PREVALENCE OF GENITOURINARY CANDIDIASIS AMONG PATIENTS WITH OR WITHOUT MEDICAL IMPLANTS ATTENDING GYNAECOLOGY CLINICS IN ZARIA, NIGERIA

BY

JIMOH OLANREWAJU

DEPARTMENT OF MICROBIOLOGY

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA.

DECEMBER 2014

Fly Leaf

Title Page

PREVALENCE OF GENITOURINARY CANDIDIASIS AMONG PATIENTS WITH OR WITHOUT MEDICAL IMPLANTS ATTENDING GYNAECOLOGY CLINICS IN ZARIA, NIGERIA

BY

JIMOH OLANREWAJU

MSc/SCIE/01480/08-09

A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES,

AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF SCIENCE IN MICROBIOLOGY,

DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE,

AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

DECEMBER, 2014

DECLARATION

I hereby declare that the work in this thesis titled ‘’Prevalence of genitourinary Candidiasis among patients with or without medical implant attending gynaecology clinics in Zaria’’ was performed by me in the Department of Microbiology under the supervision of Dr(Mrs) H.I. Inabo and Prof. S.E. Yakubu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

…… ……..

Signature Date

CERTIFICATION

The Thesis entitled ''Prevalence of genitourinary Candidiasis among patients with or without medical implant attending gynaecology clinics in Zaria'' by Jimoh Olanrewaju meets the regulations governing the award of the degree of Master of Science in the Department of Microbiology, Ahmadu Bello University, Zaria and it is approved for its contribution to knowledge and literary presentation.

DR (Mrs) H.I INABO Chairman, Supervisory Committee Department of Microbiology, ABU, Zaria	Signature	Date
PROF S.E YAKUBU Member, Supervisory Committee Department of Microbiology, ABU, Zaria	Signature	Date
PROF S.A. ADO Head of Department Department of Microbiology, ABU, Zaria	Signature	Date
PROF. A .Z .HASSAN Dean, School of Postgraduate Studies, ABU, Zaria.	Signature	Date

ACKNOWLEDGEMENT

Glory be to my God, the power behind my existence, the source of the joy of my salvation for the wisdom, direction and help over me and my family.

My immeasurable gratitude goes to Dr(Mrs) H.I. Inabo, my major supervisor, whose constructive criticism, objective observation and expertise advice added quality to this study despite her extremely tight schedules. May the good Lord increase you and beautify you on every side. My unreserved gratitude also goes to Prof. S.E.Yakubu, my co-supervisor whose contribution, advice, suggestions and expertise meaningfully added quality to this work. May the Lord bless you real good. My appreciation also goes to Dr. A.T. Olayinka who also contributed immensely to the success of this study through her constant professional advice, guidance, encouragement and support. May the good Lord continually glorify you.

My sincere gratitude goes to my dear wife, Dr Adenike Jimoh and my children, Ipa-Oluwa, Otitololuwa and Ire-Oluwa for their unalloyed support. May the good Lord bless you and make you great in life and destiny. Also to my Colleagues Dr. J. Ejembi, Dr. T.O. Ige, Dr M. Abdulaziz, Dr S. Aliyu and Dr D.H. Musa, I am really grateful for your support, advice and encouragement in the course of my study.

My gratitude also goes to Dr. F.J. Giwa, Dr. B.O.P. Musa, Dr. A. Ibrahim and Dr. A.S. Opaluwa for their love, encouragement and support .May you never lack helper of destiny in life. My appreciation also goes to the entire staff of the Department of Microbiology, ABU, Zaria, Department of Medical Microbiology, ABUTH, Zaria, Gambo Sawaba General Hospital, Zaria, Saint Lukes Anglican Hospital, Wusasa, Zaria, University Health Services(Sick Bay), ABU, Zaria and Major Abdullahi Memorial Hospital, Sabon-gari, Zaria. My utmost thanks go to

Mr Julius Okojokwu, Mr Charles Ayodeji, Mrs Fasila Abdulwakilu, Mr Mustapha Musbau, Miss Blessing Obasi and Mrs Esther Jonah. May the good Lord bless and increase you on every side. Lastly but not the least, my regards to all the great women that participated in the research without whom the research would not have been possible, may the Lord bless you greatly.

DEDICATION

This work is dedicated to the most high God and my late parents (Mr and Mrs Jimoh Ajide)

ABSTRACT

A cross-sectional study was carried out to determine the prevalence of genitourinary candidiasis among patients with or without medical implants attending four Gynaecology Clinics in Zaria, Nigeria. A total of 400 High Vaginal Swabs (HVS) and urine samples were collected from patients who presented to the four selected hospitals in Zaria, Nigeria with features suggestive of genitourinary candidiasis. The specimens were analyzed using direct microscopy, culture on sabouraud dextrose agar, CHROMAgar (Oxoid) and carbohydrate utilization test .Antifungal sensitivity testing was performed using discs diffusion methods. Out of the four hundred (400) samples examined, one hundred and sixty three (163) *Candida* isolates were obtained giving 40.8% prevalence. Out of the one hundred and sixty three (163) isolates, one hundred and forty three (143) were obtained from high vaginal swabs while the remaining twenty (20) isolates were from urine specimens. A total of fifty three (53) patients were with medical implants while three hundred and forty seven (347) patients were without medical implants. Prevalence rate of 37.7% was observed among patients with medical implants while 41.2% was observed among patients without medical implants. Overall, no significant association was found between patients on medical implants and those not on medical implants (χ^2 = 0.23, df=1, p=0.63). Chi square analysis was used to determine association of the infection with clinical and demographic factors. A significant association was found in patients with co-morbid condition (χ^2=14.757, df=3, p=0.002). There was also an association between pattern of vaginal discharge and the species isolated (χ^2=8.198, df=3, p=0.042). There was no statistical association between age, occupation, marital status, antibiotic usage and development of genitourinary candidiasis. *Candida albicans* was observed to be the most prevalent (19.75%) species among the four species isolated but overall the *non-albicans* (*C.parapsilosis 12.75%, C.tropicalis 4.5% and C. glabrata* 3.75%) were on the increase. The four species were all susceptible to the commonly used azoles (miconazole, fluconazole and clotrimazole) which are a good sign that there is no problem of resistance yet in the populace.

TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS

ABUTH	-	Ahmadu Bello University Teaching Hospital
AIDS	-	Acquired Immunodeficiency Syndrome
CLSI	-	Clinical Laboratory Standard Institute
HVS	-	High Vaginal Swab
IUCD	-	Intrauterine Contraceptive Device
MSU	-	Midstream Urine
VVF	-	Vesico Vaginal Fistula
SDA	-	Sabouraud Dextrose Agar

CHAPTER ONE

1.0 INTRODUCTION

Candida species are versatile microorganisms which reside normally in the skin, mouth, gastrointestinal tract, and genitourinary tract. In healthy people, *Candida* species usually live as benign commensals and produce no disease. However, *Candida* species are the most common cause of fungal infections in immunosuppressed individuals (Achkar and Fries, 2010), leading to a range of non life threatening mucocutaneous disease to threatening invasive systemic disease. Among *Candida* spp, *Candida albicans* is the most common infectious agent. In the 1980s, *Candida albicans* accounted for more than 80% of all *Candida* isolates from nosocomial yeast infections but the frequency of isolation of *Candida krusei, Candida glabrata, C. tropicalis* and *C.parapsilosis* is steadily increasing globally (Nadeem *et al.,* 2010).

Although published works in the Northern part of Nigeria in relation to genitourinary Candidiasis are scarce, Okungbowa *et al*. (2003) reported that *Candida glabrata* is more predominant among symptomatic individuals, following a survey of seven cities in the Southern part of Nigeria in contrast to the existing belief that *Candida albicans* is the most prevalent.

The pathogenesis of *Candida* infections is extremely complex and probably varies with each species. Adhesion of *Candida* to the epithelium of the gastrointestinal or urinary tract is critically important. *Candida* species commonly colonize the mucosal surfaces, and their ability to invade and cause infection is first dependent on binding (Forbes *et al*, 2007). Fibronectin, a component of the host extracellular matrix, may play a role in the initiation and dissemination of *Candida albicans* infections. Three distinct aspartyl proteases have been described in *Candida albicans*, and strains with high levels of proteases have been shown to have an increased ability to cause

disease in experimental animal model (Forbes *et al.,* 2007). Hydrophobic molecules present on the surface of *Candida* species also appear to be important in pathogenesis, and there is a strong correlation between adhesion and surface hydrophobicity. Other factors linked with pathogenecity include: high level of phospholipase and phenotypic switching (ability to produce hyphae and pseudohyphae) (Forbes *et al.,* 2007).

Mucocutaneous candidiasis can be divided into non-genital disease and genitourinary disease. Among non genitourinary candidiasis, oropharyngeal manifestations are the most common and usually are diagnosed in immunocompromised patients, such as HIV infected individuals (de Repentiguy *et al.,* 2004). The most frequent manifestation of genitourinary Candidiasis includes VulvoVaginal Candidiasis (VVC) in women, balanitis and balanoposthitis in men, and candiduria in both sexes (Achkar and Fries, 2010). These diseases are remarkably common but occur in different populations, in the immunocompetent as well as in the immunocompromised. While VVC affects mostly healthy women, candiduria is commonly diagnosed in immunocompromised patients or neonates. In the majority of women, a diagnosis of VVC is made at least once during their childbearing years. Among the many causes of vaginitis, VVC is the second most common after bacterial vaginosis and is diagnosed in up to 40% of women with vaginal complaints in the primary care setting (Anderson *et al.*, 2004).

It is estimated that at least 75% of healthy adult women will experience one episode of *Candida* vulvovaginitis during their reproductive lives and that 5% will have recurrent infectious episodes(Achkar and Fries,2010). *Candida albicans* is responsible for infection in 80 to 90% of cases, although the incidence of VVC due to *non- albicans Candida s*uch as *C. glabrata* has increased steadily over the past few decades (Marot –Leblond *et al.*, 2009).

The risk factors for genitourinary candidiasis may be classified into: Host related factors and behavioral risk factors. Host related risk factors include: indiscriminate use of antibiotics, uncontrolled diabetes mellitus, conditions associated with high reproductive hormone levels and genetic predispositions. Behavioural risk factors that have been related to a higher incidence of vaginal candidiasis include frequent sexual intercourse and use of high oestrogen oral contraceptives, intrauterine device, condom and spermicides. Other risk factors include: anatomic urinary tract abnormalities, co-morbidities, indwelling urinary drainage devices, abdominal surgery, increased age and female gender. These favour the development of candiduria (Achkar and Fries, 2010)

In vaginal candidiasis, itching, soreness and non-homogeneous white discharge accompany typical white lesions on the epithelial surfaces of the vulva, vagina and cervix. Sometimes the mucosa simply appears inflammed and friable. The perivulval skin may become sore and small satellite pustules may appear around the perineum and natal cleft. Some women experience recurrent episode. Soreness and irritation may lead to dyspareunia (Greenwood *et al.*, 2007).

Fungal urinary tract infections are mostly asymptomatic. This means that majority of patients have neither fever, nor dysuria, nor any other urinary tract related complaints. *Candida albicans* urinary tract infections are commonly catheter associated. Urinary catheters are hollow, latex or silicone implants that are passed through the urethra into the bladder to collect urine during surgery, in unconscious patients or in controlling urinary incontinence (Inabo, 2006).The isolation of *Candida* species from urine specimen of patients may represent several clinical states such as urethritis, cystitis and Candidaemia. Candiduria occurs late, following prolonged hospitalisation. Although, candiduria may not be a specific marker for disseminated candidiasis in all cases, it has been proposed as an indicator of poor prognosis in patients with advancing age

because of the multiple serious underlying diseases found in this population (Arjuna *et al.*, 2005).

1.1 Statement of the Problem

Vaginal discharge is a common symptom in primary health care and is often the second most common gynecological problem after menstrual disorders (Akingbade *et al*.,2013). Most women regard any secretion from the vagina as abnormal discharge and the first task for primary health care providers is to ascertain whether it is pathological or physiological (Akingbade *et al*.,2013) Some women would have used different chemicals, (antimicrobial agent, douching agents, antiseptic soap), due to intense pruritus prior to consultation. This promotes development of resistance to the common antifungal agents available. Majority of women are unaware that prolonged antibiotic use could also predispose them to genitourinary candidiasis. Some isolates of non *Candida albicans* are resistant to the azole group of antifungal drugs which are commonly used. *Candida krusei* has been found to be innately resistant to Fluconazole, *C.glabrata* has been reported to acquire resistance *in vitro* and *in vivo* and *C.dubliniensis* isolates have been observed to rapidly develop resistance to fluconazole (Arjuna *et al.,* 2005). Due to the changing epidemiology of *Candida* and availability of newer antifungal drugs with different antifungal spectra, many physicians may no longer be able to make therapeutic decisions based on broad identification of fungi as yeasts and molds but may need to go to species level so as to enhance proper treatment(Nadeem *et al.,* 2010).

1.2 Justification

The symptoms of genitourinary candidiasis may cause great discomfort, resulting in time loss from work, low self esteem, and marital dispute. Vulvovaginal complaints are the most common

reasons for gynaecological consultation (Achkar and Fries,2010). In the United States of America prior to the availability of over the counter treatment (OTC), approximately thirteen million cases of vulvovaginal candidiasis occurred annually accounting for ten million visits to gynaecology units. In 1995 alone, the annual cost of vulvovaginal candidiasis was estimated to be $1.8 billion with approximately half of this amount consisting of charges for clinicians. It was also reported by industry sources that OTC sales of vaginal antifungals were the largest component of the feminine health care sales (Achkar and Fries, 2010). This is expected to be higher in Nigeria where literacy level is low and most people patronize drug vendors who have shallow knowledge on vulvovaginal candidiasis

In addition, vaginal candidiasis is not life threatening and is treatable. However, when left untreated, is a possible risk for acquisition of HIV/AIDS due to mucosal or cutaneous barrier distruption as well as other complications (Nwadioha *et al.*,2010, Namkinga,2013).

1.3 Aim

The aim of this research was to determine the occurence of genitourinary candidiasis among patients with or without medical implants attending some gynaecology clinics in Zaria.

1.4 Objectives

1. To determine the prevalence of different *Candida* species among gynaecological patients with or without medical implants in Zaria, Nigeria.
2. To study the pattern of distribution of different *Candida* species among enrolled gynaecological patients.

3. To establish the risk factors associated with genitourinary candidiasis among patients with or without medical implants.

4. To determine antifungal susceptibility patterns of *Candida* species isolated from patients.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Yeasts are a common cause of nosocomial infection in hospitalized patients. *Candida* species as yeasts are reported as the fourth most commonly isolated pathogens from blood cultures in hospitalized patients (Adam *et al*., 2010). Candidiasis is an infection generally caused by *Candida albicans*, occasionally by non- *albicans* species and may cause lesions in mucous membranes of the mouth (thrush),skin (dermatitis),nails (paronychia and onychia),vagina(vulvo-vaginitis) and bronchopulmonary tracts and rarely endocarditis, meningitis,pyelitis and mycaemia (Dey *et al*.,2006). Infections due to *Candida* spp. and other fungi have increased dramatically in recent years and are of particular importance because of the rising number of immunocompromised patients. *Candida albicans* accounts for approximately 90% of *Candida* spp. isolated from yeast-infected patients; however, *Candida tropicalis*, *Candida parapsilosis*,*Candida glabrata*, and *Candida krusei* are of increasing significance as they tend to be more resistant to antifungal agents (Cooke *et al*., 2002).

2.2 Background History

The renowned Greek physician, Hippocrates (460–370 BC) first mentioned oral thrush: aphthae in the mouth (Barnett, 2008). Between 1839 and 1844, three independent workers namely Fredrik Berg at Stockholm, David Gruby in Paris and John Bennett in Edinburgh reported a cryptogam as the cause of buccal thrush in infants. Berg in 1841 recognized the cause of thrush to be a mould-like fungus, the filaments of which spread between epithelial cells (Barnett, 2008). Legenback in 1893 demonstrated a yeast-like fungus in the lesion of thrush. Charles-Phillipe

Robin in 1853 named the organism *Oidium albicans* and published good drawings of its cells and an extensive description of the organism (Barnett, 2008).The organism was called by Zopf in 1890 as *Monilia albicans*. In 1937, Donald Martin and his colleagues at Duke University School of Medicine made a very careful study of 153 meticulously purified strains, mostly from clinical sources, and considered the mode of growth on Sabouraud Dextrose Agar (SDA), Blood agar, Cornmeal agar and also fermentation reactions. The generic name *Candida* was suggested by Berkhout to include fungi which reproduced by budding and developed pseudomycelia. Benham in 1930 distinguished between *Candida* (*Monilia*) *albicans*,*C. tropicalis* (*Monilia candida*), *C.* (*Monilia*) *parapsilosis* and *C*. (*Monilia*) *krusei*, using as criteria; (a) the appearance of the cells grown under differing conditions; (b) fermentation reactions; (c) serological behaviour; and (d) pathogenicity for rabbits.

Identification of antifungal agents began in late 1940s with discovery of nystatin by Hazen and Brown in 1950 (Calderone, 2002).Vandeputte and Gold (1954) later discovered amphotericin B and 5-fluoroctosine was later suggested as a potential antifungal drug from studies of Grunberg, Titsworth and Bennett (1963). The first azole, miconazole was subsequently discovered by Janssen (Calderone, 2002)

2.3 Epidemiology of Genitourinary Candidiasis

Candida species, mostly *C. albicans*, can be isolated in the vaginal tracts of 20 to 30% of healthy asymptomatic non pregnant women at any single point in time and in up to 70% if followed longitudinally over a 1-year period (Achka and Fries, 2010). If the balance between colonization and the host is temporarily disturbed, *Candida* can cause disease such as VVC, which is associated with clinical signs of inflammation. Such episodes can occur sporadically or often can

be attributed to the presence of a known risk factor, e.g., the disturbance of local microbiologic flora by antibiotic use. It is estimated that around 75% of all women experience at least one episode of vulvovaginal candidiasis during their childbearing years, of which about half have at least one recurrence. However, in United States of America, vulvovaginal candidiasis is the second most common cause of vaginitis in women (Sobel and Chaim, 1996). Persons at the extremes of age (neonates and adults >65 y) are most susceptible to candidal colonization. Mucocutaneous candidiasis is also more prevalent in neonates and older adults. Very-low-birth-weight and extremely-low-birth-weight infants are at high risk for blood culture–proven late-onset candidiasis (defined as sepsis that develops 72 hours after birth, (Sobel and Chaim, 1996)

2.4 Risk Factors for Genitourinary Candidiasis

Candida is ubiquitous yeast that resides harmlessly on skin and mucous membranes until dampness, heat, and impaired local and systemic defenses provide a fertile environment for it to grow. The risk factors include the following; hot weather, restrictive clothing, poor hygiene, Infrequent diaper or undergarment changes in children and elderly patients, altered flora from antibiotic therapy and inflammatory diseases (eg, psoriasis) that occur in skinfolds (Mirela *et al*.,2010). Others are immunosuppression resulting from corticosteroids and immunosuppressive drugs, pregnancy, diabetes, endocrinopathies (eg, Cushing's disease, hypoadrenalism, hypothyroidism), blood dyscrasias, or T-cell defects.

2.5 Clinical Manifestations of Candidiasis

Predominant symptoms of vulvovaginal candidiasis include intense vulva pruritus, and abnormal vaginal discharge (which may be minimal, a thick “curd-like” material, or a watery secretion). The vulva may be erythematous, oedematous, and contain satellite lesions (Peng, 2008). There

may be burning sensation with urination. Rarely is vulva candidiasis seen without concomitant vaginal candidiasis (Peng, 2008)

2.5.1 *Candida* balanitis

Patients report penile pruritus along with whitish patches on the penis. *Candida* balanitis is acquired through direct sexual contact with a partner who has Vulvovaginal Candidiasis. Physical examination initially reveals vesicles on the penis that later develop into patches of whitish exudate. The rash occasionally spreads to the thighs, gluteal folds, buttocks, and scrotum (Mandell *et al*., 2010).

2.5.2 *Candida* cystitis

Many patients are asymptomatic. However, bladder invasion may result in frequency, urgency, dysuria, hematuria, and suprapubic pain. *Candida* cystitis may or may not be associated with the use of a Foley catheter. Physical examination may reveal suprapubic pain; other findings are unremarkable.

2.5.3 Asymptomatic candiduria

Most catheterized patients with persistent candiduria are asymptomatic, similar to non catheterized patients. Most patients with candiduria have easily identifiable risk factors for *Candida* colonization. Thus, invasive disease is difficult to differentiate from colonization based solely on culture results because approximately 5-10% of all urine cultures are positive for *Candida* (Cook and Zumla, 2003; Mandell *et al*.,2010).

2.5.4 Oropharyngeal candidiasis

This infection is seen in all countries, particularly in infants, the elderly and immunocompromised patients, including AIDS (Cook and Zumla, 2003). Thrush can occur on the tongue, lips, gums or palate. It is a patchy to confluent, whitish pseudomembranous lesions composed of epithelial cells, yeast and pseudohyphae (Mitchell, 2010). There are a number of different clinical types of oropharyngeal candidiasis. These are largely distinguished by their chronicity and clinical presentation. Acute pseudomembranous candidiasis presents with white plaques on the epithelium that are inflamed and easily detached. The scattered nature of the appearance is suggestive of speckling on a thrush's breast, hence its common name 'thrush'.

In patients with chronic pseudomembranous candidiasis the condition is often persistent and refractory to therapy. In some individuals, plaques are not formed but the mucosal surface appears red and glazed-acute erythematous candidiasis, also known as acute atrophic oral candidiaisis. This may occur in AIDS patients. In patients presenting with inflammatory changes and oral discomfort associated with dentures (denture sore mouth), persistent erythema associated with Candida is a common feature of chronic erythema candidiasis (Cook and Zumla, 2003; Mandell *et al*., 2010).

2.5.5 *Candida* intertrigo

The skin is only indirectly affected in vaginal infection when there is spread of infection to the vulva and perineum. In *Candida* intertrigo, there appear a prominent red rash in the groin and on the upper surface of the thigh together with satellite pustules and papules (Cook and Zumla 2003).There may be similar presentation beneath the breast and around the umbilicus.

2.5.6 *Erosio interdigitalis blastomycetica*

This term applies to Candida infection occurring between the fingers or toe. It has a red base, may extend onto the sides of the digits, is painful, and is predisposed to maceration (Mandell *et al*., 2010).

2.5.7 *Candida* infection and nappy dermatitis

Nappy rash in infants is a form of irritant eczema which is often secondarily infected with, among other organisms *Candida* species. The presence of yeasts may be suspected by the appearance of satellite pustules and is confirmed by culturing the organism from the swab area (Cook and Zumla, 2003).

2.5.8 Candidiasis of the nails

Paronychia is an acute or chronic infection of the nail folds caused by *Candida* species such as *Candida albicans* or *Candida parapsilosis.* This occurs commonly in the tropics and in patients who are likely to immerse their hands frequently in water or whose occupations involve cooking. In addition to swelling of nail fold, pain and intermittent discharge of pus, the lateral border of the nail may be undermined with onycholysis (Mandell *et al.*, 2010).

2.6 Laboratory Diagnosis of Candidiasis

2.6.1 Specimens

The specimen used for diagnosis depends on the site affected. These specimens include swabs and scrappings from superficial lesions, blood, spinal fluid, tissue biopsies, urine, exudates and material from removed intravenous catheters (Cheesbrough, 2005, Winn *et al*., 2006 Brooks *et al*., 2010)

2.6.2 Microscopic examination of specimen

Centrifuged fluid, swabs, tissue biopsies and other specimens may be examined by direct wet mount, Gram stained smears or histopathological slides for pseudohyphae and budding cells. Skin or nail scrapings are first placed in a drop of 10% potassium hydroxide and calcoflour (Cheesbrough, 2005; Winn *et al*., 2006; Brooks *et al*., 2010).

2.6.3 Cultural methods for the isolation of *Candida* species

All specimens are cultured on fungal or bacteriological media at room temperature or at 37^0C.Yeast colonies are examined for the presence of pseudohyphae (Brooks *et al*., 2010). *Candida albicans* is identified by the production of germ tubes or chlamydospores. The interpretation of positive cultures varies with the specimen. Positive cultures from normally sterile body sites are significant. The diagnostic value of a quantitative urine culture depends on the integrity of the specimen, hence the need for proper sample collection. Positive blood cultures may reflect systemic candidiasis or transient candidaemia due to a contaminated intravenous line (Brooks *et al*., 2010).

Most clinically significant yeast isolates grow within 36 – 72 hours in culture on sheep blood agar and most other non selective primary isolation media. The colonies are typically white or yellow –white with a smooth or pasty consistency (Winn *et al.*, 2006).

2.6.4 Germ tube test

Candida species can be identified using germ tube test. The test is the most generally accepted and economical method used in the clinical laboratory for the identification of yeasts (Forbes *et al*., 2007). A germ tube is defined as a filamentous extension from a yeast cell that is about half the width and three to four times the length of the mother cell (Winn *et al*., 2006). The true germ

tube produced by *Candida albicans* has no constriction at the neck. The germ tube is a true hyphal structure and therefore does not have the constriction characteristic of pseudohyphae. Germ tube test is carried out by suspending a small portion of an isolated colony of the yeast in 0.5ml of rabbit or human plasma or serum. The test tube is incubated at 35-37^{0}C for no longer than 2-3hours.Then a drop of the yeast –serum suspension is placed on a microscope slide, overlaid with a coverslip, and examined microscopically for the presence of germ tubes. Germ tube test should be read after 2-3hours to prevent clumping of hyphal initials and to reduce the number of false positives. (Winn *et al*., 2006; Forbes *et al*., 2007; Howell and Hazen, 2011).

2.6.5 Yeast on Cornmeal Agar

Candida albicans can be identified based on the morphology of the species grown on cornmeal agar. Chlamydospore is produced on the cornmeal agar by *Candida albicans* which can be viewed microscopically. The patterns of growth on cornmeal agar are helpful in making a presumptive identification and in serving as a quality –control check on species identification provided by the biocodes indicated by automated and kit systems.

2.6.6 CHROMagar as a differential culture medium

CHROMagar is a chromogenic differential culture medium being used in many laboratories to facilitate the isolation and identification of certain clinically important yeast species. Observation of colonial morphology and colour changes are being used to differentiate different *Candida* species. CHROMagar has been found to be economically useful and less time consuming in identification of yeast (Winn *et al*., 2006; Forbes *et al*., 2007).

Willinger and Manafi, (1999) reported a sensitivity of 98.8% and specificity of 100% for *Candida albican*,66.7% and 99.8% for *Candida tropicalis*,100% specificity and sensitivity for

Candida krusei, and finally specificity and sensitivity of *98%* and *95.7%* for *glabrata* respectively. A specificity and sensitivity above 99% was observed by Odds and Bernaert,(1994) for the three *Candida* species. Evaluation of cost effectiveness and time advantage of CHROMagar in comparison with Sabouraud Dextrose Agar (SDA) was done by Ainscough and Kibbler as documented by Winn *et al*. (2006). An overall sensitivity of 95.2% was observed in the study of 21 yeast isolates recovered from 298 clinical samples from patients with neutropenia and those with AIDS. CHROMagar was found to be 100% sensitive and specific for *Candida albicans.*

2.6.7 Carbohydrate utilization test

Fermentative yeasts recovered from clinical samples produce carbon dioxide and alcohol; therefore, production of gas is indicative of fermentation. The pH of the medium may not change. Rarely are fermentation studies needed to identify most of the common yeasts if the mycologist is familiar with typical morphology on cornmeal agar (Howell and Hazen, 2011).The test is most helpful in differentiating the various species of *Candida*, *Cryptococcus* and *Rhodotorula* which are non fermentative. There are several reliable commercial kits such as API20C AUX (bioMerieux SA) and API ID32C (Howell and Hazen, 2011).

2.7 Treatment of Genitourinary Candidiasis.

The commonly used antifungal agents for candidiasis are the azoles. These consist of the imidazoles and the triazoles. Conventionally used agents are clotrimazole, miconazole, fluconazole, itraconazole and ketoconazole. Newer triazoles consist of voriconazole, posaconazole and the most recent ravuconazole. The azoles act by distrupting the integrity of the fungal cell membrane by interfering with the synthesis of ergosterol (Forbes *et al*., 2007).

Nystatin (Polyene macrolide antifungal), an antifungal agent produced by *Streptomyces noursei*, is not absorbed by the gastrointestinal tract .It is principally used locally to treat oral or vulvovaginal candidiasis. Other polyene macrolide antifungal agents include Amphotericin B and Griseofulvin (Forbes *et al*., 2007).Additional antifungal agents used include, the anti-metabolite (Flucytosine) and Echinocandins (Caspofungin,micafungin and anidulafungin).

2.8. *Candida* Infection of Medical Device

At least half of all cases of nosocomial infections are associated with medical devices (Kojic and Darouiche, 2004) . The medical consequences of device-related infections can be disastrous; they include potentially life-threatening systemic infections and device malfunction that may require device removal, often complicated by tissue destruction. An increasing proportion of device-related infections, particularly those involving the bloodstream and urinary tract, are being caused by *Candida* spp. *Candida* infections of the urinary tract are strongly associated with the presence of a urinary catheter. A significant proportion of human infections involve biofilms. Microbial biofilms develop when organisms adhere to a surface and produce extracellular polymers that provide a structural matrix and facilitate further adhesion. Organisms in biofilms behave differently from freely suspended microbes and have been shown to be relatively refractory to medical therapy. Therefore, biofilm-associated infections of retained devices may recur after cessation of antibiotic therapy and hence may necessitate device removal. *Candida* species are emerging as important nosocomial pathogens, and an implanted device with a detectable biofilm is frequently associated with these infections (Kojic and Darouiche, 2004) Most *Candida* spp. have been shown to produce biofilm in vitro to various degrees. An in vitro study showed that *C.parapsilosis*, *C. pseudotropicalis*, and *C. glabrata* produced significantly

less biofilm on polyvinyl chloride disks than did the more pathogenic *C. albicans*, as determined by dry-weight, colorimetric, or radioisotope assays.

2.9 Antifungal Susceptibility of *Candida* Isolates

Antifungal susceptibility tests exist; they are not routinely done in most medical laboratories in the tropics. They are designed to provide information that will allow the physician to select the appropriate antifungal agent useful for treating a specific infection (Forbes *et al.*, 2007). The Clinical Laboratory Standards Institute (CLSI) provides documents and sets standard for antifungal susceptibility. The current guidelines for antifungal susceptibility testing are the M27-A2 document and M-44A. Methodology and interpretation of antifungal susceptibility tests are still evolving. Antifungal susceptibility tests are expensive and time consuming tests, but may be of value in the following circumstances;

- When the antibiogram determination for isolate within an institution is required.
- Management of patients with refractory oropharyngeal candidiasis.
- Management of patients who have invasive candidiasis where the use of the azoles is questioned in infections caused by non – *Candida albicans.*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area and Population

The study was conducted in Zaria, Kaduna State, Nigeria. Zaria is located on a plateau at a height of 2,200 feet above sea level. It is situated in the centre of Northern Nigeria. The population of Zaria was 406,990 according to 2006 census figure.The indigenous Hausas predominantly inhabit the old city and the adjacent tudun wada neighbourhood. Other neighbourhood include Samaru, Wusasa and Sabon gari inhabitants.

Target Population included symptomatic (vaginal discharge, severe itching) patients presenting at Gynaecology clinic in four selected hospitals in Zaria, Nigeria (ABU Sick Bay, Saint Luke's Hospital, Wusasa, General Hospital, Sabon- Gari and Gambo Sawaba Hospital, Zaria).

3.2 Study Design

It was a hospital based descriptive cross sectional study conducted from February 2012 to March, 2013 at selected hospital in Zaria.

3.3 Ethical Approval of the Study

Approval for the study was obtained from Kaduna State Ministry of Health and the Ethical Committee of the hospitals concerned. Informed written consent was obtained from all the patients.

3.4 Sample Size

The sample size was determined using the following equation as described by Araoye (2004).

$$n = Z^2Pq/d^2$$

Where:

n= the desired sample size.

Z=the standard normal deviate at 1.96 which correspond to 95% confidence level.

P=proportion of population with genitourinary candidiasis (assumed to be 50% since no previous published study in Nigeria was found)

q=1-p

d=degree of accuracy desired (tolerable error of 5%) =0.05

At 5% tolerable error margin i.e., d=5%; Local Genitourinary candidiasis (p) =50%

Substituting from above equation gives;

$n= 1.96^2 \times 0.5\times (1-0.5)/0.05^2$

$n=1.96^2\times 0.5 \times 0.5/0.0025$

$n=3.8416 \times 0.5\times 0.5/0.0025$

$n=3.8416\times 0.25/0.0025$

n=384.Approximately 400

Sample size=400

3.5 Data Collection

Structured questionnaires were designed and administered to patients to obtain relevant information with regards to prevalence of genitourinary candidiasis (Appendix II)

3.6 Inclusion and Exclusion Criteria

Inclusion criteria were all symptomatic (vaginal discharge, severe itching) out-patients attending Gynaecology clinic, including patient on Intrauterine contraceptive device (IUCDs) and In-patients on urethral catheters who were not on antifungal therapy. Exclusion criteria were all asymptomatic patients who present at the Gynaecology clinic for other clinical problems apart from vulvovaginitis. All other patients who presented to other clinics in the hospital within the study period and those who declined to participate though symptomatic and were on antifungal therapy were excluded.

3.7 Sample Collection and Handling.

Three hundred and forty seven High Vaginal Swabs (HVS) and Fifty three Mid Stream Urine (MSU) specimens were collected from each consenting patient. Ten (10)mls MSU was obtained in sterile bottle. Each sample was labeled appropriately with prepared codes. A convenience sampling process was used to source and obtain the samples as they present. The sample were transported to the laboratory within one hour of collection and for any delay envisaged the samples were refrigerated at 4^0C to 8^0C

3.8 Direct Microscopy:

3.8.1 Wet mount preparation of urine samples

Ten milliliters (10ml) of well mixed urine was aseptically transferred to a labeled test tube using a sterile pipette; and centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded and the sediment re-mixed by tapping the bottom of the tube. One drop of the well mixed sediment was transferred with a dropper to a slide and a cover slip was placed on the glass slide. The preparation was examined microscopically using ×10 and ×40 objectives with condenser iris closed sufficiently to give good contrast. Yeast cells were identified by characteristic spherical oval cells with budding.

3.8.2 Wet mount preparation for high vaginal swabs

Paired -swabs were collected from each patient, one was used for wet mount preparation while the other swab was used for culture to minimize contamination. The swab was emulsified in 1-2drops saline solution on a slide, then covered with a cover slip. Yeast cells were *identified* as large oval cells at ×40 magnification.

3.9 Cultivation on Solid Media

3.9.1 Cultivation of *Candida* species on Sabouraud Dextrose Agar

The remaining sediment of centrifuged samples and the second swab stick were inoculated on Sabouraud Dextrose Agar (SDA).The inoculated medium was incubated at 37^{o}C for 48 hours. *Candida* species were identified by their usual spherical oval cells with terminal, subterminal or multipolar budding or sometimes hyphae under the × 40 magnification.

3.9.2 Cultivation on CHROMagar

Approximately 15.6grams of dehydrated powder was suspended in 500mls of distilled water and one vial of Brilliance^{Tm} *Candida* selective supplement (SRO23/E) was reconstituted by dissolving in absolute ethanol. The mixture was boiled and later cooled to 45^0C in water bath. Approximately 15mls each of the medium was then poured into sterile petri dishes. One to two colonies of growth on SDA was inoculated on CHROMagar medium and then incubated at 30 - 35^0C over 48 -72 hours. The result was interpreted using the colour codes and colonial morphology. (Oxoid)

3.10 Carbohydrate Utilization and Germ Tube Tests

3.10.1 Carbohydrate utilization test for the identification of *Candida* species

A complete identification of *Candida* species was done by means of sugar assimilation test .The yeast was grown on a basal carbohydrate free medium supplemented with essential vitamins and the test sugar. One percent each of the seven test sugars namely, glucose, galactose, maltose, sucrose, cellobiose, lactose and raffinose was dispensed into each tube. Plates were incubated at 35°C for 48 h. Growth produces opacity in the medium and indicates the ability of the species to utilize a sugar.

3.10.2 Germ tube test

Germ-Tube test was performed to differentiate *Candida albicans* from other *Candida spp*. This was done by suspending a very small inoculum of yeast cells obtained from isolated colonies in 0.5ml of plasma (sheep or rabbit); the tubes were incubated at 35^0C to 37^0C for 2 hours. After incubation, a drop of the suspension was placed on a glass slide and covered with a cover slip.

The preparation was examined for germ tube at ×10 and × 40 magnifications (Cheesbrough, 2000). *Candida albicans* produced germ tubes usually within 2hours (Forbes *et al.,* 2007)

3.12 Antifungal Susceptibility Testing

The discs used for antifungal susceptibility test included Clotrimazole (10ug), Fluconazole (25ug),Flucytosine(1µg),AmphotericinB(20ug),Miconazole(10µg) (MAST DIAGNOSTICS, Mast groups Ltd, Merseyside, United Kingdom)

The disc diffusion procedure of the Clinical and Laboratory Standards Institute (CLSI) reference test similar to the standardized procedure used for antibacterial agents was employed. Two to Three (2- 3) colonies of isolated organisms were selected and suspended in physiological saline. The suspension was equated with 0.5 Macfarland standard. It was then streaked on Mueller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue. *Candida parapsilosis* (ATCC 22019) was used as control. The discs containing antifungal agents were placed on the medium. The plates were incubated at 35- 37^0C for 24-48hours.The diameter of the inhibition zone was measured with metric rule and recorded.

3.13 Data Analysis

Statistical package for the social sciences software version 20 was used to analyze the data. Data were examined for outliers, missing values and cleansed. Analysis was performed according to the aims of the research. The chi-square test was used to assess the unadjusted significance of associations between the categorical variable. A P-value of 0.05 or less was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Social Demographic Characteristics of Study Population

Of the four hundred patients recruited for the study, age group 21-30 years was the highest accounting for 196(49%) of all and the least represented age group was 61 years and above accounting for 1(0.3%).Many of the subjects were married 274(68.5%) and divorcee were the least represented 5(1.3%). Regarding education,129(32.6%) had up to tertiary education,124(31.3%),50(12.6%) and 28(7.1%) had no formal education. In relation to ethnicity, combination of other minority ethnic group was the highest accounting for 149(37.3%), Hausa Fulani 144(36%), Yoruba 71(17.8%) and Igbo ethnic group were the least represented 36(9%).A little above half of the subjects were unemployed 208(52%), student 134(33.5%) and 58(14.5%) were gainfully employed.

The cultural characteristics of different *Candida* species on Sabouraud Dextrose Agar and CHROMagar are shown in Table 4.2. Majority of the *Candida* species were white, smooth, glistening, while some were dull and rough on Sabouraud Dextrrose Agar. Although it is difficult to speciate *Candida* species using colonial morphology alone on Sabouraud Dextrose agar, CHROMagar (a differential medium) was used. Green, smooth and dry colonies on CHROMagar were identified as *Candida albicans*, while, discrete, blue and smooth colonies were *Candida tropicalis*. In contrast to this, *Candida glabrata* colonies were cream to light yellow on CHROMagar while *Candida parapsilosis* were beige and smooth colonies on the agar. Plates I to III show different *Candida* species on CHROMagar. Out of one hundred and sixty three (163)

Candida isolated on the CHROMagar,79(48.5%) were identified as *Candida albicans*, *Candida parapsilosis* 51(31.3%) ,*Candida tropicalis* 18(11.0%) and *Candida glabrata*15(9.2%).

Table 4.2 also shows production of germ tube in relation to the *Candida* species isolated. It was observed that 48.5% of the *Candida* species were positive for germ tube production while 51.5% were negative for germ tube production. *Candida albicans* remain the most prevalent being germ tube producing species. Over all, germ tube negative species were more prevalent. There was a statistically significant difference between germ tube producing *Candida* species and those not producing germ tube (χ^2=163.0,df=3,p=0.00)

Table 4.3 shows sugar assimilation tests of *Candida* species isolated from patients in the study area. The result identified 79 isolates as *Candida albicans*, 18 Isolates of *Candida tropicalis* , 15 isolates of *Candida glabrata* and 51 isolates of *Candida parapsilosis*. Definitive diagnosis was arrived at by combining the outcome of colonial characteristics on the CHROMagar with the outcome of germ tube production and carbohydrate assimilation tests.

Table 4.4 shows the occurrence of *Candida* species obtained from High Vaginal Swabs and Urine specimens of patients with or without medical implants. Of four hundred specimens examined, one hundred and forty three (143) were from high vaginal swabs as compared to twenty (20) species which were obtained from urine samples. The prevalence due to various Candida species are; *Candida abicans* 19.75%, *Candida parapsilosis*12.75%, *Candida tropicalis* 4.50% and *Candida glabrata* 3.75%. The overall prevalence of genitourinary urinary candidiasis among gynaecological patients with or without medical implant in Zaria was approximately 40.8%.

Table 4.1: Social demographic characteristics of study population

Variables	Frequency	Percentage (%)
Age (Years)		
<20	64	16
21-30	196	49
31-40	111	27.8
41-50	26	6.5
51-60	2	0 .5
>61	1	0 .3
Marital status		
Single	121	30.3
Married	274	68.5
Divorced	5	1.3
Educational status		
Primary	50	12.6
Secondary	124	31.3
Tertiary	129	32.6
None	28	7.1
Ethnicity		
Hausa-fulani	144	36
Igbo	36	9
Yoruba	71	17.8
Others	149	37.3
Occupation		
Unemployed	208	52
Gainfully employed	58	14.5
Student	134	33.5

Table 4.2: Cultural characteristics of *Candida* species on Sabouraud Dextrose Agar and CHROMAgar.

Colonial colour and Morphology of colonies on SDA	**Colonial colour and morphology of colonies CHROMagar**	**Germ tube**	**Frequency and percentage (%) n=163**	**Identity of *Candida* species**
White, smooth, glistening colonies, some dull rough colonies seen.	Green, smooth and dry colonies.	Positive	79 (48.5)	*Candida albicans*
White, smooth, glistening colonies, some dull rough colonies seen	Blue, discrete, smooth colonies	Negative	18(11)	*Candida tropicalis*
White, smooth, glistening colonies, some dull rough colonies seen	Cream, shining and smooth colonies	Negative	15(9.2)	*Candida glabrata*
White, smooth, glistening colonies, some dull rough colonies seen	Beige, and smooth colonies.	Negative	51(31.3)	*Candida parapsilosis*

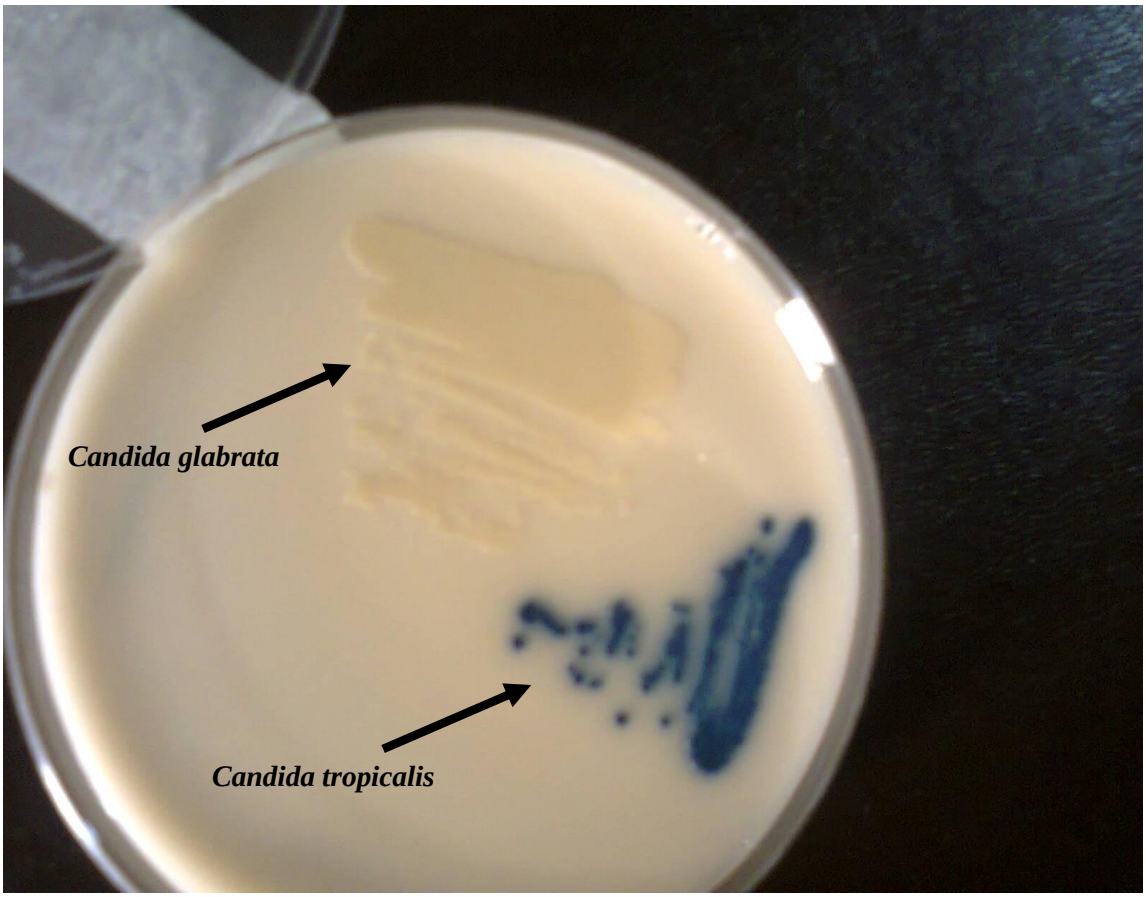

Plate I: Colonies of *Candida* species on CHROMagar. *Candida tropicalis*

Cream ---------- *Candida glabrata*

Blue ---------- *Candida tropicalis*

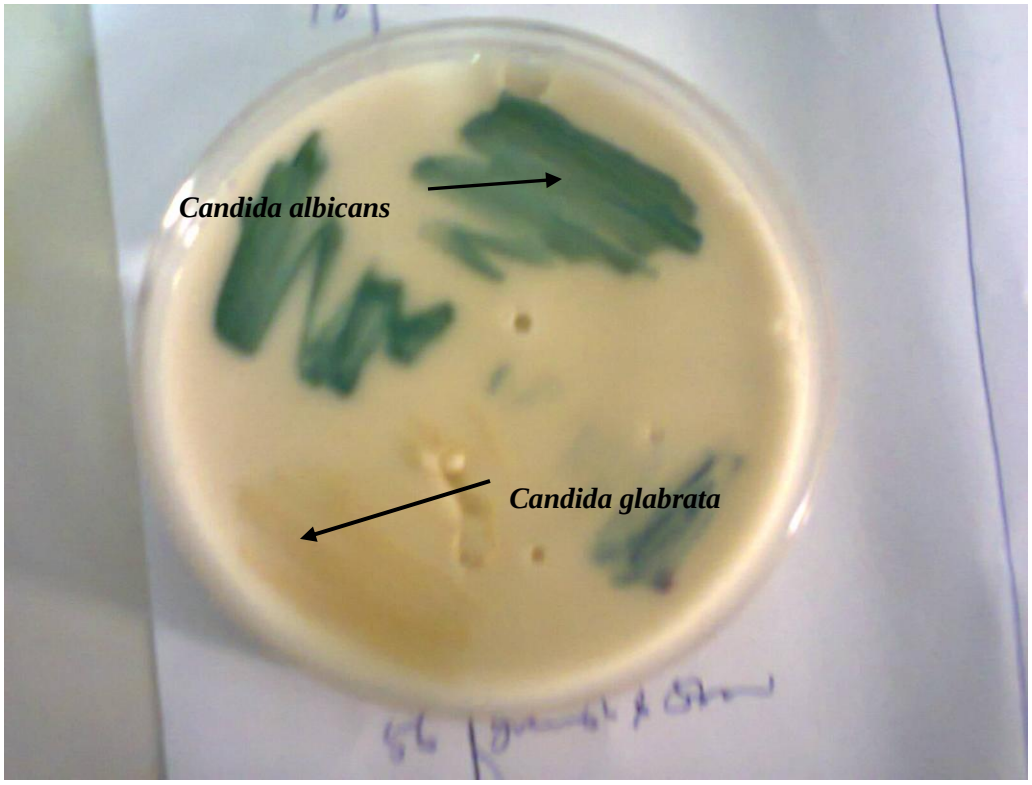

Plate II: Cultural characteristics of some *Candida* species on CHROMagar.

Key:

Green ---------- *Candida albicans*

Cream ---------- *Candida glabrata*

Blue ---------- *Candida tropicalis*

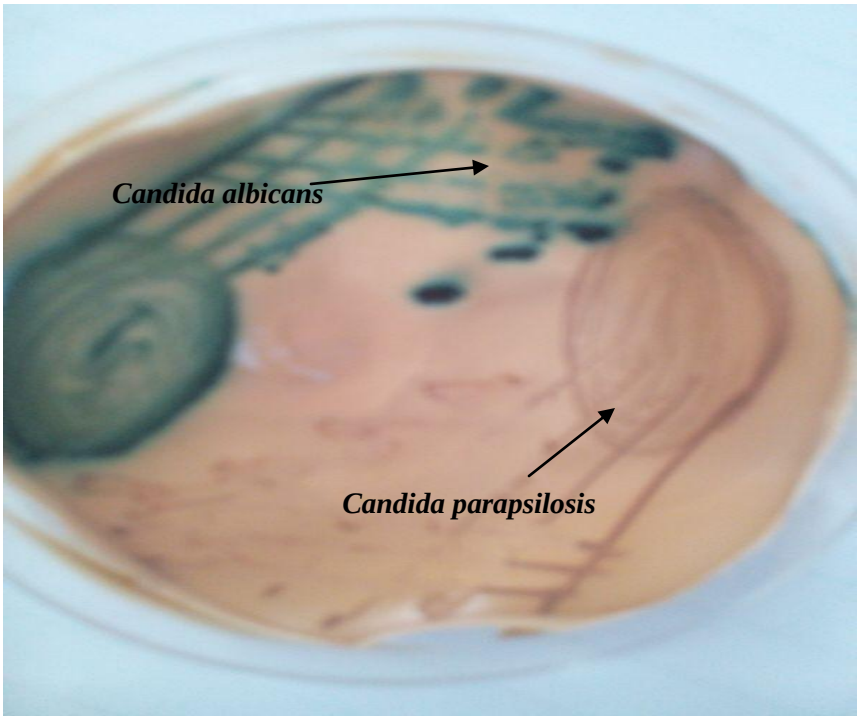

Plate III: Cultural characteristics of some *Candida* species on CHROMagar.

KEY

Green ---------- *Candida albicans*

Beige---------- *Candida parapsilosis*

Table 4.3: Sugar assimilation tests of *Candida* species isolated from patients in the study area.

Organism	Glucose	Maltose	Sucrose	Lactose	Galactose	Raffinose	Cellobiose	Total
C.albicans	P	P	P	N	P	N	N	79(48.5)
C.tropicalis	P	P	P	N	P	N	P	18(11.0)
C.glabrata	P	N	N	N	N	N	N	15(9.2)
C.parapsilosis	P	P	P	N	P	N	N	51(31.3)
Total								163(100)

Key: P –*Candida* species that assimilated sugar

N- Candida species did not assimilate sugar

Table 4.4: Occurrence of *Candida* species in the genitourinary system of Patients in the study

***Candida* species**	**High Vaginal swab**	**Urine**	**Prevalence (n=400)**
Candida albicans	63(15.75)	16 (4)	79(19.75)
Candida tropicalis	18(4.50)	0(0)	18(4.5)
Candida glabrata	15(3.75)	0(0)	15(3.75)
Candida parapsilosis	47(11.75%)	4(1)	51(12.75)
Total	143(35.75)	20(5)	163(40.75)

Figures in parastheses represent percentage

The analysis of the data in Table 4.5 in relation to age group shows that the age group 21-30 years had the highest prevalence of genitourinary candidiasis (18.5%) followed by patients in the age group 31-40years (14%). Subjects in the age group 50years and above had the least prevalence of genitourinary candidiasis (0.25%). There was no statistical association between the genitourinary candidiasis and age (χ^2=8.445,df=5, p= 0.133).

Table 4.6 shows the prevalence of genitourinary candidiasis by marital status. A higher prevalence of 29% was observed among married women when compared to single women (11.25%). However, the lowest prevalence was recorded with those who are divorced. No significant association was observed between marital status and development of genitourinary candidiasis. (χ^2=2.611, df=2, p=0.271).

Table 4.7 shows the relationship between educational status and genitourinary candidiasis. The highest prevalence of 13.25% was observed among patients who attained tertiary educational status, followed by those with secondary education with a prevalence of 11.75%.Patients who had quranic education had a prevalence of 7.75%, followed by patients who had primary education with the prevalence rate of 4.5%. Patients without any formal education had the least prevalence of 3.5%.There was no statistically significant association between development of genitourinary candidiasis and educational status of patients (χ^2=2.781, df=4, p=0.595).

In relation to ethnicity, in Table 4.8, a higher prevalence of 15.5% was observed among the minor ethnic groups. This is followed by Hausa- Fulani ethnic group with a prevalence rate of 14.25% while Yoruba and Igbo ethnic groups had rates of 7.75% and 3.25% respectively. There was no statistically significant association between species isolated and ethnicity (χ^2=4.505, df=3, p=0.212).

The results were also analyzed according to occupation of the participants as shown in Table 4.9. The unemployed patients had the highest prevalence of 22% followed by students with a prevalence of 7.75% while the gainfully employed patient had the lowest prevalence of 3.75%. There was no statistically significant association between occupation of a patient and development of genitourinary candidiasis (χ^2 =3.841, df =2, p=0.147).

The results of occurrence of vaginal discharge in relation to *Candida* species isolated are also presented in table 4.10 and 96.3% of patients had vaginal discharge while 3.7% had no vaginal discharge. However, there was no significant association between vaginal discharge and *Candida* species isolated (χ^2=4.932,df=3,p=0.177).

The results were also analyzed in relation to the frequency of occurrence of vaginal discharge in Table 4.11. This shows that 23.6% had vaginal discharge once, 35.7% had twice, and 25.5% had thrice, while the remaining 15.3% had recurrent *Candida* infection (more than four times in a year). The difference observed was not statistically significant (χ^2=6.709, df=9,p=0.667).

Table 4.12 shows characteristics of vaginal discharge in relation to *Candida* species identified, 92% of patients with vaginal Candidiasis had whitish and curdlike discharge while 8% had discharge that was not whitish and curdlike. There was a significant association between characteristic of vaginal discharge and the *Candida* species isolated (χ^2=8.198, df=3, p=0.042).

Table 4.5: Age –related prevalence of genitourinary candidiasis among patients with or without medical implants attending gynaecology clinic in Zaria.

Age in years	Organism isolated				Total
	Candida albicans	***Candida tropicalis***	***Candida parapsilosis***	***Candida glabrata***	
< 20	9(14.1)	4(6.2)	8(12.7)	3(4.7)	24(6)
21-30	33(16.8)	8(4.1)	24(12.2)	9(4.6)	74(18.5)
31-40	31(27.9)	6(5.4)	16(14.4)	3(2.7)	56(14)
41-50	5(19.2)	0(0)	3(11.5)	0(0)	8(2)
51-60	1(50)	0(0)	0(0)	0(0)	1(0.25)
≥61					

χ^2=8.445, df =5, p= 0.133. Figures in parentheses represent percentage

Table 4.6: Prevalence of genitourinary candidiasis in relation to marital status of the enrolled subjects in the study area.

***Candida* spp isolated**	**Marital status**		
	Single	**Married**	**Divorced**
C. albicans	18(14.9)	60(21.9)	1(50)
C. tropicalis	7(5.8)	10(3.7)	1(50)
C. parapsilosis	16(13.3)	35(12.8)	0(0)
C. glabrata	4(3.3)	11(4)	0(0)
Total	45(11.25)	116(29)	2(0.5)

χ^2=2.611, df=2, p=0.271. Figures in parentheses represent percentage

Table 4.7: Educational Status-related prevalence of *Candida* species

***Candida* spp isolated**	**Education status**					**Total**
	Primary	**Secondary**	**Tertiary**	**Quranic**	**None**	
C.albicans	7(14.0)	25(20.2)	28(20.2)	11(16.9)	8(28.6)	79(19.75)
C.tropicalis	2(4.1)	5(4.1)	7(5.5)	3(4.6)	1(3.6)	18(4.5)
C.parapsilosis	9(18)	13(10.6)	14(10.1)	12(18.5)	3(10.7)	51(12.75)
C.glabrata	0(0)	4(3.2)	4(3.1)	5(7.7)	2(7.1)	15(3.75)
Total	18(4.5)	47(11.75)	53(13.25)	31(7.75)	14(3.5)	163(40.75)

χ^2=2.781, df=4, p=0.595. Figures in parentheses represent percentage

Table 4.8: Ethnicity -related prevalence of genitourinary candidiasis among patients with or without medical implants

***Candida* species**	**Ethnicity**			
	Hausa-fulani	**Igbo**	**Yoruba**	**Others**
C. albicans	24(16.7)	4(11.1)	18(25.4)	33(22.1)
C. tropicalis	7(4.9)	1(2.8)	2(2.9)	8(5.4)
C. parapsilosis	19(13.2)	6(16.7)	10(14.1)	16(10.8)
C. glabrata	7(4.9)	2(5.6)	1(1.4)	5(3.4)
Total	57(14.25)	13(3.25)	31(7.75)	62(15.5)

χ^2=4.505, df=3, p=0.212. Figures in parentheses represent percentage.

Table 4.9: Prevalence of genitourinary candidiasis based on occupation of the patients in the study area.

Candida species	Occupation		
	Unemployment N=208	**Gainfully employed N=58**	**Student N=134**
C. albicans	39(18.8)	21(36.2)	19(14.2)
C. tropicalis	9(4.4)	3(5.2)	6(4.5)
C. parapsilosis	29(13.9)	7(12.1)	15(11.3)
C. glabrata	11(5.3)	0(0)	4(3)
Total	88(22)	31(7.75)	15(3.75)

χ^2 =3.841, df =2, p=0.147. Figures in parentheses represent percentage

Table 4.10: Occurrence of vaginal discharge in relation to genitourinary candidiasis

Candida species	**Occurrence**		**Total**
	Present	**Absent**	
Candida albicans	77(49.0)	2(33.3)	79(48.5)
Candida tropicalis	18(11.5)	0(0)	18(11.0)
Candida parapsilosis	49(31.2)	2(33.3)	51(31.3)
Candida glabrata	13(8.3)	2(33.3)	15(9.2)
Total	157(96.3)	6(3.7)	163(100)

χ^2=4.932, df =3, p=0.177

Figures in parentheses represent percentage

Table 4.11: Distribution of *Candida* species identified in relation to number of occurrence of vaginal discharge per annum

***Candida* species**	**No of times per annum**			
		Twice	**Thrice**	**More than four times**
Candida albicans	15(15.3)	24(18.6)	23(26.4)	15(21.7)
Candida tropicalis	5(5.2)	6(4.7)	5(5.9)	2(2.9)
Candida parapsilosis	13(13.4)	20(15.5)	11(12.6)	5(7.2)
Candida glabrata	4(4.1)	6(4.7)	1(1.1)	2(2.9)
Total	37(9.25)	56(14.0)	40(10)	24(6)

Figures in parentheses represent percentage

Table 4.12: Characteristics of vaginal discharge in relation to *Candida* species identified

Candida species	Characteristics		Total
	Whitish and curdlike	Not whitish and curdlike	
Candida albicans	75(50)	4(30.8)	79(48.5)
Candida tropicalis	17(11.3)	1(7.7)	18(11.0)
Candida parapsilosis	47(31.3)	4(30.8)	51(31.3)
Candida glabrata	11(7.3)	4(30.8)	15(9.2)
Total	150(92.0)	13(8)	163(100)

χ^2=8.198, df=3, p=0.042

Figures in parentheses represent percentage

Table 4.13 shows the relationship between antibiotics used by the patients and the *Candida* species isolated .A higher prevalence of 55.8% was observed with patients on antibiotics compared to the patients who were not on antibiotics (44.2%). Prevalence of *Candida albicans* was 48.6% among patients who were not on antibiotics while it was found to be 48.4% in patients that were on the antibiotics. Over all there were no statistically significant association between antibiotic usage and *Candida* infection (χ^2 =0.376, df= 3, p= 0.945).

The results were also analyzed in Table 4.14 according to presence of co morbid conditions and those without co morbid conditions. Though the prevalence of genitourinary candidiasis among patients with comorbid condition was only 8.5% however there was statistical significant relationship between the two variables (χ^2=14.757,df=3,p=0.002).Associated comorbid condition were shown in table 4.15 pelvic inflammatory disease accounted for 58.3% while vesicovaginal fistula was 41.7%.

Table 4.16 shows that patients on contraceptives had lower prevalence (20.7%) of genitourinary candidiasis compared to those who were not using contraceptives (79.3%). Patients on contraceptives had lower prevalence rate of *Candida albicans* infection (48.4%) compared to those who were not on contraceptives(50.4%).The difference observed in prevalence were not statistically significant.(χ^2=3.846,df=3, p=0.279)

Table 4.17 shows relationship between *Candida* species identified and medical implants. The prevalence for patients on medical implants was 37.7% while that for patients who were not on medical implants was 41.2%.There was no significant association observed between presence of medical implants and genitourinary Candidiasis (χ^2=0.23,p=0.631, df=1)

Table 4.13: Profile of patients on antibiotics before presentation with genitourinary candidiasis

Candida species	Antibiotics		Total
	Used	Not used	
Candida albicans	44(48.4)	35(48.6)	79(48.5)
Candida tropicalis	9(9.9)	9(12.5)	18(11.0)
Candia parapsilosis	29(31.9)	22(30.6)	51(31.3)
Candida glabrata	9(9.9)	6(8.3)	15(9.2)
Total	91(55.8)	72(44.2)	163(100)

χ^2=0.376, df= 3, p= 0.945
Figures in parentheses represent percentage

Table 4.14: Prevalence of organism isolated in relation to co morbid condition

***Candida* species**	**Comorbid condition**		**Total**
	Present	**Absent**	
Candida albicans	2(16.7)	67(51.5)	69(48.0)
Candida tropicalis	1(8.3)	11(8.5)	12(8.5)
Candida parapsilosis	4(33.3)	42(32.3)	46(32.4)
Candida glabrata	5(41.7)	10(7.7)	15(10.6)
Total	12(8.5)	130(91.5)	142(100)

χ^2=14.757,df=3,p=0.002.

Figures in parentheses represent percentage

Table 4.15: Associated co morbid condition

Comorbid condition	Frequency	percentage
Pelvic inflammatory disease	7	58.3
Vesicovaginal fistula	5	41.7
Total	12	100

Table 4.16: Contraceptive usage against species isolated among patients with genitourinary Candidiasis.

***Candida* species**	**Use of contraceptive**		**Total**
	Contraceptive	**No contraceptive**	
Candida albicans	15(48.4)	60(50.4)	75(50)
Candida tropicalis	4(12.9)	9(7.6)	13(8.7)
Candida parapsilosis	7(22.6)	41(34.5)	48(32)
Candida glabrata	5(16.1)	9(7.6)	14(9.3)
Total	31(20.7)	119(79.3)	150(100)

χ^2=3.846, df=3, p=0.279.

Figure in parentheses represent percentage.

.

Table 4.17: Relationship between *Candida* species isolated and medical implant

Implant	*Candida* species Isolated	No *Candida* species isolated	Total
Medical implant	20(37.7)	33(62.3)	53(13.3)
No Medical implant	143(41.2)	204(58.8)	347(86.7)
Total	163(40.8)	237(59.2)	400(100)

χ^2=0.23 p = 0.63, df=1

Table 4.18 shows that the *Candida* species were not resistant to any of the commonly used azole drugs and Amphotericin B. Ninety-eight (98.2) percent of the organisms were resistant to Flucytosine. The commonly used azoles for genitourinary Candidiasis were tested against the isolated *Candida* species. Sensitivity of isolated species to azole was 100%

Table 4.18: Antifungal Susceptibility testing of *Candida* isolates

Species	Fy		Amp		Mcl		Flu		Cl	
	S ≥20 mm	**R ≤11 mm**	**S ≥15 mm**	**R ≤10 mm**	**S ≥20 mm**	**R ≤11 mm**	**S ≥19 mm**	**R ≤14 mm**	**S ≥20 mm**	**R ≤11 mm**
Candida albicans	0	79	79	0	79	0	79	0	79	0
Candida tropicalis	1	17	18	0	18	0	18	0	18	0
Candida parapsilosis	1	50	51	0	51	0	51	0	51	0
Candida glabrata	1	14	15	0	15	0	15	0	15	0
Total	3	160	163	0	163	0	163	0	163	0

KEY

FY –Flucytosine

Amp – Amphotericin B

Mcl –Miconazole

Flu – Fluconazole.

Cl -Clotrimazole

CHAPTER FIVE

5.0 DISCUSSION

In this study, combination of colonial characteristics on Sabouraud Dextrose Agar and CHROMagar *Candida* was used for the identification of four *Candida* species from the study population. The outcome of the cultural characteristics was further corroborated with the findings of production of germ tube and the result of carbohydrate assimilation test. A prevalence rate of 40.8% was arrived at using the above method of identification and *Candida albicans* was found to be more prevalent. Whong *et al*. (2005) found a prevalence rate of 55% in Zaria, higher than the prevalence obtained in this study. This may be due to the number of patients they recruited for their own study which is smaller than what was used in this study. Enweani *et al*. (2001) reported a prevalence of 51.5% among patients using contraceptives and those without contraceptives (40.6%) in Edo State Nigeria. *Candida albicans* was also found to be more prevalent (89%) among *Candida* species in a survey carried out in United States of America. (Achkar and Fries, 2010). Other prevalences as documented by Achkar and Fries (2010) include: Italy – 77%, Austria – 88%, Turkey – 44% and India -47%. The result of this study is different from those of Okungbowa *et al*. (2003) who found *Candida glabrata* to be more predominant following a survey of symptomatic female subjects in seven cities in the southern part of Nigeria. The difference may be due to geographical location and cultural difference of the study population. A higher prevalence (80.95%) of *Candida* was also reported among referrals to the gyneacology centre in Babol, Iran by Esmaeilzadeh (2009).

A higher percentage of the *Candida* species isolated were germ tube negative. This signified that non *albicans Candida* are becoming more prevalent in contrast to the previous findings. Other

studies have also documented similar observation (Arzeni, 1997; Mohanty, 2007). These may be due to some conditions that lead to immunosuppression such as AIDS, malignancy, use of corticosteroid and Diabetes mellitus.

Age distribution of Candidiasis showed a higher prevalence of vulvovaginal candidiasis among the age group 21- 30years**.** This probably could be due to ovarian activity as well as sexual activity which peaks in women of 20-30 years of age. During this period, the ovary produces adequate amount of estrogen, which favours *Candida* growth by maintaining the acidic pH in the vagina and enhancing the yeast adherence to vaginal epithelial cells (Akortha *et al*., 2009; Adetunde *et al*.,2011). This also explains why there is lower prevalence at age 50years and above. The age distribution of genitourinary candidiasis observed in this work had a similar pattern with the findings of Okungbowa *et al.* (2003) in research carried out in southern part of Nigeria which showed highest incidence at age 26-30years. Nwadioha *et al.* (2010) also observed higher prevalence between age 21-30years in a research conducted in Jos.

Prevalence in relation to marital status showed a higher prevalence of genitourinary Candidiasis among married women compared to the single and the divorced subjects. Not much information is available with regards to prevalence of genitourinary candidiasis and marital status. The report by Eweani *et al.* (1987) as cited by Okungbowa *et al.* (2006), is in consonance with the outcome of this research which showed that there was no association between marital status and occurrence of genitourinary candidiasis. Though not significant, these results beg the idea of higher regular sexual activity among the married as well as the probability of psychological stress in marriage which could predispose to hormonal changes suited to the growth of *Candida*.

The prevalence with regards to educational status showed the highest prevalence among the patients who attained tertiary education, which is followed by the patients who attained secondary education. These are the people who are likely going to buy 'over the counter' (OTC) drugs especially antibiotics without consultation therefore predisposing them to recurrent genitourinary candidiasis. There may also be possibility of stress predisposing to vulvovaginal candidiasis. Overall no statistically significant relationship was found between educational status and genitourinary candidiasis. This is a subject for further study.

Distribution of genitourinary Candidiasis in relation to ethnic groups showed that minority ethnic groups had the highest prevalence, followed by Hausa, then Yoruba with the Igbo having the least prevalence. This difference may be due to the fact that the total number of the minority tribes that seek medical care may be more than the major ethnic groups. The Hausa-Fulani ethnic group that followed in prevalence may be as a result that the research was conducted in Zaria that is Hausa Fulani Dominated. Overall, there was no statistical relationship between genitourinary candidiasis and ethnicity from the outcome of this work.

The distribution of Candidiasis showed a higher prevalence among the unemployed individuals compared to students and the gainfully employed individuals. The higher prevalence among the unemployed individual may be due to low socioeconomic, status which may also affect the hygienic status of the individual. There was no significant association between the occurrence of genitourinary candidiasis and occupation of the patients. This agrees with the research conducted by Jombo *et al.* (2010) in Gboko where applicants had the highest prevalence of genitourinary candidiasis.

A high percentage of patients studied presented with vaginal discharge which is the commonest presentation of vulvovaginal candidiasis. This is in consonance with observation of Adetunde *et al.*,(2011). A significant association was observed between the vulvovaginal candidiasis and characteristic of the discharge.

In relation to species isolated and number of occurrence of vaginal discharge, no statistically significant association was observed but a significant percentage of the women reported one episode of vaginal candidiaisis per annum which agrees with the findings of Peng,(2008) and Achkar and Fries, (2010).

There was no statistically significant association found between use of antibiotics and genitourinary Candidiasis, although, patients that used antibiotics had higher prevalence of genitourinary candidiasis compared to those without antibiotics. Prolonged use of antibiotic therapy had been implicated as a predisposing factor to development of genitourinary candidiasis which have been documented by different authors (Prescott *et al*., 2008; Achkar and Fries., 2010; Umeh *et al.*, 2010) but this was not significantly substantiated by this work.

In relation to co-morbid condition and development of genitourinary candidiasis, a statistically significant association was observed. Even though the numbers of individuals with comorbid conditions were fewer than those without, the presence of these conditions played a significant role in promoting genitourinary candidiasis. Some of the patients had associated comorbid conditions such as Vesicovaginal Fistula, Pelvic inflammatory disease which may also be predisposing factors to genitourinary Candidiasis because these may alter the immune status of the patients. de Leon *et al.* (2002) observed an association between diabetes (co-morbid conditions) and genitourinary candidiasis, which is similar to our findings.

There was no statistically significant association between use of contraceptive and *Candida* species isolated; this may be due to fewer numbers of patients on contraceptives in this work. This result obtained on contraceptives and *Candida* isolates differs from that of previous study conducted by Eweani *et al*. (2010).

Medical implants such as Urethral catheter, IUDs have been implicated as predisposing factor to genitourinary candidiasis (Brooks, 2010; Peng, 2010). This research found no significant association between the genitourinary candidiasis and medical implant. This may be due to open drainage method of catheterization employed for patients on prolonged urethral catheter or possibility of regular changing of catheters.

In relation to antifungal susceptibility and *Candida* species isolated, most of the species isolated were susceptible to common azoles especially fluconazole. This implies there is no resistance to azoles yet and indicates that most of the current infection were due to re infection and not resistance to drugs. Sexual behaviour, poor personal hygiene, wrong diagnosis, use of inappropriate drugs could be predisposing factors. Kikani *et al.* (2010) observed 91.8% susceptibility to fluconazole using CLSI M44 disk diffusion method. This method is easier and less cumbersome compared to broth diffusion for routine laboratory use.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The result obtained from this study indicated a prevalence rate of 40.8% which still support the fact that genitounitourinary candidiasis occur frequently among women. There was an association observed between co- morbid condition as a factor and development of genitourinary Candidiasis. Association was also observed with vaginal discharge characteristics and genitourinary candidiasis. There was no association observed between use of medical implants and development of genitourinary candidiasis. This study observed an increase in non albicans *Candida* as a cause of genitourinary candidiasis which is an important factor due to their propensity to cause resistance to antifungal drugs especially azoles. The result of antifungal drug susceptibility pattern is encouraging due to the fact that most of the *Candida* species identified are still susceptible to commonly used antifungal drugs.

6.2 RECOMMENDATIONS

Infection with Candidiasis is a common occurrence among women and more especially sexually active women. It is important to reduce the occurrence and the discomfort they experience using the following strategies.

1. Indiscriminate use of antibiotics should be discouraged, since *Candida* infection was more prevalent among antibiotic users in this study though not significantly.

2. A Proper algorithm for diagnosis and treatment of genitourinary conditions should be adhered to so as to prevent growth and establishment of *Candida* infections

3. Young ladies should be counseled on the symptoms of candidiasis especially in relation to vaginal discharge so as to know that not all discharges are due to pathological conditions and to reduce antibiotic abuse.

4. There is need to counsel the health professional on the need for proper investigation of patients before prescription of drugs.

5. There should be a standard operational procedure on use of implants especially urethral catheters in hospital based patients to prevent development of complication which can favour candidiasis.

6. There is a need to create awareness with regards to increasing emergence of non albicans *Candida* and the propensity for antifungal drug resistance.

7. This study was hospital based, therefore population based studies should be carried out to reveal more information which could provide important insights to the dynamics of this infection.

REFERENCES

Achkar, J.M. and Fries, B.C. (2010). *Candida* Infections of the genitourinary tract. *Clinical Microbiology Reviews*,253 -273.

Adam, H.J., Richardson, S.E., Roscoe, M., Boroumandi, S., Gris, M. and Yau, Y.C.W. (2010). An Implementation Strategy for the Use of Chromogenic Media in the Rapid, Presumptive Identification of *Candida* Species. *The Open Mycology Journal***,** 4*:*33-38.

Adetunde, L.A., Glover, R.K.L. and Awo, O. (2011). The Investigation of *Candida albicans* infections among teenagers (between the ages of 13-19) reporting at the Tamale Teaching Hospital (TTH) with vaginal discharge problems. *International Journal of Research in Environmental Science and Technology*, 4: 47-51.

Akingbade OA, Akinjinmi AA, Awoderu OB, Okerentugba PO Okonko IO.(2013). Prevalence of *Candida albicans* Amongst Women Attending Health Centres In Abeokuta, Ogun State, Nigeria**.** *New York Science Journal* 6(9):53-59

Akortha EE, Nwaugo VO, Chikwe NO (2009).Antifungal resistance among *Candida* species from patients with genitourinary tract infection isolated in Benin City, Edo state, *Nigeria African Journal of Microbiology Research* 3(11) : 694-699 .

Anderson, M.R., Klink, K. and Cohrssen, A. (2004). Evaluation of vaginal complaints. *Journal of American Medical Association*, 291:1368–1379.

Araoye, M.O. (2004). Research Methodology with Statistics for Health and Social Sciences. 2004; Nathadex publishers,Ilorin, Pp118.

Arzeni, D., Del Poeta, M., Simonetti, O., Offidani, A.M., Lomura, L., and Balducci, M. *et al.*, (1997). Prevalence and antifungal susceptibility of vaginal yeasts in outpatients attending a gynecological center in Ancona, ltaly. *European Journal of Epidemiology*, 13(4): 447-450.

Bankar, S.M., Powar, R.M., Patil, S.A. and Kalthur, S.G. (2012). Prevalence of non-albican *Candida* infection in Maharashtrian women with leucorrhea. *Annal of Tropical Medicine Public Health*, 5:119-23.

Barnett, J.A. **(**2008**)** A history of research on yeasts 12: medical yeasts part 1, *Candida albicans. Yeast*, 25: 385–417.

Brooks, G.F., Carroll, K.C., Butel, J.S., Morse, S.A. and Mietzner, T.A. (2010). Jawetz, Melnick & Adelberg's Medical Microbiology. 25th ed: Pp647-648 McGraw-Hill companies, New York.

Calderone, R.A. (2002). Candida and Candidiasis, ASM Press, Washington, DC. Pp2-35

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. Part 2, Pp243-244, Cambridge University press,UK.

Cook, G.C. and Zumla, A. (2003). Manson's Tropical Diseases. 21st edition,1176-1177.Saunder,Elsevier Science, London

Cooke, V.M., Miles, R.J., Price, R.G., Midgley, G., Khamri, W. and Richardson, A.C. (2002). New Chromogenic Agar Medium for the Identification of *Candida* spp. *Applied and Environmental Microbiology*, 68(7):3622–3627.

de Leon, E.M., Jacober., S.J., Sobel, J. and Foxman, B. (2002). Prevalence and risk factors for vaginal *Candida* colonization in women with Type 1 and Type 2 diabetes. *BMC Infectious Diseases* 2:1-6.

de Repentgny, L., Lewanclowski,D. and Jolicoeur,P. (2004). Immunopathogenesis of oropharyngeal candidiasis in human immunodeficiency. *Clinical Microbiology Review*, **17**:729-759

Dey, N.C., Grueber, H.L.E., and Dey, T.K. (2006) Medical Mycology. pp 156-169.New Central Book agency (P) limited,India.

Edward, J.E. (2010). *Candida* species. In: Mandell, Douglas, and Bennet's Principles and Practice of infectious diseases.(Ed :Mandell GL, Bennett JE, Dolin R.).7th edition ,3225-3239.

Ellepola, A.N.B. and Morrison, C.J. (2005). Laboratory Diagnosis of invasive candidosis. *Journal of Microbiology*, 43:65-84.

Enweani, I.B., Gugnani, H.C., Okobia, R. and Ojo, S.B. (2001). Effect of contraceptives on the prevalence of vaginal colonization with *Candida* species in Edo State, Nigeria. *Review of Iberoamerican Micology*, 18: 171-173.

Enweani, I.B., Ogbonna, C.I. and Kozak, W. (1987). The incidence of candidiasis amongst the asymptomatic female students of the University of Jos, Nigeria. *Mycopathologia*, 11:135-141.

Esmaeilzadeh, S., Omran, S.M. and Rahmani, Z. (2009). Frequency and Etiology of Vulvovaginal Candidiasis in Women Referred to a Gynecological Center in Babol, lran. *International Journal of Fertility and Sterility*, 3(2):74-77.

Federal Government of Nigeria official gazette (FGP71/52007/2500(OL-24):Legal notice on publication of details of the break of national and state provisionals

Forbes, B.A., Sahm D.F. and Weissfeld, A.S.(2007). Bailey and Scott's Diagnostic Microbiology, 12th edition.629-713

Geiger, A.M., Foxnan, B. and Gillespie, B.W. (1995). The Epidemiology of Vulvovaginal Candidiasis among University Students. *American Journal Health*, 85: 1146-1148.

Greenwood, D., Slack, D., Peutherer, J. and Barer, M. (2007). A Guide to Microbial Infections; Pathogenesis, Immunity, Laboratory Diagnosis and Control, 17th edition.602-603.

Grunberg, E., Titsworth, E. and Bennett, M. (1963). Chemotherapeutic activity of 5-fluorocytosine. In: *Antimicrobial Agents Chemotherapeutic,* P. 566. University of Michigan Press, Ann Arbor.

Howell, S.A. and Hazen, K.C. (2011). Cryptococcus, and other yeast of medical importance in Manual of clinical Microbiology.(Eds : Versalovic J, Carroll KC. Funke G, Jorgensen JH., Landry ML. Warnock DW),10th edition, 2:1793-1821.ASM Publishers,Washington DC.

http://www.merckmanuals.com/professional/dermatologic_disorders/fungal_skin_infections/candidiasis_mucocutaneous.html.accessed 15/9/12

Inabo, H.I. (2006). The significance of *Candida* infections of medical implants. *Scientific Research and Essays*, **1**(1): 008-010.

Jombo, G.T.A., Akpera, M.T., Adebisi, S.I. and Bolarin, D.M. (2010). Symptomatic urogenital candidiasis and therapeutic management on women taking oral contraceptive pills. *Journal of Microbiology and Antimicrobials,* 2(9):118-122.

Jose, A. and Hidalgo, J.A.,(2012) Candidiasis; http://emedicine.medscape.com/article/213853-overview#a0199.Accessed 15/9/12

Kikani, K.M., Koshi, P.J., Mehta, S.J., Kikani, B.A., Aring, B.J. and Kamothi, M.N. (2010). Species Distribution and Antifungal Susceptibility Pattern in the cases of Vaginal Candidiasis in Saurashtra Region of Gujarat. *Electronic Journal of Pharmacology and Therapy*, 3: 9-12.

Kojic, E.M and Darouiche R.O. (2004) *Candida* Infections of Medical Devices. *Clinical Microbiology Reviews,* 17(2):255–267

Mandell,G.L, Bennett,J.E, Dolin, R.(2010). Principles and Practice of infectious diseases.7th edition, Churchill Livingstone Elsevier,Philadelphia,United State. 3225-3239.

Marot-Leblond, A., Nail –Billaud, S., Pilon, F., Beucher, B. and Poulain, D. (2009). Efficient Diagnosis of vulvovaginal candidiasis by use of Rapid immunochromatography test. *Journal of Clinical Microbiology*, 47(12):3821-3825.

Mitchell, T.G. (2010) in Jawetz,Melnick,and Adelberg's Medical Microbiology,25th edition(Ed:Brooks GF, Butel JS, Morse SA).625 -648.

Mirela,B and Mirsada, H (2010) *Candida albicans* and *non-albicans* species as etiological agent of vaginitis in pregnant and non pregnant women. *Bosnian Journal of Basic Medical Sciences*; 10 (1): 89-97.

Mohanty, S., Xess, I., Hasan, F., Kapil, A., Mittal, S. and Tolosa, J.E. (2007). Prevalence and susceptibility to *fluconazole* of *Candida* species causing vulvovaginitis. *India Journal of Medical Research*, 126(3): 216-219.

Nadeem, S.G., Hakim, S.T. and Kazmi, S.U. (2010). Use of CHROMAgar *Candida* for the presumptive identification of *Candida* species directly from clinical specimens in resource poor setting. *Libyan Journal of Medicine,* 5**:** 2144 - 2150

Namkinga,L.C.(2013).Diversity of pathogenic Candida species colonizing women with and without Candida vaginitis in Dares salaam region,Tanzanian;Journal of Biology and lifescience,4(1):153-170

Novkora, N., Yassievich, E. and Mardh, P. (2002). Microscopy of stained smears of Vaginal Secretion in the diagnosis of recurrent vulvovaginal candidosis. *International Journal of STD and AIDS*, 318-322.

Nwadioha, S.I., Egah, D.Z., Alao, O.O. and Iheanacho, E. (2010). Risk factors for vaginal Candidiasis among women attending primary health care centers of Jos, Nigeria. *Journal of Clinical Medicine and Research*, 2(7):110-113.

Odds, F.C and Bernaert R.(1994) Chromagar Candida, a new differential isolation medium for presumptive identification clinically important Candida species. *Journal of .Clinical Microbiology* 32:1923 – 1929.

Okungbowa, F.I., Isuehuemhen, O.S. and Dede, A. (2003). The distribution frequency of *Candida* species in the genitourinary tract among symptomatic individuals in Nigeria cities. *Revista. Iberoamericana de Micol*ogia, 20:60-63.

Okungbowa, F.I, Dede, PA, Isikhuemhen, OS and Okungbowa, M.O. (2006). Age and Marital Distributions of Genitourinary Candidiasis among Symptomatic Women in Nigeria. *Medical Journal of Islamic World Academy of Sciences*, 16(2):67-69.

Peng, N.L. (2008). Management of recurrent vulvovaginal Candidiasis. *Singhealth Polyclinic-Tampine,* 34(1): 61-68.

Prescott, Harley, and Klein's Microbiology 2008 7th ed.(Eds.Willey JM, Sherwood LM, Woolverton CJ).1017-1018.McGraw-Hill companies, New York.

Rylander, E., Berglund, A.L., Krassny, C. and Petrini, B. (2004).Vulvovaginal *Candida* in a young sexually active population: prevalence and association with oro-genital sex and frequent pain at intercourse. *Sexually Transmitted Infection,* 80:54–57.

Sobel, J.D and Chaim, W. (1996).Vaginal Microbiology of Women with Acute Recurrent Vulvovaginal Candidiasis. *Journal of clinical microbiology*, 2497–2499

Umeh, E.U., Umeakanne (2010). HIV/Vaginal *Candida* coinfection. *Journal of microbiology and Antimicrobial*, 2(3):30-35.

Vandeputte, P.T. and Gold, B.W. (1954). Amphotericin B, its production and its salts. Retrieved on 13th September, 2013 from www.google.com/patent/us2908611#forward-citations.

Willinger, B and Manafi M. (1999).Evaluation of Chromagar *Candida* for rapid screening of clinical specimens for Candida species. *Mycoses;* 61 – 65.

Winn, W., Jr., Allen, S., Koneman, P.G., Schreckenberger, P. and Woods, G. (2006). Koneman's Color Atlas and Textbook of Diagnostic Microbiology.6Th Edition; Pp1219-1223.Lippincott William & Wilkins,Philadelphia.

Whong, C.M.Z., Lawong, M.M., Ekpo, G.U. and Shittu, S.O.(2005)Prevalence of *Candida* species among women in Zaria. *Nigerian Journal of Pharmaceutical Sciences*, 6(1):57-62.

Appendices

Appendix I: Informed Written Consent

I……

……..of……

…….. (Address). Agree to participate in the study of prevalence of genitourinary candidiasis among patients with or without medical implant attending gynaecology clinic in Zaria. I understand that a sample of my urine and vaginal swab will be taken for the test and if I so wish, the results will be communicated to me in confidence.

I make this consent willfully without being subjected to any pressure.

Participant's Name: …… Signature: ……..

Witness Name: …… Signature ……

Researcher's Name: …….. Signature ……..

Appendix II: Structured Questionnaire

Please fill in the blank space and tick the correct option appropriately.

1.0 . SOCIO- DEMOGRAPHIC DATA

1.1 AGE: ,(≤20),(21-30),(31-40),(41 -50),(51-60),>61

1.2 Marital Status; Single ( ) Married( ) Divorce ( )

1.3 Family setting; Monogamy ( ) Polygamy ( )

1.4 If married, a .1st husband ( ),b.2nd husband,c.3rd husband. d. husband>4

1.5 Educational Status: primary ( ) Secondary ( ) Tertiary ( ) Quranic ( ) None ( )

1.6 Ethnicity: Hausa-Fulani ( ) Igbo ( ) Yoruba ( ) Others ( )

1.7 Occupation: unemployed ( ) gainfully employed ( ),student()

2.0 VAGINAL SYMPTOMS:

2.1 Have you ever had vaginal discharge? Yes ( ) NO ( )

2.2 If yes, how many times per annum? Once ( ) twice ( ) thrice ( ), ≥ 4 ( )

2.3 Characteristics of the discharge include; whitish and curd like, ( ), greenish ( ), Yellowish ( )

2.4 Other associated symptoms include; severe itching ( ), abdominal pain ( ), fever ( )

2.5 Drug was prescribed by a. Doctor ( ) b. Chemist ( ) C. Traditional healer ( ), d. Nurse ( ), e. Self ( )

2.6 Specify the name of the drug given……...

2.7 Is there any co-morbid condition? If yes, state the condition.

2.8 Are you on regular medication for the co morbid condition? If yes, state the drugs.

3.0 OTHER RISK FACTORS

3.1 Are you on any contraceptive? (Yes or No) If yes, state the type.

3.2 For how long?

3.3 For how long have you been on urethral catheter?a.48-72hr.b.1wk C.2wks d.>1mth

3.4 How often do they change it? a.24-72hrs,b.1wk,c.2wks,d.3wks e.>4wks

3.5 Are you on antibiotics? (Yes or no) If yes, for how long? ……

4. Does this condition affect your relationship with your spouse/partner? Yes or No.

4.1 If

yes,……..

……

……..…….

Appendix III: Summary of Distribution of Total Study Population

VARIABLES	Frequency	PERCENTAGE (%)
Age (Years)		
<20	64	16
21-30	196	49
31-40	111	27.8
41-50	26	6.5
51-60	2	0 .5
>61	1	0 .3
Marital status		
Single	121	30.3
Married	274	68.5
Divorced	5	1.3
Educational status		
Primary	50	12.6
Secondary	124	31.3
Tertiary	129	32.6
None	28	7.1
Ethnicity		
Hausa-fulani	144	36
Igbo	36	9
Yoruba	71	17.8
Others	149	37.3
Occupation		
Unemployed	208	52
Gainfully employed	58	14.5

Student	134	33.5
Symptoms		
Vaginal discharge	383	95.8
No discharge	17	4.2
Occurrence\annum		
Once	98	25.6
Twice	129	33.7
Thrice	87	22.7
>4times	69	18.0
Other Symptoms		
Severe itching	324	85.5
Abdominal pain	53	14.0
Fever	2	0.5
Use of antibiotic		
Antibiotic	234	58.5
no antibiotic	166	41.5
Comorbid condition		
Present	25	7.4
Absent	314	92.6
Contraception		
Use contraceptive	**71**	**19.5**
No contraceptive	294	80.5
Types of contraceptive		
Injectibles	4	6.3
Hormonal pills	33	52.4
IUD	26	41.3

***Candida* isolated**		
Growth occur	163	40.8
No growth	237	59.2
Germ Tube Test		
Positive	79	48.5
Negative	84	51.5
Species isolated		
Candida albican	79	48.5
Candida parapsilosis	51	31.3
Candida tropicalis	18	11
Candida glabrata	15	9.2
Antifungal susceptibility		
Fluconazole		

Appendix IV: Ethical approval