ISOLATION, IDENTIFICATION AND SUSCEPTIBILITY PROFILE OF *CANDIDA* SPECIES TO ANTIFUNGAL AGENTS IN PREGNANT WOMEN IN THIKA DISTRICT HOSPITAL, KENYA

CHENGO NELSON MENZA (B.Sc. KU)
Reg. No: I56/10808/2008

A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (INFECTIOUS DISEASES) IN THE SCHOOL OF HEALTH SCIENCES, KENYATTA UNIVERSITY

DECLARATION

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

Name: Chengo Nelson Menza

Signature: ..... **Date:** 08/08/2011

Supervisors:

This thesis has been submitted for review with our approval as University supervisors.

1. Dr. Margaret W. Muturi

Department of Medical Laboratory Science

Kenyatta University, Kenya

Signature: ..... **Date:** 09/08/2011

2. Dr. M. W. Wanyoike

Department of Botany

Jomo Kenyatta University of Agriculture and Technology, Kenya

Signature: ..... **Date:** 08/08/2011

DEDICATION

I dedicate this thesis to all my family members, friends, relatives and all the pregnant women who participated in this study. May God bless you

ACKNOWLEDGEMENT

I wish to acknowledge first that all the glory belongs to God. I acknowledge all the people who had anything to do with this project. The list is long and the gratitude immense. I would like to thank; Kenyatta University for the approval of this study, my hardworking Principal supervisor Dr. Margaret W. Muturi in the Department of Medical Laboratory Sciences of Kenyatta University, Co-supervisor Dr. Wanjiru Wanyoike in the Department of Botany, Jomo Kenyatta University of Agriculture and Technology for their encouragement, advice, support and for standing by me at the time of hardship during the course of my work. May God bless the work of your hands.

I would like to thank Prof. Eucharia Kenya in the Department of Biochemistry and Biotechnology of Kenyatta University for her strong advice, guidance and support during the course of this work. Irene Mutuku, Mary Kigotho and all the staff members in the Microbiology bench of Thika District Hospital for their contribution during the course of this project.

Finally, I highly acknowledge the National Council for Science and Technology (NCST) for giving a grant for this project (grant number NCST/5/003/PG/86). May God bless you and give you more resources to support more research work in the Kenya.

TABLE OF CONTENTS

TITLE **PAGE**

CHAPTER THREE

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
HIV	Human Immunodeficiency Virus
I.V	Intravenous
MIC	Minimum Inhibitory Concentration
NCCLS	National Committee for Clinical Laboratory Standards
NCST	National Council for Science and Technology
RPMI	Roswell Park Memorial Institute
SDA	Sabourauds Dextrose Agar
SPSS	Statistical Package for Social Sciences
KOH	Potassium Hydroxide
USA	United States of America

ABSTRACT

Vaginal candidiasis is relatively higher in pregnant women particularly in the third trimester compared to non pregnant women. Infection with *Candida* species causes choriomnionitis which is associated with pre-term delivery and death of the infant. Therefore, epidemiological monitoring of vaginal candidiasis infections is highly desirable for continuous determination of the existing causative species and the disease trends. Determination of susceptibility profile will be of importance in the appropriate selection of drugs for effective treatment of the infection in pregnant women. The objectives of the study were to determine the prevalence of vaginal candidiasis and identify the *Candida* species. The study also aimed at determining the susceptibility profile of the *Candida* species to antifungal drugs prescribed to the pregnant women with symptoms of the infection. A cross sectional study design and purposive sampling techniques were adopted in this study. Vaginal swabs were collected from the pregnant women attending the antenatal clinic of Thika District hospital between the months of June and August, 2010. The samples were gram stained and inoculated on Sabourands Dextrose Agar (SDA). Isolates from SDA were plated on CHROMagar for detection of mixed cultures. Germ tube, chlamydospore formation and sugar assimilation tests were performed for identification of isolates. Susceptibility profile was done using broth microdilution minimum inhibiting concentration method based on the approved NCCLS, 2002. The prevalence of vaginal candidiasis was 42.7%. The distribution of vaginal candidiasis had the highest incidence of 60% in women aged 26-35years followed by those aged 15-25years who were 26%. Twelve per cent (12%) of the women infected were aged 36-45 years. The women aged 46 and above years were the least infected (2%). This study showed that *Candida albicans* was the most common vaginal *Candida* species causing vaginal candidiasis in all the age groups. It was also found to be the most abundant species of all *Candida* species isolated. The women in their 3rd trimester had the highest infection occurrence of 68.09% followed by those in the 2nd trimester with 21.28% while those in their first trimester of pregnancy (10.63%) were least infected. *Candida albicans* was the most isolated species with 63.8%, followed by *Candida glabrata* 29.79%, *Candida tropicalis* 3.19%, *Candida krusei* 2.13% and *Candida parapsilosis* was the least with 1.06%. Most of the vaginal *Candida* species were isolated in 60% women who were 26-35 years, followed by 26% of those between 15-25 years. Only 2% of the women with 46years and above years were infected. The azole antifungal agents (fluconazole, ketoconazole, itraconazole and clotrimazole) used showed high efficacy against all the vaginal *Candida* species isolated except *Candida krusei. Candida krusei* showed 100% resistance to fluconazole, clotrimazole and a 50% resistance to ketoconazole. It was only susceptible (100%) to itraconazole. The other non-*albicans Candida* species were susceptible to the azole antifungal drugs. *Candida albicans, Candida glabrata* and *Candida krusei* showed moderate susceptibility (66.67%, 57.14% and 50%) respectively to topical nystatin. *Candida parapsilosis* was 100% susceptible to the drug. Nonetheless, the susceptibility of vaginal *Candida* species to the azole drugs and topical nystatin observed in this study supports the continued use of azole drugs but not topical nystatin for the treatment of vaginal candidiasis in the pregnant women attending the Thika District Hospital.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Candida is a gram positive, oval, budding yeast cell that produces pseudohyphae both in culture, tissues and exudates. It is a member of the normal flora of the mucous membranes in the gastro intestinal, upper respiratory and female genital tracts (Prescott et al., 2008). *Candida* is the agent most frequently implicated in the invasive vaginal candidiasis (Trick, 2002). The most common *Candida species* causing vaginal candidiasis is primarily *Candida albicans* followed by *Candida glabrata*, *Candida tropicalis* and *Candida parapsilosis* (Brousse, 2004).

Vaginal candidiasis affects approximately 75% of women of child bearing age (Kaufman, 1986). Factors that predispose women to vaginal candidiasis include hormonal fluctuation especially during pregnancy, luteal phase of menstrual cycle, antibiotic uses and use of oral contraceptives (Gloria et al., 2008). Another 5-10% of seemingly healthy women suffer recurrent vaginal candidiasis without any predisposing factors (Brousse, 2004). It is much more common in pregnant women than in non pregnant women. Moreover, a large proportion of women with chronic recurrent candidiasis first present with the infection during pregnancy (Kent, 1991).

In pregnant women, vaginal candidiasis has been related to emotional stress and suppression of immune system, which steps up the risk of *Candida* species overgrowth and become pathogenic (Sobel et al., 2004). Other risk factors are associated with the eating habits of pregnant women of sugar rich containing food. Pregnancy induced

hormonal modifications alter the vaginal environment and make *Candida* more likely to grow beyond acceptable boundaries (Monif, 2001). In developing countries, there is scanty data regarding the magnitude of *Candida*'s role in vaginal candidiasis in pregnant women along with *Candida species* distribution and antifungal susceptibility (Pfaller and Diekema, 2002). There is limited data that documents the prevalence of vaginal candidiasis in Kenyan pregnant women. Data that documents the current status of antifungal susceptibility trends of vaginal *Candida* species isolated from pregnant women is limited (Bii et al., 2002).

1.2 Statement of Problem

Vaginal candidiasis is one of the most common infections seen in general practice (Meisozo, 2008). Up to three quarters of all women suffer at least one episode of this condition during their lifetime; about half of them may suffer a further episode. Vaginal candidiasis is much more common in pregnant women where it causes spontaneous abortions, chorioamnionitis and it has been associated with premature delivery and death of the infant (Meisozo, 2008). Most cases of vaginal candidiasis in the tertiary hospital care are caused by *Candida albicans,* although studies in other parts of the world have shown that other *Candida* species are becoming increasingly significant in causing the infection in pregnant women. In Kenya, the prevalence of vaginal candidiasis among pregnant women and the causative *Candida* species are not documented. Information on the current status of antifungal drug sensitivity profile on *Candida* species is also limited. The present study therefore intends to determine the prevalence of vaginal candidiasis among pregnant women, the causative *Candida* species and the antifungal susceptibility

profile of the identified vaginal *Candida* species.

1.3 Justification of the Study

Pregnancy is a predisposing factor to vaginal candidiasis. This is due to emotional stress and suppression of immune system, which steps up the risk of *Candida* species overgrowth and therefore become pathogenic (Okungbowa et al., 2003). The increase in the incidence of *Candida* species over the past two decades is significant and non-*albicans Candida* species especially *Candida glabrata* continue to replace *Candida albicans* in causing vaginal candidiasis in pregnant women (Parveen, 2008). *Candida glabrata* has been shown to cause premature rapture of uterine membrane leading to preterm delivery and death of the infant (Salvatore, 2001). Therefore, determination of the prevalence of vaginal candidiasis and identification of vaginal *Candida* species in pregnant women will be of importance in management of the infection and giving better antenatal services in the country. Information on the antifungal drug sensitivity will facilitate appropriate treatment of vaginal candidiasis in pregnant women. This will go a long way to improve on maternal health care services countrywide.

1.4 Research Questions

1. What is the prevalence of vaginal candidiasis among pregnant women attending the antenatal clinic of Thika District Hospital?
2. What are the vaginal *Candida* species isolated from pregnant women attending the antenatal clinic of Thika District Hospital?

3. What is the antifungal susceptibility profile of vaginal *Candida* species isolates from pregnant women attending the antenatal clinic of Thika District Hospital?

1.5 Hypotheses

1. All pregnant women attending the antenatal clinic of Thika District Hospital do not have vaginal candidiasis.
2. The vaginal *Candida* species isolated from pregnant women attending the antenatal clinic of Thika District Hospital are not resistant to the antifungal agents used.

1.6 Objectives

1.6.1 General Objective

To isolate, identify vaginal *Candida* species and their susceptibility profile to antifungal agents in pregnant women attending the antenatal clinic of Thika District Hospital.

1.6.2 Specific Objectives

1. To isolate and identify vaginal *Candida* species in pregnant women attending the antenatal clinic of Thika District Hospital.
2. To determine the frequency of vaginal candidiasis in pregnant women attending the antenatal clinic of Thika District Hospital.
3. To determine the antifungal susceptibility profile of the identified vaginal *Candida* species in pregnant women attending the antenatal clinic of Thika District Hospital.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Candidiasis

Candidiasis is a fungal infection (mycosis) by any of the *Candida* species of which *Candida albicans* is most common (Pappas, 2006). Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life threatening diseases. *Candida* infections of the latter category are also referred to as candidemia and are usually confirmed in severely immunocompromised persons such as cancer, transplant and AIDS patients. *Candida* is listed by the Center for Disease Control (CDC) as a cause of sexually transmitted disease (Prescott et al., 2008). Sexual intercourse with an infected person is the most common mode of spread of genital candidiasis (Tatfeng and Nwobu, 2004). There is no other mycotic pathogen that produces a diverse spectrum of opportunistic disease in human as does *Candida*.

Candida species are part of the normal flora of the gastro intestinal tract, mouth, vagina, and skin (Akortha et al., 2009). They cause infection when some change in the body (rising glucose levels from diabetes mellitus; lowered resistance from an immunosuppressive drug, radiation, aging, or a disease, such as cancer or human immunodeficiency virus [HIV] infection) permits their sudden proliferation or when they're introduced systemically by intravenous or urinary catheters, drug abuse, hyper alimentation, or surgery (Tatfeng and Nwobu, 2004). However, the most common predisposing factor remains the use of broad-spectrum antibiotics, which decrease the number of normal flora and permit an increasing number of Candidal organisms to

proliferate. The vagina of a mother with vaginal candidiasis can cause oral thrush to infant while passing through the birth canal (Pappas, 2006). Thrush is also found in many infants who are breast-fed due to lack of pro-biotic bacteria that fight against *Candida*. The incidence of candidiasis is rising because of wider use of intravenous therapy and a greater number of immunocompromised patients, especially those with HIV infection (Pappas, 2006).

Candidiasis infection can be acute or chronic, localized or systemic. Disseminated candidiasis is frequently life threatening. There are different types of candidiasis including oral candidiasis, genital, balanitis and vaginal candidiasis among others (Akortha et al., 2009). Oral candidiasis affects majorly children and the immunocompromised patients such as the HIV/AIDs patients (Okungbowa et al., 2003). Vulva pruritis is the dominant feature of vulvovaginal candidiasis. Women may complain of dysuria, soreness, irritation, dyspareunia, suprapubic pains, and haematuria, white and clumpy vaginal discharge. The discharge is classically described as thick, adherent and 'cottage cheese like' with a pH of 4.0 – 4.5 (Tatfeng and Nwobu, 2004). Vaginal candidiasis is much more common in pregnant women. The infection causes spontaneous abortions, chorioamnionitis and it has also been associated with premature delivery and death of the infant (Meisozo, 2008). Balanitis is a *Candida* infection of male glans penis and occurs primarily in uncircumcised males (Prescott et al., 2008).

2.1.1 *Candida* Species

The great majority of candidiasis infection is caused by *Candida albicans* (Akortha et al., 2009). Its rate of isolation in swabs from patients with candidiasis is between 80% and 95% (Oriel et al., 2008). *Candida glabrata* is the second most common, but other species such as *Candida krusei*, *Candida parapisilosis*, *Candida tropicalis*, *Candida guilliermandii*, *Candida kufyr* were also identified as causes of candidiasis (Kent, 1991 and Akortha et al., 2009).

2.1.2 Diagnosis

The diagnosis is confirmed by finding the organism on a wet mount of the discharge using potassium hydroxide (KOH). Microscopy may be negative in up to 50% of patients confirmed with genitourinary candidiasis (Akortha et al., 2009). Swabs are cultured on fungal media such as Saborauds dextrose agar (SDA) at room temperature or at 37°C for 48 hours. Yeast colonies are extracted and examined under the microscope for the presence of pseudohyphae. *Candida albicans* is identified by germ tube method and production of chlamydospores. Other *Candida* isolates are identified with a battery of biochemical reactions including sugar fermentation test and sugar assimilation test (Kent, 1991). This is where different sugars are used including glucose, galactose, lactose, maltose, sucrose, raffinose and trehalose. The presence of growth in sugar media indicates the ability of the isolate to assimilate a sugar. The sugar assimilation pattern for individual species is used to identify the *Candida* species (Jawetz et al., 2001). Commercial latex agglutination tests are very useful rapid tests for detecting *Candida*

albicans antigens in vaginal secretions (Kent, 1991).

2.1.3 Treatment

Treatment first aims at improving the underlying condition that predisposes the patient to candidiasis, such as controlling diabetes or discontinuing antibiotic therapy and catheterization, if possible. The many drugs that are available at present to treat candidiasis infection can be divided into four broad groups on the basis of their mechanism of actions (Akortha et al., 2009). These antifungal agents inhibit macromolecule synthesis (flucytosine), impair membrane barrier function (polyenes) and inhibit ergosterol synthesis (azole derivatives, marpholines, allylamines and thiocarbamates) or interact with microtubules (Vanden, 2007). Nystatin is an effective antifungal for superficial candidiasis. Clotrimazole, fluconazole, ketoconazole, and miconazole are effective in mucous-membrane and vaginal candidal infections. Ketoconazole or fluconazole is the treatment of choice for chronic candidiasis of the mucous membranes. Treatment for systemic infection consists of intravenous amphotericin B or fluconazole (Akortha et al., 2009)

2.2 Vaginal Candidiasis

Vaginal cadidiasis is a fungal yeast infection of the vulva and/or vagina. It causes smelly, thick, white yellow discharge that might be accompanied by itching and burning. It can also make walking, urinating or sex very painful (Sobel, 2000). Vulvovaginal candidiasis is the most common cause of vaginitis in Europe and the second most common cause of vaginitis in the United States (Scenmach and Hillier, 2000). In the developed countries,

there is a great deal of data that shows the magnitude of vaginal candidiasis especially in pregnant women (Gloria et al., 2008) but in the developing countries there is limited data that document the prevalence of vaginal candidiasis especially in pregnant women (Gloria et al., 2008).

Up to three quarters of all women will suffer at least one episode of this condition during their lifetime, around half of them suffering a further episode (Sobel, 2000). Under some conditions such as reduced immunity, prolonged antibiotic therapy, use of steroids and oral contraceptives with a high estrogen content, diabetes, malnutrition, pregnancy, obesity, poor hygiene, use of immunosuppressant drugs and HIV/AIDs infection where there is disruption of the natural balance of the vaginal environment and cause *Candida* infection (Lisiak et al., 2000). Wearing of tight fitting pants and reaction to chemical ingredients found in soaps and detergents alters the natural organism in the vagina facilitating the growth of *Candida* leading to vaginal candidiasis (Rylander and Berglund, 2004; Akortha et al., 2009).

Vaginal candidiasis can be an occasional problem for even the healthiest women. However, it's more common and severe in women with a weakened immune system (Sobel, 1992). For many, a repeating or worsening vaginal yeast infection is the first symptom of HIV infection (Sobel et al., 2004). The incidence of vaginal candidiasis is almost doubled in pregnant women particularly in the third trimester compared with non pregnant women. There also seems to be a trend for it to occur during pregnancy as a result of the increased levels of estrogen and corticoids reducing the vaginal defense

mechanisms against such opportunistic infections as *Candida* (Rylander and Berglund, 2004).

2.2.1 Vaginal *Candida* species

Vaginal candidiasis is caused by the fungus *Candida* (Mitchell, 2004). *Candida* is found in vagina of 35-50% of healthy women (Okungbowa et al., 2003). Under some conditions such as reduced immunity, prolonged antibiotic therapy, use of oral contraceptives, diabetes, pregnancy, obesity and immune suppressant drugs that alter the environment in the vagina, and thus *Candida* becomes pathogenic and cause vaginal candidiasis. *Candida albicans* causes about 90% of the cases of vaginal candidiasis (Monif, 2001). Other *Candida* species include *Candida glabrata* and *Candida tropicalis*. The spore form of *Candida albicans* spread the infection and the infection is asymptomatic. The mycelia form of *Candida albicans* induces symptoms. *Candida glabrata* and *Candida tropicalis* do not have mycelia (Lisiak et al., 2000). However, *Candida glabrata* which is a *non-albicans Candida* species has been documented to be replacing *Candida albicans* in causing vaginal candidiasis in pregnant women (Salvatore, 2001). It has also been shown that it causes premature rapture of the uterus membrane leading to preterm delivery (Parveen, 2008).

2.2.2 Clinical Presentation

Most women with vaginal candidiasis complain of intense vulval and vaginal pruritus with or without vaginal discharge (Tatfeng and Nwobu, 2004). 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 (El-Din et al., 2001). Dysuria and dyspareunia are common. Vulval erythematic with fissuring is the most common clinical finding (Schenmach and Hillier, 2000). This is often isolated to the microcutaneous 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 classical signs of flavid vaginal candidiasis in pregnant women. Often the discharge is thick and white, but it can be thin or even purulent (Sobel et al., 2004).

2.2.3 Treatment

Topical treatments (active only on the area where it's applied) are the first choices for yeast infections and these generally work for mild to moderate cases. These include vaginal creams and suppository tablets. Many are available over the counter in a drug store (Odds et al., 2003). Most topical treatments are put into the vagina once or twice a day for 3 days or once a day for 7 days. Longer courses (7-14) days) may be more effective in HIV- positive women (Okungbowa et al., 2003). Generally, topical treatments do not cause side effects, but in a small number of women, they may lead to vaginal burning, itching or skin rush. Topical treatments are highly recommended for pregnant woman for the treatment of vaginal candidiasis (Akortha et al., 2009). A few women have experienced cramps or headaches. Oil based vaginal creams should be used with caution as they may weaken latex condoms and diaphragms (Otero et al., 1999). Currently, azole drugs are increasingly used to treat vaginal candidiasis. These include

fluconazole, ketoconazole, clotrimazole, itraconazole and are marketed as creams while isoconazole is marketed as pessaries (Vanden, 2007).

Itraconazole and fluconazole have been licensed for the short term oral treatments of vaginal cadidiasis. Fluconazole is given as a single dose of 150 mg and itraconazole as two doses of 200 mg eight hours apart with food. These drugs are more expensive than topical preparations, but patient compliance is improved (Higgins and Wolley, 1993). Nystatin was the first polyene antifungal to be applied to treat vaginal candidiasis. It is still one of the cheapest agents for the treatment of vaginal candidiasis but it requires a longer treatment period (two weeks) and has a lower cure rate than the topical or oral azoles (Mathema et al., 2001).

2.3 Antifungal Susceptibility Profile

Azole antifungal agents have therapeutic activity against different *Candida* species especially *C. albicans* (Kangogo et al., 2008). Among azole drugs, fluconazole is more tolerated with wider spectrum of efficiency (Kangogo et al., 2008). During the last decade, the higher incidence of fungal infections in hospitalized patients has resulted in the use of systemic antifungal agents especially fluconazole which remains a first line antifungal agent (Redding et al., 1994). However in the recent years increasing resistance to fluconazole has appeared and antifungal resistance is quickly becoming a major problem in immunocompromised patients (White and Marr, 1998). Majority of non *albicans Candida* are susceptible to fluconazole. However, a marked resistance is emerging among *C. glabrata* and *C. krusei.* It is well established that *C. krusei* is

intrinsically resistant to all azole drugs except itraconazole (Okungbowa et al., 2003).

Candida glabrata exhibits low resistance to azole antifungal drugs (Kangogo et al., 2008). It is known that *C. glabrata* can develop resistance to azole drugs especially fluconazole as a primary or secondary mechanism (White and Marr, 1998). Other non *albicans* like *Candida tropicalis, Candida parapsilosis* and *Candida famata* are susceptible to azole drugs (Kangogo et al., 2008). A great majority of *Candida* species exhibit or are susceptible to tropical nystatin compared to azoles drugs. This is related to the development of resistance of *Candida* species to the drug. This is because the drug is cheap and available on the counter and it's likely to be misused resulting in development of drug resistance (Kent, 1991). Emergence of drug resistance among *Candida* isolates and consequent increase in serious vaginal candidiasis have been reported (Odds et al., 2003). The antifungal drug sensitivity profile is not routinely done and therefore the present status of fungal resistance to convectional antifungal drugs in Kenya is limited (Bii et al., 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted at Thika District Hospital. The hospital is located in Thika town in Central province of Kenya (Appendix VI).

3.2 Study Population

All pregnant women aged 15 years of age with symptoms of vaginal candidiasis attending the antenatal clinic of Thika District Hospital from the month of June 1 to August 30, 2010 were enrolled into the study.

3.3 Study Design

A cross sectional study among pregnant women with symptoms of vaginal candidiasis attending the antenatal clinic of Thika District Hospital was adopted.

3.4 Sample Size Determination

The minimum sample size was determined by the formula of Alakpa and Fagbeuro (2000) using the vaginal prevalence rate of 8% (Alakpa and Fagbeuro, 2000).

$N= Z^2 \times P (1-P)/ E^2$

Where:

N= Desired minimal sample size.

Z= Standard normal deviation = 1.96 (from the tailed normal table).

P= Prevalence rate

E= The desired degree of accuracy at 95% confidence level= 0.05

N= 1.96^2 x 0.08 (0.92)/0.05^2 = 102.4 ≈ 104 samples.

3.5 Informed Consent

The consent form was read and explained to the pregnant women. After understanding and accepting participation, they signed it (Appendix IV).

3.6 Sampling Technique

Purposive sampling technique was used to enroll the pregnant women with symptoms of vaginal candidiasis in the study.

3.7 Inclusion and Exclusion Criteria

All pregnant women with symptoms of vaginal candidiasis aged 15 to 50 years were enrolled in this study. All pregnant women without symptoms of vaginal candidiasis aged below 15 years and above 50 years were excluded from participation in this study.

3.8 Laboratory Procedures

3.8.1 Sample collection

Vaginal swabs were collected from pregnant women with symptoms of vaginal candidiasis who attended the antenatal clinic of Thika District Hospital. Sterile speculum and artificial swab sticks were used for the collection of the swabs. The pregnant women were deemed symptomatic if they presented with itching, difficult in walking, dysuria and presence of thick adherent plaques on the vulval, vaginal or cervical epithelium.

Samples collected were accompanied with a request form (appendix V) with age, pregnancy trimester, history of using antibiotics, antifungal drugs and contraceptives information and were taken to the Hospital Laboratory for vaginal *Candida* species analysis. The women were stratified into four groups according to their ages as follows: 15-25, 26-35, 36-45, and over 46 years. They were also stratified according to the trimester of the pregnancy as first, second and third trimester.

3.8.2 Gram Stain

The test was carried out essentially according to the procedure of Chander (2002). Smears were made from the vaginal swab and stained using the Gram staining procedure. Gram stained smears were used to examine the presence of gram positive budding yeast cells with pseudohyphae. Specimen was considered as acceptable when 25 or more polymorphonuclear leukocytes were seen on low power field (100X) with few (less than 10) squamous epithelial cells (Chander, 2002).

3.8.3 Growth on Culture Media

Samples were cultured on Saborauds dextrose agar (SDA) containing two percent chloramphenicol. Inoculated plates were incubated at 37°C and examined after 48 hours for cream coloured pastry colonies and budding yeast cells suggestive of *Candida* species (attached appendix I, plate 1). Isolates from SDA were inoculated on CHROM agar (CHROM agar *Candida*, Difco) using an inoculating needle and incubated at 37°C for 72 hours to ensure detection of mixed cultures by color changes (attached appendix I, plate 4). The method is based on the differential release of chromogenic breakdown products

from various substrates by *Candida* species following differential exoenzyme activity (Baker, 2002). This test was used for presumptive identification of *C. albicans, C. tropicalis* and *C. parapsilosis.*

3.8.4 Chlamydospore Formation Test

All *Candida* isolates were tested for the production of chlamydospores in corn meal agar with Tween 80 (Baker, 2002). The isolates were inoculated in cornmeal agar. The test involved streaking and stabbing the media with a 48 hour old yeast colony and, covered with sterile cover slip and incubated at 25°C for 72 hours. Chlamydospore production was examined after staining with lactophenol cotton blue (Dalmau morphology method, Baker, 2002). The isolates were categorized as chlamydospore positive or negative (attached appendix II, plate 3). The test was used as a presumptive confirmatory test for the identification of *Candida albicans.*

3.8.5 Germ Tube Test

This method was used as a presumptive test for identification of *Candida albicans*. Procedure of Baker (2002) was used in carrying out the test. A single colony of the test yeast cells from a pure culture was inoculated in human serum and incubated at 37°C for 2-4 hours. A drop of the incubated serum was placed on a microscope slide and covered with a cover slip. The wet mounts were examined under the microscope for the presence of germ tube using the 40X objective (Dalmau morphology method, Baker, 2002). The isolates were classified as either germ tube positive or germ tube negative (attached appendix I, plate 2).

3.8.6 Sugar Assimilation Test

The test was carried out essentially using the procedure of Lodder (1970). The assessment of the ability of yeast to utilize carbohydrates was based on the use of carbohydrate-free yeast nitrogen base agar (Lodder, 1970). Observation for the presence of growth around carbohydrate impregnated filter paper discs was done after incubation for 18 hours at 30°C. Carbohydrates used were glucose, galactose, lactose, maltose, sucrose, raffinose, trehalose and cellobiose. Presence of growth in the medium indicated the ability of the isolate to assimilate a sugar (Lodder, 1970). The *Candida* species were identified using the sugar assimilation patterns for individual species in the Table of Lodder (1970) shown below.

Table 3.1: Sugars Assimilation Reactions Patterns.

	Carbohydrates							
Organism	**Glucose**	**Galactose**	**Maltose**	**Sucrose**	**Lactose**	**Raffinose**	**Trehalose**	**Cellobiose**
Candida albicans	+	+	+	+	-	-	+	-
Candida steallatoidea	+	+	+	-	-	-	+	-
Candida tropicalis	+	+	+	+	-	-	+	-
Candida pseudotropicalis	+	+	-	+	+	+	-	-
Candida parapsilosis	+	+	+	+	-	-	+	-
Candida guilliermondii	+	+	+	+	-	+	+	+
Candida krusei	+	-	-	-	-	-	-	-
Candida glabrata	+	-	-	-	-	-	+	-

KEY

\+ Positive (presence of growth) - Negative (no growth)

3.8.7 Antifungal Susceptibility Testing

This test was carried out on the isolates using broth micro-dilution minimum inhibitory concentration method of Hace et al (2005) and based on the approved National Committee for Clinical Laboratory Standards (NCCLS) guidelines for a broth micro-dilution reference method (2002). This method recommends the use of RPMI - 1640 medium (with glutamine and phenol red, without bicarbonate (Sigma R-7755, St. Louis, U.S.A.). The RPMI - 1640 medium was supplemented with 0.2 % glucose and buffered to a pH of 7.0 with 0.165 mol / L Mops (3-[N-morpholino] propanesulfonic acid) (Sigma M-6270). Fluconazole, ketoconazole, itraconazole, clotrimazole and topical nystatin antifungal agents were employed to determine the susceptibility trends of *Candida* species.

Two fold serial dilutions of each antifungal agent were prepared as outlined in approved NCCLS (2002) document. Seven different concentrations/ dilutions of each drug were prepared and tested as follows; 0.1, 0.5, 1.0, 5.0, 10, 50, and 100µg/ml for fluconazole and 0.01, 0.05, 0.10, 0.50, 1.0, 5.0 and 10µg/ml for ketoconazole, itraconazole, clotrimazole and topical nystatin (NCCLS, 2002), (attached appendix II, 2). The different antifungal drug dilutions/concentrations were dispensed into U-shaped microtiter plates (Greiner Bio-one, Holland). The test isolates were suspended in RPMI medium usually matched to 0.5 McFarland standard suspensions. Zero point one milliliter (0.1ml) of the mixture was inoculated into each microtiter well containing different dilutions of the test drug diluting the drug ten times to the required concentration. The inoculated microtiter plates were incubated at 35°C for 48 hours. An optical clarity (no growth) and turbidity (presence of growth) was examined at each set of well for all the seven concentrations of

each antifungal agent and only the *Candida* species isolates that showed optical clarity (no growth) at each concentration was recorded (NCCLS, 2002). The test was done in duplicate in each concentration of the drugs and the mean was recorded. The Minimum Inhibitory Concentration (MIC) was regarded as the lowest antifungal concentration that inhibited fungal growth. A susceptible interpretation was given to any species for which the MIC of fluconazole was ≤10µg/ml and for ketoconazole, itraconazole, clotrimazole and topical nystatin was ≤5µg/ml and the antifungal concentration that inhibited growth to a high number of a *Candida* species isolates was regarded as the single optimal dosage (NCCLS, 2002).

3.8.7.1 Quality Control Strains

Prior to antifungal susceptibility testing each isolate was sub-cultured at least twice on SDA for 24 hours before use. This was to obtain a pure culture of each isolate. The Quality Control strain, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258, were included in each batch of susceptibility tests to ensure quality control and the results were accepted only when the MIC of the QC strain were within the NCCLS limits.

3.9 Data Analysis

All collected data was entered into Microsoft® excel data sheet. Prevalence was calculated using the formula of Le and Boen (1995) $\{\frac{0}{P} x\ 100\%\}$, Where: O = The number of individuals with the disease, P = The population at risk of having the infection during the study period. The relationship between the occurrences of vaginal *Candida*

species in the various age groups of the pregnant women was calculated using Pearson moment correlation test. The susceptibility of vaginal *Candida* species to the antifungal drugs was calculated using ANOVA to establish any variations. Chi-square test was used to test the susceptibility association of the two groups of the antifungal drugs used to the vaginal *Candida* species isolated. All the statistical analysis was done using MINITAB and Stastical Package for Social Sciences (SPSS) version 13.0 computer package.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic Data of Patients and Prevailing symptoms

A total of 104 pregnant women with symptoms of vaginal candidiasis were examined. Forty eight percent (48%) of the women had only one of the symptoms (itching, burning or dysuria and thick adherent plaques on the vaginal, vulval or cervical epithelium). The remaining 52% had two or more of the symptoms of the infection. Fifty six (60%) of the women were in the age bracket of 26-35 years, 24 women (24%) were in the age bracket of 15-25 years and 12 women (12%) were in the age group of 36-45 years. The age group 46-50 years had the least with 2 patients. Sixty four pregnant women (68%) were in their 3rd trimester of pregnancy, 20 (21%) in their 2nd trimester while 10(11%) were in their 1st trimester. For fertility protection, 80% of the women had a history of using contraceptives before they got pregnant. Seventy percent (70%) of the women had been using over the counter antifungal drugs and antibiotics for the treatment of the infection before attending the clinic.

4.2 Isolation and Identification of Vaginal *Candida* Species

Five (5) C*andida* species were isolated and identified. These were *Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei* and *Candida parapsilosis. Candida albicans* 60 (63.83%), *Candida glabrata* 28 (29.79%), *Candida tropicalis* 3(3.19%), *Candida krusei* 2 (2.13%) and *Candida parapsilosis* 1 (1.06%) as shown in Figure 4.1 below.

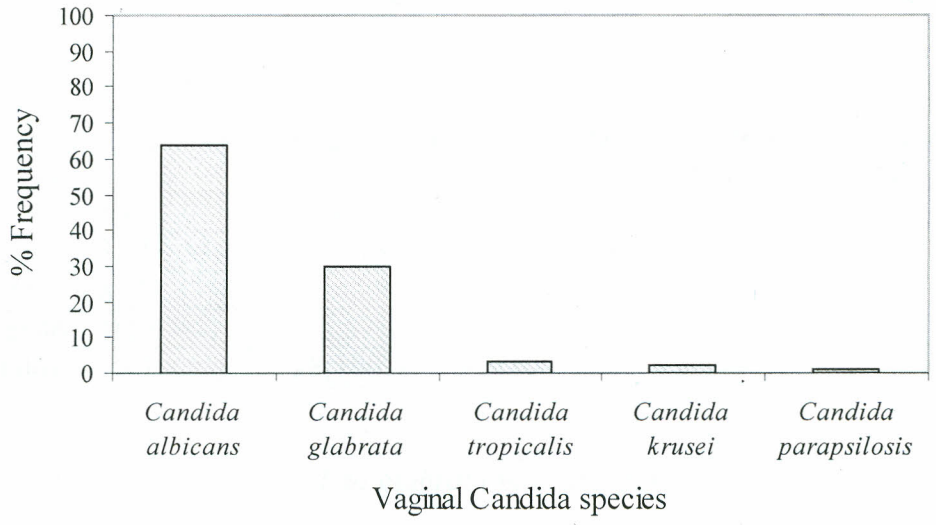


Figure 4.1: The percentage occurrence of vaginal *Candida* species in pregnant women.

To determine the occurrence of the different vaginal *Candida* species isolated, One-way ANOVA was used to analyze the data. The results showed that there was a significant difference in the occurrence of the *Candida* species causing vaginal candidiasis in pregnant women ($F = 616.62$, $df = 4$, $P = 0.000$, $P < 0.01$). This result incriminated *Candida albicans* as the most common vaginal *Candida* species causing vaginal candidiasis among pregnant women attending antenatal clinic of Thika District Hospital.

4.2.1 The distribution of vaginal *Candida* species within different age groups of the pregnant women.

The results showed that most of the vaginal *Candida* species were isolated from the pregnant women at the age brackets of 26-35 years with a total of 56 (60%) isolates. The youngest group of 15-25 years followed with 24 (26%) isolates. The age group of 36-45 years had 12 (12%) isolates and the age group of over 46 years had the least number of isolates with 2 (2%) as shown in Table 4.1 below.

Table 4.1: The distribution of vaginal *Candida* species within different age groups of the pregnant women.

Age group (years)	***C. albicans* (%)**	***C. glabrata* (%)**	***C. tropicalis* (%)**	***C. krusei* (%)**	***C. parapsilosis* (%)**	**Total (N)**
15-25	19(31.67%)	3(10.71%)	1(33.33%)	1(50%)	0(0%)	24
26-35	32(53.33%)	20(71.43%)	2(67.67%)	1(50%)	1(100%)	56
36-45	8 (13.33%)	4(14.29%)	0(0%)	0(0%)	0(0%)	12
Over 46	1(1.67%)	1(3.57%)	0(0%)	0(0%)	0(0%)	2
Total(n)	60(100%)	28(100%)	3(100%)	2(100%)	1(100%)	94

Candida albicans and *Candida glabrata* were the most isolated vaginal *Candida* species in the age bracket 26-35 years with *Candida albicans* being the most prevalent *Candida* species causing vaginal candidiasis in all the age brackets of the pregnant women (Table 4.1 above). It was observed that all the vaginal *Candida* species were isolated in the age group 26-35 years. The data showed that the occurrence of vaginal *Candida* species increased with age in pregnant women below 35 years ($r = 0.351$, $P = 0.394$). However, in those women above 35 years of age, there was a decrease in occurrence of vaginal *Candida* species ($r = -0.496$, $P = 0.060$) as shown in Figure 4.2 below.

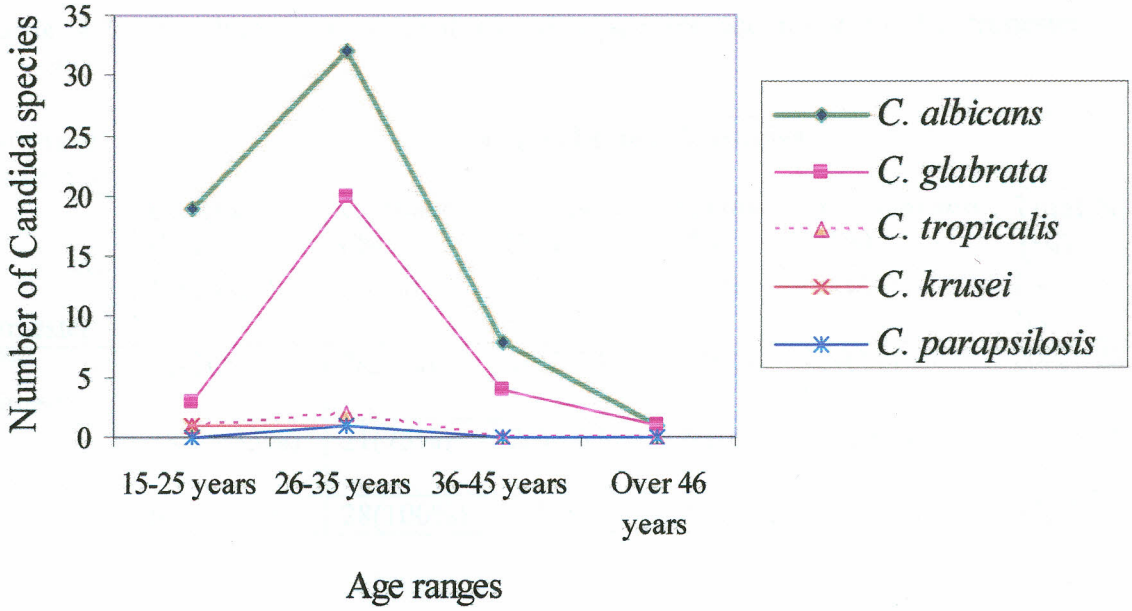


Figure 4.2: Vaginal *Candida* species isolated within different age groups of the pregnant women.

4.2.2 The distribution of vaginal *Candida* species according to the trimester of pregnancy.

The results showed that the 3rd trimester had the highest number of vaginal *Candida* species isolated with 64 (68.09%), followed by 2nd trimester 20 (21.28%) isolates. The 1st trimester had the least number of species with 10 (10.63%) as shown in Table 4.2 below.

Table 4.2: Distribution of vaginal *Candida* species according to the trimester of pregnancy.

Pregnancy trimester	**Vaginal *Candida* species**					
	***C. albicans* (%)**	***C. glabrata* (%)**	***C. tropicalis* (%)**	***C. krusei* (%)**	***C. parapsilosis* (%)**	**Total(N) (%)**
1st trimester	10(16.67%)	0(0%)	0(0%)	0(0%)	0(0%)	10(10.64 %)
2nd trimester	12(20%)	7(25%)	1(33.33%)	0(0%)	0(0%)	20(21.28 %)
3rd trimester	38(63.33%)	21(75%)	2(67.67%)	2(100%)	1(100%)	64(68.08 %)
Total(n)	60(100%)	28(100%)	3(100%)	2(100%)	1(100%)	94(100%)

The 3rd trimester had all the five (5) vaginal *Candida* species isolated. *Candida albicans* was the only species isolated in all the three (3) trimesters of pregnancy. It had the highest occurrence rate than all the other vaginal *Candida* species especially in the 3rd trimester of pregnancy. Therefore, it was incriminated as the most common vaginal *Candida* species causing vaginal candidiasis in all the 3 trimesters of pregnancy. *Candida glabrata* was isolated in 2nd and 3rd but not in the 1st trimester. It was the second most common species isolated in the 3rd trimester. *Candida tropicalis* was isolated in 2nd and 3rd but not in the 1st trimester. *Candida krusei* and *Candida parapsilosis* were the least isolated and were only isolated in the 3rd trimester as shown in Table 4.2 above.

4.3 Prevalence of vaginal candidiasis in pregnant women attending the antenatal clinic of Thika District Hospital.

One hundred and four (104) pregnant women with symptoms of vaginal candidiasis visiting the antenatal clinic of Thika District Hospital participated in this study. Ninety four, 94 (90.38%) of the pregnant women tested positive while 10 (9.62%) tested

negative for vaginal candidiasis infection in the laboratory.

Table 4.3: Prevalence of vaginal candidiasis in pregnant women attending the antenatal clinic of Thika District Hospital.

Status	No. of pregnant women	Percentage (%)
Positive	94	90.38
Negative	10	9.62
Total	104 (N)	100

The number of positive pregnant women in relation to the population at risk involved at the period of the study was calculated to give the percentage prevalence of vaginal candidiasis among the pregnant women.

Using the formula ($\frac{0}{P}$ x 100%),

Where: O = The number of individuals with the disease.

P = The population at risk during the study period.

Percentage prevalence = 94/ 220 x 100% = 42.7%

4.3.1 The percentage distribution of vaginal candidiasis within different age ranges of the pregnant women.

The percentage distribution of vaginal candidiasis among the different age groups were as follows; 56 (60%) of the women were 26-35 years of age, 24 (26%) were in the ages of 15-25 years and 12 (12%) were 36-45 years while the least number 2 (2%) of patients with infection were over 46 years of age as shown in Figure 4.3 below. The study noted that frequency of vaginal candidiasis significantly increased with the ages in pregnant women below 35 years ($r = 1.00$, $P = 0.00$, $P < 0.05$). However, in pregnant women above 35 years of age, there was a significant decrease in frequency of vaginal

candidiasis (r = - 1.00, P = 0.00, P < 0.05).

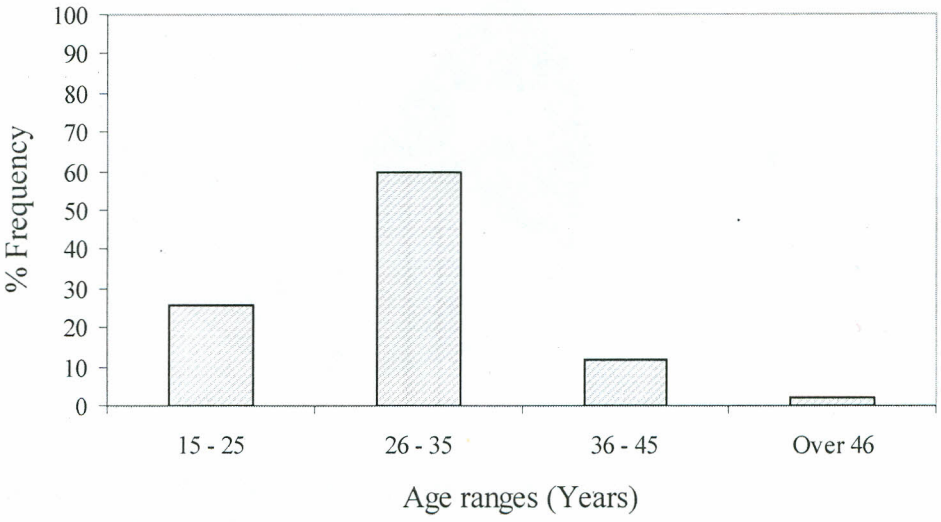


Figure 4.3: The percentage distribution of vaginal candidiasis within different age ranges of the pregnant women.

4.3.2. The distribution in percentage of vaginal candidiasis according to the trimester of the pregnancy.

The 3rd trimester had the highest number of patients 64 (68.09%), followed by 2nd trimester with 20 (21.28%) while 1st trimester had the least number of patients 10 (10.63%) as shown in Figure 4.4 below.

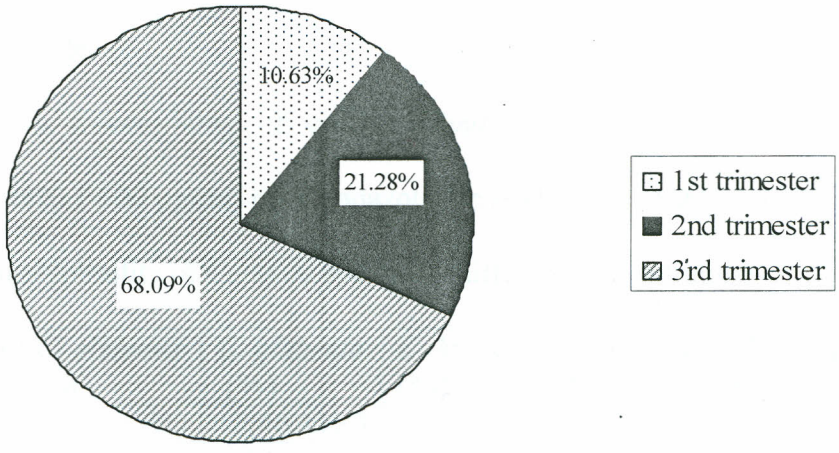


Figure 4.4: The distribution in percentage of vaginal candidiasis according to the trimester of the pregnancy.

The occurrence of vaginal candidiasis infection in pregnant women was significantly different in the three trimesters ($F = 103.17$, $df = 2$, $P = 0.002$, $P < 0.01$ $P < 0.05$).

4.4 Susceptibility of vaginal *Candida* species to different antifungal agents.

4.4.1. Fluconazole

The results on susceptibility of vaginal *Candida* species isolates against different concentrations (0.1-100 µg/ml) of fluconazole are as shown in Table 4.4 below.

Table 4.4: Susceptibility of *Candida* species to different concentrations of fluconazole.

Fluconazole µg/ml	***C. albicans* (%)**	***C. glabrata* (%)**	***C. krusei* (%)**	***C. tropicalis* (%)**	***C. parapsilosis* (%)**
0.1	14(23.33%)	13(46.43%)	Nil	Nil	Nil
0.5	27(45%)	4(14.29%)	Nil	Nil	Nil
1.0	10(16.67%)	8(28.57%)	Nil	3(100%)	1(100%)
5.0	5(8.33%)	3(10.71%)	Nil	Nil	Nil
10.0	2(3.33%)	Nil	Nil	Nil	Nil
50.0	1(1.67%)	Nil	1(50%)	Nil	Nil
100	1(1.67%)	Nil	1(50%)	Nil	Nil
Total	60(100%)	28(100%)	2(100%)	3(100%)	1(100%)

Nil = No organism was tested at that concentration.

Fifty eight (58) *Candida albicans* isolates gave optical clarity at lower concentrations ≤10µg/ml and were regarded as susceptible (Table 4.4) above. The remaining 2 *Candida albicans* isolates which gave optical clarity at higher concentrations >10µg/ml were regarded as resistant. The single optimal dosage that most of the isolates gave optical clarity was 0.5µg/ml with 27 isolates. *Candida glabrata* was 100% susceptible to fluconazole since all the isolates 28 showed optical clarity at concentrations ≤10µg/ml. Its single optimal dosage was at concentration of 0.1µg/ml with 13 isolates. High resistance (100%) was recorded for *Candida krusei* since all the 2 isolates had their optical clarity at concentrations >10µg/ml and its optimal dosage were at both 50µg/ml and 100µg/ml (Table 4.4) above. *Candida tropicalis* and *Candida parapsilosis* were all

susceptible to fluconazole.

The proportion of *Candida albicans* that was susceptible to fluconazole was 96.67% and only 3.33% were resistant. *Candida glabrata* was 100% susceptible to this drug. High resistance (100%) was recorded for *Candida krusei* while *Candida tropicalis* and *Candida parapsilosis* were all 100% susceptible to fluconazole as shown in Figure 4.5 below.

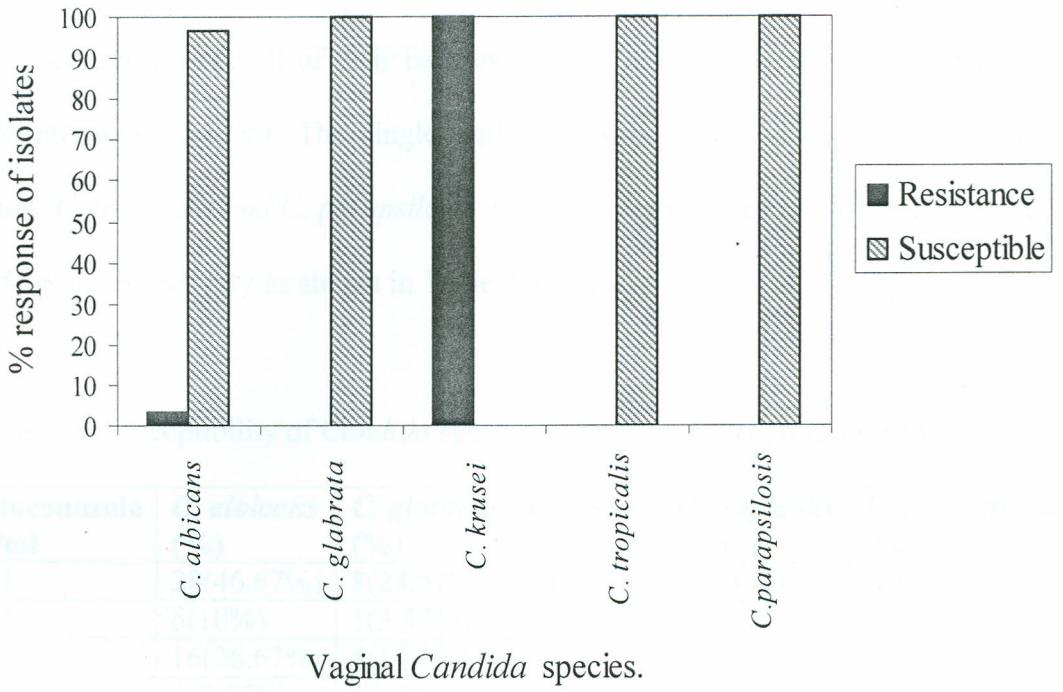


Figure 4.5: Percentage response of vaginal *Candida* species isolates to fluconazole.

4.4.2. Ketoconazole

Candida albicans was highly susceptible to ketoconazole as 57 isolates showed optical clarity at concentrations ≤5µg/ml. Three (3) isolates showed optical clarity at concentrations >5µg/ml and were considered resistant. *Candida glabrata* too, showed a high susceptibility to the drug with 27 of the isolates showing optical clarity at concentrations ≤5µg/ml. The remaining one (1) isolate showed optical clarity at concentrations >5µg/ml and was regarded as resistant. *Candida krusei* showed moderate resistance since only 1 of the 2 isolates showed optical clarity at higher concentrations >5µg/ml. *Candida tropicalis* and *Candida parapsilosis* showed the highest susceptibility to ketoconazole since all of their isolates 3 and 1 respectively showed optical clarity at concentrations ≤5µg/ml. The single optimal dosage for *C. albicans, C. glabrata, C. krusei, C. tropicalis and C. parapsilosis* were at concentrations of 0.01, 0.5, 0.5, 0.01 and 0.05µg/ml respectively as shown in Table 4.5 below.

Table 4.5: Susceptibility of *Candida* species to different concentrations of ketoconazole.

Ketoconazole µg/ml	***C. albicans* (%)**	***C. glabrata* (%)**	***C. krusei* (%)**	***C. tropicalis* (%)**	***C. parapsilosis* (%)**
0.01	28(46.67%)	8(28.57%)	Nil	3(100%)	Nil
0.05	6(10%)	1(3.57%)	Nil	Nil	1(100%)
0.1	16(26.67%)	4(14.29%)	Nil	Nil	Nil
0.5	4(6.67%)	14(50%)	1(50%)	Nil	Nil
1.0	2(3.33%)	Nil	Nil	Nil	Nil
5.0	2(3.33%)	1(3.57%)	Nil	Nil	Nil
10	3(5%)	1(3.57%)	1(50%)	Nil	Nil
Total (n)	60(100%)	28(100%)	2(100%)	3(100%)	1(100%)

NIL = No organism was tested at that concentration.

The percentage response of vaginal *Candida* species isolates to ketoconazole is as shown in Figure 4.6 below.

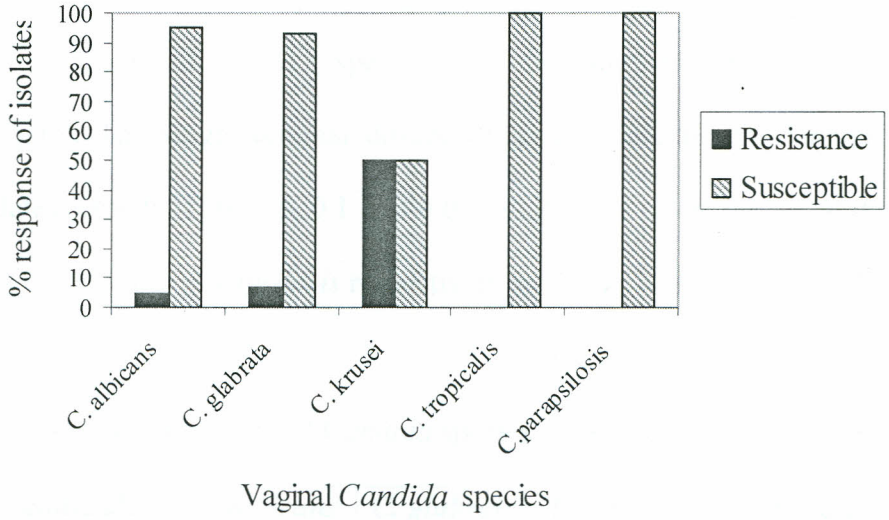


Figure 4.6: Percentage response of vaginal *Candida* species isolates to ketoconazole.

Candida albicans showed a high susceptibility to ketoconazole (95.0%) and only 5.0% of the isolates were resistant (figure 4.6) above. Ninety six point four percent (96.43%) isolates of *Candida glabrata* were susceptible while 3.57% of its isolate was resistant. *Candida krusei* isolates showed a 50% susceptibility to ketoconazole as 50% (one of its isolate) was resistant. All the 3 isolates of *Candida tropicalis* and 1 isolate of *Candida parapsilosis* showed 100% susceptibility to ketoconazole as shown in Figure 4.6 above.

4.4.3. Itraconazole

High susceptibility was recorded for *Candida albicans* to itraconazole with 56 isolates showing optical clarity at concentrations ≤5µg/ml; whereas 4 isolates showed optical clarity at concentrations >5µg/ml and were regarded as resistant. *Candida glabrata* and

Candida krusei isolates showed 100% susceptibility to itraconazole as all the 28 and 2 isolates respectively showed optical clarity at concentrations ≤5µg/ml. Similarly, *Candida tropicalis* and *Candida parapsilosis* isolates showed susceptibility of 100% in all the isolates (3 and 1) respectively as they showed optical clarity at concentrations ≤5µg/ml. The single optimal dosage of itraconazole for the vaginal *Candida* species isolates were 0.01, 0.5, 5, 0.1 and 0.01µg/ml for *C. albicans, C. glabrata, C. krusei, C. tropicalis* and *C. parapsilosis* respectively as shown in Table 4.6 below.

Table 4.6: Susceptibility of *Candida* species to different concentrations of itraconazole.

Itraconazole concentrations µg/ml	***C. albicans* (%)**	***C. glabrata* (%)**	***C. krusei* (%)**	***C. tropicalis* (%)**	***C. parapsilosis* (%)**
0.01	27(45%)	8(28.57%)	Nil	Nil	1(100%)
0.05	3(5%)	3(10.71%)	Nil	Nil	Nil
0.1	5(8.33%)	2(7.14%)	Nil	3(100%)	Nil
0.5	15(25%)	14(50%)	Nil	Nil	Nil
1.0	3(5%)	1(3.57%)	Nil	Nil	Nil
5.0	3(5%)	Nil	2(100%)	Nil	Nil
10	4(6.67%)	Nil	Nil	Nil	Nil
Total (n)	60(100%)	28(100%)	2(100%)	3(100%)	1(100%)

NIL = No organism was tested at that concentration.

Candida albicans isolates showed 93.33% susceptibility to itraconazole and only 6.67% of its isolates were resistant to the drug. *Candida glabrata, C. krusei, C. tropicalis* and *C. parapsilosis* isolates showed 100% susceptibility to itraconazole as shown in Figure 4.7 below.

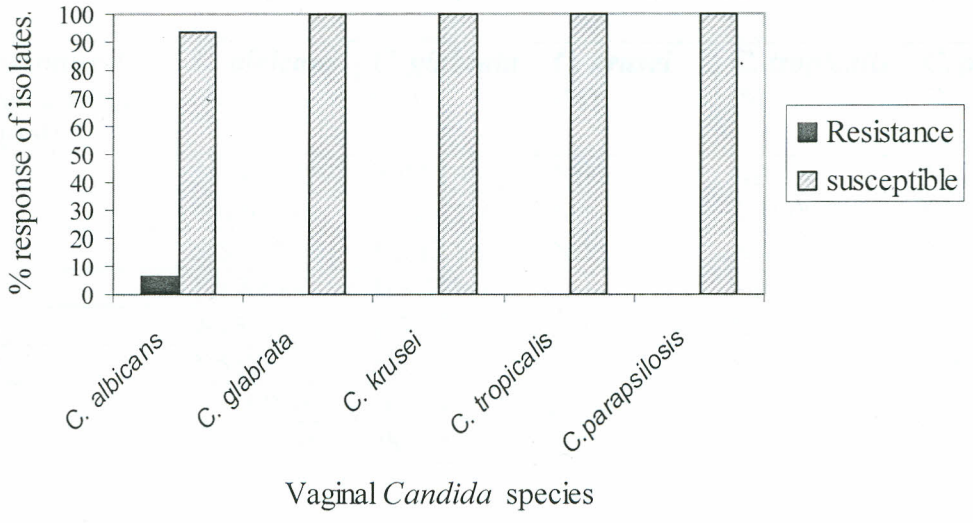


Figure 4.7: Percentage response of vaginal *Candida* species isolates to itraconazole.

4.4.4. Clotrimazole

Candida albicans 58 isolates were susceptible to clotrimazole with optical clarity at concentrations ≤5µg/ml. The remaining two (2) isolates were resistant as they showed optical clarity at concentrations >5µg/ml (Table 4.7). Twenty six (26) isolates of *Candida glabrata* were susceptible as they showed optical clarity at concentrations ≤5µg/ml while two (2) isolates showed optical clarity at concentrations >5µg/ml and were regarded as resistant. One hundred percent (100%) resistance of *Candida krusei* to clotrimazole was observed as all the two (2) isolates had optical clarity at concentrations >5µg/ml. *Candida tropicalis* and *Candida parapsilosis* was 100% susceptible to the drug for the 3 and 1 isolates respectively had optical clarity at concentrations ≤5µg/ml. The single optimal dosage of this drug to the different vaginal *Candida* species isolated was as follows: *Candida albicans* (0.01), *Candida glabrata* (0.1), *Candida krusei* (10), *Candida tropicalis* (0.05) and *Candida parapsilosis* (0.1µg/ml) as shown in Table 4.7 below.

Table 4.7: Susceptibility of *Candida* species to different concentrations of clotrimazole.

Clotrimazole concentrations (µg/ml)	***C. albicans*** (%)	***C. glabrata*** (%)	***C. krusei*** (%)	***C. tropicalis*** (%)	***C. parapsilosis*** (%)
0.01	25(41.67%)	9(32.14%)	Nil	Nil	Nil
0.05	4(6.67%)	3(10.71%)	Nil	3(100%)	Nil
0.1	5(8.33%)	12(42.86%)	Nil	Nil	1(100%)
0.5	12(20%)	1(3.57%)	Nil	Nil	Nil
1.0	9(15%)	1(3.57%)	Nil	Nil	Nil
5.0	3(5%)	Nil	Nil	Nil	Nil
10	2(3.33%)	2(7.14%)	2(100%)	Nil	Nil
Total	60(100%)	28(100%)	2(100%)	3(100%)	1(100%)

Nil = No organism was tested at that concentration.

Ninety six point six seven (96.67) % of *Candida albicans* isolates were susceptible to clotrimazole and 3.33% isolates were resistant. Ninety two point eight six percent (92.86%) isolates of *Candida glabrata* were susceptible while 7.14% isolates were resistant. All the *Candida krusei* isolates were 100% resistant, while all *Candida tropicalis* and *Candida parapsilosis* isolates were 100% susceptible to clotrimazole as shown in Figure 4.8 below.

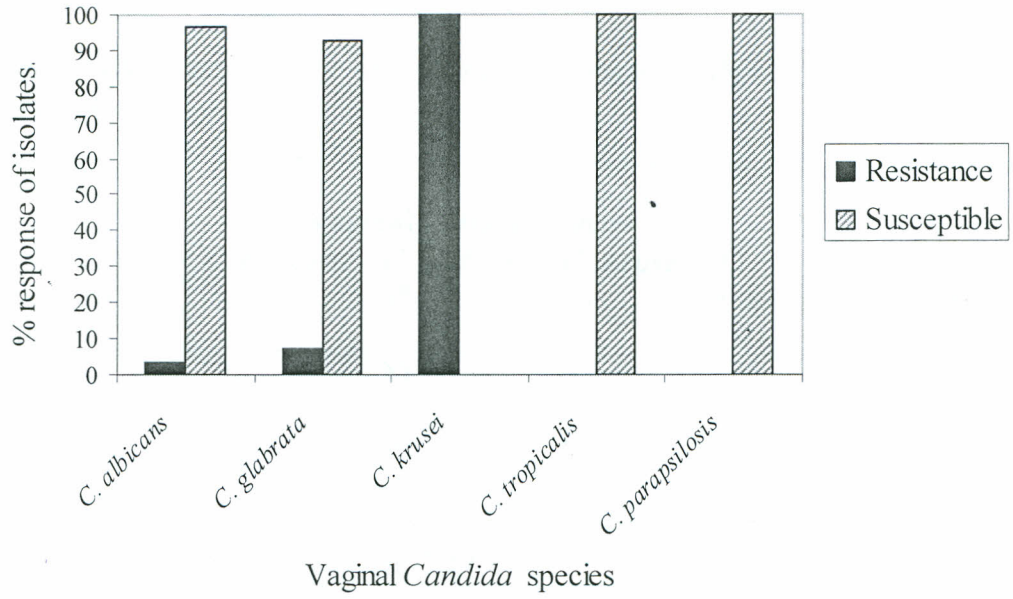


Figure 4.8: Percentage response of vaginal *Candida* species isolates to clotrimazole

4.4.5. Topical nystatin

A moderate susceptibility was recorded for both *Candida albicans* and *Candida glabrata* as 40 and 16 isolates respectively had optical clarity at concentrations ≤5µg/ml. However, 20 isolates of *Candida albicans* and 12 *Candida glabrata* isolates showed optical clarity at concentrations >5µg/ml and were regarded as resistant. *Candida krusei* had a moderate resistance to topical nystatin as one (1) of its two (2) isolates showed optical clarity at concentrations >5µg/ml. *Candida tropicalis* isolates showed low susceptibility to the drug. Only one (1) of its isolates showed optical clarity at concentrations ≤5µg/ml. The remaining 2 isolates recorded resistance to topical nystatin as they showed optical clarity at concentrations >5µg/ml. *Candida parapsilosis* isolate was 100% susceptible since it had optical clarity at concentrations ≤5µg/ml as shown in Table 4.8.

Table 4.8: Susceptibility of *Candida* species to different concentrations of topical nystatin.

Topical nystatin dilutions/concentrations (µg/ml)	Vaginal *Candida* species				
	C. albicans (%)	***C. glabrata*** (%)	**C. krusei** (%)	***C. tropicalis*** (%)	***C. parapsilosis*** (%)
0.01	Nil	Nil	Nil	Nil	Nil
0.05	4(6.67%)	1(3.57%)	Nil	Nil	Nil
0.1	5(8.33%)	1(3.57%)	Nil	Nil	Nil
0.5	7(11.67%)	2(7.14%)	Nil	1(33.33%)	Nil
1.0	9(15%)	3(10.71%)	Nil	Nil	Nil
5.0	15(25%)	9(32.14%)	1(50%)	Nil	1(100%)
10.0	20(33.33)	12(42.86)	1(50%)	2(66.67%)	Nil
Total(n)	60(100%)	28(100%)	2(100%)	3(100%)	1(100%)

Nil = No organism was tested at that concentration.

A moderate susceptibility against *Candida albicans* was recorded where 66.67% of its isolates were susceptible while 33.33% isolates were resistant to the drug. *Candida glabrata* too showed a moderate susceptibility of 57.14% and 42.86% of its isolates were resistant. Out of the two isolates of *Candida krusei*, 50% isolates were susceptible while the other isolate (50%) were resistant. *Candida tropicalis* isolates had a low susceptibility as only 33.33% of the isolates were susceptible while the 66.67% of the isolates were resistant to the drug. *Candida parapsilosis* was the only isolate that had a 100% susceptibility to this drug, Figure 4.9 below.

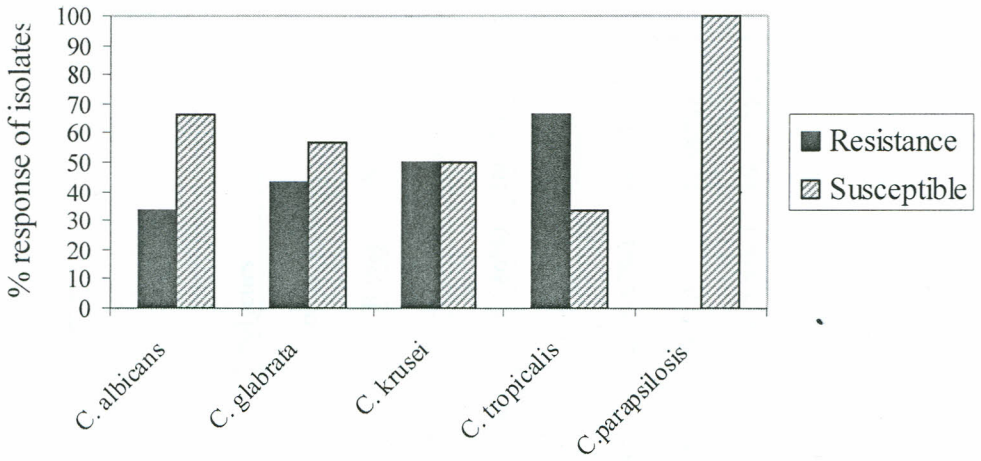


Figure 4.9: Percentage response of vaginal *Candida* species isolates to topical nystatin.

4.4.6. A summary of the susceptibility profile of vaginal *Candida* species isolates to antifungal drugs.

The summary of susceptibility of vaginal *Candida* species isolates to azoles and polyene drugs is shown in Table 4.9 below.

Table 4.9: A summary of susceptibility profile of vaginal *Candida* species isolates to antifungal drugs.

Vaginal *Candida* species isolates	**Antifungal agents**									
	Antifungal group									
	Azoles								Polyenes	
	Fluconazole		Ketoconazole		Itraconazole		Clotrimazole		Topical Nystatin	
	No. R (%)	No. S (%)	No. R (%)	No. S (%)	No. R (%)	No. S (%)	No. R (%)	No. S (%)	No. R (%)	No. S (%)
C. albicans	2(3.33%)	58(96.67%)	3(5%)	57(95%)	4(6.67%)	56(93.33%)	2(3.33%)	58(96.67%)	20(33.33%)	40(66.67%)
C. glabrata	0(0%)	28(100%)	2(7.14%)	26(92.86%)	0(0%)	28(100%)	2(7.14%)	26(92.86%)	12(42.86%)	16(57.14%)
C. krusei	2(100%)	0(0%)	1(50%)	1(50%)	0(0%)	2(100%)	2(100%)	0(0%)	1(50%)	1(50%)
C. tropicalis	0(0%)	3(100%)	0(0%)	3(100%)	0(0%)	3(100%)	0(0%)	3(100%)	2(66.67%)	1(33.33%)
C.parapsilosis	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)
Total	4(4.26%)	90(95.74%)	6(6.38%)	88(93.62%)	4(4.26%)	90(95.74%)	6(6.38%)	88(93.62%)	35(37.23%)	59(61.77%)

KEY
R - Resistance S - Susceptibility

The results on the susceptibility of *Candida* species to the azoles antifungal drugs used indicated that there was no significant difference in activity of these drugs against the vaginal *Candida* species isolated (χ^2 = 16.000, P = 0.593, P > 0.05). Vaginal *Candida* species were less susceptible to the polyene antifungal drug used (mean = 61.43 ± 11.08%) than the azoles (mean = 85.87 ± 7.02%). A comparison between the azoles and the polyene antifungal drugs used showed that there was no significant difference in activity against the vaginal *Candida* species isolated (χ^2 = 16.146, P = .096, P > 0.05).

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Occurrence of Vaginal *Candida* Species in pregnant women

The results of this study identified *Candida albicans* as the most common vaginal *Candida* species causing vaginal candidiasis among the pregnant women attending the antenatal clinic of Thika District Hospital. This is probably due to the fact that in the general population, *Candida albicans* predominates over other species. The results in this study are consistent with previous studies by Akortha et al (2009) and Abu-Elteen and Abdul Malek (1998). Akortha et al (2009) reported *Candida albicans* are the most prevalent followed by *Candida glabrata* with prevalent rates of 64% and 32% of *Candida albicans* and *Candida glabrata* respectively. Abu-Elteen and Abdul Malek (1998) documented 63% and 30% prevalent rates of *Candida albicans* and *Candida glabrata* respectively.

The current findings however contradict the earlier report by Okungbowa et al (2003) who reported *Candida glabrata* as the most common *Candida* species among the symptomatic pregnant women in Nigeria cities. This may probably be due to high abundance of *C. glabrata* in Nigeria than in Kenya. Reports from this study showed similar observations other studies (Hollandia and Young, 2003; Spinillo et al., 1997). An overall non *albicans* percentage of 24, 17 and 32 respectively were reported by each of these researchers. Therefore, non-*albicans Candida* species are emerging significant pathogens (Moran et al., 2003). This variation in reports may be attributed to the different sample sizes used in the studies.

5.1.2 Prevalence of Vaginal Candidiasis

In the present study, a prevalence of 42.7% of vaginal candidiasis in pregnant women attending the antenatal clinic of Thika District Hospital was reported. Use of contraceptives by the women before they got pregnant and the abuse of antimicrobial agents (antibiotics and antifungal agents) leads to the destruction of normal flora (bacteria) by altering the vaginal P^H to alkaline resulting in reduction of vaginal immunity. These factors could have contributed to the increase of the infection in the pregnant women. This is in agreement with other findings by Feyi and Amadi (2001) in Tanzania where he reported a prevalence of 42.9% of the infection in pregnant women.

5.1.3 Distribution of Vaginal Candidiasis According to Age groups and Occurrence of *Candida* Species

A higher frequency (60%) of vaginal candidiasis was observed in pregnant women aged 26-35 years compared to the other age groups. This is because pregnancy is common in women in this age group (Rylander and Berglund, 2004) and thus most of the pregnant women who participated in this study were in this age group compared to the other age groups studied. Sexual promiscuity which is probably common in this age group increases the risk of contracting the infection from an infected sexual partner and therefore they are vulnerable to vaginal candidiasis (Akortha et al., 2009). The observation in this study is consistent with reports of other researchers (Okungbowa et al., 2003; Akortha et al., 2009). Fifty five percent (55%) incidence rate was reported within age group 26-35 years in Benin City by Okungbowa et al (2003) while Akortha et al (2009) reported 57% within age bracket 26-35 in Benin City, Edo state in Nigeria. A low frequency of the infection in pregnant women above

35 years of age was reported in this study. This could be because pregnancy is uncommon in women in this age ranges (Rylander and Berglund, 2004) and thus therefore pregnant women who participated in this study were few in numbers. The finding is in line with a previous report by Okungbowa et al (2003) who reported a prevalence rate of 10% and 2% within the age groups of 36-45years and over 46 years respectively.

Candida albicans was the most *Candida* species isolated in all age groups in this study. *Candida glabrata* was the second after *Candida albicans* in isolation in all the age groups. This may be as a result of *Candida albicans* predominates over other species in the general population followed by *Candida glabrata* (Kent, 1991). Vaginal *Candida* species were isolated at a high prevalent within the age group 26-35 years while the least isolates were isolated in pregnant women aged 35 years and above (Figure 4.2). Women in these age groups below 35 years group of age were many compared to those above 35 years of age. They also use antimicrobial drugs indiscriminately and contraceptives especially emergency pills to prevent pregnancy than those above 35 years of age and therefore, they are vulnerable to vaginal *Candida* species. These factors could have probably contributed to the different rates of *Candida* species isolation in the age groups. This observation is consistent with reports of other researchers (Sehgal, 1990 and Okungbowa et al., 2003).

5.1.4 Distribution of Vaginal Candidiasis According to Trimester of Pregnancy and Occurrence of *Candida* Species

The 3rd trimester had the highest prevalence rate of vaginal candidiasis (68.09%), followed by 2nd (21.28%) and the 1st trimester was the least with (10.63%) as shown in Figure 4.4. The infection was at a higher frequency in the 3rd trimester than the 2nd and 1st trimesters of pregnancy ($r = 2.00$, $P = 0.00$, $P < 0.05$). Pregnant women in the 3rd trimester of pregnancy is the age of pregnancy where most of pregnant women attend clinics in Thika District Hospital as they are about to deliver (Hospital health records, 2009). Therefore, most of the pregnant women who participated in this study were in this trimester of pregnancy compared to the other trimesters of pregnancy. These results are in agreement with a previous study by Sobel (1997) who reported the highest prevalence of 67% in the 3rd trimester of pregnancy.

In the present study, the 3rd trimester of pregnancy had the highest rate of occurrence of vaginal *Candida* species (68.09%) compared to the 2nd and 1st trimesters. All the vaginal *Candida* species identified in this study were isolated in this stage of pregnancy. Pregnant women in their 3rd trimester were many in this study and thus a high rate of *Candida* species isolation was reported in this study. It is also documented that women in this trimester have increased levels of estrogen and corticoid hormones (Sobel, 2000). This body physiological change makes women in this trimester vulnerable to *Candida* species infections. This factor could probably have contributed to the highest occurrence of vaginal *Candida* species in this trimester. *Candida albicans* was the most frequently isolated species in all the trimesters followed by *Candida glabrata.* This may be due to the fact that the two *Candida* species are the most common vaginal *Candida* species in a population (Otero

et al., 1999). This is in line with a previous study by Sobel (1997) that isolated *C. albicans* and *C. glabrata* at the rates of 80% and 57% respectively in his study. *Candida krusei* and *Candida parapsilosis* were very few and were only isolated in the 3rd trimester of pregnancy. This is because these two *Candida* species are commonly found in blood samples and in patients who use hospital equipments examples catheters and dialysis machines and uncommon in vaginal swabs (Otero et al., 1999).

5.1.5 Susceptibility Profile of the Identified Vaginal *Candida* Species to Different Antifungal Agents

Most of *Candida albicans* (96.67%) isolates were susceptible to fluconazole. This is due to the fact that fluconazole is more tolerated with a wider spectrum of efficiency against *Candida albicans* and it remains the first line antifungal agent against *Candida* species (Redding et al., 1994). The high fluconazole susceptibility rate (96.67%) in *C. albicans* is consistent with other reports. Ogunbayo (1998) reported a fluconazole susceptibility of 96.3% among *Candida albicans* isolates while another study by Akortha et al (2009) reported a susceptibility of 95.7% among the *Candida albicans* isolates. No fluconazole resistance was reported among yeast isolates in earlier works on vulvovaginitis conducted in the U.S., England and Brazil (Lynch and Sobel, 1994; Ribeiro et al., 2000; El-Din et al., 2001; Sobel et al., 2004).

However, 3.33% of the *C. albicans* isolates were resistant to fluconazole (Table 4.4). Incomplete therapy which leads to incomplete eradication of the organism resulting to emergence of resistant strains could have probably contributed to the resistance of some of the isolates to the drug. Overgrowth of resistant strains, colonization and subsequent infections with a resistant organism makes the selection of drug resistant

to fungal organisms more likely to occur (Pfaller, 1995). This could have also contributed to the resistance of some of the *C. albicans* isolates to fluconazole. The findings are consistent with other research findings. A U.S. study reported fluconazole resistance in 3.6% *Candida albicans* isolates (Sobel et al., 2004) while a 4.3% *C. albicans* resistance rate was reported in Edo state in Nigeria by Akortha et al (2009) and a 2.1% *C. albicans* resistance rate reported in New York by Mathema et al (2001).

Similar susceptibility pattern was observed in ketoconazole as that of fluconazole. This is because the two antifungal drugs are in the same group of antifungal drugs (azole drugs). The 95% susceptibility and 5% resistance of the drug to *C. albicans* observed for ketoconazole in this study were also consistent with previous studies (White and Marr, 1998; Sobel et al., 2004) who also reported the same susceptibility and resistant patterns.

Most of the *Candida albicans* isolates were susceptible (93.33%) to itraconazole with MICs $\leq$10µg/ml. Itraconazole has a wider spectrum of efficiency against *C. albicans* and it is among the first line antifungal drugs against *Candida* species (Redding et al, 1994). However, 6.67% of isolates were resistant (Figure 4.7). Incomplete therapy by the pregnant women which results in incomplete eradication of the organism resulting in emergence of resistant strains could have probably contributed to the resistance of some isolates of *C. albicans* to this drug. The resistance could be as a result of overgrowth of resistant strains, colonization and subsequent infections with a resistant organism which makes the selection of drug resistant to fungal organisms (Pfaller, 1995). Also as prophylactic use of azoles increases, the incidence of azole resistant in

Candida is also likely to increase (Pfaller, 1995). The finding is consistent with previous studies by scientists whereby itraconazole resistant *C. albicans* accounted for 6-12% of the isolates from women with vaginal candidiasis (Newman, 1994). A U.S. study reported itraconazole resistance of 6% to *C. albicans* isolates (Sobel et al., 2004). A 5% *Candida albicans* resistance rate was reported in New York by Mathema et al (2001).

Clotrimazole is a well-tolerated and frequently administered drug for vaginal candidiasis. Clotrimazole is approved for both treatment and prevention of vaginal candidiasis. A susceptibility of (96.67%) of *C. albicans* to clotrimazole was observed in this study. Clotrimazole has been shown to inhibit the major fungi causing systemic infection, vaginal candidiasis at a concentration of 1µg/ml with efficacy against *Candida* (Grahame-Smith and Arsonson, 1992). Although favorable results from systemic treatment of candidiasis and vaginal candidiasis have been described, some *Candida albicans* strains still show high MICs to clotrimazole. Despite the high MICs to clotrimazole, the drug is extensively used in Kenya for management of vaginal candidiasis and for dermatological conditions (Bii et al., 2002). The high clotrimazole susceptibility of (96.67%) in *C. albicans* in this study is consistent with other studies by Kangogo et al (2008) and Bii et al (2002) that *Candida albicans* and most of the non-*albicans Candida* species are susceptible to clotrimazole.

However, 3.33% of the *Candida albicans* isolates were resistant (Figure 4.8). This might probably be due to change of vaginal pH which destroys normal flora (especially bacteria) and thus lowering vaginal immunity and thus gives a favourable environment for the overgrowth of *Candida* species and become pathogenic. It also

alters the antifungal activity of the drug and become less sensitive resulting to resistant to *Candida* species. The finding is in line with another report by Ogunbayo (1998) who reported clotrimazole resistance in 3.5% among his *Candida albicans* isolates.

Topical nystatin was the only polyene antifungal drug tested in this study. The drug is cheaper than the azole drugs and is available over the counters. Therefore, pregnant women can easily afford the drug. This leads to misuse by women which may results to development of resistance among *Candida* species. *C. albicans* showed a moderate susceptibility of (66.67%) to the drug. The resistant rate was 33.33% (Figure 4.9). These findings are in agreement with a previous observation that emerging resistance of *C. albicans* to topical nystatin is in the increase (Law and Moore, 2000).

Candida glabrata was among the non *albicans* species that was (100%) susceptible to fluconazole in this study. This finding is similar to findings by Otero et al (1999). However, the species recorded a marked resistance to ketoconazole. This is due to the fact that the species develops resistance rapidly when ketoconazole is used for treatment (Akortha et al., 2009). This is similar to past findings by Pfaller et al (1999) that there has been an emerging resistance of *C. glabrata* to ketoconazole. *C. glabrata* was 100% susceptible to itraconazole. This is because the *Candida* species is intrinsically susceptible to itraconazole (Sobel et al., 2004). Most of *C. glabrata* isolates (92.86%) were susceptible to clotrimazole. However, a resistant rate of 7.14% of the species to the drug was recorded (Figure 4.8). This is because it is among the non albicans species that develop resistance rapidly when the drug is used for treatment. This is in agreement with past studies by Pfaller (1999). *Candida glabrata*

was (57.14%) susceptible to topical nystatin while 42.86% of the isolates were resistant. This is probably due to the misuse of the drug by the pregnant women which resulted to development of resistance by the species.

Candida krusei isolates were 100% resistant to fluconazole, clotrimazole and 50% resistant to ketoconazole. This is because *C. krusei* is naturally resistant to the drugs even at high doses (Akortha et al., 2009). The results are consistent with other research reports (Goa and Barrdell., 1995; Klastersky, 1995; Akortha et al., 2009). *Candida krusei* was 100% susceptible to itraconazole. This is in agreement with a past study by Sobel et al (2004) and Akortha et al (2009) that *C. krusei* is intrinsically susceptible to itraconazole than other azole antifungal drugs. *Candida krusei* had a moderate susceptibility to topical nystatin (50%). *Candida krusei* is among the non *C. albicans* species that are less susceptible to topical nystatin (Law and Moore, 2000).

The other non *albicans Candida* species that were isolated in this study were *C. tropicalis* and *C. parapsilosis.* The two species were 100% susceptible to all the azole drugs tested. This is because the species are intrinsically susceptible to azole drugs (Kangogo et al., 2008). This is in agreement with past studies (Pfaller et al., 1999; Kangogo et al., 2008 and Akortha et al., 2009). *C. tropicalis* showed the lowest percentage of susceptibility of (33.33%) to the drug. This is because the species is among the non *C. albicans* species that are less susceptible to topical nystatin (Law and Moore, 2000). However, *Candida parapsilosis* was 100% susceptible to topical nystatin. The species is among the non *Candida albicans* that are intrinsically susceptible to topical nystatin (Kangogo et al., 2008). The finding is in agreement with a previous study (Bii et al., 2002; Moran et al., 2003; Akortha et al., 2009).

Comparison in susceptibility between the azole drugs and the only polyene drug used in this study showed that vaginal *Candida* species were less susceptible to the polyene drug than the azole drugs, however the difference was not statistically significant (χ^2=16.15, P=0.096, P < 0.05). Azoles drugs interact with the sterol synthesis enzyme and inhibit sterol synthesis of fungal cells which is difficult to be altered and affect the drug activity than polyene drugs which interact with membrane sterols and forms pores that altered cellular permeability. This can easily change when there is a slightly ergosterol membrane alteration resulting to interference of ergosterol topical nystatin interaction resulting in decrease in polyene activity (Pappas, 2007). This makes vaginal *Candida* species less susceptible to polyene drug used than the azole drugs (Pappas, 2007). The findings are in agreement with the report by Okungbowa et al (2003) that *Candida* species are more susceptible to azole antifungal drugs than polyenes drugs commonly used. Fungal infections are often challenging to manage and caution has to be exercised in the use of antifungal drugs in order to arrest any further increase in resistance. They have to be taken only under a clinician's prescription.

5.2 Conclusions

1. The prevalence of vaginal candidiasis in the pregnant women was high especially in the age ranges 26-35 years and at the third trimester of pregnancy. Therefore identification of vaginal *Candida* species accompanied by susceptibility tests is necessary before treatment is given to the women in this age group and trimester of pregnancy.

2. *Candida albicans* was the most prevalent vaginal *Candida* species across the age groups of the pregnant women and thus identified as the most common species causing vaginal candidiasis in pregnant women attending the antenatal clinic of Thika district hospital. However, non-*albicans Candida* species were also isolated and identified which indicates their importance as opportunistic pathogens in pregnant women.

3. Most vaginal *Candida* species isolates were susceptible to the antifungal drugs tested in this study. The difference in susceptibility of vaginal *Candida* species between azoles and polyene drug was not significant. However, *Candida* species were more susceptible to the azole drugs than the polyene drug used.

5.3 Recommendations

1. Clinicians in Thika District Hospital should continue prescribing the azole drugs (fluconazole, ketoconazole, itraconazole and clotrimazole) but not polyene drug (topical nystatin) to pregnant and non pregnant women for the treatment of vaginal candidiasis.

2. Antifungal drugs susceptibility tests should be carried out in all hospitals in order for the clinicians to prescribe appropriate and accurate treatment for the infections.

3. Further investigations on fungal resistance to antifungal agents are required especially in HIV/AIDS patients.

4. Further molecular studies need to be carried out in order to understand the mechanisms of resistance of *Candida* species to commonly used antifungal drugs.

REFERENCES

Abi-said, D., Uzun, O. and Vartivarian, S. (1997). The Epidemiology of Hematogenious Candidiasis caused by different *Candida* Species. *Clin. Infect. Dis.* **2**: 1122-1128.

Abu-Elteen, K. and Abdul Malek, W. (1997). Prevalence and Susceptibility of vaginal Yeast Isolates in Jordan. *Mycoses*, **40**(5-6): 179-185.

Akortha, E., Chikwe, O. and Nwaugo, O. (2009). Antifungal resistance among *Candida* species from patients with Genitourinary Tract Infection isolated in Benin City, Edo estate, Nigeria. *Afri. J. of Microbiol. Research*, **3**(11): 694-699.

Alakpa, A. and Fagbeuro, B. (2000). Prevalence rate of Vaginal Candidiasis. *Eur. J. of Scient. research,* **3**: 264-278.

Baker, F. 2nd ed. (2002). Handbook of Bacteriological Technique. New York Butterwarth and Co. Ltd. Pg. 415- 421.

Barg, N. (2003). An introduction to Molecular Epidemiology. *Infect. Contr. Hosp. Epidemiol.* **14**:395-396.

Brousse, T. (2004). Prevalence of Vaginal Candidiasis in both pregnant and health women. *Sex transm. Infect.* **66**:179-183.

Bergan, T. and Vangdal, M. (1983). *In vitro* activity of antifungal agents against yeast species. *Chemother.* **29**: 104 - 110.

Bii, C., Ouko, T., Amukoye, E. and Githinji, W. (2002). Antifungal Drug Susceptibility of *Candida albicans*. *East Afri. Med. J.* **79**(3): 143-145.

Carrol, C., Hurley, R. and Stanley, V. (1973). Criteria for diagnosis of *Candida* Vulvovaginitis in pregnant women. *J. of Obstet. and Gynaecol. of the British Commonwealth,* **80**: 258-263.

Chander, J. 2nd ed. (2002). A Textbook of Medical Mycology. New York Butterwarth and Co. Ltd, Pg. 212-227.

Colombo, A. and Dark, S. (2006). Epidemiology of Candidemia in Brazil. A Nationwide Sentinel Surveillance of Candidiasis in Eleven Centres: *J. of Clin. Microbiol.* **44**: 2816-2823.

Dixon, D., McNeil, M., Cohen, M., Gellin, B. and La Montagne, J. (1996). Fungal infections: A growing threat. *Public Health Rep.* **11**: 226 -235.

El-Din, S., Reynolds, M., Astibee, H., Barton, R. and Evan, G. (2001). An Investigation into Pathogenesis of Vulvovaginal Candidiasis. *Sex transm. Infect.* **77**: 179-183.

Enweani, I. and Ojo, S. (2000). Effect of contraceptives on the prevalence of vaginal colonization with *Candida* species in Edo state, Nigeria. *Rev. Iberoam Mycol.* **18**:171-173.

Feyi, P. and Amadi, A. (2001). The Prevalence and Pattern of vaginal Candidiasis in pregnancy in Abia. *J. of Med. Investign. and Pract.* **2**: 25-27.

Finney, D. and Latscha, R. (1963). Tables for Testing significance in a 2x2 Contingency Table. Cambridge, mass, USA: Cambridge University Press, 59.

Garcia, H. and Famighetti, A. (2006). Prevalence of Vaginal Candidiasis in women; Identification of Yeast and Susceptibility to Antifungal agents. *Rev. Argent Microbiol. Jan- Mar;* **38**(1): 9-12.

Gloria, M., Elizondo, M. and Ayala, J. (2008). Trends in Species Distribution and Susceptibility of Blood stream Isolates of *Candida* collected in Monterrey Mexico to Seven Antifungal Agents. *J. of Clin. Microbiol.* **46**(9): 2902-2905.

Goa, K. and Barrdell, E. (1995). Fluconazole and Update of its Pharmacodynamic and Pharmacokinetic properties and Therapeutic use in major Superficial and Systemic mycoses in Immunocompromised patients. *Drugs*, **50**: 658-690.

Grahame-Smith, D. and Arsonson, J. (1992). Oxford textbook of Clinical pharmacology and drug therapy. 2nd edition. New York: *Oxford University Press Inc.*pp. 559.

Gudlangssun, O., Pfaller, M. and Diekema, D. (2003). Attribute mortality of vaginal Candidiasis in pregnant women, revisited. *Clin. Infect. Dis.* **37**: 1172-1177.

Hace, K., Noskin, G., Traka, K. and Hiririchi, S. (2005). Invasive infection due to *Candida krusei* in immunocompromised patients not treated with Fluconazole. *Clin. Infect. Dis.* **20**: 342-347.

Hajjeh, R. and Warnock, D. (2004). Incidence of Blood Stream infection due to *Candida species* and in Vitro Susceptibilities of isolates collected from 1998 to 2000 in a Population-based active surveillance. *J. of Infect. Dis.* **120**: 24 – 27.

Hamilton, M. (1972). A comparative *in vitro* study of Amphotericin B, Clotrimazole and 5-Fluorocytosine against clinically isolated yeasts, **10**: 276 – 283.

Harvey, K., Donovan, J. and Drach, G. (1987). *Candida glabrata* Urinary tract infection. *J. of Urol.* **139**: 1245-1249.

Higgins, S. and Woolley, P. (1993). Pharmacological treatment of acute Vulvovaginal Candidiasis. *Intn. J. on STD and AIDS,* **4**: 261- 265.

Hoeprich, P. and Huston, A. (1976). Effect of culture media on the antifungal activity of miconazole and amphotericin B methyl ester. *J. of Infect. Dis.* **134**: 336 - 341.

Hollandia, J., and Young, M. (2003). Vulvovaginal carriage of Yeasts other than *Candida albicans. Sex transm. Infect.* **79**: 249.

Holt, R. (1972). Laboratory Assessment of the antimycotic drug Clotrimazole. *J. of Clin. Pathol.* **3**: 23-24.

Hussain, Q., Flournoy, D., Qadri, S. and Ramirez, E. (1986). Susceptibility of clinical isolates of yeasts to antifungal agents. *Mycopathol.* **95**: 183 - 187.

Jawetz, E., Melmic, L. and Adelberg, E. (2001). Medical Microbiology. 22nd ed. New York. MacGraw-Hill Ltd, Pg.1017.

Jonson, V. and Nickerson, W. (2001). Introduction, Morphogenesis and Germination of Chlamydospores of *Candida albicans. J. of Bacteriol.* **1970**: 910-921.

Kaufman, R. (1986). Vulvovaginal Candidiasis. Asymposium. *J. on reprod. Med.* **31**: 639-672.

Kangogo, M., Wanyoike, W. and Bii, C. (2008). Characterization of *Candida* species from Clinical sources in Nairobi, Kenya. *East Afri. Med. J.* **12**: 34-42.

Kent, H. (1991). Epidemiology of Vaginitis. *Amer. J. of Obstet. and Gynaecol.* **165**: 1168-1176.

Klastersky, J. (1995). Prevention and Therapy of Fungal infection in Cancer patients. A review on recently published Information. *Support care cancer*, **3**: 393- 410.

Laupland, K. and Gregson, D. (2005). Invasive *Candida* species Infection; A 5 year Population based Assessment. *J. of Antimicrob. Chemother.* **56**: 532-537.

Law, D. and Moore, C. (2000). High prevalence of antifungal resistance in *Candida spp.* from patients with AIDS. *J. of Antimicrob. Chemother.* **34** (5): 659-668.

Le, C. and Boen, J. (1995). Health and numbers: Basic biostatical methods. John Wiley, Chichester. *J. of Clin. Nursing*, **9**: 178-198.

Lisiak, M., Klyszejko, C. and Marcinkowski, Z. (2000). Vaginal candidiasis; Frequency of Occurrence and Risk factors. *Gynaecol. pol.* **71**: 964-970.

Lodder, J. (ed). (1970). The Yeasts. North- Holland publishing Co. Amsterdam. Pg. 356-358.

Lynch, M. and Sobel, J. (1994). Comparative in vitro activity of Antimycotic agents against pathogenic vaginal Yeast isolates. *J. of Mycol.* **32**: 267-274.

Marichal, P. and Gorrens. J. (1995). Origin of difference in susceptibility of *Candida krusei* to azole Antifungal agents. *Mycoses* **38**(3-4): 111-117.

Mathema, B., Cross, E. and Perlin, D. (2001). Prevalence of vaginal colonization by drug resistant *Candida* species in college-age women with previous exposure to over the counter azole antifungals. *Clin. Infect. Dis.* **33**: 23-27.

Meisozo, M. (2008). Intrauterine Candidiasis: Report of four cases. *Arch Gynaecol. Obstet.* **278**: 173-176.

Mitchell, H. (2004). Vaginal discharge, Causes, Diagnosis and Treatment. *BMJ,* **328**:1306-1308.

Monif, G. (2001). Diagnosis of Infectious Vulvovaginal Disease. *Infect. Med.* **18**: 532-533.

Moran, G., Sullivan, D., and Coleman, D. (2003). Emergence of non-*Candida albicans* species as pathogens. In *Candida* and *Candidiasis.* Ed, R. A. Calderone. New York, *Amer. Society for Microbiol.* Pg. 37 - 53.

Mori, T., Matsumura, M., and Kanamaru, Y. (1997). Myelofibrosis complicated by infection due to *Candida albicans*: emergence of resistance to antifungal agents during therapy. *Clin. Infect. Dis.* **25** (6): 1470-1471.

Myers, R. (2006). Immunization and Anticrobial agents. New York, 4th ed. Livingstone, Pg.162.

National Committee for Clinical Laboratory Standard (2002). Reference Method for Broth dilution antifungal susceptibility testing of yeasts. Approved Standard M27-A2, 2nd ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.

Newman, S. (1994). Clinically significant mucosal Candidiasis resistant to Fluconazole treatment in patients with AIDS. *Clin Infect. Dis.* **19**: 684 - 686.

Nyirjesey, P., Grody, H. and Jordan, C. (1995). Chronic fungal Vaginitis: The value of cultures. *Amer. J. Obstet. Gynaecol.* **173**: 820-823.

Odds, F., Brown, A. and Gow, N. (2003). Antifungal agents, Mechanisms of action. *Trends in Microbiol.* **6**: 276-279.

Ogunbayo, B. (1998). Isolates of yeast from male contacts of women with vaginal Candidosis. *Genit. urin. Med.* **64**: 135-136.

Okungbowa, F., Isuehuemhen, O. and Dede, A. (2003). The distribution frequency of *Candida* species in the Genitourinary tract among symptomatic individuals in Nigeria cities. *Rev. Iberoam. Mycol.* **20**: 60-63.

Oriel, J., Denny, M., Coleman, J. and Patridge, B. (2008). Genital Yeast infections. BMJ. **4**:761-764.

Otero, L., Fleiter, A. and Mendez, F. (1999). Susceptibility of *Candida* species. Isolated from female prostitutes with valvovaginitis to antifungal agents and boric acid. *Eur. J. of Clin. Microbiol. and Infect. Dis.* **18**(1): 59-61.

Pappas, P. (2006). Invasive Candidiasis. Clinical North America. *J. of Infect. Dis.* **20**: (3): 485-506.

Parveen, N. (2008). Frequency of Vaginal Candidiasis in pregnant women attending routine antenatal Clinic. *J. on Reprod. Med.* **40**: 325-328.

Pfaller M. (1995). Epidemiology of Fungal infections: The promise of molecular typing. *Clin. Infect. Dis.* **20**: 1535 -1539.

Pfaller, M. and Diekema, D. (2002). Role of Sentinel Surveillance of Candidemia. Trends in Species Distribution and Antifungal Susceptibility. *J. Clin. Microbiol. Dis.* **40**:3551-3557.

Pfaller, M., Messer, R. and Hajjeh, A. (1999). Trends in species distribution and susceptibility to fluconazole among blood stream isolates of *Candida* species in United States. *Diagn. Microbiol. Dis.* **33**: 217-222.

Pfaller, M. (1996). Application of CHROMagar Candida for Rapid screening of Clinical specimens for *Candida albicans, Candida tropicalis, Candida krusei and Candida glabrata, J. of Clin. Microbiol.* **34**: 58-61.

Pinho, R., De Resende, M. and Saliba, J. (2002). Prevalence of *Candida* species in hospitalized patients and their risk factors. *Mycoses* **45**: 306.

Plempel, M., Bartmann, K., and Regel, E. (2000). A new orally applicable antifungal substance with broad-spectrum activity. *Antimicrob. Agents Chemother.* **9**: 271 - 274.

Powderly, W. (1994). Resistant Candidiasis. *AIDS Resistance Hum. Retroviruses* **10**: 925-929.

Prescott, J., Harley, J. and Klein, D. (2008). Microbiology, 7th ed. New York, McGraw Hill, Pg. 23-25.

Redding, S., Smith, G., Farinacci, M., Rinaldi, A., Forthegill, J. Rhine-Chalberg, and Pfaller, M. (1994). Development of resistance to fluconazole by *Candida albicans* during treatment of oropharyngeal candidiasis in AIDS: Documentation by in vitro susceptibility testing and DNA subtype analyses. *Clin. Infect. Dis.***18**: 240 - 242.

Rex, J., Pfaller, M., Galgiane, M., Bartlett, A., Espinel-Ingroff, M., Ghannoum, M., Lancaster, F., Odds, M., Rinaldi, T. and Barry, A. (1997). Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of *in vitro-in vivo* correlation data for fluconazole, itraconazole, and *Candida* infections. *Clin. Infect. Dis.* **24**: 235 - 247.

Rex, J., Rinaldi, M. and Pfaller, M. (1995). Resistance of *Candida* species to fluconazole. *Antimicrob. Agents chemother.* **39**: 1-8.

Ribeiro, M., Dietze, R., Paula, C. and Colombo, A. (2000). Susceptibility profile of vaginal yeast isolates from Brazil. *Mycopathol.* **151**: 5-10.

Richardson, M. and Warnock, D. (2003). Fungal infection, Diagnosis and treatment, 3rd ed, Blackwell Publishing UK. Pg. 38 – 43.

Riley, L., Chaturredi, V., Perlin, D. (2001). Prevalence of Vaginal colonization by drug resistant *Candida* species in college age women with previous exposure to over the counter azole antifungals. *Clin. Infect. Dis.* **33**: 23-27.

Ritcher, S., Galask, R., Messer, S. and Pfaller, M. (2005). Antifungal Susceptibilities of *Candida species* causing Vulvovaginitis and Epidemiology of recurrent cases, *J. of Clin. Microbiol.* **43**(5); 2155-2162.

Rylander, E. and Berglund, A. (2004). Vulvovaginal *Candida* in a young sexually active population: prevalence and association with oro-genital sex and frequent pain at intercourse. *Sex. Transm. Infect.* **50**: 54-57.

Salvatore, F. (2001). Effects of *Candida glabrata* on pregnant women; causes premature rapture of uterus membrane leading to preterm delivery. *J. of Clin. Microbiol.* **43**(5): 2155-2162.

Sandhu, G., Cline, B., Stockman, L. and Roberts, G. (1995). Molecular probes for diagnosis of fungal infections. *J. of Clin. Microbiol. Rev.* **33**: 2913 - 2919.

Sanduen, P., Bevanger, A. and the Norwegian Yeast study Group (2006). Candidemia in Norway (1991-2003); Results from a Nationwide Study. *J. of Clin. Microbiol.* **44**:1977-1981.

Schenmach, D. and Hillier, S. (2000). Advances in Diagnostic testing for Vaginitis and Cervitis. *J. on Reprod. Med.* **34**: 555-564.

Sehgal, S. (1990). Epidemiology of male urethritis in Nigeria. *J. of Trop. Med. Hyg.* **93**: 151-152.

Sim, E. (1998). *Candida* Species from Genital Sites: their identification and susceptibility to fluconazole. *Brit. J. of Biomed. Sci.* **55** (4): 264-267).

Sobel, J. (1996). Symptomatic vulvovaginitis due to fluconazole-resistant *Candida albicans* in a female who was not infected with human immunodeficiency virus. *Clin. Infect. Dis.* **22**(4): 726-727.

Sobel, J. (1997). Vaginitis. New England Journal of Medicine: **337** (26): 1896-1903.

Sobel, J. (2000). Epidemiology and Pathogenesis of Recurrent Vulvovaginal Candidiasis. *Amer. J. Obstet. Gynaecol.* **152**: 924-935.

Sobel, J. (1992). Pathogenesis and Treatment of Recurrent Vulvovaginal Candidiasis. *Clin. Infect. Dis.* **14**(supp; 1): S148-153.

Sobel, J., Wiesentld, H., Marteus, M., Sperling, H. and Chu, T. (2004). Maintenance of fluconazole therapy for Recurrent Vulvovaginal Candidiasis, *N. Engl. J. Med.* **351**: 876-883.

Spinillo, A., Colonna, L. and Piazzi, G. (1997). Prevalence of and risk factors for fungal vaginitis caused by *non-albicans* species. *Amer. J. of Obstet. Gynaecol.* **176**: 138-141.

Tatfeng, Y. and Nwobu, G. (2004). *Candida Albicans* in urinary tract or seminal sac. *Onl. J. of Hlth. Allied Sci.* **20**: 4-5.

The U.S. Public Health Service Guidelines for the prevention of Opportunistic Infectious (2002). Guidelines recommendation about using antifungal drugs during pregnancy.

Trick, W., Gayness, P. and Edwards, R. (2002). Secular Trend of Hospital acquired Candidemia among Intensive Care Unit Patients in the United States during 1959-1999. *Clin. Infect. Dis.* **35**: 627-630.

Vanden, B., Marichal, P. and Odds, F. (2007). Mechanism of antifungal resistance. *Trends Microbiol.* **14**: 44-49.

White, L. (2000). Fluconazole treatment for Histoplasmosis in patients with the Acquired Immuno Deficiency Syndrome. *Amer. J. Med.* **98**: 336 - 342.

White, T. and Marr, K. (1998). Clinical cellular and molecular factors that contribute to Antifungal drug resistance. *Clin. Microbiol. Rev.* **11**(2): 382-402.

APPENDICES

Appendix I

Plates

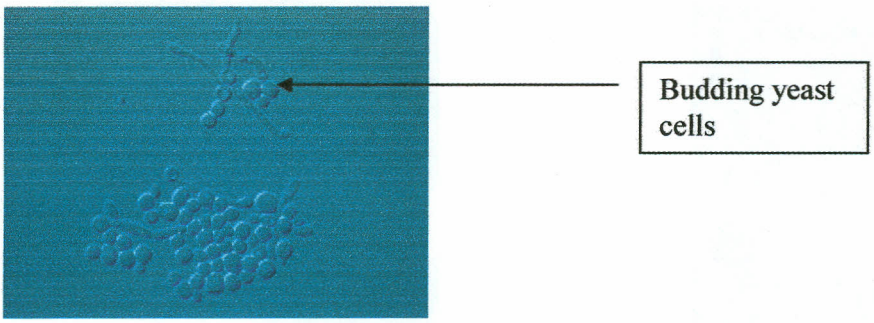


Plate 1: Budding yeast cells of *Candida* species on SDA (Magnification X40)

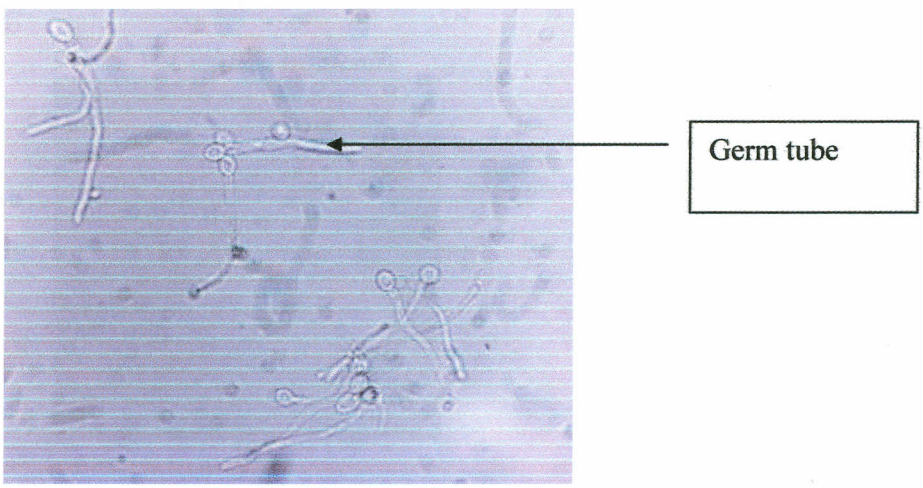


Plate 2: Germ tube positive *C. albicans* after incubation for 3 hours at 37°C (Magnification X40)

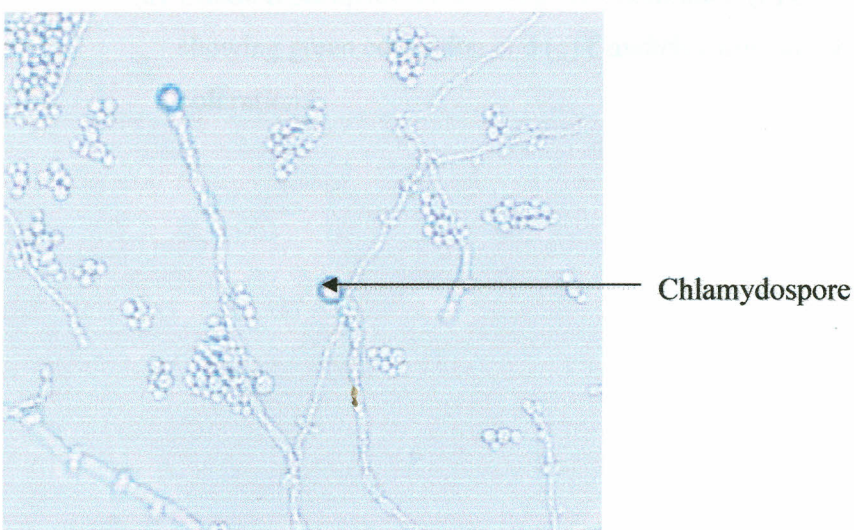
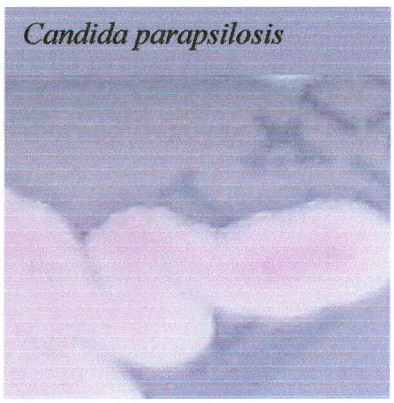
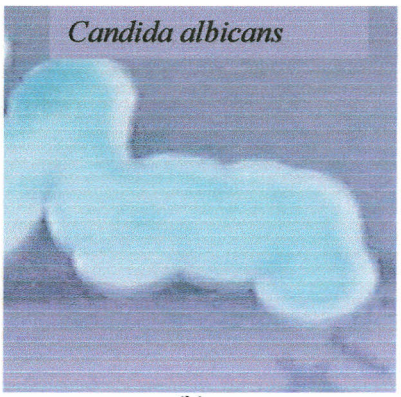


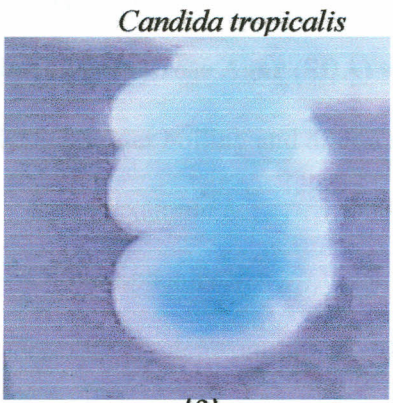
Plate 3: *Candida albicans* with abundant chlamydospores and pseudohyphae on Corn Meal Agar (Magnification X40).



(a)



(b)



(c)

Plate 4: Different *Candida* species on CHROMagar *Candida* after incubation for 48 hours;

(a) *Candida parapsilosis* showing pink coloration (b) *Candida albicans* showing green coloration and (c) *Candida tropicalis* showing blue coloration.

Appendix II

Media and Antifungal agents Preparations

1. Media preparation.

a) **Saboraud Dextrose Agar (SDA) (Emmons Modification)**

Dextrose.....20g

Peptone.....10g

Agar.....17g

Distilled Water.....1,000 ml

Final pH 6.9

Saboraud Dextrose Agar (SDA) with Antibiotics

i) To SDA after boiling and after autoclaving, add

Cyclohexamide 500mg

Chloramphenicol.....50mg

ii) Dissolve Cyclohexamide in 10 ml of acetone and add it to the medium after autoclaving at 121 °C for 15 minutes. Mix well.

iii) Dissolve the Chlorampenicol in 10 ml of 95 % ethanol add it to the medium after autoclaving at 121 °C for 15 minutes.

iv) Mix well.

v) Dispense approximately 20 ml into sterile petri dishes and leave them at room temperature.

b) Corn Meal Agar

Corn Meal.....40 g

Agar..... ... .20 g

Tween 80 (Polysorbate 80).....10 ml

Distilled Water.....1,000 ml

i) Mix Corn Meal Agar (40g) well with 500 ml of water; heat to 65 °C for 1 hour.

ii) Filter through gauze and then paper until clear, restore to original volume. Adjust to PH 6.6 - 6.8.

iii) Add agar dissolved in 500 ml of water. Add Tween 80.

iv) Autoclave at 121 °C for 15 minutes.

v). Allow the media to solidify for at least 30 minutes.

c) CHROMagar (CA)

Dehydrated CA.....47.7g

Distilled water.....1 litre

i) Reconstitute 47.7 grams of dehydrated CA in 1litre of distilled water.

ii) Bring it to boil by repeated heating and then cool it.

iii) Approximately 20 ml of the media, dispense into sterile petridishes.

iv) Allow the media to solidify for at least 30 minutes.

2. Antifungal agents preparation

Standard powders of the following antifungal agents were obtained from their respective manufacturers: Fluconazole ketoconazole, itraconazole, clotrimazole (Sigma, St. Louis, and U.S.A) and Topical nystatin (MP Biomedicals, Inc, France).

a) Fluconazole

i) Weigh 16mg of the powder.

ii) Dissolve in 1.5ml of sterile Dimethylformamide.

iii) Dilute the mixture in 23ml of RPMI medium to make an initial concentration of 100µg/ ml.

iv) Serially dilute to give a dilution range of 0.01-10µg / ml i.e., (100, 50, 10, 5.0, 1.0, 0.05 and 0.1).

b) Ketoconazole, Itraconazole and Clotrimazole.

i) Weigh 1.8mg of each of the drugs powder.

ii) Dissolve in 0.6 ml ethanol.

iii) Dilute the solution with 10.70 ml of RPMI - 1640 to make an initial concentration of 10µg / ml.

iv) Serially dilute to give a dilution range of 0.001-1µg / ml i.e., (10, 5.0, 1.0, 0.50, 0.10, 0.05 and 0.01).

c) Topical Nystatin.

i) Weigh 2700ug/ml of the drug.

ii) Dissolve in 0.540ml of Dimethyl Sulfoxide (DMSO).

iii) Dilute in 15ml of RPMI - 1640 to make an initial concentration of 10µg / ml.

iv) Serially dilute to give a dilution range of 0.001-1µg / ml i.e., (10, 5.0, 1.0, 0.50, 0.10, 0.05 and 0.01).

Each drug range was prepared as recommended by NCCLS document, 2002.

Appendix III

Approval Letter

KENYATTA UNIVERSITY
SCHOOL OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCE

New Arts Complex Rooms 2005 & 2006
Tel. 8710901/19 Extn: 3630

P.O. Box 43844, Nairobi
e-mail: chairman-medical@ku.ac.ke

Date: June 14, 2010

Medical Superintendant
Thika Level 5 Hospital
THIKA

14/6/2010

MEDICAL SUPERITENDENT
TEL: 067-21621
24 JUN 2010
P.O. BOX 227-01000 THIKA
THIKA LEVEL 5 HOSPITAL

Dear Sir/Madam,

RE: CHENGO NELSON MENZA- REG. NO. 156/10808/08

The above mentioned is an M.sc. (Infectious Disease) student in this department. He has now completed the entire course requirement.

His proposal topic has changed from diarrohoea to Vaginal Candidiasis. Kindly allow him to use Thika hospital to collect process and analyse the samples.

Thank you.

14 JUN 2010

J.J.N. Mbithi, *Dip.Bact (UK), M.Sc. (UK), Ph.D (Canada)*
Chairman, Department of Medical Laboratory Science

Appendix IV

Consent Form

Kenyatta University

Jomo Kenyatta University of Agriculture and Technology
Thika District Hospital
Prevalence of vaginal candidiasis and Susceptibility Profile of Candida Species to Antifungal Agents in Pregnant Women in Thika, Kenya

Investigators	Role on Project	Institution
Dr. Margaret Muturi	Principal Supervisor	Kenyatta University
Dr. Wanjiru Wanyoike	Co – Supervisor	Jomo Kenyatta University of Agriculture and Technology
Nelson Menza	Researcher	Kenyatta University

Investigators Statement

This is an educational as well as a research study which will be carried out by a researcher from Kenyatta University. This consent form should provide for you information that you will need to help you decide whether to will participate in the study or not. Please read it carefully.

You may ask any question concerning the purpose of the research, procedures that will be followed, your rights as a participant in the study, risks and benefits of the study.

Purpose of the Study

The purpose of the study is to determine the prevalence of vaginal candidiasis, isolate and identify vaginal *Candida species* and their antifungal susceptibility profile in pregnant women attending the antenatal clinic of Thika District Hospital. Therefore, identification of vaginal *Candida species* and the information on the antifungal drug sensitivity among pregnant women will facilitate diagnosis and appropriate treatment of vaginal candidiasis. This will go a long way to improve on maternal health services

country wide.

Procedures

If you agree to take part in the study, the age, trimester of pregnancy, history of using antibiotics, antifungal drugs, contraceptives and the area of residence will be recorded and a vaginal swab will be collected. The sample will be taken to the Hospital Laboratory for analysis.

Risks

The vaginal swab that will be taken may make you feel uncomfortable.

Benefits

The information you provide to the investigator will shed light on the prevalence of vaginal candidiasis and sensitivity of antifungal agents to vaginal *Candida species.* Identification of vaginal *Candida species* and the information on the antifungal drug sensitivity will facilitate correct diagnosis and appropriate treatment. This will go a long way to improve on maternal health services country wide.

The clinics that you are attending will be given your results and this will help them in better management of your current condition.

Other Information

Participation in the study may involve loss of privacy. Any information given to the study will be kept private. Your name will not be used in any report coming from this study. The consent form will be safely kept where only the study staff will have access to the information.

Signature of investigator Date.....

Subject Statement and Signature

The study has been explained to me. I volunteer to take part in this study. I have had a chance to ask questions. If I have more questions, I can ask one of the investigators listed.

Name of the participant.....

Signature or fingerprint of participant.....

Appendix V

Request/ Referral Form

MOH 240H

No.

MINISTRY OF HEALTH

Specimen Ref No.....

SAMPLE AND SPECIMEN REFERRAL FORM

Note: incompletely filled forms will not be processed

I. Patient and Specimen details

IP/OP No:.....

Patient's Name..... Age(yrs/months).....Sex M ☐ F ☐

Residence.....Postal address.....

Sample / specimen and description.....Source.....

Collection date (dd/mm/yyyy) ___/___/_____ Time (24hrs).....

Date of preservation.....Method of preservation.....

II. Referring Lab (name and address).....

Reasons for Referral.....

III. Details of Person Referring sample

Name.....Designation.....Mobile.....

Email..... Signture.....

IV. Investigations Requested.....

V. Lab referred to (name and address).....

VI. Details of the Person Receiving sample

Name..... .....Designation.....Mobile No. .....

Email.....Signature.....

VII. Condition of Sample:

Accepted ☐ Rejected ☐ (specify reason)_____

Appendix VI

Map of Thika

Source: Map data © 2011Google, Tracks