SPECIES DISTRIBUTION AND ANTIFUNGAL SENSITIVITY PATTERNS OF VAGINAL YEASTS AT THE AGA KHAN UNIVERSITY HOSPITAL

A dissertation submitted in part fulfillment of the requirements of the Masters of Science in Medical Microbiology

Department of Medical Microbiology, University of Nairobi

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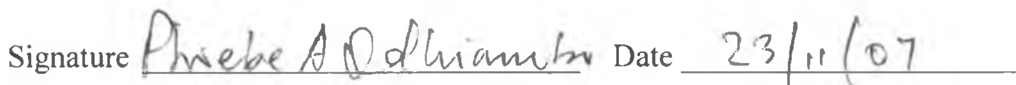

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DEDICATION

To Fred, for all your support

To my children Sally and Peter, for all the sacrifices you made

To Sarah, without whom this may never have happened

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LIST OF ABBREVIATIONS

AFST.......... Antifungal susceptibility testing

AIDS.......... Acquired Immune Deficiency Syndrome

AKUH .... Aga Khan University Hospital

API .... Analytical Profile Index

ATB .... Antibiogram

CDC .... Centers for Disease Control and Prevention

CLSI .... Clinical and Laboratory Standards Institute

CMI .... Cell mediated immunity

DNA ....Deoxyribonucleic acid

GTT .... Germ tube test

HIV............ Human Immunodeficiency Virus

HVS .... High vaginal swab

INT .... Nit1 & Nit 2 reagents

KNH .... Kenyatta National Hospital

$\mu g ml^{-1}$ .... Micrograms per milliliter

MIC .... Minimal inhibitory concentration

NCCLS...... National Council for Clinical Laboratory Standards

ND............ Not defined

OTC .... Over-the-counter

QC............ Quality control

RNA .... Ribonucleic acid

RVVC .... Recurrent vulvovaginal candidiasis

SDA .... Sabouraud dextrose agar

SDD.......... Susceptible dose dependent

SPSS .... Statistical Package for Social Sciences

STD/ STI... Sexually transmitted disease/ infection

VVC......... Vulvovaginal candidiasis

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ABSTRACT

Background: Species-level identification of yeasts and antifungal susceptibility testing is not a common practice in Kenya. Thus there is limited information on the distribution of yeast species causing vaginal infections and their susceptibility patterns to antifungal agents.

Objectives: To identify yeast isolates in vaginal specimens to species level and determine their antifungal susceptibility patterns to flucytosine (5FC), amphotericin B (AMB), fluconazole (FCA) and itraconazole (ITR).

Design: Cross-sectional in vitro study

Setting: Aga Khan University Hospital (AKUH), Nairobi

Method: One hundred and one yeasts isolated from women with vaginal discharge were identified to species level using the API Candida system. The isolates were then subjected to broth microdilution susceptibility testing and data was analyzed using SPSS for Windows version 12.0.

Results: *C.albicans* was the prominent species (69.3%) followed by *C.glabrata* (12.9%), *C.famata* (5.0%), *C.krusei* (3.0%), *C.parapsilosis* (1.0%), unidentified *Candida* species (3.0%), *Trichosporon* species (3.0%) and *S.cerevisiae* (3.0%). The percentages of *C.albicans* susceptible to flucytosine (5FC), amphotericin B (AMB), fluconazole (FCA) and itraconazole (ITR) were 94.3, 92.9, 100 and 90 respectively; that of non-albicans isolates were 93.5, 80.6, 77.4 and 29 respectively.

There was no significant difference ($p>0.05$) between the susceptibility of *C albicans* and non-albicans isolates to 5FC and AMB, however there was a significant difference ($p<0.05$) to FCA and ITR.

Conclusion: *C.albicans* is still the predominant species causing vulvovaginal candidiasis and demonstrates good susceptibility to all antifungal agents tested. Non-albicans yeasts are a significant cause of vulvovaginal candidiasis and demonstrated reduced susceptibility to all drugs, especially the azoles which are commonly used for treatment of vaginal candidiasis. The isolation of non-albicans yeasts may have clinical implication given their reduced susceptibility to antifungals.

1. INTRODUCTION

1.1 Background of the study

Vaginal candidiasis, also known as "a yeast infection", moniliasis or vulvovaginal candidiasis (VVC), is a common fungal infection that causes considerable distress to women, their partners and, to a lesser extent, their doctors, yet continues to receive very little attention from public health authorities, funding agencies and researchers. VVC is caused by an overgrowth of 'yeast-like fungi' of the genus *Candida*, which is usually present in the lower female genital tract in small amounts, being part of the normal flora in 10-20% of healthy asymptomatic women (1).

Candida albicans is the most frequent colonizer of the lower female genital tract and is responsible for most cases of VVC (2, 3). Other *Candida* species e.g. *C. glabrata*, *C. tropicalis* previously only caused vaginal candidiasis rarely; but there have been reports demonstrating an increase in the number of cases caused by non-albican species (4). Seventy five percent (75%) of all women develop VVC in their lifetime, 90% of which are caused by *C. albicans*. Five percent (5%) of women with VVC may develop recurrent vulvovaginal candidiasis (RVVC), defined as four or more episodes of VVC in one year. Thirty three percent (33%) of recurrent infections are caused by non-albican *Candida* (5).

Multi-drug resistant microorganisms are a major challenge worldwide and fungi have not been left behind. The number of antifungal agents continues to increase in the setting of a shift of candidal infections to those caused by non-albican species. Due to this, identification to species level and increased use of susceptibility testing has become necessary in order to appropriately select the agent to use (6). Decreased susceptibility of non-albican species of *Candida* and development of resistance by *C. albicans* to azoles led to the development of antifungal susceptibility testing (AFST) (6). The goal of AFST is to predict clinical response, or at least to forecast treatment failure. AFST can be used:

- To develop local antibiograms to aid in empirical selection of antifungals
- In testing of isolates to aid in selection of long-term therapy
- In testing of isolates from recurrent mucosal disease to aid in selection of

alternative regimens

However, antifungal drug sensitivity profiles are not routinely carried out in Kenya and therefore the present status of fungal resistance to conventional antifungal drugs is unknown.

This study is designed to determine the species of yeasts causing vaginal infection and the current antifungal susceptibility patterns in our set up so as to make appropriate recommendations for the management of vaginal candidiasis.

2. LITERATURE REVIEW

2.1 History

Written descriptions of thrush date back to the early 1800s. In 1839 Bernard Langenbeck described the organism he found in oral lesions of a patient as "Typhus-Leichen" (typhus bodies). By 1841 Emil Berg established the fungal etiology of thrush by infecting healthy babies with what he called "aphthous membrane material". In 1843 Charles Robin gave the organism its first name, *Oidium albicans*. More than 100 synonyms have been used for this fungus since but *Candida albicans*, the name proposed by Roth Berkhout in 1923, has persisted. Historically, the most interesting period for candidiasis research coincided with the introduction of antibiotics (7).

2.2 Features and characteristics

Candida is a genus of 'yeast-like fungi', which has more than 100 species that exist in nature though only a few of these are recognized causes of disease in humans (8). The medically significant species and their prevalence are as shown below:

- *C. albicans* 50-60%
- *C. glabrata* 15-20%
- *C. parapsilosis* 10-20%
- *C. tropicalis* 6-12%
- *C. krusei* 1-3%
- *C. kefyr* <5%
- *C. guilliermondi* <5%
- *C. lusitaniae* <5%
- *C.dubliniensis* which is primarily recovered from patients who are HIV positive.

C. albicans is normally present in the mouth, intestine and vagina and disturbances in local conditions of these sites or impairment of local or systemic host defenses results in infections.

C. albicans normally grows as a thin-walled, non-capsulated oval yeast 2-4µm in diameter. In tissues it may appear as yeasts or pseudohyphae and also in cultures when aeration is poor. The yeast and the pseudohyphae readily stain gram positive with the Gram stain. At temperatures below 26°C in nutritionally poor media like cornmeal agar it characteristically produces thick-walled resting cells 7-17µm in diameter called chlamydospores. No sexual forms are known (9).

C. albicans also characteristically produces curved, elongated germ tubes or blastoconidia within three hours when the yeast is transferred from a peptone-containing medium to mammalian serum at 37°C. In order to differentiate the germ tube from the blastoconidial germination of *C. tropicalis*, the blastoconidia of *C. tropicalis* has constriction at the neck while that of *C. albicans* does not have a constriction (10).

Growth requirements are simple and *Candida* species grows well on Sabouraud agar and blood agar. Colonies have a distinctive yeast smell and are usually 0.5mm in diameter after 18 hours and develop into high convex, off-white colonies 1.5mm in diameter after two days (11). The primary identification procedure involves presumptive differentiation of *C. albicans* from other *Candida* species with the germ tube test. Germ tube negative strains may be further identified biochemically (12). The germ tube test is considered presumptive because not all isolates *of C. albicans* will be germ tube positive and false positive results will be obtained especially with *C. tropicalis*. *C. dubliniensis* is also germ tube positive (10). On microscopic observation of the preparation, the short hyphal initials produced by *C. albicans* are not constricted at the junction of the blastoconidium and the germ tube. *C. tropicalis* also produces hyphal initials but the blastoconidia are larger than those of *C. albicans* and there is a definite constricture where the hyphal initial joins the blastoconidium.

C. krusei is important due to its intrinsic resistance to ketoconazole and fluconazole and it is also less susceptible to all other antifungals, including itraconazole and amphotericin B. *C. lusitaniae* on the other hand shows frequent resistance to amphotericin B but remains susceptible to all azoles and echinocandins (8).

2.3 Pathogenesis

Colonization of the female genital tract by *C.albicans* is aided by the ability of the yeast to adhere to mucosal cells (12). Mannoprotein components of the outer fibrillar surface of the organism are implicated as the adhesion and fibronectin and other components of the extracellular matrix as the receptors (8). These mediate binding.

Colonization of mucocutaneous membranes is the first step in the development of infection. This colonization is however kept under control by the bacterial normal flora, especially the lactobacilli which suppress the growth of the yeast by maintaining a low pH in this area. Use of broad spectrum antibiotics suppresses the normal bacterial flora resulting in an overgrowth of the *Candida* and infection. This can also result from use of vaginal antiseptic and vaginal douching which also cause changes in normal mucosal pH.

The pathogenicity of *C.albicans* is strongly associated with a shift from the yeast form to the hyphal form. During the hyphal phase there is appearance of virulence factors associated with adherence and invasion. The less virulent *Candida* species do not form hyphae. *C.tropicalis* has virulence at least equal to that of *C.albicans* although it does not form hypae. The hyphae may be involved in penetration of mucosal barriers and spread of the yeast. Liberation of extracellular enzymes such as proteases and elastases, are also associated with virulent and invasive species. These digest the epithelial cells and facilitate invasiveness and may also play a role in adherence.

Some isolates of *C. albicans* are unusually hydrophobic and adhere tenaciously to catheters and other plastic surfaces. Other virulence factors include a receptor analogous to complement receptor 3 which binds inactivated C3b (iC3b) (13), phenotypic switching and surface variation (10).

2.4 Predisposing factors

Vaginal candidiasis is not usually considered a sexually transmitted infection (STI) due to several reasons: it has a mild and transitory nature and is frequently isolated from healthy women with no apparent risk (5, 14) as normal flora and also affects celibate women.

VVC is, however, included in the CDC guidelines for treatment of STI because it is often diagnosed in women being evaluated for STIs (1). However, the extent to which *C. albicans* can be regarded as a normal flora of the genital tract is unclear. Several studies describe *C. albicans* in vaginal swabs taken from women without symptoms or clinical signs of vulvar or vaginal infection (14, 15, 16). One study found that clinical signs of infection could be detected in all pregnant women from whom the fungus was isolated, even in those without symptoms (17). These observations suggest that changes in the host vaginal environment are required before the fungus can exert its pathological effects. The changes that allow symptomatic vaginal infection with *C. albicans* to occur are unknown, but the condition has been associated with several precipitating factors (18):

- Antibiotics are frequently implicated as a cause of VVC especially in recurrent infections. Frequent antibiotic use results in a decrease in protective vaginal flora and allows for colonization by *Candida* species. The risk of a yeast infection increases with the duration of antibiotic use, but no specific antibiotic has been shown to be more likely to cause yeast infection (19). Vaginal douching or use of vaginal antiseptic or antibiotic preparations also alters the composition of the microorganisms in the vagina and may reduce the protective lactobacilli resulting in proliferation of the *Candida*.
- Diabetes mellitus is often considered a predisposing factor for RVVC. Hyperglycemia enhances the ability of *C. albicans* to bind to vaginal epithelial cells due to greater production of the surface adherence proteins in the presence of high glucose concentrations (12). However, RVVC is not a classical symptom for diagnosis of diabetes and patients with RVVC are rarely found to be diabetic (20).
- Contraceptive methods, hormonal therapy and pregnancy may also promote recurrences of vulvovaginal candidiasis. Spermicidal gels and creams alter vaginal flora and increase the adhesion of *Candida* organisms. Women who take oral contraceptive pills have a higher rate of VVC (4). One theory suggests *Candida* cells have estrogen and progesterone receptors that, when stimulated, increase fungal proliferation (20). Vaginal candidiasis is much more common in pregnant women. Moreover, a large proportion of women with chronic recurrent candidiasis first present with the infection during pregnancy (18).

- Both humoral immunity and cell-mediated immunity (CMI) are important in defense against *Candida* infections (12). With humoral immunity, the yeasts are opsonized by antibody and complement then readily phagocytosed and killed by neutrophils. In the absence of antibody, the process is less efficient. Hyphae are too large to be phagocytosed and are killed by neutrophils when they attach to the hyphae and discharge metabolites produced through the oxidative metabolic burst. Hence a deficit in neutrophils or neutrophilic function is related to serious *C. albicans* infection. The CMI is a very important arm of the immune system in *Candida* infections. There is an increased frequency of vaginal candidiasis in AIDS patients, which suggests even superficial infections involve T-lymphocyte mediated immune responses. Use of cytotoxics and steroid therapy also suppresses the immune system and as a result may cause an increased frequency of vaginal candidiasis.
- There is a common belief that wearing of nylon underwear or tights predisposes to VVC. This remains unproven (4) but may be explained by the inability of this material to absorb moisture providing suitable growth conditions for *Candida*.

In contrast to women who have infrequent episodes of vaginal candidiasis, women with chronic or recurrent infections seldom have recognizable precipitating or causal factors (5). These women tend not to use oral contraception or to have been frequently prescribed broad-spectrum antibiotics; they also tend to have normal results in glucose tolerance tests.

2.5 Clinical presentation

Most women with vaginal candidiasis complain of intense vulval and vaginal pruritus with or without vaginal discharge. The condition often develops quickly, and in women who are not pregnant it tends to begin during the week before menstruation. Some women complain of recurrent or increasing symptoms preceding each menstrual period. Dysuria and dyspareunia are common (18).

Vulvar erythema with fissuring is the most common clinical finding. This is often localized to the mucocutaneous margins of the vaginal introitus and the fourchette, but it can spread to affect the labia majora and the perineum. Vaginitis with discharge is often

commonly found. Thick white adherent plaques on the vulval, vaginal, or cervical epithelium are the classic signs of florid vaginal candidiasis. Often the discharge is thick and white, but it can be thin or even purulent.

On the basis of clinical presentation, microbiology, host factors and response to therapy, VVC can be classified as either uncomplicated or complicated (Box 1). Approximately 10-20% of women will have complicated VVC, suggesting diagnostic and therapeutic considerations (1).

Box 1. Classification of vulvovaginal candidiasis (VVC). (1)

Uncomplicated VVC	**Complicated VVC**
• Sporadic or infrequent VVC	• Recurrent VVC
OR	**OR**
• Mild-to-moderate VVC	• Severe VVC
OR	**OR**
• Likely to be *C.albicans*	• Non-albicans candidiasis
	OR
OR	• Women with uncontrolled diabetes, debilitation, or immunosuppression or those who are pregnant
• Non-immunocompromised women	

2.6 Differential diagnosis

There are several conditions that cause vaginal discharge and/or pruritus which underlines the importance of proper diagnosis before starting treatment. Other conditions that also present with vaginal discharge include trichomoniasis, bacterial vaginosis, chlamydial infections and gonococcal infections. Mucosal pruritus may also be caused by herpes infection, contact dermatitis, psoriasis and allergies (including reactions to topical antifungal treatments) (18).

Widespread use of over-the-counter (OTC) antimycotics is resulting in a rapid increase in the amount of drug resistance in *Candida* (5). OTC drugs favor short, inappropriate courses of therapy, which results in elimination of more sensitive species (*C. albicans*) and selection of more azole-resistant species (non-albican *Candida*). Resistance to azole antifungals also continues to be a significant problem with the common fungal pathogen *C.albicans* (21). However, a study on women with previous exposure to OTC drugs (22) has shown no correlation between previous OTC drug exposure and colonization of drug-resistance *Candida* in the vagina. Only a small number of isolates obtained from women with history of multiple exposures to the OTC antifungals showed resistance.

In general practice, symptoms such as pruritus and vaginal discharge are commonly the basis for diagnosing candidiasis, sometimes without even a genital examination. Patients' assessment of symptoms of discharge is not, however, a reliable method of diagnosing candidiasis. The complaints are non-specific and there are other conditions that present with similar symptoms hence it is important to confirm the actual cause of the vaginal discharge and/ or pruritus. Eckert *et al* (23) looked at this diagnosis problem in 774 women visiting a STD clinic. Of the 545 women with symptoms of either increased vaginal discharge or vulvar pruritus or burning, only 28% had positive cultures for *C.albicans*; 53% had other sexually associated infections. Of 356 women with history of vaginal yeast infections and symptoms of vulvar pruritus, burning or increased vaginal discharge only 29% had positive cultures for *C.albicans*; 47% had other STIs or bacterial vaginosis. Out of 107 women with, symptoms of vulvar pruritus or burning without increased vaginal discharge, 36% had positive cultures for *C.albicans*. In total, 186 women had *C.albicans* by culture. Of these 56% had positive potassium hydroxide (KOH) wet mounts. Fifteen women with positive cultures for species other than *C.albicans*; all had negative KOH wet mount.

C.albicans is still the commonest cause of VVC, but there has been a demonstrable increase in the number of cases caused by other species of *Candida*, some of which have reduced susceptibility to antifungal drugs (4, 6).

The ARTEMIS DISK Global antifungal surveillance study (24) was carried out in 39 countries between June 1997 and December 2003 and studied 140,767 yeast isolates from various body sites. The range of species distribution was *C.albicans* 58.5-69.8%, *C.glabrata* 9.2-11.5%, *C.famata* 0.1-0.4%, *C.krusei* 1.6-3.1%, *C.parapsilosis* 4.0-7.0%, *C.tropicalis* 4.4-7.2%, *Trichosporon* species 0.3-0.9% and *Saccharomyces cerevisiae* 0.2-0.7%. Susceptibility to fluconazole was *C.albicans* 97.8% (1.3% resistant), *C.glabrata* 66.7% (16.6% resistant), *C.famata* 79.8% (11.9% resistant), *C.krusei* 9.4% (77.2% resistant), *C.parapsilosis* 93.2% (3.6% resistant) and *S.cerevisiae* 86.9% (6.8% resistant).

Namkinga *et al* (25) carried out species identification of 272 isolates from pregnant women and found *C.albicans* 66.2%, *C.tropicalis* 4.7%, *C.glabrata* 7.35%, *C.famata* 2.2%, *C.parapsilosis* 2.2% and *C.lusitaniae* 0.37% using API Candida. The identification kit could not speciate 15.8% of isolates.

Spinillo *et al* (19), in a study carried out between 1991 and 1997 on 2043 patients found 34.1% positive for *Candida* species; 89.2% were *C.albicans*, 5.6% *C.glabrata*, 2.6% *C.krusei*, 0.7% *S.cerevisiae*, 1% *C.tropicalis* and 0.9% other *Candida* species.

Verghese *et al* (26) studied 326 HVS specimens and isolated *Candida* in 12.9% of them. Of these, 40.5% were *C.albicans*, 30.1% *C.glabrata*, 14.3% *C.tropicalis* and 7.1% *C.krusei*. All isolates were susceptible to amphotericin B; 16.7% of isolates demonstrated resistance to fluconazole, 45.2% to itraconazole and 9.5% to nystatin.

Galle *et al* (27) carried out a similar study on 250 vaginal swabs and isolated *Candida* in 27.6% of samples. Of these, 74% were *C.albicans*, 14.5% *C.glabrata*, 7.3% *C.tropicalis* and 4.3% *C.parapsilosis*. All *C.albicans* were susceptible to amphotericin B, 84.3% susceptible to fluconazole and one isolate was resistant to itraconazole. For the non-albicans species, 11.8% were resistant to fluconazole and 23.5% to itraconazole, only one sample showed amphotericin B MIC of $2 \mu g ml^{-1}$.

Richter *et al* (28) studied 593 vaginal yeast isolates between January 1998 and March 2001. 70.8% were *C.albicans*, 18.9% *C.glabrata*, 5.1% *C.parapsilosis*, 2.0% *C.krusei*, 1.35% *C.tropicalis*, 0.2% *Trichosporon* species and 1.5% *S.cerevisiae*. Flucytosine had 3.3% *C.albicans* resistant, 51.8% *C.glabrata* and 3.3% *C.parapsilosis* susceptible-dose dependent (S-DD), 8.3% *C.krusei* resistant and 50% S-DD and 100% *Trichosporon* species resistant. Itraconazole resistance was demonstrated by 74.1% *C.glabrata*, 3.4% *C.parapsilosis*, 58.3% *C.krusei* and 55.6% *S.cerevisiae*. Overall, 3.7%, 3.0% and 16.2% of isolates demonstrated resistance to fluconazole, flucytosine and itraconazole respectively.

Nawrot (29) studied 206 yeast isolates from various clinical specimens between December 1999 and February 2001. Overall 57.3% were *C.albicans*, 14.1% *C.glabrata*, 11.2% *C.krusei*, 2.4% *C.tropicalis* and 0.5% *C.parapsilosis*. Overall susceptibility to flucytosine and amphotericin B was 83% and 99%. *C.albicans* demonstrated 92% and 75% susceptibility and 4% and 11% resistance to fluconazole and itraconazole respectively.

Comert *et al* (30) carried out a similar study on 320 isolates between 2002 and 2005 and found 65.5% *C.albicans*, 11.3% *C.parapsilosis*, 8.8% *C.glabrata*, 7.8% *C.tropicalis* and 1.25% *C.famata*. Overall susceptibility to fluconazole was *C.famata* 100%, *C.parapsilosis* 94.4%, *C.albicans* 92.5%, *C.glabrata* 85.7% and *C.tropicalis* 83%.

2.7 Antifungal agents

2.7.1 Mechanisms of action

There are five classes of antifungal agents based on the mechanism of action of the drug (31):

- Polyenes, which kill the fungal cell by binding primarily to ergosterol, the major sterol found in fungal cellular membrane. This destroys the osmotic integrity of the membrane resulting in leakage of intracellular potassium, magnesium, sugars and metabolites resulting in cell death.

The two drugs in this group, Amphotericin B and Nystatin, are active against most strains of *Candida*

- Azoles, which are fungistatic and cause partial inhibition of fungal cells by inhibition of cytochrome P- 450 14α-demethylase which functions in synthesis of ergosterol from lanosterol. This results in termination of fungal cell membrane synthesis. There are two different classes of azoles:
    a) Imidazoles which consist of an azole ring with 2 nitrogens. Ketoconazole belongs to this group and has limited activity against *Candida* species compared to triazoles
    b) Triazoles which consist of an azole ring with 3 nitrogens. Fluconazole is one of the most commonly used drugs against vaginal candidiasis and is active against most species of *Candida*.

       Itraconazole is another antifungal agent in this class

       Candida isolates resistant to one drug in this group are more likely to show cross- resistance with other azoles.

       C. krusei has intrinsic resistance to fluconazole. Acquired resistance to fluconazole, even among *C. albicans*, may occur following long-term prophylaxis especially in patients with AIDS.

       Some *C. glabrata* species have dose-dependent susceptibility while about 15% exhibit true resistance.
- Antimetabolites to which flucytosine belongs but the drug has no antifungal activity until it is converted to 5-fluorouracil in the fungal cells. 5-fluorouracil then acts by disrupting pyrimidine metabolism and therefore disrupts synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins in the fungal cells. Primary resistance or reduced susceptibility in *Candida* strains is not uncommon.
- Allylamines include terbinafine and naftifine and act by inhibiting ergosterol biosynthesis through inhibition of squalene epoxide enzyme resulting in accumulation of squalene which increases membrane permeability and causes cell death. This class is active against various *Candida* species.

- Echinocandins include caspofungin and anidulafungin. They are lipopeptides with broad-spectrum antifungal activity but have limited activity against *C. parapsilosis* and *C. guilliermondii*. They inhibit glucan synthesis resulting in cessation of fungal cell wall synthesis

2.7.2 Mechanisms of resistance to antifungal agents

Fungal infections are opportunistic infections associated with immune suppression/dysfunction. The frequency of patients with immune suppression/dysfunction has increased especially due to AIDS. The rise in fungal infections has, at least in part, resulted in an increased use of antifungal drugs both for localized and systemic fungal infections. This expanded use of antifungal drugs has accelerated the development of antifungal drug resistance (32).

Several factors have been implicated in this development of resistance, including:

A: Clinical components

a) Host factors

- The host immune status normally works together with the drugs in controlling the infection. Infections in patients with immune dysfunction are more likely to be recalcitrant to treatment since the drug must control the infection on its own
- Site of the infection: Protected sites or sites inaccessible to drugs contribute to clinical resistance for not achieving therapeutic concentrations
- Severity of the infection: Larger concentrations of fungal cells require higher concentrations of the drug and / or longer treatment
- Patient compliance: non-compliance reduces the effectiveness of the drug and fails to clear the infection allowing organisms resistant to the drug to persist and become the dominant strains

b) Drug characteristics

- Fungistatic drugs, such as azoles, do not kill the cells hence are more likely to allow cells to develop resistance
- Absorption, distribution and metabolism of the drug: If this is not adequate, it affects the overall effectiveness of the drug at the site of infection

- Improper dosing in terms of quantity, frequency, fails to clear the infection allowing persistence of more resistant strains
- Drug-drug interactions can alter the effectiveness of the antifungal drugs: cyclosporine increases the effectiveness of flluconazole against *C. albicans*.

c) Fungal factors

- The species and strain determine which drugs should be used. For example, *C. krusei* has intrinsic resistance to fluconazole
- The cell type: Yeasts, hyphae, chlamydospores, conidia and mycelia can have a specific susceptibility to antifungal drugs
- Different phenotypes and serotypes vary in susceptibility to antifungal drugs
- Cell biofilm: The cells form a plaque or mat on a surface which has altered antifungal susceptibility
- Genome stability of the fungus: the development of resistance is common in haploid *C. glabrata* but infrequent in diploid *C. albicans*
- Presence of a large population of organisms increases the chance of a random mutation which will result in a resistant fungal cell in the infection
- Iatrogenic factors: Replacing an infected catheter in a patient with a systemic fungal infection on antifungal treatment results in colonization of the new catheter by fungal cells already exposed to the drugs hence the cells may be resistant
- MIC for the strain tested in vitro can predict resistance in vivo by defining breakpoints but this may not always be the case.

B. Intrinsic resistance

a) Intrinsic azole resistance found in *C. krusei* and *C. glabrata*, which are increasing in frequency as causative agents of vaginitis.

b) Intrinsic Amphotericin B and 5-flurocytosine resistance is commonly observed in some non-albicans Candida and other yeasts. *C. lusitaniae* has intrinsic resistance to Amphotericin B. Intrinsic resistance to flucytosine is common in many fungi.

C. Acquired resistance:

Azole resistant strains of *C. albicans* causing oral candidiasis are becoming a significant problem. Unlike oral isolates, vaginal isolates have not shown an increased MIC to azoles, even after long-term exposure. *C. dubliniensis,* which is more common in patients with HIV, has an increased ability to develop resistance to azole drugs. The development of azole-resistance is most likely to result from a commensal strain that is resistant or that develops resistance. A resistant strain can also be acquired by nosocomial transmission or intimate contact.

D. Cellular mechanisms

a) Change to a more resistant species or strain as a result of prolonged exposure to antifungal agents or spontaneous mutations. The resulting resistant strain will then persist in an infection and selectively out-grow the other cells under drug pressure.
b) Transient resistance refering to resistance induced in the presence of a drug, either by gene activation or repression which persists in the presence of the drug and the cells revert to a susceptible phenotype in the absence of the drug.
c) Heterogenous antifungal resistance is an unusual phenomenon in which a small percentage of cells in a population are resistant. When this resistant population is selected and grown in culture, only a small percentage of the cells in culture remain resistant. This has been observed for azoles in *C. albicans* Strains that exhibit this phenomenon are susceptible when antifungal susceptibility is determined by the standard methods of determining MICs.

E. Molecular mechanisms: This is mainly through alterations in ergosterol biosynthesis by alterations in the target enzyme or other enzymes in the pathway, as well as import, metabolism, accumulation, modification, activation, and efflux of the drug.

2.8 Problem Statement

The prevalence of VVC in Kenya is approximately 10% (33) and increases to 50% if pregnant women are included (34). VVC causes a lot of distress to women with the discomfort of vaginal discharge and an intense itch. It also causes marital problems with women being accused of infidelity though it is not classified as a sexually transmitted infection.

In most instances, cases are diagnosed based on the patient's description of symptoms, laboratory diagnosis being very rarely carried out. The symptoms of curd-like vaginal discharge and vaginal pruritus are non-specific and cannot be used to conclusively diagnose vaginal candidiasis. Many patients self-diagnose and buy over-the-counter drugs for treatment.

Majority of clinicians are aware of the existence of only one species of *Candida*, *C. albicans* which is the commonest species isolated and generally susceptible to most antifungal agents. However, studies have reported emergence of "new" *Candida* pathogens that previously did not cause infection now causing infection (4). These include *C. glabrata, C. tropicalis* and *C. krusei* among others and have been noted to have decreased susceptibility to antifungals (6). *C. albicans* has also developed resistance to azole derivatives.

Despite the reported magnitude of VVC in Kenya, relatively little is known about the predominant species and their susceptibility patterns to anti-fungals. In this country, species identification and antifungal drug sensitivity profiles are not routinely carried out, as a matter of fact no research efforts are dedicated to clinical mycology. As a result, management of VVC has been limited to empiric approach of clinically suspected cases of VVC. An updated information on species distribution and their susceptibility to antifungals is necessary to help set up guidelines in managing patients with vulvovaginal candidiasis.

2.9 Research Questions

In view of the above, some questions were raised:

1. What is the proportion of *C.albicans* vaginal infection and non-albicans infection?
2. What are the antifungal susceptibility patterns

2.10 Justification of the study

The results of this study may benefit the following groups:

a) Clinicians

- The study will create awareness on the existence of other yeasts as causes of vulvovaginal candidiasis, apart from the widely know *C albicans*. Awareness will also be created on the antifungal susceptibility patterns of these vaginal yeasts to commonly prescribed azole derivatives.
- The study will also emphasize on the importance of laboratory testing especially in patients with recurrent or refractory vulvovaginal candidiasis

b) Researchers and postgraduate students

- The study will form a base-line and encourage more studies on yeast species distribution and the current status of drug susceptibility in the country and assist in the development of local antibiograms

c) The Ministry of Health

- The study will create awareness that changes need to be made in the algorithm for management of patients with suspected vulvovaginal candidiasis

d) The patient

- With the application of the study to the diagnosis in patients with suspected vulvovaginal candidiasis, the patient will be able to get better diagnosis of the condition and appropriate treatment

2.11 Objectives

2.11.1 General Objectives

To identify the species of yeasts in vaginal discharge and their antifungal sensitivity patterns

2.11.2 Specific Objectives

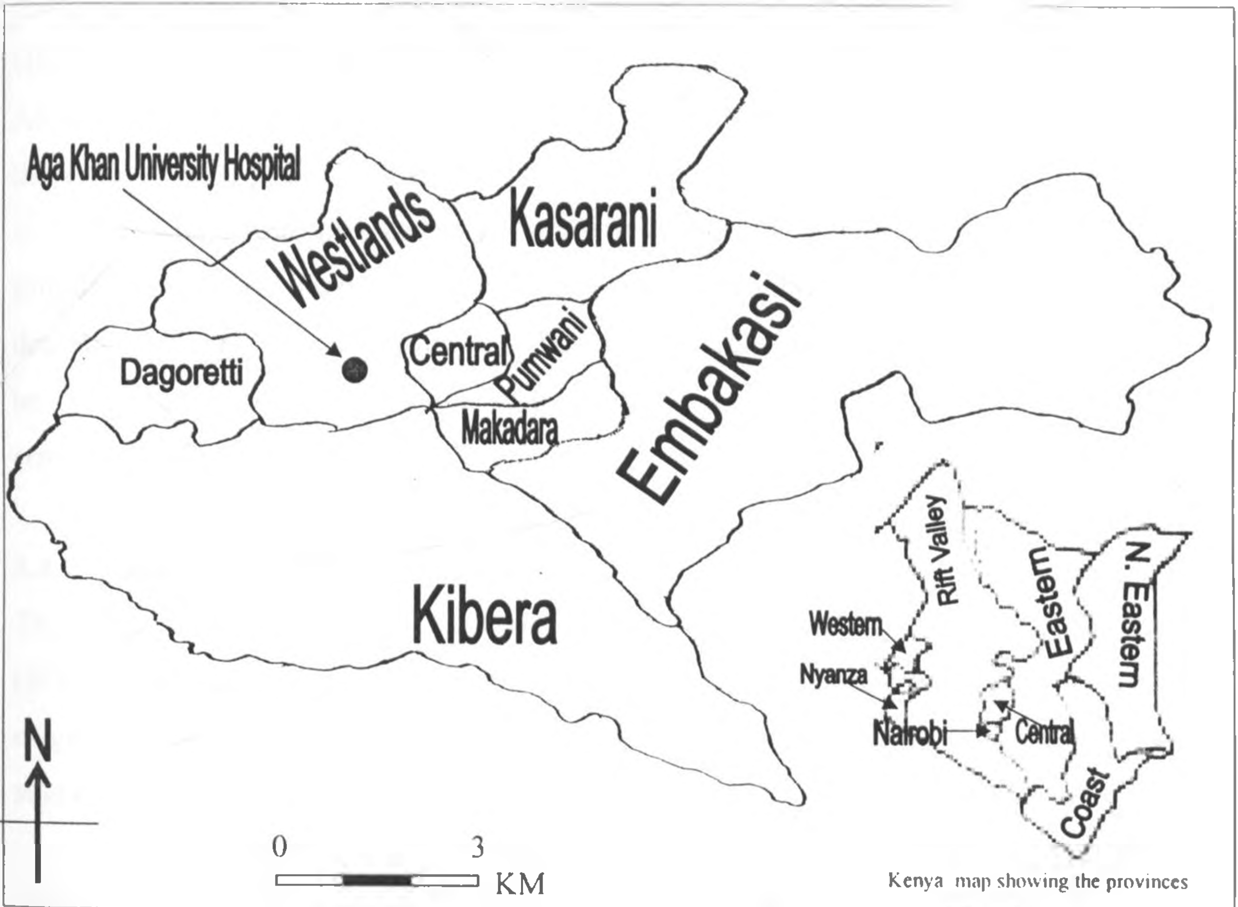
- To determine the proportions of *C. albicans* and non-albicans yeast infections
- To determine the antifungal susceptibility patterns of vaginal yeasts

3. DESIGN AND METHODOLOGY

3.1 Area of study

The study was carried out at the Aga Khan University hospital (AKUH), a university affiliated hospital located in Nairobi, Kenya. Specimens were analyzed in the microbiology laboratory of the hospital

Map showing the location of the Aga Khan University Hospital. Inset showing the position of Nairobi



Source: NAIROBI and ENVIRONS EDITION 3. Published by Survey of Kenya, 1978.

3.2 Research design

A cross-sectional in vitro study was carried out between November 2006 and January 2007. The study design was chosen mainly due to limitation of time available to carry out the study. The study analyzed data collected on a group of yeast isolates over this period of time.

3.3 Target population

All yeast isolates from high vaginal swabs received at the microbiology laboratory of AKUH .

3.4 Sampling procedure

High vaginal swab specimens collected from women with vaginal discharge and sent to AKUH Microbiology lab for microscopy, culture and sensitivity were followed up and those from which yeasts were isolated recruited to the study. Only those that satisfied the inclusion criteria were included in the study by consecutive sampling. This consisted of sampling each specimen which met the defined eligibility criteria until the pre-determined sample size was achieved. The sampling technique offered less bias that may be introduced through unintentional manipulation and results in extremely low sampling error.

3.4.1 Inclusion criteria

The yeasts that demonstrated moderate or heavy growth on Sabouraud dextrose agar (SDA) were included in the study. In those with light growth, the gram stain from the original specimen was examined and only those with pus cells $\geq 2+$ were included in the study.

3.4.2 Exclusion criteria

All yeasts that demonstrated light growth on culture with pus cells $< 2+$ on the original gram stain were excluded from the study.

3.4.3 Sample size determination

According to the study by Fonck K *et al* (34), the prevalence of vaginal candidiasis was 50% if pregnant women are not excluded. The sample size was determined using the formula (35)

$\mathbf{n = \pi (1 - \pi)/e^2}$ where π = proportion of the target population having desired characteristic = 0.5

e = required standard error = 0.05

Therefore $n = \frac{0.5 \times 0.5}{0.05^2} = 100$

Hence the minimum desired number of samples to be analyzed was 100.

3.5 Laboratory procedures

3.5.1 Equipments and reagents

Petri dishes, wire loops, test tubes, sterile pipette tips, glass slides, incubator (35°C ± 2), crystal violet stain, Lugol's iodine, acetone-alcohol decolorizer, neutral red, Sabouraud dextrose agar with chloramphenicol, API® Candida system (BioMérieux, Marcy-l'Etoile, France), ATB® Fungus 2 INT kit (BioMérieux, Marcy-l'Etoile, France), human serum, mineral oil, McFarland Standard 2 and 3, oil of immersion, normal saline, distilled water, Bunsen burner, micropipettes, gloves, microscope, sterile stocking vials, sterile cotton swabs

3.5.2 Specimen collection and processing

All high vaginal swabs received at the laboratory for microscopy, culture and sensitivity were followed up. All the original gram stain slides were retained until after the cultures were assessed. Cream colored colonies with a distinctive yeast smell were selected for further processing. The colonies were examined in a drop of normal saline for budding cells. Gram stain was also carried out to confirm the presence of yeast cells

Gram stain technique

A drop of normal saline was placed on a clean slide. A wire loop was then flamed and cooled then used to pick a colony of the suspected yeast isolate. This was then emulsified with the drop of normal saline and spread on the slide and allowed to

air dry. Two drops of methanol were dropped onto the smear and allowed to air dry. The fixed slide was then covered with crystal violet stain for 30-60 seconds after which the stain was washed off with clean tap water and all the water tipped off. The smear was then covered with Lugol's iodine for 30-60 seconds then washed off with clean water. Acetone -alcohol was used to decolorize the smear for a few seconds and washed off immediately with water and the smear covered with neutral red for 2 minutes then washed off with clean water. The slide was then blotted dry and examined microscopically, first with the 40 X objective to check the staining and the material distribution and then with the oil immersion objective with the condenser iris fully open to check for yeast cells

3.5.3 Species identification

The species-level identification was performed using the API Candida system

API Candida testing for isolates

The API Candida strip consists of 10 tubes containing dehydrated substrates, which enable the performance of five sugar acidification tests (glucose, galactose, saccharose, trehalose and raffinose) and seven ,enzymatic reactions (β-maltosidase, α- amylase, β-xylosidase, β-glucuronidase, urea hydrolysis, N-acetyl-β-glucosaminidase and β-galactosidase). The reactions produced during incubation are revealed by spontaneous color changes.

A) Preparation of the tray.

The incubation box (tray and lid) was laid out and 5ml of distilled water distributed into the honey-combed wells of the tray to create a humid atmosphere.

The strain reference was recorded on the elongated flap of the tray. The strip was removed from its individual packaging and placed on the incubation tray and the desiccant sachet discarded

B) Preparation of the inoculum.

An ampoule of NaCl 0.85% medium (2ml) was opened. One or several well-isolated identical colonies were then picked using sterile cotton swab and a suspension made with the NaCl medium to a turbidity equivalent of McFarland Standard 3. The yeast suspension was then homogenized

C) Inoculation of the strip

The prepared yeast suspension was then distributed into the tubes, avoiding formation of bubbles by positioning the pipette on the edge of the cupules. The first five tests (GLU to RAF) and the last test (URE) were covered with mineral oil immediately after the strip inoculation. The incubation box was then closed and incubated for 18-24 hours at 35-37°C in aerobic conditions.

D) Reading the strip

After the 18-24 hours of incubation, the reactions were read by referring to the table below and recorded as + or – on the result sheet. Reactions that appeared doubtful after the first 18-24 hours were re-incubated for a further 24 hours and the reactions read again.

E) Interpretation

READING TABLE			
TESTS	REACTIONS	RESULTS	
		NEGATIVE	POSITIVE
1) GLU	GLUcose (Acidification)	violet/grey-violet	yellow green/grey
2) GAL	GALactose (Acidification)	"	"
3) SAC	SACcharose (Acidification)	"	"
4) TRE	TREhalose (Acidification)	"	"
5) RAF	RAFfinose (Acidification)	"	"
6) βMAL	beta-MALtosidase	colorless	pale yellow-bright yellow
7) αAMY	alpha-AMYlase	colorless	pale yellow-bright yellow
8) βXYL	beta-XYLosidase	colorless- very pale yellow/blue/green	pale yellow-bright yellow
9) βGUR	beta-GlUcuRonidase	colorless/blue/green	pale yellow-bright yellow
10) URE	UREase	yellow-pale orange	red
11) NAG	N-Acetyl-beta-Glucosaminidase	colorless/ yellow	blue/ green
12) GAL	beta-GALactosidase	colorless/ yellow	blue/ green

The results were transformed into a numerical profile which was compared to those given in the profile list in the package insert.

3.5.4 Antifungal susceptibility testing

The susceptibility of the yeast isolates to four antimycotics: amphotericin B (AMB), 5-flucytosine (5FC), fluconazole (FCA) and itraconazole (ITR) was tested using the broth microdilution method using ATB® Fungus 2INT (BioMérieux, Marcy-l'Etoile, France). The kit contains the following drug dilution ranges: AMB 0.5-16mgl^{-1}, 5 FC 0.5-64 mgl^{-1}, FCA 0.25-128 mgl^{-1} and ITR 0.125-4 mgl^{-1}.

The strip consists of 16 pairs of cupules. The first pair does not contain any antifungal agent and is used as a positive growth control. The next 15 pairs contain the 4 antifungal agents (5FC, AMB, FCA and ITR) at several concentrations, enabling MIC determination.

A) Preparation of the strip

The strip was removed from its packaging and the code of the isolate to be tested recorded on the elongated flap of the strip

B) Preparation of the inoculum

An ampoule of API NaCl 0.85% medium was opened and inoculated with several colonies to give turbidity equivalent to McFarland Standard 2. Using a pipette, 20µl of the suspension was transferred immediately into an ampoule of ATB F2 medium

C) Inoculation of the strip, incubation and reading of results

The ATB F2 medium was homogenized then the strip inoculated by dispensing 135µl of ATB F2 medium into each cupule. A lid was then placed on the strip and the strip placed in an air-tight jar. The isolate was incubated for 24 hours at 35-37°C in aerobic conditions. If after 24 hours the growth was insufficient in the control cupules the strips were incubated for a further 24 hours under the same conditions. Each cupule was then observed for growth after placing the strip on a black background to make visual reading easier. The reading was started with the lowest concentration and the growth score recorded on the result sheet for each of the cupules compared with the control cupule

D) Result interpretation

1. MIC determination

Definition	Score
No reduction in growth	4
Slight reduction in growth	3
Distinct reduction in growth	2
Very weak growth	1
No growth	0

-For Amphotericin B, the MIC was interpreted as the lowest concentration enabling complete growth inhibition (score = 0). Presence of one or several isolated colonies or signs of growth at the periphery of the cupule was interpreted as score of "1" (as per manufacturer's instructions)

-For Fluconazole, Itraconazole and Flucytosine, due to the possibility of trailing growth, the MIC was interpreted as the lowest concentration of the antifungal agent with which a score of "2", "1" or "0" was obtained. Any sign of growth at

the periphery of the cupule was given a score of "0" or "1" (as per manufacturer's instructions)

2. Guidelines for interpreting MICs as clinical categories (S, I or R): Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards [NCCLS]) recommended breakpoints (in μgml^{-1}) for *Candida* species

	Sensitive (S)	Intermediate (I)	Resistance (R)
Flucytosine	≤ 4	8 - 16	≥ 32
Amphotericin B*	ND	ND	ND
Fluconazole	≤ 8	16 - 32	≥ 64
Itraconazole	≤ 0.125	0.25 – 0.5	≥ 1

ND: Not defined

*: For Amphotericin B, a MIC ≥ $2mgl^{-1}$ was interpreted as resistant.

*. For Fluconazole and Itraconazole the intermediate (I) category was classified as susceptible-dose dependent (S-DD)

3.5.5 Stocking of the isolates

Each pure culture was stocked in a sterile vial containing sterile distilled water for future studies. Each vial was labeled clearly with the specimen number and the date the test was requested.

3.6 Quality control

A. Performance of media and instruments

- Media was prepared following the manufacturers' instructions closely.
  Accuracy was maintained in the measuring of liquid and dry ingredients and excess heat avoided in the mixing and solubilization of the ingredients (35)
- Media was then labeled with the preparation date and stored in the dark at 2 - 8°C in a refrigerator. Before culturing, each media was checked to verify it was not

dehydrated, hemolyzed or filled incorrectly. Cracked petri dishes or media that had changed color was not used. Any media that had colony growth was considered contaminated and discarded.

- All culture plates were warmed to room temperature before inoculation with the specimens
- Instruments were checked daily to ensure they were in good working condition and set at the required temperatures.

B. Purity of *Candida* cultures

Sabouraud dextrose agar with chloramphenicol was used. The additive antibiotic inhibited bacterial growth and some fungi hence achieving selectivity.

C. Quality control (QC) stock cultures

C.tropicalis ATCC 7349 and *C. glabrata* ATCC 2001 were to be used as stock cultures but were not available in the country and could not be procured from the source due to financial constraints. Known in-house susceptible strains from old stocks were instead used to control tests.

D. Pre-testing the study instruments

Five percent of the sample size (5 isolates) was taken to test the laboratory tests data analysis methods chosen. The results obtained were not used in the final data analysis.

3.7 Data collection methods

Yeast isolates were collected and processed in the laboratory to get further data which was then analyzed.

3.8 Data recording

All information collected from the laboratory procedures, the GTT results, the yeast species identified and the antifungal susceptibility were recorded into the entry sheets and the information entered into MS Excel.

3.9 Data cleaning

All entry sheets were checked for errors and omissions in recording. Entry of each isolate into MS Excel was counter-checked to ensure it matched with what was on the entry sheet. Recording of results was flagged to ensure each result was in the correct row or column.

3.10 Data analysis

Frequency tables and graphs were used to summarize the data. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA). The susceptibility data was analyzed by the non-parametric Fisher's exact test. $P < 0.05$ was considered statistically significant.

3.11 Study limitations

- Due to the short time available to carry out the study, a cross-sectional in-vitro study was carried out without correlating with clinical outcomes.
- Due to financial constraints we could not procure chromogenic media which would have aided in the identification of specimens with mixed yeast infections.
- Sample size was small due to the high cost of the identification and susceptibility test kits. The number of isolates in some of the species was small limiting comparison conclusions.
- The data was derived from a hospital-based population and hence cannot be extrapolated to the community.
- HIV testing was not carried out due to financial constraints. This implied that any differences in species distribution and antifungal sensitivity related to the immune status of the patient were not identified.
- The choice of drugs was based on what was available in the kit used. It would have been better to choose antifungal agents based on the most commonly used for treatment of VVC but this would have increased the costs of the study.

3.12 Ethical considerations

Prior to the study, the protocol was availed and clearance sought from the Department of Medical Microbiology, University of Nairobi and the Ethical and Research Committees of the Kenyatta National Hospital and the Aga Khan University Hospital. The approval was on the agreement that good laboratory practice was ensured and the findings treated with utmost confidentiality and for the purpose of this research only. The final report will be submitted to these bodies.

4. RESULTS

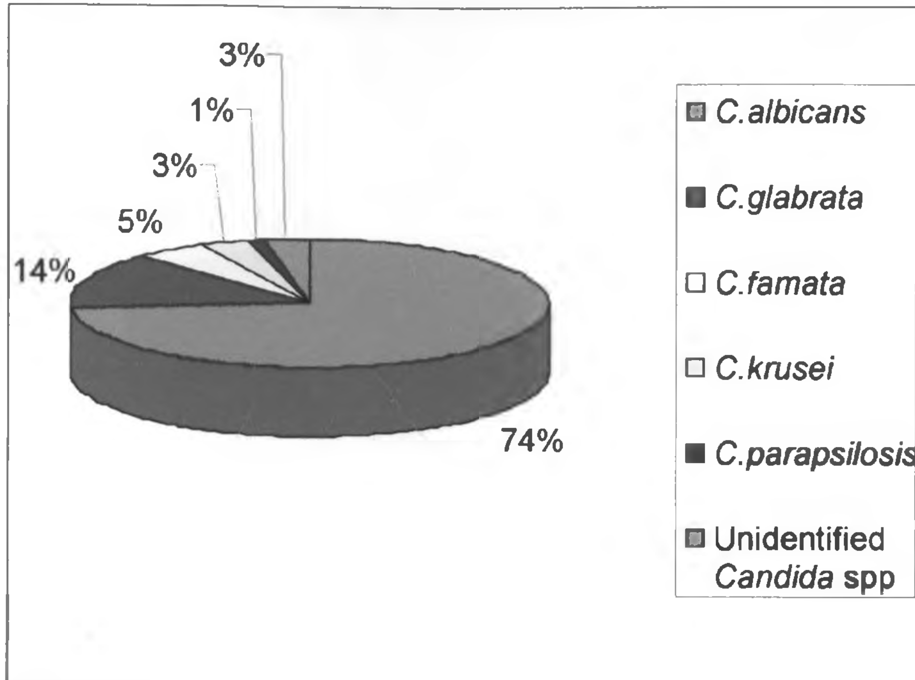
4.1 Species identification and distribution

A total of 101 yeast isolates were collected and tested between November 2006 and January 2007 (Table 1). *Candida* species accounted for 94.1% of the isolates with *C.albicans* being the most common (73.7% of all *Candida* species) (Fig.1). Among the non-albicans species, *C.glabrata* (13.7% of all *Candida* species) and *C.famata* (5.3%) were the most commonly identified species. Six isolates were identified as non-*Candida* yeasts (Fig.2).

Table 1: Species distribution of vaginal yeasts isolated from women with vaginal discharge at the AKUH

Species	No. (%) isolates
C.albicans	70 (69.3)
C.glabrata	13 (12.9)
C.famata	5 (5.0)
C.krusei	3 (3.0)
C.parapsilosis	1 (1.0)
Unidentified *Candida* species (*C.glabrata/ C.famata*)	3 (3.0)
Trichosporon species	3 (3.0)
S.cerevisiae	3 (3.0)

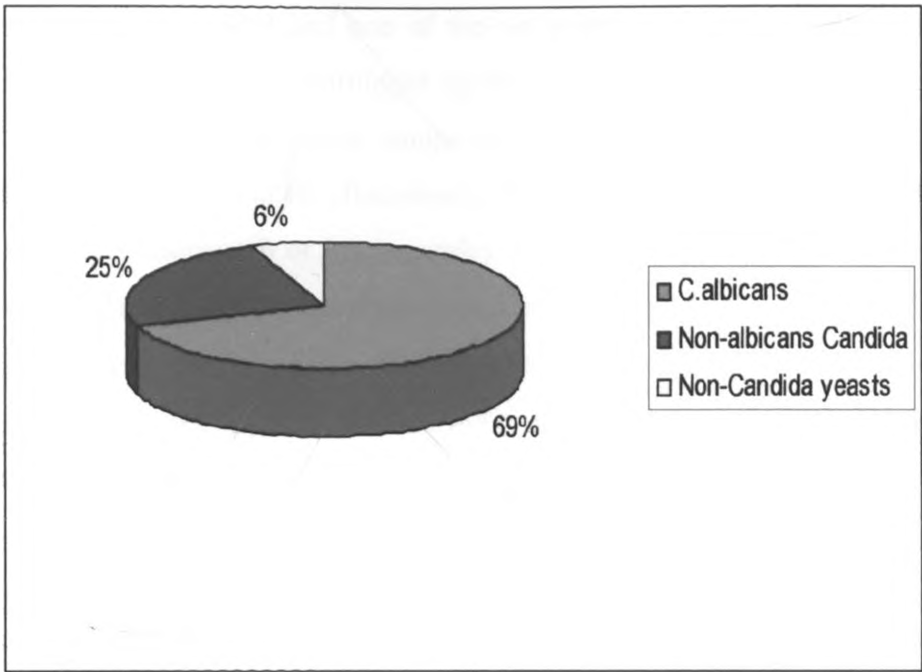
Figure 1: Percentage distribution of *Candida* species isolated from women with vaginal discharge at the AKUH



KEY:

Unidentified *Candida* species: *C.glabrata/ C.famata*

Figure 2: Percentage distribution of yeast species isolated from women with vaginal discharge at the AKUH



KEY:

Non-albicans *Candida* species: *C.glabrata*, *C.famata*, *C.krusei*, *C.parapsilosis*, *C.glabrata*/ *C.famata*

Non-*Candida* yeasts: *Trichosporon* species, *S.cerevisiae*

4.2 Antifungal susceptibility testing

All isolates were tested for susceptibility to flucytosine, amphotericin B, fluconazole and itraconazole. Overall, 55 of the 70 *C.albicans* (78.6%), six of the 25 non-albicans *Candida* strains (24%) and one of the six strains of non-candidal yeasts (16.7%) were susceptible to all tested antifungal agents. Resistance to one drug was demonstrated by 20% of the *C.albicans* strains (amphotericin B or fluconazole or itraconazole), 52% of the non-albicans *Candida* (flucytosine or itraconazole) and 66.7% of the non-candidal yeasts (amphotericin B or itraconazole). 1.4% of *C.albicans* strains showed resistance to two of the tested agents (amphotericin B and itraconazole) while 8% of the non-albicans *Candida* and 16.7% of the non-candidal yeasts showed resistance to three of the antifungal agents (amphotericin B, fluconazole and itraconazole). No isolate tested was resistant to all tested antifungal agents.

Table 2: In vitro susceptibilities of 101 vaginal yeasts to four antifungal agents as determined by MIC_{90}

Species / Drug and breakpoints		*C.albicans* (n=70)	Non-albicans *Candida*(n=25)	Non-candidal yeasts (n=6)
Flucytosine (MIC >16)	MIC_{90}	2	4	4
	% resistant	5.7	4	0
Amphotericin B (MIC $\geq$ 2)	MIC_{90}	0.5	1	16
	% resistant	7.1	8	66.7
Fluconazole (MIC > 32)	MIC_{90}	1	32	128
	% resistant	0	8	16.7
Itraconazole (MIC > 0.5)	MIC_{90}	0.125	4	4
	% resistant	10	56	33.3

MIC_{90} and MIC in μgml^{-1}

Ninety-five of the 101 yeast isolates (94.1%) were susceptible to flucytosine. One isolate (1.0%) showed intermediate susceptibility and five isolates (5.0%) were resistant. Sixty-six of the 70 *C.albicans* strains (94.3%), 23 of the 25 non-albicans *Candida* (92%) and all the non-candidal yeasts were susceptible (Figure 3). The percentage of resistant strains among the isolates (CLSI breakpoint MIC >16μgml^{-1}) was 5.7% for *C.albicans* and 4% for non-albicans *Candida* (Table 2). The susceptibility patterns to flucytosine did not differ significantly between the *C.albicans* and the other isolates (p=0.09) (Table 3)

Of the 101 isolates, 90 (89.1%) were susceptible to amphotericin B and 11 (10.9%) resistant. Sixty-five (92.9%) of *C.albicans* strains, 23 (92%) of the non-albicans *Candida* and two (33.3%) of the non-candidal yeasts were susceptible (Figure 4). The percentage of strains resistant to amphotericin B (MIC≥2μgml^{-1}) was 7.1% of *C.albicans*, 8% of the non-albicans *Candida* and 66.7% of the non-candidal yeasts (Table 2). There was no significant difference between the susceptibility patterns of *C.albicans* and the other isolates (p=1.00) (Table 3).

Ninety-four of the isolates (93.1%) were susceptible to fluconazole. Four isolates (4.0%) were susceptible-dose dependent while three (3.0%) were resistant. All *C.albicans* strains were susceptible; 19 (76%) of the non-albicans and five (83.3%) of the non-*Candida* yeasts were also susceptible (Figure 5). One of the three *C.krusei* strains was susceptible; the other two were susceptible-dose dependent. This is however not an important finding clinically since the species must be considered clinically resistant to fluconazole as per CLSI recommendations (36). The percentage of resistant strains for fluconazole (CLSI breakpoint MIC >32μgml^{-1}) were 8% non-albicans *Candida* and 16.7% for non-*Candida* yeasts (Table 2). There was significant difference between the susceptibility patterns of *C.albicans* and the other yeast isolates to fluconazole (p=0.00) (Table 3).

Itraconazole demonstrated the lowest susceptibility overall with only 72 of the 101 isolates (71.3%) being susceptible, 23 isolates (22.8%) were resistant. *C.albicans* was the species with the highest susceptibility at 90% (Figure 6); the lowest was demonstrated by *C.parapsilosis* (0%), *C.glabrata* (15.4%) and *C.famata* (20%). The percentage of

resistant strains to itraconazole (CLSI breakpoint MIC >0.5μgml^{-1}) was 10% for *C.albicans*, 56% for non-albicans *Candida* and 33.3% for non-candidal yeast isolates (Table 2). There was significant difference between the susceptibility patterns of *C.albicans* and the other yeast isolates to itraconazole (p=0.00) (Table 3).

Table 3: Antifungal sensitivity patterns of vaginal yeasts isolated from women with vaginal discharge at the AKUH to amphotericin B, flucytosine, fluconazole and itraconazole

VARIABLE (DRUG)	SPECIES		Statistical tests
	C. albicans	Non albicans	Fisher Exact test
Amphotericin B			
Sensitive[a]	**92.9%**	**80.6%**	$\chi2$= 3.30: 1df
Resistant[b]	**7.1%**	**19.4%**	P>0.05 (0.09)
Flucytosine			
Sensitive[c]	**94.3%**	**93.5%**	$\chi2$= 0.02: 1df
Intermediate[d] & resistant[e]	**5.7%**	**6.5%**	P>0.05 (1.00)
Fluconazole			
Sensitive[f]	**100.0%**	**77.4%**	$\chi2$= 16.98: 1df
S-DD[g] & Resistant[h]	**0.0%**	**22.6%**	P<0.05 (0.00) significant
Itraconazole			
Sensitive[i]	**90.0%**	**29.0%**	$\chi2$= 39.02: 1df P<0.05
S-DD[j] & Resistant[k]	**10.0%**	**71.0%**	(0.00) significant

KEY: S-DD= Susceptible-dose dependent [a] MIC<2μgml^{-1}, [b]MIC≥2μgml^{-1}, [c]MIC≤4μgml^{-1}, [d]MIC =8-16μgml^{-1}, [e]MIC≥32μgml^{-1}, [f]MIC≤ 8μgml^{-1}, [g]MIC=16-32μgml^{-1} [h]MIC≥64μgml^{-1}, [i]MIC≤0.125μgml^{-1}, [j]MIC=0.25-0.5μgml^{-1}, [k]MIC≥1μgml^{-1}

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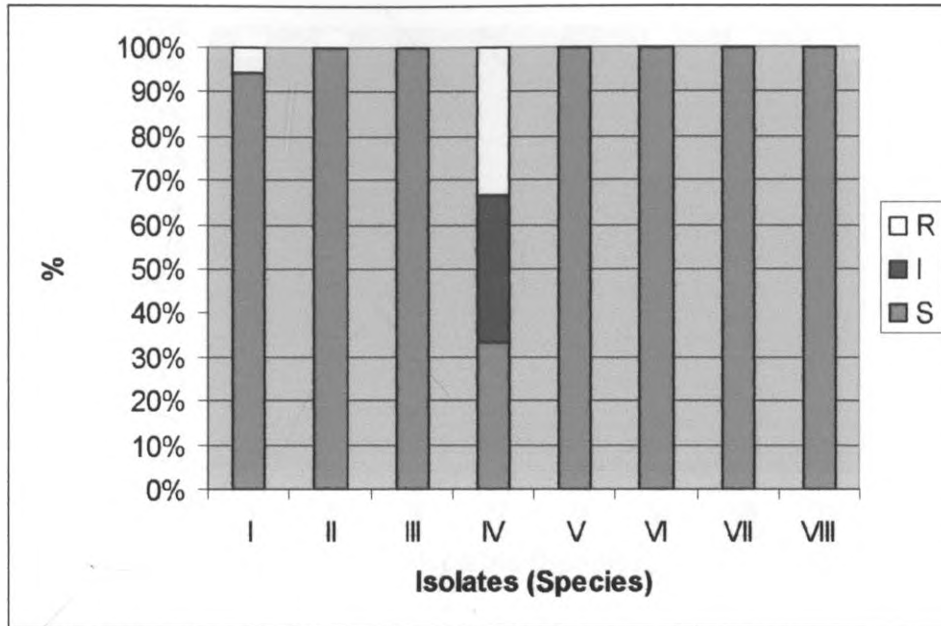
Figure 3: In vitro susceptibility of vaginal yeasts isolated from women with vaginal discharge at the AKUH to flucytosine as determined by MICs



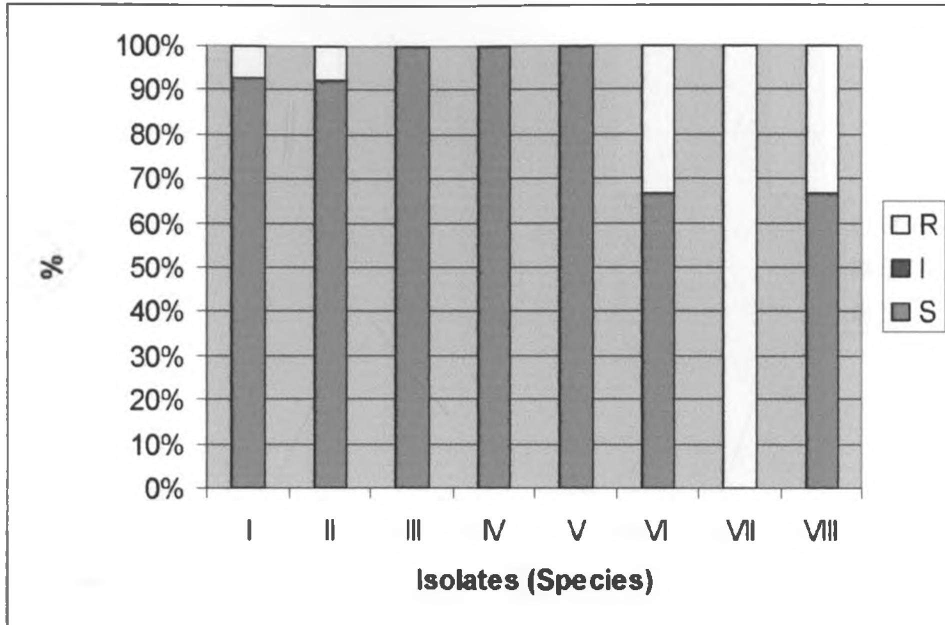
KEY: **I** – *C.albicans* (n=70), **II** – *C.glabrata* (n=13), **III** – *C.famata* (n=5),

IV – *C.krusei* (n=3), **V** – *C.parapsilosis* (n=1), **VI** – *Candida* spp. (n=3)

VII – *Trichosporon* spp. (n=3), **VIII** – *S.cerevisiae* (n=3)

S = MIC $\leq 4\mu gml^{-1}$, **I** = MIC $8\text{-}16\mu gml^{-1}$, **R**= MIC $\geq 32\mu gml^{-1}$

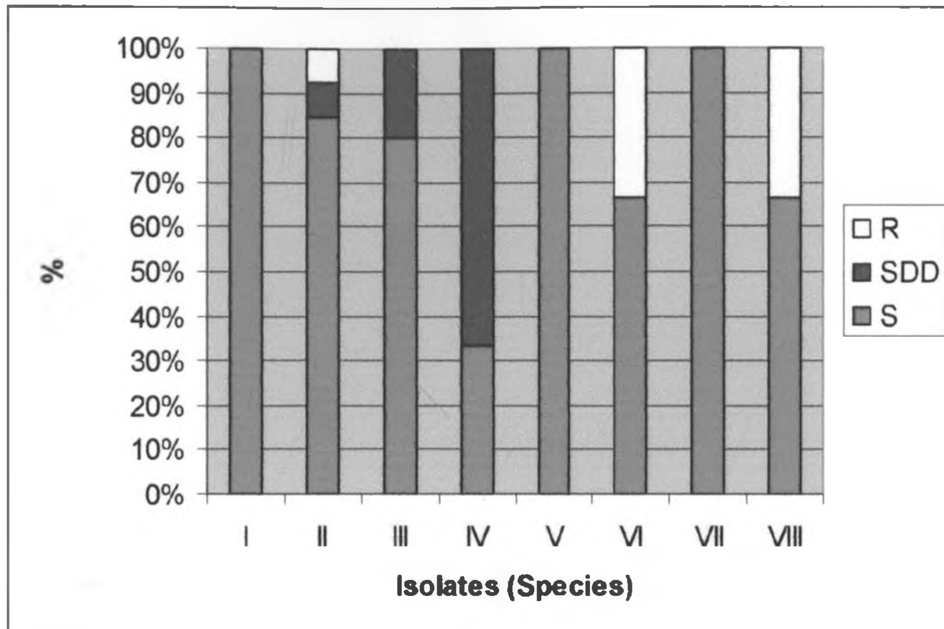
Figure 4: In vitro susceptibility of vaginal yeasts isolated from women with vaginal discharge at the AKUH to amphotericin B as determined by MICs



KEY: **I** – *C.albicans* (n=70), **II** – *C.glabrata* (n=13), **III** – *C.famata* (n=5), **IV** – *C.krusei* (n=3), **V** – *C.parapsilosis* (n=1), **VI** – *Candida* spp. (n=3) **VII** – *Trichosporon* spp. (n=3), **VIII** – *S.cerevisiae* (n=3)

S = MIC < $2\mu gml^{-1}$, **R** = $\geq 2\mu gml^{-1}$

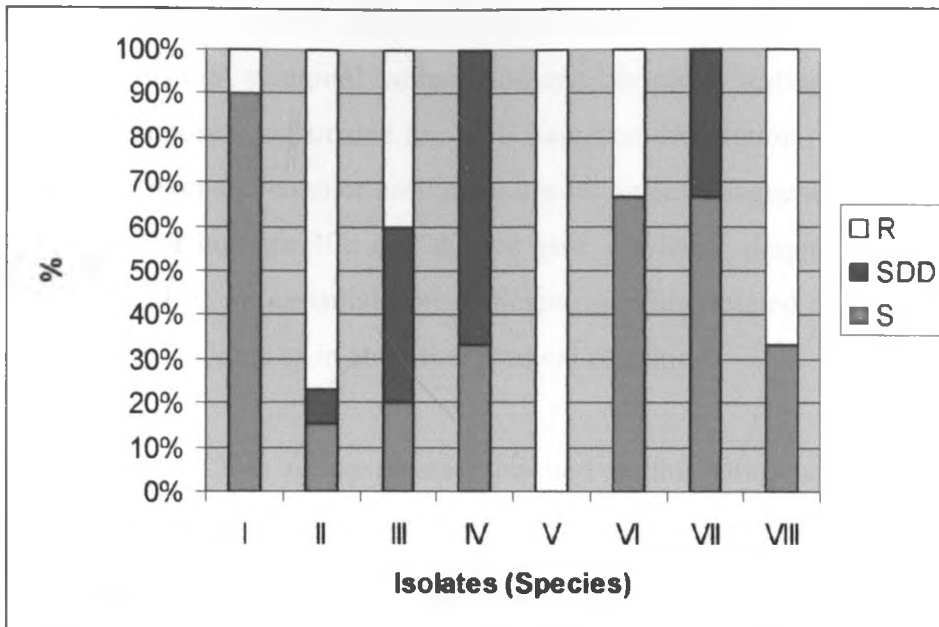
Fig. 5: In vitro susceptibility of vaginal yeasts isolated from women with vaginal discharge at the AKUH to fluconazole as determined by MICs



KEY: **I** – *C.albicans* (n=70), **II** – *C.glabrata* (n=13), **III** – *C.famata* (n=5), **IV** – *C.krusei* (n=3), **V** – *C.parapsilosis* (n=1), **VI** – *Candida* spp. (n=3) **VII** – *Trichosporon* spp. (n=3), **VIII** – *S.cerevisiae* (n=3)

S = MIC $\leq 8\mu gml^{-1}$, **S-DD** = MIC $16\text{-}32\mu gml^{-1}$, **R** = MIC$\geq 64\mu gml^{-1}$

Fig. 6: In vitro susceptibility of vaginal yeasts isolated from women with vaginal discharge at the AKUH to itraconazole as determined by MICs



KEY: **I** – *C.albicans* (n=70), **II** – *C.glabrata* (n=13), **III** – *C.famata* (n=5), **IV** – *C.krusei* (n=3), **V** – *C.parapsilosis* (n=1), **VI** – *Candida* spp. (n=3) **VII** – *Trichosporon* spp. (n=3), **VIII** – *S.cerevisiae* (n=3)

S = MIC $\leq$ 0.125µgml^{-1}, **S-DD** = MIC 0.25- 0.5µgml^{-1}, **R** = MIC $\geq$ 1µgml^{-1}

5. DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

Vulvovaginal candidiasis (VVC) is a common condition with at least 75% of women developing it in their lifetime (5). The diagnosis of VVC should routinely involve taking of a thorough history, a vaginal examination and laboratory testing. Unfortunately, many patients are diagnosed and treated for VVC based on description of symptoms (4); many more purchase over-the-counter antifungal agents on self-diagnosis. The symptoms have been found to be non-specific and do not give a reliable diagnosis (23). Appropriate laboratory testing is an essential part of diagnosis when related to the patient's history and the physical findings as in any other medical condition.

Although most (69.3%) of the species isolated in this study were *C.albicans* and a significant percentage (30.7%) were non-albicans yeasts (Table 1). The non-albicans species isolated were mainly *C.glabrata* (12.9%) and *C.famata* (5.0%). Only two species of non-candidal yeasts were isolated: *Trichosporon* species and *S.cerevisiae*. *C.albicans* was the predominant species isolated in other studies (19, 24-30) which concurs with the findings in this study. The distribution of the non-albicans species differed in different studies. The most commonly isolated non-albicans species differed as *C.glabrata* (19, 24-29) and *C.parapsilosis* (30). The second commonest non-albicans species differed in the different studies as *C.glabrata* (30), *C.tropicalis* (24, 26, 27), *C.parapsilosis* (28), *C.famata* (25) and *C.krusei* (19). Comparing the findings in the current study with those of some of the studies (19, 24-30) which demonstrated *C.albicans* in 40.5-82.9% of isolates, *C.glabrata* in 5.6-30.1%, *C.famata* in 0.1-2.2%, *C.krusei* in 1.6-11.2%, *C.parapsilosis* in 0.5-11.3%, *C.tropicalis* 2.4-7.8%, unidentified *Candida* species in 0.9-7.5%, *Trichosporon* species in 0.3-0.9% and *S.cerevisiae* in 0.2-0.7% of isolates, most of the findings in this study were within these ranges except for *C.famata*, *Trichosporon* species and *S.cerevisiae* which were more frequent in this study. *C.tropicalis* was also isolated in these studies but was not found in the current study.

API Candida system did not give exact numerical profiles for eleven isolates (10.9% of the isolates); some of these were identified further using microscopic features. The kit could not speciate three isolates whose profiles fell between *C.famata/ C.glabrata/ Geotrichum* species. *Geotrichum* species was ruled out using microscopic features and the isolates recorded as unidentified *Candida* species since the additional required test (sorbital assimilation) was not available. Namkinga *et al* (25) demonstrated inability of API Candida system to speciate 15.8% of isolates which is slightly higher than that in this study. The sensitivity of API Candida system with and without additional tests is given as 97.4% and 75.2% by Hélène *et al* (38) and 91.4% and 71.7% by Bernal *et al* (39).

The in vitro activities of four antifungal agents - flucytosine, amphotericin B, fluconazole and itraconazole - were evaluated in this study against vaginal yeasts. Flucytosine is an antimetabolite analog of cytosine with antifungal activity against *Candida* and *Cryptococcus neoformans*. The drug was initially used as a monotherapy in the treatment of cryptococcal meningitis but was noted to result in the development of flucytosine-resistant isolates (40). It has also been successfully utilized in the treatment of cryptococcal pneumonia but occasional patients who were severely immunocompromised developed cryptococcal meningitis after treatment of pneumonia with flucytosine alone. Flucytosine is also used as therapy in some life-threatening *Candida* infections such as endocarditis, meningitis, and hepatosplenic disease (41). High resistance to flucytosine may, however, result from secondary resistance which develops by a single-step mutation in the permease or any of the intracellular targets of the drug (12); this mutation occurs during therapy. Flucytosine used in combination with amphotericin B results in synergy with some advantages: there is reduced development of flucytosine resistance and lower doses of amphotericin B can be used. Amphotericin B has been successfully used in the treatment of all forms of cryptococcosis, from pneumonia to meningitis (40). The drug is associated with significant toxicity such as nausea and vomiting and dose-limiting nephrotoxicity (41). In combination with flucytosine, the antifungal activity of amphotericin B is enhanced therefore its dose and duration of treatment is reduced and this results in a reduction in its toxicity. Formulations of the drug either in liposome-encapsulated or lipid-complexed preparations has also enabled higher doses to be used

with lower toxicity. The drug may also be used in the treatment of systemic candidiasis, histoplasmosis, systemic aspergillosis, and other systemic fungal infections. The azole antifungal agents are commonly used due to their limited toxicity and ease of administration (41). They also have a broad spectrum of activity against a wide range of fungal pathogens including *Candida* species, *C.neoformans*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, among others. Fluconazole and itraconazole are however better tolerated and more effective than ketoconazole. The current management of cryptococcal meningitis is followed by several months of fluconazole (itraconazole in individuals unable to tolerate fluconazole) suppressive therapy for HIV negative patients and life-long maintenance therapy for HIV positive patients (42). These agents have two potential limitations: their frequency of interactions with concomitantly administered drugs resulting in adverse clinical consequences, and the emergence of resistance especially of *Candida* species (41). Drugs that interact with azole derivatives include antacids, H_2 receptor antagonists, anti-TB drugs (isoniazid and rifampin), warfarin, digoxin, and antiretroviral agents (zidovudine, ritonavir) among others. Chronic use of azoles for long-term suppressive therapy may result in selection of cryptococcal isolates and normal flora *Candida* resistant to the drugs.

In the current study, flucytosine showed an overall susceptibility of 94.1%; 5.0% of the isolates were resistant (Figure 3, Table 2). The tested strains demonstrated a 0-5.7% resistance except for *C.krusei* which had a resistance of 33.3%. The high resistance rate in this species may be explained by the low rate of isolation of *C.krusei* (three strains). Nawrot *et al* (29) found a low frequency (0-3%) of resistance of tested strains while Richter *et al* (28) found an overall resistance of 3% with resistance of tested strains in the range 0-100%. The 100% resistance was found in the strains isolated in small numbers in that study (28) – *C.lusitaniae* and *Trichosporon* species which had one isolate each. A study on isolates of *Cryptococcus neoformans* from clinical sources from Nairobi (43) found a 21.2% resistance to flucytosine. This is a drug currently being used in this country for treatment of cryptococcosis and it has been recommended for use in combination with amphotericin B. It was not established whether that is actually being

done in the current study. It was also not established whether the patients with flucytosine-resistant isolates had previously been on treatment with the drug.

Amphotericin B, which is the drug of choice for cryptococcal meningitis, demonstrated an overall susceptibility of 89.1% in this study, with 10.9% of isolates being resistant (Figure 4, Table 2). The susceptibility testing of this drug is still a controversial area; CLSI breakpoints have not yet been defined. Though some studies use a breakpoint of $1\mu gml^{-1}$, strains in this study with $MIC>2\mu gml^{-1}$ were considered resistant according to the manufacturer's instructions. There is limited information on amphotericin B susceptibility in this country except for cryptococcal infections. Bii *et al* (43) found a 0% resistance of the drug to *C.neoformans* with a low MIC_{90} of $0.5\mu gml^{-1}$. In the current study, though the resistance was much higher, a similar MIC_{90} was found for *C.albicans* with a resistance of 7.1%; the other species had higher MIC_{90} values (Table 2) of $1\mu gml^{-1}$ for non-albicans *Candida* (8% resistant) and $16\mu gml^{-1}$ for non-candidal yeasts (66.7% resistant). Other studies (28, 29, 31) also demonstrated a low frequency of resistance to amphotericin B (0-1%). The use of amphotericin B for treatment of cryptococcal meningitis, which is an opportunistic infection that has increased especially in the wake of the HIV/AIDS pandemic, may result in development of resistance in the normal flora *Candida* species found in the vagina and in the mouth and if these then cause endogenous infection will demonstrate resistance to the drug. The resistant strains can also circulate in the community and may have far-reaching implications especially in the event of systemic candidiasis.

Fluconazole demonstrated an overall susceptibility of 93.1% and 3.0% resistance in the current study (Figure 5, Table 2). Among the isolates in this study, *C.albicans* had a 100% susceptibility which is in agreement with the results obtained in the study by Pfaller *et al* (24) that demonstrated *C.albicans* susceptibility of approximately 94-100% though with some geographical differences. In the same study, the *C.albicans* isolates from South Africa had a susceptibility of 99.4% (0.3% resistant). In other studies, *C.albicans* demonstrated fluconazole susceptibility of 84.3% (27), 92% (29) and 92.5% (30). *C.krusei* has intrinsic resistance to fluconazole while *C.glabrata* shows reduced

susceptibility (24). The *C.glabrata* isolates in the current study demonstrated a susceptibility of 84.6% (7.7% S-DD, 7.7% resistant). The ARTEMIS Global Antifungal Surveillance study demonstrated *C.glabrata* susceptibility in the range 29.2-100%; the isolates from S. Africa had a susceptibility of 49.6% (21.1% resistant). The MIC_{90} of fluconazole for *C.albicans* in the current study (Table 2) was $1\mu gml^{-1}$ (range: 0.25-8) which was similar to that obtained by Comert *et al* (range: 0.125-8). The MIC_{90} for *C.glabrata* in the current study was $32\mu gml^{-1}$ (range: 0.25-128) which was much higher than that for the same species in the study by Comert *et al* of $8\mu gml^{-1}$ (range: 0.125-16). The elevated MIC_{90} for *C.glabrata* in this study may have been due to either the small number of *C.glabrata* isolates with a wide range of MIC distribution or to a rising resistance of the isolates in this study. Bii *et al* (43) in the study on *C.neoformans* demonstrated a resistance to fluconazole of 11.2% with a MIC_{90} of $64\mu gml^{-1}$. The MIC_{90} of *C.neoformans* in that study was higher than that of *Candida* in the current study hence *C.neoformans* demonstrated a higher resistance rate compared to *Candida*.

Itraconazole had the lowest overall susceptibility (71.3%) in this study with a resistance rate of 22.8% (Figure 6, Table 2). *C.albicans* was the species with the highest susceptibility (90%) to this drug with that of the other isolates ranging between 0% (for *C.parapsilosis* which had only one isolate) and 100%. The resistance rate for *C.albicans* was 10% which is in agreement with that found by Nawrot *et al* of 11%. Non-albicans *Candida* had a resistance rate of 50% in the current study which was higher than that found by Galle *et al* of 23.5%. The study by Bii *et al* (43) showed itraconazole resistance rate of 6.3% (MIC_{90} $0.5\mu gml^{-1}$) which is much lower compared to that of *Candida* isolates in the current study. All the isolates resistant to fluconazole were also found to be resistant to itraconazole supporting the possibility of cross-resistance between the azoles. This cross-resistance may be due to a common mode of action of the azole derivatives.

Isolates inhibited by fluconazole and itraconazole at concentrations of $16\text{-}32\mu gml^{-1}$ and $0.25\text{-}0.5\mu gml^{-1}$ respectively are placed in a category known as susceptible-dose dependent (S-DD). This category identifies yeast isolates with intermediate susceptibilities, between fully susceptible and fully resistant. Isolates in this category will

respond to fluconazole if higher doses are administered and to itraconazole if higher plasma levels are achieved (6). The definition of fluconazole susceptible and resistant does not apply for *C.krusei* since the organism is considered to have intrinsic resistance to the drug (36).

Identification of yeast isolates to species-level may aid in guiding the clinician on therapeutic options. VVC may be classified into complicated and uncomplicated forms (Box 1). The uncomplicated form is caused by *C.albicans*, which is usually susceptible to all antifungal agents, and responds to short-course oral or topical treatment (44). The complicated form is recurrent, caused by non-albicans species of *Candida* and requires treatment for ≥7 days. Patients with recurrent VVC are also more likely to acquire resistant *Candida* species due to frequent exposure to antifungal agents for the recurrent infections. Approximately 33% of RVVC is also known to be caused by non-albicans *Candida* species some of which are inherently less susceptible to common antifungal agents making them less useful in their treatment (5). The optimal treatment of non-albicans *Candida* species remains unknown (1) but they frequently respond to topical boric acid or topical flucytosine (44). Isolation of *C.krusei* in any specimen rules out the use of fluconazole for the treatment of the patient, even when the isolate tests susceptible in vitro, since the species has intrinsic resistance to this antifungal agent while *C glabrata* shows reduced susceptibility to the same agent. *C.lusitaniae* has been associated with amphotericin B resistance. Susceptibility testing is especially useful in infections due to non-albicans *Candida* species (44). In the setting of candidemia and deep infections susceptibility testing may be of benefit especially in cases where initial therapy has failed, the results may guide on suitable adjustment of therapy.

It is however important for the clinician to understand that in vitro susceptibility results are supposed to be guidelines to the treatment of patients since they may not always predict the success of a particular treatment. However, when the infection is caused by a resistant isolate, the tests may predict the possibility of failure for a particular agent or the dosage. The clinician must also understand that in vivo response to a drug is a combination of several factors: drug factors, host factors, site of the infection and fungal

factors (6). Drug factors include the dosage (quantity, frequency and cumulative dose), pharmacokinetics (absorption, distribution, metabolism and excretion), pharmacodynamics and other concomitantly administered drugs (drug-drug interactions). Host factors include the immune system which works with the drug to clear the infection, the site and severity of the infection and patient compliance to the given treatment. Site of the infection factors include the source of the infection, drug penetration and the presence of a foreign body which acts as a foci for infection. Fungal factors include the species, cell type, the size of the microorganism population, virulence factors, evasion of host inflammatory response and biofilm formation. All these factors have a role to play in the final resolution of the infection and should be taken into consideration when starting the patient on treatment.

The current study had limitations in time and cost. Due to these, a small sample size of 101 yeast isolates was analyzed. This resulted in some of the yeast species being isolated in very small numbers. The antifungal susceptibility testing was also to very few antifungal agents and none of the new agents in the market were tested e.g. voriconazole. This study therefore paves the way for more studies with larger sample sizes and in different parts of the country in order to understand the local epidemiology of yeast species and to study the local epidemiological susceptibility patterns. These would then be used to come up with local antibiograms that will aid clinicians in the selection of effective antifungal agents that can be used empirically. Larger studies to assess the clinical correlation of MIC and outcome are also required to enhance the understanding of the susceptibility testing in our set up. These studies would then form a baseline from which surveillance of antifungal drug resistance can be monitored both in hospitals and in the community.

5.2 CONCLUSION

This study observed that:

- *C.albicans* is the prominent cause of vulvovaginal candidiasis in our set up
- Non-albicans yeasts are a significant cause of vulvovaginal candidiasis
- *C.albicans* still demonstrates susceptibility to fluconazole and itraconazole
- There is a higher resistance to fluconazole and itraconazole by non-albicans yeasts than *C albicans*.

5.3 RECOMMENDATIONS

- Species identification in patients with recurrent or refractory vulvovaginal candidiasis may guide on management options. This can utilize any species identification method – biochemical methods like API Candida or cornmeal agar for morphology , which is a cheaper option
- There is a need to conduct a study to identify the species distribution and antifungal sensitivity pattern differences in HIV positive and HIV negative patients
- More elaborate studies on yeast susceptibility to antifungal agents with a wider panel tested to give a more complete susceptibility profile
- A methodical surveillance needs to be instituted in hospitals to monitor the trend of antifungal susceptibility/ resistance patterns. The current on-going HIV/AIDS pandemic makes this an urgent requirement. The surveillance should also be extended to include data of trends in the community.

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APPENDIX III: WORK PLAN

Time / Task	Jan 06	Feb	Mar	Ap	May	Jun	Jul	Au	Se	Oc	Nov	Dec 06	Jan 07	Feb	Mar	Ap
Proposal Writing	**	**	**	**												
Dept. presentation					*											
Ethical approval					**	**										
Data collection							**	**								
Proposal expansion									**							
Ethical approval (KNH & AKUH)										**						
Data collection, cleaning & entry											**	**	**			
Data cleaning, analysis & report writing														**	**	
Defense of thesis																**

APPENDIX IV: BUDGET

Category	No. of units	Cost/unit	Total cost (Ksh)
1. Services			
Photocopy			3000
Internet search/ typing			2000
2. Operating expenses			
Printing			3000
Binding			3000
Stationery			2000
Data analysis			6000
3. Apparatus/ Reagents			
Sterile swabs in tube	200	20	4000
Sabouraud dextrose agar with chloramphenicol	1	6600	6600
Glass slides	2pkts	150	300
Cover slides	2	60	120
Petri dishes	1	2400	2400
Gloves	2	100	200
Crystal violet	1	420	420
Lugol's iodine	1	570	570
Acid-alcohol	1	520	520
Neutral red	1	520	520
Pipette tips	1	750	750
API Candida	10	6100	61000
ATB Fungus 2 INT	4	10970	43880
Mineral oil	1	1025	1025
McFarland set	1	2250	2250
Miscellaneous			40000

Contingency (15% of total Cost)			27533.25
TOTAL			**211088.25**

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP", Nairobi.
Email: KNHplan@Ken.Healthnet.org

Ref: KNH-ERC/ 01/3893

Date: 9th November, 2006

Dr. Florence Mutua
Dept. of Medical Microbiology,
UNIVERSITY OF NAIROBI..

Dear Dr. Mutua,

AMMENDMENT OF PROPOSAL "SPECIES DISTRIBUTION AND ANTIFUNGAL SENSITIVITY PATTERNS OF VAGINAL YEASTS AT THE KENYATTA NATIONAL HOSPITAL NAIROBI, KENYA" (P74/2/2006)

Your letter dated 18th September, 2006 refers.

The KNH Ethics and Research Committee has considered and approved the change of the study site from Kenyatta National Hospital to Aga Khan University Hospital and the study methodology to start from vaginal isolates of candida instead of high vaginal swabs.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH-ERC

c.c. Prof. K.M. Bhatt, Chairperson, KNH-ERC

The Deputy Director CS, KNH

The Dean, Faculty of Medicine, UON

Supervisors: Dr. G. Revathi, Aga Khan University Hospital

Dr. P. A. Odhiambo, Dept. of Medical Microbiology, UON

Dr. M. Machoki, Dept. of Obs & Gynae, UON

THE AGA KHAN UNIVERSITY

Faculty of Health Sciences

Post Graduate Medical Education

18th. October, 2006

Dr. Florence Mutua
Dept. of Microbiology
University of Nairobi
NAIROBI

Dear Florence,

RE: Species Distribution and Antifungal Sensitivity Patterns of Vaginal Yeasts at the AKUHN in Nairobi

Thank you for submitting the proposal entitled " **Species Distribution and Antifungal Sensitivity Patterns of Vaginal Yeasts at the AKUHN in Nairobi** " to the AKUHN Institutional Review Committee. This proposal was discussed and the comments of the committee are stated below:

1. It is a good prospective study and the committee feels it is reasonably well written.
2. The results are likely to be very significant in contributing to the body of knowledge of practicing clinicians
3. Please include a statement in your budget on how the costs will be met without over taxing the patient, for research benefit.

Please address the budgetary issue clearly and your response will be appreciated. We shall be very grateful if you could please avail to us your response as soon as possible. In the mean time please be assured that the study project has been approved and you may commence the project as you address the minor issue indicated.

DR. M. S. ABDULLAH,
CHAIRMAN, SCIENTIFIC COMMITTEE
AGA KHAN UNIVERSITY HOSPITAL

UNIVERSITY OF NAIROBI
MEDICAL LIBRARY

3rd Parkland Avenue, The Aga Khan University Hospital, P.O. Box 30270 - 00100 - GPO, Nairobi, Kenya.
Telephone: (254 20) 3662000, 3662106, 3740000 Fax: (254 20) 3743935, 3741749

LISTS OF TABLES AND FIGURES

Table 1: Species distribution of vaginal yeasts isolated from women with vaginal discharge at the AKUH

Table 2: In vitro susceptibilities of 101 vaginal yeasts as determined by MIC_{90} to four antifungal agents

Table 3: Antifungal sensitivity patterns of vaginal yeasts isolated from women with vaginal discharge at the AKUH to amphotericin B, flucytosine, fluconazole and itraconazole

Figure 1: Percentage distribution of *Candida* species from women with vaginal discharge at the AKUH

Figure 2: Percentage distribution of yeast species from women with vaginal discharge at the AKUH

Figure 3: In vitro susceptibility of vaginal yeasts isolated from women with vaginal discharge at the AKUH to flucytosine as determined by MICs

Figure 4: In vitro susceptibility of vaginal yeasts isolated from women with vaginal discharge at the AKUH to amphotericin B as determined by MICs

Figure 5: In vitro susceptibility of vaginal yeasts isolated from women with vaginal discharge at the AKUH to fluconazole as determined by MICs

Figure 6: In vitro susceptibility of vaginal yeasts isolated from women with vaginal discharge at the AKUH to itraconazole as determined by MICs