SPECIES SPECTRUM AND SUSCEPTIBILITY PROFILES OF YEASTS ISOLATED FROM CRITICAL CARE UNIT PATIENTS IN A UNIVERSITY HOSPITAL

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(H56/35361/2019)

A research dissertation submitted to the University of Nairobi in partial fulfillment of the requirements for the award of a Master of Science degree in Medical Microbiology, University of Nairobi.

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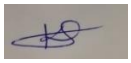
Signature: ……  …… Date: December 7, 2023

SUPERVISOR APPROVAL

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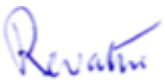
Signature:  Date December 7, 2023

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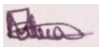
Signature:  Date December 7, 2023

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LIST OF ABBREVIATIONS

AKUHN	Aga Khan University Hospital, Nairobi
ATCC	American Type Culture Collections
BSI	Blood Stream Infection
CAP	College of American Pathologists
CCU	Critical Care Unit
CDC	Centre for Disease Control and Prevention
CFU	Colony-Forming Unit
CI	Confidence-Interval
CLSI	Clinical and Laboratory Standards Institute
CT ICU	Cardiothoracic Intensive Care Unit
CVC	Central Venous Catheter
HDU	High Dependency Unit
HCWs	Healthcare Workers
HSCT	Hematopoietic Stem Cell Transplantation
ICU	Intensive Care Unit
IPC	Infection Prevention and Control
NAC	Non-albicans *Candida*
NICU	Newborn Intensive Care Unit
OR	Odds ratio
SPP.	Species
TPN	Total Parenteral Nutrition
USA	United States of America

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ABSTRACT

Background

With the worldwide increase in yeast infections, especially in high-risk patients, comes an increase in varying patterns of antifungal drug resistance among yeast, specifically *Candida* species, which becomes an obstacle to effective therapy. This underscores the need for more information on etiological agent and species distribution that could drive treatment recommendations, given the differences in susceptibility to antifungal armamentarium among yeast species. This study aimed to identify the species spectrum and antifungal susceptibility profiles of isolated yeast and assess potential factors associated with colonization and/or infections among critically ill patients.

Methodology

This was an 11-month retrospective cohort study performed using isolated yeast organisms from patients admitted to the Critical Care Units (CCU) at a university hospital. Standard microbiological techniques were performed on all archived samples from those patients for laboratory culture and determination of yeast identity. Antifungal susceptibility testing was conducted using the VITEK 2 compact system to fluconazole, voriconazole, amphotericin B, flucytosine, caspofungin, and micafungin. Medical records were reviewed retrospectively. Data analysis was done using the R software.

Results

Among the 250 enrolled critically ill patients, 180 yeast isolates (from carriage and clinical samples) were recovered. Non-albicans *Candida* species were the most frequent isolates (86.7 percent), followed by *Candida albicans* (12.2 percent), and yeasts other than *Candida* (1.1 percent). A noteworthy resistance pattern to fluconazole and voriconazole was seen among *Candida parapsilosis*; overall resistance to the other tested antifungals was low. Previous antibiotic therapy (aOR=1.89,95%CI 1.06-3.39, P= 0.032) was identified as an independent risk factor for colonization while previous antifungal therapy (aOR=4229.22 ,95%CI 120.89-6346317.47, P= 0.001) and colonization (aOR=13.86 95% CI 1.59-528.43, P=0.049) were significantly associated with infection. Compared with non-colonized non-infected patients, independent risk factors associated with colonized-infected patients were CCU length of stay (OR=1.08,95% CI 1.01-1.16,

P=0.023), prior antifungal therapy (OR=172.76,95% CI 18.07-12678.34, P<0.001), and neoplasm (OR=27.41,95% CI 2.36-2310.28, P=0.030).

Conclusion

With shifting patterns of epidemiology, this study emphasizes the importance of continued surveillance, antifungal stewardship, and infection prevention and control measures, a timely reminder that pathogenic yeasts deserve equal attention in the new era of emerging infectious diseases.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

In recent decades, there has been a progressive increase in the incidence of invasive fungal infections, with yeast species of the genus *Candida* being the primary threat(N. A. Chow et al., 2018; Paramythiotou et al., 2014; Pfaller et al., 2019). Other yeasts, including *Rhodotorula* spp., *Geotrichum candidum, Malassezia* spp., *Trichosporon* spp. ,and *Saccharomyces* spp., have also been implicated in invasive fungal infections but are still relatively uncommon(Brown et al., 2012; Lin et al., 2019). Such infections have been seen more frequently in patients who have spent much time in the hospital as inpatients, patients exposed to multiple invasive medical procedures, parenteral nutrition, immunosuppressive therapy, and antibiotics (McCarty & Pappas, 2016; Trick et al., 2002). More so, over time, the extension and density of fungal colonization may influence the risk of infection (Pittet et al., 1994). Numerous studies have shown that yeast infections affect the prognosis of critically ill patients by increasing morbidity and varying mortality rates ranging from 10–50 percent, lengthening intensive care unit (ICU) stays, and incurring substantial additional expenditures. Despite this, invasive fungal infections are challenging to diagnose (Cleveland et al., 2012; Strollo et al., 2017; Voss et al., 1997).

The epidemiology of *Candida* infections varies geographically and has been extensively studied in high-resource setting countries compared to low-resource setting countries like Kenya. With the notable shift away from *Candida albicans*, in Asia and Latin America, the most predominant species of non-albicans *Candida* (NAC) associated with fungemia are *Candida tropicalis* and *Candida parapsilosis* (Hinrichsen et al., 2008; Morii et al., 2014), while *Candida glabrata* is common in Central and North Europe, as well as the United States of America (USA), particularly among the elderly. (Blot et al., 2001). Africa, where *Candida parapsilosis* and *Candida albicans* are the main species, presents a slightly different picture. Although they are more common in South Africa, figures vary depending on whether the facilities are public or private. In private hospitals, *Candida parapsilosis* (53 percent) predominates over *Candida albicans* (26 percent) while *Candida albicans* (46 percent) and *Candida parapsilosis* (35 percent) in public hospitals(Govender et al., 2016). However, the main concern regarding this switch from the

previous dominance of *Candida albicans* to the current increase in NAC observed is the altered antifungal susceptibility associated with these pathogens.

Fluconazole prophylactic usage regularly and an uncontrolled distribution of antifungals have been correlated to reduced antifungal susceptibility in yeasts species (Lamoth et al., 2018; Rocco et al., 2000). A significant barrier to prophylactic and empiric therapeutic strategies is the intrinsic and emergence of azole resistance by *Candida* species, the preferred treatment for fungal infections in most healthcare facilities in Africa. Since the progressive loss of echinocandin activity has also been documented, this problem is not exclusive to azole antifungals (Bassetti et al., 2020; Castanheira et al., 2020). Additionally, newly discovered species that resist treatment with antifungal medications currently in use are continuously being isolated making it is essential that laboratories provide up-to-species-level identification.

Notably, as a 'call to arms', the World Health Organization (WHO) is currently defining a fungal pathogen priority list, in line with its bacterial counterpart and includes yeasts such as *Candida parapsilosis, Candida tropicalis* and *Candida auris*. It's crucial to conduct regional and local surveillance studies to track antifungal resistance. Global surveillance is particularly effective at identifying and classifying emerging threats, while local studies offer pertinent information to guide empirical therapy and support antifungal stewardship initiatives(Pfaller et al., 2019). Against this background, this study aimed to assess the spectrum of yeast species, antifungal susceptibility patterns, and risk factors for colonization and/or infection due to yeast species.

1.2 Problem statement and study justification

Yeast species have emerged from organisms of questionable pathogenicity to infectious agents, posing a formidable threat to hospitals worldwide (Cortegiani et al., 2018; Guinea, 2014). Due to both the emergence of resistance in the context of the pressure of antimicrobials and the transmission of drug-resistant strains in healthcare settings, antifungal resistance is rising along with the global rise of fungal infections (Pfaller, 2012). The limited choice of antifungal drug classes and data available to inform therapy, makes choosing an antifungal treatment more difficult. Significant yeast infections resistant to the drug are common in critically ill individuals and have a high crude death rate of up to 50 percent (Alfouzan et al., 2020; Chalmers et al., 2011).

Geographic differences in rates and epidemiology of yeast infections have been documented, indicating the necessity of surveillance to track trends. Because of the patient characteristics from which yeast species is isolated, it might be challenging to differentiate between infection and colonization (Lau et al., 2015). Although colonization is thought necessary for infection, it has not yet been established how often colonized patients become infected (Charles et al., 2005). In order to avoid propagating antimicrobial resistance and to inform decisions about hospital infection control strategies, it is crucial to clinically differentiate between colonization and infection. Making an accurate diagnosis and fungi pathogen identification is essential since these rare species have become significant opportunistic pathogens. However, many laboratories in Kenya lack the capacity to perform yeast identification to species level.

Yeast species recognition as an emerging cause of several infections across the world, mainly among critically ill patients, accentuate the importance of vigilance and additional studies concerning its epidemiology, especially in Kenya, where data is limited. It is crucial to determine antifungal resistance to inform on IPC measures, policy, funds allocation, and implementation of efficient treatment guidelines. Drug resistance poses deleterious consequences for patient care, healthcare costs, and clinical outcomes. Understanding the contribution of colonization status is pivotal to the development of appropriate screening and the implementation of contact precautions in patients with colonization, thus containing any spread as well as preventing hospital outbreaks.

1.3 Research questions

i. What is the spectrum and the antifungal susceptibility profiles of clinical and carriage yeast species isolates from Aga Khan University Hospital, Nairobi (AKUH, N) critical care unit patients?

ii. What are the potential factors associated with yeast species colonization and infection status in AKUH**,** N critical care unit patients?

1.4 Study Objectives

1.4.1 General objective

1. To determine the spectrum and antifungal susceptibility profiles of yeast species from critical care unit patients admitted at AKUH, N.

1.4.2 Specific Objectives

1. To identify the spectrum of clinical and carriage yeast species isolates from AKUH, N critical care unit patients.
2. To describe the antifungal susceptibility profiles of isolated yeast species at AKUH, N.
3. To assess potential factors associated with yeast species colonization and/or infection among AKUH, N critically ill patients.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology and yeast species burden

2.1.1 The Fungi

The taxonomy of yeasts is constantly evolving, and currently yeasts that are of medical importance belong to two classes: the Saccharomycetes, which contains *Candida* species, and the Tremellomycetes, which contains the basidiomycetous fungi *Trichosporon* and *Cryptococcus* (Howell et al., 2015).In the genus *Candida*, greater than 200 species exist. *Candida* species are the yeast identified most commonly in the mycology laboratory and responsible for most opportunistic infections. The epidemiological infections trends caused by different *Candida* species have been investigated in several studies in which 95 percent of all invasive *Candida* infections are attributed to *Candida albicans* complex, *Candida tropicalis, Candida glabrata* complex (*Candida metapsilosis, Candida nivariensis, Candida bracarensis, Candida glabrata), Candida parapsilosis* complex (*Candida orthopsilosis, Candida parapsilosis*) ,and *Candida krusei* (Castanheira et al., 2014; Pfaller, Messer, et al., 2011). The *Candida* species distribution varies by region, factors like the patient risk factors, the history of antifungal usage patterns as well as clonal outbreaks—that is, outbreaks involving a specific molecular strain of a particular species of *Candida* that is unique to the healthcare setting (Hajjeh et al., 2004).

A majority of species of NAC was observed in a 2019 research of patients at a prominent medical center in North America; *Candida glabrata* and the other species of NAC including *Candida tropicalis* and *Candida parapsilosis* came in second, despite the fact that *Candida albicans* was the most frequent isolated species (D. L. Horn et al., 2009). An analysis revealed that *Candida parapsilosis, Candida albicans*, and *Candida glabrata* were responsible for more than half of the cases of candidemia in European countries, with *Candida albicans* taking the lead (Tortorano et al., 2006). Changes of epidemiology have also been observed in Latin American countries. In Chile, the prevalence of *Candida albicans* has reduced from 55 percent to 39 percent with a progressive increase of NAC from 45 percent to 61 percent between 2000 and 2017; *Candida parapsilosis* was the most frequent species, followed by *Candida glabrata* and *Candida tropicalis* (Santolaya et al., 2019; Siri et al., 2017). *Candida parapsilosis* makes up for 15.7 percent of

Candida isolates in North America ,10.3 percent in Europe ,and 26.5 percent in Latin America, dominated by *Candida albicans* (45.6 percent, 51.5 percent and 37.6 percent respectively) and *Candida glabrata* (26 percent) in North America (Chalmers et al., 2011; D. L. Horn et al., 2009; Nucci et al., 2013).

Partial analyses of the SENTRY study have shown that some species are endemic to particular regions. For instance, a Brazil-wide sentinel surveillance survey revealed that *Candida pelliculosa* had a prevalence of 6.2%, placing it fourth among isolated species. *Candida glabrata* (4.9%) and *Candida krusei* (1.1%) were less common than *Candida pelliculosa* (Colombo et al., 2007). *Candida auris* global spread is a highly concerning trend (Chowdhary et al., 2017; Lockhart et al., 2017). Considering local distributions rather than continental ones may be necessary according to this. In Kenya, *Candida* infections have been documented in the past. According to these reports, the species that was isolated most frequently was *Candida albicans* (Kangogo et al., 2011; Ooga et al., 2011). Nevertheless, based on many laboratory findings from the ICU patients and high dependency unit (HDU), in a university hospital in Nairobi, Kenya, reports of *Candida auris* have emerged (Adam et al., 2019; Cortegiani et al., 2018).

2.1.2 The Disease

Numerous studies have investigated the epidemiology of yeast species infections over the years to characterize the scope of worldwide antifungal resistance and fungal burden. Most clinical sites of disease including urinary tract infections, post-operative site infections, and oropharyngeal infections, have demonstrated a rising incidence of *Candida*, although candidemia is particularly impacted (Nucci et al., 2010). According to reports from European countries, the incidence rates of non-albicans candidemia infections caused by *Candida glabrata* were 14 percent, *Candida tropicalis* were 7 percent, and *Candida krusei* were 2 percent (Tortorano et al., 2006). In Brazil, *Candida albicans* accounted for 40.9 percent of cases, followed by *Candida tropicalis* (20.9 percent*), Candida parapsilosis* (20.5 percent), and *Candida glabrata* (4.9 percent), according to the Brazilian network candidemia research (Nucci et al., 2010).

Candida species are the overall fourth most significant reason for candidemia and third-most common in patients who are critically ill (Sandt et al., 2003). These point to an adult death rate of 15 percent-35 percent and a neonatal mortality rate of 10 percent-15 percent in tertiary care

hospitals around the world (Al-Obaid et al., 2017). According to research conducted in the United States on a number of hospitalized patients including patients in the ICU, candidemia contributed to a mortality rate of 38 percent (Gudlaugsson et al., 2003; Strollo et al., 2017). Two-thirds of invasive infections in the USA are attributable to *Candida* species. Increased healthcare expenses result from these infections; in the USA, the management of one candidemia case might cost between $35,000 and $68,000 (Strollo et al., 2017).

Among the *Trichosporon* species with the ability to cause disease are *T. asteroides* ,*T. asahii*, and *Cutaneotrichosporon mucoides* (*Trichosporon mucoides*), which are the main causes of trichosporonosis and significant opportunistic infections(H. Li et al., 2020).

The table below presents the predominance of various yeast species linked to particular clinical outcomes. However, it is crucial to stress that isolation of yeast species ,specifically *Candida* species, varies significantly based on the patient population and geographical region, with some NAC species being more common than *Candida albicans* in some nations (Colombo et al., 2006).

Table 1.A selection of epidemiological research on the distribution of isolates from species of yeasts related to particular clinical conditions.

Clinical description	**Number of isolates analyzed**	***Candida albicans* (%)**	**Other yeast species (%)**	**Year of Study**	**Region/ Country**	**Reference**
Candidemia	126	21	*C. parapsilosis (12), C. glabrata (3), C. tropicalis (38)*	_	USA	(Hazen et al., 1986)
	_	59	*C. parapsilosis (11), C. glabrata (12), C. tropicalis (10) C.krusei (0.7), other NAC species (<1)*	1989-1999	USA	(Trick et al., 2002)
	_	42	*C.tropicalis (16), C. parapsilosis (33), C. glabrata (2), C. krusei (2), C. guillermondii (2)*	2004-2005	Brazil	(Miranda et al., 2009)
	_	26.3	*C.glabrata (10.5), C. pelliculosa (17.6), C. guillermondii (30.4)*	_	India	(Chakrabarti et al., 2009)
	473	53	*C.tropicalis (7), C. glabrata (14), C. parapsilosis (14)*	1997-1999	Europe	(Tortorano et al., 2006)
	1239	50	*C.tropicalis (9.8), C. parapsilosis (17.4), C. glabrata (17.4), C. krusei (18)*	2008-2009	Europe/Asia/America	(Pfaller et al., 2010)

Candiduria	389	68.4	*C. parapsilosis (0.5) C. glabrata (8.2), C. tropicalis (36)*	1998-1999	Spain	(Álvarez-Lerma et al., 2003)
Oropharyngeal candidiasis	65	88.2	*C. parapsilosis (4.4), C. glabrata (27.8)*	2006	Australia	(Chen et al., 2008)
	53	79	*C.tropicalis (4.8), C. parapsilosis (6.5), C. glabrata (4.8)*	2005-2006	Portugal	(Martins et al., 2010)
Vulvovaginal candidiasis	177	81	*C.tropicalis (14.1), C. krusei (5.6), C. glabrata (22.5)*	2008	Ethiopia	(Mulu et al., 2013)
	191	67	*C.tropicalis (6.8), C. parapsilosis (1.6), C. glabrata (18.3), C. krusei (5.8), C. guillermondii* (0.5)	2006-2008	Iran	(Mahmoudi Rad et al., 2011)
	87	58.6	*C.krusei (17.2), C. glabrata (3.4), C. tropicalis (2.3), C. dubliniesis(9.2),C. parapsilosis (2.3)*	2015-2016	Ethiopia	(Bitew & Abebaw, 2018)
	63	60.3	*C. glabrata (12.7), C. krusei (7.9), C. parapsilosis (7.9), C. tropicalis (6.3)*	–	Egypt	(ElFeky et al., 2016)
	101	69.3	*C.glabrata (12.9), C. famata (5.0), C. krusei (3.0) C. parapsilosis (1.0)*	2006-2007	Kenya	(Mutua et al., 2010)

Nosocomial trichosporonosis	133	-	*T. asahii (81.2), T. dermatis (5.3), T. asteroides (3.8), T. inkin (3.8), T. dohaense (2.3), T. jirovecii (0.7)*	2009-2016	China	(Guo et al., 2019)
	22	-	*T. asahii (68), T. asteroides (23)*	1995-2004	Brazil	(Chagas-Neto et al., 2009)

2.2 Risk factors for infection and colonization by yeast species.

The rising incidence of infections due to *Candida* species among terminally ill patients can be attributed to a variety of primarily iatrogenic interventions or the disease state or intrinsic factors to the host. Immunosuppressive conditions, dialysis, necrotizing pancreatitis, total parenteral nutrition, recent major surgery, exposure to broad-spectrum antibacterial agents, long-term ICU stay with or without assisted ventilation ,and presence of an indwelling central venous catheter are among the most frequent individual risk factors (Lau et al., 2015; Ostrosky-Zeichner et al., 2007). A retrospective study of the medical records of 286 persons who received parenteral nutrition for more than 72 hours revealed that 4.9 percent had new onset candidemia. With a median of 17 days compared to 8 days in the non-candidemia group, parenteral nutrition was administered much longer in the candidemia group (Stratman et al., 2010).

The occurrence of colonization at numerous sites is a candidemia-independent predictor, and at least 60-70 percent of patients in the ICU who develop candidemia are colonized with the same *Candida* species, according to estimates (Hegazi et al., 2014; Vogiatzi et al., 2013). Monitoring for rising colonization in severely unwell children may enable early interventions for candidemia prevention. Also, colonization has been incorporated into scoring systems aimed at candidemia risk prediction in adults. Unfortunately, hospital laboratories face a significant workload challenge in monitoring colonization with different yeast species, and in many clinical settings, this task may not be cost-effective or feasible.

Some risk factors for infection may co-exist. According to the study by Blyth et al. in Australia, hematological malignancy and neutropenia were significant risk factors in both adults and children. Further analysis of the data in this study identified ICU admission and prematurity as substantial risk factors in neonates, whereas recent surgery, hemodialysis, renal disease, and diabetes mellitus were risk factors in adults (Blyth et al., 2009). A particularly significant risk factor appears to be using a central venous catheter (CVC) or vascular access device. In 70 percent of children infected, according to the same Australian study, a vascular access device was responsible for the infection. This number was even more remarkable in newborns (58 percent) and (44percent) in adults who had access to devices (Blyth et al., 2009). A number of researchers hypothesized that extensive fluconazole usage would lead to selection of yeast species like *Candida glabrata, Candida tropicalis,* or *Candida krusei* that are less susceptible to fluconazole or intrinsically resistant (Abi-Said et al., 1997; White, 1997). There was a significant shift in the incidence of infections during the study period at San Martino hospital brought on by the majority of non-albicans *Candida* species. These modifications took place at the same time as a four-fold increase in fluconazole usage. (Pelz et al., 2001).

The probability of nosocomial infections and external transmission in particular populations may be influenced by the characteristics of certain *Candida* species. The most prevalent species of *Candida* found on healthcare workers (HCWs) hands is *Candida parapsilosis*. In multicenter prospective research of newborn candidiasis carried out in the USA, *Candida parapsilosis* was isolated in 19 percent of 2989 cultures obtained from HCW's hands (Saiman et al., 2001).A similar study conducted in ICUs in Brazil reported a prevalence of 44.59 percent colonization among CCU HCWs' (da Silva et al., 2021). The ability of *Candida parapsilosis* to form biofilms may help to

explain why it frequently results in nosocomial candidemia outbreaks associated with CVCs (Trofa et al., 2008). Additionally, parenteral nutrition use has been linked to outbreaks of *Candida parapsilosis* candidemia, which may be due to the organisms' ability to grow specifically in glucose-rich hyperalimentation solutions (Trofa et al., 2008). Among 72 patients with invasive *Candida parapsilosis*, a Spanish study found that risk factors for infection included vascular catheterization (97 percent), prior antibiotic therapy (91percent), parenteral nutrition (54 percent), prior surgery (46 percent), and initial immunosuppressive therapy (38 percent). Other risk factors were neutropenia (12 percent) , transplant recipient (percent), and malignancy (27 percent) (Rodríguez et al., 2010).

Candida glabrata appears to be most commonly isolated from people who have prior exposure to fluconazole, prior exposure to surgery, solid organ transplant patient, cancer patients, and in elderly patients (Malani et al., 2005; Pfaller, 2012). In the presence of neutropenia and mucositis, *Candida tropicalis* is being isolated more frequently from patients with hematologic malignancies and hematopoietic stem cell transplant (HSCT) recipients. In these patients, colonization is a good indicator of future infection (Sipsas et al., 2009). Hematologic malignancies patients and HSCT recipients who have neutropenia, have been exposed to corticosteroids, and have previously taken fluconazole and antifungal medications have frequently been reported to have *Candida krusei* colonization (Azie et al., 2012; Lau et al., 2015; Pfaller, 2012; Sipsas et al., 2009). Other NAC species, including *Candida rugosa* (Minces et al., 2009) and *Candida guilliermondii* (Masala et al., 2003), which are relatively resistant to fluconazole, have also been linked to nosocomial epidemics, some of which involved intravascular catheters.

Trichosporon species infections have recently increased as a result of a number of factors, including an increase in the prevalence of malignant diseases and an increase in the number of patients receiving immunosuppressant, chemotherapy, invasive procedure, broad-spectrum antibiotic and organ transplant treatments(H. Li et al., 2020). Usually, but not always, immunological impairment results in infection with the yeast *Cryptococcus* spp.

2.3 Pathogenesis and spectrum of disease.

There have been more studies looking into host-pathogen interactions for some yeast species as a result of the rise of non-albicans *Candida* species, namely *Candida glabrata* and *Candida parapsilosis.* Although common features of host immunity to these species and *Candida albicans* have been published (Linden et al., 2013) to establish the mechanisms by which these new species of *Candida* influence change in the cellular and molecular components of protective immunity, more research is needed.

The transition of *Candida* species from commensalism to opportunism is linked to the induction of essential virulence factors when host immunity is compromised and/or mucosal microbiota is disturbed (Bennett, 2010; De Pauw et al., 2008). In particular, three main factors lead to invasive infection. The first is the use of extended or frequent broad-spectrum antibiotics which promote higher *Candida* species gut colonization. Because commensal gut microbiota species play a key role in triggering the release of anti-*Candida* species protective factors from the mucosa, antibiotics give *Candida* species a selective advantage over bacteria. As a result, depleting these microbiota species makes it possible for *Candida* species overgrowth. A toll like receptor 4-dependent mechanism is used by epithelial cells to react to the overgrowth of *Candida* species. This results in the activation of JUN (also known as activator protein-1) and nuclear factor-kB, and this reaction is unaffected by the morphology of the fungus. In response to a change in *Candida* morphology, epithelial cells generate cytokines (IL-8, IL-1and CCL20) by activating FOS-related pathways and mitogen-activated protein kinase 1 (MAPK1). These cytokines activate host immune cells. Additionally, β-defensins are released by epithelial cells for anti-*Candida* purposes, which, in response to IL-22 generated by TH17 cells or innate lymphoid cells (ILCs), show potent antifungal activity. (De Pauw et al., 2008; McCarty & Pappas, 2016). Another factor that makes commensal *Candida* species more likely to enter the bloodstream is mucositis induced by cytotoxic chemotherapy. Mucositis is responsible for the breaching of the gastrointestinal and cutaneous barriers (aggregation of the gastrointestinal lumen mucosa), central venous catheters and/or gastrointestinal surgery or perforation. The third factor is iatrogenic immunosuppression, which weakens innate immune defenses in tissues and allows *Candida* species in the bloodstream to invade organs like the liver, kidneys, brain, heart, and spleen. Examples of iatrogenic immunosuppression include corticosteroid therapy and chemotherapy-induced neutropenia.

Previous research has linked radiation's direct effects on lymphocyte depletion. Direct radiation damage will decrease the number of T-cells in circulation (McCarty & Pappas, 2016). As compared to invasive *Candida* species infection, effective immunity is dependent on myeloid phagocytes (mononuclear phagocytes, that is, dendritic cells ,macrophages and monocytes , and neutrophils), not lymphocytes, in contrast to mucosal candidiasis where T lymphocytes of the T helper 17 (TH 17) cell differentiation program are essential for host defense (Puel et al., 2012; Strollo et al., 2017).

Candida species' ability to evade host defenses, as well as the predictions of tissue-damaging hydrolytic enzymes (e.g., hemolysins, phospholipases and proteases), and the formations of adhesion and biofilm on host tissue and medical devices are just a few of the virulence mechanisms that contribute to their pathogenicity. By and large, *Candida albicans* is the most deleterious species; yet, in some places, *Candida* species as a whole may account for more than 50% of bloodstream isolates.(McCarty & Pappas, 2016; Wisplinghoff et al., 2004). The *Candida parapsilosis* complex, *Candida krusei, Candida tropicalis,* and *Candida glabrata* complex are the NAC species most commonly isolated in association with certain clinical circumstances. The vascular system, vagina, gastrointestinal tract, oral cavity, and skin are just a few of the anatomically diverse sites where these *Candida* species might colonize and cause illness. They must evade the immune system, ensure survival, reproduce in the environment of the host, and, in the case of systemic infection, migrate to different organs and tissues to establish infection.

In patients undergoing HSCT, *Candida glabrata* is a significant clinical pathogen, and as patients get older, their relative contribution to candidemia cases rises.(Guinea, 2014). It contributes to 20 percent of bloodstream infections (BSIs) in the United States of America (12-37 percent), 15 percent in Europe, in Asia it accounts for 10 percent, and in Latin America ,5 percent (Pfaller et al., 2004). Disseminated disease, endocarditis, and meningitis are other severe infections from which *Candida glabrata* has been isolated. Approximately 70 percent of those with oral candidosis have been reported to have mixed species infections by the *Candida* species, namely, *Candida albicans* and *Candida glabrata* (Redding et al., 2002). Every year, *Candida glabrata* is reported to cause vulvovaginal candidiasis in millions of women (Gonçalves et al., 2016; White, 1997). Even though the presence of *Candida glabrata* in patient flora is well established, little is known about the hospital reservoirs for this pathogen. It is likely spread through a complex interaction

between human and environmental reservoirs. Research has also identified hand contact with hospital staff as a possible infection source (Isenberg et al., 1989). Therefore, like other nosocomial infections, they can be acquired through contaminated environmental surfaces either indirectly or directly.

Because of its propensity to colonize the skin, a significant pathogen in newborns and babies receiving complete parenteral nutrition who have CVC is *Candida parapsilosis*. It was first discovered in 1940 to be the underlying cause of an intravenous drug user's fatal case of endocarditis. In some hospitals, *Candida parapsilosis* has surpassed *Candida albicans* as the most prevalent species of *Candida* among children. It is particularly prevalent in patients who have catheter-related candidemia and in infants under 12 months of age (Miguel et al., 2005; Puig-Asensio et al., 2014). In addition, this fungal species is the most frequently isolated from human hands, and the second most commonly isolated *Candida* species from normally sterile body sites in inpatients (Bonassoli et al., 2005).Invasive ocular diseases associated with *Candida parapsilosis* include keratitis and endophthalmitis (especially postoperative infection) (P.-H. Li et al., 2016). Otomycosis, onychomycosis, and peritonitis due to *Candida parapsilosis* infection have been documented (Trofa et al., 2008).

Candida krusei accounts for less than 3 percent of candidemia cases. The clinical outcome could be significantly impacted by its appearance, though. Comparative research on fungemia in immunocompromised patients revealed a mortality rate of 49 percent for *Candida krusei* and a rate of just 28 percent for *Candida albicans* (Mahmoudi Rad et al., 2011). According to several studies, the gastrointestinal and respiratory tracts are the two most common sites of *Candida krusei* colonization, with 70 percent of individuals being colonized before symptoms appear (Hong Nguyen et al., 1996; R. Horn et al., 1985). Fluconazole prophylaxis has been associated with an increased colonization and infection of *Candida krusei* in patients with granulocytopenia (Pelz et al., 2001). Various other NAC species have been identified in infections and should be considered when evaluating an isolated yeast culture. These comprise of *Candida auris, Candida dubliensis, Candida famata, Candida lipolytica, Candida lusitaniae, Candida ciferii, Candida haemulonii, Candida guillermondii, Candida kefyr, Candida utilis* (Deorukhkar et al., 2014).

Besides the prevention of NAC infections and early detection of the infection, a critical clinical issue in the NAC infections treatment is the rapid initiation of suitable systemic antifungal therapy. Because invasive candidiasis has no rapid diagnostic assays for NAC, the majority of clinicians base their diagnoses on standard fungal cultures and empirical evidence. This strategy can result in the inappropriate use of antifungals in people who do not have invasive candidiasis and can also delay the commencement of effective therapy with the antifungal in infected individuals. These delays in diagnosis and intervention may result in significantly worse clinical outcomes which appears to be related to the responsible NAC species. A total of 2019 individuals of all ages who have a confirmed candidemia in 23 North American sites, enrolled in the PATH Alliance database had an overall crude 12-week death rate of 35.2 percent (D. L. Horn et al., 2009). When compared to individuals infected with other *Candida* species, in individuals with *Candida parapsilosis*, the lowest rate of mortality of (23.7 percent) was seen. Crude mortality was higher in patients with *Candida krusei* candidemia at 52.9 percent.

2.4 Antifungal susceptibility of yeast species

Because of the increasing antimicrobial resistance and the occurrence of strains and species resistant to almost every drug currently on the market, yeast species are receiving significant attention. The mechanism of resistance to antifungal can either be through induction of resistance in isolates from species that are usually susceptible or by the species' selection with intrinsic resistance or, depending on the drug and the yeast species.(Pereira et al., 2010). The rise of *Candida glabrata* following the introduction of fluconazole and of *Candida parapsilosis* in environments where there was increasing usage of echinocandins serve as examples of how common the induction of resistance in isolates from species that are normally susceptible is (Arendrup & Perlin, 2014; Lortholary et al., 2011). More so, the development of resistance has been linked to inadequate dosing of azoles (Shah et al., 2012). Based on biological, epidemiological, or methodological factors, resistance rates can differ by country and hospital.

These drugs are currently categorized depending on their activity; these include echinocandins such as micafungin, anidulafungin, and caspofungin, polyenes such as amphotericin B, azoles like fluconazole and itraconazole, and analogs of purines such as flucytosine. Due to the easy accessibility of antifungal medications in some countries, these microorganisms have become more resistant (Rodríguez et al., 2010; Rodríguez-Tudela et al., 2007). *Candida tropicalis* and *Candida parapsilosis* are often azole-susceptible; although, to fluconazole, *Candida tropicalis* is less susceptible than *Candida albicans*. Fluconazole resistance is intrinsic in *Candida glabrata*, and infections induced by this species are closely related to prior neutropenia and fluconazole prophylaxis. Despite being susceptible to azoles, *Candida lusitaniae* exhibits a higher amphotericin B intrinsic resistance. This species accounts for 1-2 percent of all candidemia (Cruciani & Serpelloni, 2008).

Amphotericin B, which is commonly believed to command the largest antifungal activity spectrum, is utilized, for instance, to treat systemic infections in hospitalized patients with severe and invasive *Candida* infections. Although reports of NAC isolates with raised minimum inhibitory concentrations (MIC) have increased, amphotericin B resistance is still very rare throughout treatment (Pfaller, 2012; Pfaller, Moet, et al., 2011). It has already been proven that *Candida lusitaniae* exhibits frequent phenotypic shifts from being susceptible to amphotericin B to resistance when exposed to the drug (Yoon et al., 1999). In the context of the use of nystatin

prophylaxis and cases of breakthrough fungemia, *Candida rugosa* has also been isolated in patients receiving amphotericin B treatment (Lopes Colombo et al., 2003).

The extensive usage of azole antifungals is a result of their improved therapeutic options for infections caused by fungi and lower toxicity in the host. It is, therefore, likely not shocking that resistance to these medications, notably fluconazole, has been observed, given their extensive use. Aside from *Candida glabrata* (9 percent) and *Candida krusei* (40 percent), fluconazole resistance with MIC ≥ 64 µg/ml (susceptible MIC range: ≤ 8 µg/ml ,susceptible-dose dependent range is16 to 32 µg/ml) was observed in less than 3 percent for all species examined among 13,338 BSI isolates of *Candida* at the University of Lowa between 1992 and 2004 (Pfaller et al., 2006). *Candida glabrata* continues to be a source of concern regarding fluconazole resistance due to its prevalence as an invasive candidiasis cause in many settings. Significant differences and higher fluconazole levels have also been seen in isolates of *Candida famata* (9.8 percent to 47.4 percent), *Candida rugosa* (14.3 percent to 66.0 percent), and *Candida guilliermondii* (6.3 percent to 26.1 percent) (Pfaller et al., 2005), Such conclusions are typically drawn from the findings of a very limited number of isolates. Isolates of *Candida* species were shown to have varying drug resistance patterns of 48 percent in the disc test and 26 percent using E-test toward fluconazole in a research done in Kenya (Ooga et al., 2011).

Studies have reported that *Candida glabrata* isolates show cross-resistance to extended-spectrum triazoles (itraconazole, voriconazole, and Posaconazole). This resistance shows correlation with a rise in gene expression that encodes the CDR efflux pumps (Pfaller et al., 2004). Since azole exposure in the past may make triazole antifungal drugs safe and effective treatment alternatives for other azoles, this effect is unpredictable and needs to be confirmed by antifungal susceptibility testing that is “real-time” (Panackal et al., 2006; Spellberg et al., 2006). Fluconazole resistance in *Candida krusei* is well recognized. Contrary to *Candida glabrata, Candida krusei* does not frequently exhibit in-vitro cross-resistance to voriconazole (Pfaller et al., 2006) because in *Candida krusei*, the cytochrome P-450 isoenzyme target is significantly more efficiently bound by voriconazole than by fluconazole (Ostrosky-Zeichner et al., 2003). Additionally, it seems that voriconazole's in vitro effectiveness translates into positive clinical outcomes in C. krusei infection patients (Ostrosky-Zeichner et al., 2003). Posaconazole has fungistatic activity against some NAC species, including *Candida tropicalis*, and *Candida parapsilosis* and less inhibition against

Candida lusitaniae and *Candida glabrata* isolates as compared to voriconazole(Greer, 2007; Sóczó et al., 2007).

By and large, echinocandins, which include micafungin, caspofungin, and anidulafungin, are the most recent addition to the antifungal arsenal and have excellent fungicidal action against most *Candida* species, including those that are azole resistant (Bayegan et al., 2010). Multicenter surveys conducted by Pfaller et al. (Pfaller et al., 2005) and Ostrosky-Zeichner et al. (Ostrosky-Zeichner et al., 2003) demonstrated the superior efficacy and range of the three echinocandins against more than 4,000 BSI isolates of different *Candida* species. *Candida glabrata, Candida tropicalis,* and *Candida albicans* were highly susceptible to all three agents, but *Candida guillermondii* and *Candida parapsilosis* have increased MICs of up to 4 g/ml (normal susceptible MIC range: $\leq 2\mu g/ml$). It is significant to note in light of these results that caspofungin has been demonstrated to have no fungicidal effect against *Candida parapsilosis* and *Candida guillermondii* (Barchiesi et al., 2006). During the course of treating esophagitis (Hernandez et al., 2004), candidemia (Krogh-Madsen et al., 2006; Walker et al., 2010), and endocarditis (Moudgal et al., 2005), reports of caspofungin resistance were documented, thus raising concerns. *Candida glabrata* is over-represented among isolates that are echinocandin-resistant, with reported resistance rates of 2 to 5 percent and up to 8–12 percent at some tertiary care facilities. Echinocandins-acquired resistance has also been reported for *Candida kefyr, Candida krusei , Candida tropicalis, Candida lusitaniae,* and *Candida dubliensis* (Arendrup & Perlin, 2014). Echinocandin resistance can evolve and spread quickly within a setting, as seen by a study from a single U.S. hospital. There has been a recorded increase in the recovery of *Candida parapsilosis* that is multiechinocandin- and multiazole-resistant from hospital burn patients (Moudgal et al., 2005). The Infectious Diseases Society of America (IDSA) supports the use of echinocandins but advises that individuals whose clinical condition has improved following initial echinocandin therapy step-down to voriconazole or fluconazole within 5-7 days, a recommendation for patients who have a proven history of clearing *Candida* from their bloodstream and have an infection that can be treated with voriconazole or fluconazole (McCarty & Pappas, 2016).

Flucytosine has a limited range of activity and is typically combined with other medications such as amphotericin B and fluconazole because of the several phases in its mode of action, which include transport into the cell and deamination of the active molecule (Vermes et al., 2000).

Clinicians frequently hesitate to utilize it because of worries regarding toxicity and/or primary/secondary resistance, despite the fact that there is a consensus regarding its clinical efficacy when administered in combination (Medoff & Kobayashi, 1980; Vermes et al., 2000). Studies from Canada (Eggimann et al., 1999), the US (Hajjeh et al., 2004), Italy (Rocco et al., 2000), and Spain (Cuenca-Estrella et al., 2001) have estimated flucytosine resistance to be between 0 percent and 0.6percent for *Candida albicans* and between 0.6 percent and 6 percent for all combined *Candida* species.

Knowledge of antifungal susceptibility patterns of local yeast species can help guide the management of suspected invasive candidiasis because infections treatment caused by hospital-acquired yeast species may be complex, given the chances of being resistant.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

A single-center retrospective, cohort study was performed at Aga Khan University Hospital, Nairobi between November 2021 and October 2022.

3.2 Study population and site

Yeast species isolated from patients admitted at the critical care unit in AKUH, N from November 2021 to October 2022 were included in this retrospective study. Colonization was assessed in all consented patients through axilla and groin swabs collected in a study that was ongoing (2019/IERC-87). At that time, the ongoing study evaluated *Candida auris* colonization alone; no other yeasts were processed further**.** The colonization study samples were obtained within 48 hours of admission to the CCU and twice a week thereafter, as long as the patient stayed in the unit. Subsequent yeast infection development for every colonized patient was assessed. The possibility of colonization was taken into account for patients from whose yeast species were obtained at various evolutive moments from all the archived swabs. Medical records were then retrospectively reviewed for data abstraction.

AKUH, N is 300-bed tertiary care, a university-affiliated hospital in Nairobi, Kenya, with about 50 critical care beds, including ICU, coronary care unit (CCU), cardiothoracic ICU (CT ICU), newborn ICU (NICU) and high dependency unit (HDU). The clinical departments are supported by state-of-the-art diagnostic solutions provided by the well-equipped ultra-modern radiology and pathology departments.

The majority of the patients were from the upper middle and high socioeconomic classes, with the majority being native African Kenyans, Kenyans of Asian origin, and white people.

3.3 Criteria for study inclusion

- All isolates of yeast recovered from carriage samples between the period November 2021 to October 2022 were included. Significant yeast infections were considered if from sterile sites such as blood or tissue.

- Yeast clinical isolates from the genital tract, urine, sputum, and other non-sterile sites without site-specific symptoms were considered colonizers.

3.4 Criteria for study exclusion

- Replica isolates of those from the same species and the same site of a certain patient with the same resistant or susceptible biotype profile isolated at different times.

3.5 Definitions

Patients were classified as non-colonized and non-infected if yeast species were not isolated from them. Patients were classified as infected or colonized when one or more yeast species isolates were identified from samples, with colonization being the exposure and the outcome being infection status. In the absence of disease symptoms and signs, colonization was defined as the yeast species' isolation from the non-sterile sites. Colonization was assessed through axilla and groin swab samples obtained from an ongoing carriage study at then and clinical non-sterile samples. Patients with fungal infection caused by yeast species were defined by the growth of yeast species in sterile sample cultures in individuals exhibiting infection signs and symptoms.

The possibility of infection or colonization was taken into account for patients from whose yeast species were obtained at various evolutive moments. If it was considered colonization in all of them, the case was included only the first time, analyzing the parameters associated with the first positive culture. If one isolate corresponded to infection, the case was considered infection and the variables associated with that moment were analyzed. Infections were categorized as invasive if a yeast species was cultured from a sterile sample, or non-invasive for the remaining infection sites. During the same hospitalization, patients who experienced a second infectious episode were not considered different cases since the second infection was interpreted as a complication of the primary one.

3.6 Sample size determination

The sample size was calculated based on the primary outcome of the main study and on the data available in the microbiology laboratory of AKUHN. Analysis was made using Openepi software package; EpiTable | Sample | Sample Size | Cohort Study (https://www.openepi.com/SampleSize/SSCohort.htm).

To achieve 80 percent power to detect a relative risk greater than 2 with an allocation ratio of non-exposed to exposed subjects being 1.358, a percent of exposed with outcome being 16.04 and an α error of 0.05, EpiTable determined that at least 156 subjects were needed.

$$N_{Fleiss} = \frac{[z_{\alpha/2}\sqrt{(r+1)p(1-p)} + z_{\beta}\sqrt{rp_0(1-p_0) + p_1(1-p_1)}]^2}{r(p_0 - p_1)^2}$$

Where: $Z_{\alpha/2}$ = standard normal deviate for two-tailed test based on alpha level (relates to the confidence interval level)

Z_{β}=standard normal deviate for one-tailed test based on beta level (relates to the power level)

r = ratio of unexposed to exposed

p_1 = proportion of exposed group

p_0 = proportion of unexposed group

The sample size formula for the method described was based on formulas in Fleiss, Statistical methods for rates and proportions formulas. Upon tabulation and considering continuity correction, EpiTable determined that at least 156 subjects were needed (66 subjects in the exposed arm and 90 non-exposed subjects).

However, a total of 250 subjects were used in this study. This larger sample size ensured ample sample size for statistical testing.

3.7 Sampling procedure

All preserved yeast isolates obtained from the then ongoing study on fungal carriage and/or infection over the said period were included in the study.

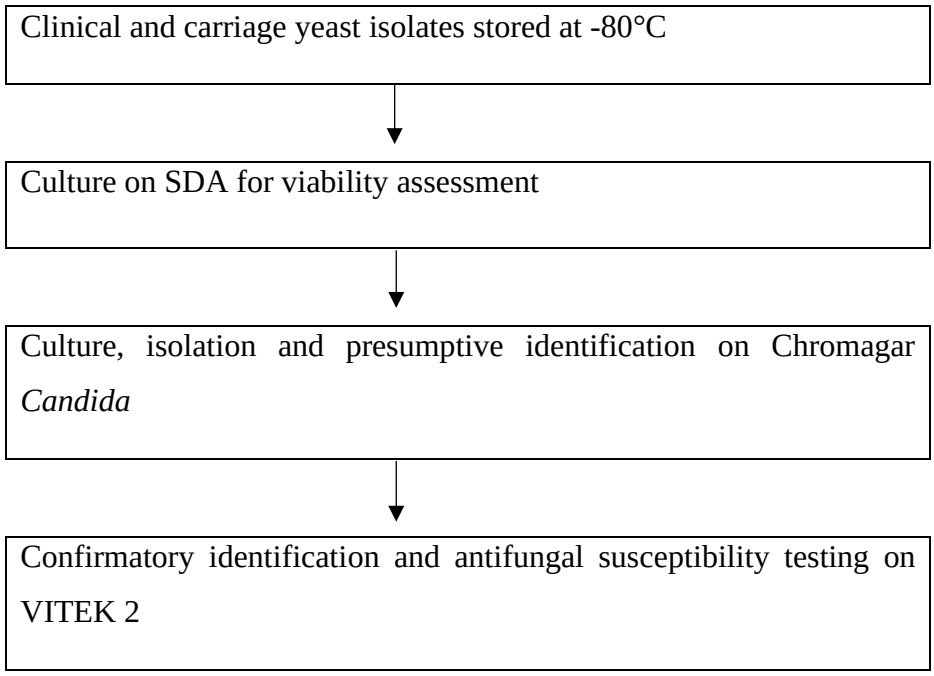
3.8 Variables

Variables likely to influence yeast species colonization or infection were collected retrospectively from the electronic medical record (EMR). Written records for the patients were reviewed to find information not found in the electronic medical record. Inter-reviewer accuracy was verified by comparing redundantly abstracted charts. A case report form was completed, and the data collected included; demographics data (ethnicity, sex, and age), hospitalization unit (classified as ICU, HDU, CCU, NICU), any known underlying conditions, exposure to invasive medical procedures, history of antibiotics or antifungal treatments, laboratory test results and colonization data. (Appendix 1).

3.9 Laboratory procedures

The viability and purity of yeast cultures preserved in brain heart infusion with 10% glycerol in secure -80°C freezers were assessed by plating them on Sabouraud dextrose agar (Basingstoke, Oxoid) for inoculation. The cultures were then incubated at 37°C-38°C in air and monitored for yeast growth for up to 48 hours. Cultures were observed after incubation using standard routine mycology methods such as colony morphology, wet preparation microscopy, gram stain, and other necessary and relevant bench tests as per requirement. On Chromagar™ *Candida*, isolates that fitted the description of yeasts were sub-cultured for presumptive identification using morphological traits like the shape of cells, size of colonies, and color. As per the recommendations from the manufacturer, the identification of yeasts was done using VITEK 2 YST ID cards, and yeast susceptibility was tested using VITEK 2 AST YS08 cards. In essence, sterile saline inoculum suspensions for the VITEK 2 testing were standardized to a turbidity of 2.0 McFarland standards, as measured using a densicheck instrument (Biomerieux). Every standardized inoculum suspension was loaded onto the VITEK 2 instrument in a VITEK 2 cassette with a polystyrene test tube, a yeast identification card, and a susceptibility card. The prepared culture suspension was automatically dispensed into the YST ID and AST YS07 cards, which were then sealed and incubated by the VITEK 2 instrument for approximately 9 to 33 hours, depending on the sample. Quality control was done using the standard strains *Candida krusei* ATCC® 6258™ and *Candida parapsilosis* ATCC® 22019™. The VITEK 2 compact system's excellent and very good ratings for each isolate's identification up to the species level were considered correct identifications. The isolates' susceptibility to antifungals was done on a panel of 6 drugs - voriconazole, Amphotericin B, flucytosine, fluconazole, micafungin, and caspofungin.

Figure 1.A flow diagram showing the laboratory procedure



3.10 Materials

The AKUHN pathology department was accredited by the College of American Pathologists (CAP). The microbiology laboratory used modern technology such as the fully automated VITEK 2 Compact (France, Marcy-etoile, Biomerieux) for pathogen identifications and routine antimicrobial susceptibility testing. All procedures were under strict internal and external quality controls.

3.11 Biosafety issues

The general laboratory biosafety and waste disposal guidelines were followed during all procedures. These included working using a level 2 biosafety cabinet, wearing safety apparel, and autoclaving all biological waste before disposal.

3.12 Ethical considerations

Ethical approval was sought from The Aga Khan University, Nairobi Scientific and Ethics Review Committee(2023/ISERC-06/v1), Appendix 4. Besides, the study was granted approval by Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-UoN ERC-P34/01/2023), Appendix 5, before commencement of the study.

3.12.1 Consent process

The researcher requested a waiver for individual informed consent per the exemption procedure for selected studies by AKU-ISERC (Exempt category 4). The study did not involve human subjects directly, and no intervention was done. Archived isolates were used. The waiver did not have a negative impact on the participants' rights and well-being. All information required was obtained from electronic medical records and written records. Any patient identifiers were removed to preserve confidentiality.

3.12.2 Confidentiality

Patient confidentiality was ensured at all times during the study. Unique coded participant identifiers were employed to guarantee data confidentiality. All records were secured in restricted lockable cabinets before archiving as per Aga Khan University's procedures. A password-protected computer was used to store the electronic data as password-encoded files. Only the principal investigator and co-investigators had access to retrieve identifiers. No identifying details have been disclosed in this work's final reports and publications.

3.12.3 Patient risks to participants

Apart from the minimal risk of loss of confidentiality of participants' data, the researcher had anticipated that there were no intended risks to the participants' as archived isolates were used instead.

3.13 Data Management

3.13.1 Data entry and storage

Participants' information was retrieved from the electronic medical records, and written records were reviewed to find information not found in the electronic medical records. The information was then entered on a Microsoft Excel spreadsheet and stored in a password-protected computer. Data on the lab testing results were stored in a locked cabinet in the department of pathology. Access to data was restricted only to the study team to ensure data security and confidentiality.

3.13.2 Data analysis

Categorical variables were displayed as frequencies and percentages for each study group (yeast species infection, yeast species colonization, colonized infected and, non-colonized non-infected). Continuous variables were expressed as the mean and standard deviation (SD) when the data followed a normal distribution or as the median and interquartile range (25th and 75th percentile) (IQR) when the distribution deviated from normality. Normality was assessed using Kolmogorov Smirnov test.

To compare the demographic and clinical characteristics of patients with and without infection and/or colonization, the chi-square test for categorical variables and the Kruskal-Wallis test or student t-test for continuous variables was used as appropriate. Pairwise multiple comparisons were performed when a test was statistically significant using Scheffe's method for means, since it can examine all possible linear combinations and shows that comparing groups when there is inequality in variance has less of an influence, and by decomposing the likelihood ratio statistics for the percentages (Allen, 2017).

Patients with yeast species infection vs. species colonization and non-colonized non-infected individuals were the dependent variables in logistic regression models with a backward stepwise selection procedure that included significant bivariate analysis variables. To prevent the discovery of spurious associations, the models consisted of variables that were a priori considered clinically relevant. The results are shown as 95% confidence intervals, odds ratios, and p-values. P value < 0.05 was set as the statistical significance. R statistical package (R version 4.2.3), was used for data analysis.

3.14 Dissemination of research findings

To increase the utility of data from the study to the scientific community, for clinical management, and to the general public, general findings were communicated through various platforms such as academic presentations, workshops, conference presentations, publication in open-access journals, and deposition of the final thesis publicly in the Universities' database.

CHAPTER FOUR

4.0 RESULTS

4.1 Characteristics of study population

During the 11-month study period from November 2021 to October 2022, 250 patients admitted to the critical care units (CCU) of the Aga Khan University Hospital, Nairobi were enrolled - 155 patients to the HDU, 42 to the ICU, 27 to the coronary care unit, and 26 to more than one CCU. The study participants were classified into four groups based on colonization and/or infection status - non-colonized non-infected (n=129), colonized (n=116), infected (n=17), and colonized infected (n=12). The mean age of the study participants was 51.25 years (SD ±19.696); this did not differ significantly between the four groups (p=0.650) (Table 2). Overall, the majority of the patients were male at 137 (54.8 percent), while females were 113 (45.2%); the age distribution did not differ significantly between the four groups (p=0.919) (Table 2).

An independent-samples t-test was conducted to compare CCU length of stay in the study groups. There was a significant difference in the colonized infected (Mean±SD;13.5±10.7) and non-colonized non-infected (4.16±7.74) cohorts' t (12) =2.97, p=0.01. These results suggest that colonization and infection status does have an effect on patients' length of stay at the CCU. Specifically, our results suggest that when patients are colonized and get infected during hospitalization, their stay at the CCU increases.

Overall, the majority of the study participants had no previous exposure to antifungal agents. This was similar to the findings in the study groups except for the infection and colonized infected groups in which most participants had an unknown status of previous exposure to antifungal agents. A chi-square test of independence was performed to assess the relationship between the other variables including radiotherapy, presence of a urinary catheter, CVC, renal replacement, previous antibiotic therapy, diabetes, TPN, surgical procedures, and steroid use, but they did not differ across the study groups.

Among the colonized, 83.6 percent of patients were already colonized with yeasts prior to admission to the critical care unit, and 16.4 percent became colonized after admission. Unifocal yeast species colonization was seen in 109 patients (94 percent) and multifocal colonization in 7 patients (6 percent). Proven yeast infection was diagnosed in 17 patients (6.8 percent), with 12 (4.8 percent) of them colonized and five (2 percent) non-colonized. Six patients developed candidemia, two candiduria, five oropharyngeal candidiasis, three cutaneous candidiasis, and one vulvovaginal candidiasis.

Table 2.Characteristics of the total study participants divided into cohorts of colonization and infection.

Variables	non-colonized non-infected (n=129)	Colonization (n=116)	Infection (n=17)	colonized infected (N=12)	Overall participants (N=250)	P-value
Age in years						
Mean (SD)	52.5 (20.1)	50.3 (18.4)	48.5 (24.3)	51.9 (19.4)	51.2 (19.7)	0.919[#]
Median [Min, Max]	53.0 [5.00, 94.0]	51.0 [3.00, 90.0]	48.0 [4.00, 90.0]	50.5 [15.0, 90.0]	51.0 [3.00, 94.0]	
Sex						
Females	61 (47.3%)	49 (42.2%)	7 (41.2%)	4 (33.3%)	113 (45.2%)	0.650[$]
Males	68 (52.7%)	67 (57.8%)	10 (58.8%)	8 (66.7%)	137 (54.8%)	
Hospitalization unit						
CCU	74 (57.4%)	11 (9.5%)	0 (0%)	8 (66.7%)	27 (10.8%)	0.398[$]
HDU	29 (22.5%)	79 (68.1%)	10 (58.8%)	1 (8.3%)	155 (62.0%)	
ICU	10 (7.8%)	13 (11.2%)	1 (5.9%)	3 (25.0%)	42 (16.8%)	
More than one unit	16 (12.4%)	13 (11.2%)	6 (35.3%)	0 (0%)	26 (10.4%)	
CCU length stay, days						

Variables	non-colonized non-infected (n=129)	Colonization (n=116)	Infection (n=17)	colonized infected (N=12)	Overall participants (N=250)	P-value
Mean (SD)	4.16 (7.74)	6.77 (7.40)	15.0 (16.9)	13.5 (10.7)	5.66 (8.61)	**0.012**[#]
Median [Min, Max]	2.00 [1.00, 77.0]	4.00 [1.00, 44.0]	9.00 [1.00, 69.0]	9.50 [3.00, 40.0]	3.00 [1.00, 77.0]	
Chemotherapy						
No	127 (98.4%)	109 (94.0%)	15 (88.2%)	10 (83.3%)	241 (96.4%)	**0.009**[$]
Unknown	2 (1.6%)	2 (1.7%)	1 (5.9%)	1 (8.3%)	4 (1.6%)	
Yes	0 (0%)	5 (4.5%)	1 (5.9%)	1 (8.3%)	5 (2.0%)	
Radiotherapy						
No	128 (99.2%)	114 (98.3%)	16 (94.1%)	11 (91.7%)	247 (98.8%)	0.028[$]
Yes	0 (0%)	0 (0%)	0 (0%)	1 (8.3%)	1 (0.4%)	
Unknown	1 (0.8%)	2 (1.8%)	1 (5.9%)	0 (0%)	2 (0.8%)	
Any other immunosuppression						
None	129 (100%)	116 (100%)	17 (100%)	12 (100%)	250 (100%)	**<0.001**[$]
Urinary catheter						
No	63 (48.8%)	48 (41.4%)	2 (11.8%)	2 (16.7%)	111 (44.4%)	0.102[$]
Yes	66 (51.2%)	68 (58.6%)	15 (88.2%)	10 (83.3%)	139 (55.6%)	
CVC						
No	112 (86.8%)	94 (81.0%)	11 (64.7%)	9 (75.0%)	208 (83.2%)	0.532[$]
Yes	17 (13.2%)	22 (19.0%)	6 (35.3%)	3 (25.0%)	42 (16.8%)	
Renal replacement						
No	127 (98.4%)	114 (98.3%)	17 (100%)	12 (100%)	246 (98.4%)	0.996[$]

Variables	non-colonized non-infected (n=129)	Colonization (n=116)	Infection (n=17)	colonized infected (N=12)	Overall participants (N=250)	P-value
Unknown	1 (0.8%)	1 (0.9%)	0 (0%)	0 (0%)	2 (0.8%)	
Yes	1 (0.8%)	1 (0.9%)	0 (0%)	0 (0%)	2 (0.8%)	
Previous antibiotics therapy						
No	64 (49.6%)	33 (28.4%)	2 (11.8%)	2 (16.7%)	97 (38.8%)	0.091[$]
Yes	65 (50.4%)	80 (69.0%)	0 (0%)	10 (83.3%)	150 (60.0%)	
Unknown		3 (2.6%)	15 (88.2%)	–	3 (1.2%)	
Previous antifungal therapy						**<0.001**[$]
No	125 (96.9%)	101 (87.1%)	2 (11.8%)	2 (16.7%)	226 (90.4%)	
Yes	0 (0%)	12 (10.3%)	1 (5.9%)	1 (8.3%)	21 (8.4%)	
Unknown	4 (3.1%)	3 (2.6%)	14 (82.4%)	9 (75.0%)	3 (1.2%)	
Diabetes						0.991[$]
No	99 (76.7%)	86 (74.1%)	13 (76.5%)	9 (75.0%)	189 (75.6%)	
Yes	30 (23.3%)	30 (25.9%)	4 (23.5%)	3 (25.0%)	61 (24.4%)	
Total Parenteral Nutrition						0.628[$]
No	125 (96.9%)	103 (88.8%)	13 (76.5%)	11 (91.7%)	230 (92.0%)	
Unknown	2 (1.6%)	6 (5.2%)	2 (11.8%)	1 (8.3%)	9 (3.6%)	
Yes	2 (1.6%)	7 (6.0%)	2 (11.8%)	0 (0%)	11 (4.4%)	
Surgical procedures						0.290[$]
No	93 (72.1%)	83 (71.6%)	9 (52.9%)	5 (41.7%)	180 (72.0%)	
Unknown	7 (5.4%)	7 (6.0%)	1 (5.9%)	1 (8.3%)	14 (5.6%)	
Yes	29 (22.5%)	26 (22.4%)	7 (41.2%)	6 (50.0%)	56 (22.4%)	

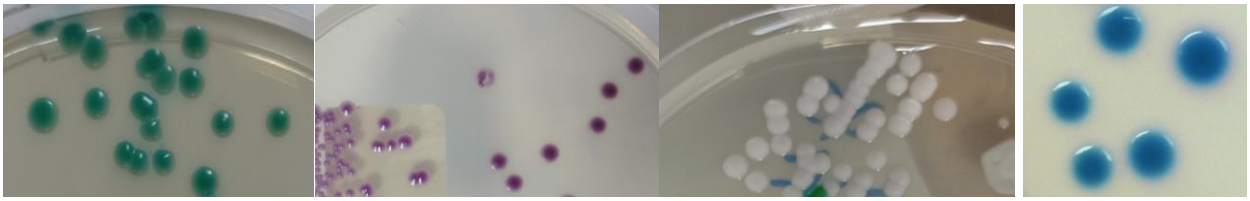
Variables	non-colonized non-infected (n=129)	Colonization (n=116)	Infection (n=17)	colonized infected (N=12)	Overall participants (N=250)	P-value
Neoplasm						
No	117 (90.7%)	95 (81.9%)	8 (47.1%)	5 (41.7%)	215 (86.0%)	**<0.001**$
Unknown	0 (0%)	20 (17.2%)	8 (47.1%)	1 (8.3%)	34 (13.6%)	
Yes	12 (9.3%)	1 (0.9%)	1 (5.9%)	6 (50.0%)	1 (0.4%)	
Steroids						
No	102 (79.1%)	79 (68.1%)	9 (52.9%)	6 (50.0%)	184 (73.6%)	0.093$
Unknown	1 (0.8%)	4 (3.4%)	2 (11.8%)	1 (8.3%)	6 (2.4%)	
Yes	26 (20.2%)	33 (28.4%)	6 (35.3%)	5 (41.7%)	60 (24.0%)	

Note: The bold p values represent the significant results among the non-colonized non-infected vs. colonized infected #-represents the independent T-test and $-represents the independent Chi-square test.

4.2 Yeast species distribution

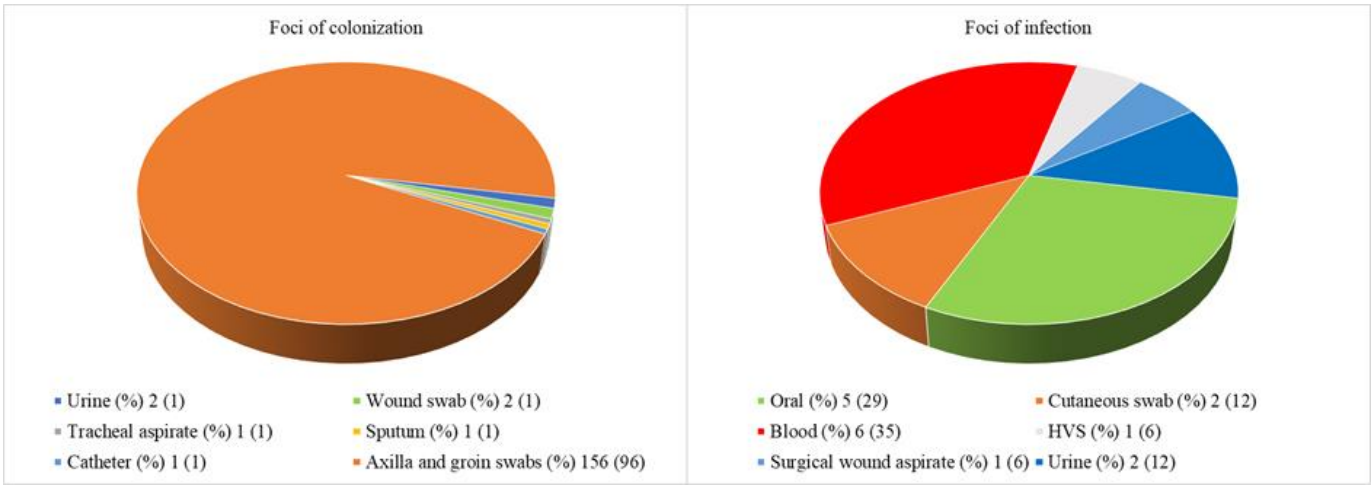
A total of 180 isolates from 250 patient samples were available for analysis, and they were all included in the revival process. The yeast isolates archived at -80 °C in brain heart infusion with 10% glycerol were thawed and revived on SDA plates. All isolates were viable after culture. After incubation at 37 °C for 24 hours, the isolates were presumptively identified on Chromagar *Candida* subcultures based on their colonial morphology. *Candida albicans* isolates gave distinctive blue-green colonies that were not seen with any of the other yeast isolates. The color of *Candida parapsilosis* colonies were also distinctive but variable. The isolates formed large to small colonies with a pale color, subjectively described as "dirty pink" to mauve. *Candida tropicalis* colonies appeared dark blue in contrast to *Candida duobushaemulonii* colonies which were white, while most of the other isolates formed colonies with a color that ranged from white to pink to mauve. (Figure 2). VITEK®2 confirmatory ID of the presumptive yeasts on culture plates identified 8 types of yeast organisms for which majority were consistent with the Chromagar *Candida* identification.

Figure 2. Growth of the presumptive yeast spp. on Chromagar *Candida*



Out of these yeast isolates, 156 (86.67 percent) were from carriage samples obtained from composite axilla and groin swabs. The remaining 24 (13.33 percent) isolates were mostly recovered from blood (n=6), oral (n= 5), urine (n=4) wound and cutaneous swabs (n=2) and one (1) each of sputum, tracheal aspirate, catheter, and high vaginal swab (Figure 3). Yeast clinical isolates from seven (7) of the 24 samples were considered colonizers, with 17 causing yeast infections (Figure 3). The distribution of yeasts recovered in carriage and clinical samples was variable.

Figure 3. Distribution of various samples for screening of yeast colonization and infection.

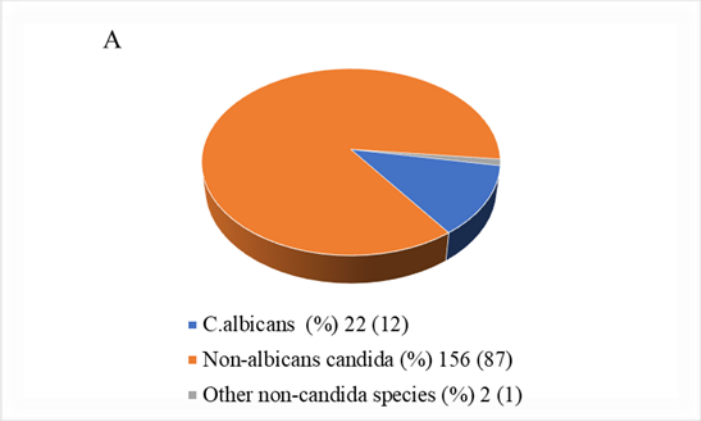


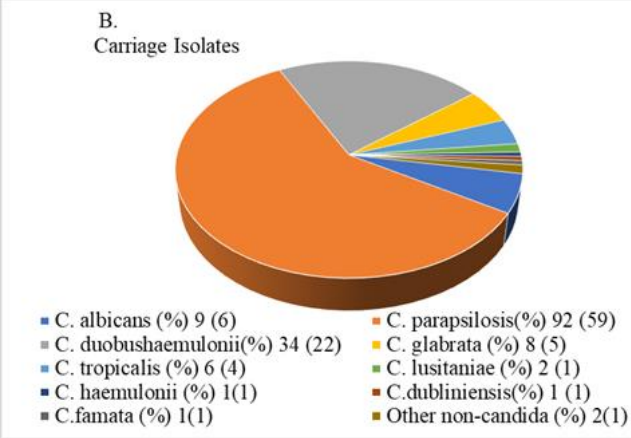
Overall, non-albicans *Candida* species were the most frequently isolated (86.7 percent) followed by *Candida albicans* (12.2 percent) and yeasts other than *Candida* (1.1 percent) as shown in Figure 4A. Among NAC species, *Candida parapsilosis* (53.89 percent), *Candida duobushaemulonii* (19.44 percent) and *Candida glabrata* (6.11 percent) were the most commonly recovered species. Of the yeasts other than *Candida*, only *Trichosporon inkin* was identified (1.11 percent).

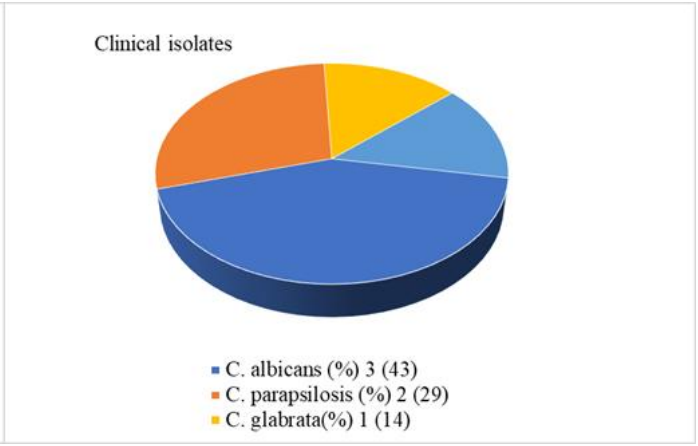
Among the colonized group, majority of the isolates were recovered from carriage samples with *Candida parapsilosis* being isolated in 59 percent of the carriage samples, followed by *Candida duobushaemulonii* in 34 percent and *Candida albicans* in 5. 8 percent. In clinical samples, *Candida albicans* was recovered in 2.6 percent of colonized cases, and non-albicans *Candida* in 3.4 percent, all from non-sterile sites without site-specific symptoms (Figure 4B).

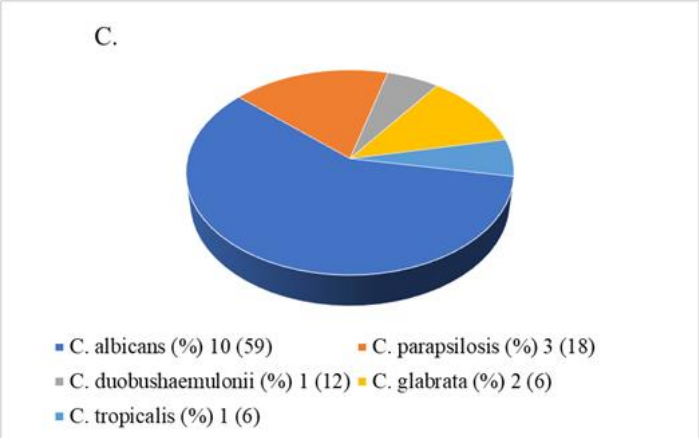
Candida albicans was isolated from 58.82 percent of clinical isolates in the infected group (Figure 4C). Blood samples showed the highest percentage of NAC species in positive samples. When the distribution of the infection agents according to the frequently seen yeast infection was studied, three (3) cases of candidemia was due to *Candida parapsilosis,* two (2) of *Candida glabrata* and one (1) *Candida duobushaemulonii*. *Candida albicans* was identified in all cases of oropharyngeal candidiasis, vulvovaginal candidiasis and candiduria.

Figure 4. Graphical depiction of (A) *C. albicans* vs. non-albicans *Candida* distribution in the entire study population, (B) *Candida* species distribution in the colonized group, and (C) *Candida* species distribution in the infected group.









4.3 Antifungal susceptibility patterns

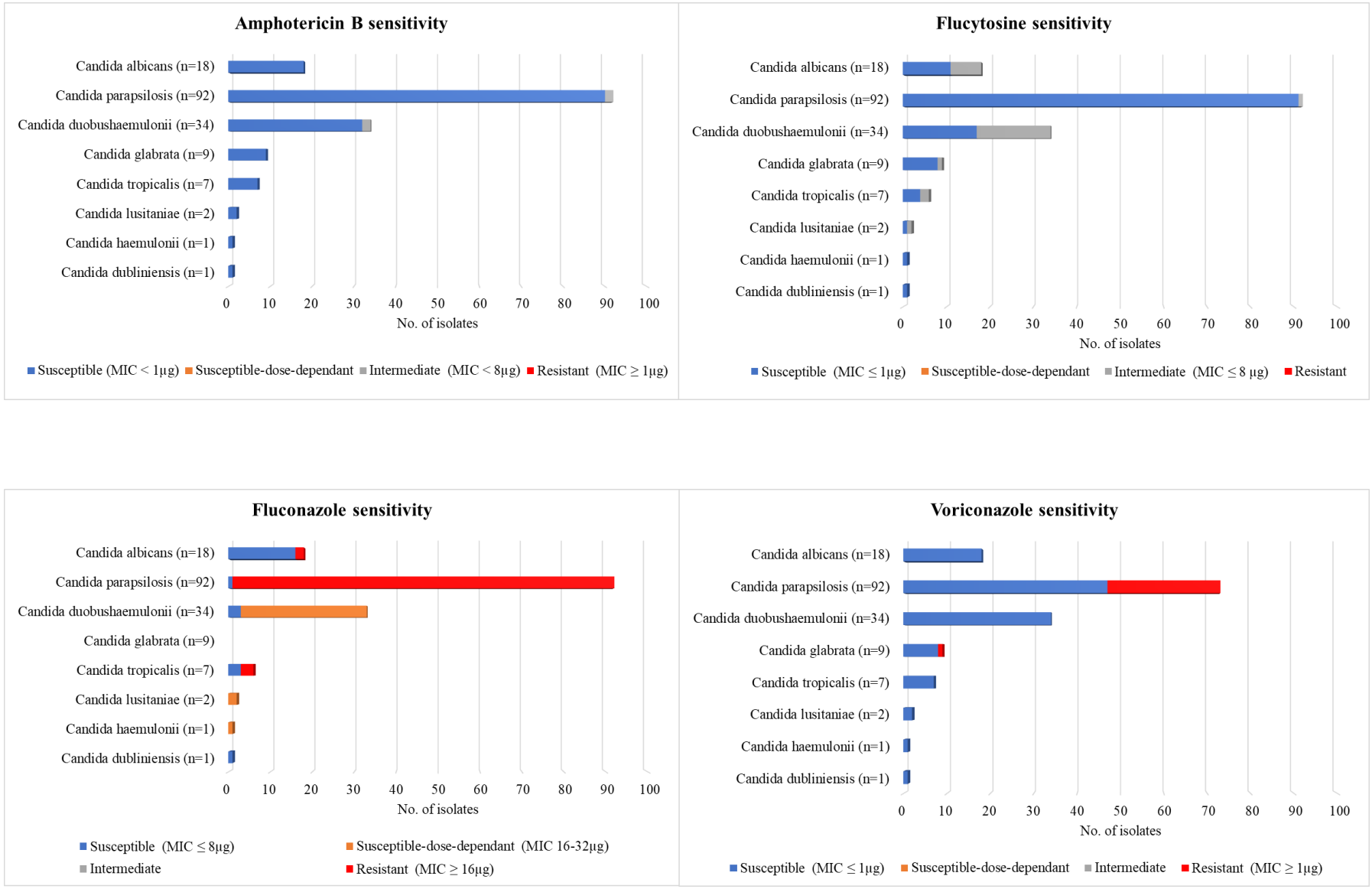
Antifungal susceptibility tests were conducted for 167/180 isolates against Amphotericin B, Flucytosine, Fluconazole, Voriconazole, Caspofungin, and Micafungin, covering the four drug classes of antifungal drugs. The antifungal susceptibility testing was performed using a commercial testing method, VITEK 2, and interpreted based on CLSI guidelines. The resulting output was the minimum inhibitory concentration (MIC), which was then interpreted as susceptible, susceptible-dose dependent, intermediate, or resistant.

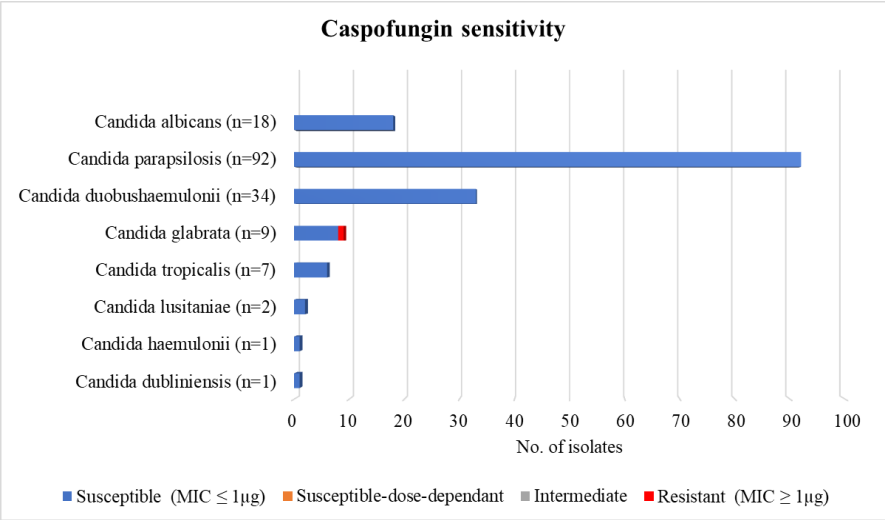
Echinocandins, caspofungin, and micafungin, showed the greatest antifungal activity against most yeast isolates being susceptible. The prevalence of fluconazole resistance was the highest among *Candida parapsilosis* (98.9 percent) followed by voriconazole resistance (35.6 percent) as shown in Figure 5. The highest MIC value was observed in fluconazole at 32µg/ml for *Candida duobushaemulonii*. Meanwhile, micafungin had the lowest MIC value at ≤0.06µg/ml as detailed in Table 3. Clinical and colonizing isolates' susceptibility patterns to all tested antifungals were comparable. However, the VITEK system was unable to report the MIC values of *Candida famata*, *Trichosporon inkin*, and for some antifungal/organism combinations like fluconazole/*Candida glabrata* and micafungin/*Candida duobushaemulonii*.

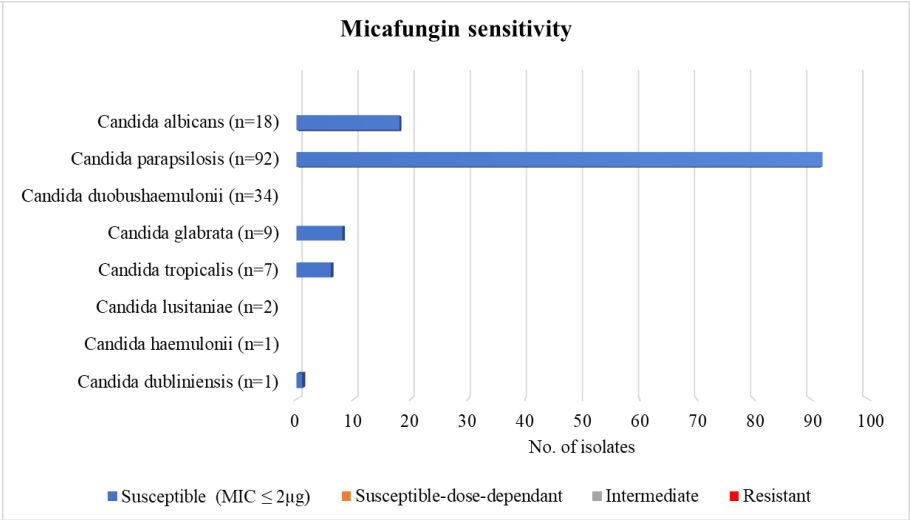
Table 3.MIC distributions for antifungal agents tested against yeast isolates

MIC (µg/ml) / Antifungal	≤ 0.06	0.12	0.25	0.5	1	2	4	8	12	16	32	Total(n)	MIC range(µg/ml)
Amphotericin B			88 (53.7%)	21 (12.8%)	51 (31.1%)	1 (0.6%)		3 (1.8%)				164	≤ 0.25-8
Flucytosine					62 (38.0%)	64 (39.3%)	8 (4.9%)	21 (12.9%)		8 (4.9%)		163	≤1-16
Fluconazole				6 (3.9%)	11 (7.2%)	1 (0.7%)			80 (52.3%)	16 (10.5%)	39 (25.5%)	153	≤ 0.5-32
Voriconazole		31 (21.4%)	8 (5.5%)	31 (21.4%)	48 (33.1%)		27 (18.6%)					145	≤ 0.12-4
Caspofungin		30 (18.5%)	2 (1.6%)	73 (45.1%)	39 (24.1%)	5 (3.1%)			1 (0.6%)			162	≤0.12-2
Micafungin	31 (24.8%)	2 (1.6%)	1 (0.8%)	60 (48.0%)		31 (24.8%)						125	≤0.06-2

Figure 5. Antifungal susceptibility profiles of isolated colonizing and infecting yeast species.




Caspofungin sensitivity
Candida albicans (n=18)
Candida parapsilosis (n=92)
Candida duobushaemulonii (n=34)
Candida glabrata (n=9)
Candida tropicalis (n=7)
Candida lusitaniae (n=2)
Candida haemulonii (n=1)
Candida dubliniensis (n=1)
0 10 20 30 40 50 60 70 80 90 100
No. of isolates
Susceptible (MIC ≤ 1µg)
Susceptible-dose-dependant
Intermediate
Resistant (MIC ≥ 1µg)


Micafungin sensitivity
Candida albicans (n=18)
Candida parapsilosis (n=92)
Candida duobushaemulonii (n=34)
Candida glabrata (n=9)
Candida tropicalis (n=7)
Candida lusitaniae (n=2)
Candida haemulonii (n=1)
Candida dubliniensis (n=1)
0 10 20 30 40 50 60 70 80 90 100
No. of isolates
Susceptible (MIC ≤ 2µg)
Susceptible-dose-dependant
Intermediate
Resistant

4.4 Risk factors associated with colonization and/or infection

A backward stepwise regression analyses was performed to determine the risk factors associated with colonization and/or infection out of the following candidate variables: age, sex, CCU length of stay, presence of a urinary catheter, previous antibiotic and antifungal therapy, diabetes, neoplasm, steroid use and colonization status. Variables with the lowest Akaike Information Criterion (AIC) were included in the final model.

The most significant independent risk factor associated with colonization was previous antibiotic therapy (aOR=1.89, 95% CI 1.06-3.39, P= 0.032). These suggests that patients admitted to the CCU who had history of antibiotic therapy were associated with an increase in the likelihood of being colonized (Table 4).

Previous antifungal therapy (aOR=4229.22, 95% CI 120.89-6346317.47, P= 0.001) and colonization (aOR=13.86, 95% CI 1.59-528.43, P=0.049) were significantly associated with infection in the multiple logistic models. A colonized patient was 13 times more likely to develop an infection. Likewise, patients with prior antifungal therapy history were associated with a higher rate of developing an infection (Table 4).

In the logistic regression analysis, compared with non-colonized non-infected patients, colonized-infected patients were likely to stay longer in the CCU (aOR=1.08, 95% CI 1.01-1.16, P=0.023), to have had a prior antifungal therapy (aOR=172.76, 95% CI 18.07-12678.34, P<0.001), and to have had neoplasm (OR=27.41%, 95% CI 2.36-2310.28, P=0.030) (Table 4).

Table 4.Analysis to determine the risk factors for colonization and/or infection among participating patients.

Variables	**Univariate logistic regression**						**Multiple logistic models**					
	Colonization		**Infection**		**Colonized infected**		**Colonization**		**Infection**		**Colonized infected**	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**
Age in years	1.00 (0.98-1.01)	0.475	0.99 (0.97-1.02)	0.555	1.00 (0.97-1.03)	0.919						
Sex												
Female	1.00		1.00		1.00							
Male	1.25 (0.76-2.07)	0.382	1.19 (0.44-3.38)	0.730	1.79 (0.54 - 7.00)	0.359						
CCU length stay in days	1.03 (1.00-1.08)	0.079	1.07 (1.03-1.11)	0.002	**1.07 (1.02 - 1.15)**	**0.019**	1.02 (0.99-1.06)	0.183	1.07 (0.98-1.15)	0.060	1.08 (1.01 - 1.16)	**0.023**
Urinary catheter												
No	1.00		1.00		1.00				1.00		1.00	
Yes	1.26 (0.76-2.08)	0.371	6.59 (1.81-42.43)	0.014	**4.77 (1.20 - 31.85)**	**0.049**			242.70 (3.15-2162866.6)	0.098	18.32 (0.51 - 7534.48)	0.177
CVC												
No	1.00		1.00		1.00							
Yes	1.33 (0.69-2.61)	0.395	2.98 (0.98-8.38)	0.042	2.20 (0.45 - 8.24)	0.272						

Previous antibiotics therapy												
No	1.00		1.00		1.00		1.00		1.00		1.00	
Yes	2.03 (1.21-3.44)	**0.007**	5.44 (1.49-35.05)	**0.027**	**4.92 (1.24 - 32.85)**	**0.045**	**1.89 (1.06-3.39)**	**0.032**	0.03 (0.00-1.06)	0.071	0.08 (0.00 - 2.51)	0.165
Previous antifungal therapy												
No	1.00		1.00		1.00				1.00		1.00	
Yes	1.60 (0.65-4.07)	0.306	150.67 (39.56-778.08)	<0.001	**93.75 (20.41 - 581.10)**	**<0.001**			**4229.22 (120.89-6346317.47)**	**0.001**	**172.76 (18.07 - 12678.34)**	**<0.001**
Diabetes												
No	1.00		1.00		1.00							
Yes	1.16 (0.65-2.07)	0.617	0.95 (0.26-2.81)	0.931	1.10 (0.23 - 3.96)	0.891						
TPN												
No	1.00		1.00									
Yes	2.09 (0.61-8.14)	0.250	3.32 (0.48-14.40)	0.146								
Surgical procedures												
No	1.00		1.00				1.00		1.00			

Yes	1.00 (0.55-1.82)	0.996	2.63 (0.91-7.20)	0.062			0.72 (0.37-1.38)	0.323	30.05 (1.45-3213.50)	0.077		
Neoplasm												
No	1.00		1.00		1.00		1.00		1.00		1.00	
Yes	1.79 (0.86-3.79)	0.121	7.08 (2.46-20.14)	<0.001	**9.75 (2.68 - 36.14)**	**<0.001**	1.54 (0.73-3.34)	0.263	4.12 (0.50-34.26)	0.172	**27.41 (2.36 - 2310.28)**	**0.030**
Steroids												
No	1.00		1.00		1.00							
Yes	1.58 (0.88-2.84)	0.127	1.81 (0.60-4.99)	0.264	2.83 (0.78 - 9.60)	0.096						
Colonization												
No			1.00	1.00								
Yes			**2.98 (1.07-9.60)**	**0.047**					**13.86 (1.59-528.43)**	**0.049**		

Note: OR represents the odds ratio. **95% CI** represents a 95% Confidence Interval

CHAPTER FIVE

5.0 DISCUSSION

This study provides information regarding the species spectrum and antifungal susceptibility profiles of yeasts isolated from critically ill patients using data from a single center over 11 months. Yeasts particularly *Candida* species have increased in the last few decades, especially in critically ill patients, and have been responsible for infections indicating the importance of rapid and accurate identification of species involved to guide antifungal therapy (Girão et al., 2008; Lopes Colombo et al., 2003). Taking into account the data from the current study, we have demonstrated that there continues to be a need for continued and extensive surveillance as a tool to assess the clinical relevance and the variables associated with the isolation of these species.

Amongst the 250 studied critically ill patients, almost 46 percent were colonized with at least one yeast species, and 6.8 percent developed an infection, even though the diagnosis of invasive yeast infection was made in less than 5 percent of the cases, with *Candida* species being the causative agent. Non-albicans *Candida* was the most frequent species representing approximately 86 percent of the isolates, and *Candida albicans* was the second most common species involved in infection and colonization. Our finding of higher NAC frequency was consistent with previous studies conducted in Ethiopia (41.4 percent), Egypt (65 percent), Nigeria (51.5 percent), India (67.6 percent), Iran (33 percent), and Greece (24.4 percent), highlighting the shift in *Candida* species trends towards NAC species which could be due to true prevalence change or improved detection rate of NAC species (Seyoum et al., 2020). *Candida parapsilosis* was the most common NAC isolate, as depicted in another study conducted by Sahal and Bilkay (Sahal & Bilkay, 2018). Several virulence mechanisms of *Candida parapsilosis* have been proposed, such as adhesion, biofilm formation, and dissemination which could explain its preponderance. Species other than *Candida* accounted for one percent of the isolates.

Philippe Eggimann et al. determined the role of *Candida* colonization in the development of subsequent infection in critically ill patients and documented a rate of infection of 1.7 percent, although this rate increased to 38 percent in patients at high risk defined by the intensity of *Candida* colonization (Eggimann et al., 1999; Eggimann & Pittet, 2014). In our study, candidemia was reported in six patients (2.4 percent), one in the non-colonized group, and five colonized cases. All

cases were caused by NAC species, with *Candida parapsilosis* causing BSI in three of the patients and the others caused by *Candida glabrata* (2) and *Candida duobushaemulonii*, which could show that NAC infections are on an upward trend (Pfaller & Diekema, 2007).

Of note, *Candida parapsilosis* is likely the most common species implicated in infection in some countries and is isolated across all age strata, whilst, in other countries, its frequency is heavily in infant candidiasis (Nucci et al., 2013). *Candida glabrata* on the other hand is emerging as a significant opportunistic pathogen worldwide. It is the second most common yeast isolated as part of normal flora, and its pathogenic role was only recently recognized (Pfaller, 2012). However, *Candida* spp. distribution varies across the globe. In North America, *Candida albicans* is the most prevalent cause of invasive candidiasis, just as seen in North Europe, while the species landscape of South Europe is more akin to that in South America, where *Candida parapsilosis* is the most common NAC species. In Central Europe, *Candida glabrata* is of increasing prominence. Across West Asia, *Candida tropicalis* might be the primary etiologic agent (Boonsilp et al., 2021; Hesstvedt et al., 2017; Puig-Asensio et al., 2014; Yamin et al., 2020). This variation is not readily explained but may be due to differences in the cohort mix. In our cohort of infected patients, colonization status was considered. Across Africa, species and antifungal susceptibility data are comparatively scarce and, thus, the utility of our study results. Some studies have reported that *Candida parapsilosis* caused more than 20 percent of candidemia cases in South Africa (Daneshnia et al., 2023), while *Candida albicans* caused the majority of non-invasive candidiasis in Ethiopia (Bitew & Abebaw, 2018; Mulu et al., 2013)

Herein, we found *Candida albicans* accounting for all oropharyngeal candidiasis (n=5), vulvovaginal candidiasis (n=1) and candiduria (n=2) cases. This finding is consistent with previous findings of *Candida albicans* as the most prevalent yeast causing particular infections in Kenya (Kangogo et al., 2011; Mutua et al., 2010; Ooga et al., 2011). Among the rarely encountered species, *Candida dubliniensis*, *Candida famata*, *Candida lusitaniae*, and *Trichosporon inkin* were isolated. Despite not being implicated in any infection in our study, BSI due to uncommon *Candida* species is emerging, and local epidemiological trends have important implications for clinical management (Blyth et al., 2009; H. Li et al., 2020). Variation in species distribution was noted among different samples. For screening patients for colonization of *Candida* species, CDC recommends axilla, groin, urine, nose, throat, perianal swab, rectal swab, or stool samples

(Fasciana et al., 2020). In our study, composite axilla & and inguinal swabs were used as they have been reported to have the highest positivity rate for colonization, and *Candida parapsilosis* was the highest recovered colonizing isolate. Interestingly, all isolates from clinical samples (sputum, tracheal aspirate, urine, and catheter) implicated in colonization were similar to those recovered from carriage swabs. Among the 4.8 percent of colonized infected patients, half were of the same species. The choice of surveillance sample would be ideal when assessing the intensity of colonization or *Candida* colonization index.

Early identification of people with a yeast infection or colonization can allow for intervention strategies such as basic infection control measures. CHROMagar *Candida* allowed for presumptive identification of some *Candida* species through observation of contrasting colony morphology and color resulting from reactions of species-specific enzymes with proprietary chromogenic substrate (Odds & Bernaerts, 1994). It has been shown to be useful in the differentiation of *Candida auris* from *Candida haemulonii* complex species (Garcia-Bustos et al., 2021); however, the utility of CHROMagar with *Candida parapsilosis* is more limited given the range of colony colors and morphologies (Hospenthal et al., 2006). We used the VITEK 2 automated system to identify and determine the susceptibility of isolated yeast since it is reliable and demonstrates excellent reproducibility, which underscores its excellent level of standardization. Also, the spectrophotometric readings remove subjectivity from the MIC determination as well as reduce the time necessary for optimizing antifungal treatment decisions (Berkow et al., 2020). However, VITEK misidentifies rare and emerging clinical isolates of the closely related *Candida haemulonii* complex species as *Candida haemulonii*, and *Candida duobushaemulonii* (Černáková et al., 2021), which could explain the high isolation rate of *Candia duobushaemulonii* and the need for confirmatory methods like molecular methods or matrix-associated laser desorption/ionization-time of flight (MALDI-TOF).

Understanding the local epidemiology and antifungal susceptibility patterns is of utmost significance for patient management, especially with the emergence of predominant NAC species. There are various antifungal classes available: azoles, polyenes, and echinocandins. Azoles, specifically fluconazole, was the primary treatment option for invasive yeast infections for many years, but the continuing emergence of resistance has now limited its clinical utility (Bassetti et al., 2018; Pappas et al., 2016). A noteworthy finding of our study was the increased resistance to

azoles. High-level azole resistance was mainly observed in *Candida parapsilosis*, with resistance rates of 98.91 percent to fluconazole, and 35.6 percent to voriconazole, all higher than those seen in a surveillance study in South Africa (Govender et al., 2016). Also, in agreement with our study, Dina Yamin reports the emergence of *Candida parapsilosis* drug resistance (Yamin et al., 2022). Whether this emergence and difference is because of strain types, clinical practice issues, or both is unclear.

A small proportion of *Candida albicans* isolates were resistant to fluconazole at 11.1 percent and none were resistant to voriconazole. A laboratory-based study conducted in Kenya earlier showed a resistance pattern of *Candida albicans* towards fluconazole at a rate of 26 percent (Ooga et al., 2011). Resistance to fluconazole by *Candida albicans* has mostly been reported among HIV-infected patients with oropharyngeal candidiasis receiving prolonged fluconazole treatment, affecting up to 21 percent of these patients (Sangeorzan et al., 1994). Resistance to fluconazole was also observed in some *Candida tropicalis* isolates, which is consistent with previous reports that *Candida tropicalis* exhibit intrinsically lower susceptibility to the azole class with prevalence and resistance prevalence varying with geographic region (Jin et al., 2018; Oxman et al., 2010). Nearly all *Candida duobushaemulonii* isolates exhibited susceptible dose dependence and revealed elevated MIC for fluconazole (32 µg/ml). Broth microdilution and E-tests should be used, when possible, to evaluate for resistance since its detection is important because it can cause invasive infections. The MIC for *Candida glabrata*, *Trichosporon inkin*, and *Candida famata* to fluconazole and some tested drugs were not provided by the VITEK.

Polyenes, once the mainstay antifungal class for invasive candidiasis treatment, acting as fungicidal were reserved for specific conditions due to their toxicity (Ben-Ami, 2018). In the present study, amphotericin B displayed good antifungal activity against all *Candida* species tested, as shown in another study (Maraki et al., 2019). On the other hand, flucytosine, a nucleoside analog, has in-vitro activity against many *Candida* species isolates, but it is not widely used due to drug toxicity as well as the frequent development of resistance when used as a single agent. In our study, 82.2 percent of the yeast isolates tested showed susceptibility while 17.8 percent were intermediate with an MIC of $\leq$ 4µg/ml and 8-16 µg/ml respectively. In contrast, higher rates of

resistance were reported in *Candida albicans* and *Candida glabrata* among clinal isolates in Italy (Barchiesi et al., 2000).

Echinocandins such as caspofungin and micafungin are the first-line therapy drugs against several forms of candidiasis, given their fungus-specific target of β-D-glucan-synthesis inhibition and improved safety and toxicity profiles. The in-vitro susceptibility of the yeast isolates was 100 percent and 99.4 percent to both micafungin and caspofungin, respectively. Notably, one colonizing *Candida glabrata* isolate was resistant to caspofungin in this study. Previous studies have reported that echinocandin resistance in susceptible *Candida* species arises after repeated or long-term exposure (Lortholary et al., 2011; Perlin et al., 2017). Multiple studies have reported the isolation of resistant *Candida* species to echinocandins across various geographical regions, with the highest level of resistance reported in India (Kordalewska & Perlin, 2019). No MIC values were obtained for *Trichosporon inkin* and *Candida famata* to echinocandins, and *Candida haemulonii* complex species to micafungin.

Several studies in different regions determined different possible risk factors for developing *Candida* species colonization and/or infection. These included prolonged stay in the ICU, increased exposure to antimicrobial agents, use of CVC, corticosteroids exposure, TPN, surgical intervention, and co-morbidities such as diabetes and lung disease (Lau et al., 2015; Ostrosky-Zeichner et al., 2007; Stratman et al., 2010), some of which we assessed in the current study. In our study, previous antibiotic therapy (OR 1.89) was significantly associated with colonization. Previous antifungal therapy (OR=0.001) and colonization (OR=0.049) were both associated with a significant increase in infection. In a previous study of candidiasis in critically ill patients admitted to the ICU, previous antifungal treatment was the only independent risk factor for the isolation of *Candida* species (Álvarez-Lerma et al., 2003). Although not always found, prior *Candida* colonization was considered an important risk factor for yeast infections in several studies. A study by Pittet et al. showed that the intensity of *Candida* colonization assessed by systematic screening helps in predicting subsequent infections in critically ill patients (Pittet et al., 1994). To find out any significant attributable risk factor for colonized infected CCU patients, we performed a risk factor comparison analysis with the non-colonized non-infected group. CCU length of stay (OR=0.023), prior antifungal therapy (OR=<0.001), and neoplasm (OR=0.030) were

independent variables significantly associated with higher rates of yeast species colonization infection. This was in agreement with findings from other studies (Chen et al., 2008; J. K. Chow et al., 2008; Peres-Bota et al., 2004). However, we found no significant differences between colonization and/or infection groups with respect to demographics or classic risk factors including TPN and steroids use in contrast to data reported by others probably due to the sample size (Beck-Sagué et al., 1993; McKinnon et al., 2001).

Our study has certain limitations, primarily among them its retrospective nature, the interrelated problems of monocentric design, and the relatively small sample size which might explain the absence of significant p-values for some variables. In addition, due to limited resources, we could not use molecular sequencing of targets or MALDI-TOF MS to differentiate the *Candida haemulonii* complex species, rather than using commercial biochemical systems that misidentifies the closely related species (Černáková et al., 2021). However, all the isolates have been stocked for possible molecular typing in the future. This knowledge will help in investigating any future outbreaks of *Candida haemulonii* complex infections at the university hospital by determining whether such infections are nosocomial or not. Nevertheless, this study does provide important epidemiological findings which pave the way for more in-depth studies that will help establish improved antifungal stewardship in our institutions.

5.1 CONCLUSION

The present study has provided updated information on the species spectrum, and antifungal susceptibility profiles of *Candida* species at a university hospital, demonstrating a change in the species spectrum landscape from previous dominance of *Candida albicans* to NAC, in particular, an increasing contribution of *Candida parapsilosis*. Overall resistance of the study isolates to flucytosine, echinocandins and Amphotericin B remained low. However, *Candida parapsilosis* exhibited reduced susceptibility to azoles, particularly fluconazole which may demonstrate the unfortunate realities in the majority of *Candida parapsilosis* infections. Previous antibiotic therapy was identified as an independent risk factor for colonization while previous antifungal therapy and colonization were significantly associated with infection. Compared with non-colonized non-infected patients, independent risk factors associated with colonized-infected patients were CCU length of stay, prior antifungal therapy and neoplasm.

With shifting patterns of epidemiology, this study emphasizes the importance of continued surveillance, antifungal stewardship, knowledge of risk factors for yeast infection amongst critically ill patients and application of infection control measures, a timely reminder that pathogenic yeasts deserve equal attention in the new era of emerging infectious diseases.

5.2 RECOMMENDATIONS

- The results presented in this dissertation represent findings from one hospital setting, in the private sector. For overall inclusion and/or in policy and patient management guidelines, a multicenter study would be necessary to assess these variables in different settings.
- The observed change in trends in species distribution and antifungal susceptibilities warrant surveillance. For instance, regular monitoring would promptly identify rising trends in azole resistance in *Candida* parapsilosis, especially in settings that use fluconazole for prophylaxis
- More elaborate knowledge of etiologic agents with regular and accurate identification of an antifungal susceptibility patterns are necessary for high-risk settings, such as critical care units. This will require equipping laboratories and training personnel on mycological diagnostic techniques.

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APPENDICES

Appendix 1: Study Case Report Form

Participant No:

Age:

Sex:

Race:

Hospitalization unit:

CCU length of stay, days:

Any underlying condition: Chronic liver disease:
Cardiovascular disease:
Chronic renal failure:
Hematologic malignancy:
Respiratory disease:
Neurologic disorder
HIV infection:
Other:

Chemotherapy:

Radiation therapy:

Any other immunosuppression:

Urinary catheter:

CVC:

Renal replacement therapy:

Previous antibiotic therapy:

Previous antifungal therapy:

Duration and indication for antibiotic and antifungal therapy:

Mechanical ventilation:

Total parenteral nutrition:

Major surgical procedures:

Neoplasm:

Steroids:

Laboratory test results

Isolated yeast Species	Specimen	Colonization status	Infection status
Candida tropicalis			
Candida parapsilosis			
Candida glabrata			
Candida lusitaniae			
Candida dubliensis			
Candida duobushaemulonii			
Other species (*Candida krusei*, *Trichosporon spp* or any other)			

Susceptibility testing results

Drug	Susceptible	Intermediate	Susceptible dose-dependent	Resistant
Flucytosine				
Amphotericin B				
Micafungin				
Caspofungin				
Voriconazole				
Fluconazole				

Appendix 2: Budget

Item	**Unit cost (Kshs.)**	**Quantity**	**Total cost (Kshs.)**
Sabouraud dextrose agar	4,200	3	12,600
Chromagar *Candida*	150,000	1	150,000
Sterile plastic Petri dishes	18,560	1 box	18,560
Microscope slides and covers, paper towels, gauze	10,000	1	10,000
Nitrile gloves	1,300	2	2600
VITEK 2 YST ID	12,500	10(for 200 tests)	125,000
VITEK saline solution	25,000	1 box	25,000
VITEK unsensitized	12,000	1 carton	12,000
Sterile swabs	24,000	1	24,000
VITEK 2 AST-YST 08	22,500	10(for 200 tests)	225,000
Stationery and printing	N/A	N/A	20,000
Publication fees	N/A	N/A	200,000
Miscellaneous	N/A	N/A	50,000
Grand total	N/A	N/A	874,760

Appendix 3: Principal Investigator's approval letter.

THE AGA KHAN UNIVERSITY

10th February 2023.

Dr Christopher Opio
Chairman ERC AKU
Research Office Secretariat

RE: APPROVAL FOR USE OF ARCHIVED SAMPLES.

Dear Dr Opio,

This is to confirm that I permit **Mr. Kipkoech Kevin; Reg No. H56/35361/2019**, Department of medical microbiology, University of Nairobi to use archived yeast isolate samples from the primary study (whose details are below as his primary supervisor on his Master's project. He was a volunteer microbiology technical support on the primary study. He is currently employed by AKU on one of my projects (AMR Surveillance, Fleming Fund Country Grant)

Primary Study title: Candida auris transmission dynamics at Aga Khan University Hospital in Nairobi, Kenya (AKUH, N)

AKU ERC Approval Ref: 2019/IERC-87 (v2)

Principle Investigator: Dr. Gunturu Revathi

Custodian of archived samples: Dr. Gunturu Revathi

Archived samples storage conditions: Under -80°C at AKUH, N microbiology laboratory as per their Standard operating procedure (SOP).

The archived samples are to be used for the nested study titled: Species spectrum and susceptibility profiles of yeasts isolated from critical care unit patients in a university hospital.

Should your office have any questions regarding the study, please feel free to contact me.

Sincerely,

Revathi

Dr. Gunturu Revathi
Dept. of Pathology
Principal Investigator Ref 2019/IERC-87 (v2)

Appendix 4: AKU-ISERC approval letter.

THE AGA KHAN UNIVERSITY

Faculty of Health Sciences
Medical College

Ref: 2023/ISERC-06 (v1)
March 2, 2023

Prof. Revathi Gunturu –Principal Investigator
Associate Professor and Head of Clinical Microbiology
Department of Pathology, Aga Khan University, Nairobi.

Kevin Kipkoech -Msc. Medical Microbiology,
University of Nairobi

Dear Dr Revathi, Mr. Kipkoech and team,

Re: Species Spectrum and Susceptibility Profiles of Yeasts Isolated from Critical Care Unit Patients in A University Hospital.

The Aga Khan University, Nairobi Institutional Scientific and Ethics Review Committee (ISERC), is in receipt of your protocol uploaded on Infonetica. The ISERC has reviewed and approved this project *{as per attached official stamped protocol and attachments - version Ref:* 2023/ISERC-06 (v1). You are authorized to conduct this study from **March 2, 2023**. This approval is valid until **March 1, 2024** and is subject to compliance with the following requirements;

1. The conduct of the study shall be governed at all times by all applicable national and international laws, rules and regulations. ISERC guidelines and Aga Khan University Hospital policies shall also apply, and you should notify the committee of any changes that may affect your research project (amendments, deviations and violations)
2. Researchers desiring to initiate research activities during COVID-19 pandemic must comply with the COVID-19 SOPs for Research as well as submit to the Research Office a Request Form to Initiate, Reinstate or Continue Research During COVID-19 Pandemic.
3. **Prior** to human subjects enrolment you must obtain a research license from the National Commission for Science, Technology and Innovation (NACOSTI), *where applicable*, site approvals from the targeted external site(s) and file the copies with the RO.
4. *As applicable*, **prior** to export of biological specimens/data, ensure a Material Transfer Agreement (MTA)/Data Transfer Agreement (DTA), is in place as well as seek shipment authority/permit from the relevant government ministry. Copies of these approvals, should be submitted to the RO for records purpose.
5. All Serious Adverse Events and the interventions undertaken must be reported to the ISERC as soon as they occur but not later than 48 hours. The SAE shall also be reported through the AKUHN quality monitoring mechanism(s) at Client Relations Department of the Chief of Staff's Office.
6. All consent forms must be filed in the study binder and where applicable, patient hospital record.
7. Further, you must provide an interim Progress Report Form **60 days before expiration** of the validity of this approval and request extension if additional time is required for study completion; as well as submit the completed Self-Assessment Tool -Monitoring Ethical Compliance in Research. You must advise the ISERC when this study is complete or discontinued and a final report submitted to the Research Office for record purposes.
8. The Aga Khan University Hospital management should be notified of manuscripts emanating from this work.

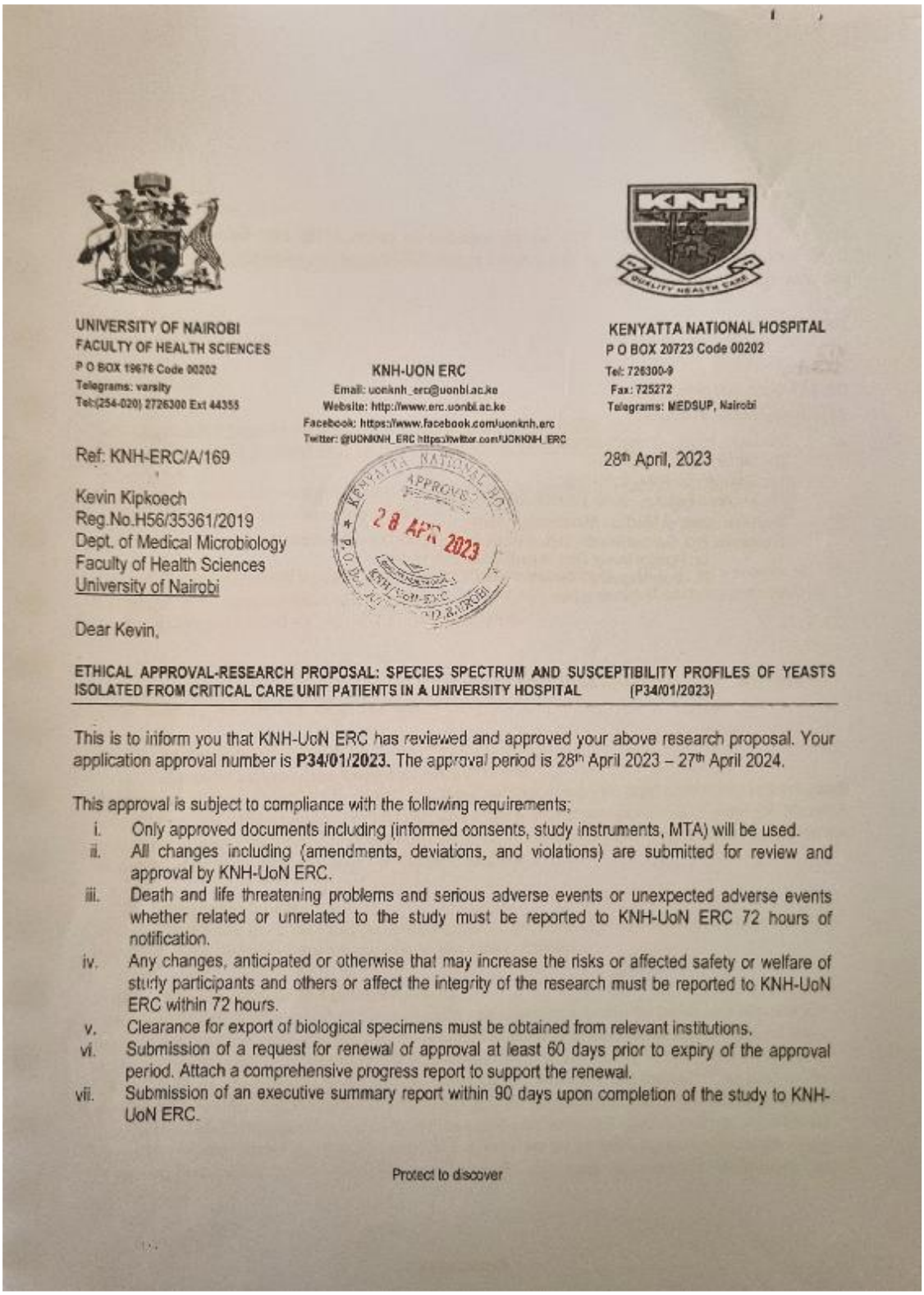
If you have any questions, please contact Research Office at AKUKenya.ResearchOffice@aku.edu or 020-366 2148/1136.

With best wishes,

Dr. Christopher Opio,
Chair - Institutional Scientific and Ethics Review Committee (ISERC)
Aga Khan University, (Kenya)
Copy: Co-Investigators

1

Appendix 5: KNH-UoN approval letter



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KENYATTA NATIONAL HOSPITAL
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Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/169

28th April, 2023

Kevin Kipkoech
Reg.No.H56/35361/2019
Dept. of Medical Microbiology
Faculty of Health Sciences
University of Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 28 APR 2023 P.O. BOX 20723 KNH-UON ERC NAIROBI

Dear Kevin,

ETHICAL APPROVAL-RESEARCH PROPOSAL: SPECIES SPECTRUM AND SUSCEPTIBILITY PROFILES OF YEASTS ISOLATED FROM CRITICAL CARE UNIT PATIENTS IN A UNIVERSITY HOSPITAL (P34/01/2023)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P34/01/2023.** The approval period is 28th April 2023 – 27th April 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.

Protect to discover

Appendix 6: Anti-plagiarism certificate

SPECIES SPECTRUM AND SUSCEPTIBILITY PROFILES OF YEASTS ISOLATED FROM CRITICAL CARE UNIT PATIENTS IN A UNIVERSITY HOSPITAL.

ORIGINALITY REPORT

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Dr Gunturu Revathi 7th September 2023

Dr. Florence Mutua September 7, 2023