PHENOTYPIC, GENOTYPIC, VIRULENCE AND AZOLE RESISTANCE CHARACTERIZATION OF CANDIDA SPECIES FROM CLINICAL SPECIMENS IN NAIROBI COUNTY, KENYA

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A Thesis Submitted to the School of Public Health, Biomedical Sciences and Technology in Partial Fulfilment for the Requirements of the Award of the Degree of Doctor of Philosophy in Medical Laboratory Sciences and Technology (Medical Mycology) of Masinde Muliro University of Science and Technology.

November, 2024

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DEDICATION

I dedicate this thesis to my loving parents, wife and children for encouragement and perseverance during long hours away working on this project.

ACKNOWLEDGEMENT

I would like to express my immeasurable gratitude to my supervisors Prof Tom Were and Dr. Christine Bii for their supervisory and advisory support towards completion of this work. I am deeply grateful to the Kenya Medical Research Institute, Centre for Microbiology and Mycology Laboratories for their technical support. Much appreciation goes to my siblings, for their inspiration and encouragement. My sincere gratitude goes to all those who assisted me in one way or the other. Above all, I thank the almighty God for his grace, love, protection and good health throughout the study period.

ABSTRACT

Invasive and systemic candidiasis are prevalent mycoses complicating immune-suppressive conditions and leading to high mortality. The development and spread of azole antimycotic drug resistance hampers their effective utilisation in therapy and prophylaxis of candidiasis. *Candida albicans* (CA), consisting of genotypes A-H with varying virulence, is the most prevalent species. Several virulence factors including biofilm formation, haemolysins, proteases, and phospholipase (PLs) activities confer the fungi with invasiveness and dissemination of disease. This study determined the distribution and anti-azole resistance of CA and non-*albicans* candida (NAC), including genotypes and virulence factor expression in CA isolates. This cross-sectional research was conducted on clinical samples (n=141) of patients presenting with different candidal mycoses at various metropolitan health facilities in Nairobi city, Kenya. Candida isolate identification was done via mycological culture, morphological, biochemical, and VITEK® 2-YST methods. Itraconazole (ITZ) and voriconazole (VOR) resistance were evaluated by the disk diffusion and broth micro-dilution tests. Genotyping and virulence factor expression were determined for the CA isolates (n=94) using PCR-RFLP and virulence expression culture methods, respectively. Overall, 141 isolates were recovered, consisting of CA (66.7%) and NAC (*C. glabrata*, 16.3%; *C. parapsilosis*, 7.8%; *C. tropicalis*, 3.6%; *C. krusei*, 2.8%; *C. guilliermondii*, 1.4%; and *C. famata*, 1.4%). Anti-azole sensitivity was low for ITZ (21.3%) and VOR (24.5%) with high cross-resistance (74.5%) in the CA isolates. In the NAC isolates, sensitivity to ITZ, VOR, and cross-resistance were: *C. glabrata* (8.7%, 13.0% and 87.0%); *C. parapsilosis* (36.4%, 45.5% and 54.5%); *C. tropicalis* (40.0%, 60.0% and 40.0%); *C. krusei* (0.0%, 33.3% and 66.7%); *C. famata* (50.0%, 50.0% and 50.0%); and *C. guilliermondii* (0.0%, 50.0% and 50.0%), respectively. The mean (range) mg/ml minimum inhibitory concentrations (MIC) at MIC_{50} and MIC_{90} of sensitive CA isolates were 0.125 and 0.250 (0.031-0.500) for ITZ; and 0.250 and 0.250 (0.031-1.000) for VOR, respectively. For NAC, the MIC_{50} and MIC_{90} mean (range) mg/ml were 0.063 and 0.125 (0.025-0.500) for ITZ; and 0.094 and 0.250 (0.031-1.250) for VOR, respectively. CA A (66.0%) versus B (4.3%), and C (19.1%) genotypes was more frequent. All CA isolates had higher activities of biofilm production (75.5%) and PLs (73.4%) compared to haemolysin (21.3%) and proteinase (36.2%). Higher expressions of biofilm (95.2% vs. 42.9%; $P<0.0001$), PLs (96.8% vs. 28.6%; $P<0.0001$), and proteinase (52.4% vs. 9.5%; $P=0.001$) activities were also noted in A relative to non-A genotypes, respectively. Additionally, PLs activity was higher in ITZ resistant vs. sensitive strains (84.8% vs. 61.1%; $P=0.044$). Altogether, these results demonstrate that CA and its genotype A are the most prevalent isolates. Candida isolates exhibit low sensitivity rates to ITZ and VOR. Production of biofilm and PLs are the most expressed virulence factors in CA isolates linked to genotype A and ITZ resistance. Implications of these results include adoption of a combination and/or use of different antifungal agents for candidiasis treatment and prophylaxis.

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ABBREVIATIONS AND ACRONYMS

25s rDNA. 25s ribosomal deoxyribonucleic acid

25s rRNA. 25s ribosomal ribonucleic acid

AIDS. Acquired immunodeficiency syndrome

AMR. Antimicrobial Resistance

APUA. Association for Prudent use of Antimicrobials

AST. Antifungal susceptibility test

ATCC. American type culture collection

CA. Candida albicans

CMR. Centre for Microbiology Research

CSF. Cerebrospinal fluid

DM. Diabetes mellitus

EUCAST. European Committee on Antimicrobial Susceptibility Testing

HIV. Human immunodeficiency virus

HVS. High vaginal swabs

IBM/SPSS. International Business Machines/Statistical Package for the Social Sciences

ITZ. Itraconazole

KEMRI. Kenya Medical Research Institute

LMIC. Low- and middle-income countries

MIC. Minimum inhibitory concentrations

NAC. Non-albicans candida

PCR. Polymerase chain reaction

PLs. Phospholipase

PLWH. People living with HIV infection

RFLP. Restriction fragment length polymorphisms

SDG. Sustainable development goals

SSA. Sub-Saharan Africa

STI. Sexually transmitted infections

VOR. Voriconazole

VVC. vulvovaginal candidiasis

WHO. World health organization

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CHAPTER ONE: INTRODUCTION

1.1. Background Information

Among the estimated 6.5 million annual incidence of fungal infections in the world, 1,565,000 million are attributable to invasive candidiasis and candidaemia, leading to 995,000 deaths (63·6% of the total fungal-related deaths) (Denning, 2024). Immunocompromised individuals are the most affected. For instance, about 50% of people living with HIV infection (PLWH) in Africa die from mycosis-related complications including invasive candidiasis (Okoye et al., 2022). In addition, 23% of diabetic foot ulcer patients had candida infections, 8% of individuals living with HIV had oral thrush and vaginal yeast contributed 25% of sexually transmitted infections (STI) during pregnancy (Chepkondol et al., 2020; Musyoki et al., 2022; Warr et al., 2019). In Kenya, ~6,328,294 persons, translating to 11.6% of the population suffer severe fungal infections comprising 1,006,954 cases (2.1%) of candida infections (Ratemo & Denning, 2023). A majority of the fungal disease burden is attributable to the increasing cases of immunosuppression, improper use of antifungal agents, and development and spread of antifungal drug resistance (Dovo et al., 2022; Dunaiski et al., 2024; Musyoki et al., 2022).

The high burden of invasive candidiasis globally and locally is attributable to the increasing population of elderly, immunosuppressed or debilitated patients, the change from *C. albicans* (CA) to non-albicans candida (NAC) infections, and the development and spread of multi-antifungal drug resistance (Lamoth et al., 2018). Invasive candida mycoses remain a significant public health problem, because of their severity, prolonged hospital stays, cost of treatment, and high morbidity and mortality. Therefore, to improve clinical management and outcomes of invasive

candidiasis, several challenges ought to be subverted including availability of epidemiologic data, improving diagnosis and strategies of antifungal therapy (Soriano et al., 2023). In Africa, invasive candidiasis is widespread and contributes significant ill health and fatalities in both paediatric and adult populations (Bongomin et al., 2022). Candida spp. are frequently isolated organisms that are responsible for many types of fungal infection in children and adults including hospitalized patients (Gebremicael et al., 2023; Reda et al., 2023). Although CA is the common cause of invasive candidiasis, the risk of opportunistic infections caused by NAC such as *C. krusei*, *C. tropicalis*, and *C. famata* are increasingly becoming prevalent in sub-Saharan Africa (SSA) (Dangarembizi et al., 2023; Kibwana et al., 2023).

Candida species cause a variety of diseases, including oral thrush and invasive disease. However, the underlying variation in severity and predisposition to antifungal drugs is not clear. Azole antimycotic agents are common medicines routinely used in therapy and prophylaxis of candidoses (Canadian Paediatric Society, 2007; Cornely et al., 2012). However, high rates of resistance hamper the effective utilisation of these drugs in the treatment and prevention of candidoses. Therefore, it is valuable to isolate and characterise the candida spp. prevalent in different regions and associated clinical conditions. In addition, determine the sensitivity of the isolated fungi to regularly used azole medications such as itraconazole and voriconazole.

Characterisation of CA using the 25S rRNA (rDNA) gene produces A, B, C, D, and E genotypes (McCullough et al., 1999; Tamura et al., 2001). Genotyping of CA from around the world and Africa, including Kenya, show that the most prevalent genotype is A in various clinical specimens from patients with invasive candidiasis and healthy

individuals (Abraham et al., 2020; Amanloo et al., 2022; C. Bii et al., 2009; Chemutai et al., 2008; Gharaghani et al., 2021; Jafarian et al., 2021; Kianifar et al., 2022; Mahmoodi et al., 2023; Mashaly & Zeid, 2022; Zida et al., 2018). Altogether, these studies illustrate that A is the widely prevalent genotype causing invasive candidoses globally.

The pathogenicity of candida species is determined by adhesive and hydrolytic enzyme expression (Talapko et al., 2021). The CA expresses proteinase and phospholipase (PLs) activity, leading to host-cell lysis and tissue penetration (Jørgensen, 2024). Likewise, CA haemolysins disrupt mucosal epithelium initiating cell signalling, inflammatory mediator production and leucocyte recruitment (Naglik et al., 2017). Moreover, CA biofilm formation enables adherence to mucosal surfaces (Jørgensen, 2024). As such, this study evaluated candida spp. diversity, azole resistance, and CA genotypes and virulence factor expression to understand the establishment and dissemination of CA leading to the invasive mycoses.

1.2. Statement of the problem

Globally, the problem of candidiasis is increasing, with the increasing number of individuals with immune-compromised states. About 1,565,000 people suffer invasive candidiasis, translating to 995,000 deaths in the world (Denning, 2024). In Kenya, over 1 million people (or 2.1% of the population) suffer candidiasis each year, translating to 2.1% of the country's population suffering candidiasis (Ratemo & Denning, 2023). Candidiasis is routinely diagnosed based on patients' clinical presentation, with little or no mycologic cultures and antifungal drug sensitivity performed to guide treatment. In addition, it is now known which Candida spp. are

accountable for the great number of mycoses in immunosuppressed patients. Likewise, treatment and prophylaxis of candida fungal infections is based on empiric prescriptions largely of the commonly used azole drugs with no susceptibility testing performed, leading to poor clinical outcomes, mainly from presence resistant strains (Dabas, 2013). Although it is known that CA is the most likely species in clinical samples, the genotypes of this yeast along with the production of the virulence markers is only partially elucidated in the country, yet it is paramount to identify patients with more pathogenic variants for focused intervention.

1.3. Research questions

1) What is the range of Candida spp. in clinical specimens from tertiary health facilities in Nairobi County?
2) What are the sensitivity profiles of candida isolates to itraconazole and voriconazole in clinical specimens from tertiary health facilities in Nairobi County?
3) What are the ABC genotypes in *C. albicans* isolated from clinical specimens from tertiary health facilities in Nairobi County?
4) What are the virulence factor production profiles in *C. albicans* isolated from tertiary health facilities in Nairobi County?

1.4. Objectives of the study

1.4.1. Broad Objective

To characterise candida spp. diversity, azole susceptibility, CA ABC genotypes, and virulence factor expression in clinical specimens from tertiary health facilities in Nairobi County.

1.4.2. Specific Objectives

1) To determine candida spp. diversity in clinical specimens from tertiary health facilities in Nairobi County.
2) To evaluate itraconazole and voriconazole resistance in candida species from clinical specimens from tertiary health facilities in Nairobi County.
3) To characterise *C. albicans* genotypes in clinical specimens from tertiary health facilities in Nairobi County.
4) To determine the production patterns of virulence markers amongst CA isolated from tertiary health facilities in Nairobi County.

1.5. Null Hypotheses

1) There are no significant differences in candida species diversity in clinical specimens (mycoses) from tertiary health facilities in Nairobi County.
2) There are low rates of itraconazole and voriconazole resistance in candida isolates from clinical specimens from tertiary health facilities in Nairobi County.
3) There are no differences in CA ABC genotype rates in clinical specimens (mycoses) from tertiary health facilities in Nairobi County.
4) There are no differences in azole resistance and virulence factor expression rates amongst CA genotypes from clinical specimens from tertiary health facilities in Nairobi County.

1.6. Justification of the study

The burden of candidiasis is not yet fully known in Kenya. With the increasing cases of immunosuppressed individuals, opportunistic candidiasis is becoming a clinical

focus as it complicates treatment outcomes. As such, the range of candidoses compounded by a huge number of resistant strains to first-choice azole medicines, including the presence of species and genotypes expressing certain virulence factors that enhance colonisation and dissemination of the infection, it is imperative that structured mycological studies be routinely done to guide treatment. In Kenya, only a few studies were performed to determine Candida spp. diversity, anti-azole drug resistance, genotypes and virulence factor expression. Evidence in the past from elsewhere in the world indicates that CA is the most prevalent and pathogenic species in clinical specimens, with high rates of anti-azole resistance and particularly genotype A as well as expression of the biofilms and hydrolytic enzyme virulence factors. Given the current trends, it is possible that highly virulent genotypes based on their ability to express azole resistance and virulence factors will continue increasing and spreading, reaching unmanageable levels. The present study presents results on Candida spp. diversity and their anti-azole resistance profiles including CA genotypes and virulence factor expression in isolates from clinical specimens. These results will inform policies on better diagnosis and detection of candida species causing invasive and disseminated candidiasis, and guide treatment, prophylaxis through selection of effective anti-azole drugs, as well as design and implementation of surveillance programs for candidiasis prevention.

1.7. Significance of the study

The results of this research will contribute to the United Nations (UN) sustainable development goal (SDG) 3: "*good health and well-being: better, more accessible health systems to increase life-expectancy by identifying the risk factors and clinical presentation of the disease which can be used for designing better intervention*

strategies" (United Nations, 2023); and the Kenya vision 2030 pillars on health (MoH Kenya, 2020) by promoting strengthening of effective prevention and treatment of candidiasis, an infectious disease prevalent in immunocompromised populations ultimately improving their quality of well-being. The findings of this research will also guide policy formulation targeting base-line evaluation and monitoring of candidiasis treatment and prophylaxis in immunocompromised individuals. The study results will also provide guidance on the design of surveillance and research initiatives on the epidemiology of candidiasis in Kenya.

1.8. Scope of the study

This research evaluated archived clinical specimens previously collected from individuals presenting with candidiasis at referral health facilities in the metropolitan city of Nairobi for the presence of candida spp. and for itraconazole and voriconazole resistance. In addition, genotypes and virulence factor expression were evaluated in CA isolates.

1.8.1. Delimitations of the study

Although candidiasis in the study setting is a common opportunistic fungal infection (Guto et al., 2016), the study was limited to analysing archived genito-urinary, respiratory, oral-pharyngeal, skin, blood and CSF specimens with no additional clinical information to delineate the clinical features of the infection. Besides, patient demographic and health history information were not available for identifying the associated risk factors of candidiasis in the patients. As such, the risk factors and modes of acquisition of the various Candida species were not examined due to the use of the archived specimens. Molecular characterisation of CA yielded high rates of the

A genotype with also B and C genotypes isolated. However, additional subtyping of the A genotype, e.g., using the RPS sequences in the ALT gene (Sawadogo et al., 2019), the use of additional genotyping approaches such as sequencing and whole genome analysis can further identify the strains and azole resistance A types in the study area. For instance, the multi-loci four microsatellite variable-number tandem repeat analysis has previously been used to identify more than 50 different genotypes of CA in Gabon (Bignoumba et al., 2023). An additional limitation of the current research is the fact that MIC testing was conducted for only 43 and 23 of CA and NAC isolates that were susceptible, the lack of clinical information to link resistance and/or susceptibility to patient outcomes, and the fact that only two azole antifungal agents, itraconazole and voriconazole were evaluated. In addition, molecular identification and profiling of azole resistance genes would have provided additional information on the genetic patterns of resistance in this population. Finally, virulence by CA in the archived specimens can be attenuated by successive sub-cultures (Hebeka & Solotorovsky, 1965), but information on the number of successive subcultures was not available.

1.8.2. Limitation of the study

This research utilised archived samples and a cross-sectional approach to answer the research questions and accept the null-hypotheses that there are no differences in the diversity, azole resistance, genotypes and virulence factor expression amongst the candida isolates. Standard mycological phenotypic, biochemical and molecular techniques were used for identifying the candida isolates, azole sensitivity profiles, and CA genotypes and virulence factor expression. Both phenotypic, biochemical, and molecular methods were utilised in evaluating the candida isolates, azole

resistance, and virulence factor expression in CA, the most widespread and pathogenic candida species. Even though NAC are emerging as important causes of candidiasis, they were not genotyped nor their virulence factor expression evaluated because this is thought to be similar to that of CA.

1.9. Conceptual framework

The conceptual framework shows the interrelationship between candida species diversity, mycoses, genotypes and virulence factor expression (Figure 1.1).

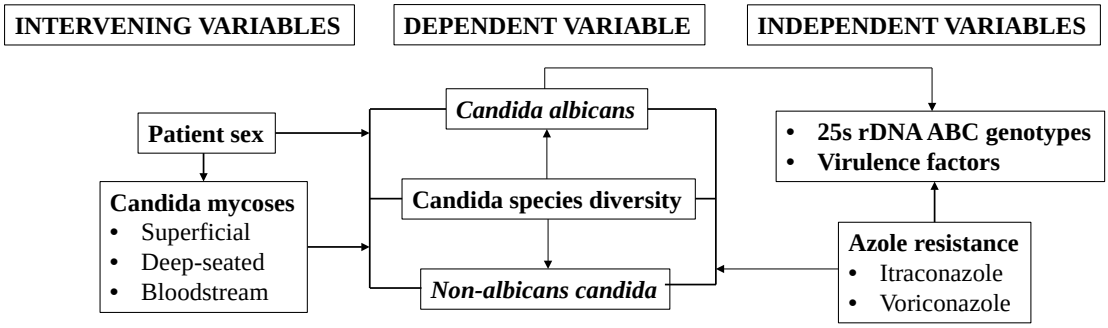

Figure 1.1: Conceptual framework

Source: designed by the researcher from literature review, 2023

1.10. Operationalization of Terms

Candidiasis. A type of mycosis resulting from infection by the fungi of the genus candida.

Non-albicans candida infection. Candidoses that are caused by Candida species other than *C. albicans*.

Dysbiosis. Imbalance the microbial community such as on the skin, gut, and vagina that is associated with disease.

Genotype. Any of the ABC variants of the 25s rDNA *C. albicans* gene.

Mycoses. Infections caused by fungi.

Anti-azole resistance. The capacity of candida isolates to grow in media containing itraconazole and voriconazole azole antifungal agents.

Morphological features. The capacity of candida colonies to produce budding cells, germ tubes, and/or chlamydospores.

Biochemical characteristics. The ability of candida colonies to produce pink, blue or apple green colour on CHROM agar, and/or grow at 45^{o} C.

Candida species diversity. Isolation of *C. albicans* and/or non-*albicans candida* species from clinical specimens.

CHAPTER TWO: LITERATURE REVIEW

2.1. Burden of candidiasis

Candidiasis also known as candidosis or moniliasis is an infection aetiologically resulting from yeast fungi of the genus Candida. It causes superficial infections of the skin and mucous membranes as well as deep invasive diseases, for example, fungal sepsis, endocarditis and meningitis. Candidiasis is a fungal disease accounting for significant morbidities and deaths globally. An estimated one million five-hundred and sixty-five thousand people experience candida bloodstream infections or invasive candidiasis annually, leading to nine-hundred and ninety-five thousand deaths, which translates to 63·6% of the infection of candidiasis cases (Denning, 2024). Estimates for invasive fungal diseases from eight African countries indicate a total of 13,300 cases of candidaemia with a mortality rate of 41.4% for the 715 cases on which mortality data was available (Bongomin et al., 2022). Additional estimates indicate a prevalence of 29.2% for vulvovaginal candidiasis (VVC) with a higher occurrence in Eastern Africa (35%) compared to Western Africa (28%) and Northern Africa (15%) (Osman Mohamed et al., 2022). Likewise, estimates in SSA shows that the rates of oral candidiasis range from 7.6 to 75.0% in PLWH infection (Mushi et al., 2017), while oesophageal candidiasis prevalence occurs in 12% of PLWH experiencing end stage disease (Olum et al., 2020). In Kenya, an estimated 1,006,954 cases of candidiasis occur each year, translating to 2.1% of the country's population suffering candidiasis (Ratemo & Denning, 2023). These cases comprised an incidence of 161 for oesophageal candidiasis (87,945 cases) in PLWH infections, 5 for candidaemia (2,804 cases) in cancer (1,914 cases) and intensive care (820 cases) patients, 0.8 for candida peritonitis (410 cases), and 123 for oral candidiasis (67,312 cases) per 100,000 population; and a prevalence of 3,349/100,000 population for recurrent

vaginal candidiasis (915,795) in females each year (Ratemo & Denning, 2023). From the aforementioned, it is evident that invasive candidiasis is a widespread problem on the African continent, including Kenya.

2.2. Aetiology and risk factors of candidiasis

Candida spp. form part of the normal microbiota in humans residing on the skin, oropharyngeal, oesophageal, gastrointestinal, and vaginal mucosae. There are several candida species causing various forms of candidiasis. The most prevalent species causing human candidoses include CA, and the NAC species such as *C. glabrata, C. guilliermondii, C. kefyr, C. krusei, C. lusitaniae, C. parapsilosis,* and *C. tropicalis* (Dabas, 2013; R & Rafiq, 2024). *Candida albicans* is responsible for 50-90% of candidoses and is the major cause of opportunistic infections especially amongst immunocompromised individuals in Africa (Okoye et al., 2022). The major risk determinants of candidoses comprise underlying malignancies such as leukaemia and lymphoma, and the use of corticosteroid treatments suppressing neutrophil, macrophage, and T-cell functions; cytotoxic therapies causing depletion of leukocytes; and prolonged poly-antibiotic treatment causing disturbances in the balance of mucosal microflora; endocrine diseases such as diabetes mellitus, myxoedema, hypoparathyroidism, and Addison's disease, as well as infectious diseases like HIV infections and tuberculosis as well as nutritional deficiencies including vitamin A, B6, or iron deficiencies, pregnancy, and use of oral contraceptives are associated with immunosuppression; cigarette smoking, xerostomia; poorly maintained dentures, intravenous tubes, invasive surgical techniques are associated with physical and psychological stresses (Jenkinson & Douglas, 2002; R & Rafiq, 2024). Besides, the use of prosthetic devices, especially

intravascular catheters and prostheses (e.g., heart valves) create biofilm sites for colonization; while old age, and infancy are linked to weak immune systems (Jenkinson & Douglas, 2002; R & Rafiq, 2024). In addition, surgical trauma, catheterisation, prosthetic implantations, and the wearing of prosthetic devices e.g., dentures can cause polymicrobial infections (Jenkinson & Douglas, 2002). Altogether, CA is the most prevalent cause of candidiasis largely amongst immunocompromised individuals.

2.3. Clinical phenotypes and pathogenesis of candida mycoses

The mycoses caused by candida spp. are classified into superficial, deep-seated, and systemic or invasive candidiasis. Superficial mycoses affect the cutaneous or mucocutaneous tissues and include oropharyngeal candidiasis, vaginitis, conjunctivitis, oesophagitis, or gastrointestinal candidiasis. Systemic mycosis, involves multiple organs and endocarditis, pyelonephritis, meningitis, and disseminated candidiasis, leading to candidaemia and candida septicaemia (Lu et al., 2023). Invasive candidiasis and candidaemia are widespread and attributable mortality is about 30% (Lass-Flörl et al., 2024). Other studies amongst cancer patients in Egypt show that candida species account for about 3.1% of bloodstream infections (El-Mahallawy et al., 2023), and incidence rate is 0.8/1000 admissions of children with candida bloodstream infections in South Africa (Gebremicael et al., 2023). Neonatal invasive candidiasis is responsible for about 22% of deaths in neonates with sepsis largely from CA in LMIC (Cook et al., 2023). Other forms of invasive candidiasis such as VVC, oesophageal candidiasis, oral candidiasis, candidal meningitis are also widespread, especially in immunosuppressed individuals (Lu et al., 2023).

The pathogenesis of candidiasis from foci of normal flora domicile is determined by several factors including dysbiosis, suppression of host protective factors, and expression of virulence factors (Oliva et al., 2023). Figure 2.1 summarises the pathogenesis of invasive candidiasis. Candida spp. are normal flora yeasts in the oral, gastro-intestinal, and genito-urinary mucosae, and skin. Invasion is initiated following antimicrobial-induced dysbiosis, iatrogenic, and/or medical interventions that damage the muco-cutaneous layer and/or impair host defences (Lopes & Lionakis, 2022). Subsequently, the yeast becomes an opportunistic pathogen causing muco-cutaneous and/or life-threatening systemic disease (Lopes & Lionakis, 2022; Oliva et al., 2023).

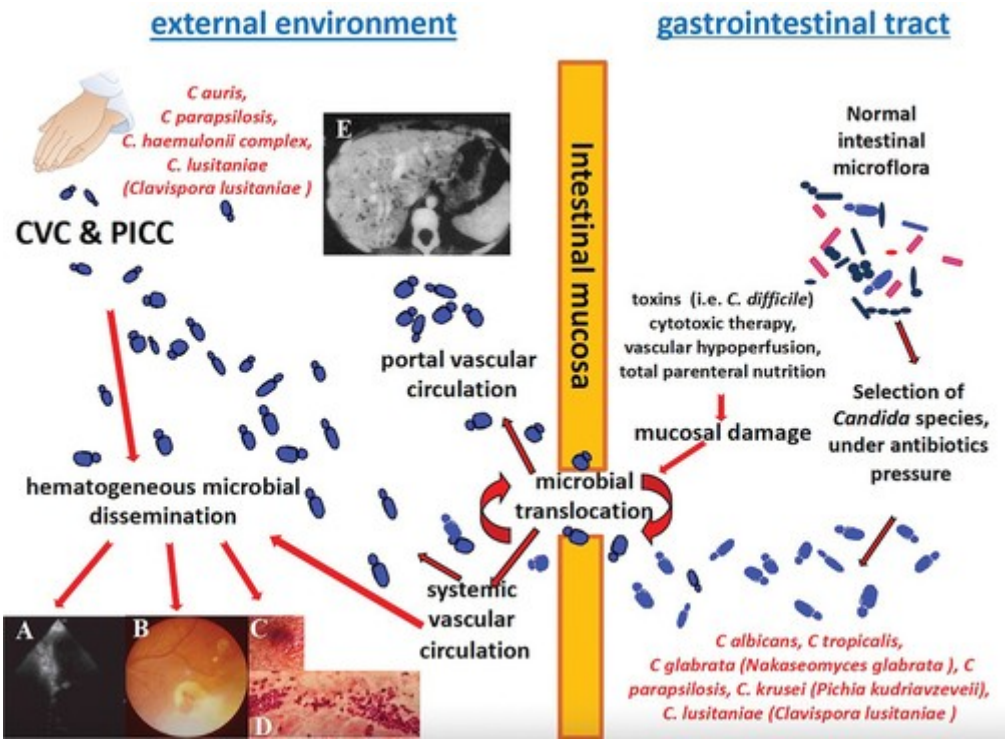

Figure 2.2: Pathogenesis and haematogenous dissemination routes of candida species and possible secondary infections

Adapted from (Oliva et al., 2023)

2.4. Genotypes of *Candida albicans*

Characterisation of CA 25S rRNA (DNA) gene in the transposable group-1 intron generates five genotypes based on PCR product sizes: 450 bp (A), 840 bp (B), 450 and 840 bp (C), 1,080 bp (D), and 1400 bp (E) (McCullough et al., 1999; Tamura et al., 2001). Numerous studies from across the world and Africa, including Kenya, indicate a predominance of the A genotype over genotypes B and C in CA isolates from several clinical phenotypes including from healthy individuals (Abraham et al., 2020; Amanloo et al., 2022; C. Bii et al., 2009; Chemutai et al., 2008; Gharaghani et al., 2021; Jafarian et al., 2021; Kianifar et al., 2022; Mahmoodi et al., 2023; Mashaly & Zeid, 2022; Zida et al., 2018). However, another study detected higher rates of genotype C, over B and A in females with CA VVC (Gharaghani et al., 2022). Altogether, these studies illustrate that genotype A is the most predominant on the African continent, and elsewhere in the world, but local differences may occur in the distribution of the genotypes.

2.5. *Candida albicans* virulence factors

The virulence and pathogenicity of candida spp. is dependent upon their adhesive properties, hydrolytic enzyme production, biofilm formation, and phenotype shifting (Talapko et al., 2021). *Candida albicans* express biofilm formation, hydrolytic enzymes such as haemolysins, PLs and proteinases. The CA proteinases, and phospholipases hydrolyse cell membrane phospholipids resulting in host cell lysis and penetration into tissues (Jørgensen, 2024). *Candida albicans* haemolysins interrupt the mucosal epithelium triggering signal transduction and production of inflammatory cytokines and leukocyte recruitment (Naglik et al., 2017). In addition, CA undergoes dimorphic switching from yeast and hyphal growth in the biofilms, creating a basal

layer of yeast cells that is thinner and a hyphal layer that is thicker for adherence to mucosal surfaces (Jørgensen, 2024). Furthermore, some studies illustrate higher rates of CA genotype A in more invasive isolates (Karahan et al., 2004), suggesting that the A genotype is highly virulent. In support of this assertion, previous studies indicate that genotype A is associated with a high level of phospholipase and haemolysin activity (Abraham et al., 2020). Likewise, genotype A is associated with higher rates of fluconazole resistance in clinical isolates from Burkina Fasso (Zida et al., 2018). Thus, it is important to evaluate virulence factor expression to understand the establishment and dissemination of CA leading to invasive mycoses.

2.6. Azole antifungal drug resistance

Azole anti-mycotic drugs have a five-membered ring with two or three nitrogen molecules (imidazoles and triazoles, respectively). Azoles of clinical importance include the imidazoles (clotrimazole, econazole, miconazole, and ketoconazole) and triazoles (itraconazole, fluconazole, and voriconazole) (Sharma et al., 2024). Azole drugs are routinely used in the therapy and prophylaxis of candidiasis (Canadian Paediatric Society, 2007; Cornely et al., 2012). However, high rates of resistance, including cross-resistance, hamper the effective utilisation of these drugs in the management and prevention of candidiasis. Azoles mainly inhibit the cytochrome P_{450}-dependent enzyme lanosterol 14α-demethylase involved in conversion of lanosterol to ergosterol, thus interfering with cell membrane sterol formation (Sharma et al., 2024). The modes of candida spp. resistance to azole and other anti-mycotic drugs include the propensity of the fungus to alternate between yeast and hyphae morphotypes and biofilm formation (Kaur & Nobile, 2023; Sharma et al., 2024), as well as intensified drug efflux from the cell, and by inhibiting the rate-limiting

enzyme for sterol synthesis, 14-α-demethylase (Lee et al., 2023; Zavrel et al., 2017). In addition, primeval studies demonstrated that tolerance of candida isolates to an azole compound was correlated with cross-resistance to further azole antifungal drugs (Odds, 1993). Subsequent studies indicated cross-resistance amongst the azoles, fluconazole, itraconazole, ketoconazole, and voriconazole in isolates from paediatric PLWHI and presenting with oro-pharyngeal candidiasis (Müller et al., 2000). It was argued that the high cross-resistance was ascribed to the more recent generation of azoles (itraconazole, fluconazole, and voriconazole) selectively binding the cytochrome P450-dependent 14α-sterol demethylase enzyme (Müller et al., 2000). As such, it is vital to determine the anti-azole susceptibility of candida isolates from patients for effective utilisation of these drugs.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study area

This research was carried out at the Kenya Medical Research Institute (KEMRI), Centre for Microbiology Research (CMR), Medical Mycology Laboratories, Nairobi, Kenya. The centre conducts microbiological research in collaboration with several local and international organisations, thus expanding the research capabilities. The CMR also houses the regional reference laboratory for Antimicrobial Resistance (AMR) Testing and Surveillance, including the local chapter of the Association for Prudent use of Antimicrobials (APUA). The CMR Research, in addition, has a state-of-the-art mycology laboratory that conducts research and referral diagnosis of fungal and opportunistic infections, including chronic and infectious diseases.

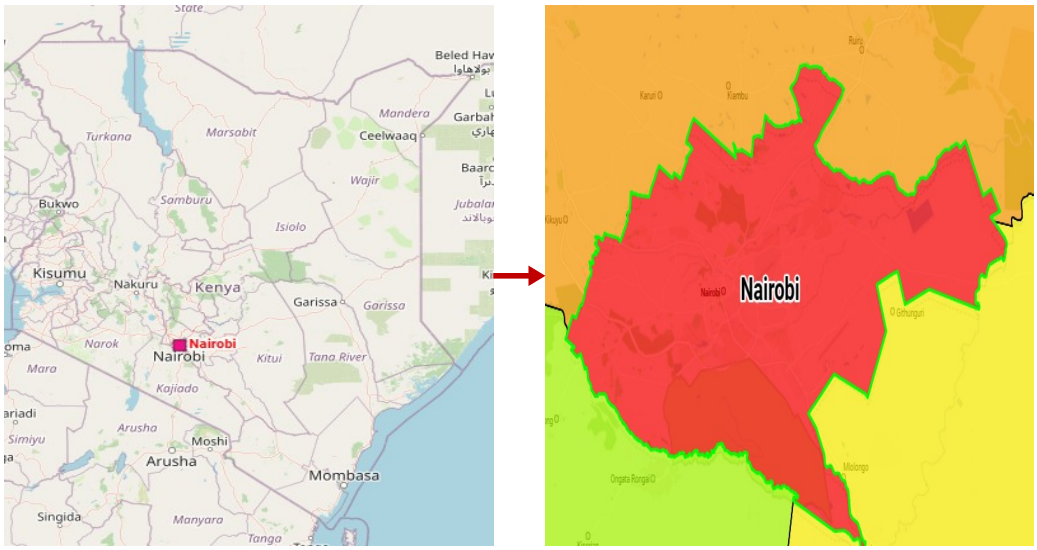

Figure 3.3: Map of Nairobi County, Kenya

Source: https://www.citypopulation.de/en/kenya/admin/Nairobi/

3.2. Study Design

This research utilised a cross-sectional descriptive approach to examine the phenotypic, molecular, virulence, and antifungal azole susceptibility in candida spp. previously isolated in clinical specimens from metropolitan Nairobi city.

3.3. Study variables

3.3.1. Dependent variables

Candida species diversity.

3.3.2. Independent variables

Phenotypic features, triazole resistance, genotypes, and virulence factors.

3.4. Study population

The clinical samples were previously obtained from patients presenting with different indications of candidosis and/or having a fungal culture request, and visiting various referral hospitals in Nairobi metropolitan city clinics for care. The mycological studies were performed on a total of 141 specimens (Table 3.1). The clinical specimens comprised of high vaginal swabs, skin secretions, oral swabs, urine, sputum, tracheal aspirates, catheter tip, pharyngeal exudates, cerebrospinal fluid, and blood.

Table 3.1: Number of the study participants

Specimen	Number
High vaginal swabs (HVS)	45
Skin secretions	4
Oral swabs	2
Urine	47
Sputum	24
Tracheal aspirates	8
Catheter tip	3
Pharyngeal exudates	3
Cerebrospinal fluid (CSF)	1
Blood	4

Phenotypic itraconazole and voriconazole susceptibility testing was performed on *C. albicans* (n=94) and non-albicans candida (n=47) isolates; while minimum inhibitory concentrations (MIC) were determined for isolates sensitive to itraconazole (*C. albicans*, n=20; and non-albicans candida, n=9). Genotyping and virulence factor characterisation was performed only for *C. albicans* isolates (n=94).

3.4.1. Inclusion criteria

Only archived samples from individuals who had consented for future use of their specimens were included in this research.

3.4.2. Exclusion criteria

Archived samples from individuals who at the time of specimen collection had declined consent, those who were pregnant (since pregnancy is an immunosuppressive state associated with higher prevalence of VVC) or who had a history of vaginal haemorrhage were excluded from this study.

3.5. Sampling techniques

The samples were selected from the specimen archives using simple random sampling technique.

3.6. Determination of sample size

The sample size (n) was calculated according to Cochran formulae (Cochran, 1977):

$n=Z^2pq/e^2$

Where;

e is the desired precision level (i.e., error margin) at 95% confidence interval of 0.05,

p is the (estimated) proportion of the Kenyan population suffering candidiasis of 9.6% (Guto et al., 2016).

q is 1 – p = 1-0.096 = 0.904

Therefore;

$n=Z^2pq/e^2$

$n = [(1.96)^2 (0.21) (0.79)) / (0.05)^2] = 133.30$

Adding 5% attrition gives sample size of 141 samples.

3.7. Data collection

3.7.1. Demographic characteristics

Only information on gender was available from the specimens, and was recorded on the data capture forms.

3.7.2. Laboratory procedures

3.7.2.1. Sample processing

All clinical specimens were cultured on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (50 mg/ml) (Oxoid, Hampshire, UK) plates at 35°C for 48 hours. Isolates obtained from positive cultures were archived suspended in 20% glycerol v/v until used for further analysis. Sub-cultures of stored isolates were performed on chloramphenicol-free SDA (Oxoid, Hampshire, UK) media at 35°C for 18 to 24 hours for optimal growth characteristics. Identification was then performed using the CHROMagar Candida (Oxoid, Hampshire, UK) and the API 20-C AUX identification system (Biomerieux, Lyon, France) using the manufacturer's protocols, and conventional methods (Warren, N.G. & Hazen, K.C., 2003).

3.7.2.2. Germ-tube test for *Candida albicans*

The Germ-tube test was done by pipetting three serum drops into clean test tubes using the Pasteur pipette. With a sterile applicator stick, a colony of the yeast isolates was briefly touched, suspended in the serum, and incubated at 35°C for 3 hours. Subsequently, one drop of the inoculum was deposited on a clean microscope slide on which a clean cover slip was placed over, and examined at 40X objectives for identification of *C. albicans* based on the presence or absence of germ tubes (Kali et al., 2013).

3.7.2.3. Dalmau chlamydospore identification technique

The Dalmau's technique for identification of chlamydospores was performed on corn meal agar (Oxoid, Hampshire, UK) culture plates as previously described (B et al., 2020). Briefly, light inocula were made by suspending a few colonies in distilled

water and scratching slightly the surface of corn meal agar. Flamed sterilised cover slips were positioned on the agar surface covering the scratches and the plates incubated for 5 days at 26°C, then examined *in situ* microscopically at 40X power objectives for the presence of chlamydospores.

3.7.2.4. *Candida albicans, C. glabrata, C. krusei,* and *C. tropicalis* identification

Candida albicans, C. glabrata, *C. krusei*, and *C. tropicalis* were differentiated by simultaneously growing all the yeasts isolated on SDA on Pal's agar, CHROM agar, and CHROM agar supplemented with Pal's agar (Oxoid, Hampshire, UK). The plates were cultured at 30°C (Pal's agar or CHROM agar supplemented with Pal's agar) or at 37°C (CHROM agar), and colony features documented at 24-, 48-, and 72-hours following incubation (Appendix VI). These agars contain enzymatic substrates coupled to chromogenic compounds. These substrates are hydrolysed by various enzymes from the *Candida* species resulting in colour changes that were used for the presumptive differentiation of the yeasts. *Candida albicans, C. glabrata*, *C. krusei*, and *C. tropicalis* were identified based on the formation of steel blue, green, rough/matted/rose-coloured or dark-violet colonies, respectively, as per the manufacturer protocols (Kali et al., 2013).

3.7.2.5. Analytical profile index test

Confirmation of the suspected yeasts was performed using the API 20C Aux and VITEK®-2 YST identification systems (BioMerieux, Lyon, France). This system is based on the principle of standard carbohydrate (sugar) assimilation. The strips consist of dehydrated carbohydrate substrates that are assimilated by the test organisms. The test yeasts were inoculated on the API strip and incubated for 24 to 72

hours at 37°C. Following incubation, the analytic index (numeric profile) was calculated from the reaction patterns and used to record the identification results which were confirmed by comparing the analytical profile index with the reference API index (Hata et al., 2007).

3.7.2.6. Genotyping of *Candida albicans*

1.7.2.6.1. Extraction of *Candida albicans* genomic DNA

DNA was extracted from candida isolates using methods of (Harju et al., 2004). Briefly, a colony of *C. albicans* was inoculated in 2 ml of yeast peptone dextrose broth media (Oxoid, Hampshire, UK) and grown in a shaking incubator at 30°C for ~48 hours. The cultures were then centrifuged in microcentrifuge tubes and the pellets re-suspended in 200 µl of lysis buffer containing 100 mM sodium chloride, 10 mM tris-hydrochloric acid (HCL), 1% sodium dodecyl sulphate, 2% triton X-100, and 1 mM ethylenediaminetetraacetic acid (EDTA) at pH 8.0 (Hoffman & Winston, 1987). The tubes were then frozen at -80°C, then thawed for 1 minute on a 95°C hotplate. The tubes were again thawed one more time followed by vigorous vortexing for 30 seconds. 200 µl of chloroform was added to the tubes which were then vortexed for 2 minutes followed by centrifugation at 20,000 × g at room temperature for 3 minutes. The aqueous layer was pipetted into a tube with 400 µl of ice-cold 100% ethyl alcohol. The samples were then precipitated at room temperature for 5 minutes followed by centrifugation. Subsequently, the DNA pellets were washed with 0.5 ml of 70% ethyl alcohol, dried, and re-suspended in 20 µl of TE buffer containing 10 mM Tris-HCL and 1 mM EDTA, pH 8.0 (Harju et al., 2004).

1.7.2.6.2. Genotyping of *Candida albicans* isolates

Candida albicans was genotyped the 25s rDNA gene intronic region specific primers CA-INT-L (5'-ATAAGGGAAGTCGGCAAAATAGATCCGTAA-3') and CA-INT-R (5'-CCTTGGCTGTGGTTTCGCTAGATAGTAGAT-3') as previously described (Hattori et al., 2006). Briefly, PCR amplifications were performed in a 25.0 μl reaction mixture containing 2.0 μl (10-100 ng) genomic DNA template, 10X PCR buffer, 1.75 mM MgSO4, 10 mM deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP) mix, 2.5U Taq DNA polymerase (Hylabs ltd., Israel), and 10 μmol of forward and reverse primers (Hylabs ltd., Israel), and double distilled deionised water. The PCR conditions were: 96°C preheating for 2 minutes, followed by 35 cycles of denaturation at 96°C for 30 seconds, annealing at 65°C for 30 seconds, elongation (extension) at 72°C for 60 seconds, and final elongation at 72°C for 5 minutes. All reactions were performed in a thermal cycler (TC-Plus, Techne, UK). Electrophoresis of the PCR products was performed on 1.0% agarose gel in tris-borate-EDTA (TBE) buffer to identify the five CA genotypes based on amplicon sizes (A, 450; B, 840; C, 450 and 840; D, 1040; and E, 1080 bp; Appendix VIII). Genotype D is specific for *C. dubliniensis* (Hattori et al., 2006; McCullough et al., 1999). Following staining with 0.5 μg/ml ethidium bromide, DNA bands were visualized by Spectroline™ ultraviolet transilluminator (Fisher Scientific, Loughborough, UK), photographed, and gel photos archived on the computer.

3.7.2.7. *Candida albicans* expression of virulence factors

Virulence factor expression was performed on CA (n=94) isolates. The virulence factors examined in the study included biofilm production, as well as alpha-haemolysin, proteinase, and phospholipase activities.

1.7.2.7.1. Biofilm production

Formation of biofilms by *C. albicans* isolates was evaluated using a modified tube method of (Yigit et al., 2011). Inocula of test organisms were prepared from SDA colonies in saline and incubated at 37°C for 24 hours. About 0.5 mL of the saline inocula suspension was added to 5 mL of glucose (8%)-enriched Sabouraud dextrose broth (Oxoid, Hampshire, UK) in screw-capped conical polystyrene tubes. The suspension was incubated at 35°C for 48 hours, then the broth aspirated gently with Pasteur pipettes. The tubes were then washed in distilled water two-times and stained with 2% safranin for 10 minutes, after which the excess stain was removed by rinsing by means of distilled water. The presence of obvious adherent film at the bottom and on the walls of the tubes was interpreted as biofilm formation. However, the presence of a ring at the liquid interface was not designated as production of biofilm (Deorukhkar et al., 2014). In each experiment, *Candida albicans* ATCC 90028 were included as positive controls.

1.7.2.7.2. Haemolysin production

Candida albicans haemolysin production was screened on sheep blood-SDA plates using a modified method by Manns et al (1994). About 10 µL of candida inoculum (10^8 cells/mL) were inoculated aseptically on to the media. The plates were then incubated for 48 hours at 37°C. In each test, *Candida albicans* ATCC 90028 and *Streptococcus pyogenes* (Lancefield group A) were included as the positive control for *alpha*- and *beta*-haemolysis, respectively.

1.7.2.7.3. Proteinase activity

Candida albicans proteinase activity was determined with modifications of the methods by (Staib, 1966). This procedure measures the proteinase activity via degradation of bovine serum albumin (BSA). About 10 µL of candida inoculum (10^6 cells/mL) was inoculated aseptically on a 1% BSA plate. The plates were incubated at 37°C for 5 days. In each experiment, *C. albicans* ATCC 90028 was included as the positive control. After the 5-day incubation, 20% trichloroacetic acid was added to inhibit further proteinase activity, after which the plates were stained with amidoblack (1.25%). The presence of amidoblack unstained zone of proteolysis around the colony indicated proteinase activity. The proteinase activity was measured as proteinase index (Prz) by recording the ratio of the colony to unstained zone diameters. The proteinase activity was then scored as: Prz value of 1 denoted no proteinase activity; while Prz <1 indicated the presence of proteinase expression in isolates based on previous studies showing that the lower the Prz value, the higher the proteinase activity (Deorukhkar et al., 2014). All assays were performed in duplicate on three separate occasions for each isolate to minimise experimental error.

1.7.2.7.4. Phospholipase activity

Phospholipase activity in *C. albicans* was examined using previous methods with modifications (Samaranayake et al., 1984). About 5 µL of inoculum (10^8 cells/mL) of test organisms were inoculated aseptically on to egg yolk agar (Oxoid, Hampshire, UK). The plates were dried at room temperature and incubated for 48 hours at 37°C. After incubation, the plates were checked for a precipitation zone (Pz) surrounding the colony, designating the expression of the phospholipase enzyme. *Candida albicans* ATCC 90028 was included in each experiment as the positive control

organism. Phospholipase activity was scored as phospholipase index (Pz) which was defined as the ratio of colony diameter to total colony plus precipitation zone diameters. Phospholipase index values of 1 indicated lack of phospholipase activity; whereas Pz <1 denoted phospholipase production in the isolates based on previous observations indicating that the lower value of Pz, the greater the phospholipase activity (Deorukhkar et al., 2014). The tests were done in duplicate at three different times to minimise experimental errors.

3.7.2.8. Disk diffusion and microdilution testing for azole drug susceptibility

Azole sensitivity testing and interpretation for itraconazole and voriconazole was performed based on the EUCAST guidelines (Arendrup et al., 2020). Briefly, inocula were prepared from overnight cultures on chloramphenicol-supplemented SDA (Oxoid, Hampshire, UK) and incubated for 24-48 hours at 37°C. The inocula were prepared by suspending one to three distinct colonies in physiological saline. The inoculum concentration was standardised by measuring absorbance at a wavelength of 530 nm to yield a 0.5 McFarland standard (Thermo Fisher Scientific Remel Products, Lenexa, USA) stock inocula. The stock solution was diluted with physiological saline at 1:10 for making the final inocula. A hundred (100) µl of the final inocula were added to the microtiter wells (Greiner Bio-One, Kremsmünster, Austria) containing 100 µl of 2-fold serial dilutions of the drugs in RPMI-1640 (Biochrom Ltd., Cambridge, UK). The plates were then incubated for 24 hours at 37°C and end-point absorbance recorded by the automated SpectraMax® 340PC384 plate reader (Molecular Devices, San Jose, USA). The antifungal drug concentration that decreased growth by 50% and 90% of the drug-free control was recorded as MIC, respectively (Appendix VII). The MIC break-point interpretations for itraconazole

and voriconazole were performed as described in the EUCAST guidelines and (Pfaller et al., 2006) protocols. *Candida parapsilosis* ATCC 90018 was run alongside the test organisms for quality control. Fifty percent of all experimental set-ups were randomly chosen and independently re-examined by two different microbiologists to ensure the validity of the results.

3.8. Data analysis

Entry, cleaning, and coding, and statistical analysis of data was conducted using the IBM/SPSS Statistics software for Windows, version 25.0. (IBM Corp, New York, USA). The proportions of distribution of candida species, azole resistance, genotypes and virulence factors were generated for the isolates in the different mycoses and clinical specimens. Data was summarised as proportions for genotypes, virulence factor expression and phenotypic resistance, and means (±standard deviation) for MIC levels for each antifungal agent. The distribution of CA and NAC isolates and genotypes across the mycoses was determined using the χ^2 test. The rates of virulence factor expression across mycoses were determined by one sample trend t-test. The Fisher's exact tests were used to compare rates of virulence factor expression and azole resistant and sensitive isolates between the A *vs*. non-A CA genotypes and CA *vs*. NAC isolates, respectively. Independent student t-tests were used to examine means in MIC_{50} and MIC_{90} for each antifungal agent between the CA and NAC isolates, genotypes and virulence factor expression. All tests were two-tailed with statistical significance interpreted at a *P*-value of 0.05.

3.9. Ethical considerations

The Helsinki's declarations on human subjects research (WMA, 2013) were followed in this study. Ethical approvals were obtained from the MMUST (MMU/COR:403012(16); Appendix I) and KEMRI/Scientific and Ethics Review (KEMRI/RES/7/3/1; Appendix IV and V) committees. The research was authorised by the Kenya National Commission for Science, Technology and Innovation (NACOSTI/P/17/73416/16038; Appendix II), and the Nairobi City County Health Service; Appendix III) . A written consent was obtained from all individuals satisfying the inclusion criteria followed by counselling prior to collecting the specimens in a private room. Confidentiality was maintained throughout the study by excluding all personal identifiers and using codes for study participant records. All study forms and data records were archived in secure cabinets and offices, with only the investigator having access to the data. The participants were provided with free health education on risk factors for candidiasis and hygiene practices.

CHAPTER FOUR

4.1. Clinical specimen distribution

One-hundred and one candida isolates were characterised in the present study. Most of the specimens in females were obtained from HVS (45.0% vs. 0.0), while in males they were obtained from tracheal secretions (7.0% vs. 42.5%). Other specimens were sputum (3.0% vs. 0.0%) relative to urine (3.0% vs. 12.5%), blood (2.0 vs. 5.0%), pharyngeal exudates (2.0% vs. 2.5%), and catheter tips (0.0% vs. 7.5%) in females and males, respectively (Figure 4.1).

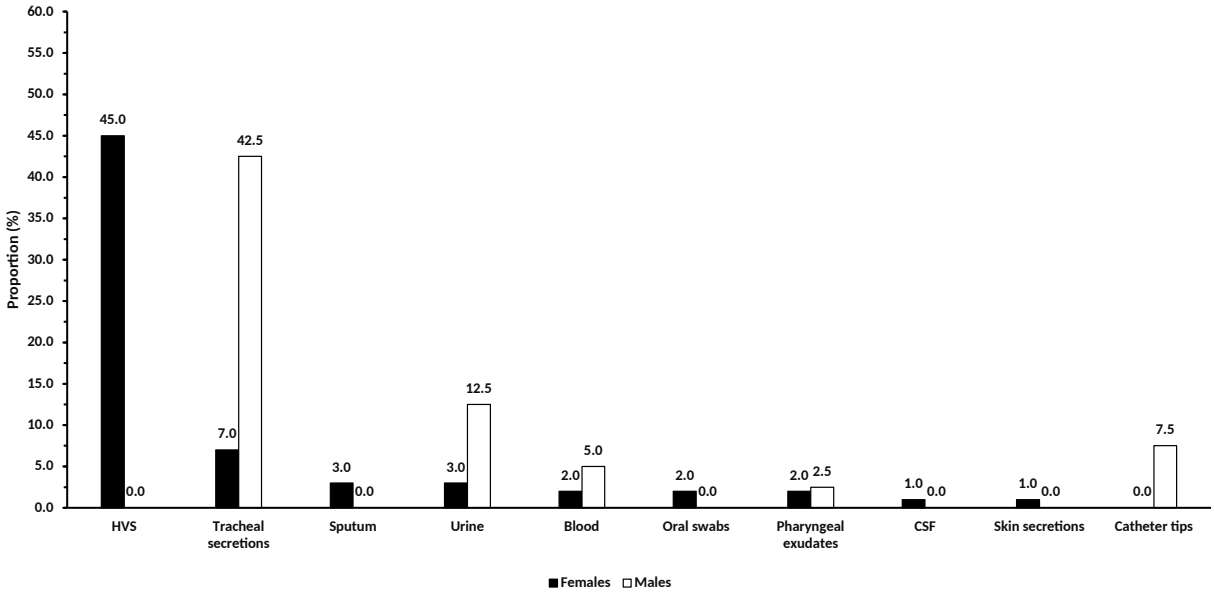

Figure 4.1: Clinical specimen distribution in the different genders

The 141 candida isolates from the different clinical samples (Figure 4.2; and Table 4.1), comprised of *C. albicans* (n=94; 66.7%) and non-*albicans* candida (n=47; 33.3%; χ^2=2.095; degrees of freedom, df=2; *P*=0.351). Overall, *C. albicans* was the prevalent isolate in the females (65.0%) and males (72.5%), *C. glabrata* and *C. parapsilosis* were obtained more frequently in the females (19.0% and 9.0%) than males (10.0% and 5.0%), respectively. The *C. tropicalis* (5.0% vs. 0.0%), *C. famata*

(1.0% vs. 2.5%), *C. krusei* (1.0% vs. 5.0%), and *C. gulliermondii* (0.0% vs. 5.0%) occurred at ≤5.0% in either of the genders.

4.2. Candida species diversity

4.2.1. Candida species diversity in the clinical specimens

The analysis based on tissue source of the clinical specimens, illustrated (Table 4.1) that majority of the candida spp. were isolated from urine (33.3%), HVS (31.9%), sputum (17.0%), and tracheal secretions (5.7%). There were also yields of positive candida cultures from blood (2.8%), skin secretions (2.8%), catheter tips (2.2%), pharyngeal exudates (2.1%), oral swabs (1.5%), and CSF (0.7%), respectively. *Candida albicans* (66.7%) was the predominant species obtained from the cultures, whereas *C. glabrata* (16.3%), and *C. parapsilosis* (7.8%) were the frequent NAC species isolated. Other, NAC isolated were: *C. famata* (1.4%), *C. guilliermondii* (1.4 %), *C. krusei* (2.8%), and *C. tropicalis* (3.6%). Most of the CA were got from urine (22.0%), HVS (19.1%), sputum (10.6%), tracheal secretions (5.0%), blood (2.8%), and skin secretions (2.1%) cultures. The yield of CA from catheter tips (1.4%), pharyngeal exudate (1.4%), oral swabs (1.4%), and CSF (0.7%) was low. The NAC, *C. glabrata* and *C. tropicalis* were isolated from HVS (7.8% and 1.4%), urine (4.3% and 1.4%) and sputum (4.3% and 0.7%), respectively. The four *C. krusei* species were each isolated from urine, HVS, sputum, and skin secretions. *Candida parapsilosis* was isolated from urine (n=5), HVS (n=4), sputum, tracheal secretions (n=1) and pharyngeal exudates (n=1). The two *C. famata* isolates were identified from urine and catheter tips, respectively. Finally, *C. guilliermondii* was isolated each from urine and HVS.

4.2.2. Candida species diversity in the different mycoses

The NAC consisted of *C. famata* (1.4%), *C. glabrata* (16.3%), *C. guilliermondii* (1.4%), *C. krusei* (2.8%), *C. parapsilosis* (7.8%), and *C. tropicalis* (3.6%) (Table 4.1). The overall distribution of the isolates as aetiologies for the different mycoses was as follows: superficial (n=51; 36.2%), deep-seated (n=86; 61.0%), and bloodstream (n=4; 2.8%) mycoses. *Candida albicans* isolation in the mycoses was: superficial (62.7%), deep-seated (67.4%), and bloodstream (100.0%), while that for NAC was: superficial (37.3%), deep-seated (32.6%), and bloodstream (0.0%) mycoses.

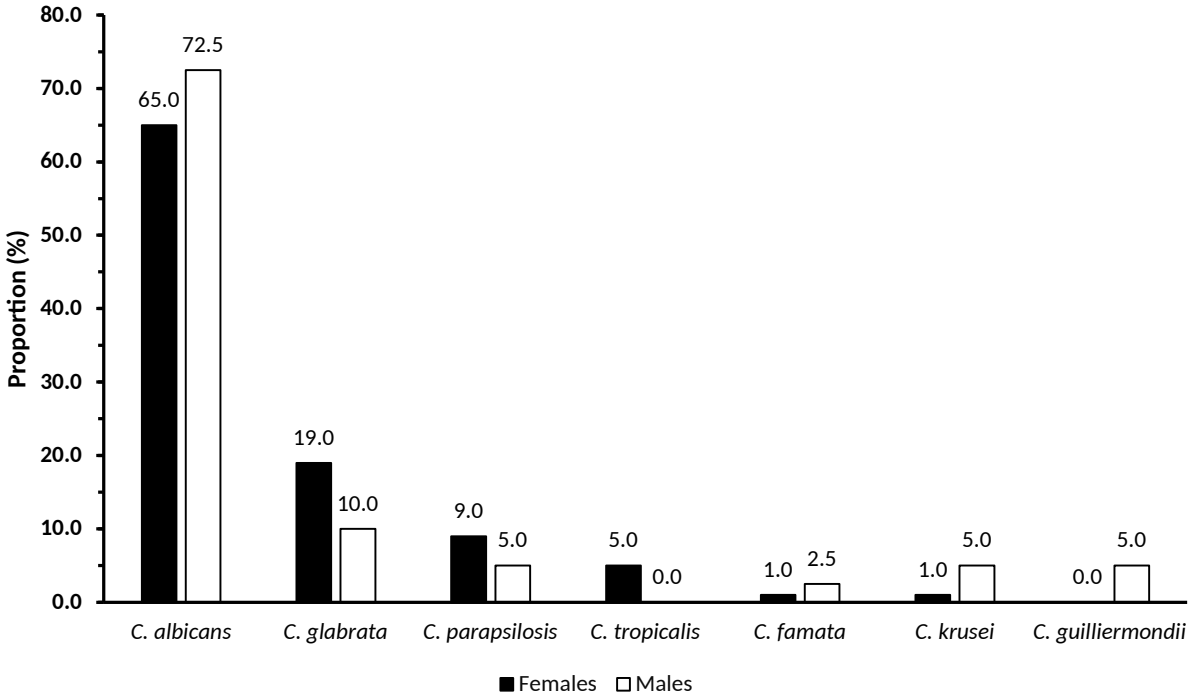

Figure 4.2: Candida isolate rates in the different sexes

Table 4.2: Diversity of Candida species isolated from different mycoses and clinical specimens

Type of mycoses, *n (%)*	***CA***	***NAC***	***CG***	***CP***	***CT***	***CK***	***CF***	***CG***	**Total isolates**	**χ^2; df; *P***
Superficial	***32 (62.7)***	***19 (37.3)***	***11 (21.6)***	***4 (7.8)***	***2 (3.9)***	***2 (3.9)***	***0 (0.0)***	***0 (0.0)***	***51 (36.2)***	
HVS	27 (52.9)	18 (35.3)	11 (21.6)	4 (7.8)	2 (3.9)	1 (2.0)	0 (0)	0 (0)	45 (31.9)	
Skin secretions	3 (5.9)	1 (2.0)	0 (0)	0 (0)	0 (0)	1 (2.0)	0 (0)	0 (0)	4 (2.8)	
Oral swabs	2 (3.9)	0 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.4)	
Deep seated	***58 (67.4)***	***28 (32.6)***	***12 (14.0)***	***7 (8.1)***	***3 (3.5)***	***2 (2.3)***	***2 (2.3)***	***2 (2.3)***	***86 (61.0)***	
Urine	31 (36.0)	16 (18.6)	6 (7.0)	5 (5.8)	2 (2.3)	1 (1.2)	1 (1.2)	1 (1.2)	47 (33.3)	2.095 ; 2; 0.351
Sputum	15 (17.4)	9 (10.5)	6 (7.0)	0 (0)	1 (1.2)	1 (1.2)	0 (0)	1 (1.2)	24 (17.0)	
TA	7 (8.1)	1 (1.2)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	8 (5.7)	
Catheter tip	2 (2.3)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.2)	0 (0)	3 (2.1)	
PE	2 (2.3)	1 (1.2)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	3 (2.1)	
CSF	1 (1.2)	0 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	
Bloodstream	***4 (100.0)***	***0 (0.0)***	***0 (0.0)***	***0 (0.0)***	***0 (0.0)***	***0 (0.0)***	***0 (0.0)***	***0 (0.0)***	***4 (2.8)***	
Blood	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.8)	
Total isolates	94 (66.7)	47 (33.3)	23 (16.3)	11 (7.8)	5 (3.6)	4 (2.8)	2 (1.4)	2 (1.4)	**141 (100.0)**	

Results are summarized as number (n) and proportion (%) of isolates. CA, *Candida albicans*. NAC, non-*albicans* candida. GB, *C. glabrata*; CP, *C. parapsilosis*; CT, *C. tropicalis*; CK, *C. krusei*; CF, *C. famata*; and CG, *C. guilliermondii*. HVS, high vaginal swab. TA, tracheal aspirates. PE, pharyngeal exudates. CSF, cerebrospinal fluid. Distribution of CA and NAC in the mycoses was performed using the chi (χ^2)-square test.

4.2.3. Morphological and biochemical features of the isolates

Morphological and biochemical evaluation (Table 4.2) showed that all the isolates grew budding on SDA agar, and all isolates except colonies of *C. albicans* (apple green), and *C. guilliermondii* (pink-purple) and *C. tropicalis* (blue) had pink colonies on CHROM agar™. Only isolates of *C. albicans* exhibited positive growth at 45° C and positive Germ-tube tests. Although growth on corn meal Tween-20 agar was absent for *C. famata*, *C. glabrata*, and *C. parapsilosis* yeasts, chlamydospores of *C. albicans*, blastoconidia pseudohyphae for *C. tropicalis*, and pseudohyphae with spores of *C. guilliermondii* and *C. krusei* were observed. VITEK®-2 YST, a biochemical reaction, positively identified all the isolates.

Table 4.3: Candida morphological and biochemical differentiation

Candida species	**Isolates, n (%)**	**SDA agar**	**CHROM agar™**	**Growth at 45°C**	**Germ tube test**	**Corn meal-Tween-80 agar**	**VITEK® 2 YST**
C. albicans	94 (66.7)	Budding cells	Apple green	+	+	Chlamydospores	*C. albicans*
C. glabrata	23 (16.3)	Budding cells	Pink	-	-	-	*C. glabrata*
C. tropicalis	5 (3.6)	Budding cells	Blue	-	-	Blastoconidia pseudohyphae	*C. tropicalis*
C. parapsilosis	11 (7.8)	Budding cells with hyphae	Pink	-	-	-	*C. parapsilosis*
C. krusei	4 (2.8)	Budding cells with hyphae	Pink	-	-	Pseudohyphae with spores	*C. krusei*
C. famata	2 (1.4)	Budding cells with hyphae	Pink	-	-	-	*C. famata*
C. guilliermondii	2 (1.4)	Budding cells with hyphae	Pink purple	-	-	Pseudohyphae with spores	*C. guilliermondii*

Culture phenotypic patterns shown as morphological and biochemical characteristics of isolates. SDA, Sabouraud dextrose agar. CHROMagar, chromogenic agar. VITEK® 2 YST, automated yeast fluorescent identification and susceptibility system.

4.3. Azole antifungal resistance profiles in candida isolates

4.3.1. Phenotypic azole antifungal resistance profiles

Evaluation of resistance amongst the isolates showed higher rates of resistance for both itraconazole (*P*=0.860) and voriconazole (*P*=0.425) across the CA and NAC (Table 4.3). These antifungal resistance results illustrated increased rates of resistance among the CA to both itraconazole (78.7%) and voriconazole (75.5%), respectively. Equally, the NAC had raised rates of resistance ranging from 50.0% to 100.0% for itraconazole (*C. famata*, 50.0%; *C. tropicalis*, 60.0%; *C. parapsilosis*, 63.6%; *C. glabrata*, 91.3%; *C. krusei*, 100.0%; and *C. guilliermondii*, 100.0%); and 40% to 87.0% (*C. tropicalis*, 40.0%; *C. famata*, 50.0%; *C. guilliermondii*, 50.0%; *C. parapsilosis*, 54.5%; *C. krusei*, 66.7%; and *C. glabrata*, 87.0%) for voriconazole. In addition, cross-resistance rates for both antifungal agents were also high (*C. albicans*, 74.5%; *C. glabrata*, 87.0%; *C. krusei*, 66.7%; *C. parapsilosis*, 54.5%; *C. famata*, 50.0%; *guilliermondii*, 50.0%; and *C. tropicalis*, 40.0%; *P*=0.092).

Table 4.4: Antifungal sensitivity profiles of fungal isolates

Isolate (n)	**Itraconazole, n (%)**		**Voriconazole, n (%)**		**Cross-resistance, n (%)**
	Sensitive	*Resistant*	*Sensitive*	*Resistant*	
C. albicans (94)	20 (21.3)	74 (78.7)	23 (24.5)	71 (75.5)	70 (74.5)
NAC (46)	9 (19.6)	37 (80.4)	14 (30.4)	32 (69.6)	32 (69.6)
C. glabrata (23)	2 (8.7)	21 (91.3)	3 (13.0)	20 (87.0)	20 (87.0)
C. parapsilosis (11)	4 (36.4)	7 (63.6)	5 (45.5)	6 (54.5)	6 (54.5)
C. tropicalis (5)	2 (40.0)	3 (60.0)	3 (60.0)	2 (40.0)	2 (40.0)
C. krusei (3)	0 (0.0)	3 (100.0)	1 (33.3)	2 (66.7)	2 (66.7)
C. famata (2)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
C. guilliermondii (2)	0 (0.0)	2 (100.0)	1 (50.0)	1 (50.0)	1 (50.0)
P	0.860		0.425		0.092

Results are shown as number (n) and proportions (%) of subjects. NAC, non-albicans candida. Fisher's exact tests were used for comparison of distributions for resistance and cross-resistance rates between the *C. albicans* (n=94) and NAC (n=46) isolates. Cross-resistance; isolates that are resistant to both itraconazole and voriconazole.

4.3.2. Minimum inhibitory concentrations

Evaluation of the MIC for the sensitive isolates against two of the most commonly used first line antifungal drugs indicated non-significantly (Table 4.4; $P<0.05$) higher MIC_{50} and MIC_{90}, range (mg/ml) in CA (0.125, 0.031-0250 and 0.250, 0.125-0.500) for itraconazole and (0.250, 0.031-0500 and 0.250, 0.250-0.100) for voriconazole (Table 4.4). In comparison to the CA, the MIC_{50} and MIC_{90}, range (mg/ml) in NAC were lower for both itraconazole (0.063, 0.025-0.500 and 0.125, 0.125-0.500) and voriconazole (0.094, 0.031-1.250 and 0.250, 0.250-1.250), respectively.

Table 4.5: Mean MIC in azole sensitivity isolates

Isolate (n)	Itraconazole (mg/ml)			Isolate (n)	Voriconazole (mg/ml)		
	MIC range	MIC_{50}	MIC_{90}		*MIC range*	MIC_{50}	MIC_{90}
C. albicans (20)[a]	0.031-0.500	0.125	0.250	*C. albicans* (23)[a]	0.031-1.000	0.250	0.250
Non-*albicans* candida (9)[b]	0.025-0.500	0.063	0.125	Non-*albicans* candida (14)[b]	0.031-1.250	0.094	0.250
P	…..	0.559	0.181	*P*	…..	0.966	0.632

Results shown are range and median concentrations of the antifungal minimum inhibitory concentrations (MIC). Un-paired t-test was used for comparing the antifungal MIC levels between the *C. albicans* and non-*albicans* candida spp. [a]n=20 for itraconazole, and n=23 for voriconazole. [b]The isolates *C. famata* (1), *C. glabrata* (2), *C. parapsilosis* (4), and *C. tropicalis* (2) were tested for itraconazole (n=9); while *C. famata* (1), *C. guilliermondii* (1), *C. krusei* (1), *C. glabrata* (3), *C. tropicalis* (3), and *C. parapsilosis* (5) were tested for voriconazole (n=14).

4.4. Genotypic characteristics of *Candida albicans*

4.4.1. *Candida albicans* genotypes

Molecular analysis of the CA isolates (n=94; Table 4.5) detected genotypes A (n=62; 66.0%), B (n=4; 4.3%), and C (n=18; 19.1%), with 10 (10.6%) of the isolates being unknown as shown in Table 4.5. The distribution of the genotypes was similar in superficial (A, n=20, 62.5%; B, n=2, 3.4%, and C, n=8, 25.0%); deep-seated (A, n=39, 67.2%; B, n=2, 3.4%, and C, n=10, 17.2%); and bloodstream (A, n=3, 75.0%; B, n=1, 25.0%, and C, n=0, 0.0%) mycoses (genotype A vs. non-A (combined B and C) genotypes: χ^2=0.883; df=2; *P*=0.643).

Table 4.6: *Candida albicans* genotypes in different types of mycoses

Type of mycoses	Genotypes				Total	χ^2; df; *P*
	A, n=62	B, n=4	C, n=18	Unknown, n=10*		
Superficial, n (%)	***20 (62.5)***	***1 (3.1)***	***8 (25.0)***	***3 (9.4)***	***32 (34.0)***	
High vaginal swabs	17 (63.0)	1 (3.7)	7 (25.9)	2 (7.4)	27 (28.7)	
Skin secretions	2 (66.7)	0 (0.0)	0 (0.0)	1 (33.3)	3 (3.2)	
Oral swabs	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	2 (2.1)	
Deep seated, n (%)	***39 (67.2)***	***2 (3.4)***	***10 (17.2)***	***7 (12.1)***	***58 (61.7)***	
Urine	22 (71.0)	0 (0.0)	8 (25.8)	1 (3.2)	31 (33.1)	0.883; 2; 0.643
Sputum	9 (60.0)	0 (0.0)	0 (0.0)	6 (40.0)	15 (16.0)	
Tracheal aspirates	5 (71.4)	0 (0.0)	2 (28.6)	0 (0.0)	7 (7.4)	
Pharyngeal exudates	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (2.1)	
Catheter tips	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (2.1)	
Cerebrospinal fluid	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	
Bloodstream infections, n (%)	***3 (75.0)***	***1 (25.0)***	***0 (0.0)***	***0 (0.0)***	***4 (4.3)***	
Blood	3 (75.0)	1 (25.0)	0 (0.0)	0 (0.0)	4 (4.3)	
Total, n (%)	***62 (66.0)***	***4 (4.3)***	***18 (19.1)***	***10 (10.6)***	***94 (100.0)***	

Data are presented as number and (proportions) of genotypes causing the mycoses. Comparison of genotype (genotype A vs. non-A genotypes (combined B and C genotypes) distribution across the mycoses was done using the Pearson's chi-square. *Uknown genotypes were excluded from the statistical analysis.

4.4.2. Morphological and biochemical features of *Candida albicans* genotypes

Examination of relationships between the genotypes with morphological and biochemical features illustrated that the three CA genotypes (A, B, and C) had positive reactions for SDA agar, CHROM agar, growth at 45°C, germ tube test, corn meal-Tween-80 agar, and VITEK®-2 YST tests (Table 4.6).

Table 4.7: *Candida albicans* genotype morphological and biochemical characteristics

Genotypes, n (%)	SDA agar	CHROMagar ™	Growth at 45°C	Germ tube test	Corn meal-Tween-80 agar	VITEK® 2 YST
A, 62 (73.8)	62 (73.8)	62 (73.8)	62 (73.8)	62 (73.8)	62 (73.8)	62 (73.8)
B, 4 (4.8)	4 (4.8)	4 (4.8)	4 (4.8)	4 (4.8)	4 (4.8)	4 (4.8)
C, 18 (21.4)	18 (21.4)	18 (21.4)	18 (21.4)	18 (21.4)	18 (21.4)	18 (21.4)
Total, 84 (100.0)	84 (100.0)	84 (100.0)	84 (100.0)	84 (100.0)	84 (100.0)	84 (100.0)

Data presented are number and proportions of genotypes. SDA, Sabouraud dextrose agar. CHROMagar, chromogenic agar. VITEK® 2 YST, automated yeast fluorescent identification and susceptibility system.

4.4.3. Azole resistance in *Candida albicans* genotypes

Evaluation of the rates of itraconazole resistance in the genotypes indicated non-significantly higher proportions of resistant isolates in genotype A (n=52, 82.5%) compared to the non-A (B and C) genotypes (n=14, 66.7%; *P*=0.137). Likewise, the proportion of voriconazole resistance was non-significantly higher in genotype A (n=49, 77.8%) compared to non-A (B and C) genotypes (n=14, 66.7%; *P*=0.384; Table 4.7).

Table 4.8: *Candida albicans* genotypes azole resistance patterns

	Itraconazole		Voriconazole		Total
Genotypes	**Sensitive, n=18**	**Resistant , n=66**	**Sensitive, n=21**	**Resistant , n=63**	
A, n (%)	11 (17.5)	52 (82.5)	14 (22.2)	49 (77.8)	63 (75.0)
B and C, n (%)	7 (33.3)	14 (66.7)	7 (33.3)	14 (66.7)	21 (25.0)
Total, n (%)	18 (21.4)	66 (78.6)	21 (25.0)	63 (75.0)	84 (100.0)
P	0.137		0.384		

Data are presented as number and (proportions) of virulence factor expression in different genotypes (itraconazole: A, n=63; B, n=3; and C, n=18). Data analysis was performed by the Fisher's exact tests.

4.4.4. MIC for azole sensitive *Candida albicans* genotypes

A comparison of the MIC between genotype A and non-A (B and C) genotypes showed similar levels (mean±SD) mg/ml at both MIC50 and MIC90 (Table 4.8): 1) Itraconazole: genotype A (0.0881±0.0438 and 0.3523±0.1460) vs. non-A (B and C) genotypes (0.1072±0.0762; *P*=0.507 and 0.3750±0.1614; *P*=0.761), respectively; and 2) Voriconazole: genotype A (0.2166±0.1277 and 0.4107±0.2705) vs. non-A genotypes (0.2724 ±0.1756; *P*=0.414 and 0.4286±0.2782; *P*=0.889), respectively.

Table 4.9: *Candida albicans* genotype azole sensitive isolates MIC

	Itraconazole (mg/ml)		Voriconazole (mg/ml)	
Genotypes	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**
A	0.0881 (0.0438)	0.3523 (0.1460)	0.2166 (0.1277)	0.4107 (0.2705)
B and C	0.1072 (0.0762)	0.3750 (0.1614)	0.2724 (0.1756)	0.4286 (0.2782)
Total	0.0955 (0.0572)	0.3611 (0.1478)	0.2352 (0.1431)	0.4167 (0.2661)
P	0.507	0.761	0.414	0.889

Results presented are mean and (standard deviation) in different genotypes. Itraconazole and voriconazole sensitive isolates: A, n=11 and 14; B, n=1 and 1; and C, n=6 and 6, respectively. Data analysis was conducted using the independent samples student t-tests.

4.5. Virulence expression in *Candida albicans* isolates

Overall (Table 4.9), biofilm production (n=71, 75.5%) and phospholipase activity (n=69, 73.4%) relative to alpha-haemolysin factor (n=20, 21.3%) and proteinase activity (n=34, 36.2%) were the most expressed virulence factors. Most of the biofilm producing isolates were isolated from deep-seated mycoses (n=42, 59.2%) compared to superficial (n=25, 35.2%) and bloodstream (n=4, 5.6%; *P*=0.164) mycoses. Phospholipase activity was significantly higher in isolated organisms from all the mycoses (superficial, n=21, 65.6%; deep-seated, n=45, 77.6%; and bloodstream, n=3, 75.0%; *P*=0.003). Alpha-haemolysin factor expression was almost two-times significantly higher amongst isolates from superficial (n=9, 28.1%) and candidaemia (n=3, 75.0%) relative to deep-seated (n=10, 17.2%; *P*=0.019) mycoses. However, proteinase activity was about 2-times significantly higher in deep seated (n=24, 41.4%) and bloodstream (n=2, 50.0%) relative to superficial (n=8, 25.0%; *P*=0.034) mycoses.

Table 4.10: Virulence factor expression by *Candida albicans* isolates from different types of mycoses

Type of mycoses	Virulence factor				Total
	Biofilm production, n=71	Haemolysin factor, n=20	Proteinase activity, n=34	Phospholipase activity, n=69	
Superficial, n (%)	***25 (35.2)***	***9 (28.1)***	***8 (25.0)***	***21 (65.6)***	***32 (34.0)***
Oral swabs	1 (50.0)	1 (50.0)	2 (100.0)	2 (100.0)	2 (2.1)
Skin secretions	2 (66.7)	0 (0.0)	0 (0.0)	1 (33.3)	3 (3.2)
High vaginal swabs	22 (81.5)	8 (29.6)	6 (22.2)	18 (66.7)	27 (28.7)
Deep seated, n (%)	***42 (59.2)***	***10 (17.2)***	***24 (41.4)***	***45 (77.6)***	***58 (61.7)***
Urine	24 (77.4)	0 (0.0)	12 (38.7)	26 (83.9)	31 (33.1)
Sputum	10 (66.7)	4 (26.7)	7 (46.7)	11 (73.3)	15 (16.0)
Cerebrospinal fluid	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (1.0)
Pharyngeal exudates	0 (0.0)	1 (50.0)	2 (100.0)	1 (50.0)	2 (2.1)
Tracheal aspirates	6 (85.7)	5 (71.4)	1 (14.3)	4 (57.1)	7 (7.4)
Catheter tips	2 (100.0)	0 (0.0)	1 (50.0)	2 (100.0)	2 (2.1)
Bloodstream infections, n (%)	***4 (5.6)***	***1 (25.0)***	***2 (50.0)***	***3 (75.0)***	***4 (4.3)***
Blood	4 (100.0)	1 (25.0)	2 (50.0)	3 (75.0)	4 (4.3)
Total, n (%)	***71 (75.5)***	***20 (21.3)***	***34 (36.2)***	***69 (73.4)***	***94 (100.0)***
P	0.164	**0.019**	**0.034**	**0.003**	…..

Data are presented as number and (proportions) of the virulence factor expression in different mycoses. Data analysis was conducted using the one-sample t-test for trend across the rates of superficial, deep-seated and bloodstream mycoses. Significant *P*-values are shown in bold.

4.5.1. Azole resistant and sensitive *Candida albicans* virulence factor expression

Evaluation of virulence factor expression in the *C. candida* azole resistant and sensitive isolates (Table 4.10) illustrated that the rates of biofilm formation and phospholipase activity were higher in itraconazole (84.8% vs. 72.2%; *P*=0.296 and 84.1% vs. 61.1%; *P*=0.044) and voriconazole (84.8% vs. 76.2%; *P*=0.512 and 84.1% vs. 66.7%; *P*=0.116) resistant strains, respectively. Haemolysin expression rates were also higher: itraconazole (31.8% vs. 27.8%; *P*=0.999) and voriconazole (33.3% vs. 23.8%; *P*=0.587) resistant isolates. However, proteinase activity was lower in itraconazole (39.4% vs. 50.0%; *P*=0.433) and voriconazole (36.5% vs. 57.1%; *P*=0.127) in sensitive strains.

Table 4.11: Virulence factor expression in azole sensitive *Candida albicans* strains

Virulence factors	Itraconazole			Voriconazole		
	Sensitive, n=18	**Resistant, n=66**	***P***	**Sensitive, n=21**	**Resistant, n=63**	***P***
Biofilm formation	13 (72.2)	56 (84.8)	0.296	16 (76.2)	53 (84.1)	0.512
Haemolysin	5 (27.8)	21 (31.8)	0.999	5 (23.8)	21 (33.3)	0.587
Proteinase	9 (50.0)	26 (39.4)	0.433	12 (57.1)	23 (36.5)	0.127
Phospholipase	11 (61.1)	56 (84.8)	**0.044**	14 (66.7)	53 (84.1)	0.116
Total	18 (21.4)	66 (78.6)	…..	21 (25.0)	63 (75.0)	…..

Data are presented as number and (proportions) of virulence factor expression in itraconazole and voriconazole resistant and sensitive *C. candida* isolates. Data analysis was conducted by Fisher's exact tests. Significant *P*-value are indicated in bold.

4.5.2. Virulence expressing isolates azole MIC

Comparison of MIC50 and MIC90 in sensitive isolates indicated non-significant difference in the levels (mg/ml) of itraconazole and voriconazole in biofilm, haemolysin, proteinase, and phospholipase producing CA isolates (Table 4.11).

Table 4.12: Virulence factor expression and MIC of azole sensitive *Candida albicans* isolates

Virulence factors	**Itraconazole (mg/ml)**				**Voriconazole (mg/ml)**			
	MIC_{50}	***P***	**MIC_{90}**	***P***	**MIC_{50}**	***P***	**MIC_{90}**	***P***
Biofilm formation	0.0866 (0.0123)	0.298	0.3462 (0.0428)	0.506	0.2383 (0.0406)	0.120	0.4375 (0.0740)	0.196
Haemolysin	0.0688 (0.0153)	0.229	0.3750 (0.0791)	0.813	0.3500 (0.0612)	0.388	0.3500 (0.0612)	0.735
Proteinase	0.0868 (0.0154)	0.536	0.3472 (0.0791)	0.703	0.2292 (0.0362)	0.275	0.3750 (0.0653)	0.337
Phospholipase	0.0881 (0.0132)	0.507	0.3523 (0.0440)	0.761	0.2166 (0.0340)	0.166	0.4107 (0.0723)	0.217

Data are presented as mean and (standard error) in the different virulence factor producers. MIC, minimum inhibitory concentrations. Biofilm formation, n=13 and 16; haemolysin, n=5 and 5; proteinase, n=9 and 12; and phospholipase, n=11 and 14 for itraconazole and voriconazole sensitive isolates, respectively. A comparison of MIC levels between virulence factor producers and non-producers was done using the independent student t-test.

4.5.3. Virulence factor expression in *Candida albicans* genotypes

Evaluation of relationship of the genotypes and virulence factor expression (Table 4.12) revealed higher expression of biofilm production (n=60, 95.2% vs. n=9, 42.9%; *P*=0.0000008) and phospholipase activity (n=61, 96.6% vs. n=6, 28.6%; *P*=0.063) relative to alpha-haemolysin factor (n=23, 36.5% vs. n=3, 14.3%; *P*=0.001) and proteinase activity (n=33, 52.2% vs. n=2, 9.5%; *P*=0.0000000004) in the genotype A and non-A genotype (B and C) strains.

Table 4.13: Virulence factor expression amongst the *Candida albicans* genotypes

Genotypes	Virulence factor expression				Total
	Biofilm production, n=69	**Alpha-haemolysin factor, n=26**	**Proteinase activity, n=35**	**Phospholipase activity, n=67**	
A, n (%)	60 (95.2)	23 (36.5)	33 (52.4)	61 (96.8)	63 (75.0)
B and C, n (%)	9 (42.9)	3 (14.3)	2 (9.5)	6 (28.6)	21 (25.0)
Total, n (%)	69 (82.1)	26 (31.0)	35 (41.7)	67 (79.8)	84 (100.0)
	0.0000008	0.063	**0.001**	**0.0000000004**	…..

Data are presented as number and (proportions) of virulence factor expression in different genotypes. Genotypes: A, n=63; B, n=3 and C, n=18, respectively. Data analysis was conducted using Fisher's exact tests. Bolded *P*-values are statistically significant.

CHAPTER FIVE: DISCUSSION

5.1. Clinical sources and diversity of candida isolates

Accurate characterisation of candida species is critical for better-treatment outcomes and understanding the distribution of specific aetiologies of candidiasis syndromes. Routine culture of specimens from patients with suspected candidiasis, especially in those with low immunity, can greatly improve management and treatment outcomes. However, diagnosis and care of candidiasis patients is mainly dependent upon the clinical features and acumen of the clinician in discerning the presenting symptoms and signs. Furthermore, a majority of health facilities in the country lack laboratory capabilities for routine fungal culture and sensitivity testing, consequently relying on the less sensitive direct wet mount preparations. As such, to improve identification of the aetiologic agents of prevalent candidiasis, better diagnostic approaches are required. This study therefore evaluated the aetiologies and distribution of CA and NAC in clinical specimens from individuals with suspected candidiasis.

The isolation of candida yeasts mainly from HVS, tracheal secretions, urine and catheter tip specimens, suggest a high prevalence of vulvovaginal, urinary tract, and sexually transmitted candidal infections in the study area. These findings are similar to the previous high rate of isolation of CA from urine, blood, and HVS in pregnant females presenting with STI attending ante-natal care in the Gambia (Isara & Baldeh, 2021), as well as in females with VVC in Ethiopia, Namibia, and Kenya, respectively (Bitew & Abebaw, 2018; Dunaiski et al., 2022; Mutua et al., 2010). Additionally, the higher yield of positive cultures from urine and HVS specimens from this study, suggest a greater prevalence of VVC, as observed in previous published mycological

results from Cameroon, Kenya, Nigeria, and Uganda (Kengne et al., 2017; Mbakwem-Aniebo et al., 2020; Mukasa et al., 2015; Mutua et al., 2010). Likewise, studies from Nigeria also identified candida species as a frequent cause of urinary tract infection amongst asymptomatic males and females with underlying HIV infections (Omoregie & Eghafona, 2009). Besides, recovery from urine samples is analogous with observations in Ghana indicating that candida species are frequent aetiologies of urinary tract infections (Asare et al., 2023; Deininger et al., 2022). Overall, these findings allude to the fact that candida species are common uropathogens that cause candidiasis following invasion under appropriate conditions.

The high rates of candida isolation from tracheal secretions suggest pleuropulmonary candidiasis. This result mirrors studies in Brazil showing higher isolation rates of candida species from tracheal secretion specimens in male than female patients admitted at a tertiary hospital in Brazil (Furlaneto et al., 2011), and in Slovania indicating high rates of candida species recovery from tracheal aspirates, sputum, thoracic puncture and drainage fluid samples from lung cancer patients post lung cancer resection surgery (Sok et al., 2002), and from early morning sputum samples of pulmonary tuberculosis patients in South India and Kenya (Kali et al., 2013; Mwaura et al., 2013). Isolation of candida from tracheal aspirates, sputum, and pharyngeal exudates also indicates fungal respiratory and oropharyngeal thrush. Certainly, previous microbiological studies in Kenya and Uganda amongst children and adults living with HIV and presenting with pneumonia or pulmonary tuberculosis indicated high rates of pulmonary candida recovery (C. C. Bii et al., 2006; Njovu et al., 2021). Furthermore, candida spp. were recovered from pharyngeal and tracheal aspirates in Guinea-Bissau and India (Basu et al., 2003; Bento et al., 2010).

Additionally, oral swab culture candida positive findings indicate oropharyngeal candidiasis (oral thrush) as previously observed in Tanzania amongst patients on anti-cancer therapy (Kibwana et al., 2023).

The recovery of candida yeasts in blood specimens indicates candidaemia, as previously observed at a private teaching and referral hospital in Kenya, showing that candida species frequently cause nosocomial blood-stream infection (Maina et al., 2016). The study also showed that candida species were also frequently recovered in community onset bloodstream infections (Maina et al., 2016). Similar results were obtained at a tertiary hospital in South Africa illustrating that CA accounted for almost half of the cases of candidaemia confirmed through blood cultures (Hussain et al., 2022). Overall, candidaemia suggests haematogenous spread of the candida pathogens resulting in multisystemic infections and candida sepsis.

The finding of candida isolates from skin lesions indicated superficial cutaneous candida mycotic infections corresponding to previous mycological cultures in Senegal, showing that candida species accounted for at least two thirds of nail and/or skin mycoses (Diongue et al., 2018). Similarly, catheter tip culture isolation of candida species is consistent with studies from South Africa showing *C. auris* yield of 25% from catheter tips (Magobo et al., 2020). Finally, the CSF yield of candida isolates suggest candida meningitis, consistent with previous analyses of Egyptian paediatric nosocomial infections in intensive care units (El-Nawawy et al., 2006).

The overall results showed higher recovery of CA, indicating that CA is the most frequent candida species in clinical specimens. These findings are similar to previous

studies in Ethiopia, South Africa, and Nigeria illustrating a predominance of CA in various clinical specimens (Abia-Bassey & Utsalo, 2006; Mnge et al., 2017; Seyoum et al., 2020). Amongst the NAC species, *C. glabrata* was common with *C. famata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* also isolated. These findings are identical to South African studies showing a high proportion of *C. glabrata*, but diverge from the Ethiopian results illustrating *C. krusei* prevalence and greater diversity of the isolated NAC species (Mnge et al., 2017; Seyoum et al., 2020). The differences are, partly, attributable to the fact that the latter study also sampled nail and corneal scrapings that yielded higher non-*albicans* positive cultures (Seyoum et al., 2020). Altogether, these studies illustrate that candidiasis is a multisystemic invasive disease frequently causing vulvovaginal, urinary tract, and pleuropulmonary candidiasis particularly in immunosuppressed individuals.

5.2. Azole antifungal resistance profiles of candida isolates

The increased numbers of immunocompromised persons such as cancer, HIV, intensive care unit, and organ transplant immunosuppressive therapy patients, amongst others across many African countries, the cases of azole drug resistant mycoses will continue increasing (Dovo et al., 2022; Dunaiski et al., 2024; Musyoki et al., 2022). This will result in an increase in the use of antifungal medications leading to the development of resistant strains, thus underscoring the importance of antifungal susceptibility testing for effective treatment and prophylaxis. Triazole antifungal drugs including itraconazole and voriconazole are amongst the first-line antifungal agents indicated for the treatment and prophylaxis of candidiasis, particularly in immunocompromised persons. However, detection of susceptibility profiles to these drugs is normally not routinely performed prior to initiation and/or

monitoring of the response to therapy and prophylaxis. In addition, most health facilities lack laboratory capabilities for conducting point-of-care, mycological culture and susceptibility testing. Thus, mycotic treatment is often empirical. Consequently, to improve the rational use of antifungal azole agents, this study evaluated the susceptibility profiles and MIC in candida yeasts isolated in clinical samples from public health facilities in Nairobi metropolitan city.

Assessment of azole sensitivity profiles in the isolates showing phenotypic resistance rates of ~50-76% for itraconazole, ~40-79% for voriconazole and ~40-75% cross-resistance, suggests that candida spp. isolates from patients in health facilities in Nairobi Metropolitan City exhibit high levels of azole resistance. These results partially mirror studies in Ghana showing high rates of triazole antimycotic drug resistant CA and NAC isolates from VVC cases (Waikhom et al., 2020), and from recurrent vulvovaginal patients (Adjapong et al., 2017). Likewise, studies in South African candidaemia patients showed increasing azole resistance in 2016 to 2020 (Chibabhai, 2022). In addition, studies at two tertiary teaching hospitals in Nairobi city, Kenya indicated increasing burden of triazole resistance in diabetic foot ulcer type-2 diabetic patients at the Kenyatta National Hospital (Musyoki et al., 2022), and in vaginal candidiasis patients at the Aga Khan University Teaching Hospital (Mutua et al., 2010). Moreover, studies in HIV-infected patients in Cameroon and Nigeria indicated high resistance to azole antifungals in CA and NAC isolates (Ngouana et al., 2019; Nweze & Ogbonnaya, 2011). The underlying causes of the high rates of resistance include an increasing number of immunosuppressed patients leading to recurrent candidiasis necessitating frequent treatment and prophylaxis with these drugs. In addition, intrinsic resistance mechanisms observed in *C. glabrata* and *C.*

krusei (Taverne-Ghadwal et al., 2022), in part, contributed to the increased rates of azole resistance. Subsequently, this leads to drug-induced development and the spread of resistant strains.

Consistent with the phenotypic pattern of resistance, MIC testing showing higher levels in CA relative to NAC isolates for both itraconazole and voriconazole, suggests increasing resistance to azole drugs. These findings contrast with earlier studies in Kenya, Ghana, Cameroon and South African patients illustrating elevated MIC in NAC isolates in comparison to CA isolates for itraconazole and voriconazole (Abrantes et al., 2021; Adjapong et al., 2017; Mutua et al., 2010; Ngouana et al., 2019). In addition, recent antimycotic susceptibility testing of candida recovered from oncology patients in Egypt revealed higher voriconazole MIC in NAC relative to CA (El-Mahallawy et al., 2023). The reasons underlying the high MIC levels include long-term azole therapy or prophylaxis, implying that higher adjustment of treatment dosages, duration and/or combinations or adoption of a different effective antifungal drug for achieving optimal therapy and prophylaxis for candidiasis. This suggestion is partly supported by previous studies showing significant *in vitro* MIC reductions in combinations of the azoles fluconazole and itraconazole, amphotericin B and caspofungin with farnesol (Cordeiro et al., 2013), and higher MIC to fluconazole in HIV patients on >6 months of fluconazole prophylaxis (Heald et al., 1996). Furthermore, high MIC levels were also reported in stipple phenotype of CA recovered from patients with neutropenia on long-lasting fluconazole use (Kiraz et al., 2000). Understanding the antifungal characteristics of candida spp. isolates before and/or during medical therapy may be important in formulating an expectation of

opportunistic candidiasis in immunocompromised hosts, including identification of the transmission pathway of the fungus.

5.3. *Candida albicans* genotypes

The 25s rDNA (rRNA) gene internal transcribed spacer region PCR amplification with restriction digestion and sequencing classifies CA strains based on amplicon sizes into: 450 bp (A), 840 bp (B), 450 and 840 bp (C), 1,080 bp (D), and 1400 bp (E) genotypes (McCullough et al., 1999; Tamura et al., 2001). In the current research, the A genotype (66.0%) was the most frequent *C. albicans* genotype compared to B (4.3%) and C (19.1%) genotypes are, in part, similar to previous 25s rDNA genotyping studies in illustrating dominance of A genotype in Kenya (60.0%) and Burkina Faso (85.4%), but differing in the B (8.0% and 10.4%) and C (16.0% and 4.2%), respectively (C. Bii et al., 2009; Sawadogo et al., 2019; Zida et al., 2018). These results are also consistent with 25s rDNA genotyping studies on CA isolates recovered from oral cavities of paediatric patients with neutropaenia and malignancies in Iran showing similar overall rates of A (58.9%) but different rates of B (16.7%) and C (7.7%) genotypes (Jafarian et al., 2022). Besides, genotyping studies based on the 25s rDNA gene in *C. albicans* isolates from blood and vaginal specimens in Iran showed higher prevalence of genotype A (52.5%) relative to B (7.5%) and C (40.0%) genotypes (Mahmoodi et al., 2023). Furthermore, genotyping of 25s rDNA from *C. albicans* isolates in hospitalised infants, neonates, and paediatric patients presenting with candiduria in Iran indicated dominance of A (72.0%) genotype over the C (28.0%) genotype (Gharaghani et al., 2021). Altogether these studies demonstrate that the genotype A rates based on 25s rDNA genotyping are the most prevalent, but the lower rates of genotypes B and C in the current study are, in part, related to the fact

that most of the *C. albicans* yeasts analysed in the previous studies had been isolated from oral samples in which the B and C genotypes are known to be the most frequent (Jafarian et al., 2022; Zida et al., 2018).

In this study, no clear association was detected between CA isolates and their invasive body tissues. This observation parallels that of (She et al., 2008) showing no evidence of associations between genotypes and cutaneous infection foci. This observation is probably related to the fact that variations in cutaneous locations were minor, leading to a lack of differences in the genotypes. The present study is also suggestive of the existence of vaginopathic candidiasis, and extravaginal candida tropism (superficial, deep-seated and blood stream mycoses). In addition, the study implies that CA strains with prevalent genotypes are more virulent in causing candidoses. Comparing the genotypes of CA recovered from different anatomical sites in the body may help further understanding of a genetic link with the development of candidiasis. The detection of specific genotypes that correlate with severity of candidiasis is undoubtedly of diagnostic and therapeutic importance.

5.4. *Candida albicans* virulence factors

Candida albicans produce a number of virulence factors such as biofilm formation and tissue-damaging hydrolytic enzymes (e.g., proteases, haemolysins, and phospholipases) that confer ability for adhesion and invasiveness leading to establishment and dissemination of infection (Lopes & Lionakis, 2022; Pappas et al., 2018; Saiprom et al., 2023; Talapko et al., 2021). In the present research, biofilm production (75.5%), phospholipase (73.4%), proteinase (36.2%), and haemolysin (21.3%) activities were the virulence factors detected amongst the isolates in the

current study, suggesting that these virulence factors confer invasiveness and establishment of *C. candida* infections especially for superficial, deep-seated, and bloodstream mycoses. These results partly parallel previous studies in Tanzania amongst different candida species isolates showing that phospholipase (72.0%), protease (62.7%), and coagulase (53.2%) activities were frequently detected (Mushi et al., 2019). In addition, studies on denture wearers in South Africa illustrated higher rates of phospholipase (85.0% and 25.0%) and proteinase (80.0% and 60.0%) activities compared to isolates from normal healthy individuals, respectively (Mothibe & Patel, 2017). However, in comparison to the current findings, previous studies in Iran detected lower rates of phospholipase (42.4% and 40.5%) and proteinase (36.4% and 28.6%) activities in CA recovered from patients with vaginitis and healthy people, respectively (Seifi et al., 2015) and very strong haemolysin (68.4%), proteinase (36.8%), phospholipase (47.4%), Esterase (94.7%) activities in *C. albicans* isolates from oesophageal candidiasis (Jafarian et al., 2021), pointing to possible patients' age, genetic, geographical, and clinical phenotype differences in virulence factor expression in *C. albicans*.

5.5. Virulence expression and azole resistance in *Candida albicans* genotypes

Candida albicans group I intron exhibits self-splicing activity required for generation of mature 25s rRNA, which has been applied in PCR genotyping analysis depending upon amplicon size variation (Mercure et al., 1993). In addition, base analogues such as triazoles can block the self-splicing process depending on the presence or absence of group I introns (Mercure et al., 1997). However, studies by (Zhu et al., 2011) on *C. albicans* clinical isolates from China indicated that A genotype strains relative to B and C genotypes had low susceptibility to flucytosine, but no associations were found

between genotypes and susceptibility to azoles. In the current study, however, genotype A (≥79%), B (67%) and C (67%) isolates exhibited high resistance levels to both itraconazole and voriconazole. These results are in part similar to studies of *C. albicans* recovered from Palestinian women with VVC showing more fluconazole resistant genotype A strains than in genotype B and C isolates (Mohammed et al., 2015). The results are also consistent with previous studies amongst Chinese vulvovaginal patients showing higher resistance rates to itraconazole in *C. albicans* genotypes B (66.7%) compared to non-genotype B strains (Liu et al., 2009). Overall, these results suggest the existence of high levels of azole resistance in *C. candida* genotypes from patients with candidiasis.

In the current study, genotypes A, B and C mostly expressed biofilm production and phospholipase activity but had lower expression of haemolysin and proteinase activity, indicating differences in the expression of virulent factors. These results are in part similar to previous studies in Egypt amongst paediatric patients presenting with features of nosocomial infections showing that mainly the expression of biofilms, phospholipase, and proteinase enzyme activity in genotype A isolates and their lower expression in C genotype strains (Mashaly & Zeid, 2022). In contrast, studies in India amongst HIV-positive individuals presenting with oral thrush indicated that the prevalent A genotype was not associated with virulence factor expression (Mane et al., 2012). Nonetheless, studies in Brazil showed that about 50% of genotype A CA strains recovered from sub-gingival biofilms in chronic periodontitis diabetic patients had beta-haemolytic activity (Sardi et al., 2012). Since, *C. albicans* recovered from PLWH receiving HAART and presenting with vulvovaginitis had lower biofilm activity but higher proteinase activity (Zanni et al.,

2017), and findings of higher adherence capacity in strains from vaginal candidiasis and greater phospholipase activity in strains from candidaemia patients (Mandelblat et al., 2017), it is possible that the regulation of virulence factor expression is governed by interaction of host environment factors such as stress with candida virulence genes.

The higher rates of formation of biofilms in itraconazole and voriconazole resistant CA, suggest greater expression of virulence factors in azole resistant candidiasis. This finding differs from previous results showing lower rates of biofilm formation in fluconazole tolerant *C. albicans* from paediatric patients in Egypt compared to non-tolerant strains, as well as inverse correlations of proteinase and biofilm production with the degree of fluconazole tolerance (Mashaly & Zeid, 2022). Furthermore, studies in Iran showed positive and inverse correlations between biofilm formation with proteinase activity, and fluconazole minimum inhibitory concentrations, respectively, in C. *albicans* recovered from oral mucosae of diabetics and non-diabetics on haemodialysis (Mohammadi et al., 2023). Overall, these results suggest that virulence factor and azole resistant genes are co-linked and undergo similar selection pressure in the host.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

This research has illustrated that the distribution of candida species in various clinical samples is dominated by *C. albicans* with non-candida *albicans* also isolated.

This study shows high rates of azole antifungal drug resistance including cross-resistance in CA and NAC isolates from clinical specimens in Nairobi County, Kenya. The study provides evidence that there exists a high frequency of phenotypic resistance in candida recovered from candidiasis patients and that MIC levels are elevated in CA relative to NAC.

Genotyping of *C. albicans* demonstrated that the A genotype, the most frequent clinical isolate from referral hospitals in Nairobi metropolitan city, expresses significantly higher rates of biofilm production and phospholipase activities. In addition, all the A, B, and C genotypes of CA had higher rates of itraconazole and voriconazole resistance.

Biofilm formation and phospholipase enzyme activities were the predominantly expressed virulence factors in CA with haemolysin and proteinase activities also detected amongst the clinical isolates from the patients in tertiary health facilities of the metropolitan city, Nairobi.

6.2. Recommendations

The finding on the dominance of CA and a diverse NAC species in clinical specimens will be important in the guiding management and constant surveillance strategies of candidiasis in the country as the emergence and spread of new species is poorly understood.

The high level of resistance to azole antimycotic drugs in the present study justifies therapeutic use of other antifungal drug families and establishing centres of excellence incorporating molecular approaches for detection and periodic surveillance on antimycotic drug resistance for improved patient treatment outcomes.

The findings on the most widespread and pathogenic *C. albicans* A genotype indicating expression of higher rates of virulence factors and azole resistance warrants concerted efforts aimed at detection and identifying sources of these variants for prevention and control strategies that curtail their spread.

Routine cultures aimed at identifying the frequently expressed virulence factors, biofilm formation and phospholipase enzyme activities which can be targeted for more effective treatment outcomes.

6.3. Recommendation for further studies

Future studies targeting healthy individuals, environmental, and zoonotic carriage and associated risk factors will provide additional information on the diversity of Candida aetiologic agents in the country.

A prospective study incorporating all antifungal drug families (polyenes, allylamines, azoles, and echinocandins), and utilising molecular genotyping of resistance patterns will provide additional insights into the most effective drugs for therapy and prophylaxis for candidiasis.

Application of more current molecular techniques such as whole genome analysis and sequencing methods in a prospective design will go a long way in identifying all the genotypes circulating in the country and further improve surveillance and prevention of the most pathogenic variants of these fungi.

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susceptibility of clinical Candida albicans in Ouagadougou (Burkina Faso). *Medical Mycology*, *56*(7), 907–910. https://doi.org/10.1093/mmy/myx127

APPENDICES

APPENDIX I: ETHICS APPROVAL

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
Tel: 056-31375
Fax: 056-30153
E-mail: rel@mmust.ac.ke
Website: www.mmust.ac.ke
P. O. Box 190
Kakamega
50100
Kenya

Institutional Ethics Review Committee (IERC)

MMU / COR: 403012(16)
9th January, 2017

Erolls Sigei
P. O. Box 30195-00100
NAIROBI
0723-880638
Email: sigei.erolls@gmail.com

Dear Sigei,

RE: ETHICAL APPROVAL TO CONDUCT RESEARCH

The IERC received your proposal titled "***Phenotypic and genotypic characterization of candida Species from clinical isolates in Nairobi County, Kenya***". The IERC, MMUST chapter therefore grants ethical clearance for you to conduct your research as proposed. In case of any adverse reactions to the patients, please report to IERC, MMUST.

On behalf of IERC and the University Senate, receive my congratulations. We wish you success in your research endeavor.

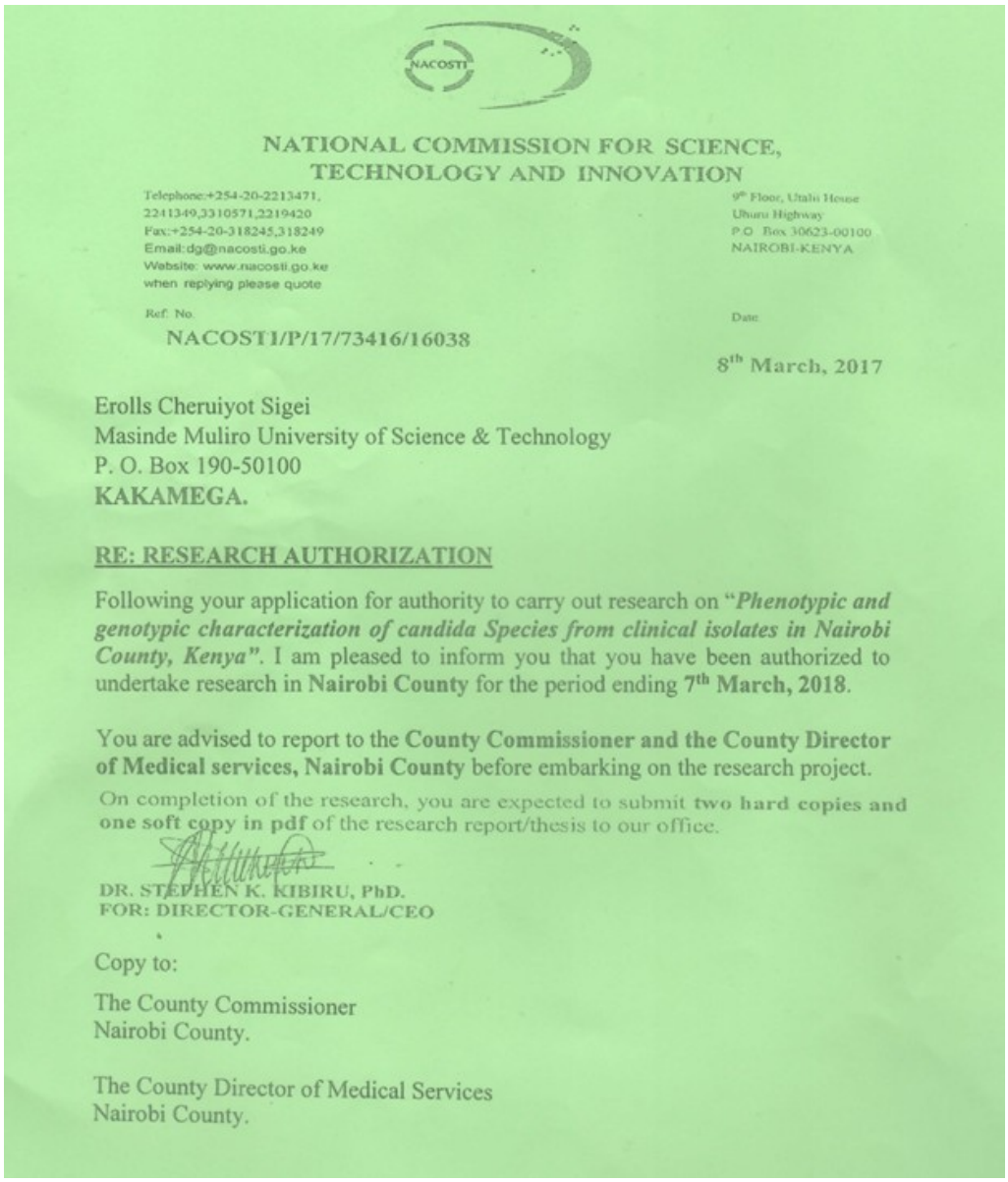
Yours faithfully,

Dr. Nguka Gordon
Chairman, Institutional Ethics Review Committee

Copy to:

- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)
- DVC (A & F)
- DVC (A&SA)

APPENDIX II: NACOSTI APPROVAL



NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email:dg@nacosti.go.ke
Website: www.nacosti.go.ke
when replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O Box 30623-00100
NAIROBI-KENYA

Ref: No.
NACOSTI/P/17/73416/16038

Date
8th March, 2017

Erolls Cheruiyot Sigei
Masinde Muliro University of Science & Technology
P. O. Box 190-50100
KAKAMEGA.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Phenotypic and genotypic characterization of candida Species from clinical isolates in Nairobi County, Kenya"***. I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for the period ending **7th March, 2018**.

You are advised to report to the **County Commissioner and the County Director of Medical services, Nairobi County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

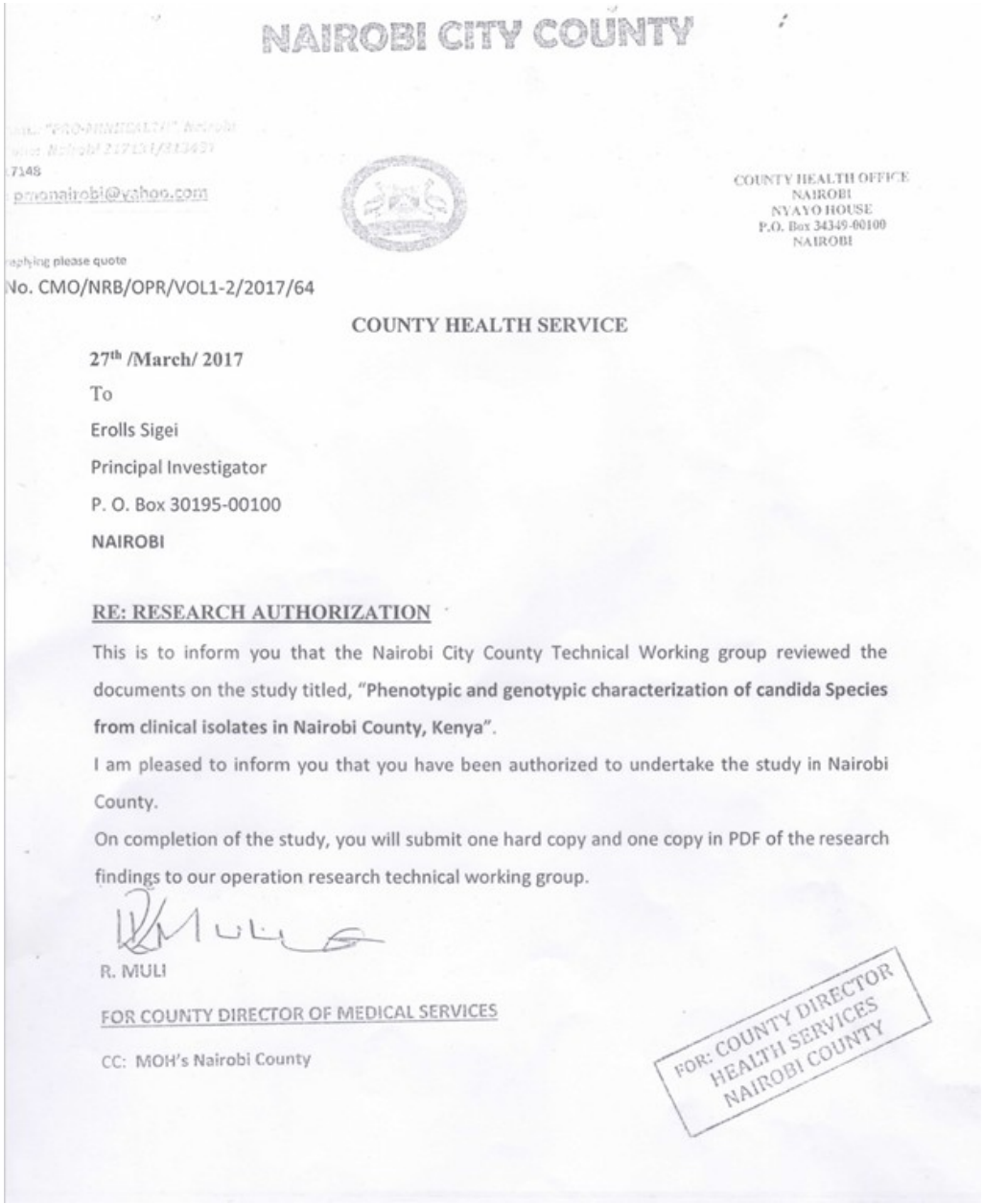
DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

The County Director of Medical Services
Nairobi County.

APPENDIX III: NAIROBI CITY COUNTY RESEARCH AUTHORIZATION



NAIROBI CITY COUNTY

7148
pmonairobi@yahoo.com

COUNTY HEALTH OFFICE
NAIROBI
NYAYO HOUSE
P.O. Box 34349-00100
NAIROBI

aphing please quote
No. CMO/NRB/OPR/VOL1-2/2017/64

COUNTY HEALTH SERVICE

27th /March/ 2017

To

Erolls Sigei

Principal Investigator

P. O. Box 30195-00100

NAIROBI

RE: RESEARCH AUTHORIZATION

This is to inform you that the Nairobi City County Technical Working group reviewed the documents on the study titled, "**Phenotypic and genotypic characterization of candida Species from clinical isolates in Nairobi County, Kenya**".

I am pleased to inform you that you have been authorized to undertake the study in Nairobi County.

On completion of the study, you will submit one hard copy and one copy in PDF of the research findings to our operation research technical working group.

R. MULI

FOR COUNTY DIRECTOR OF MEDICAL SERVICES

CC: MOH's Nairobi County

FOR: COUNTY DIRECTOR
HEALTH SERVICES
NAIROBI COUNTY

APPENDIX IV: KEMRI RESEARCH APPROVAL

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 May 19, 2017

To: MR. EROLLS C. SIGEI
PRINCIPAL INVESTIGATOR

THROUGH: ACTING DIRECTOR, CRDR,
NAIROBI

Dear Madam,

RE: PROTOCOL NO. KEMRI/SERU/CRDR/0012/3234 *(RESUBMITTED2 INTIAL SUBMISSION)*: PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF CANDIDA SPECIES FROM CLINICAL ISOLATES IN NAIROBI COUNTY- KENYA- (*VERSION 3.0 YEAR 2017*)

Reference is made to your letter dated 17th May, 2017. KEMRI/ Scientific and Ethics Review Unit (SERU) acknowledges receipt of revised study documents on 18th May, 2017.

This is to inform you that the Committee notes that the issues raised by the Expedited Review Team respectively have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day 19th May, 2017 for a period of one year. Please note that authorization to conduct this study will automatically expire on May 18, 2018. If you plan to continue data collection or analysis beyond this date, please an application for continuation approval to SERU by April 04, 2018.

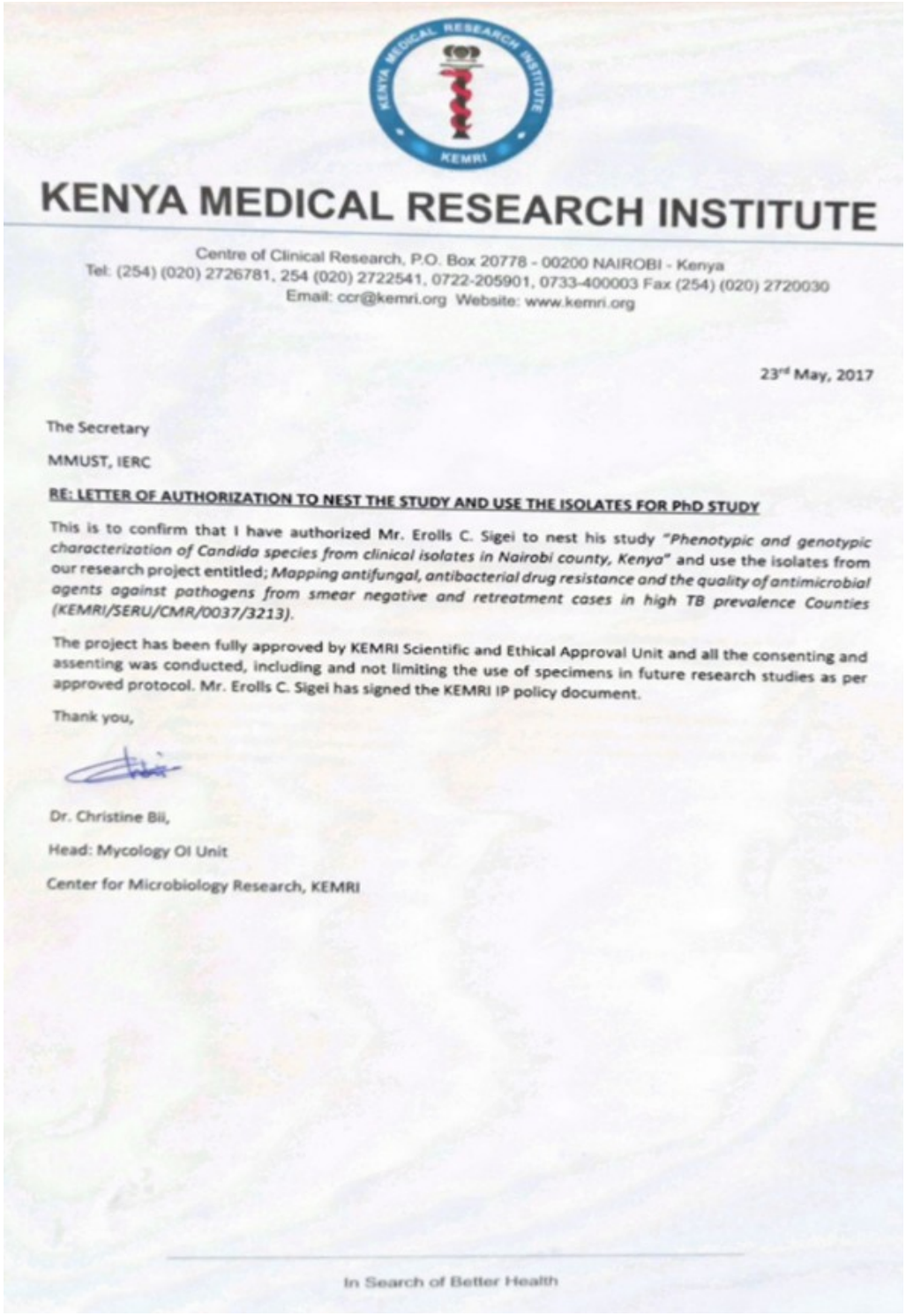
You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

FOR DR. EVANS AMUKOYE,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

APPENDIX V: KEMRI ISOLATE USE AUTHORIZATION



KENYA MEDICAL RESEARCH INSTITUTE

Centre of Clinical Research, P.O. Box 20778 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2726781, 254 (020) 2722541, 0722-205901, 0733-400003 Fax (254) (020) 2720030
Email: ccr@kemri.org Website: www.kemri.org

23rd May, 2017

The Secretary

MMUST, IERC

RE: LETTER OF AUTHORIZATION TO NEST THE STUDY AND USE THE ISOLATES FOR PhD STUDY

This is to confirm that I have authorized Mr. Erolls C. Sigei to nest his study *"Phenotypic and genotypic characterization of Candida species from clinical isolates in Nairobi county, Kenya"* and use the isolates from our research project entitled; *Mapping antifungal, antibacterial drug resistance and the quality of antimicrobial agents against pathogens from smear negative and retreatment cases in high TB prevalence Counties (KEMRI/SERU/CMR/0037/3213).*

The project has been fully approved by KEMRI Scientific and Ethical Approval Unit and all the consenting and assenting was conducted, including and not limiting the use of specimens in future research studies as per approved protocol. Mr. Erolls C. Sigei has signed the KEMRI IP policy document.

Thank you,

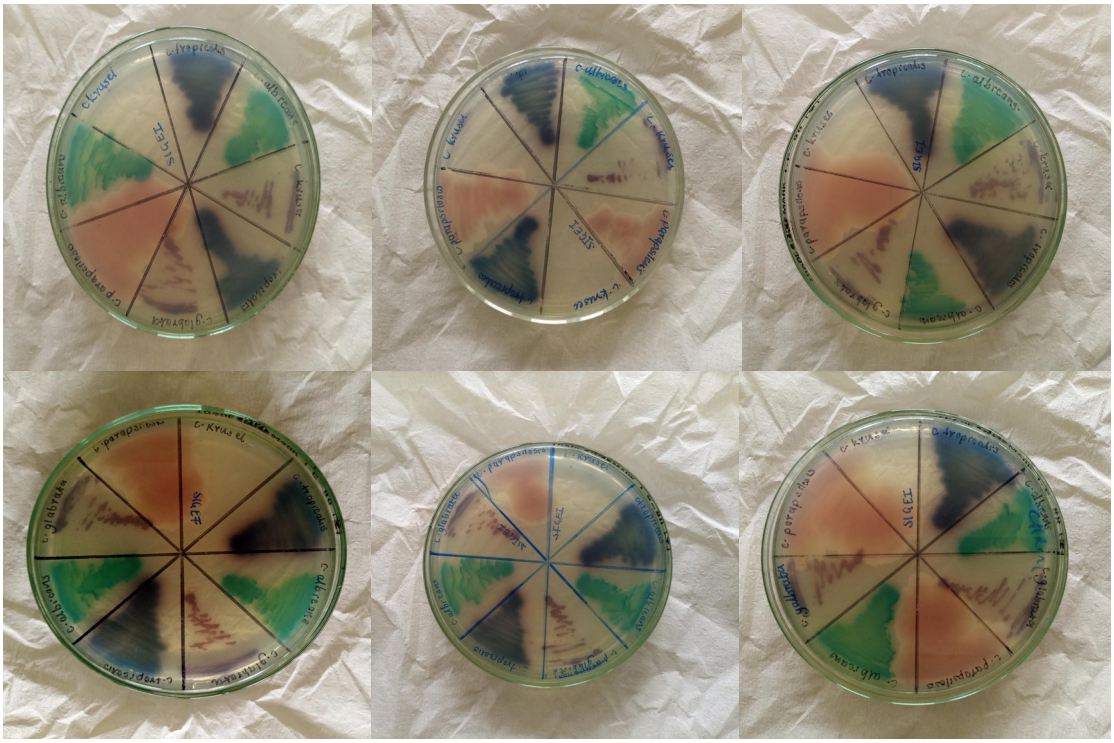
Dr. Christine Bii,

Head: Mycology OI Unit

Center for Microbiology Research, KEMRI

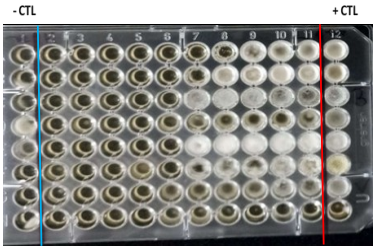
In Search of Better Health

APPENDIX VI: PHENOTYPIC MORPHOLOGICAL CHARACTERISTICS

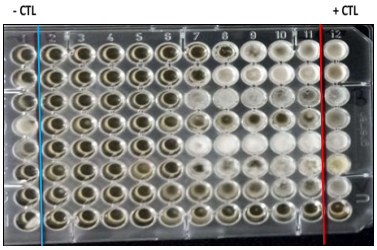


APPENDIX VII: ANTIFUNGAL SUSCEPTIBILITY TEST

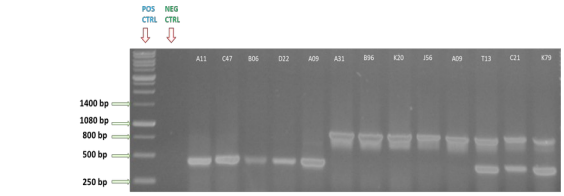
VORICONAZOLE

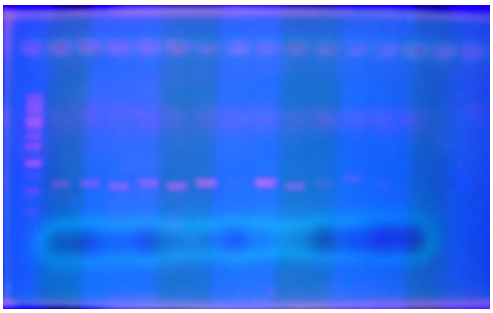


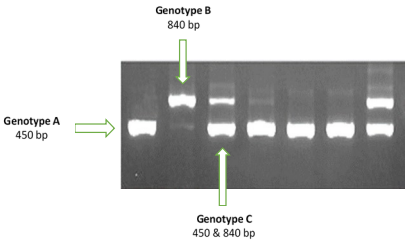
ITRACONAZOLE



APPENDIX VIII: GENOTYPIC PROFILE OF *CANDIDA ALBICANS*







APPENDIX IX: PUBLICATION-I

East African Medical Journal Vol. 100 No. 8 August 2023

CANDIDA SPECIES DISTRIBUTION IN DIFFERENT CLINICAL SPECIMENS FROM VARIOUS NAIROBI METROPOLITAN CITY PUBLIC REFERRAL HOSPITALS

Erolls Cheruiyot Sigei, Department of Medical Laboratory Sciences, Faculty of Diagnostic Sciences, Kenya Medical Training Institute, Nairobi, P. O. Box 30195-00100, Nairobi, Kenya, Munyoki Nyamai, Department of Medical Laboratory Sciences, Faculty of Diagnostic Sciences, Kenya Medical Training Institute, Nairobi, P. O. Box 30195-00100, Nairobi, Kenya, Sharon Mwikali Kitolo, Department of Medical Laboratory Sciences, Faculty of Diagnostic Sciences, Kenya Medical Training Institute, Nairobi, P. O. Box 30195-00100, Nairobi, Kenya, Vivian Patricia Okinyi, Department of Health Systems Management and Development, African Medical Research Foundation (AMREF) International University, Ministry of Education, Christine Chemutai Bii, Department of Mycology, Centre for Microbiology Research, Kenya Medical Research Institute, Ministry of Health, P. O. Box 54840-00200, Nairobi, Kenya, and Tom Were, Department of Laboratory Medicine and Human Pathology, School of Medicine, Masinde Muliro University of Science and Technology, Ministry of Education, P. O. Box 190-50100, Kakamega, Kenya.

Corresponding author: Erolls Cheruiyot Sigei, Department of Medical Laboratory Sciences, Faculty of Diagnostic Sciences, Kenya Medical Training Institute, Nairobi, P. O. Box 30195-00100, Nairobi, Kenya. Email: esigei@kmtc.ac.ke

CANDIDA SPECIES DISTRIBUTION IN DIFFERENT CLINICAL SPECIMENS FROM VARIOUS NAIROBI METROPOLITAN CITY PUBLIC REFERRAL HOSPITALS

E. C. Sigei, M. Nyamai, S. M. Kitolo, V. P. Okinyi, C. C. Bii and T. Were

ABSTRACT

***Background*: Invasive candidiasis and candidaemia are pervasive conditions complicating immunosuppressive conditions and leading to high mortality. This study aimed at determining the distribution of *C. albicans* and emergence of non-*albicans candida* species.**

***Methods*: This cross-sectional study was performed on clinical specimens previously collected from January 2017 to December 2018 from patients presenting with candidiasis at several metropolitan hospitals in Nairobi city, Kenya. The distribution of candida species in the clinical specimens was conducted using mycological culture and identification using morphological, biochemical and VITEK® 2 YST automated were used in the identification of the candida isolates.**

***Results*: The 141 isolates identified consisted of *candida albicans* (66.7%) and non-candida spp. (*C. glabrata*, 16.3%; *C. parapsilosis*, 7.8%; *C. tropicalis*, 3.6%; *C. krusei*, 2.8%; *C. famata*, 1.4%; and *C. guilliermondii*, 1.4%). A majority of *C. albicans* were obtained from urine (33.3%), high vaginal swabs (31.9%), sputum (17.0%), and tracheal aspirate (5.7%) specimens.**

***Conclusions*: These results demonstrate that *C. albicans* is the most prevalent candida species associated mainly with urogenital and respiratory candidiasis. *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* were the most common NAC.**

APPENDIX X: PUBLICATION-II

East African Medical Journal Vol. 101 No. 4 April 2024
ITRACONAZOLE AND VORICONAZOLE RESISTANCE PROFILES IN CANDIDA ISOLATES FROM NAIROBI METROPOLITAN CITY PUBLIC REFERRAL HOSPITALS
Erolls Cheruiyot Sigei, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Kenya Medical Training College, Ministry of Health, P. O. Box 30195-00100, Nairobi, Kenya, Department of Medical Laboratory Sciences, Faculty of Diagnostic Sciences, Kenya Medical Training College, Ministry of Health, P. O. Box 30195-00100, Nairobi, Kenya, Lily Alice Murugi Njue, Department of Medical Laboratory Sciences, Faculty of Diagnostic Sciences, Kenya Medical Training College, Ministry of Health, P. O. Box 30195-00100, Nairobi, Kenya, Sharon Mwikali Kitolo, Department of Medical Laboratory Sciences, Faculty of Diagnostic Sciences, Kenya Medical Training College, Ministry of Health, P. O. Box 30195-00100, Nairobi, Kenya, Vivian Patricia Okinyi, Department of Health Systems Management and Development, African Medical Research Foundation (AMREF) International University, P.O Box 27691 – 00506, Nairobi, Kenya, Erick Barasa, Department of Medical Microbiology and Parasitology, School of Medicine, Masinde Muliro University of Science and Technology, P. O. Box 190-50100, Kakamega, Kenya, Christine C. Bii, Department of Mycology, Centre for Microbiology Research, Kenya Medical Research Institute, P. O. Box 54840-00200, Nairobi, Kenya, and Tom Were, Department of Medical Microbiology and Parasitology, School of Medicine, Masinde Muliro University of Science and Technology, P. O. Box 190-50100, Kakamega, Kenya

Corresponding author: Erolls Cheruiyot Sigei, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Kenya Medical Training Institute, Nairobi, P. O. Box 30195-00100, Nairobi, Kenya. Email : esigei@kmtc.ac.ke

ITRACONAZOLE AND VORICONAZOLE RESISTANCE PROFILES IN CANDIDA ISOLATES FROM NAIROBI METROPOLITAN CITY PUBLIC REFERRAL HOSPITALS

E. C. Sigei, L. A. M. Njue, S. M. Kitolo, V. P. Okinyi, E. Barasa, C. C. Bii and T Were

ABSTRACT

***Background*: The emergence and spread of azole antifungal drug resistance including cross-resistance hinders their effective utilization in the therapy and prophylaxis of candidiasis.**

***Methods*: This study analysed the itraconazole and voriconazole anti-fungal sensitivity profiles of candida isolates from clinical specimens from candidiasis patients in Nairobi County. Antifungal susceptibility and MIC were performed using broth microdilution tests, on isolates of *C. albicans* (n=94) and non-*albicans* candida (n=46).**

***Results*: Phenotypic characterisation of antifungal resistance showed low sensitivity rates to itraconazole (21.3%) and voriconazole (24.5%) with high cross-resistance (74.5%) in the *C. candida* isolates. Among the non-*albicans* candida sensitivity for itraconazole, voriconazole, and cross-resistance were: *C. glabrata* (8.7%, 13.0% and 87.0%); *C. parapsilosis* (36.4%, 45.5% and 54.5%); *C. tropicalis* (40.0%, 60.0% and 40.0%); *C. krusei* (0.0%, 33.3% and 66.7%); *C. famata* (50.0%, 50.0% and 50.0%); and *C. guilliemondii* (0.0%, 50.0% and 50.0%), respectively. MIC**