PREVALENCE, SPECIES DISTRIBUTION, ANTIFUNGAL SUSCEPTIBILITY PROFILES OF *CANDIDA* AND RISK FACTORS ASSOCIATED WITH ISOLATION OF *CANDIDA* IN PATIENT SAMPLES SUBMITTED FOR ANALYSIS AT THE MOMBASA HOSPITAL

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Prevalence, species distribution, antifungal susceptibility profiles of *Candida* and risk factors associated with isolation of *Candida* in patient samples submitted for analysis at the Mombasa Hospital

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DECLARATION:

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

I dedicate this research work to my daughter Rahma Abdulrahman.

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I am indebted to the almighty God for the good health and positive spirit that I highly required to accomplish this research work. Secondly, my acknowledgement goes to the administrative director of The Mombasa Hospital, Mr. Abbas Nasser, for allowing me to conduct this study at the hospital and providing a conducive environment. I do appreciate Dr. Mandaliya, for his guidance and my colleagues who willingly contributed in one way or another. Special acknowledgment goes to my supervisors, Prof. Simon Karanja and Dr. Rahma Udu for their invaluable input in this task. Lastly, I sincerely appreciate my family for their endurance, encouragement and support throughout my studies.

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ABBREVIATIONS AND ACRONYMS

AST	Antibiotic sensitivity testing
AST-YS07	Antifungal susceptibility cards
ATCC	American type culture collection
BSI	Blood stream infection
CBP	Clinical break points
CLSI	Clinical and laboratory standards institute
ECV	Epidemiological cut-off values
ELISA	Enzyme linked immunosorbent assay
EUCAST	European committee on antimicrobial susceptibility testing
FDA	Food and drug administration
HIV	Human immunodeficiency virus
KOH	Potassium hydroxide
MIC	Minimum inhibitory concentration
PCR	Polymerase chain reaction
SDA	Sabouraud dextrose agar
SOP	Standard operating procedures
SPSS	Statistical package for social sciences software
TCBS	Thiosulphate citrate bile salts sucrose
XLD	Xylose lysine deoxycholate
YST	Yeasts identification cards

DEFINITION OF TERMS

Clinically Susceptible (S): A level of antimicrobial activity associated with a high likelihood of therapeutic success by applying the appropriate breakpoint in a defined phenotypic test (*European Committee on Antimicrobial Susceptibility Testing (EUCAST)(2012).*– even when a standard dosing regimen of the agent is used.

Clinically Intermediate (I): A level of antimicrobial agent activity associated with uncertain therapeutic effect. An infection due to the isolate may be appropriately treated in body sites where the drugs are physically concentrated or when a high dosage of drug can be used. It also indicates a buffer zone that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations (EUCAST, 2012).

Clinically Resistance (R): As applied to the use of antifungals, is the failure to eradicate a fungal infection despite the administration of an antifungal agent with in vitro activity against the organism attributable to the host, antifungal agent or pathogen related factors (Kanafani & Perfect, 2008). Pathogen related resistance mechanisms include; reducing the accumulation of the drug within the fungal cells, decreasing the affinity of the drug for its target and modifications of metabolism to counterbalance the drug effect (Vandeputte, Ferrari, & Coste, 2012).

Microbiological resistance (R): As applied to the use of antifungals, refers to non-susceptibility of a fungus to an antifungal agent by in vitro susceptibility testing in which the MIC of the drug exceeds the susceptibility breakpoint for that organism. It can be intrinsic (found naturally among certain fungi without prior exposure to the drug) or acquired (develops among previously susceptible strains after exposure to the antifungal agent) (Fothergill, 2012).

ABSTRACT

Candida causes mucocutaneous, potentially invasive infections and outbreaks consistent with high mortality rates. Some *Candida* exhibit resistance to certain antifungals impeding the efficacy of empirical and de-escalation treatment strategies yet most laboratories in Mombasa do not undertake *Candida* species and antifungal susceptibility testing routinely. This study sought to determine the prevalence, species distribution, antifungal susceptibility profiles of *Candida* and risk factors associated with isolation of *Candida* in patient samples at the Mombasa Hospital. In a cross-sectional study and by systematic random sampling, 384 patient samples (Urine, catheter tips, high vaginal swabs (HVS), sputum, tracheal aspirate, pus and wound swabs) were selected and cultured. Identification and antifungal susceptibilities for yeast isolates was done on Vitek 2 compact (BioMérieux). Data was analyzed by Chi-square and logistic regression analysis using SPSS version 20. Differences in parameter estimates were deemed statistically significant at $P < 0.05$. The prevalence of *Candida* isolation was 8.6%. *Candida albicans* (7.8%) was the most predominant species followed by *Candida tropicalis* (0.8%). All *Candida* isolates were susceptible to Flucytosine (100%), 96.97% were sensitive to Micafungin and Caspofungin, 93.94% were sensitive to Voriconazole and 81.82% were sensitive to Fluconazole. However, while 9.09% showed resistance and intermediate responses to Fluconazole, 15.15% were resistant to Amphotericin B hence resistance to azoles and Amphotericin B should be envisaged when starting empirical treatment.. Pregnant women ($P = 0.000$), diabetics ($P = 0.008$), chronically ill (HIV, renal disease and cancer) patients ($P = 0.026$) and catheterized patients ($P = 0.023$) should be proactively investigated for possible colonization or infections with *Candida* including their antifungal susceptibilities since they are at higher risk.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Culture reports from the Microbiology laboratory at Mombasa Hospital indicate that various *Candida* isolates are obtained from clinical specimens submitted for routine microbiological analysis and culture. Majority are isolated from patients presenting with cases of mucocutaneous forms of *Candida* infections but also isolated incidents of systemic *Candida* infections are encountered (Mombasa hospital health management information system).

Improved management of high-risk patients with novel medical and surgical interventions has partly attributed to the increase in cases of *Candida* infections in the past few decades (Diaz & Fell, 2004), (Zaragoza *et al*., 2008) and (Pappas *et al*., 2016). Prolonged hospitalization, surgery, diabetes, mechanical ventilation, central venous and peripheral catheter use, previous use of broad spectrum antibiotics, treatment with immunosuppressive medication, fungal colonization among others are risk factors implicated for predisposing patients to *Candida* infections (Muskett *et al*., 2011).

Systemic mycosis correlates with high morbidity and mortality, especially in ICU (Delaloye & Calandra, 2014) and can be diagnosed by examining human body fluids like cerebrospinal fluid, pleural effusions, peritoneal fluid, broncho alveolar lavage fluid and synovial fluid (Jones, 1990) according to the presenting signs and symptoms. *Candida* infections can be detected routinely in the laboratory by urine microscopy and culture and blood culture.

Determining the antifungal susceptibility of fungi assists in improving the clinical care of systemic mycosis necessary to save patients' lives (Parisa Badiee, 2013).

Advances in technology and commercialization of testing systems like the Vitek 2 compact (BioMérieux) has enabled clinical laboratories to perform routine identification of *Candida* and their susceptibility patterns easier and more accurately (Melhem *et al*., 2013). The Vitek 2 MIC results are available after an average of 15.5 hours of incubation for all *Candida.* It is a reliable option for testing susceptibility of yeasts to antifungal agents in addition the CLSI or EUCAST procedures (Cuenca-Estrella *et al*., 2010).

This study sought to determine the prevalence of *Candida* isolates on pathological specimens submitted at the Mombasa Hospital Laboratory for microbiological investigations. The susceptibilities of the *Candida* isolates to some commercially available antifungal agents were also determined. Risk factors associated with *Candida* infections amongst patients attending Mombasa hospital was analyzed.

In a study to establish the prevalence of vaginal *Candida* infections and to determine the occurrence of *Candida* in pregnant women in Thika District Hospital, Kenya, it was ascertained that the there was a prevalence of 42.7% of *Candida* infections with *Candida albicans* being the most commonly isolated species followed by *Candida glabrata*. Other non-*Candida albicans Candida* isolated included *Candida krusei*, *Candida tropicalis*, and *Candida parapsilosis* (Menza Nelson, Wanyoike Wanjiru2, 2013). In another study to identify the susceptibility profile of vaginal *Candida* to antifungal agents among pregnant women in the same region, it was established that *Candida albicans* was susceptible to most of the azole drugs while the other species isolated had varying responses. There is also an emerging resistance of *Candida krusei* to most of the drugs used except itraconazole (Hospital & Al, 2013).

1.2 Statement of the problem

Globally, fungal diseases kill over 1.5 million and affect over 1 billion people annually (Bongomin, Gago, Oladele, & Denning, 2017) and approximately 594,660 Kenyan women get more than 4 episodes of *Candida* vulvovaginitis annually (Guto, Bii, & Denning, 2016).

Compromised immunity in chronically ill (HIV, renal diseases and cancer) individuals predisposes them to *Candida* infections which may lead to invasive Candidiasis that is consistent with increased morbidity, mortality rates, length of hospital stay and cost of healthcare. This is occasioned by the fact that the HIV pandemic and invasive procedures for patients in critical conditions require prolonged prophylactic antifungal treatments owing to the increased risk of fungal infections in such patients including candidiasis (Loeffler J. and Stevens D., 2003).

There is an increase in the number of non-*albicans Candida* species and recently *C. auris* which has the potential to cause invasive infections and outbreaks that are consistent with high (36% – 63%) mortality rates and requires specialized laboratory methods of identification (Schelenz *et al., 2016*) and (CDC, 2018).

Whereas fungal diseases costed more than $7.2 billion in 2017 in the United States (Benedict, Jackson, Chiller, & Beer, 2019), it is estimated that in high-income countries, lost productivity due to recurrent vulvovaginal candidiasis will reach US$14·39 billion annually by 2030 (Denning, Kneale, Sobel, & Rautemaa-Richardson, 2018).

Most practitioners in Mombasa and its environs do not have capacity to undertake *Candida* identification and antifungal susceptibility tests. Antifungal agents are thus prescribed empirically, yet this may not be efficient when treating high risk and systemic *candidiasis* patients (Aguilar *et al.,* 2015); Falagas, Roussos, & Vardakas, 2010 (Pfaller

et al., 2012) Consequently, trends of increased cases of resistance of *Candida* to some antifungal agents, morbidity, mortality rates, prolonged hospital stay and cost of healthcare are observed (Bongomin *et al*., 2017) and (Benedict *et al.*, 2019).

1.3 Justification

Determination of local epidemiological patterns and *Candida* species distribution shall inform antifungal stewardship initiatives aimed at reversing the growing resistance trends to antifungal agents especially as a result of secondary resistance. This being an adjunct to the administration of appropriate and targeted treatment shall aid in reducing morbidities, mortality rates, length of hospital stay and healthcare costs as a result of *Candida* infections. Cost of treatment and hospitalization is escalating and the demands for efficiency and accountability by relevant authorities and health stakeholders is ever increasing.

As such, information on epidemiology of *Candida* infections, distribution and susceptibility to commonly prescribed antifungal agents may have possible profound implications in the management of patients reckoned to be infected with *Candida* right from the time of patient's presentation (Haddadi *et al.*, 2014).

Studies have established the importance of local epidemiological information on fungal infections and particularity the distribution of *Candida* species as a key factor in managing fungal infection efficiently. It was established that "in regions with a high incidence of *Candida* **glabrata** ***infections***, fluconazole should probably not be considered as a first-line treatment whereas in neonatal ICUs, where a significant proportion of *Candida*emia is due to *Candida* **parapsilosis**, empirical treatment with echinocandins may be withheld following data showing decreased susceptibility of this pathogen to echinocandins, especially Caspofungin" (Falagas, Roussos, & Vardakas, 2010).

The accuracy in identification of fungal infections and the provision of "real-time" antifungal susceptibility testing results aid clinicians to accurately and promptly diagnose *Candida* infections and sometimes alter treatment regimens based on the results respectively. Like in antibiotic susceptibility tests, testing for antifungal susceptibility routinely complements treatment of *Candidaemia* (Pfaller and Diekema, 2012). This has been demonstrated in a study where the susceptibility testing of *Candida glabrata* decreased cost of treatment, as a result of use of Fluconazole instead of echinocandins on *Candida glabrata* fungemia patients (Collins, Eschenauer, Salo, & Newton, 2007).

Undoubtedly, the need for monitoring the distribution of species of fungi and their susceptibility to antifungal agents is immense since an increased resistance to antifungals has been reported amongst invasive *Candida* infections (Cisterna, Ezpeleta, & Tellería, 2005).

The growing trends of isolation of non *C. albicans Candida* and resistance (intrinsic or acquired) to some common antifungal agents demonstrates the need to provide clinicians with information regarding the local epidemiological patterns so as to facilitate a more informed and evidence based treatment decision.

1.4 Research questions

1. What is the prevalence and distribution of *Candida* species in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital?
2. What are the antifungal susceptibility profiles of the *Candida* isolated from specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital?

3. What are the risk factors associated with isolation of *Candida* in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital?

1.5 Objectives

1.5.1 Broad objective

To determine the prevalence, species distribution, antifungal susceptibility profiles of *Candida* and risk factors associated with isolation of *Candida* in patient samples submitted for analysis at the Mombasa Hospital.

1.5.2 Specific objectives

1. To determine the prevalence and distribution of *Candida* species in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.
2. To determine the antifungal susceptibility profiles of the *Candida* isolated from specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.
3. To determine the risk factors associated with isolation of *Candida* in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.

1.6 Conceptual framework

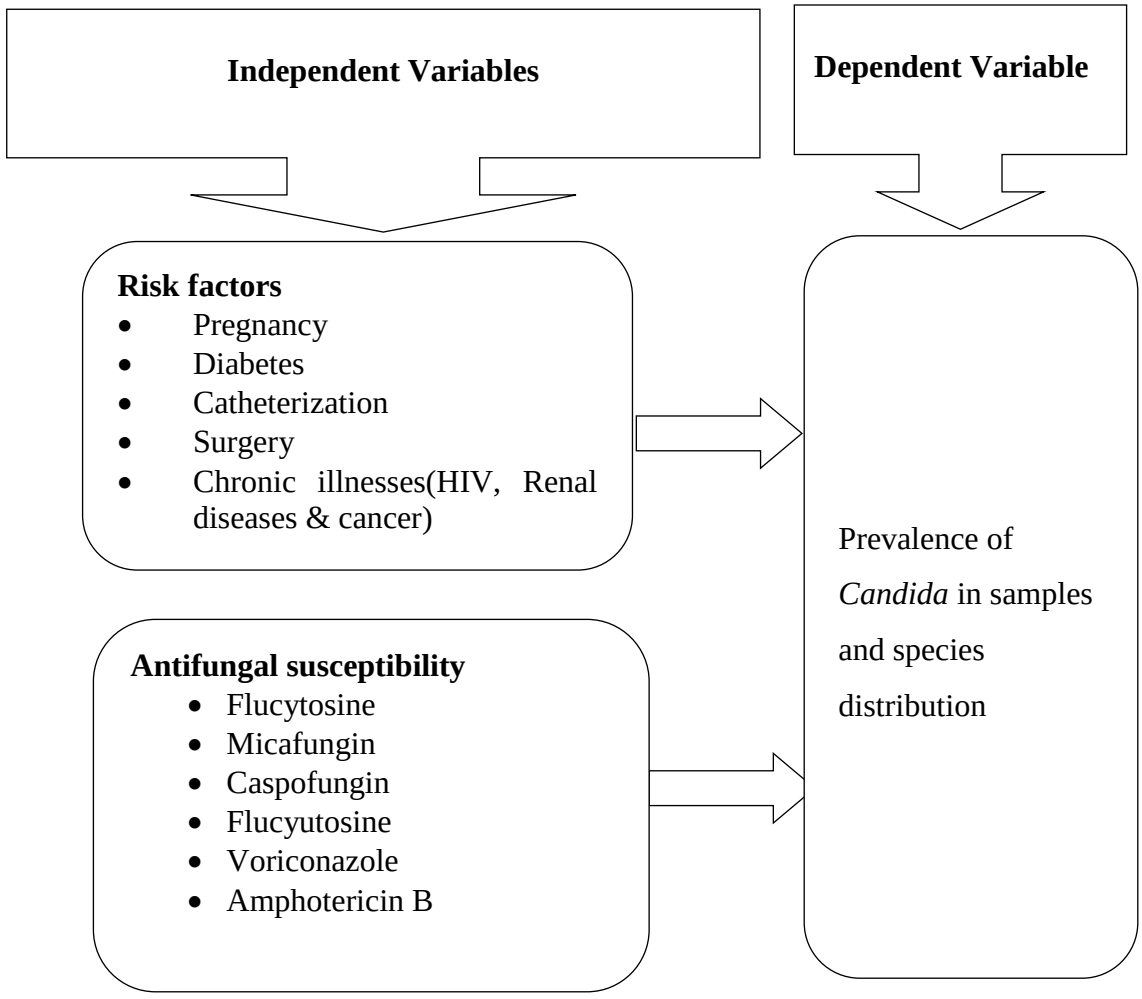

Figure 1.1 Conceptual framework

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Candida exists as a normal flora in humans. Under certain compromised circumstances, they can results into superficial infections but also present as invasive illness with multi organ involvement (Pappas *et al.,* 2004) if not appropriately well managed.

Candida albicans causes opportunistic infections like *candidiasis*, thrush and vulvo vaginitis mostly in the immunocompromised patients (Myers, 2006). These infections can occur in otherwise healthy individuals. Invasive candidiasis is associated with high mortality (Gudlaugsson *et al.*, 2003).

Candida are becoming more and more important potential aetiological agents and hence isolated in broncho-pulmonary illnesses (Kali *et al.,* 2013). The determination and monitoring of localized epidemiology is important since it has been reported elsewhere that the prevalence of *Candida*emia varies in different countries as well as responses to antifungal therapy (Colombo *et al.,* 2006).

Antifungal susceptibility testing is performed to give a scientific guide on the potential effects of the tested antifungals upon the target pathogenic fungi, to give an insight into the *in vivo* action of the antifungal and hence predict the treatment outcome, to provide a measure with which to monitor changes in susceptibilities in otherwise susceptible fungi and to assesses the efficacy of new antifungals in clinical trials (M. a. Pfaller & Diekema, 2012).

2.2 Theoretical review

2.2.1 Epidemiology of *Candida* infections

Although *Candida albicans* continues to be the most predominant in pulmonary candidiasis, several non *albicans Candida* are also reported in increasing frequency (Kali *et al*., 2013). Studies have reported an increasing prevalence of vulvo vaginitis due to non-*albicans Candida* species (V. Kumari, Banerjee, Kumar, Pandey, & Tilak, 2013). *Candida albicans* (39.2%) was the most frequent cause of *Candida*emia followed by *Candida parapsilosis* (21.6%) and *Candida tropicalis* (15.7%) and the mortality due to *Candida albicans* (37.5%) was significantly higher than the mortality due to non-*albicans* (17.7%) (Celebi, Hacimustafaoglu, Ozdemir, & Ozkaya, 2008).

In a surveillance study in Spain, it was demonstrated that 49.08% of *Candida*emia cases were attributable to *Candida albicans*, 20.73% to *Candida parapsilosis*, 13.61% to *Candida glabrata*, 10.77% to *Candida tropicalis*, 13% to *Candida krusei* and the rest (3.65%) were attributable to other species (Cisterna *et al*., 2005). Other studies reported a relative shift towards non-albicans *Candida* species and a reduction in *Candida albicans* infections. For instance *Candida parapsilosis* was reported to be the predominant species causing infections in pediatric ICU, suggesting nosocomial transmission (San Miguel *et al*., 2005). Understanding local epidemiological pattern of *Candida*emia is important since geographic variation in prevalence of *Candida*emia has been reported (Falagas *et al*., 2010).

The prevalence of superficial and invasive *Candida* infections is relatively high in Africa and Middle East region with decreasing proportion of *Candida albicans* in more recent years. The prevalence of superficial *Candida* infections (19.68%) was higher than invasive infections (12.42%) (Omrani *et al*, 2014). Diabetics are more susceptible to oral candidiasis than non-diabetics and smokers and denture wearers are at high risk of being

infected. *C. albicans* is the most prevalent species among all *Candida* species as the cause of oral candidiasis in Jordan (Abu-Elteen, Hamad, & Salah, 2006).

2.2.2 Antifungal agents

Commonly used antifungal agents are grouped into three broad categories based on their modes of actions namely those that inhibit cell wall formation, those that disrupt the fungal cell membrane and those that inhibit fungal cell division (Lewis, 2011). Although a number of formulations have been discovered that interfere with various steps in fungal cell wall synthesis with excellent antifungal activity in vitro many of which target β-glucan synthesis, the availability of such agents for commercial and clinical use remains far from realistic (Myers, 2006).

Antifungal agents that work by disrupting the fungal cell membrane target ergosterol, either by binding to the sterol, forming pores and causing the membrane to become leaky (polyene antifungals), or inhibiting ergosterol biosynthesis (azole antifungals). Amphotericin B is a polyene antifungal which binds to ergosterol in fungal membrane causing membrane to become leaky. It has been a mainstay of antifungal therapy for treating disseminated, life-threatening fungal infections. It is cytotoxic hence must be given intravenously (Laniado-Laborín & Cabrales-Vargas, 2009).

Azoles (Imidazoles and triazoles) inhibit CYP P450 14α- demethylase in fungi which is involved in the conversion of lanosterol to ergosterol. They have a lower affinity for mammalian P450's. *Fluconazole* (triazoles) is an azole, which is more potent, less toxic and provides effective oral therapy for many systemic fungal infections (Myers, 2006). It is a one-a-day tablet or suspension used to treat yeast infections of the vagina, mouth, throat, esophagus, abdomen, lungs, blood and other organs but also used to treat meningitis and for prophylaxis in high risk patients. Voriconazole is a new triazole

antifungal agent used to treat different kinds of serious fungal infections and may be used in patients who have not responded to other antifungal agents (Greer, 2003).

Nucleoside antifungal agents affect cell division by targeting the microtubule effects in forming the mitotic spindle or by inhibiting DNA transcription. Flucytosine is a pyrimidine analogue. Inside the cells, it is deaminated by fungal cytosine deaminase in the cells and incorporated into RNA hence interfering with DNA synthesis (Vermes, Guchelaar, & Dankert, 2000).

However, yeasts develop resistance to Flucytosine very quickly; hence clinicians use it only in combination with other antifungal agents, mainly amphotericin B but also itraconazole or fluconazole (Z. A. Kanafani & Perfect, 2008). Flucytosine administered in combination with amphotericin B is the standard of care for cryptococcal meningitis, and it continues to have a role in the treatment of *Candida* infections which are life threatening or in circumstances where drug penetration may be problematic, such as infections of urine, eyes, and heart valves (Hope, Tabernero, Denning, & Anderson, 2004).

Caspofungin acetate is an example of a fungal cell wall inhibitor. It is a parenteral injection effective against invasive *Candida* infections. It is a semisynthetic lipopeptide (echinocandin) derived from a fermentation product of *Glarea lozoyensis*. It inhibits (1, 3 D glucan synthesis thus disrupting the formation of β-glucan in the cell walls. Echinocandin like Caspofungin are the most effective agents against all the *Candida* species studied. They are the best antifungal agents against *Candida* colonization for pediatric patients with hematological disorders and neutropenia, followed by conventional Amphotericin B (Haddadi *et al.*, 2014).

Allylamines e.g. terbinafine and thiocarbamates e.g. tolnaftate antifungals act by inhibiting the squalene epoxidase (ERG 1) gene of ergosterol biosynthesis. Morpholines e.g. fenpropimorph and amorolfine inhibit the *ERG24* and *ERG2* genes (Prasad, Shah, & Rawal, 2016). These are however only used topically to treat dermatophytes infections such as *Tinea capitis*, *Tinea pedis* and onchomycosis owing to either their numerous side effects when administered systemically or their poor efficacy (Vandeputte *et al*., 2012).

2.3 Antifungal susceptibility testing

Antifungal susceptibility testing of *Candida* by broth micro dilution methods has been standardized and refined by both the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) organizations. It is now more valuable in managing *Candida* infections (Arendrup *et al*., 2008). 24 hour reading times for all antifungal agents has been validated. Specific epidemiological cutoff values (ECVs) for the systemically active antifungal agents for *Candida albicans* and non *Candida albicans* are now available to adequately address the previous challenge associated with the interpretation of the results of in vitro antifungal susceptibility testing.

It is now clear that antifungal susceptibility testing serves as a valuable aid in the choice of primary antifungal agents and in a de-escalation strategy for treating patients with candidiasis(M. a. Pfaller *et al.*, 2012). In a study to evaluate the use of Vitek 2 system for fungal identification and antifungal susceptibility testing, it was established that the system can reliably determine the MICs of Amphotericin B, Flucytosine, Voriconazole, and Fluconazole in 15 hours or less for the most common clinically relevant *Candida* and resistance to Flucytosine, Voriconazole, and fluconazole in quantitative and qualitative agreement with the CLSI or EUCAST broth micro dilution reference methods (Melhem *et al*., 2013).The Vitek 2 yeast susceptibility test (bioMérieux, Inc.) provides a completely automated means of performing antifungal susceptibility testing

of yeasts that has been shown to produce reproducible, rapid, and accurate results consistent with those obtained with the CLSI reference broth micro dilution (BMD) method for Amphotericin B, Flucytosine, Fluconazole, and Voriconazole (Borghi et al., 2010; M. a. Pfaller, Diekema, Procop, & Rinaldi, 2007b)a(M. a. Pfaller, Diekema, Procop, & Rinaldi, 2007a).

2.4 Resistance

Various emerging mechanisms of resistance to antifungal agents are being discovered (Spampinato & Leonardi, 2013). Primary (intrinsic) resistance is exhibited by some fungal species and does not necessarily depend on prior exposure to the antifungal hence the accurate detection of speciation of fungal isolates cannot be over emphasized. *Cryptococcus neoformans has* primary resistances to echinocandins Caspofungin and *Candida krusei* to fluconazole. Secondary (acquired) resistance is characterized by emergence of resistance to antifungals in otherwise previously susceptible fungal strains when they are exposed to the antifungal agent and is mainly as a result of distorted gene expression (Fothergill, 2012).

Individualized treatment is of fundamental importance in certain clinical presentations since clinical resistance may occur due to the use of empirical treatment strategies where there exists multiple pathogens or another diagnosis, the immune reconstitution inflammatory syndrome, infection with some fungal strains that possess more virulent characteristics, toxicities from polyenes (nephrotoxicity) and azoles (hepatitis), drug-drug interactions, the formation of biofilms on foreign bodies and suboptimal length of treatment (Z. A. Kanafani & Perfect, 2008).

2.4.1 Amphotericin B resistance.

Limited resistance to amphotericin B has been reported but severely immunocompromised patients exhibit the highest risk of resistance in *Candida* species. Development of resistance in patients previously exposed to azole antifungals probably is due to an alteration of cellular membrane components. *Candida lusitaniae* and *Candida guilliermondii* exhibit primary resistance to amphotericin B. Secondary resistance is infrequent although it has been seen in yeasts causing infections in patients with cancer. Several strains of fluconazole and amphotericin B-resistant *Candida albicans* have been found in HIV infected patients who have received prolonged courses of antifungal prophylaxis with azoles (White, Marr, & Bowden, 1998).

Earlier studies reported trends of decreased susceptibility of *Candida krusei* and *Candida glabrata* to amphotericin B (Z. A. Kanafani & Perfect, 2008).

2.4.2 Resistance to azole compounds

Azole resistance has been reported not only in mucosal candidiasis in AIDS patients usually associated with a long duration of therapy, high total cumulative doses of fluconazole and recent treatment with the antifungal, but also in non HIV, in some patients not previously exposed to antifungals and in surgical critically ill patients on Fluconazole therapy. Moreover, Infections with non-*albicans* species exhibiting primary resistant to azoles is on the rise. Reports of antifungal resistance detected in colonizing strains points a finger at mutation as a cause of resistance (White *et al*., 1998).

Main mechanisms of azoles resistance in *Candida* include decreased drug concentration at the site of action, target site alteration, increased response of target enzymes and development of by-pass pathways (Z. A. Kanafani & Perfect, 2008).

2.4.3 Flucytosine resistance.

Whereas certain strains of yeasts and molds exhibit primary resistance to Flucytosine (5-FC), non - *albicans Candida* species also register high rates of 5-FC resistance. Acquired resistance to 5-FC is mainly encountered in patients administered 5-FC monotherapy (Theodore, 1997). The prevalence of intrinsic resistance to Flucytosine by *Candida* isolates is however still minimal (Z. A. Kanafani & Perfect, 2008).

2.4.4 Resistance to echinocandins

Echinocandin are antifungals of choice in managing invasive *Candida* infections. However, increasing trends of diminishing susceptibilities of *Candida glabrata* infections to Echinocandin antifungals cannot be wished away (Alexander *et al*., 2013). Moreover, *Candida parapsilosis* infections have thrived in patients receiving echinocandins due to other indications (Z. A. Kanafani & Perfect, 2008).

2.5 Antifungal pharmacology

Amphotericin B and the echinocandins must be administered intravenously because of their relatively lower absorption from the gastro intestinal tract. Co administration of any triazole or Caspofungin with rifampin or phenytoin can lead to treatment failure due to lowered blood levels of the antifungal (Brüggemann et al., 2009). 5-FC, Fluconazole, and Voriconazole are recommended for treatment of Candidal infections of the central nervous system and optical infections due to their excellent penetration in those areas (Schwartz *et al*., 2013).

The azole antifungal agents have been a recommended treatment option for patients with invasive *Candida* infections such as mucosal infections and *Candida*emia. These drugs may be used topically or systemically because of their proven safety and efficacy

(Pappas *et al*., 2004). Triazole antifungal agents are associated with hepatotoxicity. Treatment with Voriconazole poses a risk of retinoid-like photo toxicity that is however reversible (Patel *et al*., 2010).

2.6 Prevention and control of antifungal resistance

Like many infectious diseases, the emergence of antifungal resistance poses a serious threat to the prevention and control efforts of fungal infections especially to venerable individuals and in patients who use antifungals for long period. Appropriate measures ought to be employed to reverse such trends. Prudent use of antifungals, appropriate dosing, treatment with combined antifungal agents, judicious and appropriate use of antifungal susceptibility study findings in decision making can be employed to minimize resistance (Ghannoum & Rice, 1999).

Antifungal drug therapy can be enhanced by improving the immune status of the host e.g. by use of Cytokines such as granulocyte colony-stimulating factor. Immunocompromised

patients may be administered cytokines as an adjunct to azoles in managing fungal infections. Moreover, surgical intervention minimizes the burden of fungal infections and enhances host clearance as well as improving the action of antifungal drugs (Pappas *et al*., 2009). The prolonged prophylactic use of antifungals has been associated with trends of reduced susceptibility of *Candida* to antifungals (Lortholary, Desnos-ollivier, Sitbon, & Fontanet, 2011).

2.7 Risk factors associated with isolation of *Candida* from patients

Several risk factors have been implicated for predisposing patients to invasive candidiasis. They include surgery, long-term hospitalization, mechanical ventilation,

diabetes, central venous catheter use, diarrhea, peripheral catheter use among others (Muskett *et al.*, 2011). Some of the risk factors for invasive candidiasis include the use of broad spectrum antibiotics and immunosuppressant agents, higher survival of premature infants, prolonged hospital and intensive care unit (ICU) stay, diabetes, nosocomial bacterial infection, recent surgery, mechanical ventilation, total parenteral nutrition, use of central venous catheter and shunts, transplantation and immuno compromising diseases such as cancers (Celebi *et al.*, 2008) and (Avila-Aguero *et al.*, 2005). A study in Nigeria indicated that young adults of between 21 and 30 years, pregnant mothers, immunosuppressed patients, contraceptive and broad spectrum antibiotic users were more at risk of vaginal candidiasis. In another study among pregnant women in Thika- Kenya it was concluded that those at great risk for vaginal candidiasis were aged between 26 and 35 years and in their 3rd trimester. (Menza Nelson, Wanyoike Wanjiru, 2013). Presence of central venous catheter and use of broad spectrum antibiotics were determined as risk factors for *C. auris* fungemia at a hospital in Nairobi –Kenya *(Adam et al.,* 2019)

2.8 Critique of the existing literature relevant to the study

Many of the studies on distribution of *Candida* species from clinical specimens have been done mostly in the European and Asian countries. There is scanty information on the distribution of *Candida* species and antifungal susceptibility profiles of *Candida* in developing countries especially African and in the region of this current study. Moreover, the few studies done like that of Menza *et al* (2013) in Thika district have focused on vaginal *Candida* infections in pregnant women and in *Candida*emia. The prevalence of *Candida* infections of blood and other body sites like the CSF and broncho alveolar lavage fluids ought to be investigated and understood especially in local settings. This study focuses on a wider scope and classes of the antifungal agents including some of the newer antifungal agents in the market that can be prescribed for effective treatment of systemic *Candida* infections for instance the echinocandins,

Micafungin and Caspofungin while many of the locally done antifungal profiles studies have focused on the susceptibility profiles of the traditional antifungal agents only.

2.9 Summary

Candida albicans is one of the frequently isolated fungi from various pathological samples including urine, high vaginal swabs and sputum. It is also one of the common isolates from blood cultures and cerebrospinal fluids specimens leading to systemic *Candida* infections which can be fatal especially when it is not diagnosed early in persons with compromised immunity. Several non albicans *Candida* are also reported in increasing frequency including *Candida parapsilosis, Candida tropicalis*, *Candida glabrata, Candida famata* and many others. There exists different geographic prevalence and distributions of *Candida* infections in different parts of the world hence the need for accurate identification of the *Candida* in a timely manner to allow for effective interventions especially when managing life threatening *Candida* infections. Contemporary techniques for fungal identification and antifungal susceptibility testing allow for an early, accurate and standardized diagnosis of *Candida* infections and predictability of treatment successes that permits efficiency in managing and preventing complications and mortality associated with systemic fungal infections in patients at risk.

2.10 Research gaps

Research on distribution of *Candida* species is not extensively done in Mombasa County hence the geographic distribution is not well understood.

This study focuses on both traditional and newer antifungal agent's susceptibility profiles that many of the locally done studies have not focused on and it also includes various kinds of pathological specimens other that urine, blood and vaginal specimens.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was carried out in the microbiology laboratory at Mombasa Hospital in Mombasa County in Kenya. The hospital is a level 5 private referral hospital that serves patients from Mombasa County with a catchment population of about 1 million people and also those referred from bordering counties. The Mombasa hospital cares for critically ill patients who are at risk of developing *Candida* infections hence the need to establish the local epidemiology and antifungal susceptibility profiles to aid in managing the commonly isolated *Candida b*etter. This study was piloted at Muslim Education and Welfare Association (MEWA) Hospital in Mombasa.

3.2 Study design

This study employed a cross-sectional design with a laboratory component. Through systematic random sampling, patient samples collected and submitted in the microbiology laboratory between January 2016 and June 2016 were selected and analyzed for their microbiological contents following the standard operating procedures (SOP) for acceptance, inoculation and identification of microorganisms. All isolates were identified and their antibiotic susceptibilities determined. Socio demographic information, diagnosis and treatment/intervention was sought and collected using the data collection tool (appendix 3) from consenting patients either directly or through their doctors. Further information on antifungal susceptibility results was collected from cultures that yielded *Candida*.

3.3 Study population

The study population included 384 pathological samples for microbiological culture and sensitivity testing submitted and selected at the microbiology laboratory from patients attended at the Mombasa Hospital's OPD, inpatient wing, antenatal clinic, special clinics (diabetes, HIV, oncology, urology), intensive care and renal units. These were submitted by patients with symptoms and signs of infections of the urinary, vulvo vaginal, pulmonary tracts or surgical wounds.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion criteria

Patients' specimens that were accepted and included in the study include;

1. Specimens collected from patients with clinical symptoms and signs of infections of the urinary, vulvovaginal, pulmonary tracts or those with signs of infected surgical wounds or on catheters (central venous catheter or urinary catheter).

2. Those specimens that met the samples acceptance criteria for culture and sensitivity testing as per the department's SOP between January 2016 and June 2016.
3. Those specimens obtained from patients who consented to participate in the study.

3.4.2 Exclusion criteria

1. Non consenting patients samples were not included in the study
2. Specimens submitted by clients attended at the well woman clinic (WWC) and those undergoing pre-employment medical exam.

3.5 Sample size and selection of samples

The Cochran's formula was used to determine the sample size (Cochran, 1977) at a prevalence rate of 45.4% (Mukasa *et al.*, 2015).

$$\text{Cochran's formula:-} N = \frac{Z^2 \times P(1-P)}{E^2}$$

Where:

N = required sample size.

Z = confidence level at 95% (standard value or deviation of 1.96) from the tailed normal table.

P = prevalence rate 45.4% (0.454)

E = margin of error at 5% (standard value of 0.05)

$$N = \frac{1.96^2 \times 0.454 (1- 0.454)}{0.05^2}$$

$$N = \frac{3.8416 \times 0.247884}{0.0025}$$

$$= 380.91$$

$$= 381 \text{ samples}$$

Sample size obtained was 381 patients' specimens. However, an additional three samples were included in the study since three patient's submitted more than one sample for analysis as they had more than one symptom of infection. Hence 384 patients' specimens were eventually submitted and investigated in this study during the study period. All samples submitted had an equal and independent chance of being selected.

3.6 Data collection

A total of 384 Samples including catheter tips, urine, sputum, high vaginal swabs, pus swabs, wound swabs and tracheal aspirates obtained from out patients, inpatients and patients being treated in the specialist clinics of the Mombasa hospital submitted for microbiological analysis were selected for the study. They were logged in to the samples register and cultured onto the respective agar media, incubated and analyzed appropriately. Samples that did not meet the sample inclusion criteria were rejected and excluded in the study. In addition, biographic information, diagnosis, culture and sensitivity results were collected using an itemized data collection form (see appendix 3) from consenting patients' medical record files. Other details collected included the department where the patient was treated, treatment/intervention including surgical procedures and catheterization.

3.6.1 Samples inoculations and microorganisms isolation

Appropriate differential and enriched media were used for microorganism's isolation and identification. Urine, catheter tips samples were inoculated onto blood agar (BA), Cystine Lactose Electrolyte Deficiency (CLED) and Sabouraud Dextrose Agar (SDA) media all from Himedia Laboratories Pvt. Ltd, India. Vaginal swabs, High vaginal swabs and urethral swabs were collected aseptically by a doctor, nurse or laboratory technologist and submitted for investigation in amies transport media. They were cultured immediately onto BA, Chocolate agar (CA) and SDA media as they were received in the laboratory. Sputum and aspirates samples were inoculated onto BA, Mac Conkey (MCA), Chocolate and SDA media. The inoculated plates were incubated at $37^0c \pm 1^0c$ for 18 to 48 hours.

To obtain pure organisms from mixed cultures, the colonies were sub cultured onto respective media and re- incubated. Suggestive colonies were gram stained and

confirmed microscopically for *Candida*. McFarland adjusted standard (turbidity level of 1.8 – 2.20) suspensions in sterile saline (BioMérieux) solution (Sodium chloride 0.45%) were prepared for the identification and drug susceptibility testing using the densiCHECK plus (BioMérieux) meters. The densiCHECK plus standard kit containing a 0.0, 0.5, 2.0 and 3.0 absorbance solution were used to verify the densiCHECK plus meter measurement performance and to monitor the accuracy of organism's suspension monthly.

3.6.2 Gram staining, microscopy and identification

The culture plates were incubated aerobically for 18 to 48 hours to allow for colonies to form. Colonial morphologies observed macroscopically gave an indication of the type of organisms isolated based on the physical appearance in differential media and enzyme activities of the organisms. Thick smears of suspected *Candida* colonies and aspirates of blood culture samples obtained from positively flagged blood culture bottles were made onto clean grease free glass slide, air-dried and heat fixed. Gram staining was performed using the gram PVP kit (Quimica Clinica Aplicada S.A, Spain). The stained slides were examined microscopically under oil immersion objective to ascertain the morphological characteristics of *Candida* and other bacteria. *Candida* were seen and identified is some samples as large, oval, mostly budding gram positive organisms due to their ability to retain the crystal violet stain. Identification was done using the Vitek 2 yeasts and yeast-like identification (YST) cards that were read on Vitek 2 compact system (BioMérieux, France).

3.6.4 Yeast susceptibility testing

The identification of susceptibility patterns of isolated *Candida* to the various antifungal agents was carried out using the Vitek 2 susceptibility cards (AST-YS07) BioMérieux. The *Candida* were subjected to the recommended antifungals that are commonly used

for treatment and prophylaxis of fungal infections according to the Infectious Diseases Society of America (IDSA) Guidelines for Treatment of Candidiasis (Pappas *et al*., 2004) (Pappas *et al*., 2009) and (Pappas *et al*., 2016). In a study to determine the impact of timing of appropriate antifungal therapy in Illinois Chicago it was found out that the appropriate antifungal therapy consisted of fluconazole, Caspofungin or Micafungin, amphotericin B and Voriconazole (Grim *et al*., 2012). In the current study the antifungal drugs selected for study included; Amphotericin B, Caspofungin, Fluconazole, Flucytosine, Micafungin and Voriconazole. Isolates of *Candida* were determined as susceptible, intermediate or resistant according to the following resistance breakpoints for antifungal agents; Amphotericin B > 1.0, fluconazole ≥ 64(FDA), Flucytosine ≥ 32.0(FDA), Micafungin ≥ 2.0 (CLSI), Voriconazole ≥ 4.0 (FDA) and Caspofungin ≥ 2.0 micrograms per milliliter. *Candida parapsilosis* ATCC 22019 was used as Quality control *Candida* strains during the study period.

3.7 Data analysis and presentation

Organisms identification and drug susceptibility data obtained as Vitek 2 compact printouts and in electronic forms were entered into MS Excel spreadsheets and imported onto IBM SPSS data analysis and statistics software version 20. The prevalence of *Candida* isolates was analyzed by;

$$\frac{O}{P} \times 100\%$$

Where:

O is the number of samples with *Candida* isolates

P is the total number of samples submitted for analysis and included in the study

The data was subsequently analyzed using descriptive statistics and cross tabulations. The findings were presented in graphs and tables. Chi square test and multivariate logistic regressions statistics were done to investigate the correlations between the risk factors (independent variables) and the isolation of *Candida*.

3.8 Ethics and human subject issues

An approval to conduct this study was given by the Administrative Director of The Mombasa Hospital and Ethical approval committee at Pwani University. This study did not pose any significant health risks to the study subjects. However, strict confidentiality relating to participant's information was maintained by means of codes/laboratory accession numbers to protect the identities of the participants in all the study documentation instead of real names. Unauthorized personnel were not allowed access to the data. All regulatory requirements were observed. Strict confidentiality relating to participant's information was maintained. Participation in the study was on voluntary basis. Consent for participation was obtained from adult patients or from their doctors if they were incompetent to do so by themselves. Patients or guardians consented on behalf of minors who were provided with assent form.

CHAPTER FOUR

RESULTS

4.1 Prevalence and distribution of *Candida* species

4.1.1 Distribution of patient samples collected and investigated

A total of 384 samples met the inclusion criteria, were selected and investigated in this study between January and June 2016. Urine [257(66.9%)] samples were predominantly investigated among the seven types of samples collected. Other types of samples included pus swabs [45(11.7%)], catheter tips [25(6.5%)], sputum [22(5.7%)], high vaginal swabs (HVS)[20(5.2%)], wound swabs [8(2.1%)] and tracheal aspirates [7(1.8%)](Figure 4.1).

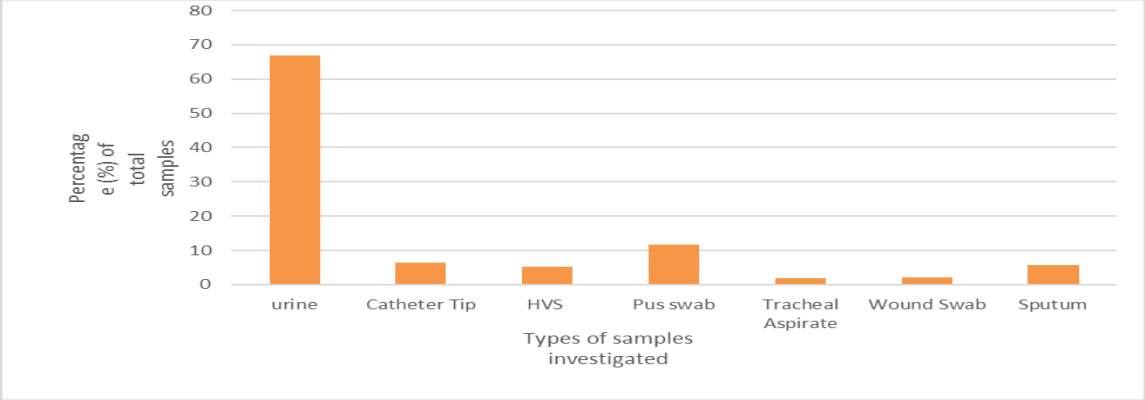

Figure 4.1 Distribution of patient samples collected and investigated at the Mombasa Hospital

4.1.2 Types of pathogens isolated from cultures of all samples

Culture results obtained in this study showed that out of the 384 samples, [226(58.9%)] did not yield any bacteria or fungi. Commensal bacteria [21(5.5%)] were isolated from some upper respiratory tract and vaginal samples, hence reported as no pathogens isolated. In total, 140 pathogens were isolated including 107 pathogenic bacteria and 33 *Candida*. Three samples yielded mixed isolates of *Candida albicans* and *Escherichia coli*, *Candida albicans* and S*taphylococcus aureus and Candida tropicalis and* S*taphylococcus aureus.* Moreover, 17 types of pathogens were isolated from the patient samples. Among them, 33 were *Candida* out of which 30(21.4%) were *Candida albicans* and 3(2.1%) *Candida tropicalis* isolates. The other 15 types of pathogens were bacteria with the most predominant ones being *Escherichia coli* [29(20.7%)], *Pseudomonas aeruginosa* [19(13.6%)], *Klebsiella pneumonia* [16(11.4%)] and *Staphylococcus aureus* [11(7.9%)] among others (Table 4.1).

Table 4.1: Types of pathogens isolated from cultures of all samples.

S/No.	Types of pathogens	Number isolated n(%)
1	Candida albicans	30(21.4)
2	Escherichia coli	29(20.7)
3	Pseudomonas aeruginosa	19(13.6)
4	Klebsiella pneumonia	16(11.4)
5	Staphylococcus aureus	11(7.9)
6	Coagulase negative S*taphylococcus*	7(5.0)
7	Proteus mirabilis	6(4.3)
8	Enterococcus faecalis	6(4.3)
9	Candida tropicalis	3(2.1)
10	Enterobacter	2(1.4)
11	Streptococcus agalactiae	2(1.4)
12	Acinetobacter	2(1.4)
13	Serratia	2(1.4)
14	Roultella ornithinolytica	2(1.4)
15	Citrobacter	1(0.7)
16	Kocuria kristinae	1(0.7)
17	Sphingomonas paucimobilus	1(0.7)
	Total number of pathogens isolated	140

4.1.3 Prevalence of *Candida* isolates from the samples submitted for analysis

4.1.3.1 Overall prevalence

Of the 384 samples investigated in this study, *Candida* was isolated from 33 samples hence the prevalence of *Candida* in samples submitted by patients for analysis in Mombasa Hospital was 8.6%.

4.1.4 Proportion of *Candida* isolates in samples collected from inpatients and outpatients

Results obtained when comparing the proportions of samples drawn and investigated from inpatients and outpatients indicated that out of the 250 samples drawn from the

inpatients, 19(7.6%) were positive for *Candida* while out of the 134 samples drawn from the outpatients, 14(10.4%) were positive for *Candida.* The results hence showed that samples drawn from outpatients had a higher likelihood of a positive *Candida* outcome than those drawn from inpatients. Moreover, Chi square (x^2= 0.901, P = 0.343, 1 df) results indicated that there was no significant difference in the proportion of *Candida* in the inpatient and outpatient hence there is no association between the presence of *Candida* and patient type (inpatient/outpatient) (Table 4.2).

Table 4.2: Proportion of *Candida* isolates in samples collected from inpatients and outpatients

Patient type	N	Candida positive samples Frequency (%)	Candida negative samples Frequency (%)	df	$x2$	p-value
Inpatients	250	19 (7.6)	231 (92.4)	1	0.901	0.343
Outpatients	134	14 (10.4)	120 (89.6)			
Total	384	33	351			

4.1.5 Proportion of *Candida* isolates in samples stratified with regard to gender

Demographic data analyzed for gender distribution showed that majority of samples were collected from female (210) patients out of which 26(12%) were positive for *Candida* and 184(88%) negative for *Candida.* Samples collected from male patients were (174) out of which 7(4%) were positive for *Candida* and 167(96%) negative for *Candida.* Chi-square statistics revealed a statistically significant association between gender and the isolation of *Candida* among patients attending Mombasa Hospital (x^2 = 8.462, P = 0.004, 1 df) (Table 4.3).

Table 4.3: Proportion of *Candida* isolates in samples stratified with regard to gender

Patients' gender	n	Candida positive samples Frequency (%)	Candida negative samples Frequency (%)	df	$x2$	p-value
Male	174	7(4)	167(96)	1	8.462	0.004
Female	210	26(12)	184(88)			
Total	384	33	351			

4.1.6 Proportion of *Candida* isolates in samples stratified with regard to age (≤18 yrs. and > 18yrs)

The ages of the patients whose samples were selected in this study were broadly divided primarily into two categories namely equal to or less than 18 years old and 18 years and above (adults). Results showed that majority of the samples were collected from adults [324 (84.4%)] patients while few samples from patients in the category of equal to or less than 18years old [60 (15.6%)] were submitted for investigations. Results further showed that among the *Candida* positive samples, almost all [32 (97%)] the *Candida* were isolated from adult patients samples and [1 (3.0%)] *Candida* isolated from patients under 18 years old. Chi square statistics showed that broad division of patients into equal to or less than 18 years old and 18 years and above age categories was significantly associated with the isolation of *Candida* among the patients in Mombasa Hospital (x^2 = 4.344, P = 0.042 with 1 df) (Table 4.4).

Table 4.4: Proportion of *Candida* isolates in samples stratified with regard to age

Age structure	n	Candida positive samples Frequency (%)	Candida negative samples Frequency (%)	df	$x2$	p-value
≤ 18 years old	60	1(1.7)	59 (98.3)	1	4.344	0.042
> 18 years old	324	32 (9.9)	292 (90.1)			
Total	384	33	351			

4.1.7 Proportion of *Candida* isolates in samples stratified with regard to sample types

An analysis to determine the prevalence of *Candida* isolates per the sample types showed that urine 257(66.9%) samples were the most submitted hence investigated samples. It was observed that only 18(7%) out of the 257 urine samples investigated were positive for *Candida* indicating a relatively lower likelihood of a positive *Candida* outcome compared to the results of 6 (30%) *Candida* positive samples out of the 20 HVS and 5 (23%) *Candida* positive samples out of the 22 sputum samples investigated. Consequently, the results showed that there is a higher likelihood of a positive *Candida* outcome from HVS and sputum samples among the various sample types investigated.

Out of the 7(1.8%) tracheal aspirate samples submitted, 1 (14%) was positive for *Candida* while 1 (2%) out of the 45 submitted pus swabs was positive for *Candida*. There were no *Candida* isolated from all the 8 (2.1%) wound swabs submitted (Table 4.5). The Chi-square (x^2 = 21.466, P = 0.002, 6 df) test showed that the types of specimens investigated had significant association with the isolation of *Candida.*

Table 4.5: Proportion of *Candida* isolates in samples stratified with regard to sample types

Sample types	N	Candida positive samples Frequency (%)	Candida negative samples Frequency (%)	df	$x2$ (Fisher's Exact)	p-value
Urine	257	18 (7)	239 (93)	6	21.466	0.002
Catheter Tip	25	2 (8)	23 (92)			
HVS	20	6 (30)	14 (70)			
Pus swabs	45	1 (2)	44 (98)			
Tracheal aspirates	7	1 (14)	6 (86)			
Wound swabs	8	0 (0)	8 (100)			
Sputum	22	5 (23)	17 (77)			

4.1.8 Prevalence of *Candida* in various sample types submitted by pregnant women

This study showed that [42(10.9%)] samples were collected from pregnant women. This includes [30(11.7%)] urine samples out of all 257 urine samples investigated attributing to 71.4% pregnant women's samples and [9(45%)] HVS samples out of all 20 HVS samples investigated accounting for 21.4% of pregnant women's samples.

In total, *Candida* was isolated from 13 out of the 42 samples submitted by pregnant women hence a prevalence of 31%. The most prevalent sample types that were submitted were of the genitourinary type. Further, 8 out of the 30(27%) urine samples submitted by pregnant women grew *Candida*, predominantly *Candida albicans* and 5 out of 9 (56%)] *Candida* isolated from pregnant women's HVS samples. Whereas the results for chi-square test confirmed that pregnancy was a statistical significant risk factor in isolation of *Candida* among the patients in Mombasa Hospital (Table 4.3.7.3),

there is no statistically significant association between the type of samples collected by pregnant women and the isolation of *Candida* (x^2= 4.152, p = 0.246 6 df) (Table 4.6).

Table 4.6: Proportion of *Candida* isolates in samples submitted by pregnant women

Sample types	n	Candida positive samples Frequency (%)	Candida negative samples Frequency (%)	df	x^2 (Fisher's Exact)	P-value
Urine	30	8 (27)	22 (73)	6	4.152	0.246
Catheter Tip	0	0 (0)	0 (100)			
HVS	9	5 (56)	4 (44)			
Pus swabs	1	0 (0)	1 (100)			
Tracheal	0	0 (0)	0 (100)			
Wound swabs	2	0 (0)	2 (100)			
Sputum	0	0 (0)	0 (100)			

4.1.9 Prevalence of *Candida* isolation with regard to various risk factors

4.1.9.1 Candida isolation with regards to diabetes

Among the samples investigated, [73 (19%)] were collected from patients with diabetes while those from non-diabetic patients were [311 (81%)]. Among the *Candida* positive samples, the likelihood of *Candida* positive outcome in samples submitted by patients with history of diabetes [12 (16.4%)] is higher compared to those by non-diabetic [21 (6.8%)]. Chi-square result (x^2 = 7.0612, p = 0.008, OR = 0.37 with 1 df) showed that there is a significant association between patients with history of diabetes and the isolation of *Candida* among the patients samples investigated (Table 4.7).

Table 4.7: Proportion of *Candida* isolates with regards to diabetes

Diabetic	n	*Candida* positive samples Frequency (%)	*Candida* negative samples Frequency (%)	x^2	df	p-value	OR
Yes	73	12 (16.4)	61 (83.6)	7.0612	1	0.008	0.37
No	311	21 (6.8)	290 (93.2)				

4.1.9.2 Candida isolation with regards to surgery

Samples submitted for investigation from patients who had undergone surgery were [103 (26.8%)] while [281 (73.2%)] samples were submitted by those who were not operated on. The results show that among the *Candida* positive samples, the likelihood of a *Candida* positive outcome was higher amongst the non-operated [25(8.9%)] patients samples compared to those submitted by patients who had been operated on [8(7.8%)]. Chi square statistics indicated that there was no association between the isolation of *Candida* and surgical operation in this study (x^2=0.12, p = 0.726 with 1 df) (Table 4.8).

Table 4.8: Proportion of *Candida* isolates with regards to surgery

Surgery done	n	*Candida* positive samples Frequency (%)	*Candida* negative samples Frequency (%)	x^2	df	p-value	OR
Yes	103	8(7.8)	95 (92.2)	0.123	1	0.726	1.16
No	281	25(8.9)	256(91.1)				

4.1.9.3 Candida isolation with regards to pregnancy

Samples submitted by pregnant women were 42(20%) while those submitted by non-pregnant women were 168(80%). Among the samples submitted by women (210),

samples submitted by pregnant women 13(31%) had a higher likelihood of *Candida* positive outcome compared to those submitted by non-pregnant women 20(12%). Chi-square result (x^2 = 30.011, p = 0.000, OR = 0.14 with 1 df) showed that there is a significant association between pregnancy and the isolation of *Candida* among the patients' samples investigated (Table 4.9).

Table 4.9: Proportion of *Candida* isolates with regards to pregnancy

Patient Pregnant	**n**	**Candida positive samples Frequency (%)**	**Candida negative samples Frequency (%)**	x**2**	**df**	**p-value**	**OR**
Yes	42	13(31)	29 (69)	30.011	1	0.000	0.14
No	168	20(12)	148 (88)				
Total	210	33	177				

4.1.9.4 Candida isolation with regards to catheterization

Clinical samples submitted by catheterized patients were [109(28.4%)] while those submitted by non-catheterized patients were [275(71.6%)] from. Catheterized patients samples from whom *Candida* was isolated constituted [15(14%)] and indicated a higher likelihood of Positive *Candida* outcome compared to those submitted by non-catheterized patients samples [18(7%)]. Chi-square results (x^2 = 5.174, p = 0.023, OR = 0.44 with 1 df) showed that there is a significant association between catheterization and the isolation of *Candida* among the patients samples investigated (Table 4.10).

Table 4.10: Proportion of *Candida* isolates with regards to catheterization

Patient Catheterized	n	*Candida* positive samples Frequency (%)	*Candida* negative samples Frequency (%)	x^2	df	p-value	OR
Yes	109	15 (14)	94 (86)	5.174	1	0.023	0.44
No	275	18 (7)	257 (93)				

4.1.9.5 *Candida* isolation with regards to chronic illnesses (HIV, Renal diseases & Cancer)

Whereas [31(8.1%)] patient samples were collected from patients with history or diagnosis of HIV, renal diseases and cancer, [353(91.9)] samples were from patients with no history or diagnosis of HIV, renal diseases and cancer. Amongst the patients with chronic illnesses (HIV, renal diseases and cancer), [6(19.3 %)] had *Candida*. The proportion of patients samples with *Candida* isolates among the patients with no history of or diagnosis of HIV, renal diseases and cancer was [27(7.6%)] indicating a relatively lower likelihood of *Candida* positive outcome. Chi square statistics (x^2 = 4.971, p = 0.026, OR = 0.35 with 1 df) indicated that there was a significant association between chronic illness and isolation of *Candida* (Table 4.11).

Table 4.11: Proportion of *Candida* isolates with regards to chronic illnesses (HIV, Renal diseases & Cancer).

Chronic illnesses	n	*Candida* positive samples Frequency (%)	*Candida* negative samples Frequency (%)	x^2	df	p-value	OR
Yes	31	6 (19.3)	25 (80.7)	4.971	1	0.026	0.35
No	353	27 (7.6)	326 (92.4)				

4.1.9.6 Distribution of Candida species in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital

The analysis of types of *Candida* isolated from the 384 samples collected showed that two types of *Candida* were isolated from all samples investigated. *Candida albicans* 30 (7.8%) was the predominant species while 3(0.8%) *Candida tropicalis* were isolated (Table 4.12).

Table 4.12: Distribution of *Candida* species in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.

Candida	Number of isolates	Prevalence (%)
Individuals with *Candida albicans*	30	90.9
Individuals with Candida tropicalis	3	9.1
Total (Candida albicans + Candida tropicalis)	33	100

4.2 Antifungal susceptibility profiles of the *Candida* isolates in Mombasa hospital.

Six antifungal drugs (Fluconazole, Voriconazole, Micafungin, Caspofungin, Amphotericin B and Flucytosine) were investigated in this study by Vitek 2 compact system to determine the susceptibility profiles of the 33 *Candida* isolated. Majority of the *Candida* isolated from patients attending Mombasa Hospital responded well to the antifungal drugs investigated. All the [33 (100%)] *Candida* isolates were sensitive to Flucytosine. The study also revealed that [32 (97%)] *Candida* isolates were sensitive to Micafungin and Caspofungin but1 (3.0%) isolate showed resistance to the two drugs in vitro. [31 (93.9%)] isolates were sensitive to Voriconazole with two *Candida* isolates showing resistance. [27 (81.8%)] of the isolates were sensitive to Fluconazole while [3

(9.1%)] of the isolates showed resistance and [3 (9.1%)] intermediate responses. 5 (15.2%) *Candida* isolates showed resistance to Amphotericin B. Chi square results (x^2 = 20.165, P < 0.05) indicated that there is an association between the antifungal drug used and susceptibility of the *Candida* species isolated (Table 4.13).

Table 4.13: Response of *Candida* to the six anti-fungal drugs investigated

Antifungal Susceptibility: Frequency(%)n = 198					
Antifungal	**Sensitive**	**Intermediate**	**Resistant**	**x^2 (Fisher's Exact)**	**p-value**
Voriconazole	31(93.9)	0(0)	2(6.1)	20.165	0.028
Fluconazole	27(81.8)	3(9.1)	3(9.1)		
Micafungin	32(97)	0(0)	1(3.0)		
Caspofungin	32(97)	0(0)	1(3.0)		
Amphotericin B	27(81.8)	1(3.0)	5(15.2)		
Flucytosine	33(100)	0(0)	0(0)		

4.3 Risk factors associated with isolation of *Candida*

4.3.1 Correlation analysis

Bivariate correlation analysis was done to determine the correlation between the dependent variable (isolation of *Candida*) and the risk factors; diabetes, pregnancy, surgical interventions, catheterization and chronic illnesses (HIV, renal diseases and cancer). The results obtained showed that there is a strong positive correlation between pregnancy and the isolation of *Candida*. (Correlation is significant at the 0.01 level, 2-tailed). Hence it was discerned that pregnancy is a risk factor and hence pregnant women (p = 0.000) stand a relatively higher risk of isolation of *Candida* followed by gender (p = 0.004), diabetics (p = 0.008), catheterization (p = 0.023) and those patients with chronic

(HIV, renal diseases and cancer) illnesses (p = 0.026) (Correlation is significant at the 0.05 level, 2-tailed). No correlation was found between surgical intervention and isolation of *Candida* in this study (Table 4.14).

Table 4.14: Correlation of risk factors and isolation of *Candida*

	Risk factors associated with isolation of *Candida*						
Correlations	**Diabetes**	**Pregnant**	**Operated On**	**Catheterization**	**Chronic illnesses**	**Age (Yrs)**	**Sex**
Pearson Correlation	0.136**	0.280**	-0.018	0.116*	0.114*	-0.077	-0.148**
Sig. (2-tailed)/p-value	0.008	0.000	0.727	0.023	0.026	0.135	0.004
N	384	384	384	384	384		

NB: * Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

4.3.2 Logistic regression analysis for the risk factors

Logistic regression model was used to determine the risk factors associated with isolation of *Candida* because the response variable is binary resulting in only two outcomes namely success (isolation of *Candida*)or failure (non- isolation of *Candida*). A multiple logistic regression model is given by the following equation;

$$\pi_i = \frac{e-(\beta_0+\beta_1 x_1+...+\beta_k x_k)}{1+e-(\beta_0+\beta_1 x_1+...+\beta_k x_k)}$$

Where π_i represents the probability of success in the i^{th} observation

In logistic regression, the null hypothesis is always stated as $H_0:\beta_0=\beta_1=...=\beta_k=0$. Rejection of the null hypothesis implies that at least one of the risk factors (explanatory variables) is significant at a specified level of significance. From table 4.15, it can be seen that at 5% level of significance, pregnancy, chronic illness, diabetes and age are

statistically significant. Therefore, the probability of patient acquiring *Candida* infection and hence isolation of *Candida* keeping other factors constant is given by the following equation;

$$\pi_i = \frac{e - (1.162x_1 + 2.96x_2 + 1.176x_3 - 0.028x_4)}{1 + e - (1.162x_1 + 2.96x_2 + 1.176x_3 - 0.028x_4)}$$

Where $x_1, x_2, ...; x_4$ represents diabetes, pregnancy, chronic illness (HIV, renal diseases and cancer) and age respectively.

The logistic regression model results supported the chi square statistics in affirming that age and gender have a significant association with the isolation of *Candida* among the patients investigated in Mombasa Hospital (Table 4.15).

Moreover, the logistic regression model results were in congruence with chi square results in affirming a statistically significant association between chronic illnesses (p = 0.049) and diabetes (p = 0.024) and the isolation of *Candida* in Mombasa Hospital.
Further, the logistic regression model (Table 4.15) supported the chi square findings (Table 4.3.7.3) in asserting a statistically significant association between pregnancy (p = 0.000) and the isolation of *Candida* among patients in Mombasa Hospital.

On the other hand, findings of the chi square statistics (Table 4.8) concurred with the logistic regression model (p = 0.398) in affirming that surgery was not a statistical significant influence in determining the isolation of *Candida* among patients attending Mombasa Hospital (Table 4.15).

Finally, the logistic regression model results (p = 0.27) (Table 4.15) revealed that catheterization was not a significant factor in isolation of *Candida*. They contradicted the chi square results (p = 0.023) which showed a significant association between

catheterization and the isolation of *Candida* among the patients' samples investigated (Table 4.10).

Table 4.15 Logistic regression analysis for the risk factors

Variables in the Equation		B	S.E.	Wald	df	Sig. (p - value)	Exp(B) (Odds Ratio)
Step 1[a]	Diabetes	1.162	0.515	5.090	1	0.024	3.196
	Pregnant	2.967	0.642	21.384	1	0.000	19.439
	Operated On	-0.467	0.553	0.715	1	0.398	0.627
	Catheterisation	0.595	0.539	1.218	1	0.270	1.813
	Chronic Illnesses	1.176	0.599	3.860	1	0.049	3.241
	Age (Yrs)	-0.028	0.014	4.317	1	0.038	0.972
	Sex	-0.965	0.515	3.509	1	0.061	0.381
	Constant	-4.260	2.282	3.487	1	0.062	0.014

a. Variable(s) entered on step 1: Diabetes, Pregnant, Operated On, Catheterization, Chronic Illnesses, Age (Yrs) and Sex.

4.3.3 Risk Estimates

4.3.3.1 Risk estimates for Candida in age and gender.

Age is a significant factor in isolation of *Candida*. This study indicated there is a relatively higher likelihood (6 times) to isolate *Candida* for adult patients (18 years and above) than from patients of equal to or less than 18 years old. Results also indicated that it is highly likely (3 times) to isolate *Candida* from female patients compared to male patients.

Table 4.16: Risk estimates for *Candida* in age and gender.

	Age Structure (≤18 years/> 18 years)			Gender (Male/Female)		
Risk estimate	**value**	**95% Confidence Interval**		**value**	**95% Confidence Interval**	
		Lower	Upper		Lower	Upper
Odds Ratio	0.155	0.021	1.154	0.297	0.125	0.701
For Cohort *Candida* positive	0.169	0.024	1.212	0.325	0.145	0.730
For Cohort *Candida* negative	1.091	1.039	1.146	1.095	1.032	1.162
N of valid cases	384			384		

4.3.3.2: Risk estimates for Candida in pregnancy, catheterization, diabetes and chronic illness (HIV, Renal diseases & Cancer).

This study revealed that the likelihood of isolation of *Candida* from samples submitted by pregnant women is seven times more than those submitted by non-pregnant women while the likelihood of isolation of *Candida* from samples submitted by patients with chronic illness (HIV, Renal diseases & Cancer) is three times more compared to those without chronic illnesses (HIV, renal diseases and cancer),. The likelihood of isolating *Candida* from samples submitted by diabetic patients is almost three times more compared to those submitted by non-diabetic patients while the likelihood of isolating *Candida* from samples submitted by catheterized patients is twice as much as that from patients submitted by non-catheterized patients

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Table 4.17: Risk estimates for *Candida* in pregnancy, catheterization, diabetes and chronic illness (HIV, Renal diseases & Cancer).

	Pregnancy			**Catheterization**			**Chronic illness**			**Diabetes**		
	(pregnant / Non pregnant)			**(Catheterization / Non Catheterization)**			**(chronic illness / no chronic illness)**			**(Diabetic/Non diabetic)**		
Risk estimate	value	95% Confidence Interval		value	95% Confidence Interval		value	95% Confidence Interval		value	95% Confidence Interval	
		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
Odds Ratio	7.217	3.259	15.	2.278	1.104	4.703	2.898	1.095	7.672	2.717	1.269	5.815
For Cohort *Candida* positive	5.293	2.846	9.843	2.102	1.099	4.021	2.530	1.132	5.659	2.434	1.256	4.719
For Cohort *Candida* negative	0.733	0.598	0.900	0.923	0.851	1.001	0.873	0.733	1.040	0.896	0.806	0.996
No of valid cases	384			384			384			384		

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Prevalence of isolation of *Candida* and species distribution

No bacteria or fungi were isolated from 226 (58.9%) samples that were submitted for analysis in this study. Moreover, 21 (5.5%) samples had no pathogens isolated since bacteria that were considered normal flora in non-sterile sites like the upper respiratory tract and vagina were isolated.

Findings of the current study incriminated *Candida* as a significant causal organism of superficial infections of the genitourinary and pulmonary tracts hence predisposing patients to risk of systemic candidiasis. It concurs with studies that implicated *Candida* as a significant contributor to systemic infections and a common causative agent of nosocomial infections (Wisplinghoff *et al.*, 2004) and (Michael Pfaller, Pfaller, & Diekema, 2010).

This study indicated a lower prevalence (8.6%) of superficial *Candida* infections below the continental (19.68%) one (Omrani, Pecen, Hajek, Raghubir, & Zigmond, 2014), attributable to geographical factors (Li *et al*., 2013), (Falagas *et al.*, 2010) and (P Badiee, Alborzi, & Alborzi, 2011). However, higher prevalence (31%) was reported amongst pregnant women correlating with the Thika (42.7%) (Menza Nelson, Wanyoike Wanjiru, 2013) study carried out in antenatal mothers and (45.4%) (Mukasa *et al.*, 2015). The relatively high proportion of *Candida* positive samples in HVS (30%) resonate with those of a North India study which reported that female patients (30.6%) with complaints of vulvovaginitis grew *Candida* (Varsha Kumari *et al*., 2013).

Results of this study are further supported by previous studies that identified *Candida* as an emerging potential pathogen fungus in broncho-pulmonary diseases patients (Kali *et al.*, 2013) since it rated sputum (23%) samples as the third most prevalent source of *Candida* isolates among patients samples investigated in Mombasa Hospital after urine (30%).

Female patients submitted more samples than male patients and consequently had a higher (12%) likelihood of *Candida* positive outcome compared to males (4%). These findings correspond with a study which reported that patients who presented with candiduria were more commonly female and pregnant (Achkar & Fries, 2010).

Globally, the incidence of *Candidal* systemic mycoses in hospitalized patients is on the rise and contributes significantly to morbidity and mortality (Giannini, 2016) and suggested nosocomial transmission of *Candida* (San Miguel *et al*., 2005). On the contrary, this study reported a higher likelihood of *Candida* positive outcomes amongst samples submitted by outpatients (10.4%) compared to the inpatients (7.6%). This could be attributed to the occurrence of a lower prevalence of superficial *Candida* infection (8%) in the study area manageable in an ambulatory setup and which is subsequently much lower than the established prevalence of superficial infection (19.68%) and systemic infection (12.42%)) in Africa (Omrani *et al.*, 2014).

In this study, the proportions of isolation of *Candida albicans* [30(90.9%)] was significantly higher than that of *Candida tropicalis* [3(9.1%)]. No other *Candida* was isolated. These findings concur with previous studies which identified *Candida albicans as* the most commonly isolated *Candida* (Menza Nelson, Wanyoike Wanjiru, 2013). The current study indicated that *Candida albicans* were the most predominant *Candida* in all sample types including sputum and urine samples hence corresponding with a study in India which identified *Candida albicans* as the most predominant in pulmonary candidiasis (Kali *et al.*, 2013).

Contrary to a study which reported an increasing prevalence of vulvo vaginitis due to non-*albicans Candida* species (V. Kumari *et al.*, 2013), this study reported a significantly low prevalence of non albicans *Candida* amongst vaginal samples. This could be attributed to geographical/regional variation.

The study also corresponds with findings of a study in Thika which identified *Candida albicans* as the most common cause of vaginal candidiasis (Menza Nelson, Wanyoike Wanjiru2, 2013).

Furthermore, findings of this study are similar to those of a study in Brazil which reported *Candida albicans* 83(92.3%) as the most commonly isolated from pregnant women's samples. (Dias, Souza, Melhem, Szeszs, & Filho, 2011)

5.1.2 Antifungal susceptibility profiles

Although patterns of antifungal resistance of *Candida* differ geographically, necessitating localized surveys of the same in order to localize antifungal therapy (Li *et al.*, 2013), there has been a slow uptake of routine antifungal susceptibility testing hence the availability of scant data in some regions (Mukasa *et al.*, 2015).

This study established that majority of the *Candida* isolated responded well to the antifungal drugs investigated. However, the finding of some resistant *Candida* isolates here is supported by (Cisterna *et al.*, 2005)) and (Ingham *et al.*, 2012) who observed a trend towards increasing resistance to antifungal agents in life threatening *Candida* infections.

In the current study, 27 (81.8%) of the isolates were sensitive to fluconazole while 3 (9.1%) showed resistance and intermediate responses respectively. 31 (93.9%) isolates were sensitive to Voriconazole with two *Candida* showing resistance. Azole resistance

has been associated with the use and occasional overuse of fluconazole (White *et al*., 1998). Resistance to Fluconazole was reported elsewhere albeit at elevated prevalence (13.6 %) (Aguilar *et al.,* 2015) compared to the current findings.

The high 5 (15.2%) Amphotericin B resistance in the current study is supported by findings in a previous study that reported Fluconazole and Amphotericin B- resistance in *Candida albicans* isolated from HIV infected patients on long term azoles prophylaxis (White *et al.,* 1998)

All 33 (100%) *Candida* isolated from all patients were sensitive to Flucytosine hence it could be used as a presumptive treatment for invasive candidiasis. These findings confirm those of previous studies that Flucytosine administered in combination with amphotericin B is very useful in treating *Candida* infections (Hope *et al.,* 2004).

The absence of resistance to Flucytosine as reported in this study confirms that although there is primary resistance to Flucytosine, its prevalence remains very low in certain yeasts (1% – 2% among *Candida* isolates) (Z. A. Kanafani & Perfect, 2008). However, it should be noted that secondary resistance to Flucytosine is common in patients receiving Flucytosine monotherapy (Theodore, 1997).

The study also revealed that 32 (96.97%) *Candida* isolates were sensitive to Micafungin and Caspofungin with only 1 (3.03%) isolate showing resistance to the two drugs in vitro. The minimal resistance to Micafungin and Caspofungin as reported in this study suggest that these two drugs are effective antifungal agents. Haddadi *et al.* (2014) also established that echinocandin like Caspofungin is the most effective agent against all the *Candida* species studied and are the best antifungal agents against *Candida* colonization for specific pediatric patients with some form of suppressed immunity. However,

resistance to Echinocandins in *Candida glabrata* has been reported (Alexander *et al.*, 2013).

5.1.3 Risk factors associated with isolation of *Candida*

Improved management of high-risk patients with novel treatment methods has been partly attributed to the increase in *Candidiasis*. (Diaz & Fell, 2004), (Zaragoza *et al.*, 2008) and (Pappas *et al.*, 2016)..

5.1.3.1 Pregnancy

This study showed that 42(10.9%) of the samples were collected from pregnant women who predominantly submitted genitourinary samples including 30(71.4%) urine and 9(21.4%) HVS samples. Prevalence of *Candida in* HVS (30%) samples was highest among all sample types investigated hence the significant correlation between pregnancy and candidiasis observed. This study reported a higher likelihood of *Candida* positive outcomes amongst samples submitted by pregnant women (31%) compared to those by non pregnant women (12%) hence concurring with the Mbarara (45.4%) study in HVS samples (Mukasa *et al.*, 2015) and Nigerian (36%) (Olowe, Makanjuola, Olowe, & Adekanle, 2014).

Chi-square test confirmed that pregnancy was a significant risk factor associated with isolation of *Candida* in this study attributable mainly to a relatively reduced immunity (Nnaemeka, 2010) and (Mukasa *et al.*, 2015)) pregnancy that predisposes such women to developing candidiasis of the genitourinary tract. Although *Candida albicans* often colonizes the vagina (Apalata, B, Aw, Wh, & Moodley, 2014) (S *et al.*, 2016) without causing disease, *Candida* infections mainly occur as opportunistic infections due to altered conditions of the host (Apalata *et al.*, 2014; Okungbowa, Isikhuemhen, & Dede, 2003) (Apalata *et al.*, 2014)

Some of the altered conditions that occur during pregnancy is the change in pH (Apalata *et al*., 2014) of the vagina which facilitates thriving of *Candida* organisms. In gestation, pH of vaginal discharge acidifies to 4 or 5 hence unfortunately, favors the growth of *Candida albicans* (Kamath, Pais, & Nayak, 2013). The reduction of the vaginal acidity to pH 5.0 - 6.5, provides a conducive milieu for *Candida* colonization and infection (Nnaemeka, 2010).

During gestation, elevated levels of estrogen and glycogen in vaginal secretions enhances the risk of vulvo vaginal candidiasis in women (Kamath *et al.*, 2013, Plitteri Adele, 2007 Soong & Einarson, 2009). Likewise, (Apalata *et al*., 2014) enumerated pregnancy among other medical conditions as a risk factor associated with Vulvovaginal *Candidiasis.*

5.1.3.2 Diabetes

The current study in Mombasa Hospital established that the likelihood of *Candida* positive outcome in samples submitted by patients with history of diabetes [12 (16.4%)] is higher compared to those by non-diabetic [21 (6.8%)] patients and revealed a significant association between diabetes and isolation of *Candida*. This is because diabetics have high levels of glucose in their tissues which is an essential source of carbon that is needed by *Candida* for their active metabolism, colonization and hence infection concurred with those of other studies in recognizing diabetes as a significant risk factor associated with isolation of *Candida* in various patient sub populations. Diabetes mellitus is one among several risk factors for vaginal candidiasis with *Candida albicans* being the most frequently isolated etiologic agent (Saporiti,*et al.,* 2001.).

In a study in Pakistan involving *Candida* esophagitis, it was revealed that diabetes mellitus was one of the major risk factors for candidiasis alongside carcinomas, corticosteroid and antibiotic therapy (Yakoob *et al*., 2003).

5.1.3.3 Surgical interventions

Patients who have undergone surgery are at risk of *Candida* colonization and may subsequently develop into invasive candidiasis hence the need to ascertain the occurrence of *Candida* colonization and subsequent infection in surgical intensive care units patients (Liew *et al*., 2015) cannot be over emphasized. Only 8(7.8%) patients who had undergone surgery during their stay at the Mombasa hospital had *Candida* isolated from their samples. Surgery was not statistically a significant factor associated with isolation of *Candida* among patients attending Mombasa Hospital.

These findings were lower compared to the study of (Rasilainen, Juhani, & Kalevi, 2015) who established that 33% of critically ill surgical patients were colonized with *Candida* thereby corroborating the potential risk of fungal infections in surgical patients.

The observed difference could be due to the kind of patients samples selected. The higher prevalence may be due to the focus on critically ill surgical patients' samples in the former study while the current study has included non-critically ill surgical patients samples including those undergoing simple surgeries like caesarean section and appendectomy.

5.1.3.4 Catheterization

Fungal infection constitutes a significant proportion of all catheter associated urinary tract infection and diabetes mellitus, long term antibiotic use, immunosuppressive

therapy, and prolonged duration of catheterization are the most common risk factors (Mohammad, 2012)

The current study showed that *Candida* isolates were obtained form 15(14%) samples submitted by patients who were catheterized. Both Chi-square results (P =0.023 at 5% level of significance) and logistic regression analysis (P =0.0026) showed that catheterization is a significant risk factor associated with isolation of *Candida*.

Moreover, catheterization (central venous and urinary) has not only been associated with isolation of *Candida* among other factors in other studies but also reported as the most common predisposing factor associated with invasive candidiasis (Aguilar *et al*., 2015).

5.1.3.5 Chronic illnesses (HIV, cancer and renal diseases)

The inadvertent use of immunosuppressive drugs for life threatening health situations occasioned by a rise in Human Immunodeficiency Virus (HIV) infections has n led to more cases of severely immunosuppressed patients (Enrique & Puebla, 2012) and in whom the principal etiological agent of fungal infection is *Candida* (Enrique & Puebla, 2012). HIV pandemicity and invasive procedures for critically ill patients require the use of prolonged prophylactic antifungal treatments since such patients are more at risk of developing fungal infections including candidiasis (Loeffler J. and Stevens D., 2003).

The current study indicated that 31(8.1%) samples were submitted by patients with chronic illnesses (HIV, cancer and renal diseases). Six of them (19%) grew *Candida*. Chi-square and logistic regression revealed a correlation between chronic illnesses (HIV, renal diseases and cancer), and isolation of *Candida* concurring with a Pakistani study which revealed an association between *Candida* esophagitis, chronic diseases, treatment with corticosteroids and use of antibiotics (Yakoob *et al*., 2003).

The high risk of *Candida* positive outcome in samples submitted by chronically (HIV, renal diseases and cancer) ill patients in this study concurs with a study that revealed that *Candida* infections in the gut affects primarily patients with compromised immunity and those infected with HIV (Gupta, 2012) among other patient sub populations. Prevalence of *Candida* infections was significantly higher in HIV- infected (30.63%) than in HIV-negative patients (15.07%) (Omrani *et al*, 2014).

5.2 Conclusion

1. The prevalence of *Candida* in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital is 8.6%.
2. *Candida albicans* were the predominant *Candida* species isolated from specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital, although few *Candida tropicalis* species were also found.
3. Whereas *Candida* species are highly susceptible to Flucytosine, Micafungin and Caspofungin, they showed a relatively reduced susceptibility to Amphotericin B, Voriconazole and Fluconazole.
4. This study determined pregnancy, diabetes, chronic (HIV, renal diseases and cancer) illnesses and catheterization as significant risk factors associated with isolation of *Candida* in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.
5. Thus study provided a model equation for the estimation of the probability of a positive *Candida* outcome in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.

5.3 Recommendations

1. Empirical treatment strategies due to certain *Candida* species may be hampered by existence of intrinsic or secondary resistance to antifungal agents leading to clinical

resistance hence proper identification of fungal species and antifungal susceptibilities should be conducted from suspected infections.

2. Antifungal resistance to azoles, Amphotericin B and other antifungal agents should envisaged when considering an empirical or de-escalation treatment strategy.
3. Pregnant patients, diabetics, those with underlying chronic (HIV, renal diseases and cancer) illnesses and catheterized patients should be proactively investigated for possible colonization or infections with *Candida* since they are at higher risk.
4. Similar study should be replicated in a public facility in Mombasa.

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APPENDICES

Appendix 1 Budget

	Item	Quantity	Unit cost (Ksh)	Total cost (Ksh)
1	Internet	--	5000	5000
2	Telephone	--	5000	5000
3	Printing	--	3000	3000
4	Transport	--	800	800
5	Yeats ,Gram negative & Pos Identification Cards	20x20 packs	11500	230,000
6	Yeast, Gram Neg and Pos Sensitivity Cards	20x20 packs	11500	230,000
7	Blood Agar	1x500g	5000	5000

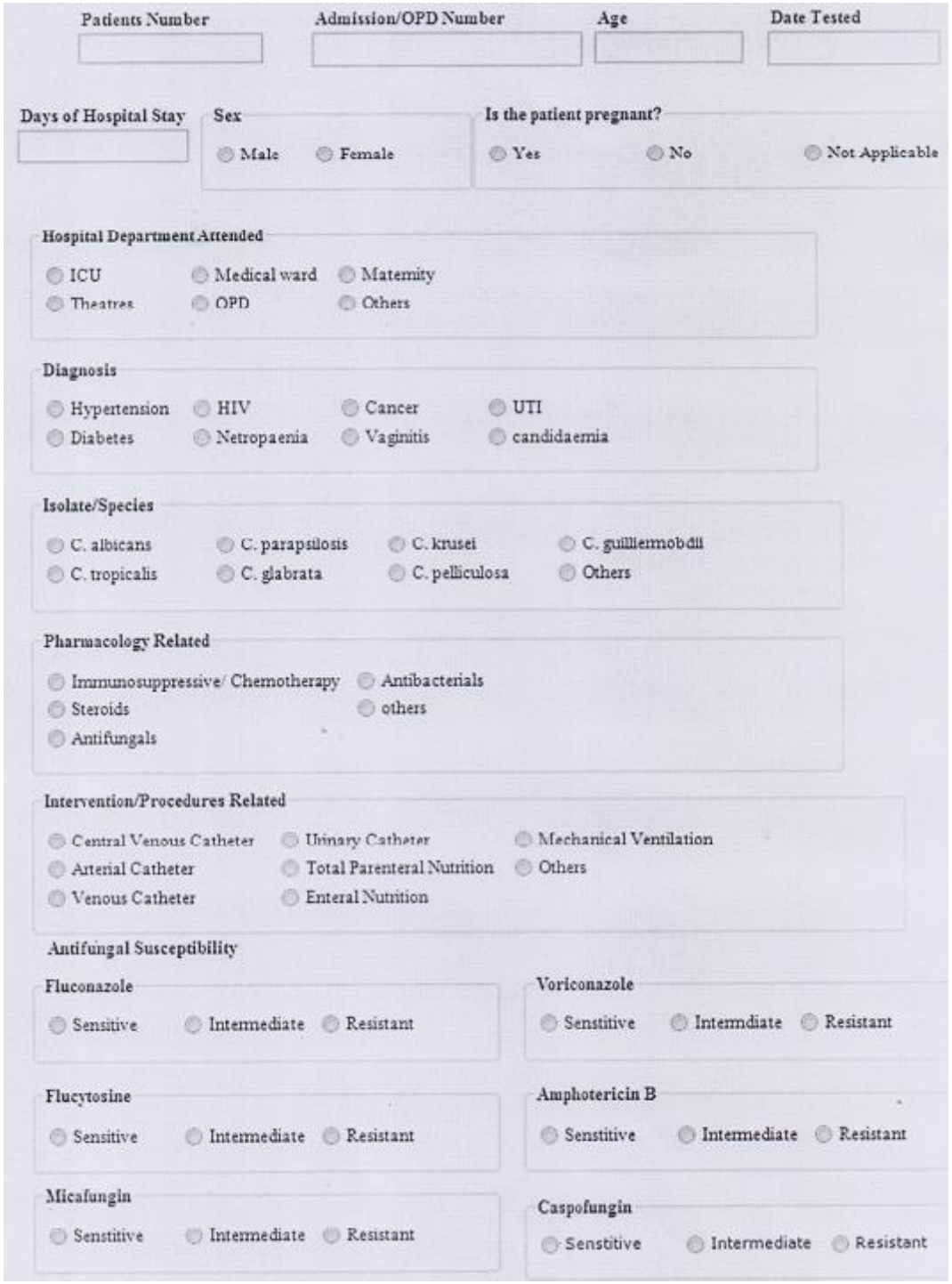
8	Sabouraud Dextrose Agar	1x500g	5000	5000
9	*Candida*krusei ATCC6258 QC strains	2	10000	20000
10	*Candida albicans* ATCC90028 QC strains	2	10000	20000
11	*Candida parapsilosis* ATCC22019 QC strains	2	10000	20000
12	Mac Farland standard kit	2 kits	5000	10000
13	Saline solutions	2 packs	8000	14000
14	Sampling bottle	25x10	15	3750
15	Disposable culture plates	1000	5.50	5500
16	Gram staining reagents (gram PVP kit)	1	3500	3500

17	Sheep red blood cells 1×10ml bottles	5	70	350
18	Oil emersion 1×100ml	2	270	540
19	Microscope Glass slides 1×50	5	20	100
20	Sterile cotton swabs	500	10	50
	Total			581, 590

Appendix 2 Plan of Activities

Activity	Feb. 2015	Mar.2015	Apr – Dec.15	Dec 2019
Submission of concept paper				
Proposal writing & literature review				
Proposal submission and presentation/Ethical review				
Validation of data collection tools /data collection & analysis				
Report writing				
Submission of the thesis				

Appendix 3 Data Collection Tool



Patients Number | Admission/OPD Number | Age | Date Tested

Days of Hospital Stay | Sex: Male, Female | Is the patient pregnant?: Yes, No, Not Applicable

Hospital Department Attended: ICU, Medical ward, Maternity, Theatres, OPD, Others

Diagnosis: Hypertension, HIV, Cancer, UTI, Diabetes, Netropaenia, Vaginitis, candidaemia

Isolate/Species: C. albicans, C. parapsilosis, C. krusei, C. guilliermobdii, C. tropicalis, C. glabrata, C. pelliculosa, Others

Pharmacology Related: Immunosuppressive/ Chemotherapy, Antibacterials, Steroids, others, Antifungals

Intervention/Procedures Related: Central Venous Catheter, Urinary Catheter, Mechanical Ventilation, Arterial Catheter, Total Parenteral Nutrition, Others, Venous Catheter, Enteral Nutrition

Antifungal Susceptibility

Fluconazole: Sensitive, Intermediate, Resistant

Voriconazole: Senstitive, Intermdiate, Resistant

Flucytosine: Sensitive, Intermediate, Resistant

Amphotericin B: Senstitive, Intermediate, Resistant

Micafungin: Senstitive, Intermediate, Resistant

Caspofungin: Senstitive, Intermediate, Resistant

Appendix 4 Consent Form (English)

This consent form explains precisely significant issues to all participants prior to their decision to take part in this study. Please read it carefully before making any decision. Feel free to discuss its contents with your trusted acquaintances including your personal doctor and family. You may ask any questions relating to this study. Kindly sign the acknowledgement and acceptance to participate section of this form if you have made up your mind to take part.

Study Title: Prevalence, species distribution, antifungal susceptibility profiles of *Candida* and risk factors associated with isolation of *Candida* in patient samples submitted for analysis at the Mombasa Hospital.
Principal Investigator: Abdulrahman Subira - Laboratory Manager, Mombasa Hospital P.O. Box 90294 G.P.O 80100 Mombasa, Kenya. **Telephone:** +254 41 2312191 Ext.222 E-mail addresses labmnager@mombasahospital.com

Supervisors: Professor Simon Karanja and Dr. Rahma Udu
Study Population: Patients who have been requested by their doctors to do a culture and sensitivity test

Aim and Objectives of the Study

The purpose of this research is to;

1. To determine the prevalence and distribution of *Candida* species in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.
2. To determine the antifungal susceptibility profiles of the *Candida* isolated from specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.
3. To determine the risk factors associated with isolation of *Candida* in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.

Procedure

1. About 384 patients will take part in this research.

2. You will also be asked a few questions as part of the usual doctor's history taking to understand your condition better. The questions may take about 5 minutes. Please provide correct information to assist us in drawing meaningful conclusions.

3. Depending on your symptoms and illness, your doctor has requested you to produce the relevant specimens and submit to the laboratory for culture and sensitivity test.

4. Your test results shall be will forwarded to the requesting clinician within 24 to 72 hours

5. Your result will be anonymously analyzed using your lab number/code and not your name to understand how the *Candida* (if isolated) responds to the standard antifungal drugs.

6. If further information is required such as the treatment provided it will be obtained through your doctor.

7. The results will then be submitted to the relevant authorities to assist in policy making with regards to treatment of *Candida* infections.

Risk/Benefit

There would be very minimal risk although not directly attributed to the study rather to the normal processes of collecting specimens for lab tests. All the samples will be collected in privacy and it is not expected that you will not endure any substantial physical and psychological discomfort.

Your acceptance and subsequent participation in this study does not entitle you to any form of remuneration. However, please note that there is an enormous gain to the general public including the invaluable health information obtained in this study that may be used by policy makers to formulate better policies and treatment guidelines.

Assurance of confidentiality

Strict confidentiality relating to your information shall be observed. The information shall be maintained by means of codes/laboratory accession numbers to protect the identities of the participants. Unauthorized personnel shall not have access to the data.

Right to Refuse or Withdraw

Participation in this study is voluntary because you have been asked by your doctor to do a culture and sensitivity test of your ……specimen(s) to find out if you have any infection that may be responsible for your illness and to determine the appropriate antibiotics to be used as part of your treatment.

The decision to participate in this study is entirely dependent upon you (individual). If you decide to participate, you are welcomed. Moreover, you are free to exit from the study at any point without necessarily giving notice. Decision not to participate or halt participation does not require any explanation nor have any consequences or loss of your compensation if entitled to.

Contact Principal Investigator

The Principal Investigator in this study is Mr. Abdulrahman Subira. He can be reached between 8 am and 5p m for clarifications on any concerns or input pertaining to the study including withdrawal from the study.

Consent

I hereby acknowledge that I have been adequately notified on the context of the study. The objectives, procedure, confidentiality and potential harm of the research have been communicated to me in a precise and comprehensive way. I have been informed that there are no direct benefits for participating as well. Likewise, all my concerns pertaining to participation have been adequately addressed to my satisfaction. I am aware that I can withdraw at any point without notice and that shall not attract any penalty or loss of compensation.

Acknowledgement and acceptance to participate

Participant

I …….. ……hereby agree to participate in this study.

Name Signature

Witness

I…….have witnessed the above acceptance.

Name Signature

Appendix 4 Consent Form (Kiswahili)

Fomu hii ina maelezo muhimu kuhusu kushiriki kwako katika utafiti huu.Tafadhali soma fomu hii kwa makinikabla ya kuchukua uamuzi wa kushiriki. Unaweza kujadilialan a na familia yako, marafiki zako au daktari wako kuhusu uamuzi wako.Jihisi kwamba uko huru kuuliza maswali yanayoambatana na utafiti huu. Ukiamua kushiriki katika utafiti huu utahitajika kuweka sahihi katika fomu hii.

Study Title: Prevalence, species distribution, antifungal susceptibility profiles of *Candida* and risk factors associated with isolation of *Candida* in patient samples submitted for analysis at the Mombasa Hospital

Mpelelezi Mkuu:

Abdulrahman Subira - Laboratory Manager, Mombasa hospital

P.O. Box 90294 G.P.O 80100 Mombasa, Kenya.

Simu: +254 41 2312191 Ext.222

Barua pepe: labmnager@mombasahospital.com

Supervisors: Professor Simon Karanja and Dr. Rahma Udu
Study Population: Patients who have been requested by their doctors to do a culture and sensitivity test

Malengo ya Utafiti

Madhumuni ya utafiti huu;

1. To determine the prevalence and distribution of *Candida* species in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.
2. To determine the antifungal susceptibility profiles of the *Candida* isolated from specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.
3. To determine the risk factors associated with isolation of *Candida* in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.

Utaratibu

1. Takriban wagonjwa 384 watashiriki katika utafiti huu

2. Utaulizwa maswali machachae na daktari ili apate ufahamu wa kina kuhusu hali yako ya afya. Maswali yatachukua takriban dakika tano kujibu.Tafadhali toa taarifa sahihi ili itusaidie katika kutoa uamuzi wa mwafaka.

3. Kulingana na dalili za ugonjwa wako, daktari atakuhitaji utoe vielelezo muhimu kwa ajiji ya kupimwa katika maabara.

4. kisha utawasilisha sampuli zako kwa maabara kwa ajili ya kupima
5. Matokeo ya vipimo vyako yatapelekwa kwa daktari wako ndani ya masaa 24-72

6. Matokeo yako yatachambuliwa ilhali jina lako limebanwa na nambari yako ya maabara ndio itakayotumiwa ili kutambua namna gani vimelea vya *Candida* (kama vimepatikana) vinavyo weza kuuliwa na dawa za antifungal

7. Matokeo ya utafiti kisha yatawasilishwa kwa mamlaka husika ili kusaidia katika kubuni sera muwafaka kwa upande wa matibabu ya magonjwa ya *Candida*.

Hatari/Faida

Kutakuwa na hatari ndogo sana ingawa haiwezi kuhusishwa moja kwa moja na utafiti huu bali ni kwa taratibu za kawaida za kukusanya vielelezo kwa vipimo vya maabara. Sampuli zote zitakusanywa katika faragha na haitarajiwi kwamba kutakuwa na usumbufu wowote mkubwa wa kimwili na kisaikolojia..

Hutopata manufaa muwafaka kwa kushiriki kwako katika huu utafiti. Hata hivyo, taarifa maridhawa zitapatikana katika utafiti huu ambazo zinaweza kutumiwa na watunga sera kutunga sera bora na miongozo ya matibabu.

Uhakika wa Usiri

Kutakuwa na usiri mkali kuhusian an maelezo yako. Maelezo yatahifadhiwa kwa njia ya nambari maalum yako ya maabara wala sio kwa jina lako ili kulinda utambulisho wa washiriki. Wafanyakazi asioruhusia hawatopata maelezo/data yak.

Haki ya kukataa au Kutoa

Kushiriki katika utafiti huu ni kwa hiari kwa sababu umetakiwa na daktari wako kufanya uchunguzi wa vimeleo na dawa muwafaka katika sampuli yako ya ..... ili kujua kama una maambukizi yoyote ambayo inaweza kuwasababisha ugonjwa ulionao na kutathmini antibiotics sahihi zitakazotumika kama sehemu ya matibabu yako.

Unaweza kuchagua kushiriki au kutoshiriki. Kama umechagua kushiriki, unaweza kubadili uamuzi wako huo na kujitoa katika utafiti huu wakati wowote. Kukataa kushiriki au kujitoa katika ushiriki kwako hakutahusishwa na adhabu yeyote au kupoteza haki zako za matibabu

Mawasiliano na Mpelelezi Mkuu

Mpelelezi Mkuu katika utafiti huu ni Bw. Abdulrahman Subira. Anapatikana kati ya 8am na 5pm kujibu maswali, wasiwasi au ulalamishi kuhusu utafiti huu au kujitoa kutoka kwa utafiti huu.

Kauli ya Ridhaa

Nimesoma maelezo katika fomu hii ya ridhaa zikiwemo hatari na faida ambazo huenda zikapatikana. Maswali yangu yote kuhusu utafiti huu yamejibiwa vilivyo na nimeridhika. Naelewa kwamba niko na uhuru wa kujitoa katika utafiti huu wakati wowote bila ya kupata adhabu au kupoteza faida zozote ambazo ni haki yangu.

Nimeridhia kushiriki katika utafiti huu.

KUKIRI NA KUKUBALI KUSHIRIKI

Mshiriki

Mimi…… …… nakubali kushiki katika utafiti huu

Jina Sahihi

Shahidi

Mimi…… …… nimeshuhudia kukubali kwa mshirika.

Jina Sahihi

Appendix 4 Assent Form

Study Title: Prevalence, species distribution, antifungal susceptibility profiles of *Candida* and risk factors associated with isolation of *Candida* in patient samples submitted for analysis at the Mombasa Hospital
Principal Investigator: Abdulrahman Subira - Laboratory Manager, The Mombasa Hospital P.O. Box 90294 G.P.O 80100 Mombasa, Kenya. **Telephone:** +254 41 2312191 Ext.222 E-mail addresses labmnager@mombasahospital.com
Supervisors: Professor Simon Karanja and Dr. Rahma Udu

Contact Principal Investigator

The Principal Investigator in this study is Mr. Abdulrahman Subira. He can be reached between 8 am and 5p m for clarifications on any concerns or input pertaining to the study including withdrawal from the study.

What is a research study?

Research studies help us learn new things or test new ideas. First, we ask a question. Then we try to find the answer.

1. About this form
    1. This form explains a research to be done and the choice that you have to take part in it.
    2. Please read it carefully before making any decision. Feel free to discuss it with your parents, doctor, colleagues, any trusted friend or family member.
    3. We want you to ask us any questions that you have in relation to this study. You can ask questions any time.

2. Important things to know
    1. You get to decide if you want to take part.
    2. You can say 'No' or you can say 'Yes'. No one will be upset if you say 'No'.
    3. If you say 'Yes', you can always say 'No' later (at anytime).
    4. We would still take good care of you no matter what you decide.

3. Purpose of this research
    1. To determine the prevalence and distribution of *Candida* species in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.

2. To determine the antifungal susceptibility profiles of the *Candida* isolated from specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.
3. To determine the risk factors associated with isolation of *Candida* in specimens submitted by patients with clinical symptoms of infection for analysis at the Mombasa Hospital.

4. What would happen if I join this research?

If you decide to be in the research, we would ask you to do the following:

1. You will be asked a few questions as part of the usual doctor's history taking to understand your condition better. The questions may take about 5 minutes. Please provide correct information to assist us in making meaningful conclusions.
2. Depending on your symptoms and illness, your doctor shall ask you to produce the relevant specimens and submit to the laboratory for testing.
3. Your test results shall be taken to your doctor within 24 to 72 hours
4. Your result will be secretly analyzed using your lab number/code and not your name to understand how the *Candida* (if isolated) responds to the antifungal medication.
5. If further information is required such as the treatment provided, it will be obtained through your doctor.
6. The results will then be submitted to the relevant authorities to assist in policy making with regards to treatment of *Candida* infections.

5. Could bad things happen if I join this research?

1. Some of the tests might make you uncomfortable or the questions might be hard to answer. We will try to make sure that no bad things happen.
2. You can say 'no' to what we ask you to do for the research at any time and we will stop.

6. Could this research help me?

This research will not help you directly apart from you knowing the cause of your illness and getting the right treatment. We however do hope to learn something from this research. And someday we hope it will help other kids who have symptoms like you do.

7. Assurance of confidentiality

No one will know about the result of your test or any information obtained from you apart from your doctor and parents/guardian. He research team will access your information in a coded way and your name shall be hidden from them. Unauthorized personnel shall not have access to the data.

8. What else should I know about this research?
    1. If you don't want to be in the study, you don't have to be.
    2. It is also OK to say yes and change your mind later. You can stop being in the research at any time. If you want to stop, please tell the research doctors.
    3. You would not be paid to be in the study.
9. Acknowledgement and acceptance to participate

If you want to be in the research after we talk, please write your name below. We will write our name too. This shows we talked about the research and that you want to take part.

Name of Participant _____

(To be written by child/adolescent)

Printed Name of Researcher

_____

Signature of Researcher

_____

Date Time

Appendix 5 Ethical Review Approval Certificate

NACOSTI ACCREDITED

ERC/MSc/039/2015

Pwani UNIVERSITY

ETHICS REVIEW COMMITTEE

ACCREDITTED BY THE NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION (NACOSTI, KENYA)

CERTIFICATE OF ETHICAL APPROVAL

THIS IS TO CERTIFY THAT THE PROPOSAL SUBMITTED BY:

ABDULRAHMAN SUBIRA

REFERENCE NO:
ERC/MSc/039/2015

ENTITLED:
Prevalence and Antifungal susceptibility profiles of various Candida species isolated from patients attending Mombasa Hospital

TO BE UNDERTAKEN AT:
MOMBASA COUNTY, KENYA

FOR THE PROPOSED PERIOD OF RESEARCH

HAS BEEN **APPROVED** BY THE ETHICS REVIEW COMMITTEE

AT ITS SITTING HELD AT PWANI UNIVERSITY, KENYA

ON THE **30th DAY OF OCTOBER 2015**

CHAIRMAN SECRETARY LAY MEMBER

PTO

Pwani UNIVERSITY

Ethics Review Committee,
Pwani University, www.pu.ac.ke, email: r.thomas@pwaniuversity.ac.ke, tell: 0719 182218.
The ERC, Giving Integrity to Research for Sustainable Development