OCCURRENCE OF *Candida* SPECIES ASSOCIATED WITH VULVOVAGINITIS AMONG PREGNANT WOMEN AND THEIR SUSCEPTIBILITY TO ANTI-FUNGAL AGENTS IN YOLA, NIGERIA

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JUNE, 2014

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THEIR SUSCEPTIBILITY TO ANTI-FUNGAL AGENTS IN YOLA, NIGERIA

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BEING A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, SCHOOL PURE AND APPLIED SCIENCES, MODIBBO ADAMA UNIVERSITY OF TECHNOLOGY, YOLA IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF M.TECH DEGREE IN MEDICAL MICROBIOLOGY.

JUNE, 2014

DECLARATION

I hereby declare that this thesis was written by me and it is a record of my own research work. It has not been presented before in any previous application for a higher degree. All references cited have been duly acknowledged.

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Signature

DEDICATION

This project is dedicated to my entire family and to all who contributed to this research.

APPROVAL PAGE

This thesis entitled "Occurrence of *Candida* species associated with Vulvovaginitis among pregnant women and their susceptibility to anti-Fungal agents in Yola, Nigeria." meets the regulations governing the award of Masters of Technology of the Modibbo Adama University of Technology, Yola and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Two hundred and seventy three high vaginal swab samples from pregnant women were studied for the presence of *Candida* species and their antifungal susceptibilities. The samples were subjected to both microscopic, cultural identification and biochemical test. Bromocresol green agar was used to identify *Candida* species. Culture of the high vaginal swab led to isolation of 105 (38.46 %) yeast cells. *Candida albicans* was the predominant species isolated (81.90 %), this was followed by *Candida tropicalis*

(15.24 %) and then *Candida krusie* (2.86 %). There was a high occurrence of *Candida* (25.64 %) in the age group, 21-30 years. The study shows that the percentage of women in the area with a second vaginal infection due to *Candida* species was 39.05 %. About 11.43 % had *Candida* infection in the first trimester, 48.57 % showed infection in second trimester while 40.00 % were infected in the third trimester of pregnancy. Seventy five point two four percent of the infected women were multigravidae. The percentage of women who experienced vaginal discharge was 67.62 %. Out of 105 of the infected women, 65.71 % used antibiotic before pregnancy while 24.76 % of the infected women were using oral contraceptives. Only 36.20 % of the *Candida* isolates were susceptible to all the antifungal drugs. Ketoconazole was active against 59.62 % isolates, Itraconazole was active against 53.33 % and Fluconazole was active against 47.62 % of the *Candida* isolates. Ketoconazole and Itraconozole showed the lowest MIC at 0.125 μg/ml for 50 isolates and 39 isolates of *Candida albicans* respectively. It is recommended that pregnant women should be educated on symptoms and dangers of infection with *Candida* species. As well as proper diagnosis and antibiotic susceptibility screening should be carried out to prevent wrong diagnosis and ensure effective treatment.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Many species of *Candida* are endosymbionts of animal host including humans, while others are commensals. Yet other species causes infection in human called candidiasis, especially in immuno-compromised patients (Douglas, 1988).

Candida albicans inhabit the oral, vaginal mucosa and gastrointestinal tract of human beings as one of the commensal organisms. It causes opportunistic infections in immuno-compromised patients producing allergic reactions and causes morbidity and mortality (Douglas, 1988).

Vulvovaginal candidiasis (VVC) is a common gynecologic ailment affecting three out of every four women in their life times. More than 40 % of affected women will have two or more VVC episodes with more infection frequently among pregnant women. The high oestrogen level and higher glycogen content in the vaginal secretion during pregnancy increases the risk of infection (Derrick and Adrienne, 2009). Symptoms include vaginal itching, white curdy discharge, swollen vulva and vagina and pain during sex (Parveen *et al.,* 2008)

Vaginal candidiasis cuts across different age group in any society whether or not a person is sexually active; though it is treatable and mild, when left untreated it is a possible risk for complications such as pelvic inflammatory disease, infertility, ectopic pregnancy, pelvic abscess, menstrual disorders, spontaneous abortion and premature birth (Nwadioha *et al.,* 2010). According to Parveen *et al.,* (2008), risks factors includes vagina douching, using tight clothing, broad spectrum antibiotics, oral and intrauterine contraceptives usage.

Various studies have revealed the incidence of vulvovaginal candidiasis prevalence among pregnant women. In Nigeria, Nwadioha *et al., (*2010) recorded 40 % prevalence in Jos, among pregnant women, Akah *et al.,* (2010) recorded 62.2 % in Enugu while Oyelewo *et al.,* (2013) recorded 70 % in Minna. Incidence of 48 % was recorded among pregnant women in Pakistan (Aslam *et al.,* 2008) and 12.5 % in United States (Akinbiye *et al.,* 2010). Infection can be transmitted from the vagina of an infected mother to the child during delivery hence given rise to congenital infection. Infants with oral thrush on the other hand give rise to nipple rash in breast feeding mothers with recurrent infection, (Omar, 2001).

In time past *Candida albicans* was the *Candida* species that held the most clinical attention, recently however infections caused by other *Candida* species such as *Candida tropicalis, Candida glabrata* and *Candida krusei* are on the rise.

Resistance to antifungal agents by these organisms necessitates the monitoring of the susceptibility of isolates from patients so as to determine strains with decreased susceptibility, that patterns are responsible for the morbidity and mortality of patients (Negri *et al.,* 2009).

1.2 Statement of the Problem

The yeast *Candida albicans* colonize the vaginal mucosa and gastro-intestinal tract of human causing opportunistic infection in immuno compromised patients and women of child bearing age (Douglas, 1988). *Candida* can be transmitted from an infected mother to the child through the vagina during delivery. Furthermore, an infant with oral thrush can cause nipple rash to the mother during breast feeding. Infection with *Candida* could lead to abortion and preterm birth in mothers with recurrent infection. In recent time, however, some strains have developed resistance to antifungal agents leading to treatment failure. Hence the need to determine the occurrence of *Candida* species associated with vulvovaginitis and their susceptibility to antifungal agents among pregnant women in Yola, Adamawa State Nigeria.

1.3 Aim of the Study

To investigate occurrence of *Candida* species associated with vulvovaginitis among pregnant women in Yola and determine their susceptibility to antifungal agents Nigeria.

1.4 Objectives of the Study

(i) To isolate and identify the *Candida* species associated with vulvovaginitis among pregnant women in Yola.

(ii) To determine the relationship of *Candida* vulvovaginitis to age, trimester of pregnancy and other factors in the affected women.

(iii) To determine the susceptibility of the isolate to antifungal agents.

1.5 Significance of the Study

This study will provide data on the prevalence of *Candida* vulvovaginitis in the study area as well as the spectrum of antifungal agents that can be used for treatment for epidemiological purposes and in treatment of the infection to mitigate its effect on the affected women.

1.6 Scope of the Study

Due to limited factors such as time and financial constraints, beyond the control of the researcher, the study will be limited to *Candida* vulvovaginitis among pregnant women attending three antenatal clinics in the study area. Federal

Medical Centre, Yola, Adamawa State Specialist Hospital, Yola and Girei Local Government Clinic Sangere.

1.7 Limitation of the Study

(i) This study was aimed at covering three hospitals under the study area in spite of many Hospitals within the study area, due to time and financial constraints.

(ii) Due to ethical reasons, specimen will be collected only from women who agree to participate in the study.

CHAPTER TWO

2.0 LITERATURE REVIEW

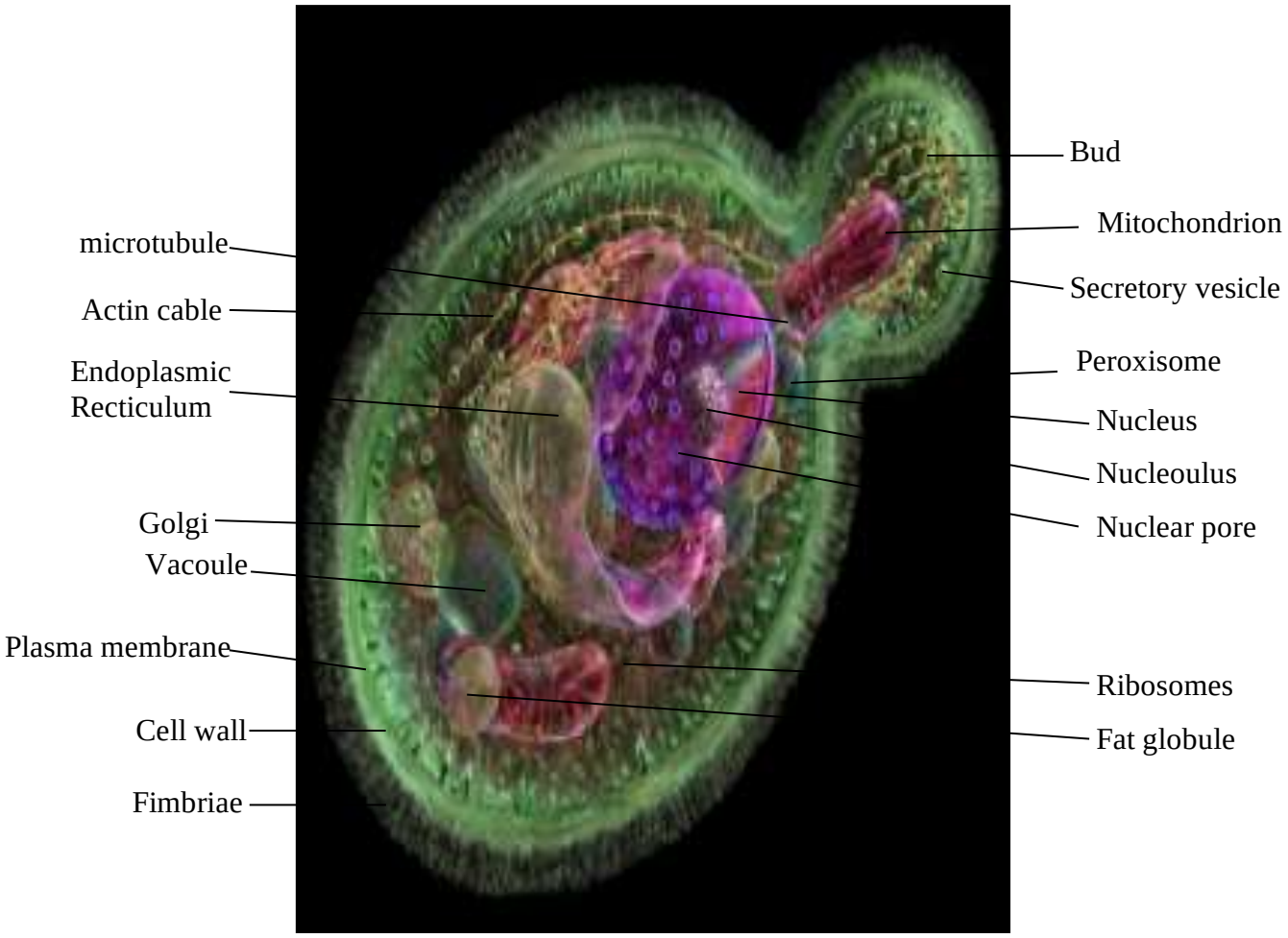
2.1 Vulvovaginitis

Vulvoginitis refers to the inflammation of the virginal and vulva. According to Omnia (2009), vulvovaginitis is the most common gyneacologic condition seen by practitioners rendering primary care to women. Traditionally, the three classical entities of vaginitis include Bacteria vaginosis, trichomonas infection and candidiasis. During the reproductive years of women, the vagina maintains a moist environment that fluctuates constantly. The secretion of an alkaline transudate from the vaginal epithelium and cervical gland maintains a pH ranging from 3.8 – 4.5. Its microflora forms a unique balance environment that changes under pressure from external stimuli but returns to normal when the stimuli are removed. These changes vary in degree during menstrual cycle, pregnancy and sexual activities. The vaginal epithelium consist of three cell layers; superficial, intermediate and basal, capable of storing glycogen under the influence of oestrogen.

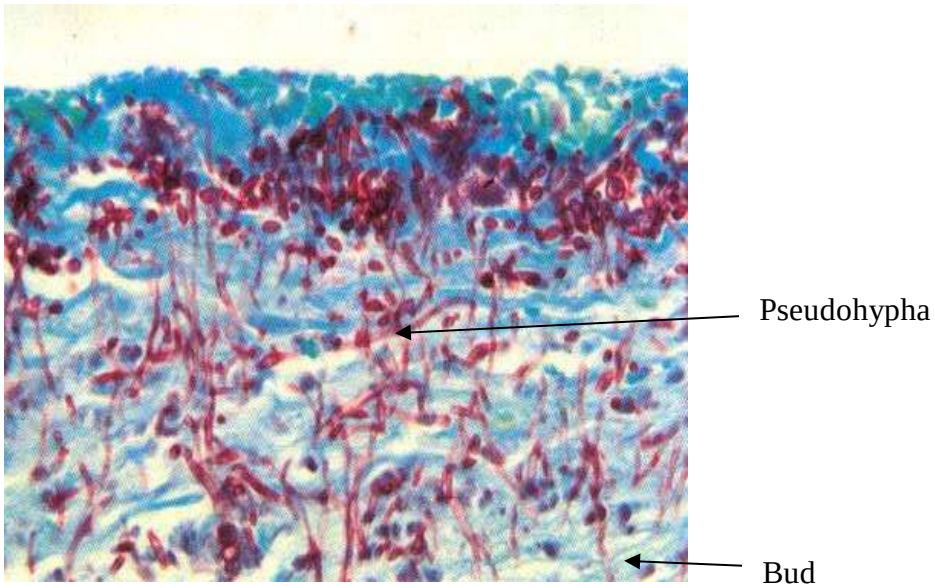
Glycogen is available in fully matured cells in the superficial layer of the epithelium. Glycogen storage thickens the epithelium when exogenous or endogenous oestrogen level is elevated, while the layers become thin when the oestrogen level diminishes (Omnia, 2009). In women of reproductive age, a healthy vagina contains both aerobic and anaerobic gram-positive and gram negative bacteria. *Lactobacillus* and *Corynebacterium* are predominant over *Streptococcus*, *Bacteroides*, *Staphylococcus* and *Peptostreptococcus*. *Lactobacillus* and *Corynebacterium* produce lactic acid and acetic acid from glycogen to help maintain the low vaginal pH.Other bacteria are kept in check by these acid producing bacteria and are rarely pathogenic. However, they may become pathogenic if environmental balance is affected. The skin of the vulva is sensitive to the vaginal environment and hormonal, metabolic and allergic influences. (Omnia, 2009).

2.2 Vulvovaginal Candidiasis

Vulvovaginal candidiasis is the second most common cause of vaginal symptoms after bacteria vaginosis. The infection affects both the vaginal and the vulva or external genitalia (Nester *et al.,* 2007). *Candida albicans* is responsible for vulvovaginal candidiasis (BCCDC., 2010).



(A) Budding yeast stage of *Candida albicans*



(B) Pseudohypha stage

Plate 1: Morphological Stages *Candida albicans* (Russell, 2012)

Candida is a gram positive, oval, budding yeast cell (Plate 1) that produces pseudohyphae both in culture and in tissue exudates. It is a member of the normal flora

of the mucous membranes. It is found in the gastrointestinal, upper respiratory and female genital tracts (Jha *et al.,* 2006).

Candida is a genus of yeasts, many of the species of the genus are endosymbionts of animal hosts including humans, while some are commensals and others have potential to cause diseases. The most significant member of the genus is *Candida albicans*, which can cause infections known as candidiasis or thrush in human and other animals particularly in immuno-compromised patients (Douglas, 1988). Yumi *et al.,* (2008) states that *Candida albicans* is a commensal yeast of the normal oral microbiota. Yet it can be predispose to develop into candidiasis due to several local and systemic factors. Candidiasis may be divided into the following types, oral candidiasis, (thrush), *Candida* vulvovaginitis, *Candida* intertrigo, diaper candidiasis, congenital cutaneous candidiasis, perianal candidiasis, *Candida* paronychia, candidiasis systemic and antibiotic candidiasis to mention but a few. Candidiasis is usually a localized infection of the skin or mucosal membranes:the pharynx, esophagus, gastrointestinal tract, the urinary bladder or genitalia (vagina and penis) (Walsh and Dixon, 1996).

2.3 Immunidogical and antigenic determinants for the pathology of infection

Symtomatic Candidiasis occurs following perturbation for loss of local defence mechanisms. The virulence factor associated with vagina Candidiasis includes adhensins, yeast to hypha transition and phenotype switching, antigenic variation and the production of enzyme especially the secreted aspartic proteinase (Sap) family (Kumomato and Vinces, 2005; Cassone *et al.,* 2007). The vaginal epithelian cell provides mechanical barrier and acts as sentinels that recognizes and process antigens and also secretes immune mediators (chemokines, cytokines and defensive peptids, these orchestrates innate immunity and regulates adaptive immunity, these are under the control of sex hormone. The epithelian cell posses anticandidal activity which is under the control of oestrogen. Oestrogen modifies the vaginal tissue and decreases the inhibitory potential of epithelian cells. It thickens the epithelian cells and keratinize it. This provide the fungus with substrete for attachment growth and biofilms formation and the production of secretory aspartic proteinase (Sap). Oestrogen also modulate cell immunity and reduce leukocyte infiltration, this favour *Candida* species and infection (Chandra *et al.,* 2001; Cassone *et al.,* 2007).

The mannose binding lectin (MBL) reduces vaginal level of polymorphism of the IL-4 (T-589) gene co-relates with high prevalence of recurrent vulvovaginitis. MBL

enhances complement activation to bind with *Candida albicans* and provides protection. Recurrent vulvovaginal Candidiasis has been associated with decreased in vivo concentration of mannose binding lectin and an increase interleukin – 4 (IL-4). Women with recurrent infection have higher variant of MBL gene. IL – 4 blocks the anti Candida response mediated by macrophage, hence and increase level of IL – 4 leads to inhibition of local defence mechanism (Omnia, 2009).

Polymorphonuclear cells (PMN) such as neutrophiles are present in the genital track and epithelian cell may remove fungal cells from the vaginal cavity PMN infiltration into the vaginal tissue when intensity correlates with fungus growth (Cassone *et al.,* 2007). Germ tubes are the precursors of hyphal form on the vaginal epithelium few hours after intravaginal challenge. Adhensin plays both direct and indirect role to the pathogenesis. The direct role is by fostering adherence to the vagina tissue and indirect role by exerting cell wall remodeling needed for hypha transition. The protein glycosylphosphatidylinosin anchored protein or β-glucanase enzyme adhensin, Sap and hypha formation seems interconnected in the Vagina environment.

The transition from hypha to commensal yeast cells to antigenic structural variation in the cell wall, avoidance of phagocyte, internalization, killing and perturbation in dendritic cell (Hoyer, 2001). The transglucosidage enzyme MP65 is a target of the human cell mediated immune response against *Candida*. The pathogenity is associated with the cell hyphal cell may be rightly impringe on multiple and redundant cell. Adhensin encoding genes are expressed during hypha formation. Dimorphic switching is associated with hypha elongation (Kumamoto and Vinces, 2005). Plate 1a, and b shows the morphology of *Candida albicans*, the budding or yeast stage and pseudohypha stages. High level of secreted aspartic proteinas (Sap) and the presence of immune deficiency virus is associated with vagina Candidiasis. An inhibitor of Sap e.g. pepstartin A, however is degraded by Sap 2 and immunodeficiency virus (Cassone *et al.,* 1987).

Antibiodies play a vital role by neutraling the virulence factor. Anti-Sap 2 FC-free domain antibodies (d-Abs) which cannot relay on help from host immune effector inhabits Sap activities and adherence of fungal to the vagina tissue (De Bernadis *et al.,* 2007). Anti- β mannose antibodies are protective, while α mannose are non protective configuration dependent epitope. Selective binding pattern may have opposite or compacting effects. The presence of protective epitopes on abundant cell molecule such

as the mannans, favours the fungus by helping it to avoid eradication by Candicidal antibodies (Cassone, *et al.,* 2007).

Non-Toll like receptors also play critical roles in fungal pathogen recognition dectin -1 for instance recognize molecular patterns common to all fungal β – glucan (Brown and Gordon, 2005). Others however bind to specific *Candida* Components example β – 1, 2 – mannosides, which are mannan-type constituent present exclusively in pathogenic *Candida* Species are recognize by the S-lectin galectin – 3 which cooperates with Toll-like receptor 2 for signaling galectin -3 binding may directly cause the death of the fungal. This anti *Candida* defence play a vital role in the epithelian cell as well as on macrophages (Joault *et al.,* 2006). T-cells have been quantified there are about 240 T hymphocytes per mm^2 of vaginal epithelia tissue CD 8^+ T cells are more than CD 4^+ T cells and the ratio is 1:25. CD 4^+ T cell appear to be abundant in the transition zone between the exocervix and endocervix little more than 100 CD 8^+ and a less than 100 CD 4^+ T-cell per mm^2 of vagina tissue are found in normal women. Majority of the T-cell have a memory C D $45RO^+$ phenotype. Most T cells migrate to the vaginal epithelium in response to a local antigenic stimuli or inflammation. (Cassone *et al.*, 2007)

Nabhan (2006) stressed that though vulvovaginal candidiasis is a non notifiable disease, it has been excluded from the rank of sexually transmitted diseases. Patients of *Candida* have over the years been categorized into two groups asymptomatic carriers of *Candida* (colonization) and symptomatic disease (*Candida* vaginitis). Recently however, vulvovaginal candidiasis has replaced these distinct categories. It is now used to emphasize the vulva dominant component of the symptomatic infection.

2.4 Etiology

Vulvovaginal candidiasis is caused by *Candida albicans,* a yeast that is part of the normal flora of the vagina. Since *Candida albicans* is a fungus, it has a eukaryotic cell structure (Nester *et al.,* 2007) Vulvovaginal candidiasis can be an acute, chronic, recurrent or persistent condition that can involve the vulva, vagina and adjacent areas. An estimated 10 – 50 % of reproductive aged American women are considered opportunistic carriers. *Candida albicans* is identified approximately 85 – 90 % of the time. In recent time other species such as *Candida glabrata, Candida tropicalis* and *Candida krusei* have been reported. This emergence may be associated with the

widespread use of over the counter drugs, long term use of suppressive azole and frequent use of short courses of antifungi drugs (Omnia, 2009).

2.5 Symptoms

Vulvovaginal candidiasis is accompanied by symptoms such as extreme itching, swollen vulva, painful urination, thick white vaginal discharge, pain during sex, redness of the vagina, (Nester *et al.,* 2007). Incubation period is unknown but generally ranges from three to ten days when associated with antibiotics; though in most cases no symptom occurs.

2.6 Predisposing Factors

During pregnancy, the body undergoes several changes, one of which is vaginal discharge known as leucorrhea a thin white or milky and mild smelling. However, abnormal discharges during pregnancy leading to candidiasis may be due to predisposing factors such as the use of contraceptives (Aslam *et al.,* 2008). Nabhan (2006) found that infection is highest in women that use oral contraceptives, especially those with high oestrogen content. The use of diaphragm, spermicidal, contraceptives sponge, and intra uterine contraceptive also posses risk due to vaginal colonization.

2.6.1 Age

Age is a major risk factor in *Candida* yeast infection. According to Nabhan (2006), infection is extremely rare before menarche but increases towards the second decade of life and picks over the next two decades. The rate of sexual activeness increases vaginal secretion and may be associated with amount of signed infection in reproductive ages. High prevalence of *Candida* infection was observed in women in the age range less than 40 years this may be due to the sexual activeness of the age group and is more prevalent in married women than non-married women (Faraji *et al.,* 2012). Feyi-Wabosom and Amadi (2001) associated it to longer sexual history.

2.6.2 Hormonal Influence

Vulvovaginal candidiasis is more common and difficult to eradiate during pregnancy. Sheary (2005) stated that high oestrogen levels make women more susceptible to vulvovaginal candidiasis as seen in pregnant women and postmenopausal women on hormone therapy. Oestrogen increases the glycogen content of the vagina and has a direct effect on *Candida* growth and increases its adherence to the vaginal epithelium.

2.6.3 Contraceptives

Numerous studies have found a significantly high risk of VVC in women who use oral contraceptives. Contraceptives with high oestrogen content pose a greater risk of infection than those with low oestrogen content. Furthermore, the use of diaphragm and spermicidals increase the rate of *Candida* colonization alongside with other contraceptives such as sponge and intrauterine contraceptive device (Sobel *et al.,* 1998; Nabhan, 2006). Oral birth control pills mostly contain the hormone oestrogen. Supplemental oestrogen in the synthetic form has been found to promote the growth of yeast. Hormones could affect intestinal bacteria. The use of copper intrauterine device (IUD) leads to the development of excessive copper in the tissue of women who use it, it therefore helps to promote yeast growth. Excess copper can depress the adrenal, thyroid and immune systems of the body. This makes it more difficult for the body to resist yeast infection. However, with the development of newer oral contraceptives with lower oestrogen dose, users are not likely to be pre-dispose to *Candida vulvovaginitis* (Omnia, 2009). Oral contraceptives with high oestrogen content (75 - 15μg) increases the rate of *Candida* colonization. The changes in the vaginal milieu are similar to those that occur during pregnancy (Fora *et al.,* 1997). Faraji *et al.* (2012) reported a significant difference between vaginal candidiasis and contraceptives methods. It was observed that women who use hormonal contraceptives such as intrauterine device (IUD) and condom had 60% prevalence of vaginal candidiasis as against 40% recorded by those who don't. This was attributed to low doses of hormones, increase in vaginal secretion and acidity of the pH of vaginal by IUD and the sensitivity of vaginal epithelial cells to condom. Ilusanya *et al.* (2012) noted that women who used condom as a source of contraceptive had high prevalence rate of vaginal infection.

Similarly, Oyewole *et al.* (2013) reported a high prevalence rate of vulvovaginal candidiasis among multigravidae, which was attributed to the use of contraceptives and antibiotics. Hence, infection doubles in those taking high dose of oestrogen which increase progestin content, this increases the risk of candidiasis (Fora *et al.,* 1997).

2.6.4 Sexual Factors

Vulvovaginal candidiasis is not considered as sexually transmitted disease since it occurs in celibate women also because *Candida* is part of the normal vaginal flora. However, vaginal sexual intercourse and other forms of sexual activities might facilitate the inversion of *Candida* into the vagina and result in local trauma, creating conditions suitable to invade tissue. Furthermore, there are remarkable increases of vulvovaginal

candidiasis when women begin regular sexual activities (Foxman, 1990). Individual episode is also not related to number of sexual partners. On the other hand, frequency in oral-genital contact increases risk of infection. Receptive oral sex has been previously reported as a risk factor for VVC. Oral sex might facilitate transmission of yeast as *Candida* often colonizes the oral cavity (Leon *et al.,* 2002).Though sexual intercourse alone have not been shown to alter vaginal *Candida* colonization (Nabhan, 2006). According to Egan and Lipsky (2000), young age at first intercourse and having intercourse more than four times per month are risk factors Jindal *et al.,* (2007) is of the view that risk is due to sexual activities in the second to fourth decade of life. While Nabhan (2006) stated that annual incidence increases dramatically towards the end of the second decade of life and picks up towards the next two decades. The genital tract of women consist of resident microflora of wide variety of species, while some play useful role to help maintain a healthy state of the vagina, others however reside as commensals but may become pathogenic if opportunity arises. This occurs when organism in the vagina ascend to the cervical area especially during sexual intercourse. This may cause infectious agents to spread through the peritoneal cavity which may produce toxins thus extra-genital effect may occur after been absorbed through the mucosa (Winn *et al.,* 2006). In view of this, *Candida* can be transmitted sexually as penile colonization is four times more frequent in male partners of women infected by *Candida* (Rodin and Kolator, 1997; Mclelland *et al.,* 2009). Furthermore, infected partners are known to carry identical strains (O'Connor and Sobel, 1986).

2.6.5 Antibiotics

Antibiotics have been identified as risk factor for vulvovaginal candidiasis in some women, though the exact mechanism is not yet known – precisely. Thus, complication may occur when antibiotic destroys both harmful and useful bacteria in the body thereby giving *Candida* a chance to multiply. A person frequently on antibiotic more than 1 course of 7 – 10 days is a prime Candidate for *Candida.* Antibiotic treatment decreases the total population levels of indigenous bacteria flora and predisposed one not only to gastrointestinal overgrowth but also to subsequent systemic dissemination to the liver, kidney and spleen by *Candida albicans* (Timothy and Birdsall, 1997). An association between lack or loss of vaginal lactobacilli, hydrogen peroxide production and susceptibility to VVC is yet to be established in women that develop infection while taking antibiotics.

2.6.6 Immune Deficiency

A weakened or under developed immune system is significant predisposing factors of candidiasis. Nutrient deficiency, steroids, stress, HIV/AIDS, mononucleosis and cancer are factors that lead to candidiasis (Odds, 1987). About 15% of people with weakened immune systems develop a systemic illness caused by *Candida* species. In extreme cases, these superficial infections of the skin or mucous membrane may enter into the bloodstream and cause systemic *Candida* infections.

2.6.7 Diabetes

This is one of the predisposing factors associated with candidiasis. Diabetes mellitus predisposes individuals to bacteria and fungi infections including those caused by *Candida* species. Vulvovaginal candidiasis occurs more frequently in diabetics on the other hand, chronic reoccurring VVC may be a marker of diabetes (Peer *et al.,* 1993). However, there is controversy as to whether diabetes leads to more symptomatic or more VVC episode. Potential risk factors for VVC include diabetes type, severity and degrees of glucose control *Candida* carriage increases with age. Women with type 1 diabetes have higher *Candida* colonization rates than those with type 2. One possible explanation is the duration of carriage. Women with type 1 and type 2 diabetes may equally acquire *Candida* but those with type 1 may be less able to clear it. The reason is that hyperglycemia in type 1 diabetes increases risk for *Candida* colonization. Hyperglycemia limits neutrophil function among persons with type 1 diabetes including neutrophils ability to phagocyte and kill *Candida* organisms. Since the oxidative killing ability of neutrophils is hindered, diabetics and non diabetics may not be able to clear pathogens. Hyperglycemic individuals may also have increased risk for *Candida* colonization because their secretion contains glucose, which provide nutrient for *Candida*. Furthermore, fucose (6-deoxy-galactose) a vaginal epithelial cell receptor which aids in adhesion of *Candida* to vaginal epithelial cells contains an isomer of glucose that acts as one form of receptor site for *Candida* adhesion. It is possible that *Candida* colonization is proportional to glucose level (Gibb *et al.*, 1995; Leon *et al.*, 2002). Akah *et al.* (2010) carried out a study to determine the prevalence of vulvovaginal candidiasis in pregnancy in a rural community reported that no glucosaria was found among the women an indication that none had diabetes. According to Ten Teachers (1997) reduced renal threshold for sugar occurs in many pregnant women with many having glucosaria without being obviously diabetic. Vaginal epithelial cells have

a greater potential to bind to *Candida albicans* in diabetic women than in non-diabetic due to increase glucose levels in the genital tissue which enhances yeast adhesion and growth. Contrary, Neheed *et al.* (2008) in a study of pregnant women attending routine antenatal clinic in Pakistan found that pregnant women with diabetics had significantly increased infection ratio and recommended routinely screening for vaginal candidiasis during pregnancy.

2.6.8 Pregnancy

One-third of pregnant women worldwide can be affected by vulvovaginal candidiasis. This is due to the high level of reproductive hormones and an increase in the glycogen content in the vaginal environment which create a favourable environment for *Candida* species. The combination of these changes provides an abundant source of carbon for *Candida* growth, germination and adherence. Furthermore, the acidity of a pregnant women's vaginal flora can suppress the growth of other micro organisms that are naturally inhibitory to *Candida*. The initial attachment of the organism occurs more readily at pH value (six to seven), germ tube formation and development of mycelia are favoured by low vaginal pH (<5) (Omnia, 2009). The high prevalence rate of microorganisms in pregnant women could be attributed to the higher oestrogen state which increases their susceptibility to microorgansms (Adinma *et al.*, 2001). The increase hormone level affects both the glycogen content and normal floral creating a favourable condition for *Candida* (Gabbe *et al.*, 1991). High prevalence of *Candida albicans* in pregnant women is more than in non-pregnant women (Agbakoba *et al.*, 2008). The incidence of candidiasis during pregnancy may be twice that in non-pregnant women and is highest during the third trimester (Slavin *et al.*, 1992).

Feyi-Waboso and Amadi (2001) observed a high prevalence of vaginal candidiasis during pregnancy among primigravidae and younger group. Women in their third trimester of pregnancy belonging to younger age group (18 – 30 years) have high prevalence rate. Furthermore, Neeheed *et al.* (2008) stated that multigravidae suffer more from vaginal candidiasis than primigravidae. This may be associated with the fact that multigravidae have longer sex history and also more number of pregnancies hence making them more prone to develop the infection than primigravidae with less sexual exposure. Similarly, Oyewole *et al.* (2013) also observed high prevalence among multigravidae attributing it to the use of contraceptives and antibiotics. Multigravidae

also have higher prevalence for recurrence infection. The highest occurrence of *Candida* was found in pregnant women in their second trimester.

2.6.9 Feminine Hygiene Products and Douching

The use of sanitary napkins or tampons has not been shown to increase risk of VVC, while the role of tight fitting clothing in precipitating episodes of VVC remains unproved (Nabhan, 2006). On the other hand, Jindal *et al.,* (2007) reveals that women of low socio-economic status, unsatisfactory genital hygiene and those using tight, poorly ventilated nylon underclothing showed significantly high incidence of VVC than those of middle class with satisfactory genital hygiene and those using well ventilated cotton under garment. The use of tight fitting, synthetic / nylon clothing could be contributing to VVC by increasing perinea moisture and temperature.

2.7 Epidemiology

The disease is generally not sexually transmitted, however the disease is said to be more common in black college women than white (Nabhan, 2006; Nester *et al.,* 2007). Approximately 75 % of women experience at least one episode of vulvovaginal candidiasis and up to 5 % of this group will have recurrent infection. Incidence of vulvovaginal candidiasis is highest in women between the ages of 20 – 40 years. It is however, rear in pre-pubertal and postmenopausal women (Sheary, 2005).Although *Candida* is very common in women, it can also occur in male. Male genital yeast infection is less common and incidence of infection is only a fraction of that in women, though yeast infection on the penis from direct contact via sexual intercourse with an infected partner is common (David *et al.,* 1997). Treating the male partner is unnecessary unless he is uncircumcised or has inflammation of the penis (Egan and Lipsky, 2000). Symptoms of the infection of male genitalia include; red patchy sores near the head of the penis, or on the foreskin, severe itching or burning sensation; *Candida* of the penis can also have a white discharge though uncommon. Omnia (2009) stated that 50% of college aged women have an episode of vulvovaginal candidiasis in the United States. While 75% of all women experience an attack of *Candida* vulvovaginitis with about half of these women having more than one episode and a few having frequent relapses.

2.8 Diagnosis

Though, vulvovaginal candidiasis could be self diagnosed, culture and light microscopic examination of high vaginal specimen are extremely valuable (Nabhan, 2006). Diagnosis is done by microscopy examination or culturing. For identification by light microscopy, high vaginal swab smear is made on a slide and viewed. Alternatively, the vaginal discharge is mixed with physiological saline and viewed under both low and high power magnification. Under microscopy the spores and mycelia are visible. The presence of yeast blastospore or pseudohyphae can be detected in 30 – 50 % of patients with the infection. Adding 10 % potassium hydroxide to solution makes the alkaline resistant branching budding hyphae of *Candida* easier to be seen. Although the sensitivity of 10 % potassium hydroxide examination is higher; at least one-third of the patient with symptomatic VVC will have negative findings with potassium hydroxide microscopy. Blastospores of non *albicans* strains do not form hyphae or pseudohyphae; this makes it difficult for recognition, vaginal pH remain normal in VVC. These two tests are inexpensive yet they have been under- used (Nabhan, 2006). Vulvovaginal candidiasis is diagnosed daily in thousands of women through a telephone conversation or self diagnosis through clinical symptoms and signs such as irritation, whitish cheesy discharge. Sheary, (2005) stressed that misdiagnosis can occur while Nabhan (2006) described this situation as unfortunate as vaginal cultures which are extremely valuable but not routinely carried out. Culture diagnosis such as germ tube test, chlamydiospore formation sugar fermentation and assimilation tests are valuable clinical diagnostic yet not carried out by most clinics except when there is suspicion of a resistant organism (Nabhan, 2006). In germ tube test, identification is carried out on Sabouraud agar and incubated at 25 – 37 ℃ for 24 – 48 hours positive colonies are inoculated in smaller tubes containing either bovine or rabbit serum. The tube is incubated at 35 – 37 ℃ for two to three hours. The presence of sprouting yeast cells with tube-like appearance shows the presence of *Candida albicans* (Cheesbrough, 2000). The presence of conidiospore and creamy colour of colonies allow initial diagnosis. Women's ability to self diagnose vulvovaginal candidiasis based on clinical symptoms shows that less than half were correct (Sheary, 2005). This is because vulvovaginal candidiasis and bacterial vaginosis (BV) may occur simultaneously (BCCDC, 2010).

2.9 Treatment by Therapeutical Agents

Table 1 indicate the various antifungal agents commonly used for treatments and their recommended dosage. (Nabhan, 2006). Topical azole which is said to be effective for

Table 1: Antifungal drugs and their recommended dosage.

Antimycotic Drugs	Formulation
Topical	
Butoconazole	2 % cream, 5 g for 3 days
Clotrimazole	1 % cream, 5 g for 7 – 14days
Miconazole	2 % cream, 5 g for 7days
	100 mg vaginal suppository, 1 suppository for 7 days
	200 mg vaginal suppository, 1 suppository for 3 days
	1200 mg vaginal suppository, 1 suppository single dose
Econazole	150 mg vaginal tablet, 1 tablet for 3 days
Fenticonazole	2 % cream, 5 g for 3 days
	6.5 % cream, 5 g single dose
Terconazole	0.8 % cream, 5 gm for 3 days
	80 mg vaginal suppository, 1 suppository for 3 days
Nystatin	100,000 units' vaginal tablet, 1 tablet for 14 days
Oral	
Ketoconazole	200 mg bid for 5 days
Itraconazole	200 mg bid for 1 day
Fluconazole	150 mg single dose

(Nabhan, 2006)

treatment in 80% of cases should be avoided in the first trimester of pregnancy (Sheary, 2005). However, centre for disease control permit its use even in the first trimester. Antimycotic drugs used for treatment include Butoconazole, clotrimazole and Econazole which are topical. Oral antimycotic drugs include ketoconazole, clotrimazole and fluconazole (Nabhan, 2006). For instance, a one time dose of fluconazole (as Diflucan 150mg tablet taken orally) is 90% effective in treating vaginal yeast infection (Moosa *et al.*, 2004). Care must be taken by those allergic to azole group of medicine. Local treatment may include vaginal suppositories or medicated douches. Omnia (2009) stated that the cell wall of the organism is a complex glycoprotein which depends on the biosynthesis of ergosterol. Azole compound found in antimycotic drugs are believed to block this step in biosynthesis. Fluconazole achieves therapeutic concentration in vaginal secretions at least 72hours after ingestion of a single 150mg tablets. Uncomplicated sporadic vaginitis is caused by strains of *Candida albicans* majority of which exhibit sensitivity to azole – based antifungal agents and thus responsive to all forms of antifungal therapy. Consideration is taken regarding drug interactions with oral usage. Hypertoxicity to ketoconazole occurs in approximately 1 in every 10,000 – 15,000 individuals exposed to the drug (Omnia, 2009).

Before 1980 topical agents approved by the US Food and Drug Administration (FDA) for the treatment of vulvovaginal candidiasis included nystatin, miconazole and clotrimazole other drugs have now been added (Nabhan, 2006). Azoles has higher rates of clinical and mycological cure (90 – 95 %) than nystatin (70 – 80 %) in comparative trials of 10 – 14 days course therapy and more effective even for shorter durations than the 14 days required for nystatin. Akah *et al.*, (2010) noted that treatment with nystatin and clotrimazole invariably showed the same efficacy. It was observed that pregnant women with vulvovaginal candidiasis who received once daily dosing of the drugs had their symptoms resolved within one week treatment. However, the repeat culture of their high vagina swab yield some growth of *Candida* (33 %) and (31 %) respectively. For pregnant women that received twice daily dosing of nystatin and clotrimazole, also had their symptoms resolved within one week but the repeat culture of their high vaginal swabs yielded only scanty growth. Hence, the amount of dosing also plays a significant role in treatment. Young and Jewel (2001) also reported that treatment with single dose of nystatin for three or four days was less effective when assessed by culture and symptoms than those carried out for seven days, stating that topical imidazole was more effective than nystatin for treatment of symptomatic vaginal candidiasis in

pregnancy lasting for seven days. Due to its efficacy and low risk profile, nystatin remains the first line treatment for *Candida* infections in the first trimester (Fan and Liu, 2010). A combined treatment of nystatin vaginal tablets and cream for six days was observed to be efficient, safe and economic option in the treatment of vulvovaginal candidiasis (Dressen *et al.,* 2012). Zarei *et al.,* (2013) recorded the susceptibility of nystatin to various species of *Candida* as 44.1 %, while dose dependent was 54.8 % and resistant was 1.1 % respectively. Sensitivity of various species of *Candida* to itraconazole was 7.5 % while 91.4 % was dose dependent and resistant was 1.1 %. Ketoconazole recorded 19.4 % sensitivity to various species of *Candida*, 53.8 % were dose dependent, while 26.9 % resistant. Sensitivity of *Candida* species to fluconazole was 12.9 %, dose dependent was 38.7 %, while resistance was 48.4 % respectively.

Fora *et al.,* (1997) stated that due to comparable efficacy of many antifungal agents, selection depends on other factors such as severity, duration of symptoms, the extent of inflammation and the causative organism versus its known susceptibility pattern. While Zarei *et al.* (2013) suggested that controlled survey must be taken to optimize antifungal therapy based on characteristics of *Candida* strains. *Candida* species such as *Candida krusei* are resistant to fluconazole, *Candida glabrata* also show some resistance to fluconazole. Inspite of this, fluconazole is a frequently choice for treatment and prevention of fungal diseases. The recent trend has been towards shorter treatment courses with higher antifungal doses resulting in a number of one day regimens. In general, shorter therapeutic courses promotes better compliance and prolongs persistence therapeutic concentration in the vaginal for several days (Fora *et al.,* 1997).

2.10 Recurrent Vulvovaginal Candidiasis

Recurrent vulvovaginal candidiasis is defined as four or more episode of vulvovaginal candidiasis in a year Sheary, (2005). In the pathogenecity of recurrent thrush, two main reasons may account for frequent recurrences. These are either due to re-infection from a sexual partner or due to reservoir of the yeast in the gut. Vaginal relapse may be due to incomplete eradication of the yeasts. Identical species have been isolated from the rectum of most women with vaginal thrush. However, an attempt to eradicate the carriage of yeast in the gut has not significantly reduced the number of symptomatic of vaginal recurrences (El-din *et al.,* 2001).

The misdiagnosis by clinician inevitably results in incorrect self diagnosis by the patients. Reasons for incorrect diagnosis include; self perpetuation of *Candida* as nonmycotic topical contact dermatitis, hyper-sensitivity reactions and chemical or allergic reactions to antimycotic therapy which frequently results in continuation of symptoms that are incorrectly thought to be caused by fungi resulting in inappropriate additional antimycotic therapy (Nabhan, 2006). In secondary vulvovaginal candidiasis, either host or microbial factors are considered the causative factor for the conditions. These include conditions affecting a patient's immunological status e.g. uncontrolled diabetes, thyroid disease and immunodeficiency virus (HIV). Microbial factors include non *albicans Candida* species, most commonly *Candida glabrata*. Resistant *Candida albicans* is uncommon (Sheary, 2005).

Timothy and Birdsall (1997) stated that there is a connection between gastrointestinal candidiasis (GIC) and recurrent vulvovaginal candidiasis. *Candida albicans* has been shown to be the cause of diarrhea. In a three year study of 854 patients, fungal proliferation was noted. The predominant fungal species isolated were *Candida albicans* (64.5 %), followed by *Candida tropicalis* (23.3 %), *Candida krusei* (6.9 %) and *Torulopsis glabrata* (1.6 %). *Trichosporon* species and *Geotrichum* species were found to be responsible for the diarrhea in 2.3 % of adults (Timothy and Birdsall, 1997). The connection between GIC and recurrent vulvovaginal candidiasis is demonstrated in the disease causing potential of *Candida albicans* infections of the gastrointestinal tract. Out of ninety-eight patient complaining of recurrent vaginal candidiasis, *Candida albicans* was found to be present both in the vaginal and fecal material in 52 % of the patients while 47 % of the patients were *Candida* free at both sites. In only one patient was *Candida* found in the stool but not in the vagina. However in no case was *Candida* previously isolated from a patient who subsequently proved to be yeast free. Behavioural factors are thought to trigger episode of vulvovaginal candidiasis. Some of which include sexual practice, clothing, habits and diet (Sheary, 2005). On the other hand, Nabhan (2006) states that the role of sexual transmission and re-infection in causing repeated episodes remains unclear as most studies failed to document that treatment of male partners puts an end to recurrent VVC. Derrick and Adrienne (2009) are of the view that wearing of tight pants and douching are contributory factors.

Pregnant patients are often present with symptomatic yeast infections hence the need for safe management of yeast infection during pregnancy in order to prevent reoccurrence. Hence the need for antifungal agent.

Antifungal: Topical formulations of imidazole antifungals (e.g. butoconazole, clotrimazole, miconazole) and triazole antifungals (e.g. fluconazole, itraconazole) are considered the therapy of choice during pregnancy due to their safety to both human as well as animals; no major malformation occurs when mothers are exposed to these drugs during pregnancy. Systemic absorption of these topical medications are minimal and pose little risk to the unborn child by means of transfer, a duration of seven days is required instead of a shorter duration for effective treatment (Derrick and Adrienne, 2009). Little evidence exists that antimicrobial resistance could be involved in recurrent vulvovaginal candidiasis though the spread of non *albicans Candida* species with intrinsic azole resistant may be an emerging concern, which may adversely influence the outcome of the therapy (Magliani *et al.,* 2002).

The need for new antifungal drugs with greater potency and broader spectrum of activity such as sordarins and caspofungin are under study (Magliani *et al.,* 2002).

Antiseptics: The use of boric acid has been studied in the treatment of VVC though not commercially available; it is an alternative to antifungal agents, though little is known about its safety to humans. There is a weak association between boric exposure during pregnancy and major malformation though of little significance. Unless the vaginal epithelium is severely excruciated, only a limited amount of boric acid is systemically absorbed. However, the amount is too little to expose the unborn foetus to risk. The dose is 600 mg intravaginally per night for 14 consecutive nights (Derrick and Adrienne, 2009). Maintenance therapy with topical has been experienced but its efficacy in the cure of vaginitis and the prevention of lapses ends with the suspension of the therapy (Magliani *et al.,* 2002).

Corticosteroids: Itchiness, redness and the use of topical corticosteroids can alleviate acute symptoms. It has been observed that mothers exposed to oral corticosteroids have no significant major malformations. Though about 3 % of topical corticosteroid dosage applied onto the skin is systemically absorbed. Fortunately, there is no increasing major malformation in babies of mothers who use the drug during pregnancy (Derrick and Adrienne, 2009).

Immunotherapeutic Strategies: Increase in percentage of vaginal infection have also been ascribed to non *albicans* species such as *Candida glabrata* and more rarely to *Candida krusei,* innately less susceptible to most antifungal agents. The increase use of over the counter antifungal treatments as well as widespread prescription of systemic oral azole have resulted in the spread of azole resistant *Candida albicans* as well as non

albicans Candida species. Innate and acquired humoral and cell mediated immune response are involved in the defence against *Candida* infection. T – cells dependent cell immune response are involved in defence against *Candida* mucocutaneous infections. Cytokine produced by monocytes during innate response may modify T cell – mediated immune response and different *Candida* immunoregulatory cytokines during infection (Magliani *et al.,* 2002).

Members of the secretory aspartyl proteinase gene family, surface mannan, a mannoprotein extract and other constituents of *Candida* cell have been proposed as immunodominant *Candida* antigen able to stimulate a potentially protective immune response against either systemic and / or mucosal candidiasis and have been considered as potential vaccine Candidates. Natural and monoclonal antibodies generated against some of those antigens proved to be protective when given before infection and therapeutic when given after infection (Magliani *et al.,* 2002).

2.11 Drug Resistance

Drug resistance may occur due to misuse and over the counter self medication, (Sheary, 2005). According to Sayyada *et al.* (2010), in resource limited countries, lack of training, improper reagents supplies and lack of equipments makes detection and rapid presumptive identification of yeast infection quite difficult. Thus making appropriate selection of antifungal therapy difficult hence prophylaxis becomes almost impossible. Increasing incidence of drug resistance is also due to non *albicans* vulvovaginal candidiasis. Misuse of over the counter antimycotic along with short causes therapy has become common resulting in resistance. In patients with recurrent or resistant vaginal candidiasis an acquired hypersensitivity reaction to *Candida albicans* has been reported. Desensitization therapy with *Candida albicans* antigen has been found to be an effective treatment (Timothy and Birdsall, 1997).

Recurrent vulvovaginal candidiasis and gastrointestinal candidiasis are connected. This is attributed to the disease causing potential of *Candida albicans* infections of the gastrointestinal tract. According to Timothy and Birdsall (1997), in study of ninety-eight consecutive patients complaining of recurrent vaginal candidiasis, *Candida albicans* was found in both vaginal and fecal material in 52% of the patients, while 47 % of the patients were *Candida* free in both sites. In no case was *Candida* previously isolated from the vaginal of a patient that was yeast free. Only in one patient was *Candida albicans* found in the stool but not in the vagina. An indication that vaginal

candidiasis does not occur naturally without the presence of *Candida albicans* within the large bowel hence cure is unlikely as long as the vagina remains the only treatment target.

2.12 Control

Approximately, 75 % of women come down with candidiasis at least once with 5 % of women with recurrent infections. The highest infection occurs in women within the range of 20 – 40 years. It is rear in pubertal and post menopausal women (Sheary, 2005). This calls for effective control measure since most pregnant women fall within this age range. Such measures include; avoid the abuse of antimycotics (Nabhan, 2006). As effective control measure, the use of douches, scented products like sprays and tampons should be avoided. Furthermore, frequent change of pad and tampons, helps to reduce colonization and reduce infection, synthetic under clothings and wet swimsuits should also be avoided. Oral contraceptives should not be used often (Jindal *et al.*, 2007; Amini *et al.*, 2009).

2.13 Bacterial Vaginosis

Bacterial vaginosis is one of the most common causes of reproductive tract infection (Amini, *et al.*, 2009). It is the most common vaginal disease of women in the United States accounting for 40 to 50 % of cases in women of child bearing age. It is termed Vaginosis rather than vaginities because inflammatory changes are absent. Pregnant women with this disease have seven fold increase in the risk of having a premature baby or other complications due to premature rupturing of membrane and pretermed labour (Egan and Lipsky, 2000; Nester, *et al.*, 2007).

Common Symptoms, include gray – white vaginal discharge with unpleasant fishy odour, the diseases is asymptomatic in up to 50% of women (Egan and Lipsky, 2000; Nester *et al.* , 2007; Botash, 2010). Though the causative agent is unknown, it is however believed to be caused by proliferation of a number of organisms such as *Gardnerella vaginalis, Mycoplasma hominis* and *Streptococcus* species. However a marked decrease in vaginal lactobacilli is a constant feature, the incubation period is also unknown (Egan and Lipsky, 2000).

The pathogenesis is unknown however, there is marked distortion of the normal vaginal epithelium in the absence of inflammation. Odour due to metabolic products of anaerobic bacteria, premature birth and complications are associated with pregnancy

(Nester *et al.,* 2007). The disease is spread through multiple sexual partners, or a new infected sexual partner and poor hygiene. It is also common in women of child bearing age, sexually abused children and rarely in virgins (Amini, *et al.,* 2009; Nester *et al.,* 2007). Bacterial vaginosis is diagnosed using whiff test that uses potassium hydroxide which gives intense amine odour. On wet preparation, clue cells are seen with paucity of white blood cells. On gram stain, clue cells are identified as epithelia cells covered by small gram negative rods. Culture is not generally recommended (Botash, 2010). Though there is no proven preventive measures, however, good hygiene is recommended. While treatment with metronidazole is considered to be effective (Nester *et al.,* 2007; Amini *et al.,* 2009).

2.14 Trichomoniasis

Trichomoniasis is ranked third after vaginosis and candidiasis among diseases that cause vaginal symptoms. It affects 180 million women worldwide and accounts for 10 – 25% of vaginal infections (Egan and Lipsky, 2000). About 5million Americans contact this sexually transmitted disease each year. In Africa, it can cause two to three fold increase in HIV transmission (Nester *et al.,* 2007). It is associated with multiple sexual partners (Botash, 2010).

In women, the disease is associated with itching, burning, swelling, vaginal redness, frothy, sometimes associated with a yellow-green discharge and burning sensation during urination (Nester *et al.,* 2007). Symptoms often picks just after menses. Infection during pregnancy is associated with premature deliveries and low birth weight infants (Botash, 2010). In men, symptoms include; discharge from the penis, burning sensation during urination, painful testes and tender prostate. In both sexes, symptoms are often asymptomatic (Nester *et al.,* 2007).

Trichomonas vaginalis, a protozoa measuring 10 by 30µm having four anterior flagellum and a posterior flagellum attached to the undulating membrane is the most common cause of vaginitis (Egan and Lipsky, 2000). Infection causes redness and slight swelling of the vaginal walls and vulva, often with scattered pinpoint hemorrhages, caused by the mechanical trauma by the moving protozoa. *Trichomonas vaginalis* is distributed worldwide as human parasite with no other reservoir. Since it lacks cyst form, it is easily killed by dryness. Transmission is by sexual contact. It can survive on moist objects such as birth tubes and towels, thereby enabling non-sexual transmission. Newborn infants contract the disease through infected mothers. Rate of infection is

highest among men and women with multiple sexual partners (Nester *et al.*, 2007). Trichomonas may be identified in 30 to 80% of the male sexual partners of infected women. It may also act as vector for other venereal diseases (Egan and Lipsky, 2000).

Motile trichomonads may be seen in wet preparation of isotonic sodium chloride solution. More than 10 white blood cells are seen on wet preparation using high power field. Accuracy may be improved by culture on diamond medium (Botash, 2010),Prevention is by abstinence, monogamy and the use of condoms. Most strains of *Trichomonas vaginalis* respond quickly to metronidazole treatment, but a few are resistant (Nester *et al.*, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Specimen Collection

Ethical permit was obtained from all the health facilities (appendices 1,2,3 and 4) while informed verbal consent of each participant was obtained before the collection of samples. A total of two hundred and seventy three (273) high vaginal swab samples were collected from consenting pregnant women attending antenatal clinic in Yola, Nigeria from April to September 2012. Thirty three (33) high vaginal swabs were collected from Federal Medical Centre, Yola. Two hundred and six (206) from Specialist Hospital, Yola and thirty four (34) from Girei Local Government Clinic, Sangere. The swab samples were collected using sterile swab sticks. Two swab samples were collected per individual. Each individual samples was accompanied by a questionnaire indicating the age, occupation, trimester stage, number of pregnancies, types of family planning method used and other information as indicated in the questionnaire about the participant. (Appendix 5).

Two high vaginal swabs using sterile cotton wool swab were taken from the pregnant women, one swab stick was immersed in Amies transport medium and protected from direct light and heat by wrapping a paper bag with a black polythene and placing it the swaps stick in it, before transporting to the laboratory for processing (Bhavan *et al.*, 2010). The other was inoculated into normal saline and transported to the laboratory.

3.2 Direct Microscopy

3.2.1 Direct Microscopy using Physiological Saline

One swab specimen was placed in 1ml sterile physiological saline upon collection and was vigorously shaken until it turned cloudy. A drop of the sample was placed on a clean glass slide covered with a cover slip and examined under a microscope using x40 objective of the microscope. Presence of yeast cells, motile cells and any morphological features was observed (Egan and Lipsky, 2000).

3.2.2 Direct Microscopy using Potassium Hydroxide (10% KOH)

A sample of the high vaginal discharge was mixed with a drop of 10% KOH solution on a slide. This was done by making a smear of the HVS on a clean glass slide in a drop of 10% KOH solution. It was covered with a cover slip and viewed microscopically under

x40 objective. A characteristic fish-smell indicates the presence of bacteria vaginosis. Budding yeast cells and branching hyphae were visible when present (Botash, 2010).

3.3 Culture

Swab samples were inoculated on Sabouraud's Dextrose Agar (SDA) containing 50 µg/ml chloramphenicol. The medium was prepared based on manufacturer's instructions. While the drug was added to the medium on cooling to 45 ℃, mixed and dispensed into Petri dishes. The plates were incubated at 37 ℃ for 48 hours. These were then examined for the growth of cream coloured pasty colonies (Bhavan *et al.*, 2010).The swab samples were also cultured on MacConkey agar to enable the identification of bacteria. The plates were incubated at 37 ℃ for 24 hours and observed for growth of microbial colonies, for morphological shapes, elevations, edge, colours and surfaces (Cheesbrough, 2000; Christopher and Bruno, 2003).

3.4 Identification of *Candida* species using *Candida* Bromocresol Green Agar

Bromocresol green agar was used as a medium to complement the identification of *Candida* species in addition to the biochemical tests.

The medium was prepared based on the manufacturer's instructions and dispensed into plates. Isolates were inoculated on Bromo cresol green agar plates by streaking and incubated aerobically at 30 ℃ for 48 hours. Colony interpretation was carried out as described by Haroid and Snyder (1968).

3.5 Gram Staining

Microbial colonies growing on Sabouraud's Dextrose Agar and MacConkey agar were gram stained. Glass slides were flamed to remove all traces of grease. A drop of normal saline was placed on a glass slide. A flamed wire loop was used to pick a test colony from a cultured plate and emulsified in the normal saline and allowed to air dry. It was then heat fixed gently over a bunsen burner flame. The slides were flooded with crystal violet and allowed to stand for 60 seconds before been washed with water. Slides were flooded with Lugol's iodine for 60 seconds, these were then washed with water and decolourised briefly using ethanol; slides were then rinsed with water and flooded with neural red for 60 seconds. The slides were washed, drained and allowed to air dry. Slides were examined microscopically under oil immersion using objective lens x100 for the observation of yeast morphology (Cheesbrough, 2000; Bhavan *et al.*, 2010).

Identification of yeast cells was done using both the morphology of the isolates on SDA which showed the growth of cream coloured pasty colonies and the microscopic examination of gram positive organisms which retains the primary colour (Cheebrough, 2000; Bhavan *et al.,* 2010). Sabouraud's Dextrose Agar Slants were used for preserving yeast isolates in refrigerator at 4^0C.

3.6 Chlamydospore Formation

Corn meal agar medium (pH 7) containing 1 % Tween 80 was deeply streaked with a 24hours culture of the yeast cells using a sterile loop and sterile glass cover slip was placed over the inoculated area to reduced oxygen tension. The inoculated plates were incubated at 25^0C for 72hours. The plates were then examined under the microscope at x40 for the presence of large, highly refractive thick walled terminal chlamydospore (Balish, 1973; Forbes *et al.,* 1998; Jha *et al.,* 2006; Bhavan *et al.,* 2010).

3.7 Germ Tube Test

This was carried out as described by Cheesbrough (2000). Five ml (5ml) of human serum was pipetted into a small test tube. The serum was inoculated with a yeast colony from the culture plate using a sterile wire loop. The tube was incubated at 37^0C for three hours. A sterile Pasteur pipette was used to transfer a drop of the serum yeast culture to a glass slide and covered with a cover slip. The preparation was examined under x10 and x40 objectives. The presence of sprouting yeast cells with a tube-like outgrowths from the cells, known as germ tube indicates the presence of *Candida albicans* isolate. Absence of germ tube indicates other yeast isolates, other than *Candida albicans*.

3.8 Biochemical Tests

3.8.1 Coagulase Test

Coagulase test was carried out on gram positive bacteria isolates as described by Cheesbrough (2000). Three slides were used one as a test slide while the second and third slides serves as positive and negative control slides. The three slides were labeled and two drops of saline was placed on each slide. A sterile wire loop was used to pick colonies and emulsified. A drop of human serum was added to the slides and mixed. The slides were gently rocked for 10 seconds. The presence of clumping observed on a test slide showed a positive coagulase factor due to the presence of thrombin.

Staphylococcus aureus served as positive coagulase control, while *Escherichia coli* served as negative coagulase control.

3.8.2 Catalase Test

Catalase test was also carried out to determine the breakdown of hydrogen peroxide to oxygen and water. Three ml (3ml) of hydrogen peroxide solution was poured into a clean test tube, a sterile applicator stick was used to pick a test colony of the organism. It was then immersed into the hydrogen peroxide and the result was observed. The presence of air bubbles shows positive result while absence of air bubbles shows negative result (Cheesbrough, 2000).

3.8.3 Indole Test

Indole test was also carried out by inoculating the test organisms into peptone water and incubated at 37^0C for 24hours. Few drops of Kovacs reagent was added and shaken, it was then observed for the presence or absence of red ring colouration. Red ring colouration shows indole positive, while the absence of red ring colouration shows indole negative (Cheesbrough, 2000).

3.8.4 Methyl Red Test

This determines the ability of bacteria to produce acid by fermenting glucose. Each bacteria isolate was inoculated in sterile screw capped bottle containing 5ml of glucose phosphate broth and incubated at 37 ℃ for two days. Five drops of 0.04% solution of alcoholic methyl red solution was added to the inoculated broth and vigorously shaken. A bright red colour indicates a positive result while a yellow colour indicates a negative result (Sharma, 2009).

3.8.5 Voges-Proskauer Test

This determines the ability of bacteria to ferment carbohydrates which result in the production of acetyl methyl carbinol (acetoin). Acetoin is oxidized to diacetyl in the presence of alkali and atmospheric oxygen to give a red colour. The bacteria isolates were inoculated in sterile crew capped bottles containing 5ml glucose phosphate broth and incubated at 37 ℃ for 48hours. One ml (1 ml) of 40 % potassium hydroxide (KOH) containing 0.3 % creatinine and three ml of five percent solution of Naphthol was

added. A pink colour indicates a positive result in two to five minutes, while yellow colouration indicate negative result (Cheesbrough, 2000; Sharma, 2009).

3.8.6 Citrate Utilization

This determines the ability of bacteria to utilize citrate as a sole source of carbon for growth. The bacteria isolates were inoculated directly on Simmon's citrate agar slants by picking a colony from the inoculated plate using a sterile straight wire, the media was stabbed and streaked, then inoculated for 24 – 48 hours. The appearance of growth and the change in colour of the media from green to blue indicates a positive change while negative result show no growth and the media retains its green colour (Sharma, 2009).

3.8.7 Urease Test

This determines the ability of an organism to produce urease enzyme. Urease in the presence of water converts urea into ammonia and carbondioxide. Ammonia makes the medium alkaline. The bacteria isolates were inoculated on urea agar slants and incubated at 37^0C and observed after 4hours and 24hours respectively. A change in colour of the media from orange to purple-pink indicates a positive result while in negative result, there was no colour change in the media (Sharma, 2009).

3.8.8 Triple Iron Sugar Test

Kligler's iron agar slants were inoculated by stabbing and streaking with a sterile straight wire in sterile screw capped bottles and incubated at 37 ℃ for 24 – 48 hours. Blackening indicates hydrogen sulphide, gas production was indicated by the presence of cracks in the media. A reddish pink colour indicates alkalinity while a yellow colour signifies acidity, the colour of the slope and butt were also observed (Cheesbrough, 2000; Sharma, 2009).

3.8.9 Mannitol Salt Agar

Bacteria isolates presumed to be Staphylococcus were streaked on mannitol agar plate and incubated at 37 ℃ for 24 hours. A change in colour from orange to yellow indicates fermentation (Sharma, 2009).

3.8.10 Sugar Fermentation Test

Glucose, sucrose, lactose, maltose, trehalose and galactose were used to determine the ability of the yeast isolates to ferment carbohydrate with the release of carbondioxide and alcohol. The yeast fermentation broth consisted of 5.5 g yeast extract, 7.5 g of peptone, 60 g of specific carbohydrate per litre of deionized water. Prior to inoculation, the medium was brought to room temperature. A suspension of a pure colony of yeast from a day old culture in sterile deionized water was prepared. Two drops of the suspension were added to each tube containing a carbohydrate. Durham tube was inverted into each tube. The tubes were tightly capped and inverted to dislodge trap bubbles. The tubes were incubated aerobically at 37 ℃ for three days. These were observed for the presence of bubbles or air space in the Durham tubes. If no bubbles was present, the tubes were gently shaken and caps loosen and observed for presence of rising bubbles in the broth. If the results are indefinite, the tubes' caps were then tighten and re-incubated and re-examined at regular intervals for three weeks (PML Microbiological, 2001; Mpofu *et al.,* 2008).

3.8.11 Sugar Assimilation Test

Glucose, lactose, maltose, sucrose, trehalose and galactose were used to determine the ability of cultured yeast to utilize sugar (carbohydrates); using a basal medium (4.5g yeast extract, 7.5g peptone and 20g of each test sugar in 1 litre of distilled water). Phenol red (1mg/ml) was used as indicator. The pH was adjusted to 7.0 – 8.0. The medium was dispensed into test tubes containing Durham tubes and sterilized by autoclaving at 121 ℃ for 15 minutes. The pure culture of the isolates was suspended in saline (NaCl). One ml of the isolates suspended in saline was added into each tube containing various test sugars. These were incubated at 30 ℃ for 72 hours. Results were indicated by change in colour from red to yellow (Tiwari *et al.,* 2007).

3.9 Susceptibility Testing using Calibrated Dichotomous Disc Diffusion Method

This was performed using the Calibrated Dichotomous Sensitivity (CDS) (Bell *et al.,* 2006).

3.9.1 Drug Stock Solution

Fluconazole, ketoconazole and itraconazole were used for the susceptibility tests. The drugs were dissolved in dimethyl sulfoxide at various concentrations, first 150 mg fluconazole capsule was dissolved in 6 ml dimethyl sulfoxide, while 200 mg of ketoconazole tablet was dissolved in 20 ml of the dimethyl sulfoxide and 100 mg of itraconazole capsule was dissolved in 10ml of the solvent. This gave a concentration of 25 mg/ml (25000 µg/ml) of fluconazole, 10mg/ml (10000 µg/ml) for ketoconazole and itraconazole respectively. A further 1:10 dilution for each of the drugs gave 2.5 mg/ml (2500 µg/ml) for fluconazole and 1mg/ml (1000 µg/ml) for ketoconazole and itraconazole. Filter papers were used to make discs, each measuring 6mm. The discs were sterilized and one ml of the various drugs concentration was added to one hundred discs (each disc containing 0.01ml of either ketoconazole, fluconazole or itraconazole). The discs were dried at 37 ℃ in hot air oven until fully dried. The final drug concentration in the discs was 25 µg for fluconazole and 10 µg for ketoconazole and itraconazole. The discs were transferred into sterile universal bottles and stored at 4 ℃ in a refrigerator.

3.9.2 Preparation of the Inoculum and Performance of the Test

The test organisms were grown on SDA at 35 ℃ and subcultured at least twice to ensure purity and viability. The inoculum suspension was prepared by picking five colonies, each at least 1mm in diameter. This was then suspended in 5ml of sterile 0.85 NaCl. The turbidity of the cell suspension measured at 530 nm and adjusted with sterile saline to match the transmittance produced by a 0.5 McFarland barium sulfate standard. This produced a cell suspension containing 1 x 10^6 to 5 x 10^6 organisms per ml. One ml (1ml) of the inoculum was inoculated on a pre-dried potato dextrose agar plate. The plates were gently rocked to ensure even distribution. Excess inoculum was removed. The plates were turned upside down for 30 minutes after placing the drug disc, each plate was labeled at the bottom to indicate the type of drug disc placed at each position. The antifungal discs were placed at sites that tallied with the label. The plates were incubated at 37 ℃ for 18 – 24 hours (Liu *et al.,* 2002; Bell *et al.,* 2006).

3.9.3 Reading of Zone of Inhibition

The annular radii was measured which indicates the zone of inhibition that is from the edge of the disc to the edge of confluent growth. The mean of four plates per isolate was taken as the zone of inhibition (Bell *et al.*, 2006).

Interpretation of Results. Disc potency in μg and its acceptance range in mm based on Calibrated Dichotomous Sensitivity (CDS) test were used for interpretation of the results i.e. 7.4 – 10 mm for fluconazole, 5.1 – 7.5mm for itraconazole and 8.6 – 12.4 mm for ketoconazole (Bell *et al.*, 2006).

3.10 Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) for the one hundred and five Candida isolates were determined by the macro broth dilution method. The MIC of the antifungal drugs was determined using a range of concentration of 256 μg/ml to 0.05 μg/ml for fluconazole and 32 μg/ml to 0.625 μg/ml for itraconazole and ketoconazole. All the drugs were dissolved in dimethyl sulfoxide for the MIC determination, 1ml of the yeast inoculum and 1ml of the appropriate dilution of the drug was added to 8ml of sterile Mueller-Hinton broth in a test tube. The tubes were shaken and incubated at 37 ℃ for 48hours. Two control tubes were included as control. One tube containing only the sterile broth medium which served as positive control, while the other tube contained inoculated broth but without the drug stock served as the negative control. The MIC was determined as the lowest antifungal drug concentration that inhibits the growth of the yeast cells. (Silva *et al.*, 2002). Interpretation of result was carried out based on the proposed National Committees for Clinical Laboratory Standard (NCCLS) criteria for broth dilution method as contain in the document M27-A2 (Silva, 2002).

According to NCCLS criteria, *Candida* species isolates for fluconazole with MIC $\leq$ 8 μg/ml are classified as susceptible (s) 16 to 32 μg/ml are termed susceptible dose dependent (S-DD) while > 64 μg/ml as resistant (R). Isolates for which itraconazole and ketoconazole with MIC $\leq$ 0.125 μg/ml are classified as susceptible, while those for which MICs are 0.25 to 0.5 μg/ml are classified as S-DD and those with MIC $\geq$ 1 μg/ml are termed resistant.

3.11 Statistical Analysis

Data were analysed using percentage, pie chart, histogram while Chi-square was used to determine the relationship between age and infection with *Candida* vulvovaginaties at P $\leq$ 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Microscopic Examination of the High Vaginal Sample from the Study Area

Table 2 shows the distribution of the various microscopic features of the samples examined. On the whole, fifty (50) of the samples yielded yeast cells, red blood cells were seen in 29 samples and pus cells were seen in 15 samples. Epithelial cells were also observed and classified based on abundance (+++), moderate (++) and scanty (+) respectively. No motile organisms signifying the presence of *Trichomonas vaginalis* was observed from the samples collected. One isolate (0.36%) gave a characteristic fish smell indicating the presence of bacteria vaginosis (Whiff test).

4.2 Occurrence of *Candida* Species in Pregnant Women from the Three Study Centres in Yola

Thirty three high vaginal samples were collected from Federal Medical Centre, Yola out of which 20 (60.60 %) were positive for *Candida* species. Two hundred and six samples were collected from Specialist Hospital, Yola out of which 64 (31.06 %) were positive for *Candida*, while 21 (61.76 %) *Candida* samples were isolated from Girei Local Government Clinic, Sangere. Plates II-V shows the morphological features of *Candida* species on sabouraud dextrose agar and Bromocresol green agar respectively. While appendix 6 shows the various sugar utilized by each of the *Candida* species. Overall prevalence isolated from culture in the study was 105 (38.46 %), (Table 3). On the other hand associated bacterial isolated along with their biochemical test are shown in appendix 6.

4.2.1 Frequency of Isolation of Candida Species

A total of 105, isolates of *Candida* species were obtained during the study. Figure 1 shows the distribution of *Candida* species isolated from HVS samples in the study. *Candida albicans* was the most frequently isolated species accounting for 86 isolates (81.90 %). This was followed by *Candida tropicalis* 16 isolates (15.24 %) and then *Candida krusei* 3 (2.86 %) isolates.

Table 2: Microscopic Examination of High vaginal samples

Cell	Health Facilities			Total	Percentage (%)
	FMC (n=33)	SHY (n=206)	Sangere Clinic (n=34)		
Epithelial cells					
+	5	27	9	41	15.02
++	11	123	15	149	54.58
+++	17	56	10	83	30.40
Pus cell	3	10	2	15	5.49
Red blood cell	2	27	0	29	10.60
Yeast like cell	6	36	8	50	18.31

Key:

0 = not seen

+ = Scanty
++ = Moderate

+++ = Abundant

FMC = Federal Medical Centre Yola
SHY = Specialist Hospital, Yola

HVS = High Vaginal Swab

n = Number of samples collected

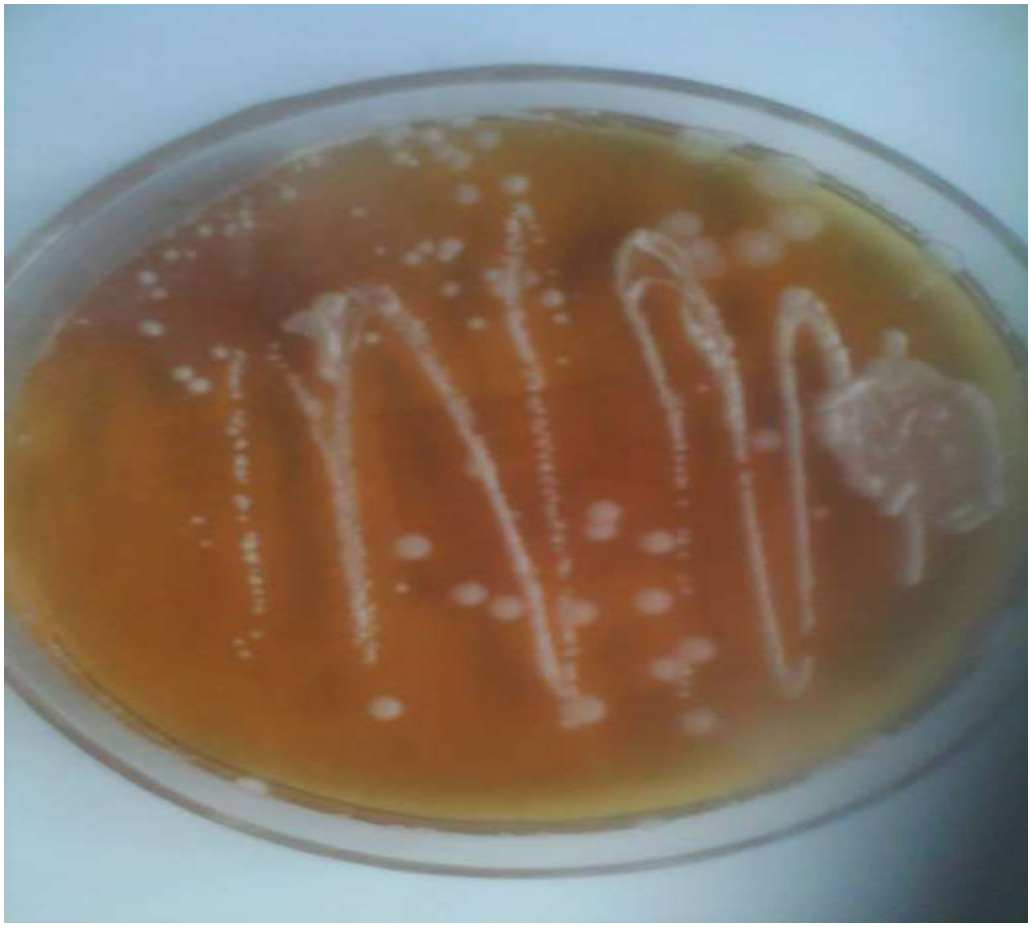


Plate II: *Candida* species on Sabouraud Dextrose Agar

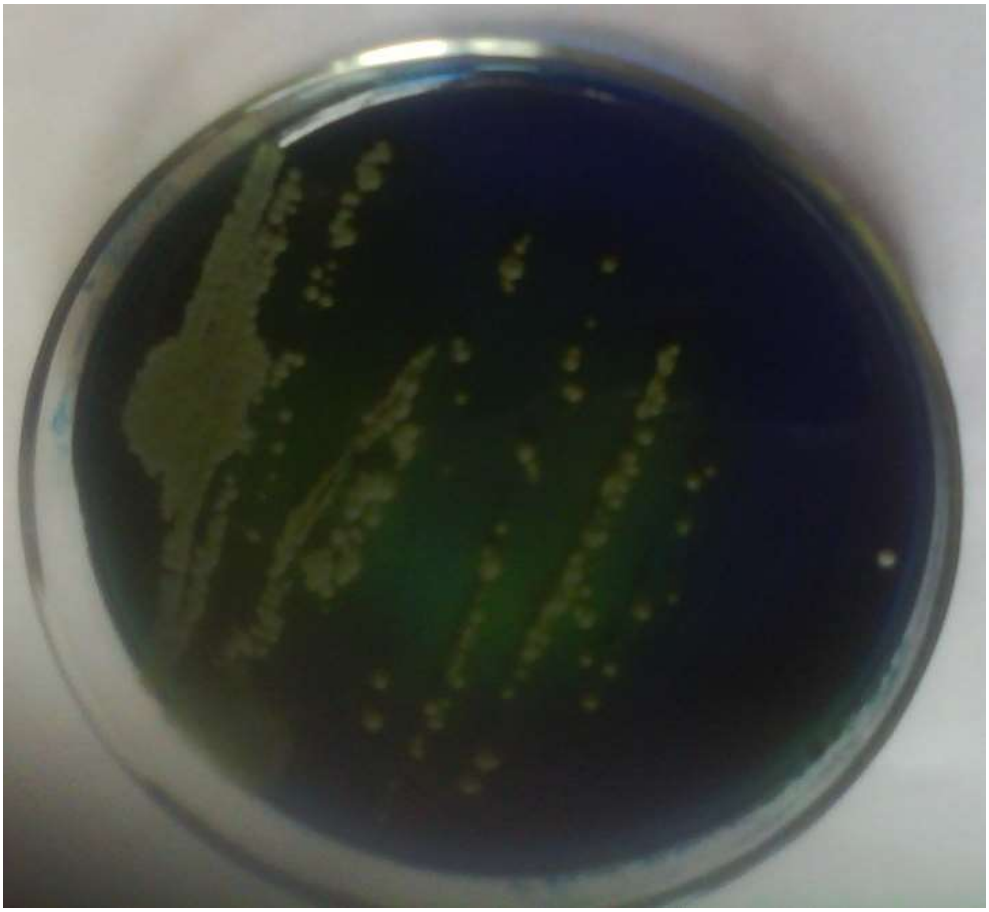


Plate III: *Candida albicans* on Bromocresol green agar

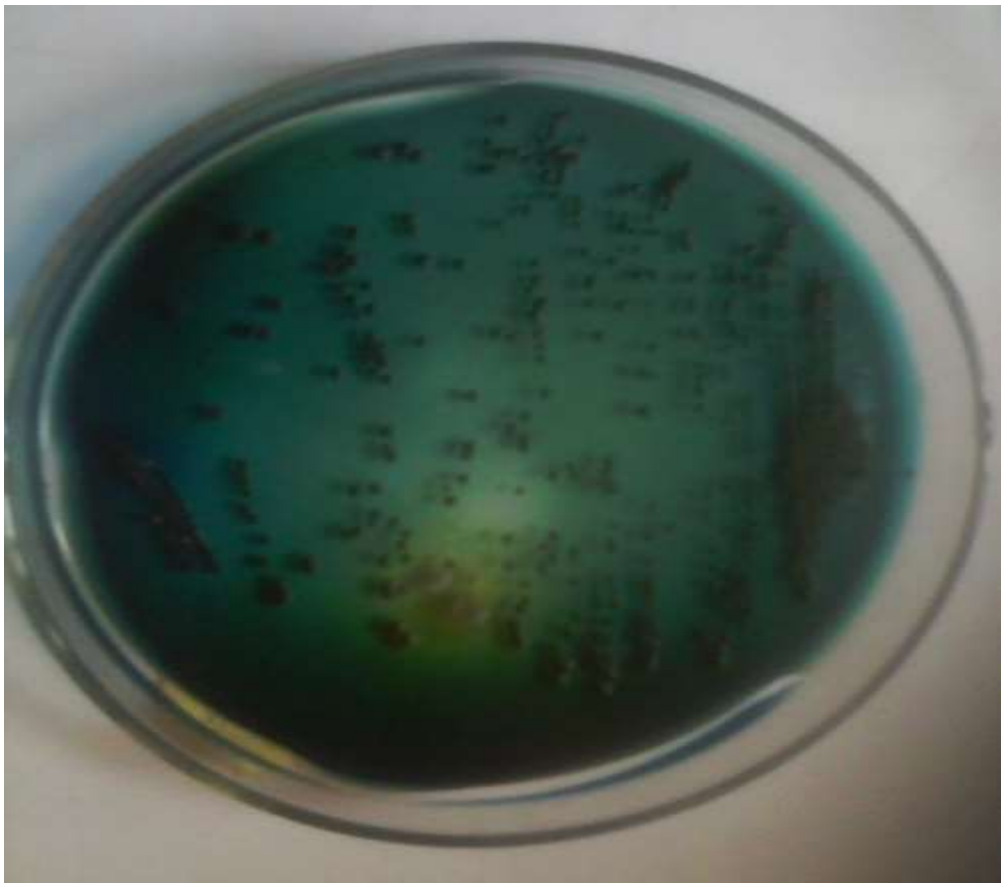


Plate IV: *Candida krusei* on Bromocresol green agar

Plate V: *Candida tropicalis* on Bromocresol green agar

Table 3: Distribution of High Vaginal Swabs collected from the Study Area

Name of Facility	Number of Samples collected	Number of Positive *Candida*	Percentage (%)
Federal Medical Centre, Yola	33	20	60.60
Specialist Hospital, Yola	206	64	31.06
Girei Local Government Clinic Sangere	34	21	61.76
Total	273	105	38.46

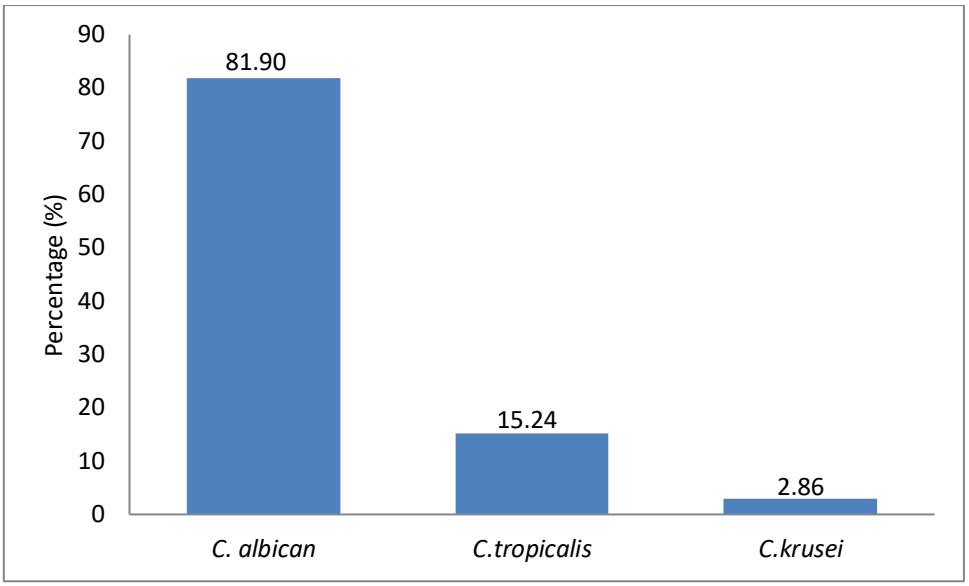


Figure I: Frequency of *Candida* species Isolated from Pregnant Women at the Three Medical Centres in Yola.

4.3 Occurrence of Species among pregnant women in Yola in Relation to age

The age of the study population ranged from less than 20 years to greater than 40 years. The highest prevalence of *Candida* infection was recorded among women in the age range 21 – 30 years with 25.64 %, this was followed by those in the age range 11 – 20 years with 6.95 %. Women in the age group 31 – 40 years and 41 – 50 years had prevalence of 5.49 % and 0.37 % respectively (Figure 2).

4.4 Prevalence of *Candida* Isolates in relation to bacteria in the study

Table 4 shows the overall occurrence of *Candida* species in relation to bacteria associated with the disease organisms in the study area. A total of 135 organisms were isolated, staphylococcus *aureus* was 17 (6.23 %), while Klebseilla aerogenes was observed to be 8 (2.93 %). Streptococcus agalactiae 1 (0.37 %), *Escherichia coli* 3 (1.09 %) and Proteus vulgaris 1 (0.37 %) were the least isolated. An isolation rate of 49.44 % was obtained for the samples. Bacteria alone accounted for 10.98 %. The isolate that gave a fish odour was identified as streptococcus. The highest percentage of isolates, 32.60 % was obtained from women in the age bracket 21-30 years. Those in the age group 11 – 20 years had 27 (9.98 %), while age group 41-50 years had 0.36 % which is the least number of isolates.

4.5 Co-infection among Pregnant Women Attending Antenatal Clinics in the Study Area

Mixed infection of organisms was observed in 8 cases studied (Table 4). Mixed infection with *Candida* and *Staphylococcus aureus* had the highest number 6 (2.19 %), infection with *Streptococcus agalactiae* and *Candida* was 1(0.37 %) and also *Staphylococcus aureus* and *Klebsiella aerogenes* had the percentage occurrence of 1(0.37 %).

4.6 Frequency of Vulvovaginal Candidiasis among women in relation to re-occurrence

Figure 3 shows the percentage of women studied based on the reported number of cases of infection. Only 28.57 % of the study population reported as first case of infection with vulvovaginitis: 39.05 % and 32.38 % of the women had cases of re-infection for the first and second time (or more) respectively.

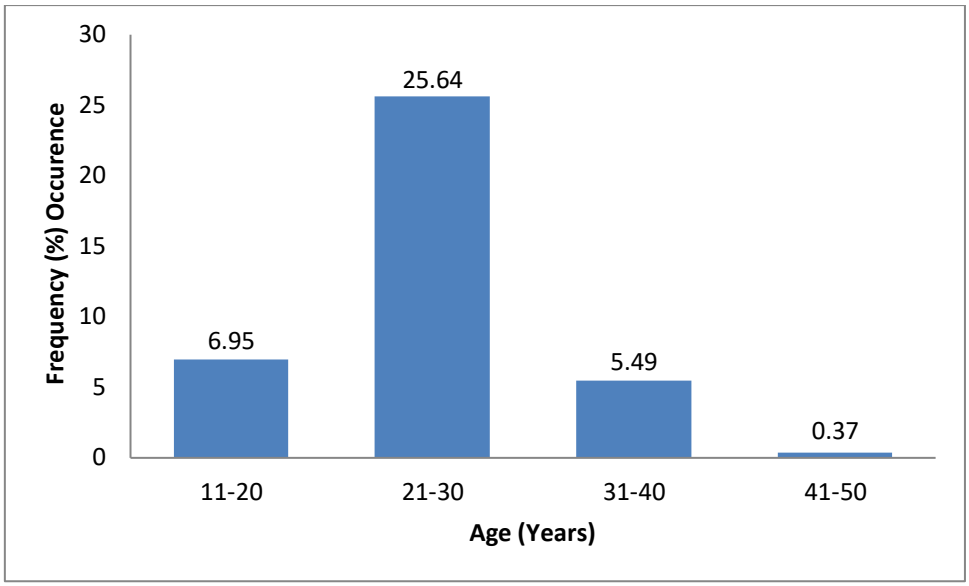


Figure 2: Prevalence of Isolation of *Candida* among Pregnant Women in relation to Age

Table 4: Occurrence of *Candida* species co-infected with bacteria in the study.

Organisms	Total Number Infected	Percentage (%)
Candida species	105	38.46
Staphylococcus aureus	17	6.23
Escherichia coli	3	1.09
Klebsiella aerogenes	8	2.93%
Streptococcus agalatiae	1	0.37
Proteus vulgaris	1	0.37
Total	**135**	**49.44**
Co-infection		
Staphylococcus aureus and *Candida* species	6	2.19
Staphylococcus Aureus and Klebsiella aerogenes	1	0.37
Streptococcus agalatiae and Candida	1	2.93
Total	**8**	**2.93**

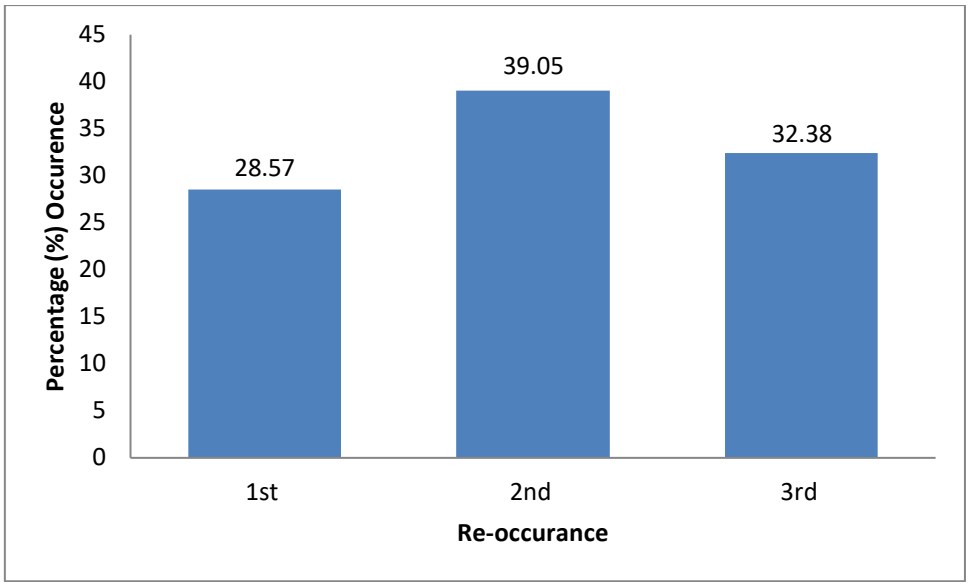


Figure 3: Frequency of Vulvovaginal Candidiasis among Women in relation to Re-occurrence

4.7 Occurrence of Vulvovaginal candidiasis in relation to gestation

Candida was most isolated in women who were in their second trimester of pregnancy (48.57 %), followed by those in their third trimester with 40 % and lastly those in their first trimester accounted for 11.43 % (Figure 4).

4.8 Incidence of Vulvovaginal Candidiasis in relation to abnormal vaginal discharge, antibiotic use and occupation

Out of the one hundred and five (105) subjects in whom *Candida* was isolated, 71 (67.62 %) had vaginal discharge while 32.38 % were asymptomatic. Sixty nine of the pregnant women (65.71 %) indicated the use of antibiotics before the pregnancy, while 39 (34.29 %) did not use antibiotics. Infection based on occupation shows that 15 (14.28 %) of the infected women were civil servants 38 (36.19 %) were business women, 42 (40 %) were full time housewives, 10 (9.52 %) were not specific (Table 5).

4.9 Family planning methods used by the infected women

Candida isolation was highest 26 (89.14 ℃) in women who used oral either pills or inserted devices for birth control. 22 (75.42 ℃) of the infected women did not specify any method of birth control. Some subjects indicated the use of family planning method before their first pregnancy (Figure 5).

4.10 Relationship between *Candida* and number of pregnancies

Table 6 shows the relationship between *Candida* vulvovaginitis and number of pregnancies (gravidae) of the women attending antenatal clinic. Multiple gravidae had the highest prevalence rate of infection 75.24% compared to primigravidae which had a lower prevalence rate of 24.76%.

4.11 Remedies used by the infected pregnant women in this Study

Twenty three (21.90 %) of the infected women indicated that they were solely using oral pills for treatment (Table 7). Other methods used for treatment include: douching (27.26 %) and use of medicinal herbs accounted for (3.81 %), while (23.81 %) of the infected women did not indicate any form of treatment.

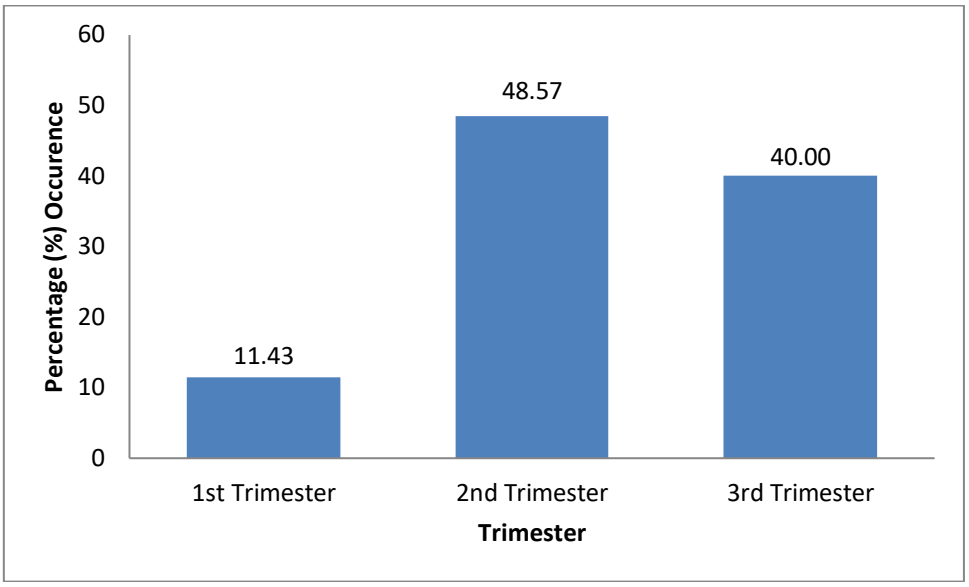


Figure 4: Prevalence of Vulvovaginal Candidiasis in relation to gestation period

Table 5: Incidence of Vulvovaginal Candidiasis in relation to Abnormal Vaginal Discharge, Antibiotics use and Occupation

Group	Incidence Rate (%)
Total number examined (105)	
Symptomatic (with discharge)	71(67.62 %)
Asymptomatic (no discharge)	34(32.38 %)
Antibiotic use	69(65.71 %)
Non-antibiotic use	36(34.29 %)
Infection based on Occupation	
Civil Servants	15(14.28 %)
Business Women	38(36.19 %)
Full time housewives	42(40.0 %)
Others	10(9.52 %)

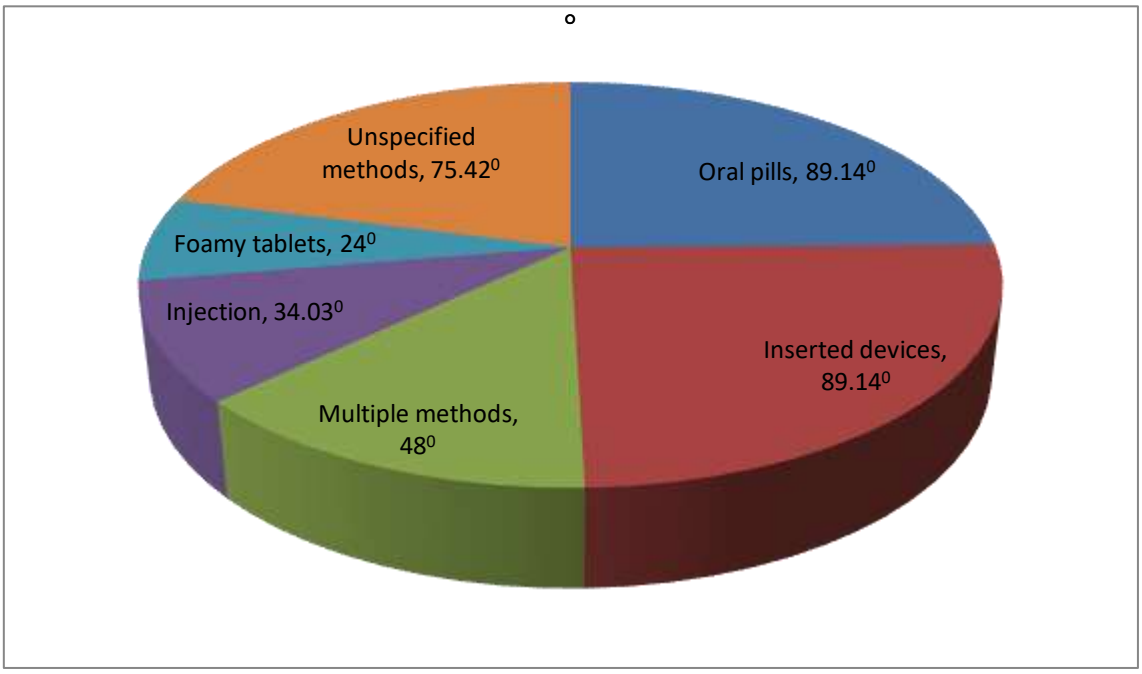


Figure 5: Types of Family Planning Methods used by the Infected Women

Table 6: Relationship between *Candida* vulvovaginitis and Number of Pregnancies (Gravidae) in Women attending Ante-natal Clinics in the Study Area

Gravidae	Number Examined	Number Infected	Percentage (%)
Primigravidae	79	26	24.76
Multiple gravidae	194	79	75.24
Total	273	105	100

Table 7: Treatment Methods used by the Infected Women

Treatment Methods	Number	Percentage (%)
Douching (salt and hot water)	29	27.62
Oral pills	23	21.90
Topical (antifungal cream)	15	14.29
Oral pills and douching	6	5.71
Oral pills and topical	3	2.86
Traditional herbs	4	3.81
Unspecified Treatment	25	23.81
Total	105	100

4.12 Susceptibility of *Candida* to antifungal agents

Sixty two (59.05 %) out of the one hundred and five isolates were susceptible to ketoconazole (10 μg), while 50 (47.62 %) isolate were susceptible to fluconazole (25 μg) and 56 (53.33 %) isolates were susceptible to itraconazole. Susceptibility based on species shows that out of the eighty six *Candida albicans* isolated, 50 (58.14%) were susceptible to ketoconazole (10 μg), 42 (48.84 %) were susceptible to fluconazole (25 μg) and 47 (54.65 %) were susceptible to itraconazole (10 μg). Analysis further shows that out of 16 isolates *Candida tropicalis*, 11 (68.75 %) were susceptible to ketoconazole (10 μg), 6 (37.50 %) were susceptible to fluconazole (25 μg), while 8 (50.00%) were susceptible to itraconazole (10μg). Out of the three *Candida krusei* isolated, the highest susceptibility was recorded by fluconazole (25 μg) that is two isolates (66.66%). Susceptibility to ketoconazole (10μg) and itraconazole (10μg) was one each (33.33 %). On the whole, 26 (24.76 %) isolates were resistant to all the three drugs, 19 (18.09 %) were susceptible to one drug, 22 (20.95 %) were susceptible to two drugs, while 38 (36.20 %) were potent to the three drugs tested (Plate VI and Table 8).

4.13 Minimum Inhibitory Concentration (MIC)

Thirty three (38.37 %) isolates of *Candida albicans* were inhibited at MIC $\leq$ 8 μg/ml, while 35 (40.69 %) isolates of *Candida albicans* were susceptible dose dependent and 18 (20.93 %) were resistant to fluconazole. For *Candida tropicalis*, 5 (31.25 %) isolates were susceptible, while 8 (50.00 %) were susceptible dose dependent and 3 (18.75 %) isolates were resistant to fluconazole. Itraconazole had its highest potency to 39 (45.34 %) isolates at MIC 0.125 μg/ml agaist *Candida albicans*. The MIC range for ketoconazole against *Candida albicans* was susceptible for 50 (58.14 %) isolates and 23(26.76 %) isolates at MIC $\leq$ 0.25 – 0.5 μg/ml while 13 (15.12 %) isolates had MIC range between 1-32 μg/ml. For *Candida tropicalis*, 5 (31.25 %) of the isolates were susceptible to Fluconazole at MIC $\leq$ 8 μg/ml, 8 (50.00 %) were susceptible dose dependent while 3 (18.75 %) were resistant. Ketoconazole on the other hand shows no susceptible at MIC $\leq$ 0.125 μg/ml but was susceptible dose dependent for 8 (50.00 %) isolates and also resistant for 8 (50.00%) respectively. Susceptibility to Itraconazole was 5 (31.25 %) at MIC 0.125 μg/ml and resistant for 11 (68.75%) isolates MIC 8-32 μg/ml. For *Candida Krusei* only 1 (33.33%) isolate was susceptible to fluconazole at MIC 0.5 μg/ml and one isolate (33.33%) to itraconazole. Two isolates (66.66%) of *Candida Krusei* had MIC of 0.125 μg/ml for ketoconazole (Table 9).

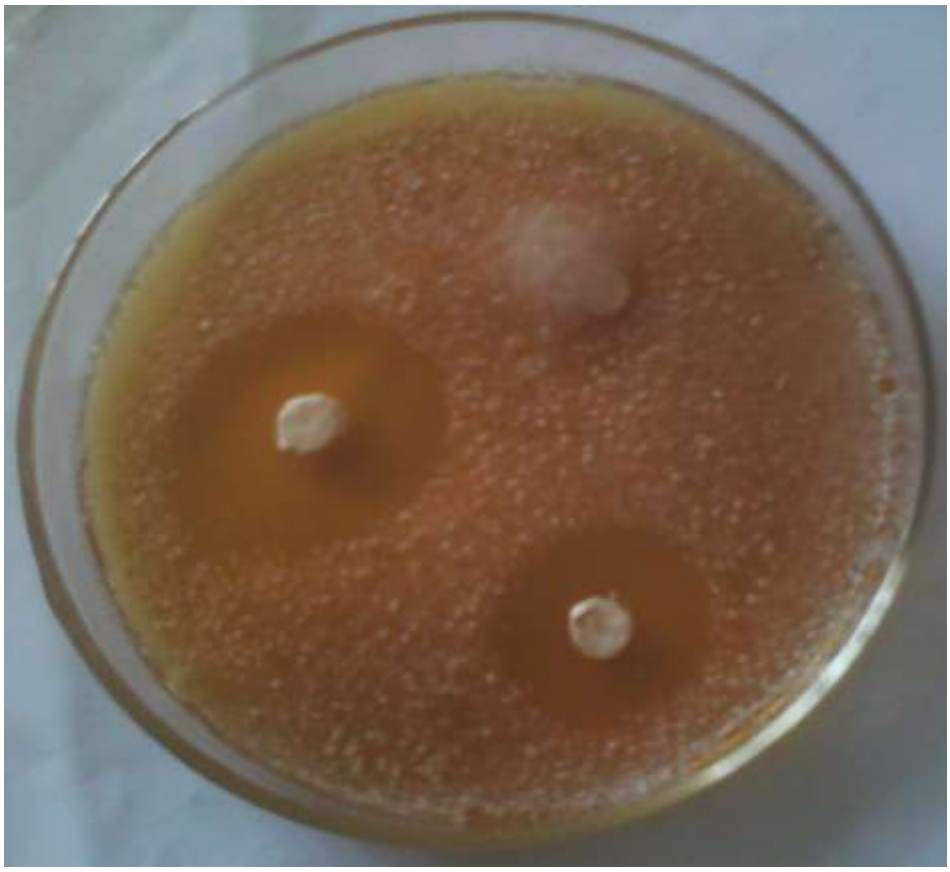


Plate VI: Susceptibility test of three antifungal drugs: Ketoconazole, itraconazole and fluconazole on *Candida albicans*.

Table 8: *In vitro* Susceptibilities for 105 Clinical *Candida* species Isolates by Calibrated Dichotomous Sensitivity Method

Organism	Antifungal	Disc Potency (µg)	Accepted Range (mm)	Susceptibility	Resistant
Candida albicans n = 86	Ketoconazole	10µg	8.6 – 12.4	50 (58.14%)	36 (41.86%)
	Fluconazole	25µg	7.4 – 10.0	42 (48.84%)	44 (51.16%)
	Itraconazole	10µg	5.1 – 7.5	47 (54.65%)	39 (45.35%)
Candida tropicalis n = 16	Ketoconazole	10µg	8.6 – 12.4	11(68.75%)	5 (31.25%)
	Fluconazole	25µg	7.4 – 10.0	6 (37.50%)	10 (62.50%)
	Itraconazole	10µg	5.1 – 7.5	8 (50.00%)	8 (50.00%)
Candida krusei n = 3	Ketoconazole	10µg	8.6 – 12.4	1(33.33%)	2 (66.66%)
	Fluconazole	25µg	7.4 – 10.0	2 (66.66%)	1 (33.33%)
	Itraconazole	10µg	5.1 – 7.5	1 (33.33%)	2 (66.66%)

Table 9: MIC Range for Antifungal Agents against *Candida* species

Candida species	Antifungal Agent	Number of Isolates with MIC (μg/ml) of											Susceptibility	Susceptible Dose Dependent	Resistant (%)
		0.125	0.25	0.5	1	2	4	8	16	32	64	128			
Candida albicans n = 86	Fluconazole	-	-	2	6	7	9	9	6	29	8	10	33(38.37%)	35(40.69%)	18(20.93%)
	Ketoconazole	50	8	15	2	3	1	2	2	3	-	-	50(58.14%)	23(26.74%)	13(15.12%)
	Itraconazole	39	12	20	1	2	4	2	4	2	-	-	39(45.25%)	32(37.21%)	15(17. 44%)
Candida tropicalis n = 16	Fluconazole	-	-	0	0	0	1	4	2	6	0	3	5(31.25%)	8(50.00%)	3(18.75%)
	Ketoconazole	0	2	6	0	0	2	4	0	2	-	-	0 (0.00%)	8(50.00%)	8(50.00%)
	Itraconazole	5	0	0	0	0	0	1	2	8	-	-	5(31.25%)	0 (0.00%)	11(68.75%)
Candida krusei n = 3	Fluconazole	-	-	1	0	0	0	0	0	0	2	0	1(33.33%)	0 (0.00%)	2(66.66%)
	Ketoconazole	2	0	0	0	0	0	0	0	1	-	-	2(66.66%)	0 (0.00%)	1(33.33%)
	Itraconazole	1	0	0	0	0	1	0	0	1	-	-	1(33.33%)	0 (0.00%)	2(66.66%)

CHAPTER FIVE

5.0 DISCUSSION

In this study, only 18.31 % of the HVs revealed the presence of yeast cell as against 38.46 % when cultured. Other cells seen in the wet mount include epithelial cells which was classified as scanty 15.02 %, moderate 54.58 % abundance 30.40 % pus cell 5.49 % and red blood cells 10.6 % respectively. Faraji *et al.* (2012) reported 9 % against 29 % when direct microscopy and culture was used for screening the presence of yeast cells, stating that culture is more sensitive than direct microscopy of wet mount. Furthermore, Aslam *et al.* (2008) revealed that 48 % of *Candida* was identified when culture was used against 38 % when gram stained smears / KOH wet mount was used.

The two hundred and seventy three (273) high vaginal swabs were cultured on various media, morphology of isolates along with biochemical test enable the identification to species level of all positive isolates; for both yeast and bacteria isolated. (*Candida* on Sabouraud's Dextrose agar).

The prevalence of vulvovaginal candidiasis among the pregnant women attending antenatal clinics in this study was 38.46 % . This is in agreement with Paveen *et al.* (2008) who recorded prevalence of 38 % among pregnant women in Pakistan. Similarly, Aboyeji and Nwabuisi (2003) in a randomize screening of asymptomatic pregnant women attending antenatal clinic at University of Ilorin Teaching Hospital, Nigeria recorded an incidence of 37.8 %. Nwadioha *et al.,* (2010) recorded prevalence of 40 % among pregnant women attending primary health care clinics in Jos, Nigeria. Aslam *et al.* (2008) recorded a slightly higher incidence of 48 % among pregnant women attending antenatal clinics in Pakistan. The result is however in contrast to a higher prevalence of 62.2 % as reported by Akah *et al.* (2010) among pregnant women in a rural community in Enugu, Nigeria. Oyewole *et al.* (2013) also reported a higher incidence of 70 % among pregnant women attending antenatal clinic at Federal University of Technology, Minna, Nigeria. A lower prevalence of 28 % was recorded among pregnant women in Peru (Garcia *et al.,* 2004). Moaledmohseni *et al.* (2012) also recorded a lower prevalence of 26.4 % in Iran. However, taking into consideration of individual health facilities in the study, Federal Medical Center, Yola and Girei Local Government Clinic, Sangere had higher incidence rate of 60.66 % and 61.76 % respectively which is higher than that of Specialist Hospital Yola with occurance of 31.06 %. The variation among the health facilities may be linked to certain factors such

as level of awareness been created before sample collection. Indecisive and shyness over the subject matter. In the case of Federal Medical Centre, Yola, participants were made up of pregnant women who had either vaginal discharge, itching or burning sensation (symptomatic). The high incidence of vulvovaginal candidiasis recorded among the subjects in Girei Local Government Clinic, Sangere, may be due to the high number of the subjects with symptoms or signs of vulvovaginalis candidiasis to make themselves available either secretly or openly after health talk.

Most clinical laboratories identify isolates using germ tube production which is regarded as presumptive test for *Candida albicans*. This is due to factors such as high cost for media, equipments and lack of expertise. Furthermore most of such test are expensive for people in developing countries (Bhavan *et al.,* 2010). In view of these, yeast identification is often limited to *Candida albicans* or non *albicans* (Nwadioha *et al.,* 2010). Identification of yeast to species level is important particularly when clinical microbiology are concerned (Oliveira *et al.,* 2006). Bhavan *et al.,* (2010) recommended that test for identification to species level to include germ tube test, chlamydospore formation, sugar assimilation and fermentation as well as the use of chromogenic agar.

Three *Candida* species were identified from a total of 105 *Candida* isolates during this study. The most frequent isolated *Candida* species was *Candida albicans* (81.90 %), followed by *Candida tropicalis* (15.24 %) and *Candida krusei* (2.86 %). Oyewole *et al.* (2013) isolated 42 *Candida*, out of a total of 60 isolates from pregnant women attending antenatal clinic at Federal University of Technology, Minna, Nigeria, out of the five *Candida* species isolated, *Candida albicans* was the most prevalent (50 %) which was lower than that obtained in this study. Other species were *Candida glabrata* (21.4 %), *Candida tropicalis* (14.3 %), *Candida krusei* (11.9 %) and *Candida pseudotropicalis* (2.4 %) respectively. Furthermore, lower prevalence of 17.5 % was recorded by Agbakoba *et al.* (2008) among pregnant women in Ibadan, Nigeria. Alo *et al.* (2012) also recorded 27.47 % prevalence among pregnant women in South-Eastern Nigeria. Pregnancy is a physiological state which produces several normal and expected changes in all the maternal organ systems. Vaginal secretions falls from a pH greater than 7 (alkaline) to pH 4 or 5 (acidic pH). This change makes the vagina resistance to bacterial invasion during pregnancy, but favours the growth of *Candida albicans.* Hence, *Candida* infection occurs more frequently in pregnant women (Plitteri, 2007).

Nwadioha *et al.,* (2010) isolated 84 % *Candida albicans* in a study carried out in Jos, Nigeria, while the remaining 16 % were attributed to non *Candida albicans* species.

This is in agreement with the findings in this study. Similarly, Faraji *et al.* (2012) carried out a study among Iranian pregnant women with candidiasis and noted that prevalence due to *Candida albicans* was 70 %, *Candida glabrata* 12.5 % and *Candida parapsilosis,* 5 %. Roberts *et al.* (2011) in a study carried out among pregnant women in Australia attributed 73 % vaginal colonization to *Candida albicans,* 3.1 % each of *Candida krusei* and *Candida tropicalis* the rest was distributed among other species. The reason for the dominance of *Candida albicans* over the other *Candida* species is because, it is the most abundant species in the environment and hence more in the human body and have the ability to survive in a depressed immune system (Nwadioha *et al.,* 2010). The first step in establishing a yeast infection is its ability to bond to the vaginal mucosa, thus *Candida albicans* may be more adhesive than non-*Candida albicans* species (Grigoriou *et al.,* 2006).

The distribution in relation to age showed that age group 21 – 30 years had the highest overall prevalence (25.64 %) and all the three species of *Candida* isolated in this study. Parveen *et al.* (2008) also recorded peak infection among pregnant women in age group 18 – 30 years. Oyewole *et al.* (2013) noted the peak infection of 59.5 % in the age group 21 – 30 years among pregnant women attending antenatal clinic at the Federal University of Technology, Minna, Nigeria. While, Moaledmohseni *et al.,* (2012) recorded 26.4 % prevalence of candidiasis among pregnant women with cervicovaginal infection in Iran. Roberts *et al.,* (2011) revealed that 72(73 %) of the pregnant women with candidiasis were colonized with *Candida albicans* with the remaining been distributed among non-*albicans* species. While age bracket 18 – 30 years suffered significantly (Parveen *et al.,* 2008). This is in agreement with this present study. Similarly, Akinbiyi *et al.* (2009) attributed 65.7 % of *Candida* infection to *Candida albicans* in pregnant women in UK and in age group 21 – 30 years, which coincided with this study. Study carried out by El-Din *et al.,* (2001) found the presence of *Candida* in vaginal washing, HVS and rectal swab. Citing *Candida albicans* as been common in rectal swabs. This may be a possible reason for the high prevalence of *Candida albicans* in this study where the common practice of clean up after using toilet is washing. Kamath *et al.,* (2013) in a study carried out to determine the risk of vagina candidiasis among pregnant women in a rural community in India noted prevalence rate of 52.8 % in age group 26 – 33 years.

According to Parveen *et al.,* (2008) the high prevalence associated with this age group in Pakistan may be attributed to factors such as early marriage, personal hygiene, high

sexual activity and inadequate information about *Candida* species. Parveen *et al.* (2008) stated that developing countries have higher prevalence than developed countries where public awareness of female hygiene and contraception is more pronounced.

Garcia *et al.* (2004) in a study of vagina candidiasis in pregnant women in Peru, reported high prevalence due to *Candida albicans* in more than 90 % of the cases studied. In a study conducted in UK among asymptomatic pregnant women, prevalence of *Candida albicans* was 12.5 % and the highest percentage 65.7 % was in the age group 21 – 30 years (Akinbiyi *et al.,* 2009). A significant relationship of a Chi–square calculated value of 34.00 at $P \leq 0.05$ was recorded for relationship between infection and age (Appendix 8).

Various microbial organisms were isolated from pregnant women in this study, *Candida* species accounted for 38.46 %, *Staphylococcus aureus* 6.23 %, *Escherichia coli* 1.09 %, *Klebsiella aerogenes* 2.93 %, while *Streptococcus agalatiae* and *Proteus vulgaris* accounted for 0.37 % each. A total of 135 organisms were isolated with prevalence of 49.44 %. Pregnant women in age bracket 21 – 30 years had the highest percentage of isolates, 32.60 %, while the least percentage of isolates was 0.36 % (Table 4). Studies have shown an increased level of vaginal pathogens during pregnancy due to increase in vaginal discharge with about 35.5 % of women having one type of cervico vaginal infection or another (Moaledmohseni *et al.,* 2012). Agbakoba *et al.,* (2008) isolated various vaginal pathogens from pregnant women with *Candida albicans* having the prevalence of 17.5 %, *Escherichia coli* 8.8 %, *Staphylococcus aureus* 3.7 %, attributing the increase in pathogens was to high oestrogen level during pregnancy, hence increasing the susceptibility of the vagina to microorganisms. Ilusanye *et al.,* (2012), isolated *Staphylococcus aureus* as the predominant organisms from pregnant women, accounting for 42.2 %. This agrees with the present study with *Staphylococcus aureus* as the second predominant isolated next only to *Candida*.

Mixed infection of organisms was observed in eight (8) of the cases studied. Co-infection with *Candida* and *Staphylococcus aureus* was 6 (2.19 %), *Staphylococcus aureus* and *Klebsiella aerogenes* 1 (0.37 %), co-infection with *Streptococcus agalactiae* and *Candida* 1 (0.37 %) respectively. Overall prevalence of co-infection was 2.93 %. Moaledmohseni *et al.* (2012) reported mixed infection between candidiasis and bacteria vaginosis with prevalence of 2.7 % among pregnant women in Iran. This is in agreement with the prevalence found in this study. Okonkwo *et al.* (2009) revealed the frequency of mixed infection between *Klebsiella* and *Staphylococcus* species among

pregnant women studied in Ibadan, Nigeria with prevalence of 5.3 %. Alo *et al.* (2012) recorded a higher prevalence of co-infection between candidiasis and trichomoniasis (21.73 %). Variation may be attributed to socio-economic status, ethnicity of the subjects and settings of the study among other factors (Al-Sibian, 2010).

In regards to frequency of reoccurrence, women experiencing the infection for the second time had the highest prevalence of 39.05 %, while those having three or more reoccurrence were next with prevalence of 32.38 % and lastly by those with first time occurrence 28.57 %. According to Foxman, (1990) one-fourth of women with urinary tract infection will have a second infection. Risk of second infection is greater than the first since the first infection sets the stage for the recurring infection. Parveen *et al.* (2008) stated that a proportion of women with recurrent infection may have their first infection during pregnancy. Recurrence occurs in 40–50% of cases (MacNeill, 2001).

Prevalence of occurrence of vaginal candidiasis in respect to gestation (pregnancy) period, shows that women in their second trimester were the most infected (48.57 %) which was slightly higher than those in their third trimester 40.00 %, while those in their first trimester had the least prevalence of 11.43 %. This result agrees with the findings of 61 %, 21.4 % and 16.7 % recorded in the second, third and first trimester respectively as reported by Oyewole *et al.* (2013) which was attributed to the foetus demand for nutrient. Lower percentage of occurrence of 32.41 %, 30.0 % and 20.41 % was recorded in the third, second and first trimester respectively by Alo *et al.* (2012). Okonkwo *et al.* (2009) recorded prevalence of 55.1 % and 41.4 % in the third and second trimester, while no prevalence was associated with the first trimester. This was attributed to the fact that incidence of *Candida* is twice commonly isolated in pregnant women than non-pregnant women due to increased hormonal level which affects glycogen content and normal vaginal floral making it more conducive for *Candida* (Fora *et al.,* 1997).

A high prevalence of 69 (65.71 %) was recorded by antibiotic users in this study against 36 (34.29 %) non-users. The use of antibiotics especially broad spectrum agents such as tetracycline and ampicillin-like agents predisposes a person to *Candida* by reducing the number of protective resident vagina bacteria (Omnia, 2009). Nwadioha *et al.,* (2010) recorded a lower incidence of 16 %, stating that broad spectrum antibiotics posses a risk of infection.

Infection base on occupations shows that civil servants were less infected than full time house wives and business women. This may be associated with personal hygiene and low socioeconomic status (Moaledmohseni *et al.,* 2012).

Women who used oral contraceptives have significantly higher risks of vulvovaginal candidiasis especially those with high oestrogen contents (Nabhan, 2006). Out of the 105 subjects, 83 (284.58^0) of the women in this study indicated the use of one form of family planning method or another, while 22 (75.42^0) did not specify any birth control method. Faraji *et al.* (2012) reported that 60 % of pregnant women infected in their study indicated the use of contraceptive against 40 % who do not use contraceptives. Oral pills and inserted devices were the predominant contraceptive used by pregnant women in this study. Intercourse with the use of a diaphragm and spermicidals increased the rate of *Candida* colonization (Monif, 1985). An increase in the risk of infection has been reported with the use of vaginal contraceptive sponge and intrauterine contraceptive devices (Sobel *et al.,* 1998). Other risk factor includes habit of washing from front to back after using toilet, the use of scented laundry soap to clean the perineum. The use of synthetic undergarment enhances the chances of acquiring *Candida* yeast infection (Kamath *et al.,* 2013).

The highest prevalence of vulvovaginal candidiasis was found in multigravidae 79 (75.24 %) than in primigravidae 26 (24.76 %). Multigravidae suffer more than primigravidae in this study. Aslam *et al.* (2008) reported prevalence of 60 % against 40 % in respect to multigravidae and primigravidae respectively in Pakistan. Similarly, Parveen *et al.,* (2008) stated that younger age group 18 – 30 years and multigravidae suffered significantly more. This was attributed to early marriage in the population, hence the women become multipara by age 30 years. Oyewole *et al.* (2013) also reported high prevalence of 59.5 % in multigravidae and 40.5 % in primigravidae. The high prevalence may be due to the use of contraceptive and antibiotic (Nwadioha *et al.,* 2010). Early marriage was observed in this study among the infected women with the youngest in the study been 17 years old and may become multi gravity by age 21 – 30 years. Kamath *et al.* (2013) also observed the same pattern in Parkistan and described multipara as risk factor.

Vaginal candidiasis is often associated with symptoms such as vaginal discharge, itching, burning and irritation. In order to find relief, various therapeutic methods are employed. In this present study, 23 (21.90 %) of the infected women stated the use of oral pills for treatment while a good percentage used douching, 23.81 % did

not specify the use of any treatment method. The distribution pattern may be associated with ignorance, poverty and inadequate knowledge on *Candida* infection. Jombo *et al.* (2011) in a study conducted in Gboko, Nigeria revealed that 94.1 % did not know candidiasis as a diseases, 95.4 % could not list a single urinogenital symptom unique to candidiasis while 83.6 % did not see the need for treatment. Similar trend was observed among the subjects in this study, hence the reason for low percentage recorded for the use of orthodox drugs for treatment: 2.85 % and 5.71 % for oral pills - topical cream and a combination of oral pills - douching respectively.

According to Robert *et al.* (2011), there is a connection between infection and preterm birth, while Derrick and Adrienne (2009) also stated the risk of cleft palate in children born by infected mothers with urinary track infection. Hence, the need to treat infection before pregnancy so as to avoid passing infection to the unborn child and infecting one's partner In this present study, two subjects, their partners and a daughter each were infected with the same species of *Candida*. One of the subject also reported two cases of pretermed birth after been infected.

The efficacy of ketoconazole, itraconazole and fluconazole to 105 vaginal *Candida* species isolates from pregnant women. *Candida albicans* 50 (58.14 %) was sensitive to ketoconazole (10 μg) while 11 (68.75 %) isolates of *Candida tropicalis* were also susceptible to ketoconazole. However only one isolate of *Candida krusei* (33.33 %) was susceptible to ketoconazole. Grigoriou *et al.,* (2005) reported 91.4 % sensitivity of *Candida* species to ketoconazole which is in agreement with the high efficacy of ketoconazole to *Candida* species in this study. Zarei, (2013) also reported a high activity of 73.2 % of ketoconazole to *Candida* species. Karuppayil *et al.,* (2012) in a study carried out in India reported that only one isolate was found resistant to ketoconazole sensitivity to fluconazole by *Candida albicans* in this study was 42 (48.84 %), (66.66 %) of *Candida krusei* however were sensitive to fluconazole (25 μg). Zarei *et al.,* (2013) reported a low sensitivity (12.9 %) by *Candida* isolates to fluconazole. Contrary to this study, Al-Mamani *et al.,* (2014) reported that of the 93 *Candida albicans* in their study, none was susceptible to fluconazole. Itraconazole was active against *Candida albicans* and *Candida tropicalis* 54.65 % and 50.00 %. This is in agreement with Zarie *et al.,* (2013) who reported that no isolate of *Candida* species only susceptible dose dependent 91.4 % to itraconazole.

Minimum inhibitory concentration showed that 50 (58.14 %) isolates of *Candida albicans* were inhibited at MIC 0.125 μg/ml while 26.74 % were susceptible

dose dependent. The highest resistant exhibited against ketoconazole was 50.00 % at MIC $\geq$ 4 μg/ml by isolates of *Candida tropicalis*. Similarly, *karuppayil et al.*, (2012) recorded 22 % resistant and 55 % susceptibility dose dependent by *Candida* isolates. Silva *et al.,* (2002) reported a high MIC of $\geq$ 32 μg/ml by ketoconazole for all *Candida* strains. The high MIC is due to a phenomenon called cross resistance. Cases of treatment failure has been reported Karuppayi *et al*., (2012). In this current study, 38.37 % and 40.69 % of *Candida albicans* isolates were susceptible and susceptible dose dependent at MIC $\leq$ 8 μg/ml and $\geq$ 16 μg/ml to fluconazole. No isolate was susceptible to fluconazole at MIC < 0.25 μg/ml, while 20.93 % isolates of *Candida albicans* and 66.66 % *Candida krusei* had MIC of 64 – 128 μg/ml respectively Fouzia and Beqai, (2010) reported an MIC of 64 μg/ml as having the maximum sensitivity against *Candida* species using fluconazole. Which is similarly to this study where increase in dosage lead to increase in susceptibility of the *Candida* species to the antifungal agents. Although fluconazole remains a drug of choice, in many regions, however susceptible dose dependent strains may acquire resistance due to repeated exposure (Karuppayil *et al.,* 2012). Itraconazole was active against *Candida albicans* with MIC of 0.125 μg/ml for 39 (45.34 %) isolates. Susceptibility of *Candida tropicalis* (31.25 %) and *Candida krusei* to Itraconazole (33.33 %). Itraconazole is among the first line antifungal drugs with wide spectrum of efficacy against *Candida* species (Nelson *et al.,* 2013). Resistance to Itraconazole by *Candida albicans* in the study was 17.44 % with 37.21 % been susceptibility dose dependent at MIC 0.25-0.5 μg/ml. Nelson *et al.,* (2013) reported 100% susceptibility of *Candida krusei* to Itraconazole which is higher than prophylactic use of azole drugs in treatment of *Candida* infection may be associated with the development of resistant Itraconazole (Nelson *et al.,* 2013). The resistance may be attributed to certain factors such as indiscriminate use of antibiotics, anti fungal drugs, socio-economic status, long history of urinary tract infection and vagina douching (Obiogbolu *et al*., 2009; Nwadioha *et al*., 2010).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

This research was aimed at finding the prevalence of vulvovaginal candidiasis among pregnant women attending ante-natal clinics in Yola and its environs. The study also aimed at finding the most effective drug against the infection.

A total of 105 isolates of *Candida* species were identified from pregnant women from the three health centres; Federal Medical Centre, Yola, 20 isolates, Specialist Hospital, Yola 64 isolates and Girei Local Government Clinic, Sangere 21 isolates. Three species of *Candida* were identified, *Candida albicans* 86 (81.90 %), *Candida tropicalis* 16 (15.24 %) and *Candida krusei* 3 (2.84 %). The prevalence of *Candida* in the study was 38.46 %. The highest prevalence of *Candida* was in age group 21 – 30 years (25.64 %), followed by 17 – 20 years (6.98 %), 31 – 40 years (5.49 %) and 41 – 50 years (0.37 %). A total of thirty bacteria were isolated in the study. Mixed infection of organisms was observed in eight of the cases comprising of *Staphylococcus aureus* and *Candida* 6 (2.19%), *Staphylococcus aureus* and *Klebsiella aerogenes* 1 (0.37 %) and *Streptococcus agalactiae* and *Candida* 1 (0.37 %).

Only 28.57 % of the infected women reported as first case with vulvovaginal candidiasis, 39.05 % and 32.38 % of the women were re-infected for the first and second or more times respectively. Women in their second trimester had the highest prevalence, followed by those in their third and first trimester with 48.57 %, 40.0 % and 11.43 % respectively. Vulvovaginal symptoms with discharge had high prevalence than in asymptomatic women. Full time housewives and business women had higher prevalence of infection than civil servants. On the other hand, women who used inserted devices and oral pills as means of birth control had higher prevalence than those who used injection. Multigravidae had higher prevalence than primigravidae in this study. Most of the subjects used douching and oral pills as a means of treatment for vulvovaginal candidiasis. In terms of susceptibility, 62 (59.05 %) isolates were susceptible to ketoconazole, 56 (53.33 %) of the isolates were susceptible to itraconazole while 50 (47.62 %) isolates were susceptible to fluconazole. On the whole, 38 (36.20 %) of the isolates were susceptible to all the three drugs tested.

In terms of MIC however, Ketoconazole was susceptible to 52 isolates of *Candida* species at 0.125 μg/ml but susceptible dose dependent to 31 at 0.25 – 0.5 μg/ml isolates of *Candida* species while itraconazole was potent at 0.125 μg/ml for 45 isolates.

6.2 Conclusion

This current study reveals a high prefonderance of occurrence of vulvovaginal candidiasis among pregnant women attending antenatal clinics in Yola. Speciation of *Candida* was accomplished within 48 hours using Bromocresol green agar against three weeks using traditional biochemical test methods. The predominant *Candida* species isolated was *Candida albicans* 81.90% followed by *Candida tropicalis* 15.24% and *Candida Krusei* 2.86%. Ketoconazole showed the highest potency to 62 (59.05%) isolates 56 (53.33%) isolates were susceptible to itraconazole while 50 (47.62%) were susceptible to fluconazole. MIC for Ketoconazole was 0.125 μg/ml for 50 isolates of *Candida albicans* while Itraconazole also showed a low MIC at 0.125 μg/ml for 39 *Candida albicans* isolates. The work further shows that combination of species identification and susceptibility test not only provide specificity only but also facilitates precise therapeutic drug for individual isolates hence reducing treatment failure and therapeutic drug resistant. Furthermore this will help to reduce incidence of recurrent infection. Preterm birth and transmission of infection from mother to foetus. This research will also provide base line epidemiological data since no research has been reported in relation to Vulvovaginitis due to *Candida* species in Yola.

6.3 Recommendations

Based on the result of the study, the following are recommended to ensure preventive and effective management of *Candidiasis*.

1. Pregnant women and women in general should be educated on proper personal hygiene by ensuring regular bathing with mild soap and clean water.
2. Pregnant women should be oriented on signs and symptoms associated with vulvovaginal candidiasis during health talk such as vaginal itching, curdy discharge, swollen virgina and vulva, pain during sex right from the unset of pregnancy, as some view it as normal signs associated with pregnancy and pay no attention to it due to ignorance until it becomes problematic.
3. Women should be educated on predisposing factors associated with the infection such as early sex due to early marriage as observed in this study with infection rate of 6.95% in pregnant women between the age brackets 17-20 years,
4. Indiscriminate use of antibiotics, oral contraceptive, intrauterine devices, vagina douching, tight clothing and using synthetic under wears should be avoided.

5. Indiscriminate use of over the counter antifungal agents and under dosage should be avoided.
6. Proper medical attention should be sought as soon as signs and symptoms are observed.
7. Sensitivity test should be carried out to ensure that the right therapies are given.
8. Pregnant women attending antenatal clinic should be screened for the presence of *Candida*.

Suggested Areas for Further Studies:

1. Comparative study of *Candida* vulvovaginatis between singles ladies and pregnant women in the study area.
2. Comparative study of *Candida* vulvovaginatis between male and female.
3. Efficacy of medicinal plants found in the study area against *Candida* species.

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APPENDIX 1

APPENDIX 2

APPENDIX 3

APPENDIX 4

APPENDIX 5: QUESTIONNAIRE ON THE DEMOGRAPHY OF THE SUBJECTS

Instruction: Please tick the correct box

Name of Health Center: …….

1. Age

   Less than 20 { }, 21 – 30 { }, 31 – 40 { }, 41 – 50 { }, 51 and above { }

2. Occupation

   Civil Servant { }, Business { }, Full Time Housewife { }, Others { }

3. Pregnancy Stage (Month)

   1 – 3 { }, 4 – 6 { }, 7 – 9 { }

4. Number of Pregnancies

   First { }, Second { }, Third { }, Fourth { }, Fifth { },Sixth { } Above six { }

5. Type of Family Planning

   Oral Pills { }, Injections { }, Insertion e.g loop { }, Foaming Tablets { },
   Others { }, Never { }.

6. Are you on Medication

   Yes { }, No { }

7. Type of Medication

   Antibiotics { }, Steroids { }, Insulin { }, Others { }

8. Reason for Medication

   Diabetes { }, Stress { }, Others { }

9. First Infection { }, Second { }, Third { }, Fourth { } with abnormal discharge?

   Yes { }, No { }.

10. Previous Treatment of Vaginal Infection.

    Never { }, One { }, Twice { }, Three times and above { }

11. Drug Used

    Oral Pills { }, Cream { }, Douching { }, Others { }

12. Who Prescribed the Drugs?

    Self { }, Clinic { }, Friends { }

13. Is your partner infected?

    Yes { }, No { }.

14. Is any of your child infected?

    Yes { }, No { }.

APPENDIX 6

Morphology and Biochemical Identification of the *Candida* Isolates

	Gram Reaction	Germ Tube	Chlamydospore Prodution	Sugar Fermentation						Sugar Assimilation						Presumptive Identification
				Glucose	Sucrose	Lactose	Galactose	Maltose	Trahalose	Glucose	Sucrose	Lactose	Galactose	Maltose	Trahalose	
1.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
2.	+	-	-	+	-	-	+	+	---	+	+	-	+	+	_	*C. tropicalis*
3.	+	+	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
4.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
5.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
6.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
7.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
8.	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	*C. krusei*
9.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
10.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
11.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
12.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
13.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
14.	+	-	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
15.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
16.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
17.	+	+	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*

18.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
19.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
20.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
21.	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	*C. krusei*
22.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
23.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
24.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
25.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
26.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
27.	+	-	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
28.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
29.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
30.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
31.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
32.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
33.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
34.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
35.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
36.	+	+	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
37.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
38.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
39.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
40.	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	*Candida krusei*

41.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
42.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
43.	+	+	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
44.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
45.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
46.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
47.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
48.	+	-	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
49.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
50.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
51.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
52.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
53.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
54.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
55.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
56.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
57.	+	-	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
58.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
59.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
60.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
61.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
62.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
63.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*

64.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
65.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
66.	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	*Candida albicans*
67.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
68.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
69.	+	-	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
70.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
71.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
72.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
73.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
74.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
75.	+	-	-	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
76.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
77.	+	-	+	+	+(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*
78.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
79.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
80.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
81.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
82.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
83.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	-	*Candida albicans*
84.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
85.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
86.	+	-	-	+	-(delays)	-	+	+	-	+	+	-	+	+	-	*C. tropicalis*

87.	+	-	-	+	+(delays)	-	-	+	-	+	+	-	+	+	-	*C. tropicalis*
88.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
89.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
90.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
91.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
92.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
93.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
94.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
95.	+	-	+	+	-	-	-	+	-	+	+	-	+	+	-	*C. tropicalis*
96.	+	+	+	+	+(delays)	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
97.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
98.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
99.	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	*Candida albicans*
100.	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	*Candida albicans*
101.	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	*Candida albicans*
102.	+	-	-	+	-	-	+	+	-	+	-	-	+	+	-	*C. tropicalis*
103.	+	+	+	+	+(delays)	-	+	+	+	+	+	-	+	+	+	*Candida albicans*
104.	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	*Candida albicans*
105.	+	+	+	+	-		+	+	+	+	+	-	+	+	+	*Candida albicans*
					-											

Keys: + Positive - Negative D - Delay in reaction (slow)

APPENDIX 7

Morphological and Biochemical Characterization of the Bacteria

Isolate Code	Gram Reaction	Shape	Catalase	Coagulase	Indole	Methyl red	Voges Proskauer	Urease	Citrate	Kliger's Iron Agar				Identification
										Slope	Butt	Hydrogen Sulphate	Gas Production	
XA_1	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XB_2	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XC_3	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XD_4	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XE_5	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XF_6	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XG_7	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XH_8	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XI_9	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XJ_{10}	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XK_{11}	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XL_{12}	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XM_{13}	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XN_{14}	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XO_{15}	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XP_{16}	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
XQ_{17}	+	Cocci	+	+	-	+	+	-	-	Y	Y	-	+	*S. aureus*
MD_1	-	Rod	-	-	-	-	+	+(slow)	+	R	Y	-	+	*Klebsiella*
MD_2	-	Rod	-	-	-	-	+	+	+	R	Y	-	+	*Klebsiella*
MD_3	-	Rod	-	-	-	-	+	+	+	R	Y	-	+	*Klebsiella*
MD_4	-	Rod	-	-	-	-	+	+	+	R	Y	-	+	*Klebsiella*
MD_5	-	Rod	-	-	-	-	+	+	+	Y	Y	-	+	*Klebsiella*
MD_6	-	Rod	-	-	-	-	+	+	+	R	Y	-	+	*Klebsiella*
MD_7	-	Rod	-	-	-	-	+	+	+	R	Y	-	+	*Klebsiella*
MD_8	-	Rod	-	-	-	-	+	+	+	R	Y	-	+	*Klebsiella*
KB_1	+	Diplococci	-	-	-	+	+	+	-	Y	Y	-	+	*Streptococcus*
LA_1	-	Rod	-	-	+	+	-	-	-	Y	Y	-	+	*E. coli*
LA_2	-	Rod	-	-	+	+	-	-	-	Y	Y	-	+	*E. coli*
LA_3	-	Rod	-	-	+	+	-	-	-	Y	Y	-	+	*E. coli*
GB_1	-	Straight rod	-	-	+	+ (slow)	-	+	-	R	Y	+	+	*Proteus vulgaris*

KEY: + = Positive, - = Negative, Y = Yellow (acidity), R = Red (alkaline).

APPENDIX 8

Chi-square Test for Infection with *Candida* vulvovaginitis in relation to Age in the Study Area among the Women attending Antenatal Clinic

Age in Years	Uninfected		Infection with *Candida*			Infection with *Candida* only		
	Observed (O)	Expected (E)	Observed (O)	Expected (E)	Total	O – E	$(O - E)^2$	$\frac{(O - E)^2}{E}$
11 – 20	99	72.61	19	45.38	118	26.38	695.9044	15.3350
21 – 30	48	72.61	70	45.38	118	24.62	606.1444	13.3570
31 – 40	11	16.0	15	10	26	5	25	2.5
41 – 50	10	6.76	1	4.23	11	-3.23	10.4329	2.4664
Total	168		105		273			34.0

χ^2_{cal} = 34.0 at degree of freedom 3 and $P \leq 0.05$

χ^2_{tab} = 7.81

Chi-square value shows a significant relationship between age and prevalence of infection.

APPENDIX 9

Antifungal Susceptibility Test for *Candida* species

Species	Classified	Fluconazole	Ketoconazole	Itraconazole
Candida albicans (n = 86)	S	33	50	39
	S-DD	35	23	32
	R	18	13	15
Candida tropicalis (n = 16)	S	5	0	5
	S-DD	8	8	0
	R	3	8	11
Candida krusei (n = 3)	S	1	2	1
	S-DD	0	0	0
	R	2	1	2

Key:

S - Susceptibility.

S-DD - Susceptibility Dose Dependent.

R - Resistant.

MIC Range for:

Fluconazole $\leq$ 8 μg/ml – Susceptible, 16 – 32 μg/ml – Susceptible Dose Dependent, $\geq$ 64 μg/ml – Resistant.

Ketoconazole $\leq$ 0.125 μg/ml – Susceptible, 0.25 – 0.5 μg/ml – Susceptible Dose Dependent, $\geq$ 1 μg/ml – Resistant.

Itraconazole $\leq$ 0.125 μg/ml – Susceptible, 0.25 – 0.5 μg/ml – Susceptible Dose Dependent, $\geq$ 1 μg/ml – Resistant.