MAKERERE  **UNIVERSITY**

COLLEGE OF HEALTH SCIENCES

School of Biomedical Sciences Department of Medical Microbiology

COLONISATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS OF *CANDIDA AURIS* AMONG PATIENTS AT INTENSIVE CARE UNITS AT MULAGO HOSPITAL

BY

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SEPTEMBER 202

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DEDICATION

This dissertation is lovingly dedicated to my beautiful wife, **Everlyne Kalungi**, whose unwavering support, patience, and encouragement have been my greatest source of strength throughout this academic journey. Your love and belief in me kept me grounded and focused, even during the most challenging times.

To my daughters and my son—your smiles, laughter, and presence have been my daily inspiration.

I also dedicate this work to my dear **brothers and sisters**, whose faith in me has never wavered and who have stood by me with encouragement and understanding.

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LIST OF ACRONYMS

AFR	Antifungal Resistance
AST	Antifungal Susceptibility Testing
C.auris	*Candida auris*
CDC	Centre of Disease Control and Prevention
CI	Confidence Interval
COVID-19 EUCAST	Corona Virus Disease European Committee on Antimicrobial Susceptibility Testing
ICU	Intensive Care Unit
IPC	Infection Prevention and Control
MALDI TOF	Matrix-assisted laser desorption/ionization time-of-flight
MIC	Minimum Inhibitory Concentration
MS	Mass Spectrometry
PR	Prevalence Ratio
SDA	Sabouraud Dextrose Agar
qPCR	Quantitative Polymerase Chain Reaction
WHO	World Health Organisation

LIST OF OPERATIONAL DEFINITIONS

1. **Antifungal Susceptibility Testing (AFST)**- A laboratory procedure used to determine the effectiveness of antifungal agents against *Candida auris* isolates, typically performed using the VITEK 2 system and interpreted using EUCAST clinical breakpoints.
2. **Candida auris (C. auris)**- A species of multidrug-resistant yeast that can cause invasive infections and is often misidentified by conventional laboratory methods.
3. **Colonisation**- The presence of *Candida auris* on a patient's body surface (e.g., axilla, groin, ear canal, or nose) without signs or symptoms of active infection.
4. **Convenience Sampling**- A non-probability sampling method in which study participants are selected based on their availability and willingness to participate during the study period.
5. **Cross-Sectional Study**- A type of observational study design that collects data at a single point in time to determine the prevalence and associated factors of *Candida auris* colonisation.
6. **Environmental Sampling**- Collection of swabs from non-patient surfaces in the ICU (e.g., bed rails, monitors) to detect potential environmental reservoirs of *Candida auris*.
7. **EUCAST Clinical Breakpoints**- Interpretive criteria established by the European Committee on Antimicrobial Susceptibility Testing used to categorize *Candida auris* isolates as susceptible (S), susceptible with increased exposure (I), or resistant (R) to antifungal drugs.
8. **Infection Prevention and Control (IPC)**- A set of practices aimed at preventing the spread of infections within healthcare settings, including hand hygiene, patient isolation, and environmental decontamination.
9. **Intensive Care Unit (ICU)**- A specialized hospital ward that provides intensive treatment and monitoring for critically ill patients.
10. **Minimum Inhibitory Concentration (MIC)**- The lowest concentration of an antifungal agent that visibly inhibits the growth of *Candida auris* in vitro.
11. **Multidrug Resistance (MDR)**- Resistance of *Candida auris* to two or more classes of antifungal agents, notably azoles, polyenes (e.g., amphotericin B), and echinocandins.
12. **Nosocomial Transmission**- The spread of *Candida auris* within a healthcare facility, particularly within ICUs, through person-to-person contact or via contaminated surfaces or equipment.
13. **Prevalence**- The proportion of ICU patients in the study who were found to be colonised with *Candida auris* at the time of sampling.

14. **Risk Factors**- Patient-related or hospital-related characteristics that increase the likelihood of colonisation with or infection by *Candida auris*. Examples include recent surgery, mechanical ventilation, and immunosuppressive therapy.

15. **VITEK 2 System**- An automated system used for yeast identification and antifungal susceptibility testing, including *Candida auris*.

ABSTRACT

Background:

Candida auris is a multidrug-resistant yeast identified as a global priority pathogen by the World Health Organization due to its ability to cause severe, difficult-to-treat infections, particularly in intensive care settings. Colonisation with *C. auris* — the presence of the organism on the skin or mucosal surfaces without overt infection — is a well-documented precursor to invasive disease. It facilitates nosocomial transmission, persistence in hospital environments, and eventual progression to bloodstream and deep-seated infections. Although multiple outbreaks have been reported globally and in neighboring countries such as Kenya and South Africa, there is limited data currently on *C. auris* colonisation or antifungal resistance in Uganda.

Objective:

To determine the prevalence of *Candida auris* colonisation, antifungal susceptibility patterns, and associated epidemiological risk factors among patients admitted to the intensive care units (ICUs) of Mulago National Referral Hospital in Uganda.

Methods:

A cross-sectional study was conducted over six months, involving 65 ICU patients enrolled through convenience sampling. Swabs were collected from the axilla, groin, ear canal, and nose, along with environmental swabs from the patients' surroundings. Samples were cultured on Sabouraud Dextrose Agar with chloramphenicol and CHROMagar Candida Plus, and incubated at both 35°C and 42°C to exploit the thermotolerance of *C. auris*. Colonies with characteristic morphology underwent Gram staining and germ tube testing before confirmation using the VITEK 2 Compact system. Antifungal susceptibility testing was performed using VITEK AST-YS08 cards, and results were interpreted using EUCAST Clinical Breakpoints (version 11.0). Patient clinical and demographic data were extracted from medical records. Associations between colonisation and potential risk factors were assessed using modified Poisson regression with robust standard errors.

Results:

Candida auris was isolated in 21 of the 65 patients, yielding a colonisation prevalence of 32.3% (95% CI: 21.9–44.9%). The most frequently colonised sites were the axilla (33.3%) and groin (28.6%), areas prone to moisture and clinical manipulation. All 21 isolates were resistant to fluconazole and amphotericin B (100%) but were susceptible to echinocandins (micafungin and caspofungin). One isolate showed intermediate susceptibility to voriconazole. Colonisation with *C. auris*, a key step preceding clinical infection, was independently associated with low

hemoglobin levels (anemia) (PR = 2.55; 95% CI: 1.09–5.99; p = 0.031), suggesting a link with underlying immunosuppression or chronic illness.

Conclusion:

The study reveals a significant burden of *Candida auris* colonisation among ICU patients in Uganda, with one in three patients colonised. The complete resistance to fluconazole and amphotericin B highlights the challenge of treating potential infections that may evolve from colonisation. The strong association between colonisation and anemia highlights the vulnerability of immunocompromised individuals. Given that colonisation precedes infection in over half of *C. auris* cases globally, early detection through routine screening and antifungal stewardship are urgently needed to prevent invasive disease and control transmission in ICU settings.

Key Words: *Candida auris*, colonisation, antifungal resistance, ICU, Mulago Hospital, VITEK 2, fluconazole, echinocandins, anemia, nosocomial infection.

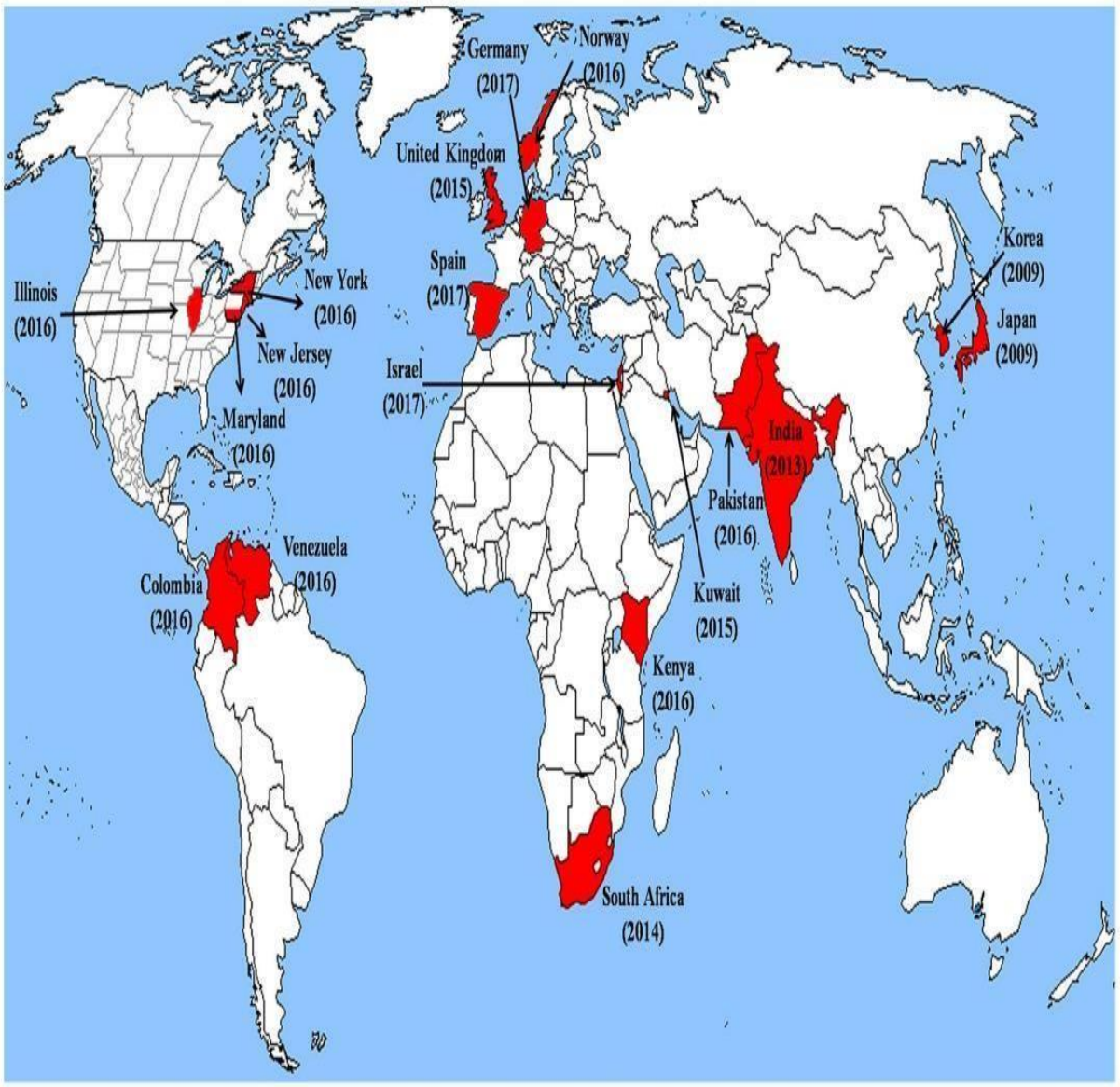


Fig 1. A global map depicting rapid emergence of multidrug-resistant clinical *Candida auris* strains in 5 continents. The value in parentheses denotes the year of report of *C. auris* from the respective country or state.

Figure 1:Map Showing the Global depiction of *Candida auris* emergence .

CHAPTER ONE:

1.0. INTRODUCTION

1.1 Background

In October of 2022, a list for priority fungal pathogens was published by the World Health Organization and one of the fungi on this list is *Candida auris* ; pathogen that has garnered traction in the recent years (WHO, 25 October 2022). On the global stage, 2009 saw isolation of a single strain of a new species of ascomycetous yeast belonging to the genus Candida discovered in the external ear of a Japanese patient and classified as *C. auris* at the taxonomic species level (Satoh et al., 2009).

In a hospital environment, C. auris colonizes the skin, axillae, groin, and other areas. and is able to be transmitted from person to person. C auris also has the ability to colonize inanimate surfaces making the organism a recognizable threat especially in the intensive care units(Briano et al., 2022). *C. auris* colonization usually precedes infection as has been demonstrated in >50% of bloodstream infections (CDC, 2023).

A single center retrospective study including all patients admitted to Intensive Care Units (ICUs) with isolation of *C. auris* in any non-sterile body site between February 2020 and May 2021 showed that 59 % of all the 157 patients became colonized with *C. auris* while in the Intensive care units (Briano et al., 2022).Genetic analyses suggested five genetically different clades of C. auris were isolated from locations throughout the world (Du et al., 2020). In the United States of America, 7413 cases of *C.auris* were recorded between 2009 and December 2021 and there was an increase in colonization screening volumes and screening cases by more than 80 % in 2019 and 200% in 2021 and the numbers of antifungal resistance to echinocandins is 3 times that in 2019 (CDC).There were 5,754 instances reported in the US from January 2022 to December 2022, according to the US Center for Disease Control and Prevention (CDC) (Prevention, May 16, 2023). The first known case of C. auris in Europe occurred in 2009 and was brought in from India (Ciurea et al., 2021). But in 2013, 12 individuals with positive microbiological clinical samples between 2009 and 2012 were found to represent the first cases of *C. auris* in India.

Spontaneous outbreaks throughout Europe were reported between April 2013 to 2017 when 50 cases in London were reported (Cortegiani *et al*., 2018). Sporadic cases were also reported in South America with the first outbreak recorded in Venezuela between March 2012 and July 2013 and later on Colombia. In the middle East, Saudi Arabia reported the initial cases and then subsequently Kuwait, Oman and the United Arab Emirates. In the African continent, the first sporadic cases occurred in South Africa and Kenya and the first four cases in South Africa were isolated between 2012 to 2013 (Cortegiani *et al*., 2018). At the beginning, the isolates of candida were grouped into four clades i.e clade 1 (south asia), clade 2 (east asia), clade 3(south Africa and clade 4 (south America). Later on a fifth clade from Iran was reported(Sanyaolu et al., 2022). The worldwide distribution of *C.auris* is depicted in Figure1.

C. auris infections have been reported from other African countries including Egypt, Algeria (Zerrouki et al., 2022) and Nigeria (Oladele et al., 2022), and (Adam et al., 2019; Ombajo et al., 2020). In Uganda, there are no reports on *C. auris*. However, the prevalence of *Candida* species colonization in Uganda ranges between 14% to 52% mostly in neonates, pregnant women and intensive care unit (ICU) patients and between 2008 and 2009 it was demonstrated that *candida* species colonization amongst the neonates was 23.5 % (Abdallah, Kaddu-Mulindwa, Nankunda, & Musoke, 2015; Bakandonda et al., 2019; Jane, Iramiot, & Kalule, 2019; Olowo et al., 2022).

Therefore this project aims to study the prevalence of colonization, Antifungal Resistance (AFR) patterns and associated epidemiological risk factors of *C. auris* isolated from patients in the intensive care units of Mulago Hospital.

1.2 Statement of problem.

Candida auris has rapidly emerged as a multidrug-resistant fungal pathogen of global concern, particularly in intensive care units (ICUs). Colonization of patients, especially in anatomical sites such as the axilla and groin, has been shown to precede invasive infections and contribute to difficult-to-control nosocomial outbreaks. Despite this growing threat, Uganda lacks local data on the prevalence of *C. auris* colonization, its antifungal susceptibility patterns, and associated risk factors among critically ill patients.

Globally, most *C. auris* isolates are resistant to fluconazole, with significant proportions also resistant to amphotericin B, leaving echinocandins as the only consistently reliable therapeutic option. In resource-limited settings like Uganda, empirical antifungal treatment often relies on fluconazole and amphotericin B, which may be ineffective against resistant

strains. This creates a risk of poor outcomes, prolonged ICU stays, and increased healthcare costs.

The absence of baseline surveillance in Uganda means that colonization and resistance dynamics remain unknown. Without such data, infection prevention and control (IPC) measures, antifungal stewardship programs, and treatment guidelines cannot be tailored to the local context.

This study therefore set out to determine the prevalence of *C. auris* colonization among ICU patients at Mulago National Referral Hospital, establish antifungal susceptibility patterns, and identify associated clinical and demographic risk factors. By addressing this knowledge gap, the study provides critical evidence to guide surveillance, IPC, and antifungal stewardship in Uganda.

1.3 Research questions

- What is the prevalence of *C. auris* colonization among patients in the ICU of Mulago Hospital?
- What is the antifungal resistance patterns of *C. auris* isolated from patients in the ICU of Mulago Hospital in Uganda?
- What are the epidemiological factors associated with *C. auris* colonization and AFR among patients in the ICU of Mulago Hospital?

1.4 Aim/ General objective

This study aims to determine the prevalence of *C. auris* colonisation, antifungal susceptibility patterns and associated epidemiological factors of the isolates among patients in the intensive care unit at Mulago National Referral Hospital, Uganda.

1.5 Specific objectives

1. Determine the prevalence of *C. auris* colonization among patients in the ICU of Mulago Hospital.
2. Determine the antifungal susceptibility patterns for *C. auris* isolated from patients in the ICU of Mulago Hospital in Uganda.
3. Describe epidemiological factors associated with *C. auris* colonization and AFR among patients in the ICU of Mulago Hospital.

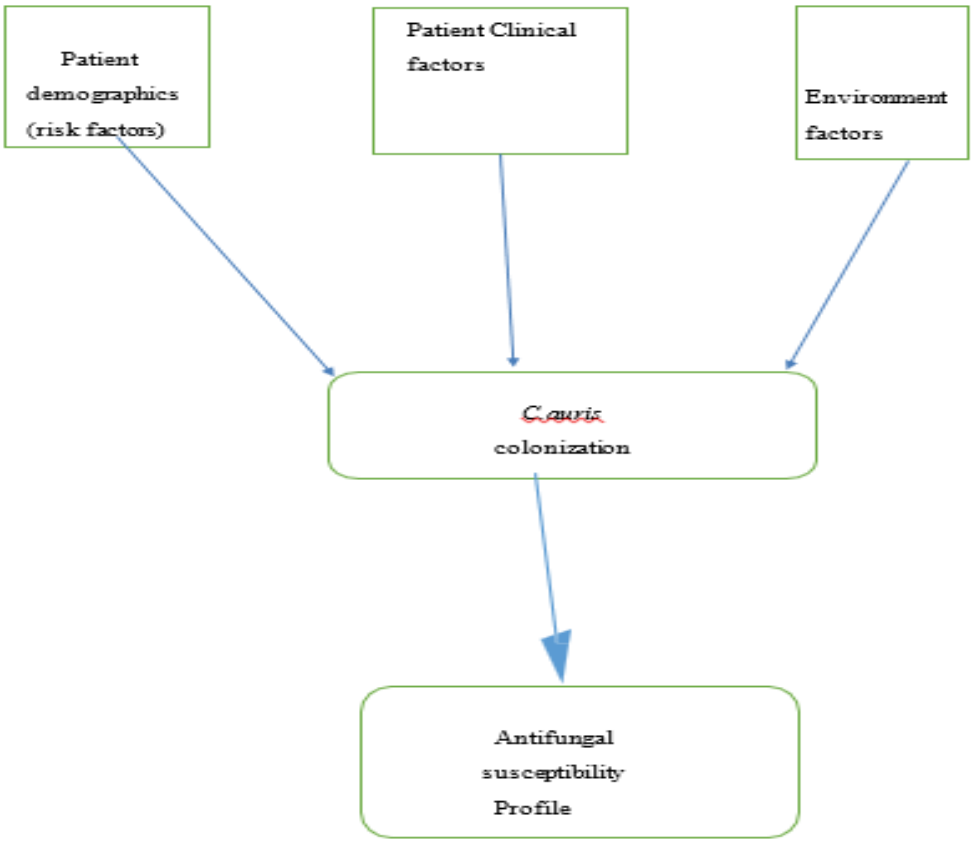
1.6 Justification

C. auris has emerged globally as a multidrug-resistant fungal pathogen associated with high morbidity and mortality in ICUs. However, no published data currently exist from Uganda, despite the high-risk environment in referral hospital ICUs where patients frequently receive broad-spectrum antibiotics, invasive medical devices, and prolonged hospital care, all known risk factors for colonization and infection.

The absence of baseline data in Uganda presents a critical knowledge gap, particularly since empirical antifungal therapy in our ICUs still relies heavily on fluconazole and amphotericin B, agents that are increasingly ineffective against *C. auris*. Without local evidence, clinicians and policymakers lack the data needed to revise treatment guidelines, strengthen infection prevention and control, or prepare for potential outbreaks.

This study is therefore justified as the first to document *C. auris* colonization prevalence, antifungal susceptibility, and associated risk factors in Uganda. The findings will provide a foundation for national surveillance systems, antifungal stewardship programs, and ICU infection control policies, aligning Uganda's response with global WHO recommendations and ensuring better patient outcomes in critical care settings.

1.7 Conceptual framework



Framework Highlights

Independent variables

- Patient factors → age, sex, comorbidities, immune status
- Clinical factors → prior antibiotic/antifungal use, ICU stay duration, invasive devices
- Environmental factors → surfaces, beddings, healthcare environment

Dependent variable

Candida auris colonisation (presence/absence in patients)

Antifungal susceptibility (MIC patterns)

CHAPTER TWO:

2.0 LITERATURE REVIEW

Candida auris, also referred to as *C.auris,* is one type of yeast that can cause serious illness and is easily spread among patients in healthcare facilities. It frequently exhibits resistance to antifungal therapies, meaning that the drugs intended to eradicate the fungus and halt infections are ineffective(Harris, 2023). On its most recent list of dangerous fungi, the World Health Organization (WHO) identified *Candida auris* as a critical priority pathogen (WHO, 25 October 2022).

2.1 History

2009 saw the first description of *Candida auris* and it was grown from a patient's external ear canal in Japan(Khan & Ahmad, 2017). The first report of blood stream infections by *C.auris* from Korea in 2011 revealed the characteristic of persistent fungaemia in patients receiving fluconazole and amphotericin B therapy. Within five years of that initial report, hospitals in India, South Africa, Kuwait, Brazil, Venezuela, the United States, the United Kingdom, and Pakistan had either reported or been notified of cases of *C.auris* fungaemia and deep-seated infections (A. Chowdhary, Voss, & Meis, 2016).

Given that *C. auris* is misidentified as *Saccharomyces cerevisiae, Candida haemulonii, Candida famata, Candida sake, and Rhodotorula glutinis* phenotypically, it is expected that it is not confined to the aforementioned nations and that its true burden has not yet been investigated(A. Chowdhary *et al*., 2016).

2.2 Epidemiology.

Since its initial discovery ten years ago, *C. auris* has rapidly emerged as a major infectious pathogen with rising global incidence. The number of clinical cases of Candida auris infections in the US increased from 476 in 2019 to 1471 in 2021, according to a Centers for Disease Control and Prevention evaluation of specimens obtained during routine care (Harris, 2023). Although the positivity rate of the screens remained constant over time at about 8%, the number of colonization cases found through screening increased by more than 200% to 4041 screening cases in 2021 (Harris, 2023) Candida auris is an emerging fungus associated with significant death rates. *C. auris* candidemia has a crude in-hospital death rate ranging from 30% to 60% overall, and infections usually happen many weeks (10–50 days) after admission(Jeffery-Smith et al., 2018; Osei Sekyere, 2018). Please refer to Figure 1 for the global map depiction for *C.auris* emergence.

2.3 “Colonization” literature

It is possible for people to harbor *C. auris* on their skin and other body parts without becoming ill or infected. Healthcare professionals call this "colonization" and even after colonization, *C. auris* can continue spread to additional patients by landing on surfaces or objects that a colonized person comes into touch with(Harris, 2023). *C. auris* generally poses no hazard to healthy individuals(Harris, 2023).

2.4 Risk factors for colonisation

According to Cortegiani et al. (2018), isolates from non-sterile bodily regions such the lungs, urinary system, skin and soft tissue, and genital apparatus are more likely to be the result of colonization than infection. When C. auris is isolated from a place with infection-causing signs and symptoms, it is possible to distinguish between simple colonization and infection, much like with other species of Candida (Pappas et al., 2016). It is critical to identify C. auris even from non-sterile body sites since colonization raises the risk of transmission and requires the adoption of infection control measures (Cortegiani et al., 2018).

Rudramurthy et al. performed a subgroup analysis and comparison of the clinical symptoms of *C. auris* and non-auris cases in 27 Indian ICUs in order to look into risk variables linked to *C. auris* infections(Rudramurthy *et al*., 2017). As per earlier research, risk factors for infection with other Candida spp. were not different(Sarma & Upadhyay, 2017). These included diabetes mellitus, length of stay in the intensive care unit, chemotherapy, blood transfusions, hemodialysis, urinary catheterization, post-operative drain placement, immunosuppressive state (Azar, Turbett, Fishman, & Pierce, 2017) and neutropenia (Mohd Tap et al., 2018), as well as prior or continuous use of broad-spectrum antibiotics and antifungal agents.

C. auris is far more common in patients with primary or acquired impaired immune response, bone marrow transplant recipients, patients receiving therapeutic care for hematologic malignancies, and patients with other illnesses needing immunosuppressive medicines (Vallabhaneni, Snigdha (2016); Vallabhaneni, S. et al., 2017). According to available data, adults are affected by most infections, with severely sick patients in intensive care unit (ICU) settings being more likely to contract them. Only South America and Asia have reported pediatric patients(Warris, 2018). *C. auris* primarily affects patients with major underlying medical conditions that necessitate advanced medical care, people who use invasive medical devices, such as feeding tubes, breathing tubes, urine catheters, and venous catheters, are more likely to contract *C. auris* infections (Preda, Chivu, Ditu, Popescu, & Manolescu, 2024).

Patients with *C. auris* infections share similar risk factors as those with other Candida spp. infections, including surgery on the abdomen (25-77%), use of broad-spectrum antibiotics (25%–100%), admission to the intensive care unit (58%), diabetes mellitus (18%), central venous catheter use (25%–94%), and cancers (11-43%). Healthy individuals are less likely to contract *C. auris* if they do not have these risk factors, such as close family members and medical professionals (Preda et al., 2024).

2.5 Drivers of clonal transmission and nosocomial outbreaks.

C. auris colonization on skin and other body locations has been seen even weeks to months after an initial infection (Kean, McKloud, et al., 2018). This can contaminate the healthcare setting and represent a danger of continued transmission (Snigdha Vallabhaneni, 2016). Moreover, samples from the chairs, windowsills, bedside tables, bed rails, and mattress have been used to isolate *C. auris*.

2.6 Pathogenesis.

The virulence factors of *Candida auris* include germination, adhesion, biofilm formation, and the generation of proteinases and phospholipases. Even while *C. auris* produces much fewer biofilms than *C. albicans*, it may nevertheless form adherent biofilm communities on a variety of therapeutically critical substrates. Larkin et al. examined the morphology and virulence factors of sixteen distinct *C. auris* isolates that were taken from patients in Germany, South Korea, Japan, and India and in contrast to *C. albicans*, *Candida auris* shows a marked decrease in its capacity to cling to catheter material and produces phospholipase and proteinase in a strain-dependent way(E Larkin et al.). Moreover, the majority of the yeast cells in *C. auris* biofilms were attached to the catheter material. On the other hand, Candida albicans displayed a remarkably diverse biofilm architecture, with both yeast cells and hyphae embedded in the extracellular matrix(E. Larkin et al., 2017). Sherry et al. reported that *C. auris* can produce biofilms that are resistant to all three major classes of antifungals (Sherry et al., 2017). According to Kean et al., these biofilms had a less sensitive phenotype than *Candida albicans* and *Candida glabrata* since they were resistant to hydrogen peroxide and chlorhexidine. In a recent study, Kean and colleagues employed a molecular method to examine the genes responsible for the resistance of *C. auris* cells within the biofilm(Kean, McKloud, et al., 2018).

Differential expression analysis demonstrated that 791 and 464 genes were upregulated in biofilm formation and planktonic cells, respectively, with a minimum twofold change. Notably, several genes encoding efflux pumps were increased in the intermediate and mature stages of

biofilm generation. These genes included the major facilitator superfamily (MFS) transporter and ATP-binding cassette (ABC), which suggests efflux-mediated resistance in *C. auris* (Kean, McKloud, et al., 2018). According to earlier research by Ben-Ami et al., isolates of *C. auris* had considerably higher ABC-type efflux activity than isolates of *C. glabrata*, as demonstrated by Rhodamine 6G transport. This suggests that *C. auris* has intrinsic efflux-mediated resistance to azoles (Ben-Ami et al., 2017; Kean, McKloud, et al., 2018).

In an immunocompetent mouse model of invasive infection, the virulence of *Candida auris, Candida haemulonii, and Candida glabrata* was recently compared with *Candida albicans* and *Candida glabrata*. According to the study's authors, C. auris' virulence appears to be comparable to that of *C. albicans and C. glabrata*, indicating that shared gene sequences may be important (Fakhim et al., 2018). According to Muñoz et al. (2018), the whole genome data of the emerging multidrug-resistant species and other related Candida showed that *C. auris* shares some important gene family expansions linked to virulence in *C. albicans* and related infections, including transporters and secreted lipases.

The pathogenicity of *C. auris* in comparison to other common pathogenic yeast species in the invertebrate *Galleria mellonella* infection showed strain-specific differences in *C. auris* behavior, and the isolates that formed aggregates were significantly less pathogenic than the isolates that did not form aggregates (A. M. Borman, Szekely, & Johnson, 2016).

Crucially, according to Borman, Szekely, and Johnson (2016), the non-aggregating isolates showed pathogenicity that was on par with that of *Candida albicans*(A. M. Borman et al., 2016). Finally, *C. auris's* resistance in hospital settings may be attributed to its capacity for salt tolerance as well as its propensity for clumping into big, difficult-to-disperse aggregates. Despite having the virulence factors, *C. auris* is not as virulent as other species, as evidenced by the fact that its expression of those factors is noticeably less than that of other species (E. Larkin et al., 2017).

2.7 Clinical picture

C. auris colonization usually precedes infection as has been demonstrated in >50% of bloodstream infections. The location and severity of the *C. auris* infection dictates the symptoms, which can also result in infections in the ears and open wounds, among other parts of the body (CDC, 2023). The signs may be similar to those of an infection caused by bacteria. There isn't a usual set of signs and symptoms specific to *C. auris* infections (CDC, 2023).

2.8. Laboratory identification.

The tests are performed on swabs from skin, throat, ears, and vaginal specimens as well as sputum, urine and stools samples, in parallel to cultures on Sabouraud agar, to aid in the diagnosis. (Marathe, Zhu, Chaturvedi, & Chaturvedi, 2022)

Enrichment Culture Methodology for Colonization Screening with *C. auris*

The CDC, regional, and state public health laboratories in the US use the Salt Sabouraud Dulcitol enrichment broth method to separate *C. auris* from clinical skin swabs and environmental sponge surveillance samples. Welsh et al. proposed using dulcitol instead of dextrose as the main carbon source in order to lessen the chance of co-incubating non-target species like *C. glabrata and C. parapsilosis*. (Welsh et al,2017)

To screen for C. auris colonization, swabs are submitted to laboratories that are modified liquid Amies medium-immersed. Salt Sabouraud Dulcitol enrichment broth is usually inoculated with 100 µl of the vortexed swab medium and then incubated at 40°C (a temperature specific to C. auris) with constant shaking at 250 rpm for at least five days.

A loop streaks each sample that turns turbid onto CHROMagar Candida. If the sample doesn't change color, an aliquot is placed on CHROMagar Candida. Every CHROMagar Candida plate is incubated for 48 hours at 37°C, and any colonies that could be indicative of *Candida auris* are screened for.(Sexton et al., 2018)

2.9 Microbiological characteristics of C. auris

2.9.1 By phenotypic methods

Candida auris exhibits distinct microbiological characteristics that contribute to its identification and differentiation from other *Candida species.* Colonies of *C. auris* grown on Sabouraud's agar appear as smooth, white cream-colored colonies, while on CHROMagar Candida medium, colonies may vary in color from pale to dark pink or occasionally beige. Notably, *C. auris* demonstrates the ability to grow at elevated temperatures, particularly at 42 °C, a feature that distinguishes it from closely related species such as *C. haemulonii*, which does not thrive at this temperature(Kathuria et al., 2015). Separating the *C. auris* from the *C. haemulonii complex* may be aided by increasing the temperature and incorporating Pal (sunflower seed extract) agar into commercial chromogenic medium (Kumar et al., 2017).

Recently, the CHROMagar™ Candida Plus was introduced as a selective chromogenic culture medium for use in the qualitative direct detection, differentiation and presumptive identification of Candida species including *C. auris(Marathe et al., 2022)*.. Results can be

interpreted after 24-48 h of aerobic incubation at 30-37 °C (Marathe et al., 2022).

Microscopically, *C. auris* cells typically appear as aggregate or nonaggregate oval-shaped yeast cells(Mahmoudi, Afshari, Gharehbolagh, Mirhendi, & Makimura, 2019) without the formation of pseudohyphae; however, under certain culture conditions, such as exposure to high concentrations of sodium chloride (10%), *C.auris* can exhibit morphological variability including round-to-ovoid and elongated forms, and even pseudohyphal-like structures(A. Borman & Szekely). Furthermore, the growth of *C.auris* is inhibited by cycloheximide at concentrations of 0.1% and 0.01%(E Larkin et al.). In elongated cells, DAPI labeling reveals several nuclei. Nevertheless, when Calcofluor White Staining is used, no septin/chitin rings are seen between conjoint cells(Spivak & Hanson, 2018).

2.9.2 Biochemical Methods.

Because of its genetic similarity to the *C. haemulonii complex, C. auris* is sometimes mistaken for other species of Candida, which presents a substantial diagnostic difficulty. According to Kim, Kweon, Kim, and Lee (2016), commercial biochemical-based tests like API AUX 20C, VITEK-2 YST, BD Phoenix, and MicroScan frequently misidentify Candida species and other genera as C. auris. These genera include *Rhodotorula glutinis, Rhodotorula mucilaginosa, Saccharomyces, C. catenulate, C. lusitaniae, C. guilliermondii, and C. parapsilosis.*

. It has been reported that VITEX 2 XL (bioMérieux version 8.01) provides a high level of accuracy in recognizing *C.auris*. Nonetheless, Ambaraghassi and colleagues showed that although this program is quite good at recognizing isolates from the South American clade, it has limitations when it comes to correctly identifying C.auris from the African and East Asian clades.(Ambaraghassi et al, 2019)

2.9.3 Molecular Methods

2.9.3.1 MALDI TOF (matrix-assisted laser desorption/ionization time-of-flight)

When the right extraction technique is chosen and the reference database contains the *C.auris* spectrum, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has proven to be an effective way to distinguish *C.auris* from other Candida species, thereby resolving the misidentification challenge mentioned above.

(Prakash et al., 2016).

MALDI-TOF mass spectrometry (MS) can distinguish Candida spp. from one another and efficiently separate *C.auris* from colonies recovered in the enrichment broth only if the *C.auris* spectra is included in the reference database (Girard et al., 2016). Right now, the most likely

technique to obtain accurate identification of *C.auris* is the FDA-approved MALDI-TOF Biotyper (Bruker-Daltonics). Additional techniques include the use of the CA System library (Version Claim 4), the updated research use only (RUO) library (Versions 2014 5627 and more recent), the FDA-approved IVD v3.2, or RUO Version 4.14 with the Saccharomycetaceae update with the VITEK (MALDI-TOF) MS (bioMérieux).

.(CDC, 2023)

2.9.4 Other Methods

Additionally, the development of specific PCR assays targeting *C.auris* and related species directly from cultured colonies holds promise for rapid and accurate identification, particularly during outbreak situations (Leach, Zhu, & Chaturvedi, 2018).

Whole genome sequencing (WGS), amplified fragment length polymorphism (AFLP), proteomic analysis, and multilocus sequence typing (MLST) can all be used to determine how related C. aureus isolates are genetically (Girard et al., 2016).

Molecular identification techniques using sequencing of genetic loci such as D1/D2, RPB1, RPB2, and internal transcribed spacers (ITS1, ITS2) have been demonstrated to effectively identify *C.auris*; however, these methods are not yet routinely employed in clinical diagnostic laboratories(Chatterjee et al., 2015). In an MLST investigation, reports state that four genetic loci—ITS, D1/D2, RPB1 and RPB2—are highly selective for strain separation between *C. haemulonii complex and C. auris.*

(Desoubeaux et al., 2018)

2.10Antifungal susceptibility profile.

Because *Candida auris* can become resistant to several antifungal medications, it presents a serious clinical problem and raises the death rates of infections brought on by this disease (Navalkele, Revankar, & Chandrasekar, 2017). Data on antifungal susceptibility reveals that different C. aureus strains have differing degrees of resistance, especially against important classes of antifungal medications such as azoles, polyenes, and echinocandins (S. R. Lockhart et al., 2017)

Resistance to fluconazole, a commonly used azole, is a notable characteristic of *C.auris*. Studies have reported high rates of fluconazole resistance in *C.auris* isolates, with MICs ranging from 32 to ≥ 64 mg/L in Indian hospital settings . However, isolated strains with lower MICs (2–8 mg/L) have been identified in India and Colombia, suggesting regional variations in fluconazole susceptibility(Mathur et al., 2018). Over the course of eight years, 350 *C.auris* isolates from ten Indian hospitals were tested for their susceptibility to antifungals. The findings showed that 90% of the isolates were resistant to amphotericin B (MIC ≥ 2 mg/L), 2.3% to

voriconazole (MIC 16 mg/L), 2% to echinocandins (MIC ≥ 8 mg/L), and 90% to fluconazole (MIC 32 to 64 mg/L)(Anuradha Chowdhary et al., 2018).

Azole resistance in C. aureus is mostly caused by mutations in the lanosterol 14 α-demethylase (ERG11) gene, which is susceptible to azole resistance. There is evidence connecting certain amino acid substitutions—Y132F, K143R, and F126L in ERG11, for example—with fluconazole resistance. Moreover, it has been noted that some strains of the fluconazole-resistant bacteria had higher copies of the ERG11 gene, which may indicate a mechanism for drug resistance. Azole resistance in C. aureus has also been linked to the upregulation of drug efflux pumps, including CDR1, CDR2, and MDR1, as a result of gain-of-function alterations in transcription factors (e.g., TAC1, MRR1)(Anuradha Chowdhary et al., 2018).

The primary mechanism of echinocandin resistance in C. aureus is due to mutations in the FKS1 gene, which codes for the drug target 1,3-beta-glucan synthase. Notably, echinocandin resistance in isolates from India has been linked to the FKS1 amino acid alteration S639F (Anuradha Chowdhary et al., 2018). Furthermore, a study revealed the clinical significance of these genetic changes by identifying *C.auris* isolates with pan-echinocandin resistance (MICs > 8 mg/L) containing the S639F mutation(Kordalewska et al., 2018).

Although the underlying molecular processes are still being investigated, resistance to the polyene antifungal drug amphotericin B is becoming a problem in *C. auris* (Meis et al., 2017; Lockhart et al., 2017). Recent research on C. aureus isolates from Colombia has revealed non-synonymous changes linked to amphotericin B resistance, indicating regional differences in resistance patterns (Escandón et al., 2019). Moreover, difficulties have been identified with amphotericin B antifungal susceptibility testing, highlighting the necessity for cautious interpretation of laboratory results (Kathuria et al., 2015)

2.11Infection prevention and control.

These consist of isolating patients and their associates, having healthcare personnel wear personal protective equipment, screening patients in afflicted wards, decontaminating skin with chlorhexidine, cleaning the environment with reagents based on chlorine, and decontaminating terminals using hydrogen peroxide vapor or ultraviolet (UV) light (Schelenz et al., 2016). It has recently been demonstrated that enhanced terminal cleaning with UV light reduces infections with numerous nosocomial pathogens and may also be useful in halting the spread of *C.auris*.

The increasing number of *C.auris* outbreaks and isolated cases highlights the necessity of effective preventative measures. Recent epidemic reports indicate that colonization is hard to

get rid of and often lasts for months (Schelenz et al., 2016). Early detection of occasional cases, reservoir identification, and timely communication are essential components of epidemic prevention. International organizations, like Public Health England (PHE-UK) (England, 2016), have produced guidelines that include suggestions for patient isolation, contact precautions, and cleaning of surroundings and equipment that come into touch with ill patients. Early detection of occasional cases, reservoir identification, and timely communication are essential components of epidemic prevention. International organizations, like Public Health England (PHE-UK)(England, 2016), have produced guidelines that include suggestions for patient isolation, contact precautions, and cleaning of surroundings and equipment that come into touch with ill patients. Policies for infection control and prevention are empirical, primarily derived from recommendations developed for containment tactics against other organisms' resistant to several drugs.

The CDC has recommended the following actions for infection Control:

- Using contact precautions and isolating the patient with *C.auris* in a separate room
- Stressing the importance of hand hygiene; using prescribed items to clean and disinfect the patient care environment on a daily and terminal basis; and screening contacts of recently diagnosed case patients to detect *C.auris* colonization

The following patients should be screened: current roommates; roommates at the current facility or other facilities within the last month (even if they have been released from the facility) in order to discover colonization among patients who may be epidemiologically connected. For the purpose of screening for C. aureus, a composite swab from the patient's groin and axilla and sites of consistent colonization should be utilized.

Additionally, it has been discovered that patients had *C.auris* colonization in their noses, external ear canals, oropharynges, urine, wounds, and rectum.

Every laboratory, particularly those that service healthcare facilities where *C.auris* cases have been found, should examine previous microbiology data to find instances of confirmed or suspected *C.auris* cases.

Prospective surveillance should be carried out to detect future cases of *C.auris*. Patients' close contacts should be screened for colonization. Teaching all medical professionals including those employed by environmental cleaning services about *C.auris* and the necessity of taking the necessary safety measures and keep an eye on compliance with infection control procedures and antibiotic and antifungal stewardship(Cortegiani et al., 2018).

Early evidence indicates that the spread of *C.auris* is mostly associated with exposure to

contaminated facilities and transmission by healthcare staff, however the precise mode of transmission still has to be determined. According to Tsay et al., hand transmission and surface pollution have been linked to persistent outbreaks(Tsay et al., 2017) .

However, it remains difficult to define the role of healthcare professionals. A recent investigation tested patients and their contacts, medical staff, and the surrounding environment in four Colombian hospitals that had previously reported *C.auris* outbreaks.

(Escandón et al., 2019). It's interesting to note that surfaces with little to no patient contact and infrequent healthcare worker contact, like closet cabinets, door handles, and alcohol gel dispensers, and surfaces with frequent patient contact but infrequent healthcare worker contact, like chairs, bed trays, and medical equipment, both yielded positive samples. The study found C.auris on a variety of items and facilities, including flooring, bedrails, a bed hand-controller, and mobile phones (Escandón et al., 2019).

As a result, as soon as *C.auris* is present in a hospital setting, environmental contamination spreads far from the patient's bedside and frequently leads to new colonizations.
A variety of wet and dry surfaces, including plastic, are suitable for *C.auris* survival, and the pathogen can live there for up to 14 days(Welsh et al., 2017).It seems that cationic surface-active therapies and quaternary compound disinfectants are ineffective against C. aureus. To get the most decrease in *C.auris* colony-forming units (CFU), Cadnum et al. advise using disinfectants with sporicidal activity and hydrogen peroxide-based solutions for cleaning surfaces and healthcare facilities(Cadnum et al., 2017). Following patient discharge, the effectiveness of hydrogen peroxide vapor, ultraviolet light, and detergents based on chlorine was shown(Sherry et al., 2017).

Nonetheless, the continued presence of *C.auris* in the hospital setting in spite of disinfection protocols also points to the pathogen-surface interaction and duration of disinfectant exposure playing a role(Kean,Sherry,etal.,2018). Authorities advise following urine catheter care bundles, central and peripheral catheter care bundles, and tracheostomy site care in order to stop transmission(England, 2016). Patients who are colonized or who have a confirmed or suspected C.auris infection should be kept in isolation and subject to stringent contact precautions until microbiological screening and diagnostic results are obtained (Schelenz et al., 2016). Patients should be screened upon transfer from institutions where C. aureus isolation has been confirmed (England, 2016). It is advised to screen in the groin and axilla, urine, nose and throat, perineal, and rectal areas.

CHAPTER THREE:

3.0 METHODOLOGY

3.1 Study design and site

This was a cross sectional study conducted over a period of 6 months in the ICUs of Mulago National Referral Hospital.

3.2 Study setting

The study setting for this study was the Intensive Care Units (ICUs) of Mulago National Referral Hospital in Uganda. Newly admitted patients in the ICUs as well as patients with medical instrumentation such as a central venous, dialysis or urinary catheter, Tracheosotomy, long ICU stay, referral in from another facility and international travel, endotracheal tube, abdominal surgery, mechanical ventilation, nasogastric tube, parenteral nutrition, use of blood products, use of broad-spectrum antibiotics and antifungals were enrolled.

3.3 Study population

The study included paediatric and adult patients admitted to the ICUs during the study period.

3.4 Inclusion and exclusion criteria

Inclusion criteria

- The study included paediatric and adult patients admitted to the ICUs during the study period. Patients who had received antifungal treatment for more than 72 hours and were still symptomatic (Symptoms were defined as persistent fever with an axillary temperature ≥37.5 °C, despite antifungal therapy) and had provided informed consent and patients who had medical instrumentation like central catheters, urinary catheters, tracheostomy, endotracheal tube, abdominal surgery, mechanical ventilation, nasogastric tube, parenteral nutrition, use of blood products, use of broad-spectrum antibiotics.

Exclusion criteria

- Inability to provide direct or surrogate consent for study participation.
- No informed consent or had withdrawn the consent.

3.5 Sample size estimation

Prevalence of Candida colonization in Uganda ranges between 14% to 52% mostly in neonates, pregnant women and intensive care unit (ICU) patients (Abdallah et al., 2015; Bakandonda et al., 2019; Jane et al., 2019; Olowo et al., 2022).

Using the Keish – Leslie formula for sample size calculation

$$n = \frac{Z^2 P\,(1-P)}{e^2}$$

where

n =Sample size

Z^2 = Abscissa of the normal curve that cuts off an area □ at the tail, i.e., the desired confidence interval at 95%

P = prevalence of *Candida* colonization among patients in Uganda (14%) (Abdallah et al., 2015; Bakandonda et al., 2019; Jane et al., 2019; Olowo et al., 2022).

e^2 = Desired level of precision, at 5% (0.05) Therefore,

Adjusting by 15% to take care of errors and non-response:

$$n = 185$$

The calculated sample size was 185 participants. However, due to study duration limits, ICU occupancy rates, and ethical approvals for patient enrollment, a total of 65 patients were included in the final analysis. This sample was considered adequate to provide baseline prevalence and resistance data for this exploratory study, though future studies with larger sample sizes are recommended for more precise estimates

The sample size for the study was 65 participants.

3.6 Sampling method

Convenience sampling was employed.

3.7 Sources of Data

1. Patient Records and Charts- Data was collected from patient medical records and charts, which contained information about patient demographics, admission details, underlying conditions, prior hospitalizations and previous antifungal use.
2. Microbiological Laboratory Records - Information on *Candida auris* isolates was obtained from clinical specimens and was crucial. This data included the date of isolation, site of isolation, and results of antifungal susceptibility testing. This was then followed by results from VITEK 2.0 Platform.
3. Antifungal Susceptibility Testing Results - Detailed laboratory data on antifungal susceptibility profiles of *Candida auris* isolates obtained by VITEK 2.0 platform were essential

for analyzing resistance patterns.

6. Environmental Sampling Data - Data from environmental sampling from immediate patient surroundings and high touch surfaces within the ICU setting to identify potential reservoirs of *Candida auris* also contributed to the data.

3.8 Sample collection and handling

A total of 325 samples were collected. For each participant, 4 samples were collected at once, a single swab from each site. 260 Swabs were taken from the patients' axilla, nares, groin and external ear and were taken for culture to confirm colonization. One environmental swab sample was taken from each patient's immediate surroundings therefore 65 environmental samples were collected. Swabs were collected with the BD ESwab Liquid Amies Collection and Transport System (Becton, Dickinson, Franklin Lakes, NJ, USA) at ambient temperature. All Samples were collected and transported to the Laboratory within 2 hours and were recorded along with the corresponding participant identifiers.

3.9 Clinical procedures

Clinical procedures included gathering of demographic data and collecting clinical samples. During the conduct of the study, we collected data on age, gender, length of hospitalization, reasons for admission, underlying comorbidities, admissions in the preceding 3 months, admission in another facility and international travel. We collected data on the presence of a central venous catheter, dialysis catheter, endotracheal tube, surgery, mechanical ventilation, urinary catheter, nasogastric tube, parenteral nutrition, use of blood products, use of broad-spectrum antibiotics and antifungals.

3.10Laboratory methods

Swabs were collected from multiple body sites including the groin, axilla, ear, nose, and patient environment. Each specimen was processed immediately or stored at appropriate temperatures for same-day processing.

3.12 Culture and Isolation of Yeasts

Swabs collected from patient skin and mucosal surfaces (groin, axilla, ear, and nose) and environmental surfaces were processed for yeast isolation using a standardized mycological workflow.

Each swab was inoculated directly onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol to suppress bacterial growth. The inoculated SDA plates were incubated at 35–37 °C for 24–48 hours, providing a general medium for yeast growth.

Following growth on SDA, colonies with yeast-like appearance were sub-cultured onto CHROMagar Candida Plus for presumptive identification of *Candida* species. CHROMagar plus plates offered differential coloration for various *Candida* species and were critical for preliminary identification.

To exploit the thermotolerance property of *Candida auris*, isolates were incubated in parallel at two temperatures: 35–37 °C and 42 °C. *C. auris* is known to grow optimally at elevated temperatures (up to 42 °C), unlike most other *Candida* species which fail to grow robustly at this range. This approach helped suppress growth of other yeasts and enriched for thermotolerant *C. auris*.

- On SDA, *C. auris* formed smooth, cream to tan-colored colonies, lacking filamentous growth or pigment production.
- On CHROMagar Candida Plus, *C. auris* colonies typically appeared light blue with a blue halo, a distinguishing characteristic.
- Other *Candida* species exhibited different colony morphologies and pigmentation: e.g., *C. albicans* often appears pale green on CHROMagar, while *C. tropicalis* may produce blue colonies with a purple halo.

This dual temperature and chromogenic plating technique improved presumptive identification accuracy and aided in early differentiation of *C. auris* from other *Candida* species. Colonies matching expected morphology and growth characteristics were flagged for further confirmatory testing and antifungal susceptibility profiling. Swabs were initially inoculated onto Sabouraud Dextrose Agar (SDA) with chloramphenicol and incubated at 35–37 °C for 24–48 hours to support general yeast isolation. For selective identification of *Candida* species, CHROMagar Candida plates were used.

Each isolate was streaked onto CHROMagar Candida and incubated in parallel at 35–37 °C and at 42 °C, taking advantage of the thermotolerance of *C. auris*. Colonies of *C. auris* typically appeared light blue with a blue halo on CHROMagar Candida Plus and cream to tan

on SDA. Other yeast species were differentiated by distinct color morphologies on CHROMagar.

Figure 2

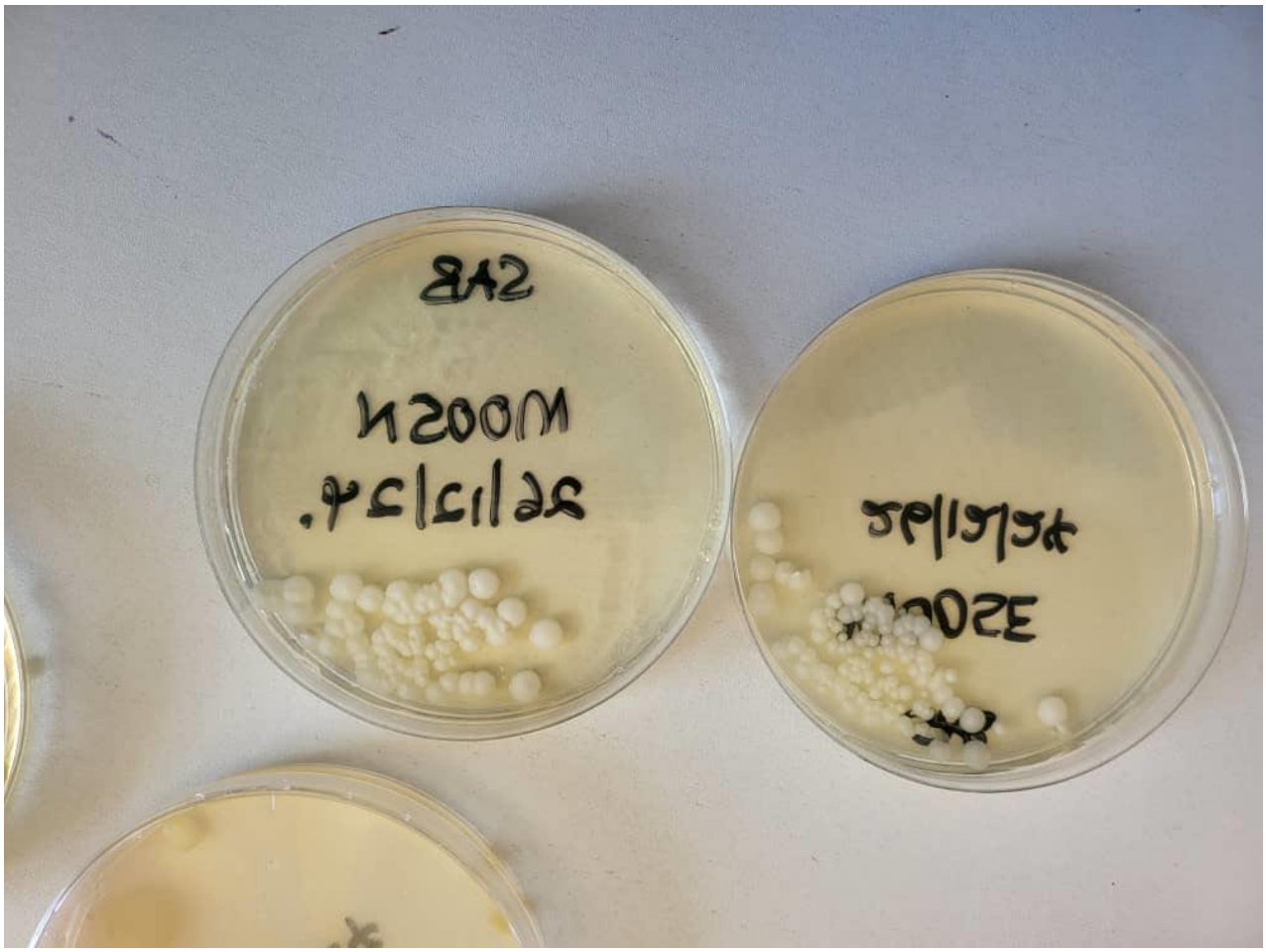


Figure 2.- Candida auris Colonies Isolated from ICU Patient Swabs Cultured on Sabouraud Dextrose Agar (SDA). Smooth, cream-colored, slightly convex colonies with moist surfaces were observed after 24–48 hours of incubation at 35°C.

Figure 3

Figure 3: Growth of Candida Isolates on Chromogenic Medium Showing Differential Colony Colorations. The grid plate demonstrates presumptive identification of Candida auris and related species based on differential color development on chromogenic agar. Positive controls include Candida auris CDC B11903, Candida albicans ATCC 60193, and other reference strains ATCC 14243, 2001, and 1369 were also included for validation.

3.13 Preliminary Identification Tests

Presumptive *Candida* colonies observed on SDA and CHROMagar Candida were subjected to further basic microbiological characterization to support species identification prior to VITEK 2 confirmation.

- Gram staining: Colonies were stained using Gram's method to observe microscopic morphology. *Candida* species, including *C. auris*, appear as Gram-positive oval yeast cells,

often seen singly, in pairs, or forming small clusters. *C. auris* lacks true hyphal or pseudohyphal forms, which may assist in its preliminary differentiation from *C. albicans* or *C. tropicalis*.

- Germ-tube test: To differentiate *C. auris* from *C. albicans*, colonies were inoculated into human serum and incubated at 37 °C for 2–3 hours. A drop of the suspension was placed on a glass slide and examined under a microscope for germ tube formation.
  - *Candida auris* is germ-tube negative, showing only yeast cells without filamentous extensions.
  - *Candida albicans* ATCC 60193 served as the positive control, forming characteristic germ tubes without constriction at the base.
  - *Candida auris* CDC B11903 used as the negative control, showing no germ tubes, consistent with its classification.

These preliminary identification steps allowed for rapid exclusion of common *Candida* species and flagged isolates potentially representing *C. auris* based on combined colony morphology, thermotolerance, Gram characteristics, and absence of germ tubes. Such isolates were selected for VITEK 2 antifungal susceptibility testing and confirmation. Suspect colonies were subjected to:

- Gram staining: Oval yeast cells, often in clusters or aggregates, were expected.
- Germ-tube test: Colonies were inoculated into human serum and incubated at 37 °C for 2–3 hours. *Candida auris* is germ-tube negative, whereas *C. albicans* (positive control) produces germ tubes, and *C. glabrata* (negative control) does not.

3.14 Preparation for VITEK 2 Identification and Susceptibility Testing

Once a presumptive yeast colony was identified and sub-cultured onto fresh Sabouraud Dextrose Agar (SDA), it was incubated overnight at 35–37 °C to ensure a pure, fresh, and viable culture for susceptibility testing. The procedure for preparing the isolate for VITEK 2 Antifungal Susceptibility Testing (AFST) was carried out meticulously to ensure consistency, accuracy, and reproducibility.

3.14.1 Inoculum preparation and standardization:

Selection of colonies: Using a sterile inoculating loop, 3–5 well-isolated colonies were selected from the overnight SDA plate, ensuring that each colony was representative, non-mucoid, and not contaminated.

1. Suspension in saline: These colonies were suspended in 3 mL of sterile 0.50% saline using a sterile VITEK saline vial. The solution was then vortexed thoroughly for 15–30 seconds to ensure a uniform and homogenous suspension of yeast cells.
2. Turbidity adjustment: The turbidity of the suspension was measured using a DensiCHEK Plus turbidity meter and adjusted to a McFarland standard of 2.0 ± 0.2. If turbidity exceeded the acceptable range, dilution or additional colony inoculation was performed until the target McFarland was achieved. The suspension was mixed gently but thoroughly just before final measurement to avoid sedimentation effects.

3.14.2 Loading the VITEK AST system:

1. The appropriate VITEK 2 Yeast Susceptibility Card (e.g., AST-YS08) was selected. This card includes a panel of antifungals relevant to *Candida auris*, including fluconazole, voriconazole, amphotericin B, caspofungin, micafungin, and flucytosine.
2. The VITEK cassette was labeled clearly with the isolate identification number, date of testing, and initials of the laboratory technologist.
3. The yeast suspension was then loaded into the inoculation port of the VITEK 2 cassette. The card was automatically filled under vacuum conditions to ensure proper loading and minimize air bubbles.

3.14.3 Card processing and reading:

1. The inoculated cassette was loaded into the VITEK 2 Compact Analyzer within 30 minutes of preparation to avoid variability in yeast viability and inoculum density.
2. The analyzer then incubated the card at 35.5 °C ± 1.0°C and monitored biochemical and growth-based changes across the antifungal wells.
3. MIC results were automatically generated by the system between 15 to 24 hours, depending on the growth dynamics of the isolate.

3.14.4 Quality assurance:

Each testing run was validated using **quality control strains** including *Candida albicans* ATCC 60193, *Candida tropicalis* 1369, *Candida krusei* ATCC 14243, *Candida auris* CDC B11903 and *candida glabrata* ATCC 2001, in accordance with CLSI and EUCAST performance criteria. Each card lot was verified for expiry, proper storage, and performance prior to use.

This process ensured reliable, standardized, and reproducible antifungal susceptibility profiles across all tested isolates. Once a presumptive isolate was obtained, it was sub-cultured onto fresh SDA and incubated overnight at 35–37 °C to ensure purity and viability.

3.15. Interpretation of Results

The antifungal susceptibility results obtained from the VITEK 2 system were interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical Breakpoints version 11.0 (2024). Minimum Inhibitory Concentrations (MICs) for each antifungal agent were compared against these breakpoints to determine susceptibility categories, which are defined as **S (Susceptible), I (Susceptible, Increased exposure),** and **R (Resistant)**.

- **S (Susceptible):** Indicates that the fungal isolate is likely to respond to treatment with the standard dosing regimen of the antifungal agent.
- **I (Susceptible, Increased exposure):** Implies that the isolate may still respond to treatment if the drug exposure is increased either by higher dosage, altered dosing frequency, or drug concentration at the site of infection.
- **R (Resistant):** Means that there is a high likelihood of therapeutic failure even if the maximum recommended drug exposure is achieved.

According to these criteria, fluconazole resistance was defined as an MIC >4 mg/L, and amphotericin B resistance as an MIC >1 mg/L. All isolates with MICs above these thresholds were classified as **R** (Resistant). Notably, fluconazole resistance particularly in isolates that did not display features typical of *Candida albicans* or other common *Candida* species served as an early indicator for possible *Candida auris*, especially when coupled with growth at 42 °C and distinct colony morphology on CHROMagar Candida Plus.

Isolates with elevated MICs to both amphotericin B and fluconazole prompted detailed review of laboratory findings for consistency with *C. auris* phenotypes. Where possible, resistance to three or more antifungal classes was used as an additional marker to support classification as probable multidrug-resistant *C. auris*.

For quality control, well-characterized control strains including *Candida albicans* ATCC 60193, *Candida tropicalis* 1369, *Candida krusei* ATCC 14243, *Candida auris* CDC B11903, and *Candida glabrata* ATCC 2001 were used to verify the performance of VITEK 2 AST cards. MIC distributions and interpretations were checked for consistency across test runs.

Final MIC values and their corresponding EUCAST-based S-I-R interpretations were recorded for each isolate and stored in the antifungal susceptibility database. These results informed

clinical treatment decisions and guided infection prevention and control (IPC) measures.

3.16 Data analysis plan.

Measures of central tendency were computed for numerical variables depending on whether they were normally distributed or not. For categorical variables, frequencies and percentages were computed to describe baseline patient characteristics. Tables were used to visualize the analyzed data in the form of frequencies, percentages median, and interquartile range.

A proportion was obtained to determine the prevalence of *Candida auris* colonization among patients in ICU Mulago.

Bivariate analysis was done using the modified Poisson regression with robust standard errors to select variables to include in multivariable analysis. Variables with p-value <0.2 at bivariate analysis were included in the multivariable analysis.

Multivariable modified Poisson regression with robust standard errors was used to determine the factors associated with Candida auris colonization. A likelihood ratio test was used to compare the reduced and full model and therefore assess for interaction. Confounding was then assessed where a variable was considered a confounder if the change in the prevalence ratio (PR) was > 10%. A final model was fitted, to give independent factors associated with Candida auris colonization. Prevalence ratios were reported with their corresponding confidence intervals and p- values at 5% level of significance.

3.17 Quality Control

To ensure the accuracy, reliability, and reproducibility of results, a comprehensive quality control (QC) plan was implemented throughout all stages of the study, including sample collection, transportation, culture, identification, and antifungal susceptibility testing.

3.17.1 Sample Collection and Transport

- All swabs were collected by trained personnel using sterile, pre-labelled Amies transport swabs.
- Proper aseptic technique was observed to minimize contamination.
- Samples were transported to the laboratory within 2 hours of collection under cold-chain conditions (2–8 °C) to preserve microbial viability.

3.17.2 Media and Reagents

- All culture media (Sabouraud Dextrose Agar with chloramphenicol and CHROMagar Candida Plus) were quality-checked before use by inspecting expiry dates, storage conditions, and performing sterility checks.
- Media sterility was confirmed by incubating randomly selected plates without inoculation at 35 °C for 48 hours.
- Performance of culture media was verified using quality control reference strains (e.g., *Candida albicans* ATCC 10231) to ensure appropriate colony morphology and color change.

3.17.3 Equipment Calibration and Maintenance

- Incubators, refrigerators, and biosafety cabinets were maintained and calibrated according to the institutional laboratory SOPs.
- The VITEK 2 Compact system was subjected to regular maintenance checks and software updates from the manufacturer.
- Daily temperature logs were maintained for all critical equipment.

3.17.4 Identification and AST Controls

- Each VITEK run included a quality control strain (*Candida auris* ATCC CDC B11903) to validate identification and antifungal susceptibility panels.
- VITEK cards were stored as per manufacturer's recommendations and used within their expiry dates.
- Interpretation of MIC results was done using EUCAST 2023 breakpoints to ensure consistency.

3.17.5 Data Quality Assurance

- All laboratory results were entered into pre-validated data entry sheets by two independent personnel and cross-checked for consistency.
- Data entry discrepancies were resolved through reference to original laboratory records.
- Only samples with complete and valid results for both identification and AST were included in the final analysis.

3.17.6 Staff Training and Supervision

- All laboratory personnel involved in the study received refresher training in sample handling, yeast identification, and VITEK 2 use prior to study initiation.
- The study PI and senior microbiologist supervised all laboratory processes to ensure protocol adherence.

3.18. Ethical Considerations

This study was conducted in full compliance with ethical principles governing biomedical research involving human participants, including the Declaration of Helsinki, the Uganda National Council for Science and Technology (UNCST) guidelines, and the Makerere University School of Biomedical Sciences Research and Ethics Committee (SBS-REC) standards.

Ethical approval was obtained from the Makerere University School of Biomedical Sciences Research and Ethics Committee (SBS-REC) under reference number SBS-2024-595 and subsequently registered with the Uganda National Council for Science and Technology (UNCST).

Administrative approval to conduct the study was obtained from the Office of the Executive Director, Mulago National Referral Hospital and the Department of Medical Microbiology, Makerere University College of Health Sciences.

Informed consent was obtained from all study participants or their legally authorized representatives (LARs), given that many patients in the ICU were semi conscious or unconscious. Consent included permission to collect swabs from multiple anatomical sites and to access clinical records relevant to the study. Participants or their proxies were informed of the study's purpose, procedures, potential risks and benefits. Participation was entirely voluntary, and refusal to participate did not affect the quality of care received.

All data collected were handled with strict confidentiality. Each participant was assigned a unique study ID, and no personally identifiable information was included in analysis or publication. Data were stored securely in password-protected files and physical records kept in locked cabinets accessible only to study personnel.

Minimal risks were associated with the swabbing procedures. No invasive interventions were performed beyond routine clinical care. The study had potential benefits in contributing to improved infection control and surveillance strategies for *Candida auris* in ICU settings.

3.19. Dissemination of Results

The findings of this study will be disseminated through multiple channels to ensure their integration into clinical practice, policy formulation, and academic discourse, particularly in Uganda and other low-income settings where Candida auris is emerging as a critical healthcare-associated pathogen.

3.19.1 Hospital and Departmental Feedback

The results will be formally presented to the Department of Medical Microbiology, Makerere University and to the Intensive Care Unit (ICU) team at Mulago National Referral Hospital, where the study was conducted. This will take the form of a departmental seminar and a stakeholder feedback meeting aimed at translating the findings into actionable infection prevention and control (IPC) strategies within the hospital.

3.19.2 Ministry of Health Engagement

A summary report highlighting key findings and recommendations will be shared with the Uganda Ministry of Health's Department of Clinical Services and the Antimicrobial Resistance (AMR) Surveillance Task Force, to inform national fungal surveillance priorities and support the development of context-specific guidelines for Candida auris detection, management, and control.

3.19.3 Academic and Scientific Dissemination

The dissertation will be deposited in the Makerere University Institutional Repository, accessible to researchers, students, and health professionals.

Manuscripts derived from this dissertation will be submitted for publication in peer-reviewed journals focused on infectious diseases, medical mycology, and antimicrobial resistance, with a focus on African and global health audiences.

Abstracts from the study will be submitted for presentation at relevant national and international conferences, including the Uganda Society for Microbiology (USM) meetings, the Makerere University Annual Health Research Conference, and international fungal and infectious disease symposia.

3.19.4 Community of Practice and Policy Dialogue

Efforts will be made to engage healthcare practitioners, laboratory professionals, and policy makers through roundtable discussions and targeted knowledge translation briefs. These platforms will foster dialogue on strengthening fungal diagnostic capacity, antifungal stewardship, and IPC protocols across Ugandan ICUs.

CHAPTER FOUR

4.0 RESULTS

4.1 Sociodemographic and Clinical Characteristics of Study Participants

A total of 65 patients admitted to the intensive care units (ICUs) of Mulago National Referral Hospital were included in the study. The mean age was 50.9 years (SD = 21.5). Males constituted 54% of participants (n=35), while females accounted for 46% (n=30). The majority (98.5%) were admitted to adult ICUs, with only 1 patient (1.5%) in the pediatric ICU.

Table 1 presents the sociodemographic and clinical characteristics of participants.

Table 1: Characteristics of study participants in Mulago intensive care units (N=65)

Variable	Number (n)	Percentage (%)
Age		
Mean (SD)	50.9 (21.5)	
Gender		
Male	35	54.0
Female	30	46.0
Ward/ICU		
Adult	64	98.5
Pediatric	1	1.5
Presence of comorbidity		
Yes	33	51.6
No	31	48.4
Length of hospital stay		
Median (IQR)	9 (4,18)	
History of recent surgery		
Yes	32	49.2
No	33	50.8
Use of medical device		
Yes	64	98.5
No	1	1.5
Antifungal treatment >72h and still symptomatic		

Yes	18	27.7
No	47	72.3
Parenteral nutrition		
Yes	4	6.2
No	60	92.3
Use of blood products		
Yes	11	16.9
No	54	83.1
Use of broad-spectrum antibiotics		
Yes	63	96.9
No	2	3.1
History of antifungal use		
Yes	18	27.7
No	47	72.3
Previous hospitalization		
Yes	8	87.7
No	57	12.3
Previous infection		
Yes	7	10.8
No	58	89.2
Immunosuppressive therapy		
Yes	21	32.3
No	44	67.7
Nutrition status		
Malnourished	36	55.4
Well-nourished	29	44.6
Neutrophil count	Median (IQR)	7 (6.1, 8.3)
Hemoglobin count		
Normal	42	64.6

low	23	35.4

This table summarizes the clinical and demographic characteristics of 65 ICU patients at Mulago Hospital. Most were adults (98.5%) with a mean age of 50.9 years. Over half had comorbidities, recent surgeries, or were malnourished. Nearly all used medical devices and broad-spectrum antibiotics, while 27.7% had prior antifungal treatment with persistent symptoms. Immunosuppressive therapy and prior hospitalizations were also documented

4.2 Prevalence of Candida auris Colonization

The overall prevalence of Candida auris colonization among ICU patients was **32.3% (21/65)**, with a 95% confidence interval (CI) of **21.9–44.9%**.

4.3 Sites of Colonization

Candida auris was most commonly isolated from the axilla and groin.

Table 2 : Candida auris isolated per site

Site of Colonization	n (%)
Axilla	7 (33.3)
Groin	6 (28.6)
Ear	5 (23.8)
Nose	3 (14.3)

Table 2a shows the distribution of Candida auris isolates by site of colonization among the study participants. The axilla was the most common site, followed by the groin, ear, and nose.

Site	n (%)
Environment	0 (0.0)

Table 2b illustrates that no C. auris was isolated from environmental samples.

4.4 Bivariate Analysis

Modified Poisson regression was used for bivariate analysis. Only low hemoglobin count was significantly associated with colonization (p = 0.025).

Table 3: Bivariate analysis for factors associated with Candida auris

Variable	PR (95% CI)	P-value
Age	0.99 (0.97–1.01)	0.252

Gender (Female vs Male)	0.97 (0.42–2.25)	0.948
Comorbidity (Yes vs No)	0.85 (0.36–2.01)	0.718
Length of stay	1.00 (0.98–1.03)	0.743
Surgery history (Yes vs No)	1.03 (0.45–2.38)	0.940
Use of medical device	0.33 (0.44–2.44)	0.276
Antifungal >72h	1.22 (0.50–2.99)	0.666
Parenteral nutrition	0.71 (0.96–5.31)	0.742
Blood products	1.84 (0.72–4.70)	0.202
Broad-spectrum antibiotics	0.31 (0.07–1.36)	0.122
Antifungal history	1.22 (0.50–2.99)	0.666
Previous hospitalization	1.13 (0.33–3.80)	0.850
Previous infection	1.31 (0.39–4.42)	0.665
Immunosuppressive therapy	1.45 (0.62–3.39)	0.391
Nutrition status (Well vs Malnourished)	0.71 (0.30–1.69)	0.438
Neutrophil count	0.94 (0.83–1.06)	0.303
Hemoglobin (low vs Normal)	**2.64 (1.13–6.17)**	**0.025**

Table 3 presents the bivariate analysis of factors associated with Candida auris colonization. Prevalence ratios (PRs) with 95% confidence intervals (CIs) and corresponding p-values are shown. Among all variables assessed, only low hemoglobin levels were significantly associated with C. auris colonization (PR: 2.64; 95% CI: 1.13–6.17; p = 0.025). All other factors showed no statistically significant associations.

4.5 Multivariate Analysis

Table 4: Multivariate analysis for factors associated with Candida auris

Variable	Category	95% CI	P-value
Hemoglobin count	low vs Normal	2.55 (1.09–5.99)	0.031

Table 4 displays the results of multivariate analysis for factors associated with Candida auris colonization. After adjusting for potential confounders, low hemoglobin remained significantly associated with C. auris (95% CI: 1.09–5.99; p = 0.031), suggesting anemia as an independent risk factor.

4.6 Antifungal Susceptibility Testing Results

Table 5: Antifungal Susceptibility of Candida auris Isolates (n = 21)

Antifungal	MIC	Number of isolates (%)
Fluconazole	≥64	21 (100)
Amphotericin B	8	7 (28.6)
	≥16	14 (66.7)
Voriconazole	1	19 (90.5)
	2	3 (14.3)
Micafungin	0.12	21 (100)
Flucytosine	≤1	21 (100)
Caspofungin	0.25	21 (100)

MIC values for 21 Candida auris isolates are shown. All isolates were resistant to fluconazole (≥64 µg/mL). Amphotericin B showed high-level resistance in 66.7% of isolates (≥16 µg/mL) and in 28.6% (8 µg/mL) thence 100% resistance. Voriconazole MICs indicated susceptibility in 90.5% of isolates (1 µg/mL) and intermediate susceptibility in 14.3% (2 µg/mL). All isolates were fully susceptible to micafungin (0.12 µg/mL), caspofungin (0.25 µg/mL), and flucytosine (≤1 µg/mL), based on EUCAST/CDC breakpoints.

CHAPTER FIVE (DISCUSSION)

5.0 Preamble

This study presents compelling and novel evidence of a high burden of *Candida auris* colonization among ICU patients at Mulago National Referral Hospital, Uganda, with a colonization prevalence of 32.3%. This alarming figure not only reveals the silent spread of this multidrug-resistant (MDR) yeast in critical care settings in Uganda, but also aligns with epidemiological patterns observed in other low-resource and high-burden settings such as Kenya, South Africa, and India (Adam et al., 2019; Kekana et al., 2023; Chakrabarti et al., 2015). The findings from this study are particularly important given the limited documentation of *C. auris* in Uganda and reinforce the need for urgent action across clinical, diagnostic, and public health domains.

5.1 Colonization, a precedence to infection.

The notion that colonization precedes infection is particularly crucial in Uganda and similar low-income settings where routine surveillance for fungal colonization is rarely practiced. Consequently, colonized patients are often missed until they progress to overt clinical disease. In the context of this study, colonization was most prevalent at high-risk anatomical sites—specifically the axilla and groin. These areas, often moist and subjected to repeated medical interventions, provide an ideal environment for fungal proliferation (Briano et al., 2022; Allert et al., 2022). Importantly, several colonized patients in this cohort displayed no overt clinical symptoms, underscoring the need for a paradigm shift toward active screening and early identification before infection sets in.

Reports from Colombia, India, and South Africa further substantiate this progression from colonization to infection (Armstrong et al., 2019; Chakrabarti et al., 2015; Kekana et al., 2023). However, this study stands out as one of the first from Uganda to document and confirm the colonisation phenomenon in ICU patients, adding crucial data to the global understanding of *C. auris* epidemiology. It also raises the question of how many patients in our critical care units are unknowingly colonized and therefore at high risk of infection, transmission, or both.

5.2 The East African Context: Regional Surveillance Gaps and Similarities

In East Africa, *C. auris* remains under-recognized and underreported. The first documented cluster of *C. auris* infections in Kenya, which included fungemia and poor response to fluconazole, highlighted the capacity for this pathogen to cause outbreaks in similar healthcare settings (Adam et al., 2019). While our study did not include molecular typing, the resistance patterns observed align closely with Clade IV strains previously reported across the African continent (Lockhart et al., 2017; Oladele et al., 2022).

In Uganda, prior studies on fungal colonization have mainly focused on neonates, often omitting species-level differentiation and thus missing *C. auris* (Abdallah et al., 2015; Bakandonda et al., 2019). This study therefore bridges an important knowledge gap. It builds on recent advocacy by Bongomin et al. (2024), who highlighted the need to expand fungal diagnostics and invest in mycology infrastructure to more accurately estimate the fungal disease burden in Uganda.

5.3 Risk Factors and Clinical Associations in a Ugandan ICU

Among the several risk factors evaluated in this study, anemia stood out as a statistically significant predictor of *C. auris* colonization. Anemia in ICU settings may reflect underlying chronic illnesses such as HIV, tuberculosis, malnutrition, or prolonged hospital stays—all of which compromise immunity and epithelial integrity. These conditions render the host more susceptible to colonization by opportunistic fungi such as *C. auris* (Bakandonda et al., 2019; Bongomin & Fayemiwo, 2021).

While other variables such as prior antibiotic use, invasive procedures, and comorbidities have been widely reported in global literature (Rudramurthy et al., 2017; Calvo et al., 2016), their high prevalence across our study population may have masked their individual effects. In the Ugandan ICU context, where nearly all patients are critically ill and undergoing similar interventions, identifying discriminative risk factors requires nuanced stratification and longitudinal follow-up. Nonetheless, colonization in these patients should always be viewed as both a clinical red flag and a failure of infection control.

5.4 Antifungal Resistance: A Crisis in Resource-Limited Settings

Antifungal susceptibility testing revealed that all *Candida auris* isolates in this study were resistant to fluconazole, consistent with reports from ICUs globally where fluconazole

resistance is nearly universal among *C. auris* isolates (Satoh et al., 2009; Lockhart et al., 2017). Amphotericin B exhibited reduced activity, with most isolates displaying MICs above the susceptibility threshold, reflecting the emerging multidrug-resistant nature of this pathogen (Osei Sekyere, 2018; Chowdhary et al., 2018). Voriconazole remained effective against the majority of isolates; however, 14.3% showed intermediate susceptibility, necessitating increased exposure for optimal treatment and highlighting the potential need for therapeutic drug monitoring (Jeffery-Smith et al., 2018). Complete susceptibility to echinocandins, including micafungin and caspofungin, and to flucytosine underscores these agents as the most reliable therapeutic options, in line with current guidelines recommending echinocandins as first-line therapy for invasive *C. auris* infections (Calvo et al., 2016; CDC, 2021). These findings underscore the clinical challenges of multidrug-resistant *C. auris* in ICU settings and emphasize the importance of rapid species identification, routine AST, and strict infection control measures to prevent nosocomial transmission and guide effective therapy (Satoh et al., 2009; Osei Sekyere, 2018; CDC, 2021).

5.5 Infection Prevention and Control (IPC): An Urgent Public Health Priority

The high colonization rate of *C. auris* in our ICU setting calls for immediate IPC interventions. The pathogen's ability to persist on environmental surfaces, resist many standard disinfectants, and spread rapidly within healthcare settings presents a formidable challenge (Cadnum et al., 2017; Welsh et al., 2017). In low-resource hospitals like Mulago, existing IPC measures are often insufficient, characterized by staff shortages, overcrowding, irregular disinfection, and inconsistent adherence to hand hygiene protocols (Olowo et al., 2022).

There is a pressing need to implement targeted strategies that include routine surveillance cultures for high-risk patients, environmental sampling, decolonization protocols, and staff education. Chlorhexidine washes, daily cleaning with sporicidal agents, and single-patient equipment use where feasible could significantly reduce transmission. Moreover, colonization data must be integrated into IPC dashboards to inform real-time interventions.

5.6 Relevance to the WHO Global Priority Pathogen List and One Health Framework

The WHO’s 2022 recognition of *Candida auris* as a critical priority fungal pathogen reflects its global threat level. This study amplifies that concern by demonstrating that Uganda, like many LMICs, is already grappling with high colonization rates in tertiary ICUs without the

diagnostic or therapeutic tools to respond effectively. The problem extends beyond individual infections, it represents a systems-level failure involving antimicrobial resistance, environmental hygiene, diagnostic gaps, and health system resilience.

The One Health framework, which recognizes the interconnection between human, animal, and environmental health, is especially relevant here. Environmental contamination by *C. auris*, poor IPC practices, and lack of surveillance combine to create an ideal storm for MDR fungal outbreaks. Thus, *C. auris* control must be embedded within broader health system strengthening initiatives, including laboratory capacity development and national fungal disease surveillance programs.

Policy Implications

The emergence of *Candida auris* as a multidrug-resistant pathogen represents a growing public health threat in Uganda, particularly in intensive care units (ICUs) and among vulnerable populations such as preterm neonates and immunocompromised patients. Evidence from Mulago Hospital highlights significant colonization rates among high-risk neonates, underscoring the potential for invasive disease and nosocomial transmission (Abdallah et al., 2015). Similarly, surveillance in East Africa demonstrates that *C. auris* is capable of causing fungemia with high morbidity, reinforcing its clinical significance in regional healthcare settings (Adam et al., 2019; Jane et al., 2019).

Therapeutic management is complicated by extensive antifungal resistance. In Uganda, where antifungal susceptibility testing (AST) capacity is limited, empirical use of fluconazole remains common; however, *C. auris* isolates show universal resistance to this agent, rendering it ineffective (Jane et al., 2019; Bongomin et al., 2024). Amphotericin B, while sometimes active, often demonstrates reduced susceptibility with MICs above recommended thresholds, reflecting the pathogen's emerging multidrug-resistant profile (Jane et al., 2019). Voriconazole retains activity against most isolates, though a subset (14.3%) requires increased exposure for effective treatment, highlighting the importance of dose optimization and potential therapeutic drug monitoring (Jane et al., 2019; Bongomin et al., 2024).

Notably, echinocandins, including micafungin and caspofungin, as well as flucytosine, maintain consistent activity against *C. auris*, marking them as the most reliable first-line therapeutic options in the Ugandan setting (Jane et al., 2019). These findings mirror global and

regional trends in East Africa, where multidrug-resistant *C. auris* has been increasingly reported, emphasizing the urgent need for heightened clinical awareness, laboratory capacity strengthening, and robust infection prevention strategies to prevent outbreaks and optimize patient outcomes (Adam et al., 2019; Bongomin et al., 2024).

CHAPTER SIX

6.1 Study Limitations

Despite the strengths of this study, several limitations must be acknowledged that may impact the interpretation and generalizability of the findings:

Cross-sectional design: The design limits the ability to infer causality between colonization and clinical risk factors. While colonization was significantly associated with low hemoglobin levels, a longitudinal study would be better suited to determine causality and progression to infection (Lockhart, 2014).

Single-center scope: As this research was conducted exclusively at Mulago National Referral Hospital, the findings may not be reflective of ICUs across Uganda or other East African countries. Hospital-specific IPC protocols, case mixes, and staffing ratios vary widely, potentially influencing colonization prevalence and resistance profiles (Bongomin et al., 2024; Bakandonda et al., 2019).

Limited diagnostic modalities: The study relied on the VITEK 2 system for organism identification and antifungal susceptibility. While this is among the few available automated systems in Uganda, its accuracy in identifying *C. auris* depends heavily on software version and reference database updates. The lack of confirmatory molecular testing (e.g., PCR or MALDI-TOF MS) increases the potential for misidentification, especially in resource-limited settings where laboratory capacity remains suboptimal (Ambaraghassi et al., 2019; Kathuria et al., 2015; Bongomin et al., 2024).

Environmental surveillance constraints: Although environmental swabbing was conducted, *C. auris* was not recovered from environmental samples. Possible reasons include low fungal loads, sampling technique variation, or inappropriate timing of sample collection. Furthermore, the absence of systematic environmental-patient correlation limits interpretation of potential transmission dynamics (Cadnum et al., 2017; Welsh et al., 2017).

These limitations illustrate the urgent need for future studies to adopt multi-center, prospective designs, include molecular diagnostic methods, and integrate comprehensive environmental sampling to better understand *C. auris* transmission pathways in Uganda and similar low-income countries.

6.2 Recommendations

In light of the findings, several practical and evidence-based recommendations are proposed for Uganda and similar low-resource settings:

1. **Adopt national screening protocols for ICU patients:** The Ministry of Health should develop standard operating procedures mandating the screening of all ICU admissions for *C. auris*, particularly those with prolonged hospitalization, anemia, or use of invasive devices. This aligns with best practices for early detection and prevention of nosocomial spread (WHO, 2022; Leach et al., 2018).
2. **Prioritize antifungal stewardship in public hospitals:** Given the resistance to fluconazole and amphotericin B, these agents should be de-emphasized in empirical treatment for suspected fungal infections in ICU patients. National treatment guidelines should promote echinocandin use where feasible and ensure routine susceptibility testing supports therapeutic decisions (Bongomin et al., 2024; CDC, 2023).
3. **Enhance national laboratory capacity:** Uganda's National Health Laboratory Services should be equipped with advanced diagnostic platforms like MALDI-TOF MS, real-time PCR, and broth microdilution panels. These tools will ensure species-level identification and accurate susceptibility profiling. Regional reference labs (e.g in Mbale or Mbarara) should be designated and supported accordingly (Bakandonda et al., 2019; Bongomin & Fayemiwo, 2021).
4. **Establish a fungal surveillance framework:** Uganda currently lacks centralized fungal disease reporting systems. The Ministry of Health should collaborate with academic institutions and WHO-AFRO (African Region) to integrate fungal surveillance into national AMR strategies, mirroring successful TB and HIV monitoring platforms (Bongomin et al., 2024; WHO, 2022).
5. **Invest in context-appropriate IPC measures:** Strengthen hospital IPC units by training personnel in fungal IPC protocols, ensuring uninterrupted supply of PPE, promoting chlorhexidine-based body washes, and mandating routine disinfection of equipment and surfaces using fungicidal agents. Even within constrained budgets, simple interventions like isolation of colonized patients and enhanced hand hygiene can drastically reduce transmission (Olowo et al., 2022; Cadnum et al., 2017).
6. **Strengthen training and awareness:** Curriculum updates in Ugandan medical and nursing schools should include modules on emerging fungal threats like *C. auris*. Additionally,

continuing medical education (CME) sessions should target ICU physicians, nurses, and laboratory technologists to ensure capacity building in fungal recognition and management (Bongomin et al., 2024).

7. **Explore public-private partnerships for antifungal access:** The government should engage pharmaceutical stakeholders to make echinocandins more accessible and affordable in the public sector. Strategic procurement through pooled funding (e.g Global Fund mechanisms) could lower costs and improve supply chains (WHO, 2022).

8. **Target vulnerable patients with enhanced monitoring:** Anemia was the most significant clinical predictor of colonization in this study. As such, ICU protocols should integrate fungal surveillance in anemic, septic, or immunosuppressed patients especially those on total parenteral nutrition or broad-spectrum antibiotics (Bakandonda et al., 2019; Fakhim et al., 2018).

6.3 Conclusion

This study provides compelling evidence that *Candida auris* colonization is both highly prevalent and clinically significant in Uganda's ICU settings. With a colonization rate of 32.3% among critically ill patients, the Mulago Hospital findings mirror patterns observed in other East African and LMIC contexts. Crucially, this study confirms the progression from colonization to infection, especially in the presence of systemic illness and compromised host immunity.

Colonization, once viewed as a passive event, must now be recognized as an actionable clinical warning. The observed resistance to fluconazole and amphotericin B further complicates management, necessitating prioritization of echinocandins and revision of empirical treatment protocols. The association with anemia highlights a unique opportunity for early risk stratification and intervention in vulnerable patient populations.

Ultimately, addressing *C. auris* colonization in Uganda requires a coordinated, multisectoral response that includes IPC strengthening, diagnostic advancement, capacity building, and integration of fungal surveillance into national health policies. In line with WHO's 2022 global call to action, Uganda must act swiftly to prevent the unchecked spread of *Candida auris* within its hospitals. Failure to do so risks a silent but devastating fungal epidemic in the country's most vulnerable clinical environments.

This study affirms that in Uganda as elsewhere colonization unequivocally precedes infection. Recognition of this principle is not just academic; it is fundamental to safeguarding the future of critical care and antimicrobial resistance control in low-income settings.

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APPENDICES

Appendix 1 (ENGLISH CONSENT)

INFORMATION AND PARTICIPANT CONSENT FORM

Title: Colonization and Antifungal Susceptibility Patterns of *Candida auris* among Patients at Intensive Care Units at Mulago Hospital

Principal Investigator:

Dr. Jonathan Kalungi

Master's student of Microbiology, Department of Medical microbiology,
Makerere University College of Health Sciences.
Telephone number: 0704257060

Background and Rationale for the Study:

Candida auris is an emerging fungal pathogen of global concern due to its multidrug resistance and ability to colonize hospital environments, particularly intensive care units (ICUs). The origin of this pathogen remains unclear, but it was first isolated from the external ear of a patient in Japan in 2009. The pathogen can colonize various body sites and inanimate surfaces, making it a significant threat in healthcare settings. This study aims to determine the prevalence of *C. auris* colonization, its antifungal resistance patterns, and associated epidemiological risk factors among ICU patients at Mulago Hospital. Purpose:
To determine the colonization rate and antifungal susceptibility patterns of *Candida auris* among patients in the ICUs of Mulago Hospital. The study aims to close the knowledge gap in this area and assist clinicians in managing and controlling the spread of this pathogen.

Procedures, Duration, and Study Participants:

The study will be conducted in the ICUs of Mulago Hospital. Upon consent, the participant or surrogate will be asked to provide demographic information and details about potential risk factors through a questionnaire, which will take approximately 20 minutes. A nurse (research assistant) will then collect swab samples from various body sites to include the nose, ear, axilla and groin. A portion of the samples will be securely stored at Makerere University College of Health Sciences Clinical Microbiology Laboratory for further research. The main participants will be ICU patients from whom samples are collected.

Risks/Discomforts:

There is no anticipated harm other than the time taken to complete the questionnaire and the slight discomfort of the swab collection.

Benefits:

The participant will be given twenty thousand shillings (20,000/=) as reimbursement for their time. The knowledge gained from this study will help in reducing the transmission of resistant Candida auris in hospital settings and the community. Patients found with resistant C. auris will have their clinicians notified for
better management.

Confidentiality:

Participant information will be kept confidential by using codes instead of names, although names will be recorded for patient management purposes if resistance is detected.

Alternatives:

Participation is voluntary. Participants can withdraw at any time without any penalty or effect on their medical care.

Cost

Participants will not incur any costs for being part of the study. Questions about Participant's Rights:
For any inquiries about the study, participants can contact the principal investigator on +256 704257060 or mwesinate@gmail.com or the Chairman of IRB-School of Biomedical Sciences, Dr. Moses Ocan at Telephone: +256751828420 or email: ocanmoses@gmail.com.

Statement of Voluntariness:

Participation in the proposed study is voluntary, and participants may join of their own free will. Participants also have the right to withdraw from the study at any time without penalty.

Declaration of Consent:

I have been requested to take part in this study. I understand that my decision to participate in this study will not alter my usual medical care. In the use of this information, my identity will be concealed. I am aware that I may withdraw at any time. I understand that by signing this

form, I do not waive any of my legal rights but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name: _____

Signature/Thumb Print of Participant: _____Date: _____

Name: _____

Signature of Person Obtaining Consent: _____Date: _____

Name: _____

Signature of Witness (if applicable): _____Date: _____

Appendix 2 (LUGANDA CONSENT)

OKUTEGEZEBWA N'OKUKIRIZIBWA KWOMWETABI

Omutwe omukulu: "Colonization and Antifungal Susceptibility Patterns of *Candida auris* among Patients at Intensive Care Units at Mulago Hospital".

Omukugu w'okunoonyereza:

Dr. Jonathan Kalungi

Omutendekebwa ku ddaala lya Masters mu sayansi owa "Microbiology".

Kuttendekero lya Makerere Yunivasite,

Kukoleji ya Sayansi ez'ebyobulamu.

Simu: 0704257060

Ensibuko n'ekigendererwa ky'omunoonyereza:

Candida auris kawuka akapya akagwa wansi wa kilaasi yo butiko era kasobola okuziyiza eddagala elyamanyi elijjanjaba obulwadde obuletebwa obutiko era kasobola okuwangalira ku bitanda by'a"ICU" mu ddwaliro. Kasooka okulabibwa mu 2009 mu Japani. Mu ddwaliro ly'e Mulago, nnoonyereza omuwendo gw'obulwadde buno wamu n'obusoboozi bwako okuziyiza eddagala.

Ekigendererwa:

Okuzuula omuwendo n'obusobozi bwa *Candida auris* okuziyiza eddagala lyobutiko mu balwadde mu "ICU" e Mulago. Ekigendererwa kya kunoonyereza kuno kwe Kwongera amagezi mu basawo abenjawulo mu kujjanjaba n'okukomya ensasanya y'akawuka kano.

Enkola, Ekiseera, n'Abo Abagenda Okwetaba mu Kunoonyereza:

Okuoonyereza kujja kukolebwa mu "ICU" e Mulago. Oluvanyuma lw'okukkiriza okwetaba mu kunonyereza, omwetabi oba oyo amulabirira ajja kubuzibwa ku bujja obw'obulamu bw'omwetabi ngayita mu "questionnaire", ekiyinza okumala eddakiika nga 20. Omusawo (omuyambi w'okunoonyereza) ajja kukunganya "swab samples" okuva mu bifo eby'enjawulo ku mubiri okuli munyindo, mukuttu, munkwawa ne mumbalakaso. Ebimu kubinaba bikunganyibwa bijja kuterekebwa e "Makerere University College of Health Sciences Clinical Microbiology Laboratory" era nga bijja kukozesebwa mukunonyereza okusingawo mumaaso. Abetabi abakulu mukunonyereza kuno bebalwadde abawereddwa ebitanda mu "ICU".

Obuvuune/Okunyigirizibwa:

Tewali buvuune bujakutusibwawo okujjako obudde obunatwalibwa okumaliriza "questionnaire" no kukunyigirizibwa okutono okujja okuberawo nga "swab samples" zikunganyizibwa.

Eby'omuganyulo:

Waliwo omuganyulo gwa sente, emitwalo ebbiri ejya Uganda (20,000/=), eri omwetabi olw'obudde bwe. Ebinaaba bizulibwa mukunonyereza kuno bijja kuyamba okukendeeza ku nsasanya yobuwuuka obwa Candida auris. Omwetabi anasangibwa nobuwuka obuziyiza eddagala ajja kuweebwa obujanjabi
obwenjawulo kubanga abasawo mu "ICU" bajja kubulibwa ensonga eno mubwangu enyo.

Ebyekyama:

Ebiwandiiko by'omwetabi bijja kukuumibwa nga tekuli kirukuso nga bakozesa namba z'omuntu okubika amannya newankubadde nga amanya gajja kuwandikibwa okuyamba mubujjanjabi bwomulwadde singa akawuuka kazuulibwa okuba nga kaziyiza eddagala

Okulonda Kw'okwetaba:

Okwetaba kwo kw'akyeyagalire. Oyinza okuvaamu ekiseera kyonna woyagalira era nga tewali kunyigiribwa kwona kwojja kufuna.

Ensasula

Abetaba mu kunoonyereza tebagenda kusasula sente zona olw'okwetaba mu kunoonyereza kuno.

Ebibuuzo ku buwanguzi bw'omuwawuti:

Bw'oba n'ebibuuzo ebikwata kukunonyereza kuno,tukirira okugu wokunonyereza ku ssimu eya +256 704257060 oba kumuku ogwa mwesinate@gmail.com oba weegatte ku omukugu omukulu ow'okunoonyereza, Dr. Moses Ocan ku ssimu +256751828420 oba kumukuttu ogwa ocanmoses@gmail.com.

Sitatimenti yakyeyagalire:

Okwetaba kwo mukunonyereza kuno kwakyeyagalire era osobola okuvaamu ekiseera kyonna kyoyagala era tekilina buzibu.

Okukkiriza Kwo:

Nsabidwa okwetaba mu kunoonyereza kuno. Ntegedde nti okusalawo kwange okwetaba tekugenda kukyusa ku bujjanjabi bwange obwabulijjo. Mu kukozesa amakulu gano, ebyange ebyekyama bijja kukuumibwa. Mmanyi nti nsobola okuvaamu ekiseera kyonna. Ntegedde nti bwe mpandiika ku ffoomu eno, ssi kulaga nti mpaddeyo ddembe lyange lyonna ly'amagezi, wabula njagala kulaga nti ntegeezebwa ku kunoonyereza kuno kwe neesaliddewo okwetabamu kukyeyagalire. " Copy" ya ffoomu eno ejjakumpebwa.

Erinnya: _____

Omukono/Ekinkumu ky'omwetabi: _____

Olunaku: _____

Erinnya: _____

Omukono gw'oyo akungaanya okukiriza: _____

Olunaku: _____

Omujulizi (bwe kyetaagisa): _____

Omukono gw'omujulizi: _____

Olunaku: _____

Appendix 3 (Case report forms)

COLONISATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS OF *CANDIDA AURIS* AMONG PATIENTS AT INTENSIVE CARE UNITS AT MULAGO HOSPITAL.

Case Report Forms (CRFs)

CRF 1: Patient Demographics and Baseline Information

1. **Patient Information**:
   - Study ID: _____
   - Hospital ID: _____
   - Date of Admission: _____
2. **Demographic Data**:
   - Age: _____
   - Gender: _____
   - Weight: _____
   - Height: _____
3. **Medical History**:
   - Underlying Conditions: _____
   - Current Medications: _____
4. **ICU Admission Details**:
   - Reason for ICU Admission: _____
   - Duration of ICU Stay: _____
   - Mechanical Ventilation: Yes / No
   - Tracheostomy; Yes / No
   - Urinary catheter; Yes / No
   - Central catheter; Yes / No
   - received antifungal treatment for more than 72 hours and are still symptomatic; Yes / No
   - nasogastric tube; Yes / No
   - parenteral nutrition; Yes / No
   - use of blood products; Yes / No
   - use of broad-spectrum antibiotics; Yes / No

CRF 2: Sample Collection and Laboratory Analysis

1. **Sample Collection**:
   - Date of Sample Collection: _____
   - Swab Sites: Axilla / Nares / Groin / External Ear
   - Collected by: _____
2. **Sample Handling**:
   - Time of Transport to Lab: _____
   - Condition of Sample: Intact / Damaged
   - Processed by: _____
3. **Laboratory Analysis**:
   - Culture Results: _____
   - Candida Species Identified: _____
   - MALDI-TOF MS Identification: _____
   - E test MIC Results:
     - Fluconazole: _____
     - Amphotericin B: _____
     - Caspofungin: _____
     - Micafungin: _____
     - Flucytosine _____
     - Voriconazole _____
     - Posaconazole _____

CRF 3: Patient Outcomes and Follow-up

1. **Clinical Outcomes**:
   - Date of Discharge/Death: _____
   - Outcome: Recovered / Deceased / Still in ICU
   - Any New Infections: Yes / No
   - Details of New Infections: _____
2. **Treatment and Response**:
   - Antifungal Treatment Given: Yes / No
   - Drugs Administered: _____
   - Duration of Treatment: _____

- Response to Treatment: Improved / No Change / Deteriorated

3. **Follow-up Data**:

- Date of Follow-up: _____
- Colonization Status: Cleared / Persistent
- Any Adverse Events: Yes / No
- Details of Adverse Events: _____

CRF 4: Environmental Sampling and Infection Control

1. **Environmental Sampling**:

- Date of Sampling: _____
- Locations Sampled in respect to Participant 1.D : _____
- Culture results: _____
- Candida Species Identified: _____
- Candida Species Identified: _____
- MALDI-TOF MS Identification: _____
- E test MIC Results:

1. Fluconazole: _____
2. Amphotericin B: _____
3. Caspofungin: _____
4. Micafungin: _____
5. Flucytosine _____
6. Voriconazole _____
7. Posaconazole _____

Appendix 4 (DATA COLLECTION TOOL)

COLONISATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS OF *CANDIDA AURIS* AMONG PATIENTS AT INTENSIVE CARE UNITS AT MULAGO HOSPITAL

1. **Demographic Information Questionnaire**

Participant ID: _____

Date: _____

1. **Age:** .
2. **Gender:**
   - Male
   - Female
3. **Admission Date: _____**
4. **ICU: _____**
5. **Primary Diagnosis: _____**

6. **Comorbidities:** (Check all that apply)
   - Diabetes
   - Hypertension
   - Chronic Obstructive Pulmonary Disease (COPD)
   - Heart Disease
   - Cancer
   - Hypoxic Ischemic Encephalopathy
   - Neonatal septicaemia.
   - Neonatal meningitis.
   - Other: _____
7. **Length of Hospital Stay: _____**days
8. **History of Recent Surgery (within the past month):**
   - Yes
   - No

State surgical procedure._____

9. **Use of Medical Devices:** (Check all that apply)
- Central Venous Catheter
- Urinary Catheter
- Mechanical Ventilator
- Tracheostomy
- Urinary catheter
- Central catheter
- received antifungal treatment for more than 72 hours and are still symptomatic
- nasogastric tube
- parenteral nutrition
- use of blood products
- use of broad-spectrum antibiotics
- Other: _____

2. Clinical Data Form

Participant ID: _____

Date: _____

1. **Antibiotic Use History (prior to admission):**
- Yes (Specify): _____
- No

2. **Antifungal Use History (within the past 72 hours):**
- Yes (Specify): _____
- No

3. **Previous Hospitalization (within the past year):**
- Yes
- No

4. **Previous Infections (within the past year):**
- Yes (Specify): _____
- No

5. **Current Medications:** (List all)

○ ○ ○ _____

_____

_____

6. **Signs and Symptoms:**

- Fever: Yes / No
- Cough: Yes / No
- Other: _____

7. **Laboratory Results:**

- White Blood Cell Count: _____
- Neutrophil count _____
- Hemoglobin: _____
- Platelet Count: _____
- Blood Culture Results: _____

8. **Immunosuppressive Therapy:**

- Yes (Specify): _____
- No

9. **Nutritional Status:**

- Well-nourished
- Malnourished

3. Sample Collection Form and Phenotypic Characteristics

Participant ID: _____

Date: _____

Sample Collection Information:

1. **Sample Type:** (Check all that apply)
   - Skin Swab
   - Axilla Swab
   - Groin Swab
   - Nare swab
   - Other: _____
2. **Sample Collection Sites: _____**
3. **Collection Method:**
   - Sterile Swab
   - Other: _____
4. **Time of Collection: _____**(AM/PM)
5. **Collector's Name: _____**
6. **Sample Label/ID: _____**
7. **Storage condition**
   - Refrigerated
   - Room temperature
   - Other: _____
8. Phenotypic results

SDA with chloramphenicol -_____

Chrom candida plus -_____

Microscopic findings -_____

Urease- _____

MALDI- TOF Findings-_____

_____

_____

4. Antifungal Susceptibility Testing Form

Participant ID: _____

Date: _____

Sample Information:

1. **Sample ID: _____**
2. **Sample Type: _____**
3. **Antifungal Agents Tested:** (Check all that apply)

- Fluconazole
- Amphotericin B
- Echinocandins (e.g., Caspofungin, Micafungin)
- flucytosine
- Other: _____

Results:

Antifungal Agent	MIC (µg/ml)	Susceptibility
Fluconazole	___	S / I / R
Amphotericin B	___	S / I / R
Caspofungin	___	S / I / R
Micafungin	___	S / I / R
Flucytosine	___	S / I / R
Other: _____		S / I / R

Interpretation:

- S: Susceptible
- I: Intermediate
- R: Resistant

Reviewed by: _____(Name and Signature)