to intrinsic resistance (Chakraborty *et al.,* 2020).

2.6.3 Polyenes Activity

Polyenes, such as Amphotericin B, are effective broad-spectrum antifungal agents derived primarily from a strain of *Streptomyces* bacterium (Waghule *et al.,* 2020). Polyenes have an isochronous amphiphilic macrolide structure that binds to ergosterol, a lipid component of the yeast cell membrane (Waghule *et al.,* 2020). This causes the yeast cell membrane to lose its integrity due to the creation of aqueous perforations, which causes depolarization and leakage of cytosolic components K^+ and Na^+ into the environment (Spampinato and Leonardi, 2013a).

2.6.3.1 Resistance to Polyenes

According to Arendrup *et al.,* (2014), polyene resistance mechanisms include changes in yeast cell membrane ergosterols, thus shielding it against oxidative destruction, enhanced catalase activity, errors in ergosterol biosynthetic genes, cell membrane fatty acid composition, and modifications in sterol-to-phospholipid ratio (Waghule *et al.,* 2020). Biofilm glucan sequestration as a multidrug resistance mechanism to Amphotericin B has been seen in *C. lusitaniae* (Spampinato and Leonardi, 2013a). *Trichosporon* species have also shown some level of resistance to Amphotericin B a drug that was very effective before year 2000 in the treatment of *Trichosporon* species infections (Spampinato and Leonardi, 2013a).

2.6.4 Flucytosine Activity

5-fluorouracil (5-FU) undergoes accelerated deamination to form 5-fluoro-deoxy uridylic acid monophosphate, which has antifungal action against yeast (Ahmed *et al.,* 2021). This by-product (5-fluoro-deoxy uridylic acid monophosphate) is a non-competitive inhibitor of thymidylate synthetase, required for yeast DNA/RNA synthesis (Ahmed *et al.,* 2021). Hence,

5-FC interferes with sensitive yeast growth by hindering the absorption of purines and pyrimidines (Ahmed *et al.,* 2021).

In addition, when coupled with azoles such as Fluconazole, 5-FC, an oral or intravenous formulation, has been proven to be effective against resistant yeast infection (Loyse *et al.,* 2013). In vivo and in vitro studies have demonstrated that 5-FC and Itraconazole interact to establish a fungicidal and fungistatic regime that is effective in treating more than 40% of yeast isolates of

Candida and *Cryptococcus* species that are resistant to Flucytosine monotherapy (Loyse *et al.,* 2013). This is due to the fact that Flucytosine, which is microscopic and hydrophilic in nature, is particularly permeable in a number of host organs and will thus improve the results of Amphotericin B administration at low concentrations, which has poor penetration (Ahmed *et al.,* 2021). This will also reduce the occurrence of yeast 5-FC resistance in the future (Loyse *et al.,* 2013).

2.6.4.1 Resistance to Flucytosine

Flucytosine has shown resistance patterns in specific yeasts, and its susceptible to resistance in patients receiving 5FC monotherapy (Loyse *et al.,* 2013). Flucytosine is therefore only used in combination with other antifungal agents (Loyse *et al.,* 2013). An example of this resistance is mismatch repair mutations in *Cryptococcus gattii* that have resulted in resistance (Loyse *et al.,* 2013).

2.6.5 Other Antifungal Agents

Squalene-epoxidase and mammalian enzyme that synthesizes ergosterol are impeded by allylamines and thiocarbamates (Spampinato and Leonardi, 2013a). Griseofulvin a product of *Penicillium* inhibits fungal mitosis by disrupting the synthesis of spindle and cytoplasmic microtubules (Spampinato and Leonardi, 2013a).

2.7 Diagnosis of Yeasts

An early diagnosis of yeast infection is done by isolating the yeast in a microbiology laboratory and characterizing it (Arendrup *et al.,* 2014). The cultivation of yeast samples confirms the final diagnosis (Rajasingham *et al.,* 2019).

2.7.1 Conventional Diagnosis of Yeasts

2.7.1.1Gram staining and Microscopy

All yeasts are gram-positive when viewed under a 100X objective microscope (Jonsson *et al.,* 2005). Hence, the technique is useful for immediate identification of yeast for further processing (Jonsson *et al.,* 2005). The technique is crucial for yeast morphological study since the organisms' shape could range from ovoid to elongated (Jonsson *et al.,* 2005).

2.7.1.1.1 Indian ink

Cryptococcus species can be identified as encapsulated yeast cells in cerebrospinal fluid (CSF) with more than 1,000 colony-forming units/milliliter using Indian ink or Negrosin stain (Rajasingham *et al.,* 2019). The polysaccharide capsule is observed encircling the organisms when the CSF is combined with India ink and observed under the microscope at 40X (Rajasingham *et al.,* 2019).

2.7.1.2 Culture

Culturing of yeasts is the gold standard for microbiological testing of yeasts, this is because it is precise in diagnosis of yeast based on the isolation and identification (Leelavathi *et al.,* 2014). Yeasts are non-fastidious organisms that are able to grow in diverse media (Kathuria *et al.,* 2015). The most utilized media for culture is Sabouraud’s dextrose agar (Solomon, 2021). Other media such as cysteine lactose electrolytes deficient (CLED), blood agar (BA) and MacConkey (MAC) can be used for culturing samples suspected to contain yeasts (Solomon,

2021). To make Sabouraud's dextrose agar selective for the growth of yeast and prevent bacterial development, it is coupled with antibiotics such as tetracycline, chloramphenicol, and/or gentamicin (Leelavathi *et al.,* 2014).

Although the addition of cycloheximide suppresses the growth of saprophytes on Sabouraud's dextrose agar, certain yeasts of *Candida*, such as *C. krusei*, *C. tropicalis*, and *C. parapsilosis*, are sensitive to cycloheximide (Leelavathi *et al.,* 2014). Therefore, it is generally not advisable to add cycloheximide on Sabouraud's dextrose agar (Solomon, 2021). The inoculum suspected to contain yeast is cultured by incubating the Sabouraud's dextrose agar plate with the organism for 24 hours at 37 °C (Leelavathi *et al.,* 2014). The use of Sabouraud's glucose agar or unselective Sabouraud's dextrose agar (without cycloheximide) is preferable for the isolation of *Cryptococcus* species from varied human samples (Kathuria *et al.,* 2015). *Cryptococcus* species are readily isolated on blood agar and Sabouraud's dextrose agar without cycloheximide as large, white, butyrous colonies (Leelavathi *et al.,* 2014). A presumptive identification of *Cryptococcus* species can be made using a quick urease positive, phenol oxidase release, and a negative nitrate test (Solomon, 2021).

Prior to subculturing on Sabouraud's dextrose agar, blood agar, or MacConkey agar, blood samples require additional culture in blood culture bottles (Leelavathi *et al.,* 2014). Blood culture has become more convenient due to the use of various methods such as lysis-centrifugation tubes and automated monitoring of blood culture bottles (Solomon, 2021). The lysis improves the sensitivity of the culture method by increasing the number of yeast species detected in the blood by using a wash solution to expel the yeast from inside the host phagocytes to the cell surface, as well as shortening the time required for growth after inoculation (Solomon, 2021).

2.7.2 Phenotypic identification

2.7.2.1 Carbohydrate Utilization

By examining a yeast's ability to utilize carbohydrates, one can identify and characterize it (Kathuria *et al.,* 2015). The biochemical identification of yeast species is enabled by a pattern of anaerobic carbohydrate fermentation and aerobic assimilation (Solomon, 2021). The auxano graphic approach is another simple way for determining the assimilation property of yeast species that outperforms the conventional method developed by Wickerham and Burton (Kathuria *et al.,* 2015).

2.7.2.2 Cryptococcal Antigen Test (CRAG) Test

The serum cryptococcal antigen (CRAG) test is a reliable and sensitive indicator of cryptococcal meningitis. A presumptive diagnosis of cryptococcal meningitis should be made if the positive titer is more than 1:8. This is because 98% of patients with culture-proven cryptococcal meningitis were also found to have positive serum CRAG levels (Chayakulkeeree and Perfect, 2007).

2.7.2.3 Germ Tube Test

The Germ tube test is a screening test used to distinguish *C. albicans* from other yeast (Kathuria *et al.,* 2015). *Candida* forms a germ tube after 3 hours of growth in human or sheep serum at 37°C, which can be observed on wet KOH films as filamentous outgrowth extending from yeast cells (Solomon, 2021). When cultivated in a medium rich in protein, isolates of both *C. dubliniensis* and *C. albicans* form germ tubes in 95–97% of cases (Kathuria *et al.,* 2015).

2.7.3 Current Methods of Yeast Diagnosis

2.7.3.1 Automated Methods

2.7.3.1.1 VITEK-2

VITEK-2 is a machine that uses pure culture inoculum to identify microorganisms (Adam *et al.,* 2019). VITEK-2 identifies microorganisms using an inoculum applied to a card in a machine by integrating a large number of databases (Nadeem *et al.,* 2010). After creating and standardizing a primary inoculum, VITEK-2 automatically completes all of the procedures crucial for identifying yeasts (Nadeem *et al.,* 2010). This technology, which has a 30-card capacity and identifies yeast using a fluorogenic method, has sped up and simplified patient diagnosis (Adam *et al.,* 2019). To be more specific, VITEK-2 has decreased inpatient stays by 16 hours for 80% of the samples when compared to conventional methods (Adam *et al.,* 2019). Lastly, the technique has proven to be cost effective over time, with valid and reliable results (Nadeem *et al.,* 2010).

2.7.3.1.2 API 20C AUX

The API 20C AUX system is a commercial micro method technique used to precisely identify yeast in diagnostic laboratories (Nadeem *et al.,* 2010). This API 20 C AUX technique assesses the assimilation ability of 19 carbohydrates and identifies them within 24 to 72 hours of incubation (Nadeem *et al.,* 2010). The API 20 C AUX strip is made up of 20 cupules that contain dehydrated substrates and allows for the performance of 19 assimilation tests (Buehler *et al.,* 2017). A semisolid minimal medium is used to inoculate the colonies (Nadeem *et al.,* 2010). The yeasts will only grow if each substrate serves as their only carbon source (Buehler *et al.,* 2017). Reading the reactions involves comparing them to growth controls (Nadeem *et al.,* 2010). The Analytical Profile Index is used to establish identification (Buehler *et al.,* 2017).

2.7.3.1.3 MALDI-TOF MS

Mass patterns by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) have recently become available for obtaining rapid and accurate identification and AST information for yeasts (Kathuria *et al.,* 2015). While this method has the advantage of quicker TAT and improved sensitivity, this platform is more expensive and may not be economically viable for some laboratories (Buehler *et al.,* 2017). MALDI-TOF MS is very handy in the accurate species identification of yeast especially *Candida* complexes since they are closely associated and often misidentified (Kathuria *et al.,* 2015).

2.7.3.2 Molecular Assay

Polymerase Chain Reaction (PCR) is a very quick and precise molecular approach for identifying yeast at the species level, although it is very expensive. PCR primers generated from yeast DNA sequences are used in the technique for genetic sequencing of the yeasts. These primers amplify the variable sequences present in the internal transcribed spacer (ITS) region. The variable sequence contains tandemly repeated 18S, 5.8S, and 26S yeast rRNA, as well as the D1/D2 area of 26S rRNA gene loci of yeast such as *Trichosporon* and *Candida* complexes. However, one drawback of the ITS region is that it is extremely similar among the numerous species in the genus *Trichosporon* (Kathuria *et al.,* 2015; Buehler *et al.,* 2017).

2.7.4 Antifungal Susceptibility Testing for Yeasts

Antifungal sensitivity in yeast can be identified using various antifungal susceptibility testing (AST) methods, which can also aid in the selection of the best course of action for a given yeast (Solomon, 2021).

2.7.4.1 MIC Determination Using E-Test Strips

E-test is a commercially available, patented method for determining an antimicrobial's susceptibility (AB Bio disk, Solna, Sweden) (Cantón *et al.,* 2010). Crossing an area of growth

inhibition with a calibrated strip placed on an agar plate containing the microbiological isolate being tested and impregnated with an antibiotic concentration gradient yields minimum inhibitory concentration (MICs) (Kathuria *et al.,* 2015). This method has been used with a variety of antifungal medications (Kathuria *et al.,* 2015). Nonuniform growth of the fungal lawn, as well as the appearance of a feathered or trailing growth edge, might make establishing the end point problematic (Cantón *et al.,* 2010).

2.7.4.2 Standard Broth Microdilution Method

The standard broth microdilution method is used to determine the minimum inhibitory concentration (MIC) of yeast isolates using the Clinical and Laboratory Standards Institute (CLSI) report M27-A2 criteria (Kathuria *et al.,* 2015). The range of antifungal drug concentrations examined is generally from 0.015 g/mL to 64 g/mL (Cantón *et al.,* 2010). The microdilution plates are then incubated for 24 and 48 hours at 35 °C before being checked for visible growth (Cantón *et al.,* 2010). The microdilution wells are scored using a magnifying mirror, and the growth in each well is compared to the growth control well (Cantón *et al.,* 2010). The MIC50 and MIC90 values are calculated using the minimum inhibitory concentrations at which 50% and 90% of the isolates become inhibited, respectively (Cantón *et al.,* 2010). When carrying out the AST test, quality control of each yeast strain is also included. Minimum inhibitory concentrations are reported with interpretative standards where applicable (Cantón *et al.,* 2010).

2.7.4.3 Agar Disk Based Susceptibility Testing

Antifungal susceptibility testing techniques based on chromogenic Chrom agar dilution at specified concentrations are intriguing since they are easy to carry out, quick, and affordable. The modifications of this technique by the addition of methylene blue (0.5 g/ml) have demonstrated significant similarity with the standard broth techniques by giving improved zone

edge delineation and making it easier to read the plate's surface. However, for this method, extensive testing of possibly resistant isolates is still required (Knabl and Lass-Flörl, 2020).

2.7.4.4 Commercial Broth-Based Minimum Inhibitory Concentration (MIC) Systems

Commercial broth-based systems such as Candifast, Sensititre, Integral Systems Yeasts, and Fungitest are antifungal susceptibility testing techniques that have recently been investigated. These minimum inhibitory concentration system kits utilize colorimetric reagents such as Alamar Blue redox marker for testing a limited concentration of antifungal drugs that have been pre-selected as key endpoint values. Although one study reported some issues with testing *C. var. neoformans*, this kit tests a wide range of drug concentrations, and rates of conformity with the reference standards of 85% are often obtained (Matar *et al.,* 2003).

2.7.4.5 Direct Quantification of the Azole Trailing Effect

The direct quantification of the azole trailing effect in yeast isolates is critical in categorizing isolates as sensitive or resistant. This direct measurement of changes in ergosterol synthesis in vitro and in vivo is important given that azole antifungal drugs work by inhibiting its synthesis. The results of the technique have been proven to be comparable to the NCCLS M27A technique for *Candida* species (Matar *et al.,* 2003).

2.7.4.6 MALDI-TOF MS and VITEK - 2

MALDI-TOF MS and VITEK-2 are extremely useful in determining the antifungal susceptibility patterns of yeast (Kathuria *et al.,* 2015). Following the preparation and standardization of a primary inoculum, VITEK-2 or MALDI-TOF MS automatically performs all of the steps required for AST of yeasts (Kathuria *et al.,* 2015).

2.7.4.7 Viability Dyes and Flow Cytometry

In studies that mostly focused on *Candida* species, flow cytometry was designed for yeasts as a potential technique for antifungal susceptibility testing (Knabl and Lass-Flörl, 2020). The use of appropriate dyes to stain yeast allows for the quick detection of damaged yeast (Knabl and LassFlörl, 2020). Recent research has investigated this idea further, demonstrating the possibility of a link between flow-cytometry techniques and the reference method (Simonsen *et al.,* 2019). Flow cytometry may be particularly helpful for identifying Amphotericin B resistance (Simonsen *et al.,* 2019). In previous studies, fluorescent viability dyes have been used to investigate the type of drug-induced harm to yeasts and to evaluate minimum inhibitory concentration and minimal fungicidal concentrations (MFCs) for yeasts (Knabl and Lass-Flörl, 2020).

2.8 Conceptual Framework

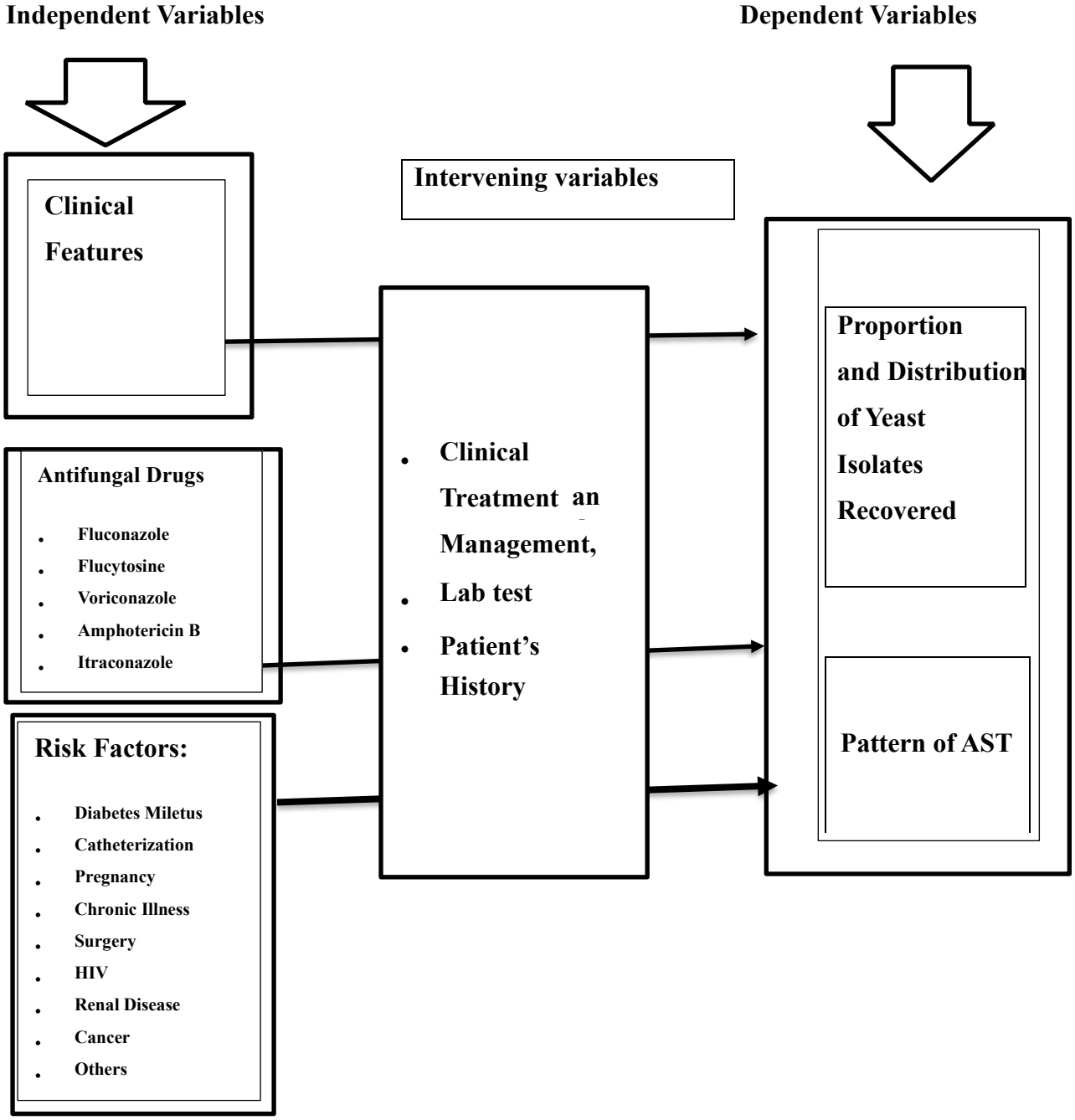

Figure 2: Conceptual Framework Model

Chapter Three: Methodology

3.1 Study Design

The study was a retrospective cross-sectional analysis of the proportion of yeast infection, the antifungal drug sensitivity profile of yeast isolates recovered from inpatients and assessment of risk factors associated with yeast infections in inpatients diagnosed with yeast infection at Nairobi South Hospital, Nairobi, Kenya from October 1st, 2018, and September 30th, 2022.

3.2 Study Area

The research study was carried out at the Nairobi South Hospital's microbiology laboratory, which is located on Muhoho Avenue, South C in Nairobi County. The laboratory is adequately equipped and processes all types of laboratory samples from patients.

3.3 Study Population

A total of 2006 data files of inpatients who had microscopy, culture and sensitivity tests performed were identified and scanned for yeast infection from the study period.

3.3.1 Patient Selection

Clinical and laboratory data for this study was extracted from Nairobi South Hospital inpatients' record system over the four-year period. Records of inpatients with microbial culture and sensitivity were eligible for the study and were further screened using the inclusion and exclusion criteria. Age, gender, length of hospital stay, antibiotics used, chemotherapy, laboratory tests, admission to the intensive care unit, mortality, and any other important clinical information for these inpatients was extracted from their files.

3.3.2 Inclusion Criteria

I. All the inpatients with positive yeast isolates from any type of specimens collected and analyzed for culture and sensitivity during the study period were recorded.

3.3.3 Exclusion Criteria

II. Any other data of different microorganism isolated other than yeast such as *Staphylococcus aureus,* and *Klebsiella pneumoniae* from inpatients files was excluded from the study.

III. Inpatient files with missing baseline information such as patient's age, sex, type of specimen, ward admitted in, name of yeast species isolated and AST results.

3.4 Sample Size

Kathuria *et al.,* (2015), recommends the use of Fisher's *et al* to calculate sample size for population estimates more than 2,000 hence, the sample size was calculated using Fisher's *et al* because the inpatients records that information on microbial culture and sensitivity done at Nairobi South Hospital were more than 2,006:

$$n = \frac{z2}{pq2}$$

Where:

n – is the sample size

p = the approximated proportion of the target population with the attributes being measured

z = is the standard normal deviation required confidence level (Solomon, 2021)

q=1-p

d – is the margin of error (Could be 0.10, 0.05 or 0.01) n= $(1.96)^2 \times 0.50 \times 0.50 / (0.05)^2$

n =3.8416×0.50×0.50/0.0025

n=0.9604/0.0025

n=**384**

Therefore, n=384 inpatients with yeast infection.

The minimum calculated sample size used was **384** in this study in order to maintain a 95% confidence interval.

3.5 Sampling Procedure

A stratified random sampling of all the data of records of microbial culture and sensitivity for inpatients from the population that were positive for yeast infections from 1st October 2018 to 30th September 2022 was included in the study.

3.5.1 File Selection Criteria

Proportionate stratification of yeast isolate records per genus among the inpatients diagnosed with yeast infection was the most suitable method used for this study to control for selection bias. *Candida, Cryptococcus,* and *Trichosporon* distribution and proportion was expected to vary between the different yeast strata. This assumption was based on a literature review of comparable fungal investigations conducted at similar level 6 facilities in Kenya, including Nairobi Hospital, Agha Khan University Hospital, and Mombasa (Giri and Kindo, 2012; Solomon, 2021). In addition, Mutua *et al.,* (2010), depicted the proportion of *Candida* species were the most predominant at 80%, followed by *Cryptococcus* species at 14% and *Trichosporon* species at 6%. Despite having non-equal sample sizes from each stratum of

yeast, this resulted in a more precise and realistic estimate of the burden of yeast infections among Nairobi South Hospital inpatients. The sample size for each yeast genus stratum was then computed using a proportional stratified method based on the total population of yeast isolates. This was accomplished by classifying the strata according to subsets that included the patients' age, gender, comorbidity, ward, and the type of sample from which the data of yeast isolate was collected that was later pooled to form a representative sample. The table 1 below shows the sampling based on all the 2006 records of microbial culture and sensitivity performed among inpatients at Nairobi South Hospital of which 308 records were positive for yeast infection over the study period.

Table 1. Yeast Isolates' Strata Sample Size Calculation

Yeast genus	**Assumed population of each yeast genus**	**Estimated proportion As per Literature %**	**Sample size of each stratum**
Candida spp.	1615	80	248
Cryptococcus spp.	270	14	41
Trichosporon spp.	115	6	19
	N=2006	**100%**	**n**=308

$$\text{Stratified sampling groups} = \frac{\text{Total sample size records of Yeast Isolates}}{\text{Entire population of records of Inpatients tested for m. c. s}} \times \text{Population of Strata of Yeast Species}$$

Candida species Sample Size of Strata $= \frac{308}{2006} \times 1615$

$$\cong 248$$

Cryptococcus species Sample Size of Strata $= \frac{308}{2006} \times 270$

$$\cong 41$$

Trichosporon species Sample Size of Strata $= \frac{308}{2006} \times 115$

$$\cong 19$$

3.6 Data Collection Instruments and Methodology

Data of inpatients with a yeast infection diagnosis was retrospectively obtained using a data abstraction tool from Nairobi South Hospital's electronic medical record system.

3.6.1 Study Variables

Dependent variable: Yeast isolate

Independent variable: Specimen type, admission ward, age, sex, and antifungal sensitivity

3.6.2 Microbiological Testing

Fungal strain identification and ASTs were performed using VITEK-2 model DL 96 fungal disc cards following the passage of quality commercial controls for the yeast species and all the five antifungal drugs: Amphotericin B, Flucytosine, Fluconazole, Itraconazole, and Voriconazole (Bio-Merieux SA, Marcy l 'etoile, France). This VITEK-2 system has a 30-card capacity and identifies yeast using a fluorogenic method. The system identifies microorganisms using a pure culture inoculum applied to a card and an integration of a large number of databases to automatically complete 14 biochemical tests for identifying yeasts (Adam *et al.,* 2019).

However, VITEK-2 can identify yeast isolates of *Cryptococcus* genus to species level, yeast of *Trichosporon* at only the genus level and yeast of *Candida* genus to species level except for the species of *Candida* complexes. The VITEK-2 system is not able to differentiate this complex's species but groups them as one *C. haemulonii* a limitation of this model (Adam *et al.,* 2019). However, we did not have any record of recovery of yeast from the *C. haemulonii* complex among inpatients at Nairobi South Hospital during the study duration.

3.6.3 Antifungal Drug Sensitivity Testing

Antifungal drug sensitivity testing was done according to the described method for all the yeast isolates against a panel of five antifungal drugs: Amphotericin B, Flucytosine, Fluconazole, Itraconazole, and Voriconazole, and the findings were reported as sensitive or resistant for each yeast species as per CLSI guidelines (Sadeghi *et al.,* 2018).

3.7 Statistical Analysis

A data abstraction tool was used to document the data from the patient's electronic file. The study's sample size was calculated to be 384 hospital records of inpatients having yeast isolates. Due to the rarity of yeast isolate recovery, only 308 hospital records of inpatients with yeast isolates with complete information were analyzed from the total 2006 microbial culture and sensitivity test done. This sample size, however, had no effect on the statistical power to answer the study's main questions and it produced statistically significant results. As a result, the missing cases were not a concern. All the information gathered from the hospital records of 308 inpatients at Nairobi South Hospital was analyzed using both Microsoft Excel and IBM SPSS software version 26 for Windows programs. As the two programs were complementary and some functions were exclusive to one. Quantitative data that were not normally distributed were presented as median and quartile ranges (M (P25, P75)). Binary data that was normally

distributed, had a comparison made between these groups using a chi-square test with the p value set at 0.05. Multivariate analysis was conducted using logistic regression analysis. For differences to be deemed statistically significant, a $p < 0.05$ had to be met.

3.8 Ethical Considerations

The study protocol was reviewed and approved by the Kenyatta National Hospital-University of Nairobi Ethics Review Committee (P747/09/2022), the National Commission for Science, Technology, and Innovation (NACOSTI/P/23/25973), and the Nairobi South Hospital Administration. Furthermore, the study was part of the routine inpatient care process. As a result, no written informed consent from the inpatient was required for participation in this study; nonetheless, the requirement of the Data Privacy and Protection Act was met by maintaining the confidentiality of the patient information.

3.9 Dissemination Plan

This study is a model for future research on hospital-based yeast infections, antifungal sensitivity profiles, and risk factors in Kenya and the rest of Sub-Saharan Africa. The findings will be presented to the Nairobi South Hospital and discussed during a Continuing Medical Education workshop with staff and policy makers. The findings will also be published as a thesis report and presented to the department of Medical Microbiology and Immunology at the University of Nairobi. Finally, presentations at conferences and seminars will be used to disseminate this knowledge both locally and publication of study results in peer-reviewed journals for scientific consumption.

Chapter Four: Results

4.1 Characteristics of Data of Inpatients' Microbial Isolates Studied

A total of 2006 inpatients who had microbial culture and sensitivity tests done were identified, 308 had yeast cultures positive giving a prevalence of 15% yeast infections in the hospital's inpatients between October 1st, 2018, and September 30th, 2022. A summary of the patient characteristics is presented in Table 2. A majority of the inpatients were female both in the overall culture and sensitivity and in patients with yeast isolates 64% (n=1293) and 64% (n=196) respectively. The patients with yeast isolate had a median age distribution of 42 years (lowest quartile = 28 and maximum quartile= 60). The age group 19 to 45 years had the highest proportion in both the overall culture and sensitivity 73% (n =1454), and in patients with yeast isolates 73% (n=226). Majority of the patients with culture and sensitivity tests were from the general wards [64% (n=1280)] similar to that of yeast isolates 41% (n=126). Generally, 57% (n =175) of yeast isolates were from urine and urinary catheter specimen type. From 2018 to 2021, the incidence of yeast isolates increased, followed by a by a slight decline in 2022. Year 2021 had the highest microbial culture and sensitivity carried out 59% (n=1188), with the highest proportion of yeast isolates at 54% (n=166). Generally, in all the categories there was no statistically significant difference in the proportion of non-yeast isolates culture and the yeast isolates culture. However, there was a significant difference in the proportion of yeast isolates among inpatients who had a prior surgery and those who did not have a history of surgery (p = 0.0050). Majority of the patients with positive culture and sensitivity tests had diabetes mellitus [18% (n= 365)] but those with yeast isolates were inpatients with systemic infection 30% (n = 94). The overall proportion of yeast isolates for the 5-year period was 15.4% (13.8-17.1) as shown in table 2.

Table 2: Baseline Characteristics Inpatients with Microbial Isolates and Prevalence of Yeasts

Categories		Overall Specimens for C/S N= 2006	Non-Yeast Isolates N=1698	Yeasts Isolates n=308	P value
Age	Median (IQR)	45 (31-60)	45 (31-60)	42 (28-60)	
Age Distribution n (%)	<0-5yrs	25 (1.2)	22 (1.5)	3 (1.0)	0.6570
	6-18yrs	127 (6.3)	112(66.0)	15 (4.9)	
	19-64yrs	1454 (72.5)	1228 (72.3)	226 (73.4)	
	>65yrs	400 (19.9)	336 (19.8)	64 (20.8)	
Sex n (%)	Female	1293 (64.5)	1097(64.6)	196 (63.6)	0.7440
	Male	713 (35.5)	601 (35.4)	112 (36.4)	
Ward Type n (%)	Covid-19	168 (8.4)	126 (7.4)	42 (13.6)	0.0005
	General	1280 (63.8)	1154 (68.0)	126 (40.9)	
	ICU and HDU	315 (15.7)	198 (11.7)	117 (38.0)	
	Maternity	81 (4.0)	63 (77.8)	18 (5.8)	
	NICU and Pediatric	162 (8.1)	157 (96.9)	5 (1.6)	
Specimen Type n (%)	Ascitic and Peritoneal Fluids	30 (1.5)	10 (33.3)	20 (6.5)	0.0005
	Blood	419 (20.9)	395 (94.3)	24 (7.8)	
	CSF	26 (1.3)	17 (65.4)	9 (2.9)	
	CVC Tip	156 (7.8)	136 (87.2)	20 (6.5)	
	Sputum and Tissue	53 (2.6)	29 (54.7)	24 (7.8)	
	Stool	37 (1.8)	28 (75.7)	9 (2.9)	
	Urine and Urinary Catheter	1177 (58.7)	1002 (85.1)	175 (56.8)	
	Wound Pus Swab	108 (5.4)	81 (75.0)	27 (8.8)	
Year n (%)	2018	46 (2.3)	40 (2.4)	6 (1.90)	0.0005
	2019	274 (13.7)	237 (14.0)	37 (12.0)	
	2020	391 (19.5)	340 (20.0)	51 (16.6)	
	2021	1188 (59.2)	1022 (60.2)	166 (53.9)	

	2022	107 (5.3)	59 (3.5)	48 (15.6)	
Prior Surgery (n (%)	Surgery Done	322 (16.1)	256 (12.8)	66 (21.4)	0.0050
	No Surgery Done	1684 (83.9)	1442 (71.9)	242 (78.6)	
Patient Comorbidity n (%)	Autoimmune	214 (10.7)	207 (10.3)	7 (2.3)	0.0000
	Cancer	25 (1.2)	16 (0.8)	9 (2.9)	
	Complicated Malaria	248 (12.4)	230 (11.5)	18 (5.8)	
	Complicated UTI	135 (6.7)	122 (6.1)	13 (4.2)	
	COVID-19	36 (1.8)	15 (0.7)	21 (6.8)	
	DM	365 (18.2)	318 (15.9)	47 (15.3)	
	Hematological Malignancy	53 (2.6)	41 (2.0)	12 (3.9)	
	Heart Failure	22 (1.1)	16 (0.8)	6 (1.9)	
	IDA	282 (14.1)	268 (13.4)	14 (4.5)	
	Liver Disease	106 (5.3)	95 (4.7)	11 (3.6)	
	Pulmonary Pneumonia	171 (8.5)	162 (8.1)	9 (2.9)	
	Renal Disorders	170 (8.5)	142 (7.1)	28 (9.1)	
	RVD	34 (1.7)	15 (0.7)	19 (6.2)	
	Systemic Infection/Sepsis	145 (7.2)	51 (2.5)	94 (30.4)	
	Proportion at 95% CI	15.4% (13.8 - 17.1)			

CI = Confidence Interval, CVC= Central Venous Catheter, CSF = Cerebral Spinal Fluid, ICU = Intensive Care Unit, HDU = High Dependency Unit, NICU= Neonatal Intensive Care Unit, IQR= Interquartile Range, C/S = Culture and Sensitivity, DM = Diabetes Mellitus, IDA = Iron Deficiency Anemia, UTI = Urinary Tract Infection, RVD = Retroviral Disease

4.2 Distribution of Yeast Isolates

4.2.1 Distribution of Yeast Isolates by Age Group, Sex, Ward, and Specimen Type

Three genera of yeast, *Candida, Cryptococcus*, and *Trichosporon*, were isolated, with yeasts of the *Candida* genus being the most common, 88% (n = 272). All three genera of yeast isolates were more predominant in the age group of 19-64 years, with yeast isolates of the *Candida* genus being the most predominant at 65% (n = 201). Yeast isolates of the *Candida* genus were also more predominant in females, 58% (n = 179), compared to males, while yeast isolates of the *Cryptococcus* genus were more predominant among males, 6% (n = 19), than females. Yeast isolates of the *Trichosporon* genus were only isolated from females, 2% (n = 6). Yeast isolates of the *Candida* genus were more in the general ward, 36% (n = 112), than any other ward, while yeast isolates of both the *Cryptococcus* 5% (n = 15) and *Trichosporon* genera, 1% (n = 4) were predominant in the intensive care unit and high dependency unit than any other ward.

Yeast isolates of both the *Candida* genus 53% (n = 162) and *Cryptococcus* 4% (n = 12) were isolated more from the urine and urinary catheter tip specimens than any other specimen, while yeast isolates of the *Trichosporon* genus were frequent in the central venous catheter (CVC) tip specimens 0.6% (n = 2). Overall, *C. albicans* was the most frequent of all yeasts isolated at 38% (n = 116), while *C. lusitaniae* was the least recovered at 1.6% (n = 5). Age group less than 5 years only had 3 isolates from *C. dubliniensis, C. guilliermondii, and C. laurentii* at less than one percent of each species. Age group 6 - 8 years had *C. dubliniensis* 2% (n = 6) as the most frequent. *C. albicans* was the most frequent isolate in both age groups, 19-64 years, 28% (n = 86) and those above 65 years 8% (n = 25. Both females and males sexes had *C. albicans* as the most frequent isolate, 24% (n = 74) and 14% (n = 42) respectively. The general ward had the most yeast isolates overall, 41% (n = 126). *C. albicans* was the most frequent isolate in the General wards 17% (n = 53), COVID-19 wards 5% (n = 15), and intensive care unit and high

dependency unit 14% (n = 43), whereas *C. dubliniensis* was the most predominant in both maternity ward 2% (n = 7) and the neonatal intensive care unit and pediatrics ward 0.6% (n = 2). The urine and urinary catheter tips specimen 57% n = 175) had the most yeast isolates overall. *C. albicans* was the most frequent isolate in urine and urinary catheter tips 23% (n = 71), ascitic and peritoneal fluids 5% (n = 14), central venous catheter (CVC) tip 2% (n = 7), and wound pus swabs 5% (n = 14). Both *C. albicans* and *C. glabrata* were most common in blood specimens with six isolates of each (2%). *C. dubliniensis* was the most frequent isolate in sputum and tissue samples at 3% (n = 9) and in stool specimen 2% (n = 5). *C. var. neoformans* was the most frequent isolate in cerebral spinal fluid (CSF) specimen, 3% (n = 8), as shown in Table 3.

Table 3: Distribution of Yeast Isolates by Age Group, Sex, Ward, and Specimen Type (n=308)

Yeast Isolate		Age Groups				Sex		Ward					Specimen							
	n (%)	<0-5yr	6-18y	19-64	>65yr	Female	Male	General	Maternity	NICU and Pediatric	COVID-19	ICU and HDU	Urine and Urinary Catheter	Ascitic and Peritoneal Fluids	Blood	CSF	CVC Tip	Sputum and Tissue	Stool	Wound Pus Swab
Total n (%)	308	3(1.0)	15(4.9)	226(73.4)	64(20.8)	196(63.6)	112(36.4)	126(40.9)	18(5.8)	5(1.6)	42(13.6)	117(38.0)	175(56.8)	20(6.5)	24(7.8)	9(2.9)	20(6.5)	24(7.8)	9(2.9)	27(8.8)
Candida genus	272(88.3)	2(0.6)	14(4.5)	201(65.3)	55(17.9)	179(58.1)	93(30.2)	112(36.4)	18(5.8)	4(1.3)	40(13.0)	98(31.8)	162(52.6)	19(6.2)	23(7.5)	1(0.3)	18(5.8)	20(6.5)	7(2.3)	22(7.1)
C. albicans	116(37.7)	0(0.0)	5(1.6)	86(27.9)	25(8.1)	74(24.0)	42(13.6)	53(17.2)	5(1.6)	0(0.0)	15(4.9)	43(14.0)	71(23.1)	14(4.5)	6(1.9)	0(0.0)	7(2.3)	4(1.3)	0(0.0)	14(4.5)
C.dubliniensis	73(23.7)	1(0.3)	6(1.9)	49(15.9)	17(5.5)	52(16.9)	21(6.8)	28(9.1)	7(2.3)	2(2.7)	13(4.2)	23(7.5)	46(15.0)	3(1.0)	5(1.6)	0(0.0)	0(0.0)	9(2.9)	5(1.6)	5(1.6)
C. glabrata	22(7.1)	0(0.0)	0(0.0)	21(6.8)	1(0.3)	17(5.5)	5(1.6)	8(2.6)	3(1.0)	0(0.0)	4(1.3)	7(2.3)	14(4.5)	1(0.3)	6(1.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.3)
C.guillermondii	6(1.9)	1(0.3)	1(0.3)	3(1.0)	1(0.3)	4(1.3)	2(0.6)	2(0.6)	0(0.0)	1(0.3)	0(0.0)	3(1.0)	1(0.3)	0(0.0)	2(0.6)	0(0.0)	1(0.3)	2(0.6)	0(0.0)	0(0.0)
C. lusitaniae	5(1.6)	0(0.0)	0(0.0)	4(1.3)	1(0.3)	5(1.6)	0(0.0)	2(0.6)	0(0.0)	0(0.0)	0(0.0)	3(1.0)	1(0.3)	1(0.3)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	2(0.6)	0(0.0)
C parapsilosis	12(3.9)	0(0.0)	1(0.3)	8(2.6)	3(1.0)	4(1.3)	8(2.6)	6(1.9)	0(0.0)	0(0.0)	3(1.0)	3(1.0)	6(1.9)	0(0.0)	0(0.0)	1(0.3)	4(1.3)	1(0.3)	0(0.0)	0(0.0)
C. tropicalis	29(9.4)	0(0.0)	1(0.3)	22(7.1)	6(1.9)	16(5.2)	13(4.2)	9(2.9)	1(0.3)	1(0.3)	4(1.3)	14(4.5)	15(4.9)	0(0.0)	3(1.0)	0(0.0)	6(1.9)	3(1.0)	0(0.0)	2(0.6)
C.krusei	9(2.9)	0(0.0)	0(0.0)	8(2.6)	1(0.3)	7(2.3)	2(0.6)	4(1.3)	2(0.6)	0(0.0)	1(0.3)	2(0.6)	8(2.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)
Cryptococcus genus	30(9.7)	1(0.3)	1(0.3)	22(7.1)	6(1.9)	11(3.6)	19(6.2)	12(3.9)	0(0.0)	1(0.3)	2(0.6)	15(4.9)	12(3.9)	0(0.0)	0(0.0)	8(2.6)	0(0.0)	3(1.0)	2(0.6)	5(1.6)
C.laurentii	21(6.8)	1(0.3)	0(0.0)	16(5.1)	4(1.3)	7(2.3)	14(4.5)	7(2.3)	0(0.0)	1(0.3)	1(0.3)	12(3.9)	12(3.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(0.6)	2(0.6)	5(1.6)
C. var. neoformans	9(2.9)	0(0.0)	1(0.3)	6(1.9)	2(0.6)	4(1.3)	5(1.6)	5(1.6)	0(0.0)	0(0.0)	1(0.3)	3(1.0)	0(0.0)	0(0.0)	0(0.0)	8(2.6)	0(0.0)	1(0.3)	0(0.0)	0(0.0)
Trichosporon genus	6(1.9)	0(0.0)	0(0.0)	3(1.0)	3(1.0)	6(1.9)	0(0.0)	2(0.6)	0(0.0)	0(0.0)	0(0.0)	4(1.3)	1(0.3)	1(0.3)	1(0.3)	0(0.0)	2(0.6)	1(0.3)	0(0.0)	0(0.0)
T. beigelii	6(1.9)	0(0.0)	0(0.0)	3(1.0)	3(1.0)	6(1.9)	0(0.0)	2(0.6)	0(0.0)	0(0.0)	0(0.0)	4(1.3)	1(0.3)	1(0.3)	1(0.3)	0(0.0)	2(0.6)	1(0.3)	0(0.0)	0(0.0)

CVC= Central Venous Catheter, CSF = Cerebral Spinal Fluid, ICU = Intensive Care Unit, HDU = High Dependency Unit, NICU = Neonatal Intensive Care Unit,

4.2.2 Distribution of Yeast Isolates by Patient Comorbidities

The 308 inpatients with yeast isolates showed a varied range of comorbidities. These included autoimmune disorders in 2%, cancer in 3%, complicated malaria in 6%, complicated urinary tract infection (UTI) in 4%, COVID-19 in 7%, diabetes mellitus (DM) in 15%, hematological malignancies in 4%, heart failure in 2%, iron deficiency anemia (IDA) in 5%, liver disease in 4%, pulmonary pneumonia in 3%, renal disorders in 9%, retroviral disease (RVD) in 6%, and systemic infection or sepsis in 30%. *C. albicans* and *C. dubliniensis* among the non - *albicans* of *Candida* species were the most predominant in patients with the varied comorbidities. *C. var. neoformans* was only isolated among inpatients with retroviral disease as shown in table 4.

Table 4: Distribution of Yeast Isolates as Per the Patient Comorbidities n=308

Associated Comorbidity	*C. albicans*	*C. dubliniensis*	*C. glabrata*	*C. guilliermondii*	*C. lusitaniae*	*C. parapsilosis*	*C. tropicalis*	*C. krusei*	*C. laurentii*	*C. var. neoformans*	*T. beigelii*
Total n (%)	116(37.7)	73(23.7)	22(7.1)	6(1.9)	5(1.6)	12(3.9)	29(9.4)	9(2.9)	21(6.8)	9(2.9)	6(1.9)
Autoimmune n = 7	1(0.3)	2(0.6)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	3(1.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Cancer n = 9	6(1.9)	1(0.3)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)	0(0.0)
Complicated Malaria n = 18	8(2.6)	3(1.0)	0(0.0)	1(0.3)	1(0.3)	0(0.0)	1(0.3)	1(0.3)	3(1.0)	0(0.0)	0(0.0)
Complicated UTI n = 13	5(1.6)	2(0.6)	3(1.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(1.0)	0(0.0)	0(0.0)	0(0.0)
COVID-19 n = 21	8(2.6)	11(3.6)	2(0.6)	0(0.0)	0(0.0)	2(0.6)	1(0.3)	1(0.3)	1(0.3)	0(0.0)	0(0.0)
DM n = 47	24(7.8)	2(0.6)	0(0.0)	0(0.0)	1(0.3)	2(0.6)	7(2.3)	0(0.0)	1(0.3)	0(0.0)	1(0.3)
Hematological Malignancy n = 12	4(1.3)	1(0.3)	2(0.6)	1(0.3)	1(0.3)	0(0.0)	0(0.0)	1(0.3)	1(0.3)	0(0.0)	0(0.0)
Heart Failure n = 6	2(0.6)	4(1.3)	0(0.0)	1(0.3)	0(0.0)	1(0.3)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
IDA n = 14	7(2.3)	4(1.3)	1(0.3)	1(0.3)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Liver Disease n = 11	5(1.6)	6(1.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	1(0.3)	0(0.0)	0(0.0)
Pulmonary Pneumonia n = 9	0(0.0)	7(2.3)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	1(0.3)	0(0.0)
Renal Disorders n = 28	9(2.9)	6(1.9)	4(1.3)	0(0.0)	0(0.0)	1(0.3)	4(1.3)	0(0.0)	3(1.0)	0(0.0)	0(0.0)
RVD n = 19	1(0.3)	6(1.9)	0(0.0)	0(0.0)	0(0.0)	2(0.6)	0(0.0)	0(0.0)	2(0.6)	8(2.6)	0(0.0)
Systemic infection/ Septic Shock n = 94	36(11.7)	18(5.8)	7(2.3)	2(0.6)	2(0.6)	4(1.3)	10(3.2)	2(0.6)	8(2.6)	0(0.0)	5(1.6)

UTI = Urinary Tract Infection, DM= Diabetes Mellitus, IDA= Iron Deficiency Anemia, RVD = Retroviral Disease, C/S = Culture and Sensitivity

4.2.3 Summary of Yeast Isolates Distribution

A total of 308 inpatients out of the 2006 examined for microbial culture and sensitivity during this time period tested positive for yeast isolates, giving an overall proportion of 15%. The current study findings revealed that there is a substantial increase in the number of yeast infection cases among inpatients at Nairobi South Hospital from the year 2018 to 2022 with yeasts of *Candida albicans* species being more common compared to *Candida* Non- *albicans*, though other yeast genus such as *Cryptococcus* and *Trichosporon* were observed though not prominent.

4.3 Antifungal Sensitivity of Yeast Isolates

4.3.1 Antifungal Sensitivity for the Yeast Species

As illustrated in Figure 3A, each yeast species displayed a distinct sensitivity pattern to the five drugs. Overall, *C. albicans* isolates were the most sensitive and most resistant to the five antifungal drugs reviewed at 40% and 36%, respectively, while *C. dubliniensis* had the most isolates with intermediate sensitivity at 29%.

The minimum inhibitory concentration (MIC)s for Amphotericin B were interpreted as sensitive (MIC range ≤0.25 – 1 µg/ml), intermediate (MIC 2 - 8µg/ml), or resistant (MIC ≥16µg/ml) (Figure 3B and Supplementary Table 1). The highest rate of resistance was observed in Amphotericin B with 91% (n = 280), of the yeast isolates showing MIC ≥16µg/ with the highest resistance observed in *C. albicans* isolates at 35% ml (Figure 3B and Supplementary Table 1). *C. dubliniensis* species had the highest proportion of sensitive isolates, 4% (MIC range of ≤0.25 - <1µg/ml). Most of the sensitive isolates, 5% (n = 14), had an MIC of 0.25µg/ml, while the intermediates had an MIC of 2µg/ml (Supplementary Table 1).

The minimum inhibitory concentrations for Flucytosine were interpreted as sensitive (MIC range ≤0.05 – 4 µg/ml), intermediate (MIC 8µg/ml), or resistant (MIC ≥16µg/ml) (Supplementary Table 1).

More than half of the isolates, 55% (n = 168), were sensitive to Flucytosine, which was the highest compared to the other drugs (Figure 3C and Supplementary table 1). The highest sensitivity and resistance to Flucytosine were observed in *C. albicans* isolates at 31% and 7% respectively. Twenty-five percent (n = 76) of all the isolates were resistant to Flucytosine, with two isolates from the *Cryptococcus* genus being intermediate to the drug at MIC of 8µg/ml, as shown in supplementary table 1. Twenty-five (n = 77) of the isolates were sensitive to Itraconazole at minimum inhibitory concentration of 0.06µg/ml (Supplementary Table 1).

The minimum inhibitory concentrations for Itraconazole were interpreted as sensitive (MIC range ≤0.03 – 0.5 µg/ml), intermediate (MIC 1 - 2µg/ml), or resistant (MIC ≥4µg/ml) (Supplementary Table 1). A total of 45% (n = 137) of all the isolates were sensitive to the drug. However, more than half of the isolates, 55% (n = 168), were resistant Itraconazole (Figure 3D and Supplementary table 1). *C. albicans* species were also both the most sensitive and resistant to Itraconazole at 15% and 19% respectively (Figure 3D).

The minimum inhibitory concentrations for Fluconazole were interpreted as sensitive (MIC range ≤0.13 – 8.0µg/ml), intermediate (MIC ≤32µg/ml), or resistant (MIC ≥64µg/ml) (Supplementary Table 1). Most isolates, 37% (n = 113) were sensitive to Fluconazole at an MIC of 0.25µg/ml, while 47% (n = 146) were resistant at an MIC of >64µg/ml, (Figure 3E and Supplementary table 1). However, yeast isolates of *C. albicans* species were both the most sensitive at 21% and resistant to Fluconazole 17% as shown in (Figure 3E).

The minimum inhibitory concentrations for Voriconazole were interpreted as sensitive (MIC range ≤0.03 – 0.5 µg/ml), intermediate (MIC 1 - 2µg/ml), or resistant (MIC ≥8µg/ml) (Supplementary Table 1). Most isolates, 45% (n = 136) were sensitive at MICs of 0.03µg/ml, while 39% (n = 120) of the isolates were resistant at MICs >8µg/ml. Yeast isolates of *C. albicans* species were also the most sensitive at 23%, intermediate at 1% and resistant to Voriconazole at 13% respectively, (Figure 3F and Supplementary Table 1).

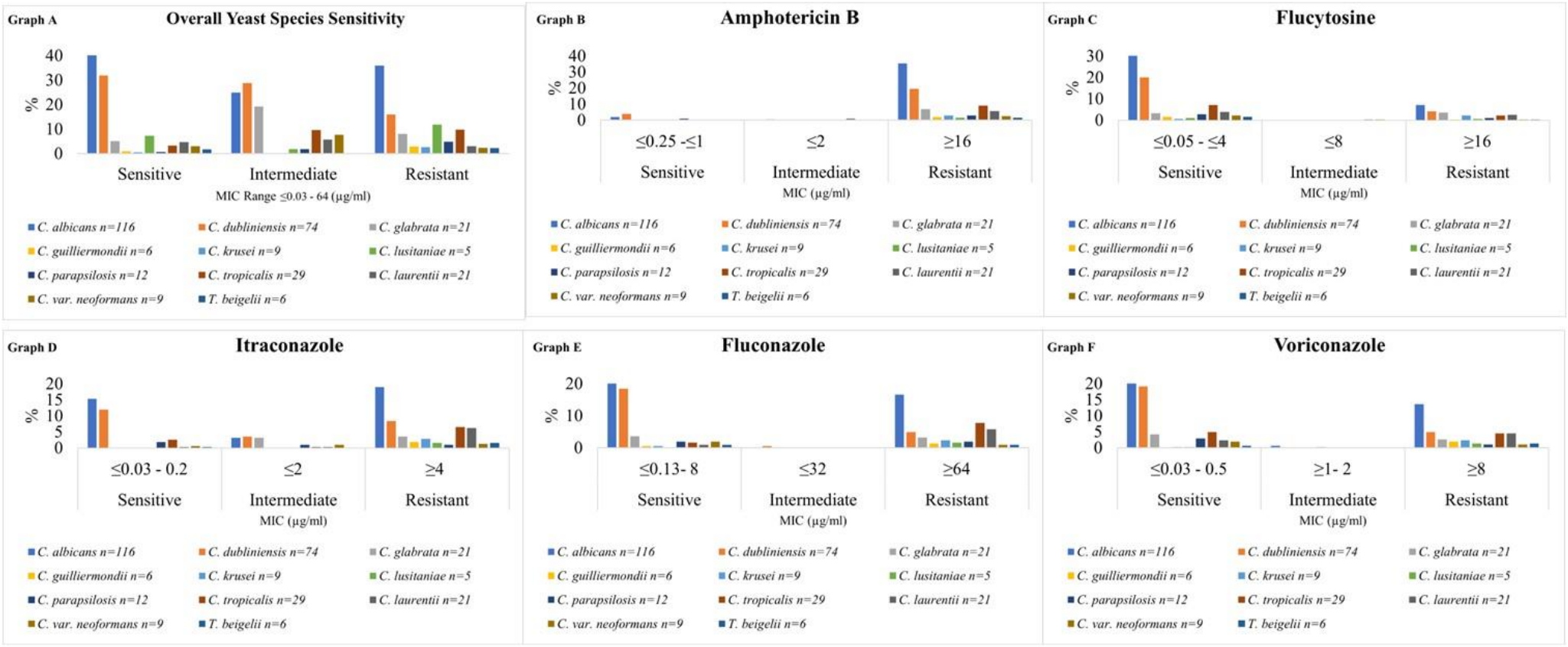

Figure 3: Antifungal Sensitivity Pattern for the Yeast Species isolated

4.3.2 Distribution of Antifungal Sensitivity Pattern by Age Group, Sex, Ward, Specimen Type and Year

Overall, based on the patients' characteristics, the yeast isolates were almost equally divided into either sensitive or resistant to the five antifungal drugs reviewed with almost negligible proportion of intermediates in each characteristic (Figure 4). However, there were still some distinct differences in the antifungal sensitivity patterns in each specific patients' characteristic. Antifungal sensitivity patterns varied between age groups; with age group 19-64 years (n = 226) having the highest number of yeast isolates, with 31% of them were sensitive, 38% resistant, and 5% intermediate (Figure 4A). However, there was no statistically significant variance in the sensitivity pattern ($p = 0.9850$) of the isolates (Figure 4A). Females (n = 196) had the most yeast isolates, with 30% of the isolates as sensitive, 32% resistant, and 4% intermediate, (Figure 4B). The sensitivity pattern was statistically insignificant ($p = 0.1110$). The general ward (n = 126) had the most yeast isolates, with 19% sensitive, 20% resistant, and 4% intermediate, while the intensive care unit and high dependency unit had patterns of sensitivity similar to the general ward. The differences in overall antifungal sensitivity patterns between the different wards were not statistically significant ($p = 0.0500$). The urine and urinary catheter tips specimen (n = 175) had the most yeast isolates when compared to the other specimens, with 30% sensitive, 28% resistant, and 3% intermediate, (Figure 4D). Antifungal sensitivity patterns in various specimens were statistically significant ($p = 0.0005$). When compared to the other years, 2021 had the most yeast isolates (n = 166), with 25% sensitive, 30% resistant, and 4% intermediate, (Figure 4E). Antifungal sensitivity patterns between the different years were statistically insignificant ($p = 0.9220$). Both sensitive and resistant isolates increased over time, from 6% in 2018 to 30% in 2021 and 5% in 2018 to 30% in 2021, respectively.

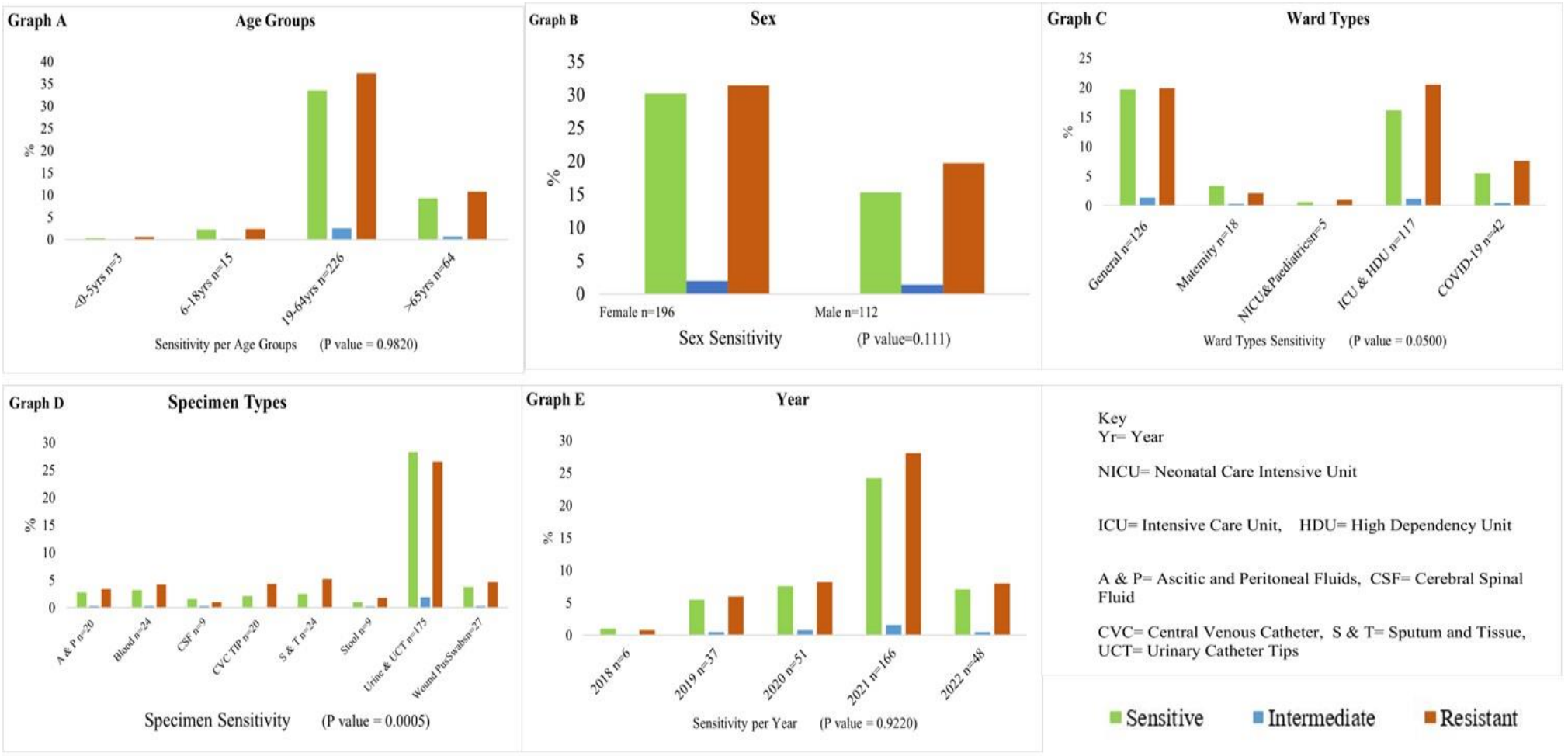

Figure 4: Distribution of Antifungal Sensitivity Pattern as per the Age Group, Sex, Ward, Specimen Type, and Year

4.3.3 Distribution of Multiple Drug Resistance in Yeast Isolates

Multiple drug resistance (MDR) was linked to the yeast species, sex, age group, specimen type, and ward type. Each yeast isolate had a significant amount of multiple drug resistant with all isolates of *C. guilliermondii*, *C. lusitaniae*, and *C. krusei* species had the highest multiple drug resistant proportion with each presenting with resistance to more than four number of drugs out of the five reviewed. Of the remaining yeast species, *C. albicans* had the highest multiple drug resistant rate at 16% most of the multiple drug resistant yeast isolates 9% (n = 27) were resistant to more than 4 drugs within that species.

As compared to males, females (n = 90) had the highest multiple drug resistant at 29% most of the multiple drug resistant yeast isolates 16% (n = 50) were resistant to more than four drugs within that sex. Age group 19–64 years (n = 111) had the highest multiple drug resistant at 36% when compared to other age groups with most of the multiple drug resistant yeast isolates 17% (n = 53) were resistant to more than four drugs within that age group. Comparing different specimen types, urine and urinary catheter tip specimens (n = 72) had the highest multiple drug resistant at 23% with most of the multiple drug resistant yeast isolates 11% (n = 35) were resistant to more than four drugs within that specimen. The differences in multiple drug resistant proportion between the specimen types were statistically significant (p = 0.020). Relative to other ward types, the intensive care unit and high dependency unit (n = 57) had the highest multiple drug resistant at 19% most of the multiple drug resistant yeast isolates 10% (n = 30) were resistant to more than four drugs within that ward. Relative to other years, the year 2021 (n = 79) had the highest multiple drug resistant at 26% most of the multiple drug resistant yeast isolates 12% (n = 37) were resistant to more than four drugs within that year. However, the differences between the number of drugs multiple drug resistant yeast isolate were resistant to was statistically significant in only the sexes and the yeast isolates types (p = 0.010; p = 0.0005) respectively as illustrated in table 5.

Table 5: Distribution of Multiple Drug Resistance in Yeast Isolates

Parameter		n (%)	NON-MDR	MDR	P Value (Non-MDR vs MDR)	No. of Drugs MDR Yeast Isolates were Resistant To			P Value of (Difference between No. of Drugs MDR Yeast Isolates were Resistant To)
						3	4	5	
Total		308(100%)	160(51.9%)	148(48.1%)		33(10.7%)	72(23.4%)	43(14.0%)	
Age Group (Years)	<0-5	3(1.0%)	1(0.3%)	2(0.6%)	0.8380	0(0.0%)	1(0.3%)	1(0.3%)	0.8320
	6-18	15(4.9%)	8(2.6%)	7(2.3%)		2(0.6%)	2(0.6%)	3(1.0%)	
	19-64	226(73.4%)	115(37.3%)	111(36.0%)		26(8.4%)	53(17.2%)	32(10.4%)	
	>65	64(20.8%)	36(11.7%)	28(9.1%)		5(1.6%)	16(5.2%)	7(2.3%)	
Sex	Female	196(63.6%)	106(34.4%)	90(29.2%)	0.4640	22(7.1%)	50(16.2%)	18(5.8%)	0.0100
	Male	112(36.4%)	54(17.5%)	58(18.8%)		11(3.6%)	22(7.1%)	25(8.1%)	
Yeast Isolated	*C. albicans*	116(37.7%)	66(21.4%)	50(16.2%)	0.0005	7(2.3%)	27(8.8%)	16(5.2%)	0.0005
	C. dubliniensis	73(23.7%)	54(17.5%)	19(6.2%)		13(4.2%)	5(1.6%)	1(0.3%)	
	C. glabrata	22(7.1%)	9(2.9%)	13(4.2%)		2(0.6%)	9(2.9%)	2(0.6%)	
	C. guilliermondii	6(1.9%)	0(0.0%)	6(1.9%)		2(0.6%)	3(1.0%)	1(0.3%)	
	C. lusitaniae	5(1.6%)	0(0.0%)	5(1.6%)		1(0.3%)	2(0.6%)	2(0.6%)	
	C. parapsilosis	12(3.9%)	9(2.9%)	3(1.0%)		0(0.0%)	0(0.0%)	3(1.0%)	
	C. tropicalis	29(9.4%)	8(2.6%)	21(6.8%)		5(1.6%)	15(4.9%)	1(0.3%)	
	C. krusei	9(2.9%)	0(0.0%)	9(2.9%)		1(0.3%)	3(1.0%)	5(1.6%)	
	C. laurentii	21(6.8%)	6(1.9%)	15(4.9%)		1(0.3%)	5(1.6%)	9(2.9%)	
	C. var. neoformans	9(2.9%)	6(1.9%)	3(1.0%)		0(0.0%)	1(0.3%)	2(0.6%)	
	T. beigelii	6(1.9%)	2(0.6%)	4(1.3%)		1(0.3%)	2(0.6%)	1(0.3%)	

Specimen Type	Ascitic and Peritoneal Fluids	20(6.5%)	9(2.9%)	11(3.6%)	0.0200	4(1.3%)	3(1.0%)	4(1.3%)	0.5330
	Blood	24(7.8%)	12(3.9%)	12(3.9%)		4(1.3%)	7(2.3%)	1(0.3%)	
	CSF	9(2.9%)	7(2.3%)	2(0.6%)		0(0.0%)	0(0.0%)	2(0.6%)	
	CVC Tips	20(6.5%)	5(1.6%)	15(4.9%)		4(1.3%)	7(2.3%)	4(1.3%)	
	Sputum and Tissue	24(7.8%)	7(2.3%)	17(5.5%)		4(1.3%)	8(2.6%)	5(1.6%)	
	Stool	9(2.9%)	4(1.3%)	5(1.6%)		0(0.0%)	3(1.0%)	2(0.6%)	
	Urine and Urinary Catheter Tips	175(56.8%)	103(33.4%)	72(23.3%)		15(4.9%)	35(11.4%)	22(7.1%)	
	Wound Pus Swabs	27(8.8%)	13(4.2%)	14(4.5%)		2(0.6%)	9(2.9%)	3(1.0%)	
Ward Type	COVID-19	42(13.6%)	17(5.5%)	25(8.1%)	0.5440	5(1.6%)	12(3.9%)	8(2.6%)	0.0640
	General	126(40.9%)	70(22.7%)	56(18.2%)		9(2.9%)	28(9.1%)	19(6.2%)	
	ICU and HDU	117(38.0%)	60(19.5%)	57(18.5%)		14(4.5%)	30(9.7%)	13(4.2%)	
	Maternity	18(5.8%)	11(3.6%)	7(2.3%)		5(1.6%)	1(0.3%)	1(0.3%)	
	NICU and Pediatric	5(1.6%)	2(0.6%)	3(1.0%)		0(0.0%)	1(0.3%)	2(0.6%)	
Year	2018	6(1.9%)	5(1.6%)	1(0.3%)	0.5960	0(0.0%)	1(0.3%)	0(0.0%)	0.4680
	2019	37(12.0%)	20(6.5%)	17(5.5%)		3(1.0%)	8(2.6%)	6(1.9%)	
	2020	51(16.6%)	25(8.1%)	26(8.4%)		8(2.6%)	13(4.2%)	5(1.6%)	
	2021	166(53.9%)	87(28.2%)	79(25.6%)		14(4.5%)	37(12.0%)	28(9.1%)	
	2022	48(15.6%)	23(7.5%)	25(8.1%)		8(2.6%)	13(4.2%)	4(1.3%)	

MDR = Multidrug Resistance, ICU = Intensive Care Unit, NICU = Neonatal Intensive Care Unit, HDU = High Dependency Unit, CSF = Cerebral Spinal Fluid, CVC = Central Venous Catheter, No = Number

4.3.4 Summary of Antifungal Sensitivity Pattern of Yeast Isolates

The current study findings revealed that *Candida albicans* was the most susceptible and the most resistant to the five antifungal medications reviewed. We found that *C. krusei* had the highest resistance to all the five antifungal drugs, (87%), while *C. dubliniensis* and *C. parapsilosis,* had the lowest levels of resistance to the five antifungal drug panels, 34% and 39%, respectively. *C. parapsilosis* and *C. dubliniensis,* on the other hand, had the least proportions of multiple drug resistant at 26% and 28%, respectively. Moreover, Flucytosine showed high potency as antifungal drugs against most yeast of all the three genus *Candida*, *Cryptococcus* and *Trichosporon* whereas Amphotericin B and the triazole (Itraconazole, Fluconazole and Voriconazole) were proved to be less effective drugs in the species of *C. lusitaniae*, *C. krusei, C. guilliermondii,* and *C. tropicalis* and *T. beigelii* which had the highest multiple drug resistance. In addition, most of the multiple drug resistant yeast isolates were resistant to more than four drugs out of the five reviewed.

4.4 Risk Factors for Yeast Infections among Inpatients

Compared to the general ward, patients in the COVID-19 and neonatal intensive care unit and pediatric ward and were about 0.3 times less likely to have yeast isolates (aOR 0.3 (95% CI 0.14–0.69)). Patients in intensive care unit and high dependency unit were about 135 times more likely to have yeast isolates than patients in the general ward (aOR 135.0 (95% CI 56.8–374.0)). Blood and urine and urinary catheter tips specimens, were about 0.1 times less likely to have yeast isolates compared to ascitic and peritoneal fluid specimens, (aOR 0.14 (95% CI 0.04–0.47)). Prior surgery patients were more likely to have a yeast infection than those who had no prior surgery (cOR 1.54 (95% CI 1.13-2.07)). However, after controlling for the covariates and confounders in the multivariable analysis, there was no significant relationship between prior surgery and yeast infection risk (P = 0.670). To control for potential confounders, before performing multiple logistic regression on potential risk factors that predispose inpatients to yeast infections, inpatients with numerous comorbidities had only the major reason for admission recorded as the risk for yeast infection. Though the research intended to assess if each inpatient's comorbidity was a risk factor for yeast infection, the numbers for each comorbidity were too small to allow for individual analysis. Instead, the comorbidities were combined and analyzed collectively to assess whether an absence or presence of a comorbidity was a risk factor for yeast infection. As demonstrated in table 6, inpatients with any recorded comorbidity were 0.8 times less likely to have yeast isolates recovered than inpatients without any recorded comorbidity (aOR 0.8 (95% CI 0.76-0.83)).

Table 6: Factors Associated with Yeast infection among Inpatients

	Univariable Analysis			Multivariable analysis		
Characteristic	**cOR**	**(95% CI)**	**P-value**	**aOR**	**(95% CI)**	**P-value**
Ward Type						
General		—		—		
COVID-19	3.05	(2.04-4.50)	0.001	0.32	(0.14-0.69)	0.004
ICU and HDU	5.41	(4.04-7.26)	0.001	135.00	(56.8-374.00)	0.001
Maternity	2.62	(1.46-4.47)	0.001	1.79	(0.68-4.63)	0.230
NICU and Paediatric	0.29	(0.10-0.65)	0.008	0.01	(0.00-0.06)	0.001
Specimen Type						
Ascitic and Peritoneal		—		—		
Fluids						
Blood	0.03	(0.01-0.07)	0.001	0.01	(0.00-0.06)	0.001
CSF	0.26	(0.08-0.78)	0.019	3.84	(0.59-23.60)	0.150
CVC tip	0.07	(0.03-0.18)	0.001	0.28	(0.06-1.29)	0.110
Sputum and Tissue	0.41	(0.16-1.03)	0.064	3.35	(0.60-18.50)	0.170
Stool	0.16	(0.05-0.45)	0.001	1.63	(0.25-9.90)	0.600
Urine and Urinary Catheter	0.09	(0.04-0.19)	0.001	0.14	(0.04-0.47)	0.001
Wound Pus Swab	0.17	(0.07-0.39)	0.001	0.43	(0.11-1.70)	0.230
Surgeries						
No Surgical Prior		—		—		
Prior Surgical	1.54	(1.13-2.07)	0.005	1.17	(0.57-2.41)	0.670
Associated Comorbidities						
Absence of Comorbidity		—		—		
Presence of Comorbidity	0.23	(0.10-0.52)	0.007	0.80	(0.76-0.83)	0.002

cOR = crude Odds Ratio, aOR = adjusted Odds Ratio, CI = Confidence Interval, CVC= Central Venous Catheter, CSF = Cerebral Spinal Fluid, NICU = Neonatal Intensive Care Unit, ICU = Intensive Care Unit, HDU = High Dependency Unit

Chapter Five: Discussion

5.0 Empirical Review

The specific objectives of this study were to describe the proportion and distribution of yeast isolates recovered from patients at Nairobi South Hospital (NSH), in Nairobi, Kenya, describe the isolates' antifungal drug sensitivity pattern, and assess the risk factors associated with yeast infection.

5.1 Distribution of Yeast Isolates

The proportion of yeast from our study was 15% out of the 2006 microbial culture and sensitivity carried out at Nairobi South Hospital within the study period. The majority of yeast isolates were found in females. This was consistent with many other research that found females have greater health seeking behavior than males and are more vulnerable to inflammation-related conditions such as yeast infection due to frequent hormonal changes and anatomical structure (Borman *et al.,* 2013; Parkes-Ratanshi *et al.,* 2015). Also, our findings could be due to the fact that there were more females than males in our study (64% vs 36%), resulting in the current study findings to skew towards the female side. In the current study, over 50% of the yeasts were found on urine and urinary catheter tips, and half of them showed both susceptibility to and resistance to the five antifungal drugs. Our findings were comparable to those of a study conducted in Iran in year 2019 on two hundred and two patients with yeast infections, in which the majority of these yeast isolates (67%) were similarly isolated from urine specimens study (Taei *et al.,* 2019). This could be as a result of the natural colonization of the urogenital tract with *Candida* species hence an overgrowth can occur (Almirante *et al.,* 2006; Hu *et al.,* 2015; Mutua *et al.,* 2010). The year 2021 had the highest peak for yeast isolates in contrast to other years; this could be attributed to the COVID-19 pandemic, which resulted in an elevated hospitalization incidence. From 2018 to 2021, the number of cases of yeast

isolates grew fivefold, followed by a little reduction in 2022. This could be due to also the comorbidities of the inpatients since most of the isolates were recovered among patients with systemic infection in this current study meaning the patients were critically ill. This concurred with Richardson's findings that among the primary risk factors for the onset of yeast infections is immune suppression (Richardson, 2005). From 2018 to 2021, we found an increase in the number of sensitive and resistant yeast isolates to the five antifungal drugs reviewed in this study, followed by a decline after the COVID-19 pandemic; nevertheless, there was no notable change in antifungal sensitivity patterns in each individual year from 2018 to 2022. These findings are consistent with reports, which stated that the years 2020 to 2021 saw the largest global hospitalization of patients owing to the pandemic, because many patients had coinfections, one of which was yeast (Hughes *et al.,* 2020).

5.1.1 *Candida albicans* Distribution

The current study identified yeasts of *Candida* with fewer *C. albicans* (38%) than non- *albicans* of *Candida* species (51%). This findings agree with many study reports that have stated *C. albicans* as the most predominant fungal isolates (Bongomin *et al.,* 2017; Guto *et al.,* 2016; Santos *et al.,* 2018). A number of studies have identified a higher prevalence *C. albicans* in premature and older patients this was different from findings of our current study where we observed *C. albicans* in all age groups except for inpatients under the age of five years. The difference could be as a result of the little sample size of patients below 5 years in the current study. The current study also identified twice as high prevalence of *C. albicans* in females than among males of which most of the *C. albicans* were isolated from urine and urinary catheter tips specimen. Other studies conducted at the Detroit Medical Center in the USA among females reported that *C. albicans* is the dominant species responsible for recurrent candidiasis among at least 70% females in all socioeconomic groups globally especially when in reproductive age (Sobel, 2016). These results are largely due to a number of risk factors,

including the beginning of menopause, hormonal replacement therapy, and increasing usage of contraceptives. *C. albicans* was also more in the intensive care unit and high dependency unit, and general wards. This is similar to other studies that stated that critically ill patients are more likely to be put on strong antibiotics that disrupt the normal flora causing infection by the opportunistic *C. albicans*. This findings agree with many study reports that have stated *C. albicans* as the most predominant fungal isolates (Bongomin *et al.,* 2017; Guto *et al.,* 2016; Santos *et al.,* 2018).

5.1.2 Non – *albicans Candida* species Distribution

Cumulatively, non- *albicans* of *Candida* species at (51%) were the majority of the yeast isolated in the current study. Besides, *C. dubliniensis, C. glabrata, C. guilliermondii, C. lusitaniae, C. parapsilosis, C. tropicalis*, and C. *krusei* were the non – *albicans* of *Candida* species isolated in the current study. Our findings differed to those of a study done from January 2016 to June 2016 among 384 patients at Mombasa Hospital in Kenya by Subira, (2018), who reported *C. albicans* as the most predominant yeast at 21% while the non -*albicans Candida* were very few at 2% overall proportion with yeast of *C. tropicalis* as the only non -*albicans Candida* species isolates (Subira, 2018). Our findings were comparable to those of a study conducted in Iran in year 2019 on two hundred and two patients with yeast infections, in which non - *albicans* of *Candida* species (60%) were more than *C. albicans* species (38%) (Taei *et al.,* 2019). *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. guilliermondii*, and *C. krusei*, were also the yeasts that caused the majority of non - *albicans* of *Candida* species infections in the study (Castanheira *et al.,* 2016; Taei *et al.,* 2019). *C. dubliniensis* was the most common, and it was reported across each ward.

However, the current study only found, *C. dubliniensis*, *C. guilliermondii*, and *C. tropicalis* yeast isolates among inpatients under the age of five and no record of *C. parapsilosis*. This finding differed from several studies that have reported *C. parapsilosis* as the most predominant

non-*albicans* of *Candida* species in neonatal intensive care units and pediatric wards (Celebi *et al.,* 2012; Hernández-Castro *et al.,* 2010; Lupetti *et al.,* 2002; Richardson, 2005). Further, other inpatients study records from a large tertiary-care hospital in Spain from 1988 to 2000 have reported *C. parapsilosis* as the most common yeast species causing infections in NICUs and pediatrics wards who have both candidemia and congenital cardiac disease as predisposing risk factors (García San Miguel *et al.,* 2004). However, our current study found only five yeast isolates, with *C. dubliniensis* being the most common species in this ward types. The difference could be due to the current study's small sample size, which makes it incomparable to the large tertiary-care hospital, which included more than 500 children in the study (García San Miguel *et al.,* 2004). Also, the time difference could have resulted in a shift in the epidemiological pattern of yeast from 1988 to date (Richardson, 2005).

5.1.3 Other Yeasts Distribution

The current study also identified yeasts of *Cryptococcus* with a higher prevalence of *C. laurentii* than *C. var. neoformans* species. Non-neoformans species of *Cryptococcus* were previously thought as non-pathogenic to humans, but there are increasing reports of their potential to cause opportunistic infections, particularly in immunocompromised patients, including HIV, malignancy, transplant patients, and in patients with endovascular catheters (Smith *et al.,* 2017). A number of studies have identified a higher prevalence *C. var. neoformans* than *C. laurentii* in HIV-positive patients (Iyer *et al.,* 2021; Lin *et al.,* 2015; Arendrup *et al.,* 2014; Pfavayi *et al.,* 2021).

In the current study, yeasts from *Trichosporon* species were identified in blood, central venous catheter (CVC) tips, sputum and tissue, ascitic and peritoneal fluids, urine, and urinary catheter tips among adult female inpatients in the intensive care unit and high dependency unit, and general ward. The yeasts of *Trichosporon* species were previously known for only causing superficial infections to humans, especially on the scalp and hair (González & Montoya, 2014).

However, the species has evolved to now cause widespread endogenous yeast infections among immunocompromised patients after *Candida* species (Chagas-Neto *et al.,* 2008; Córdoba *et al.,* 2011; Oberoi *et al.,* 2012). These findings are similar to Ruan study where they established that the presence of central venous catheters tips was a risk factor for yeast infection, especially trichosporonosis, after conducting a study on 19 patients in Taiwan (Ruan *et al.,* 2009). The current study obtained *Trichosporon* species among patients with systemic infection and diabetes mellitus comorbidities. We also found one case of fungemia as a result of the *Trichosporon* species. Other studies by Kontoyiannis *et al.,* (2004) on some of the risk factors related to yeast infection caused by *Trichosporon* species among 17 cancer patients reported that 41% of the ten patients with fungal infection was caused by yeast of *Trichosporon* species especially patients with neutropenia, hematological malignancies, catheterization, immunosuppressant and catheterization (Kontoyiannis *et al.,* 2004). Girmenia *et al.,* (2005) also performed a retrospective study data of 287 cases reported of globally to have yeast infection caused by *Trichosporon* species where they reported similar findings as Kontoyannis et al. (2004) that more than half of the patients with trichosporonosis had either hematologic malignancy, transplantation, dialysis, leukemia and retroviral disease prior to the diagnosis (Girmenia *et al.,* 2005).

5.2 Antifungal Sensitivity of Yeast Isolates

Antifungal susceptibility testing is necessary for both patient treatment and antifungal effectiveness monitoring. Testing for antifungal susceptibility is fast advancing in Africa, particularly Kenya. The M27 and M60 testing methods for determining minimum inhibitory concentration breakpoints for isolates of *Candida*, *Trichosporon*, and *Cryptococcus* are still being improved by the National Committee for Clinical Laboratory Standards (NCCLS), which yield accurate results (Espinel-Ingroff *et al.,* 2005; Ling *et al.,* 2003; Sadeghi *et al.,* 2018). Multiple research studies reported a rise in the number of resistant yeast species (Aldardeer *et*

al., 2020). Amphotericin B, Flucytosine, Fluconazole, Itraconazole, and Voriconazole were the five medications that were examined in this current study, and each yeast isolate revealed a unique sensitivity pattern to each one of them.

5.2.1 Amphotericin B

The current study identified Amphotericin B, a polyene demonstrated the highest proportion of resistance in ninety percent of all the yeast isolates species it was tested against with *C. albicans* as the species that showed the high rates of resistance to the drug. Studies done in South Africa and India reported resistance rate of 4-10% to Amphotericin B (Chellan *et al.,* 2010; Mnge *et al.,* 2017). Our findings contrast with those done elsewhere in the world that reported up to 100% susceptibility rate of *Candida* species to Amphotericin B (Musyoki *et al.,* 2022). Mini-reviews of previous research on 44 published case reports of Amphotericin B resistance among immunocompromised patients were conducted in Baltimore, USA, without including a precise minimum inhibitory concentration breakpoint for Amphotericin B in the inclusion criteria (Sterlingt & Merz, 1998). The study also found an increase in yeast species with clinically significant Amphotericin B resistance, including *Candida albicans*; however, this increase was primarily observed in non-*albicans Candida* species such as *C. lusitaniae*, *C. guilliermondii*, *C. glabrata*, and *C. tropicalis* (Sterling *et al.,* 1998). Other studies have also reported yeast isolates of *Trichosporon* species resistant to Amphotericin B (González & Montoya, 2014). The high level of Amphotericin B resistance in the current research could potentially be due to the routine utilization of the drug. Treatment failure is thought to be linked with this resistance, which appears rarely and gradually in yeast isolates from patients receiving Amphotericin B (Ellis, 2002). It has also been suggested that biofilm glucan sequestration as a multidrug resistance mechanism to Amphotericin B could be a reason for the resistance seen in *Candida* species isolates (Spampinato & Leonardi, 2013).

5.2.2 Flucytosine

The current study identified Flucytosine as having the highest sensitivity in this study, with more than seventy percent of all the yeast isolates sensitive to the drug. The current study also found some yeast isolates, such as *C. lusitaniae* and *C. var. neoformans,* displaying resistance to the drug. This was consistent with other study findings that reported an almost ninety percent proportion of yeast susceptibility to the drug, much as a few *Candida* and *Cryptococcus* strains demonstrated in-vitro resistance and intermediate resistance to the drug (Arikan, 2007; Bhattacharya *et al.,* 2020; Cuenca-Estrella *et al.,* 2001). Flucytosine is an adjunct drug with other antifungal agents for the treatment of fungal infections, and therefore almost all *Candida* species exhibit susceptibility activity to it (Musyoki *et al.,* 2022). The observed low resistance to Flucytosine in this study may be attributed to the synergistic combination of the drug with other antifungal agents for clinical use (Musyoki *et al.,* 2022). Hence, the drug's selective pressure has developed since it's rarely prescribed alone (Castelo-Branco *et al.,* 2022; Giacobbe *et al.,* 2021). Resistance recorded by yeast such as *C. lusitaniae* and *C. var. neoformans* may be linked to mutations of cytosine permease, cytosine deaminase, and uracil phosphoribosyl transferase genes associated with the active transportation of the drug into the fungal cell and the enzymatic conversion of the drug into 5-fluorouridine monophosphate or 5-fluorouracil (Arendrup & Patterson, 2017).

5.2.3 Azoles

The current study identified Itraconazole as the azole with the highest proportion of resistance in seventy percent of all the yeast isolates species it was tested against when compared to the azoles reviewed. The current study also found more than half of all the yeast isolates were resistant to Fluconazole, Itraconazole and Voriconazole. In addition, Yeast isolates of *C. albicans* species were the most resistant to the azoles when compared to other non – *albicans*

of *Candida* species in the current study. These findings contradicted previous research that found a higher rate of azole drug resistance in non-*albicans* of *Candida* species than in *C. albicans* (Mukherjee & Wang, 2009; Perlin *et al.,* 2015). Resistance to Fluconazole may be due to long - term therapy and repeated treatment. Resistance to azole antifungals is rising, posing a serious challenge in the treatment of *Candida* species infections in Kenya and maybe across developing countries. Studies on molecular resistance mechanisms have attributed the resistance to changes in the gene encoding, the ERG11 target enzyme or overexpression of efflux pump genes (CDR1, CDR2, and MDR1). Other studies observed cross -resistance with the antifungal (Fluconazole, clotrimazole, Itraconazole, and ketoconazole) (Castanheira *et al.,* 2016). According to Partha *et al.,* (2022), the increasing cases of resistances of *Candida* species to azole antifungal causes this group not to be used as empirical therapy for invasive candidiasis in non-neutropenic patients instead echinocandins antifungals are more widely used in patients with severe clinical conditions (Systemic *Candida* infection may be suspected in patients with neutropenia who remain febrile after broad-spectrum antibiotic therapy (Partha *et al.,* 2022). The antifungal caspofungin, Amphotericin B, or intravenous Voriconazole are recommended by the Infectious Disease Society of America (IDSA) as empiric therapy, though Fluconazole or Itraconazole may be used (McCarty & Pappas, 2016). Fluconazole is recommended as the first choice for treating *Candida* species infections since is a broad spectrum, high efficiency, and good bioavailability and safety profile (Partha *et al.,* 2022). The bioavailability of Fluconazole and Voriconazole range from 90%, while Itraconazole reported to have the lowest.

Among the antifungal's azole, Fluconazole is the only one eliminated via the kidney, while the rest are eliminated via the liver. Based on their ability to penetrate the CSF, the order of the best is Fluconazole, Itraconazole, and Voriconazole. In patients with resistance to Fluconazole, Voriconazole, and Itraconazole can be used as alternative therapy (Houšť *et al.,* 2020). Fluconazole is good for treating infections caused by *Candida* species, while Itraconazole and

Voriconazole have antifungal activity against *Candida* species and *Aspergillus* species (Pappas *et al.,* 2016; Partha *et al.,* 2022). The availability of Fluconazole and Itraconazole make them categorised as first-generation triazoles in Kenya. The increasing resistance to Fluconazole, necessitates an evaluation into the antifungal activity of azoles against *Candida albicans* (Murtiastutik *et al.,* 2020). Conducting a Time-kill assay (TKA) is one way of evaluating antifungal activity to get information on the speed and antifungal activity, as well as characteristics of pharmacodynamics (i.e., the relationship between drug concentration and its effect on fungal colony growth). The TKA method counts the number of fungal isolates that grow at certain intervals after exposure to antifungals with specific concentrations (Partha *et al.,* 2022).

5.2.4 Multiple Drug Resistance

About half of yeast isolates in this study were multiple drug resistant, with *C. krusei*, *C. guilliermondii*, and *C. lusitaniae* having total multiple drug resistant proportions within each species. There is not much reported about multiple drug resistant findings for *Candida albicans* species however, resistance to echinocandin-class drugs, first reported in 2005 (Park *et al.,* 2005) was relatively low, at <3% with *C. albicans* and most *Candida* species (Pfaller *et al.,* 2010). Generally, *C. albicans* are reported to have more multiple drug resistant strains within the species compared to other *Candida* species. A survey of *C. albicans* and *C. glabrata* bloodstream isolates in Switzerland pointed out that echinocandin resistance remained at a low level despite a significant rise in echinocandin use which was associated mainly with individual pre-echinocandin exposure of prolonged duration (Kritikos *et al.,* 2018). SENTRY Antimicrobial Surveillance Program reported echinocandins resistance of 8.0% –9.3% for *C. glabrata* bloodstream isolates from 2006 to 2010 (Pfaller *et al.,* 2012). In

another study at Duke hospital for a period of 10 years, echinocandin resistance increased from 2%–3% to >13% in 2009–2010 (Alexander *et al.,* 2013).

Antimicrobial resistance may be having a global based shift as evidenced by the spread in as a study of 1380 isolates of *C. glabrata* collected between 2008 and 2013 from 4 US cities (Atlanta, Georgia; Baltimore, Maryland; Knoxville, Tennessee; and Portland, Oregon) showed that 3.1%, 3.3%, and 3.6% of the isolates were resistant to anidulafungin (Pham *et al.,* 2014). Importantly, echinocandin resistance *Candida* species is often associated with cross-resistance to azole antifungals yielding multidrug-resistant strains. In yet another study, 36% of echinocandin-resistant isolates were also resistant to Fluconazole (Pham *et al.,* 2014). In many healthcare centers, the widespread use of echinocandin and azole prophylaxis has prompted an epidemiologic shift, with some *Candida* species presenting as the dominant fungal pathogen of bloodstream (Lortholary *et al.,* 2011; Perlin, 2015). Most *Candida* species that presents as multidrug-resistance, leave patients with extremely few options for the treatment of such infection (Healey and Perlin, 2018). Most multiple drug resistant *Candida* infections involve isolates belonging to species with intrinsic resistance, for example, echinocandin resistance in *C. glabrata* and *C. krusei* (Forastiero *et al.,* 2015) or infections with *C. guilliermondii* or *C. auris,* which is intrinsically multidrug resistant and currently emerging in other countries (Lockhart *et al.,* 2017).

Multidrug resistance in species that possess no intrinsic resistance is rare, as in general it requires acquisition of several resistance mechanisms and these often come at a fitness cost (Ben-Ami & Kontoyiannis, 2012; Vincent *et al.,* 2013).However, *ERG3* and *ERG2* alterations have individually been associated with azole and Amphotericin B cross-resistance in *C. albicans* and *C. dubliniensis* (Arendrup & Patterson, 2017). A stepwise development of azole, echinocandin, and amphotericin B resistance was observed in *C. albicans* from a patient with

mucosal infection over a 5-year period (Jensen *et al.*, 2015) and azole, flucytosine, and echinocandin resistance was acquired in *C. glabrata* due to acquisition of mutations in *FUR1* (*CgFUR1*) and *CgFKS2* and overexpression of *CgCDR1* and *CgCDR2* during 20 weeks of antifungal therapy in a hematopoietic stem cell transplant recipient (Arendrup & Patterson, 2017).

5.3 Risk Factors for Yeast Infections among Inpatients

Current investigation revealed that every inpatient with comorbidity recorded had an occurrence of yeast isolate recovery from microbial culture. This was in line with numerous study reports that identify immune suppression as a key element in the emergence of the majority of yeast infections (Miceli *et al.,* 2011). The incidence of yeast infections at Nairobi South Hospital is on the increase based on the current study findings specifically in inpatients with autoimmune, retroviral disease, liver disease, heart disease, septic shock, diabetes mellitus, cancer, hematological malignancies, lung diseases, or other illnesses and comorbidities.

The current study identified, patients in intensive care unit and high dependency unit were more likely to have yeast isolates than patients in the general ward. This was comparable to other studies done in 2020, amongst intensive care unit and high dependency unit patients at Nairobi Hospital, Kenya that reported a remarkable incidence of yeast infection, specifically candidemia, as a result of the patients' severe medical conditions (Solomon, 2021). The current study also found, the type of specimen and history of prior surgery was not a statistically significant risk factor for yeast infection ($p = 0.670$). This was similar to study done from January 2016 to June 2016 among 384 patients at Mombasa Hospital in Kenya by Subira, (2018), who reported that surgery had no effect on the risk of yeast infection (Subira, 2018).

However, the current research was not able to assess if each patient's condition was a risk factor for yeast infection due to too small numbers for each condition to allow for analysis. Nevertheless, after merging all the inpatients conditions into one, the current study revealed inpatients with any recorded comorbidity were less likely to have yeast isolates recovered than patients without any recorded condition. This was inconsistent with the findings of other investigations, which demonstrated that a weakened immune system, presence of comorbidities and chronic conditions was directly associated with the development of fungal infections (Rao *et al.,* 2016; Richardson, 2005; Wey *et al.,* 1989). The discrepancies in our current study findings may be due to the numbers for each comorbidity were too small to allow for analysis resulting in large confidence intervals; limitation for a retrospective study design (Chen *et al.,* 2005).

This study also demonstrated that patients in the COVID-19 wards, intensive care units (ICU), high dependency unit (HDU) and neonatal intensive care unit (NICU) were more at risk of developing yeast infection compared to patients in other wards. These findings correspond to the statement by Wudhikarn that patients in the intensive care unit (ICU) are extremely vulnerable to yeast infections, because their immune systems are compromised (Eggimann *et al.,* 2011; Wudhikarn *et al.,* 2020). Numerous risk factors, such as prior antibiotic exposure, the presence of indwelling central venous catheters (CVC), and endotracheal intubation, predispose patients to yeast infections (Gonzalez-Duarte *et al.,* 2015). This causes traumatic inoculation of yeast into the bloodstream via skin or mucous membrane breaks or wounds (Gonzalez-Duarte *et al.,* 2015).

In addition, this current study did not show any significant correlation between prior surgery and yeast infection risk after controlling for the covariates and confounders in the multivariable analysis, (P = 0.670). These findings differ with Richardson study findings that revealed a strong correlation between yeast infection and prior surgery (Richardson, 2005), due to a

variety of factors, including gastrointestinal surgery and the impact of excised parts on intestinal flora (Nagao *et al.,* 2014). However, to differentiate between risk factors and comorbidities, assessing a condition's association with a disease requires either a cohort study or a case-control study, neither of which was used in the current research's study design *(Fu et al.,* 2016).

5.4 Study Limitations

The current study, which was conducted retrospectively, was not unable to determine whether yeast isolates from non-sterile samples such as urine and urinary catheter tips, stool, wound pus swabs, and central venous catheter tips indicated a true infection or colonization. Our findings are however still important as these yeasts pose the risk of nosocomial infections. Besides, this research reviewed previously documented data on patients' preexisting conditions, which were reported at the time of admission. As a result, the study could not determine if the recovery of yeast isolates in the patient's specimen was a direct result of their conditions, if they acquired the yeasts in the hospital, or if the patient already had the yeasts at the time of admission. Also, the number of individual inpatients with pre-existing conditions were too small to carry outs multivariate regression analysis, therefore they had to be consolidated into presence or absence of a comorbidity before analysis.

Using the yeast isolate data provided by the current study's VITEK model 96-II, has low discrimination power for some yeast species. Hence, all the yeast isolates of the *Trichosporon* genus could only be categorized as *T. beigelii*. Based on the current taxonomical classification, the VITEK model database was unable to determine which specific species of the *Trichosporon* genus affected our inpatients. Some *Candida* species, such as *Candida krusei*, have shown inherent azole resistance, particularly to fluconazole. However, due to the current study's retrospective design, and the VITEK-2 model used the antifungal drug sensitivity data used for

this study did not distinguish whether these *Candida* species' resistance was intrinsic or acquired.

Although the Kenya Essential Medicines List 2019 includes other approved antifungals besides amphotericin B, itraconazole, fluconazole, flucytosine, and voriconazole, such as clotrimazole, miconazole and griseofulvin, for reproducibility, the study restricted its choice to data from the five antifungals on the test cards for the AST pattern. In addition, they were the only antifungals loaded on the VITEK DL 96 II cards. As a result, we were not able to identify the antifungal sensitivity of each antifungal available in the Kenyan market used for patient care. Lastly, considering this study was limited to a single hospital in Nairobi, Kenya, the findings may not be generalizable to other parts of the country because the proportion of yeast infections varies due to environmental and socioeconomic variables.

Chapter Six: Conclusion and Recommendations

6.1 Conclusion

The research described in this dissertation retrospectively examined the species distribution of yeast isolates from a private hospital in Nairobi, Kenya over a four-year period. Based on the findings of this study, we made a number of conclusions: Non - *albicans* of *Candida* were the most common species than *Candida albicans* and other less commonly isolated yeasts of *Cryptococcus* and *Trichosporon* species. Also, the susceptibility patterns of yeast isolates to antifungal drugs varies. In this study, flucytosine showed the highest sensitivity whereas amphotericin B and the azoles were less sensitive. Besides, all the yeast isolates showed some level of multi-drug resistance with the highest observed in *Candida lusitaniae*, *Candida krusei, Candida guilliermondii,* and *Candida tropicalis*. Lastly, the presence of comorbidities in patients is associated with an increased risk of yeast infection with patients in intensive care unit and high dependency unit were more likely to have yeast isolates than patients in the general ward.

6.2 Recommendations

Given the findings of this study we drew a number of recommendations:

I. The growing proportion of yeast infections, particularly those caused by *Candida* species, among inpatients poses a significant risk to public health. As a result, efforts to control infection and transmission should be implemented the public health officers, health practitioners, epidemiologists and any other stakeholders in order to cut down on the associated morbidity and mortality of inpatients.

II. The rising rate of antifungal drug resistance is an indication of an upsurge in yeast infections, as evidenced by a large proportion of yeast isolates resistant to several

antifungal drugs in the three *Candida*, *Cryptococcus*, and *Trichosporon* genus. Hence, it is critical that healthcare providers' choice of antifungal treatment is guided by local fungal antibiograms in this era of evidence-based medicine.

III. Owing to the increased multiple antifungal drug resistance patterns, monitoring of antifungal drug susceptibilities by health practitioners, policy makers and any other stakeholders is required for an optimal antifungal treatment regimen. This is crucial given the small number of available antifungals approved by the Kenya Essential Medicines List 2019 due to their high human toxicity.

IV. In this study, a broad review of the comorbidities as a whole found no link between the conditions of the inpatients and the risk of yeast infections. This was particularly limited by our study design. Nevertheless, there is a need to investigate locally for conditions that are risk factors for yeast infections for better patient management or to do longitudinal studies with more specific target population.

References

Adam, R. D., Revathi, G., Okinda, N., Fontaine, M., Shah, J., Kagotho, E., Castanheira, M., Pfaller, M. A., & Maina, D. (2019). Analysis of Candida auris fungemia at a single facility in Kenya. *International Journal of Infectious Diseases*, *85*, 182–187. https://doi.org/10.1016/j.ijid.2019.06.001

Aldardeer, N. F., Albar, H., Al-Attas, M., Eldali, A., Qutub, M., Hassanien, A., & Alraddadi, B. (2020). Antifungal resistance in patients with Candidaemia: A retrospective cohort study. *BMC Infectious Diseases*, *20*(1), 1–7. https://doi.org/10.1186/s12879-019-4710-z

Alexander, B. D., Johnson, M. D., Pfeiffer, C. D., Jiménez-Ortigosa, C., Catania, J., Booker, R., Castanheira, M., Messer, S. A., Perlin, D. S., & Pfaller, M. A. (2013). Increasing echinocandin resistance in Candida glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *56*(12), 1724–1732. https://doi.org/10.1093/cid/cit136

Almirante, B., Rodríguez, D., Cuenca-Estrella, M., Almela, M., Sanchez, F., Ayats, J., Alonso-Tarres, C., Rodriguez-Tudela, J. L., & Pahissa, A. (2006). Epidemiology, risk factors, and prognosis of Candida parapsilosis bloodstream infections: Case-control population-based surveillance study of patients in Barcelona, Spain, from 2002 to 2003. *Journal of Clinical Microbiology*, *44*(5), 1681–1685. https://doi.org/10.1128/JCM.44.5.1681-1685.2006

Arendrup, M. C., Boekhout, T., Akova, M., Meis, J. F., Cornely, O. A., Lortholary, O., Arikan-Akdagli, S., Cuenca-Estrella, M., Dannaoui, E., van Diepeningen, A. D., Groll, A. H., Guarro, J., Guinea, J., Hope, W., Lackner, M., Lass-Flörl, C., Lagrou, K., Lanternier, F., Meletiadis, J., … Ullmann, A. J. (2014). ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. *Clinical Microbiology and Infection*, *20*(S3), 76–98. https://doi.org/10.1111/1469-0691.12360

Arendrup, M. C., & Patterson, T. F. (2017). Multidrug-Resistant Candida: Epidemiology, Molecular Mechanisms, and Treatment. *The Journal of Infectious Diseases*, *216*(suppl_3), S445–S451. https://doi.org/10.1093/infdis/jix131

Arikan, S. (2007). Current status of antifungal susceptibility testing methods. *Medical Mycology*, *45*(7), 569–587. https://doi.org/10.1080/13693780701436794

Ben-Ami, R., & Kontoyiannis, D. P. (2012). Resistance to echinocandins comes at a cost: the impact of FKS1 hotspot mutations on Candida albicans fitness and virulence. *Virulence*, *3*(1), 95–97. https://doi.org/10.4161/viru.3.1.18886

Bhattacharya, S., Sae-Tia, S., & Fries, B. C. (2020). Candidiasis and mechanisms of antifungal resistance. *Antibiotics*, *9*(6), 1–19. https://doi.org/10.3390/antibiotics9060312

Bongomin, F., Gago, S., Oladele, R. O., & Denning, D. W. (2017). Global and multi-national prevalence of fungal diseases—estimate precision. *Journal of Fungi*, *3*(4), 57. https://doi.org/10.3390/jof3040057

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

Castanheira, M., Messer, S. A., Rhomberg, P. R., & Pfaller, M. A. (2016). Antifungal susceptibility patterns of a global collection of fungal isolates: results of the SENTRY Antifungal Surveillance Program (2013). *Diagnostic Microbiology and Infectious Disease*, *85*(2), 200–204. https://doi.org/10.1016/j.diagmicrobio.2016.02.009

Castelo-Branco, D., Lockhart, S. R., Chen, Y.-C., Santos, D. A., Hagen, F., Hawkins, N. J., Lavergne, R.-A., Meis, J. F., Le Pape, P., Rocha, M. F. G., Sidrim, J. J. C., Arendrup, M., & Morio, F. (2022). Collateral consequences of agricultural fungicides on pathogenic yeasts: A One Health perspective to tackle azole resistance. *Mycoses*, *65*(3), 303–311. https://doi.org/10.1111/myc.13404

Celebi, S., Hacimustafaoglu, M., Koksal, N., Ozkan, H., Cetinkaya, M., & Ener, B. (2012). Neonatal candidiasis: Results of an 8 year study. *Pediatrics International*, *54*(3), 341–349. https://doi.org/10.1111/j.1442-200X.2012.03574.x

Chagas-Neto, T. C., Chaves, G. M., & Colombo, A. L. (2008). Update on the genus Trichosporon. *Mycopathologia*, *166*(3), 121–132. https://doi.org/10.1007/s11046-008-9136-x

Chellan, G., Shivaprakash, S., Karimassery Ramaiyar, S., Varma, A. K., Varma, N., Thekkeparambil Sukumaran, M., Rohinivilasam Vasukutty, J., Bal, A., & Kumar, H. (2010). Spectrum and prevalence of fungi infecting deep tissues of lower-limb wounds in patients with type 2 diabetes. *Journal of Clinical Microbiology*, *48*(6), 2097–2102. https://doi.org/10.1128/JCM.02035-09

Chen, Y.-Y., Chou, Y.-C., & Chou, P. (2005). Impact of Nosocomial Infection on Cost of Illness and Length of Stay in Intensive Care Units. *Infection Control & Hospital Epidemiology*, *26*(3), 281–287. https://doi.org/10.1086/502540

Córdoba, S., Vivot, W., Bosco-Borgeat, M. E., Taverna, C., Szusz, W., Murisengo, O., Isla, G., & Davel, G. (2011). Species distribution and susceptibility profile of yeasts isolated from blood cultures: results of a multicenter active laboratory-based surveillance study in Argentina. *Revista Argentina de Microbiologia*, *43*(3), 176–185. https://doi.org/10.1590/S0325-75412011000300003

Cuenca-Estrella, M., Díaz-Guerra, T. M., Mellado, E., & Rodríguez-Tudela, J. L. (2001). Flucytosine primary resistance in Candida species and Cryptococcus neoformans. *European Journal of Clinical Microbiology and Infectious Diseases*, *20*(4), 276–279. https://doi.org/10.1007/PL00011265

Eggimann, P., Bille, J., & Marchetti, O. (2011). Diagnosis of invasive candidiasis in the ICU. *Annals of Intensive Care*, *1*(1), 1–10. https://doi.org/10.1186/2110-5820-1-37

Espinel-Ingroff, A., Barchiesi, F., Cuenca-Estrella, M., Pfaller, M. A., Rinaldi, M., Rodriguez-Tudela, J. L., & Verweij, P. E. (2005). International and multicenter comparison of EUCAST and CLSI M27-A2 broth microdilution methods for testing susceptibilities of Candida spp. to fluconazole, itraconazole, posaconazole, and voriconazole. *Journal of Clinical Microbiology*, *43*(8), 3884–3889. https://doi.org/10.1128/JCM.43.8.3884-3889.2005

Forastiero, A., Garcia-Gil, V., Rivero-Menendez, O., Garcia-Rubio, R., Monteiro, M. C., Alastruey-Izquierdo, A., Jordan, R., Agorio, I., & Mellado, E. (2015). Rapid Development of Candida krusei Echinocandin Resistance during Caspofungin Therapy. *Antimicrobial Agents and Chemotherapy*, *59*(11), 6975–6982. https://doi.org/10.1128/AAC.01005-15

Fu, J., Wang, X., Wei, B., Jiang, Y., & Chen, J. (2016). Risk factors and clinical analysis of candidemia in very-low-birth-weight neonates. *American Journal of Infection Control*, *44*(11), 1321–1325. https://doi.org/10.1016/j.ajic.2016.03.026

García San Miguel, L., Pla, J., Cobo, J., Navarro, F., Sánchez-Sousa, A., Alvarez, M. E., Martos, I., & Moreno, S. (2004). Morphotypic and genotypic characterization of sequential Candida parapsilosis isolates from an outbreak in a pediatric intensive care unit. *Diagnostic Microbiology and Infectious Disease*, *49*(3), 189–196. https://doi.org/10.1016/j.diagmicrobio.2004.03.017

Giacobbe, D. R., Magnasco, L., Sepulcri, C., Mikulska, M., Koehler, P., Cornely, O. A., & Bassetti, M. (2021). Recent advances and future perspectives in the pharmacological treatment of Candida auris infections. *Expert Review of Clinical Pharmacology*, *14*(10), 1205–1220. https://doi.org/10.1080/17512433.2021.1949285

Girmenia, C., Pagano, L., Martino, B., D'Antonio, D., Fanci, R., Specchia, G., Melillo, L., Buelli, M., Pizzarelli, G., Venditti, M., & Martino, P. (2005). Invasive infections caused by Trichosporon species and Geotrichum capitatum in patients with hematological malignancies: a retrospective multicenter study from Italy and review of the literature. *Journal of Clinical Microbiology*, *43*(4), 1818–1828. https://doi.org/10.1128/JCM.43.4.1818-1828.2005

González, G. M., & Montoya, A. M. (2014). Trichosporon spp. : an emerging fungal pathogen. *Medicina Universitaria*, *16*(62), 17–43. https://www.elsevier.es/en-revista-medicina-universitaria-304-pdf-X1665579614283703

Guto, J. A., Bii, C. C., & Denning, D. W. (2016). Estimated burden of fungal infections in Kenya. *Journal of Infection in Developing Countries*, *10*(8), 777–784. https://doi.org/10.3855/jidc.7614

Hernández-Castro, R., Arroyo-Escalante, S., Carrillo-Casas, E. M., Moncada-Barrón, D., Álvarez-Verona, E., Hernández-Delgado, L., Torres-Narváez, P., & Lavalle-Villalobos, A. (2010). Outbreak of Candida parapsilosis in a neonatal intensive care unit: A health care workers source. *European Journal of Pediatrics*, *169*(7), 783–787. https://doi.org/10.1007/s00431-009-1109-7

Houšť, J., Spížek, J., & Havlíček, V. (2020). Antifungal Drugs. *Metabolites*, *10*(3). https://doi.org/10.3390/metabo10030106

Hu, Y., Yu, A., Chen, X., Wang, G., & Feng, X. (2015). Molecular Characterization of Candida africana in Genital Specimens in Shanghai, China. *BioMed Research International*, *2015*. https://doi.org/10.1155/2015/185387

Hughes, S., Troise, O., Donaldson, H., Mughal, N., & Moore, L. S. P. (2020). Bacterial and fungal coinfection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. *Clinical Microbiology and Infection*, *26*(10), 1395–1399. https://doi.org/10.1016/j.cmi.2020.06.025

Iyer, K. R., Revie, N. M., Fu, C., Robbins, N., & Cowen, L. E. (2021). Treatment strategies for cryptococcal infection: challenges, advances and future outlook. In *Nature Reviews Microbiology* (Vol. 19, Issue 7, pp. 454–466). Nature Research. https://doi.org/10.1038/s41579-021-00511-0

Jallow, S., & Govender, N. P. (2021). Ibrexafungerp: A first-in-class oral triterpenoid glucan synthase inhibitor. *Journal of Fungi*, *7*(3), 1–19. https://doi.org/10.3390/jof7030163

Jensen, R. H., Astvad, K. M. T., Silva, L. V., Sanglard, D., Jørgensen, R., Nielsen, K. F., Mathiasen, E. G., Doroudian, G., Perlin, D. S., & Arendrup, M. C. (2015). Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance in vivo in Candida albicans orchestrated by multiple genetic alterations. *The Journal of Antimicrobial Chemotherapy*, *70*(9), 2551–2555. https://doi.org/10.1093/jac/dkv140

KEML. (2019). *Kenya Essential Medicines List 2019*. http://publications.universalhealth2030.org/uploads/KEML-2016Final-1.pdf

Kontoyiannis, D. P., Torres, H. A., Chagua, M., Hachem, R., Tarrand, J. J., Bodey, G. P., & Raad, I. I. (2004). Trichosporonosis in a tertiary care cancer center: Risk factors, changing spectrum and determinants of outcome. *Scandinavian Journal of Infectious Diseases*, *36*(8), 564–569. https://doi.org/10.1080/00365540410017563

Kritikos, A., Neofytos, D., Khanna, N., Schreiber, P. W., Boggian, K., Bille, J., Schrenzel, J., Mühlethaler, K., Zbinden, R., Bruderer, T., Goldenberger, D., Pfyffer, G., Conen, A., Van Delden, C., Zimmerli, S., Sanglard, D., Bachmann, D., Marchetti, O., & Lamoth, F. (2018). Accuracy of Sensititre YeastOne echinocandins epidemiological cut-off values for identification of FKS mutant Candida albicans and Candida glabrata: a ten year national survey of the Fungal Infection Network of Switzerland (FUNGINOS). *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *24*(11), 1214.e1-1214.e4. https://doi.org/10.1016/j.cmi.2018.05.012

Lin, Y. Y., Shiau, S., & Fang, C. T. (2015). Risk factors for invasive Cryptococcus neoformans diseases: A case-control study. *PLoS ONE*, *10*(3), 3. https://doi.org/10.1371/journal.pone.0119090

Ling, T. K. W., Liu, Z. K., & Cheng, A. F. B. (2003). Evaluation of the VITEK 2 system for rapid direct identification and susceptibility testing of gram-negative bacilli from positive blood cultures. *Journal of Clinical Microbiology*, *41*(10), 4705–4707. https://doi.org/10.1128/JCM.41.10.4705-4707.2003

Lockhart, S. R., Etienne, K. A., Vallabhaneni, S., Farooqi, J., Chowdhary, A., Govender, N. P., Colombo, A. L., Calvo, B., Cuomo, C. A., Desjardins, C. A., Berkow, E. L., Castanheira, M., Magobo, R. E., Jabeen, K., Asghar, R. J., Meis, J. F., Jackson, B., Chiller, T., & Litvintseva, A. P. (2017). Simultaneous Emergence of Multidrug-Resistant Candida auris on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *64*(2), 134–140. https://doi.org/10.1093/cid/ciw691

Lortholary, O., Desnos-Ollivier, M., Sitbon, K., Fontanet, A., Bretagne, S., & Dromer, F. (2011). Recent exposure to caspofungin or fluconazole influences the epidemiology of candidemia: a prospective multicenter study involving 2,441 patients. *Antimicrobial Agents and Chemotherapy*, *55*(2), 532–538. https://doi.org/10.1128/AAC.01128-10

Lupetti, A., Tavanti, A., Davini, P., Ghelardi, E., Corsini, V., Merusi, I., Boldrini, A., Campa, M., & Senesi, S. (2002). Horizontal transmission of Candida parapsilosis candidemia in a neonatal intensive care unit. *Journal of Clinical Microbiology*, *40*(7), 2363–2369. https://doi.org/10.1128/JCM.40.7.2363-2369.2002

McCarty, T. P., & Pappas, P. G. (2016). *Invasive candidiasis*. *Infectious Disease Clinics*. *30*(1), 103–124.

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

Mnge, P., Okeleye, B. I., Vasaikar, S. D., & Apalata, T. (2017). Species distribution and antifungal susceptibility patterns of Candida isolates from a public tertiary teaching hospital in the Eastern Cape Province, South Africa. *Brazilian Journal of Medical and Biological Research = Revista Brasileira de Pesquisas Medicas e Biologicas*, *50*(6), e5797. https://doi.org/10.1590/1414-431X20175797

Mukherjee, P., & Wang, M. (2009). Antifungal Drug Resistance. *Antifungal Therapy*, *125*(1), 63–86. https://doi.org/10.3109/9780849387869-5

Musyoki, V. M., Mutai, W., Ngugi, N., Otieno, F., & Masika, M. M. (2022). Speciation and antifungal susceptibility of Candida isolates from diabetic foot ulcer patients in a tertiary hospital in Kenya. *The Pan African Medical Journal*, *41*, 34. https://doi.org/10.11604/pamj.2022.41.34.30815

Mutua, F., Revathi, G., & Machoki, J. M. (2010). Species distribution and antifungal sensitivity patterns of vaginal yeasts. *East African Medical Journal*, *87*(4), 156–162. https://doi.org/10.4314/eamj.v87i4.62202

Oberoi, J. K., Wattal, C., Goel, N., Raveendran, R., & Datta, S. (2012). *Non- albicans Candida species in blood stream infections in a tertiary care hospital at New Delhi , India*. *December*, 997–1003.

Pappas, P. G., Kauffman, C. A., Andes, D. R., Clancy, C. J., Marr, K. A., Ostrosky-Zeichner, L., Reboli, A. C., Schuster, M. G., Vazquez, J. A., Walsh, T. J., Zaoutis, T. E., & Sobel, J. D. (2016). Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *62*(4), e1-50. https://doi.org/10.1093/cid/civ933

Parkes-Ratanshi, R., Achan, B., Kwizera, R., Kambugu, A., Meya, D., & Denning, D. W. (2015). Cryptococcal disease and the burden of other fungal diseases in Uganda; Where are the knowledge gaps and how can we fill them? *Mycoses*, *58*, 85–93. https://doi.org/10.1111/myc.12387

Partha, A. D. S. L., Widodo, A. D. W., & Endraswari, P. D. (2022). Evaluation of fluconazole, itraconazole, and voriconazole activity on Candida albicans: A case control study. *Annals of Medicine and Surgery (2012)*, *84*, 104882. https://doi.org/10.1016/j.amsu.2022.104882

Perlin, D. S. (2015). Mechanisms of echinocandin antifungal drug resistance. *Annals of the New York Academy of Sciences*, *1354*(1), 1–11. https://doi.org/10.1111/nyas.12831

Perlin, D. S., Shor, E., & Zhao, Y. (2015). Update on Antifungal Drug Resistance. *Current Clinical Microbiology Reports*, *2*(2), 84–95. https://doi.org/10.1007/s40588-015-0015-1

Pfaller, M. A., Castanheira, M., Lockhart, S. R., Ahlquist, A. M., Messer, S. A., & Jones, R. N. (2012). Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of Candida glabrata. *Journal of Clinical Microbiology*, *50*(4), 1199–1203. https://doi.org/10.1128/JCM.06112-11

Pfaller, M. A., Castanheira, M., Messer, S. A., Moet, G. J., & Jones, R. N. (2010). Variation in Candida spp. distribution and antifungal resistance rates among bloodstream infection

isolates by patient age: Report from the SENTRY Antimicrobial Surveillance Program (2008-2009). *Diagnostic Microbiology and Infectious Disease*, *68*(3), 278–283. https://doi.org/10.1016/j.diagmicrobio.2010.06.015

Pfavayi, L. T., Denning, D. W., Baker, S., Sibanda, E. N., & Mutapi, F. (2021). Determining the burden of fungal infections in Zimbabwe. *Scientific Reports*, *11*(1), 1–13. https://doi.org/10.1038/s41598-021-92605-1

Pham, C. D., Iqbal, N., Bolden, C. B., Kuykendall, R. J., Harrison, L. H., Farley, M. M., Schaffner, W., Beldavs, Z. G., Chiller, T. M., Park, B. J., Cleveland, A. A., & Lockhart, S. R. (2014). Role of FKS Mutations in Candida glabrata: MIC values, echinocandin resistance, and multidrug resistance. *Antimicrobial Agents and Chemotherapy*, *58*(8), 4690–4696. https://doi.org/10.1128/AAC.03255-14

Rajasingham, R., Wake, R. M., Beyene, T., Katende, A., Letang, E., & Boulware, D. R. (2019). Cryptococcal meningitis diagnostics and screening in the era of point-of-care laboratory testing. *Journal of Clinical Microbiology*, *57*(1), 1218–1238. https://doi.org/10.1128/JCM.01238-18

Rao, R., Advisor, M., & Gegear, R. (2016). Understanding and Managing C. albicans Infections A Thesis Submitted to the Faculty of Worcester Polytechnic Institute In partial fulfillment of the requirements for the Degree of Master of Science By Catherine G . Harwood Table of Contents. In *BMC Research Notes*.

Richardson, M. D. (2005). Changing patterns and trends in systemic fungal infections. *Journal of Antimicrobial Chemotherapy*, *56*(SUPPL. 1). https://doi.org/10.1093/jac/dki218

Ruan, S. Y., Chien, J. Y., & Hsueh, P. R. (2009). Invasive trichosporonosis caused by Trichosporon asahii and other unusual Trichosporon species at a medical center in taiwan. *Clinical Infectious Diseases*, *49*(1), 1. https://doi.org/10.1086/599614

Sadeghi, G., Ebrahimi-Rad, M., Mousavi, S. F., Shams-Ghahfarokhi, M., & Razzaghi-Abyaneh, M. (2018). Emergence of non-Candida albicans species: Epidemiology, phylogeny and fluconazole susceptibility profile. *Journal de Mycologie Medicale*, *28*(1), 51–58. https://doi.org/10.1016/j.mycmed.2017.12.008

Santos, G. C. d. O., Vasconcelos, C. C., Lopes, A. J. O., Cartágenes, M. do S. d. S., Filho, A. K. D. B., do Nascimento, F. R. F., Ramos, R. M., Pires, E. R. R. B., de Andrade, M. S., Rocha, F. M. G., & Monteiro, C. de A. (2018). Candida infections and therapeutic strategies: Mechanisms of action for traditional and alternative agents. In *Frontiers in Microbiology* (Vol. 9, Issue JUL). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2018.01351

Smith, N., Sehring, M., Chambers, J., & Patel, P. (2017). Perspectives on non- neoformans cryptococcal opportunistic infections. *Journal of Community Hospital Internal Medicine Perspectives*, *7*(4), 214–217. https://doi.org/10.1080/20009666.2017.1350087

Sobel, J. D. (2016). Recurrent vulvovaginal candidiasis. *American Journal of Obstetrics and Gynecology*, *214*(1), 15–21. https://doi.org/https://doi.org/10.1016/j.ajog.2015.06.067

Solomon, D. A. (2021). Prevalence. Antifungal Susceptibility and Molecular Characterization of Isolated Candida Species from Bloodstream of Critical Care Unit Patients in Nairobi Hospital, Kenya, 47. DOI:10.4236/ojmm.2021.111003

Spampinato, C., & Leonardi, D. (2013). Candida infections, causes, targets. *And Resistance*

Mechanisms: Traditional and Alternative Antifungal Agents, *203*.

Sterlingt, T. P., & Merz, W. G. (1998). *Resistance to amphotericin B : emerging clinical and microbiological patterns*. 161–165.

Subira. (2018). INTERNATIONAL JOURNALS OF ACADEMICS & RESEARCH (IJARKE Business & Management Journal). *Academia.Edu*, *1*(1), 185–193. https://www.academia.edu/download/64375501/08-IBMJ Vol 3 Issue 1 08.pdf

Vincent, B. M., Lancaster, A. K., Scherz-shouval, R., Whitesell, L., & Lindquist, S. (2013). *Fitness Trade-offs Restrict the Evolution of Resistance to Amphotericin B*. *11*(10). https://doi.org/10.1371/journal.pbio.1001692

Wey, S. B., Mori, M., Pfaller, M. A., Woolson, R. F., & RP., W. (1989). Risk factors for hospital-acquired candidemia. *A Matched Case-Control Study*, *149*, 2349–2353.

WHO. (2018). *The diagnosis, prevention and management of cryptococcal disease in hiv-infected adults, adolescents and children*. Mar. Available from: https://www.ncbi.nlm.nih.gov/books/NBK531449/.

Wudhikarn, K., Palomba, M. L., Pennisi, M., Garcia-Recio, M., Flynn, J. R., & Devlin, S. M. (2020). ... & Perales, M. *A. Infection during the First Year in Patients Treated with CD*, *10*(8), 1–11.

Appendix I: Supplementary Tables

Supplementary Table 1: MIC Distribution for Amphotericin B, Flucytosine, Itraconazole, Fluconazole, and Voriconazole for the Yeast Isolates from VITEK-2 at NSH

		%Mic (µg/ml) for Pure Cultivation														
		≤0.03	**0.05**	**0.06**	**0.13**	**0.25**	**0.5**	**0.6**	**1**	**2**	**4**	**5**	**8**	**16**	**32**	**≥64**
Amphotericin B						**0.25**	**0.5**		**1**	**2**		**5**	**8**	**16**		
					Sensitive					**Intermediate**				**Resistant**		
Total	n (%)					14(4.5)	6(1.9)		1(0.3)	4(1.3)		1(0.3)	2(0.6)	280(90.9)		
C. albicans	116(37.7)					4(1.3)	2(0.6)		0(0.0)	1(0.3)		0(0.0)	0(0.0)	109(35.4)		
C. dubliniensis	74(24.0)					9(2.9)	0(0.0)		1(0.3)	0(0.0)		1(0.3)	1(0.3)	62(20.1)		
C. glabrata	21(6.8)					0(0.0)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	0(0.0)	21(6.8)		
C. guilliermondii	6(1.9)					0(0.0)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	0(0.0)	6(2.0)		
C. krusei	9(2.9)					0(0.0)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	0(0.0)	9(2.9)		
C. lusitanie	5(1.6)					0(0.0)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	0(0.0)	5(1.6)		
C. parapsilosis	12(3.9)					0(0.0)	3(1.0)		0(0.0)	0(0.0)		0(0.0)	0(0.0)	9(2.9)		
C. tropicalis	29(9.4)					0(0.0)	1(0.3)		0(0.0)	0(0.0)		0(0.0)	0(0.0)	28(9.1)		
C. laurentii	21(6.8)					0(0.0)	0(0.0)		0(0.0)	3(1.0)		0(0.0)	0(0.0)	18(5.8)		
C. var. neoformans	9(2.9)					1(0.3)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	0(0.0)	8(2.6)		
T. beigelii	6(1.9)					0(0.0)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	1(0.3)	5(1.6)		
Flucytosine			**0.05**	**0.06**	**0.13**	**0.25**	**0.5**	**0.6**		**2**	**4**		**8**	**16**		
		Sensitive										**Intermediate**		**Resistant**		
Total	n (%)		2(0.6)	168(54.7)	13(4.2)	22(7.2)	12(3.9)	2(0.6)		4(1.3)	6(2.0)		2(0.6)	76 (24.8)		
C. albicans	116(37.7)		2(0.6)	77(25.0)	4(1.3)	4(1.3)	1(0.3)	1(0.3)		0(0.0)	4(1.3)		0(0.0)	22(7.1)		
C. dubliniensis	74(24.0)		0(0.0)	42(13.6)	5(1.6)	10(3.2)	4(1.3)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	13(4.2)		
C. glabrata	21(6.8)		0(0.0)	8(2.6)	0(0.0)	0(0.0)	1(0.3)	1(0.3)		0(0.0)	0(0.0)		0(0.0)	11(3.6)		
C. guilliermondii	6(1.9)		0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)	0(0.0)		4(1.3)	0(0.0)		0(0.0)	1(0.3)		
C. krusei	9(2.9)		0(0.0)	2(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	7(2.3)		
C. lusitanie	5(1.6)		0(0.0)	2(0.6)	1(0.3)	0(0.0)	0(0.0)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	2(0.6)		
C. parapsilosis	12(3.9)		0(0.0)	8(2.6)	0(0.0)	0(0.0)	1(0.3)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	3(1.0)		
C. tropicalis	29(9.4)		0(0.0)	18(5.8)	0(0.0)	2(0.6)	0(0.0)	0(0.0)		0(0.0)	2(0.6)		0(0.0)	7(2.3)		
C. laurentii	21(6.8)		0(0.0)	5(1.6)	0(0.0)	6(1.9)	1(0.3)	0(0.0)		0(0.0)	0(0.0)		1(0.3)	8(2.6)		
C. var. neoformans	9(2.9)		0(0.0)	3(1.0)	0(0.0)	0(0.0)	4(1.3)	0(0.0)		0(0.0)	0(0.0)		1(0.3)	1(0.3)		
T. beigelii	6(1.9)		0(0.0)	3(1.0)	2(0.6)	0(0.0)	0(0.0)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	1(0.3)		
Itraconazole		**≤0.03**		**0.06**	**0.13**	**0.25**	**0.5**		**1**	**2**	**4**					
		Sensitive							**Intermediate**		**Resistant**					
Total	n (%)	2(0.6)		77(25.0)	22(7.1)	32(10.4)	4(1.3)		1(0.3)	2(0.6)	168(54.5)					
C. albicans	116(37.7)	0(0.0)		37(12.0)	9(2.9)	10(3.2)	0(0.0)		0(0.0)	0(0.0)	60(19.5)					

C. dubliniensis	74(24.0)	0(0.0)	30(9.7)	7(2.3)	11(3.6)	0(0.0)	0(0.0)	0(0.0)	26(8.4)			
C. glabrata	21(6.8)	0(0.0)	0(0.0)	0(0.0)	4(1.3)	4(1.3)	0(0.0)	2(0.6)	11(3.6)			
C. guilliermondii	6(1.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(1.9)			
C. krusei	9(2.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	9(2.9)			
C. lusitanie	5(1.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	5(1.6)			
C. parapsilosis	12(3.9)	0(0.0)	5(1.6)	1(0.3)	3(1.0)	0(0.0)	0(0.0)	0(0.0)	3(1.0)			
C. tropicalis	29(9.4)	2(0.6)	3(1.0)	3(1.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	20(6.5)			
C. laurentii	21(6.8)	0(0.0)	1(0.3)	0(0.0)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	19(6.2)			
C. var. neoformans	9(2.9)	0(0.0)	1(0.3)	1(0.3)	3(1.0)	0(0.0)	0(0.0)	0(0.0)	4(1.3)			
T. beigelii	6(1.9)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	5(1.6)			
Fluconazole				**0.13**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**32**	**64**
				Sensitive							**Intermediate**	**Resistant**
Total	n (%)			3(1.0)	113(36.7)	10(3.2)	3(1.0)	5(1.6)	15(4.9)	11(3.6)	2(0.6)	146(47.4)
C. albicans	116(37.7)			1(0.3)	57(18.5)	3(1.0)	0(0.0)	0(0.0)	3(1.0)	1(0.3)	0(0.0)	51(16.6)
C. dubliniensis	74(24.0)			2(0.6)	38(12.3)	6(1.9)	1(0.3)	2(0.6)	4(1.3)	4(1.3)	2(0.6)	15(4.9)
C. glabrata	21(6.8)			0(0.0)	2(0.6)	0(0.0)	0(0.0)	3(1.0)	0(0.0)	6(1.9)	0(0.0)	10(3.2)
C. guilliermondii	6(1.9)			0(0.0)	2(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(1.3)
C. krusei	9(2.9)			0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(0.6)	0(0.0)	0(0.0)	7(2.3)
C. lusitanie	5(1.6)			0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	5(1.6)
C. parapsilosis	12(3.9)			0(0.0)	5(1.6)	0(0.0)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(1.9)
C. tropicalis	29(9.4)			0(0.0)	3(1.0)	0(0.0)	0(0.0)	0(0.0)	2(0.6)	0(0.0)	0(0.0)	24(7.8)
C. laurentii	21(6.8)			0(0.0)	2(0.6)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)	18(5.8)
C. var. neoformans	9(2.9)			0(0.0)	2(0.6)	0(0.0)	1(0.3)	0(0.0)	3(1.0)	0(0.0)	0(0.0)	3(1.0)
T. beigelii	6(1.9)			0(0.0)	2(0.6)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(1.0)
Voriconazole		**≤0.03**	**0.06**	**0.13**	**0.25**	**0.5**	**1**	**2**		**8**		
		Sensitive					**Intermediate**			**Resistant**		
Total	n (%)	136(44.2)	1(0.3)	2(0.6)	28(9.1)	18(5.8)	0(0.0)	3(1.0)		120(39.0)		
C. albicans	116(37.7)	58(18.8)	0(0.0)	0(0.0)	13(4.2)	1(0.3)	0(0.0)	2(0.6)		42(13.6)		
C. dubliniensis	74(24.0)	52(16.9)	1(0.3)	1(0.3)	2(0.6)	3(1.0)	0(0.0)	0(0.0)		15(4.9)		
C. glabrata	21(6.8)	2(0.6)	0(0.0)	0(0.0)	6(1.9)	5(1.6)	0(0.0)	0(0.0)		8(2.6)		
C. guilliermondii	6(1.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)		6(1.9)		
C. krusei	9(2.9)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.3)		7(2.3)		
C. lusitanie	5(1.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)		4(1.3)		
C. parapsilosis	12(3.9)	9(2.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)		3(1.0)		
C. tropicalis	29(9.4)	8(2.6)	0(0.0)	0(0.0)	3(1.0)	4(1.3)	0(0.0)	0(0.0)		14(4.5)		
C. laurentii	21(6.8)	3(1.0)	0(0.0)	0(0.0)	1(0.3)	3(1.0)	0(0.0)	0(0.0)		14(4.5)		
C. var. neoformans	9(2.9)	2(0.6)	0(0.0)	1(0.3)	3(1.0)	0(0.0)	0(0.0)	0(0.0)		3(1.0)		
T. beigelii	6(1.9)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)		4(1.3)		

Appendix II: Study Area

Appendix III: Data Abstraction Tool

Yeast Isolates Characterization* and *Assessment of Associated Risk Factors among Inpatients at Nairobi South Hospital

Table 1. Patient Demographic and Clinical Features

Patient Code			
Age			
Gender		**Male**	**Female**
Date of Admission		Date of Discharge	
Ward Admitted			
Length of hospital Stay (Days)			
Comorbidity on Admission			
Laboratory Tests Done	Blood Culture	**Positive [ ]**	**Negative [ ]**
	PCT _____ ng/ml		
	CRP _____ mg/ml		
	Neutrophils _____ 10^9/L		
	Creatinine _____ g/L		
	Albumin _____ g/L		
	Total Bilirubin _____		
	Levels of Lymphocytes:	**High [ ]**	**Low [ ]**
	Other Tests Done		
Antibiotics and Antifungal Administered Prior to Yeast Isolation			
Survival Status on Discharge		**Recovered** [ ]	**Expired** [ ]

Table 2. Proportion and distribution of yeast isolates recovered

Please Tick accordingly

Name of Yeast Isolated	**Sample Type**	**Date Isolation**	**Percentage of Pure Cultivation** (CFU/ml) %	**Any other Microorganism Isolated Alongside** YES/NO
Candida albicans				
Candida tropicalis				
Candida glabrata				
Candida lusitaniae				
Candida auris				
Candida dubliniensis				
Candida guilliermondii				

Candida krusei				
Candida haemulonii				
Candida parapsilosis				
Cryptococcus var neoformans				
Cryptococcus laurentii				
Trichosporon beigelii				

Table 3. The Antifungal Drug Susceptibility Pattern of Yeast Isolates

Sample						
Name of Yeast Isolated						
AST	S	MIC	I	MIC	R	MIC
Amphotericin B						
Fluconazole						
Flucytosine						
Itraconazole						
Voriconazole						

KEY WORDS

S – Sensitivity R – Resistant I – Intermediate

AST-Antifungal Sensitivity Testing MIC – Minimum Inhibitory Concentration

Table 4. Underlying Disease or Associated Comorbidity

Underlying Disease or Associated Comorbidity	*Tick All*
Diabetes Mellitus/ Hypertension	
Cancer/Malignancy	
Renal Failure/Renal Disease	
Sepsis	
Surgical Procedure	
Acute Decompensate Heart Failure (ADHF)	
Hematological Condition	
Parenteral Nutrition/ Intubation/ Catheterization	
COVID-19	
Use of Immunosuppressants	
Retroviral Disease (RVD)	
Autoimmune Condition	
Neonate/ Elderly/Pregnant	
Other Chronic Illness	

Appendix IV: Ethical Approval Letters and Waiver of Informed Consent from Nairobi South Hospital

UNIVERSITY OF NAIROBI
FACULTY OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/51

31st January, 2023

Charity Lyavuli Akweya
Reg. No. H56/38327/2020
Dept. of Medical Microbiology
Faculty of Health Sciences
University of Nairobi

Dear Charity,

RESEARCH PROPOSAL: YEAST ISOLATES CHARACTERIZATION AND ASSESSMENT OF ASSOCIATED RISK FACTORS AMONG INPATIENTS AT NAIROBI SOUTH HOSPITAL (P747/09/2022)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P747/09/2022.** The approval period is 31st January 2023 – 30th January 2024.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

DR. BEATRICE K.M. AMUGUNE
SECRETARY, KNH-UoN ERC

c.c. The Dean, Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Assistant Director, Health Information Dept., KNH
The Chairperson, KNH- UoN ERC
The Chair, Dept. of Medical Microbiology, UoN
Supervisors: Dr. Florence Mutua, Dept. of Medical Microbiology, UoN
Ms. Winnie Mutai, Dept. of Medical Microbiology, UoN

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **407709**

Date of Issue: **18/May/2023**

RESEARCH LICENSE

This is to Certify that Miss.. CHARITY Lyavuli AKWEYA of University of Nairobi, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nairobi on the topic: YEAST ISOLATES CHARACTERIZATION AND ASSESSMENT OF ASSOCIATED RISK FACTORS AMONG INPATIENTS AT NAIROBI SOUTH HOSPITAL for the period ending : 18/May/2024.

License No: **NACOSTI/P/23/25973**

407709

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013 (Rev. 2014)
Legal Notice No. 108: The Science, Technology and Innovation (Research Licensing) Regulations, 2014

The National Commission for Science, Technology and Innovation, hereafter referred to as the Commission, was the established under the Science, Technology and Innovation Act 2013 (Revised 2014) herein after referred to as the Act. The objective of the Commission shall be to regulate and assure quality in the science, technology and innovation sector and advise the Government in matters related thereto.

CONDITIONS OF THE RESEARCH LICENSE

1. The License is granted subject to provisions of the Constitution of Kenya, the Science, Technology and Innovation Act, and other relevant laws, policies and regulations. Accordingly, the licensee shall adhere to such procedures, standards, code of ethics and guidelines as may be prescribed by regulations made under the Act, or prescribed by provisions of International treaties of which Kenya is a signatory to
2. The research and its related activities as well as outcomes shall be beneficial to the country and shall not in any way;
    i. Endanger national security
    ii. Adversely affect the lives of Kenyans
    iii. Be in contravention of Kenya's international obligations including Biological Weapons Convention (BWC), Comprehensive Nuclear-Test-Ban Treaty Organization (CTBTO), Chemical, Biological, Radiological and Nuclear (CBRN).
    iv. Result in exploitation of intellectual property rights of communities in Kenya
    v. Adversely affect the environment
    vi. Adversely affect the rights of communities
    vii. Endanger public safety and national cohesion
    viii. Plagiarize someone else's work
3. The License is valid for the proposed research, location and specified period.
4. The license any rights thereunder are non-transferable
5. The Commission reserves the right to cancel the research at any time during the research period if in the opinion of the Commission the research is not implemented in conformity with the provisions of the Act or any other written law.
6. The Licensee shall inform the relevant County Director of Education, County Commissioner and County Governor before commencement of the research.
7. Excavation, filming, movement, and collection of specimens are subject to further necessary clearance from relevant Government Agencies.
8. The License does not give authority to transfer research materials.
9. The Commission may monitor and evaluate the licensed research project for the purpose of assessing and evaluating compliance with the conditions of the License.
10. The Licensee shall submit one hard copy, and upload a soft copy of their final report (thesis) onto a platform designated by the Commission within one year of completion of the research.
11. The Commission reserves the right to modify the conditions of the License including cancellation without prior notice.
12. Research, findings and information regarding research systems shall be stored or disseminated, utilized or applied in such a manner as may be prescribed by the Commission from time to time.
13. The Licensee shall disclose to the Commission, the relevant Institutional Scientific and Ethical Review Committee, and the relevant national agencies any inventions and discoveries that are of National strategic importance.
14. The Commission shall have powers to acquire from any person the right in, or to, any scientific innovation, invention or patent of strategic importance to the country.
15. Relevant Institutional Scientific and Ethical Review Committee shall monitor and evaluate the research periodically, and make a report of its findings to the Commission for necessary action.

National Commission for Science, Technology and
Innovation(NACOSTI),
Off Waiyaki Way, Upper Kabete,
P. O. Box 30623 - 00100 Nairobi, KENYA
Telephone: 020 4007000, 0713788787, 0735404245
E-mail: dg@nacosti.go.ke
Website: www.nacosti.go.ke

Muhoho Ave; South C,
P.O. Box 74079-00200
Nairobi, Kenya.

THE NAIROBI SOUTH HOSPITAL

Tel: +254 020 6001164
Mobile: 0721 700408/ 0700 100086
Fax: +254 020 601154
Email: info@nairobisouthhospital.org
www. nairobisouthhospital.org

Our Ref. NSH/ND/25/10/22

25th October, 2022

Charity Lyavuli Akweya
P.O. Box 7 - 00902
Kikuyu.

Dear Charity,

RE: "CHARACTERIZATION OF YEAST AND ASSESSMENT OF ASSOCIATED RISK FACTORS AMONG INPATIENTS AT NAIROBI SOUTH HOSPITAL.

Reference is made to your request to carry out the above study at The Nairobi South Hospital. We are pleased to advice that the approval has been granted.

In line with our Research project policy, you will be required to submit a copy of the final audit findings for our records.

Kindly take note that information/data collected and potential findings shall not be in conflict with the hospital's confidentiality policy and that "you will not without consent of the hospital disclose any of the information to anyone who is not authorized to receive them.

For: The Nairobi South Hospital,

Dr. Abdisalan Maalim.
Medical Director.

Cc. Chief Executive Officer.
Nursing Services Manager.

Exemplary Service