CHAPTER ONE

1.1 Introduction

Trichomoniasis has emerged to be one of the most common sexually transmitted infections. It is caused by a pear-shaped protozoan called *Trichomonas vaginalis*. The disease is characterized in female patients by frothy-greenish-foul smelling vaginal discharge accompanied by vulvo-vaginal irritation, dysuria and lower abdominal pains. These symptoms are usually aggravated during menses and pregnancy. Other complications include cystitis, cervicitis and urethritis (Jatau *et al*, 2006).

It has been reported that *Trichomonas vaginalis* causes discomfort and psychosocial distress in infected patients. Trichomoniasis is also reported to be a major cause of concern in obsterics and gynaecology. The disease is primarily transmitted through sexual contact, but contaminated formites such as towels and clothing have been implicated in the transmission. The incidence of trichomoniasis depends on the population screened or examined. Certain factors such as poor personal hygiene, multiple sex partners, low socio-economic status and underdevelopment are associated with high incidence of infection (Crosby *et al.,* 2002).

Trichomonas vaginalis is detectable in vaginal, prostatic or urethral secretions, semen and urine of infected individuals (Jatau *et al*., 2006). The most commonly employed diagnostic methods are: direct microscopic examinations of wet mount preparations and culture techniques. Sensitivity of microscopic observation varies (Lawing *et al.*, 2000).

Candida is a yeast fungus found as part of the muco-cutaneous flora of humans. There are approximately 200 species, among which are *Candida albicans, Candida glabrata,Candida tropicalis, Candida stellatoidea, Candida parapsilosis,Candida catemilata,Candida ciferri, Candida guilliermondii, Candida haemulonii, Candida kefyr and Candida krusei. Candida*

albicans is the most common species found in the vagina of 20-50% of healthy and asymptomatic women (Pam *et al.,* 2012). Non-albicans such as *C. glabrata* and *C. tropicalis* are also found in vaginal specimens. Although *Candida* species occur as normal vaginal flora, opportunistic conditions such as diabetes, pregnancy and other immune depressants in the host enable them to proliferate and cause infection. About 75% of adult women experience at least one episode of vulvovaginal candidiasis during their lifetime among which approximately 40-50% would experience further episodes and 5% will develop reocurrence, with at least three symptomatic episodes in one year (Jombo *et al.,* 2011; Okongbowa *et al.,* 2003). The number of episodes tends to be more in women who are sexually active, pregnant, immunocompromised or on contraceptive pills (Ogunfowaken, 2010).

Candidiasis is often associated with the production of a thick, white/cream/yellow discharge from the vagina tract. This discharge may be watery, often odourless and usually with an accompanying vulvo-vaginal itching and inflammation (Chijioke *et al.*, 2016).

Candida infections usually occur in warm and moist parts of the body. Clothing that is too tight or made of nylon materials that can trap heat and moisture may aggravate to candidiasis. Other predisposing factors such as poor personal hygiene, corticosteroids or immunosuppressive drugs, drug addiction, immunological deficiencies and systematic conditions such as vitamin B deficiency, hypothyroidism and lymphoblastoma supports the growth of *Candida* spp (Priotta and Garland, 2006).

The azole antifungals, such as the triazole fluconazole, interfere with sterol biosynthesis. They inhibit the cytochrome P450 14a-lanosteroldemethylase, encoded by the ERG11 gene, which is part of the ergosterol biosynthetic pathway. Inhibition of ERG11 depletes the ergosterol content of membranes and results in the accumulation of toxic sterol pathway

intermediates which inhibit growth (Akins, 2005; Sanglard and Bille, 2002). Mutations in ERG3, which lower the concentration of ergosterol in the membrane, cause amphotericin B resistance and also confer resistance to azoles. Multiple mechanisms have been explained for azole resistance in *C. albicans*. The drug target, ERG11 can be overexpressed or can develop point mutations that reduce fluconazole binding (Akins, 2005; Sanglard and Bille, 2002; White *et al.*, 1998). Azole-induced *C. albicans* growth inhibition is caused by reduction in the ergosterol content of membranes, and also by the accumulation of toxic ergosterol precursors such as 14a-methylergosta-8,24(28)-dien-3b,6a-diol. If ERG3 is inactivated by mutation, in the presence of fluconazole these cells accumulate the non-toxic sterol 14a-methylfecosterol.

1.2 Background of Study

Trichomonas vaginalis has been recognized as a cosmopolitan parasite of male and female genital tract. Globally, an estimated 180 million people are infected yearly. Most cases of *T. vaginalis* remain undiagnosed as it is currently not a target of sexually transmitted infections control and besides because of its asymptomatic nature in about half of infected men and women (Soper, 2004). Although the disease has been seen as a risk factor for other sexually transmitted agents such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* it has also been linked to one of the predisposing factors to HIV infection, acquired immune deficiency syndrome, and cervical cancers (Akinbo and Mokobia, 2017). Its symptoms are commonly observed in women than in men. *Trichomonas vaginalis* is the most common sexually transmitted parasitic disease in Nigeria; and studies on STPDs remain relatively scanty (Amadi *et al.*, 2013). In addition, knowledge about *T. vaginalis* and trichomoniasis is hugely lacking; and this identified gap may have impacted on the burden of infection (Omorodion, 2018).

Recent estimated burden of candida in Nigeria is 9,284 (6/100,000) (Oladele and Denning, 2014). Vulvovaginal candidiasis has a global burden of approximately 134,000,000. Tens of millions are estimated to have mucosal candidiasis. Studies have shown that mortality associated with fungal diseases is greater than 1.6 million and similar to that of tuberculosis. This estimate is 3 fold greater than that of malaria. Socioeconomic, geo-ecological characteristics and increasing number of at-risk populations are the main determinants of variations on incidence and prevalence of candidiasis (Bongomin *et al.,* 2017). However fungal diseases are often neglected by public health authorities as most deaths from fungal diseases are avoidable. *Candida albicans* is the main agent responsible for most cases of mucosal diseases. It is the main etiologic agent associated with nosocomial invasive candidiasis globally (Guinea, 2014). Resistance to fluconazole is common. This is of medical and economic importance as it is the most commonly used antifungal agent for prophylaxis and treatment of Candida infections in many parts of the world. Resistance to fluconazole is linked to the genetic flexibility of the fungus.

1.3 Statement of the Problem

Trichomoniasis is a sexually transmitted disease caused by the parasitic protozoan *Trichomonas vaginalis*. It is the most common non viral sexually transmitted disease, with an estimated 170 million cases occurring worldwide each year (Robert *et al*, 2012). This estimate may well be low. However, inapparent infection rates are as high as 50% in women and even higher in men. Trichomoniasis has been implicated in adverse pregnancy outcomes and has been associated with an increased risk of human immunodeficiency virus (HIV) transmission.

Candida infections are responsible for increased morbidity and mortality rates in at-risk patients, especially in developing countries where there is limited access to antifungal drugs

(Charlene and Pedro, 2017). In most individuals, *C. albicans* resides as a lifelong, harmless commensal. Under certain circumstances, however, *C. albicans* can cause infections that range from superficial infections of the skin to life-threatening systemic infections.

Generally, similarity in cell types makes antifungal therapy relatively difficult especially in cases of systemic mycoses. Selective toxicity is usually very low. Hence, infected individuals are placed on long regimens of antifungal drugs with low concentrations to contain the challenge. This measure not withstanding, factors such as self medication and drug abuse have over the years, enhanced increased resistance of pathogens to antimicrobials.

Sexually active women have been found to be highly vulnerable to candidiasis and the use of antifungal agents to contain this infection becomes difficult when resistance by the organism sets in. Azole drugs especially Fluconazole have been found to be highly effective against *Candida* infections. Azole drugs are relatively cheap and readily available. They bind to Ergosterol which is a sterol synthesized by *Candida albicans* and other fungi. Ergosterol is a clear target of Azole drugs because it is relatively different from cholesterol (Human Sterol). Hence, this distinction becomes a yardstick for inhibition of a fungal pathogen in a human cell with little or no harm to the human cell since both cells are Eukayotic.

Lately, owing to selective pressure and genetic flexibility, fungal cells have found a way of by passing Ergosterol synthesis. The gene ERG11 is responsible for regulating Ergosterol biosynthesis. Primarily, the expression of this gene is a pointer to prospects of Azole resistance. Hence, minimal optimal or maximal expression of this gene influences the relative availability of Ergosterol which is the binding site of most Azole drugs.

1.4 Justification of the Study

Candida species are responsible for a majority of superficial and disseminated fungal infections in humans while azole antifungals have long provided effective treatment for such infections Recent epidemiological studies indicate that intrinsic azole resistance in some *Candida* species as well as development of high level azole resistance is a problem of critical importance in the clinical setting.

The most commonly prescribed antifungal used for most *C. albicans* infections is fluconazole, a member of the azole class of antifungals (Sarah *et al.,* 2017). One mechanism of resistance identified in this species is the presence of point mutations in ERG11. Previous studies have identified amino acid substitutions that resulted in decreased fluconazole susceptibility (Flowers *et al.*, 2015).

There has been relative paucity with respect to studies on antifungal agents in Nigeria. Most reports of resistance to antimicrobial agents are of bacteriological interest. There have been extensive studies on a lot of genes responsible for bacterial resistance to antimicrobial agents and studies are still on going. A few studies in Nigeria primarily looked at resistance of *Candida* to Azole drugs especially fluconazole which is the common drug of choice in treating Candidiasis. Studies by Efunshile *et al*., (2016) recorded 0% resistance to fluconazole but studies by Adsesiji *et al.* (2011) recorded 73% resistance to Fluconazole and 61.5% resistance to Voriconazole. Only a study by Pam *et al.*, (2012) assessed the incidence of the ERG11 gene in the DNA of *Candida* species isolated from females in Lagos.

1.5 Aim of the Study

This study was aimed at determining prevalence of *Trichomonas vaginalis* and triazole resistant *Candida albicans* expressing ERG11 gene among adult females in abakaliki, Ebonyi state.

1.5.1 Objectives

The specific objectives are:

1. To determine the prevalence of *T. vaginalis* and *C. albicans* in urine and vaginal swab specimens from apparently healthy pregnant and non-pregnant women in Abakaliki metropolis.
2. To determine the susceptibility of *C. albicans* isolates to commonly available azole drugs (Fluconazole and Voriconazole).
3. To determine the minimum inhibitory concentration (MIC) of fluconazole against resistant *C. albicans* isolates.
4. To test for the expression of ERG11 gene in fluconazole resistant *C. albicans* isolates using RT-PCR.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Trichomonas vaginalis*

Trichomonas vaginalis is a flagellated parasitic protozoan, typically pyriform but occasionally amoeboid in shape, extracellular to genitourinary track epithelium with a primarily anerobic lifestyle (Harp and Chowdhury, 2011). It belongs to the domain eukaryote, Phylum; metamonada, class; parabasalia, order; trichomonadida, family; trichomonadidae and Genus: *Trichomonas.*

The individual organism is 10–20 μ m long and 2–14 μ m wide. Four flagella project from the anterior portion of the cell and one flagellum extends backwards to the middle of the organism, forming an undulating membrane. An axostyle extends from the posterior aspect of the organism. *T. vaginalis* is a highly predatory obligate parasite that phagocytoses bacteria, vaginal epithelial cells and erythrocytes and is itself ingested by macrophages. *T. vaginalis* uses carbohydrates as its main energy source via fermentative metabolism under aerobic and anaerobic conditions.

2.1.1 Epidemiology of *Trichomonas vaginalis*

Trichomonas vaginalis is likely the most common non-viral sexually transmitted infection (STI) in the world.. *T. vaginalis* is more prevalent that *Chlamydia trachomatis, Neisseria gonorrhoeae,* and syphilis combined. Variation in rates occur as most findings are derived from studies that used microscopy rather than the more sensitive nucleic acid amplification tests (NAAT) (Kissinger, 2015). With no surveillance programs in place, the epidemiology of *T.vaginalis* is not completely known. It is known, however, to vary greatly by population and geography. In the United States, two population-based studies that used PCR testing

found rates of 2.3 % among adolescents and 3.1 % among women 14–49 (Sutton *et al.*, 2007). Population-based studies in Africa show distinctly higher rates. In Zimbabwe the rate was 9.5 % among both genders using antibody testing. Using NAAT, the positivity rate among men in Tanzania was 11 % (Klinger *et al*, 2006). Women in Papau New Guinea also appear to have exceptionally high *T.vaginalis* rates ranging from 21 % in pregnant women to 42.6 % in the general population (Wangnapi *et al*., 2015). Other population-based studies that used NAAT testing among reproductive aged women in other parts of the world found lower rates (1 % in rural Vietnam and 0.37 % in Flanders, Belgium (Depuydt *et al*, 2010), 2.9 % in Shandong Province in China (Huang *et al.*, 2011)). Screening rates among women attending antenatal or family-planning clinics are often used as an indicator of the prevalence in the general population. Studies at these sites found prevalence rates from 3.2–52 % in resource limited settings and 7.6–12.6 % in the US (Johnston and Mabey, 2008). Thus, rates of *T.vaginalis* vary greatly and are dependent on the risk factor profile of the population.

In general, Africans or persons of African descent have higher rates of *T.vaginalis*, as evidenced by higher rates in Sub-Saharan Africa, and among persons of African descent such as Garifunas (Paz Bailey *et al*., 2009) and African Americans in the US. In the United States, the highest prevalence of TV infection in US women is seen among African-Americans with rates ranging from 13–51 % (Shaffir *et al.*, 2009). African American women have rates that are ten times higher than white women, constituting a remarkable health disparity. Other risk factors for *T.vaginalis* include increased age, incarceration, intravenous drug use, commercial sex work (Freeman *et al.*, 2010) and the presence of bacteria vaginosis (Rathod *et al.*, 2011).

Most recently, a study by Hamafyelto and Ikeh (2017) reported a prevalence rate of 20.5% out of 200 women from IDP camps in Maiduguri. Okojokwu *et al* (2015) reported a

prevalence rate of 4% out of 150 women in Jos. Iwueze *et al* (2015) reported a prevalence rate of 17% with some hospitals presenting with 0% prevalence. There have been studies in Abakaliki, Abeokuta and Lagos which reported prevalence rates of 2.8%, 3.3% and 20% respectively (Adeoye and Akande, 2007, Olurongbe *et al.*, 2010, Uneke *et al*., 2006)

2.1.2 Clinical Features and Pathogenesis of *Trichomonas vaginalis*

T. vaginalis primarily infects the squamous epithelium of the genital tract. It resides in the female lower genital tract and the male urethra and prostate, where it replicates by binary fission. It is transmitted among humans, its only known host, primarily by sexual intercourse (Peirera-Neves *et al.,* 2003). Infection may persist for long periods, possibly months or even years, in women but generally persists less than 10 days in males (Kissinger, 2015). The parasite does not appear to have a cyst form and does not survive well in the external environmentbut can survive outside the human body in a wet environment for more than three hours (Afzan and Suresh, 2012). While thought to be rare, evidence of non-sexual transmission via fomites and possibly water has been described (Crucitti *et al.*, 2011).

The majority of women and men with TV are asymptomatic. One third of asymptomatic women become symptomatic within 6 months (Kissinger, 2015). Among those who do have symptoms, they include urethral discharge and dysuria. Among women, common sites of infection include the vagina, urethra and endocervix. Symptoms include vaginal discharge (which is often diffuse, malodorous, yellow-green), dysuria, itching, vulvar irritation and abdominal pain. The normal vaginal pH is 4.5, but with TV infection this increases markedly, often to >5(Petrin *et al.,* 1998). Coplitis macularis or strawberry cervix is seen in about 5 % of women, though with colposcopy this rises to nearly 50 %. Other complications include infection of the adnexa, endometrium, and Skene and Bartholin

glands. In men, it can cause epididymitis, prostatitis, and decreased sperm cell motility (Kissinger, 2015).

2.1.3 Diagnosis of *Trichomonas vaginalis*

Diagnostic methods available for trichomoniasis are wet mount preparation, staining methods, culture in laboratory medium, and molecular methods. Historically, detection of the parasite is made possible by examination of urine and High Vaginal Swab (HVS) in a drop of saline or *Trichomonas* diluents for the characteristic wobbling and rotating motion. Amadi and Nwagbo (2013) reported that either urine sample or vaginal swab is insufficient for proper diagnosis of *T. vaginalis* infection and have suggested that for better results both urine and vaginal swab should be used. Diagnosis of trichomoniasis has relied mostly on wet mount demonstration and staining of the parasite in the laboratory with success rate of between 20 and 80% (Omorodion, 2018). A combination of cultural method with microscopic wet mount demonstration is now the acceptable procedure for effective diagnosis (Arora and Arora, 2005). New molecular diagnostic tests with improved sensitivity have been developed in response to the increasing recognition by stakeholders of the importance of this wide-spread STI. Thus the detection of *T. vaginalis*, including rapid antigen detection and nucleic acid amplification tests, has significantly improved the quality of diagnostics for trichomoniasis, particularly in women (Hobbs and Sena, 2013).

2.2 *Candida albicans*

Candida albicans is an opportunistic fungal pathogen that is responsible for candidiasis in human hosts. *C. albicans* grow in several different morphological forms, ranging from unicellular budding yeast to true hyphae with parallel-side wall (Sudbery, 2004). *Candida albicans* belong to the Kingdom Fungi, division Ascomycota, classSaccharomycetes, order Saccharomycetales, family Saccharomycetaceae and Genus Candida .

Typically, *C. albicans* is a harmless commensal in the gastrointestinal and genitourinary tract. There are three major forms of disease caused by *C. albicans* namely: oropharyngeal candidiasis, vulvovaginal candidiasis, and invasive candidiasis. For oropharyngeal candidiasis, infection occurs in the mouth or throat, and is identified by white plaque growth on oral mucous membranes. Vulvovaginal candidiasis or a "yeast infection" is the overgrowth of *C. albicans* in the vagina, and results in rash, itchiness, and discharge from the genital region. Lastly, invasive candidiasis occurs when the fungal pathogen enters the bloodstream and can easily spread to organs throughout the body. Invasive candidiasis is best identified when antibiotics fail to cure a patient's fever. *C. albicans* infections are usually treatable with fluconazole, while severe infections require amphotericin B.

Candida albicans is usually transmitted from mother to infant through childbirth and remains as part of a normal human's microflora. The overgrowth of *C. albicans* leads to symptoms of disease, and it occurs when there are imbalances – for example, changes in the normal acidity of the vagina. *C. albicans* infections very rarely spread through sexual intercourse. The typical reservoir for *C. albicans* is in the normal human microflora and is not found in animal vectors (Sudbery 2004).

2.2.1 Epidemiology of *Candida* Species

Several Candida species are commensal and colonize the skin and mucosal surfaces of humans. Critically ill orotherwise immunocompromised patients are more proneto develop both superficial and life-threatening Candida infections (Hasan *et al.,* 2009). Candida infections also constitute the most common fungal infections in AIDS patients (Fidel, 2006; Hasan *et al.,* 2009). These patients predominantly develop oropharyngeal candidiasis, which can lead to malnutrition and interfere with the absorptionof medication.*C. albicans* is the predominant cause of invasive fungal infections (Horn *et al.,* 2009) and represents a

seriouspublic health challenge with increasing medical andeconomic importance due to the high mortality rates and increased costs of care and duration of hospitalization (Almirante *et al.*, 2005; Lai *et al.*, 2012).

Although *C. albicans* is the most prevalent species involved in invasive fungal infections, the incidence of infections due to non-albicans species is increasing. In a study with 2019 patients at major North American medical centres, a predominance of non-albicans species was observed;although *C. albicans* was the most frequently isolated species, it was followed by *C. glabrata* and other non-*C. albicans* species (Sardi *et al*., 2013). This change in epidemiology could be associated with severe immunosuppression or illness, prematurity, exposure to broad-spectrum antibiotics and older patients (Horn *et al.*, 2009).

In European countries especially in member countries of the European confederation of Medical mycology (France, Italy, UK, Belgium, Sweden, France, Poland and Portugal), an analysis showed that more than half of the cases of candidaemia were caused by *C. albicans*, and the incidence rates for non-albicans candidaemia infections were 14% each for *C. glabrata* and *C. parapsilosis*, 7% for *C. tropicalis* and 2% for *C. krusei* (Tortorano *et al.*, 2006). Changes in the epidemiology have also been observed in LatinAmerican countries. In Chile, the prevalence of *C. albicans* has changed, and a progressive increase of non-albicansinfection has been observed; *C. parapsilosis* was the most frequent species, followed by *C. tropicalis* and *C. glabrata*.All isolates were susceptible to amphotericin B; however, 50% of the *C. glabrata* isolates were resistant to fluconazole (Ajenjo *et al.*, 2011). According to the Brazilian Network Candidaemia Study, *C. albicans* accounted for 40.9% ofcases in Brazil, followed by *C. tropicalis* (20.9 %), *C. parapsilosis* (20.5 %) and *C. glabrata* (4.9 %) (Colombo *et al.*, 2006; Nucci *et al.*, 2010). Other species have been isolated in healthy people and patients. *Candida dubliniensis* was usually found in combination with other yeast species, especially *C. albicans* (Sullivan *et al.*, 2004). A high

prevalence of *C. dubliniensis* in the oral cavities of HIV-infected and AIDS patients has also been reported (Tintelnot *et al.*, 2000; Lasker *et al.*, 2001).Since the first description of *C. dubliniensis* from the oral cavities of HIV-positive patients from Ireland (Khan *et al.*,2012), subsequent epidemiological studies have revealed that this species is prevalent globally in association with human (Loreto *et al.*,2010; Khan *et al.,* 2012) and non human habitats with a possibility of inter-host transmission(Nunn *et al.*, 2007).

The species has now been reported from other body sites/specimens, such as the vagina, urine, skin and gastrointestinal tract of both HIV-positive and HIV negative patients (Loreto *et al.,* 2010; Mokaddas *et al.*,2011; Khan *et al.,* 2012). Rarely do patients colonized with this species develop candidaemia (Loreto *et al.,* 2010). The reasons for this limited ability of *C. dubliniensis* to cause invasive disease have been the focus of recent studies (Jackson *et al.*, 2009).

It has been shown that the *C. dubliniensis* genome lacks important hypha-related virulence genes and that it has a limited ability to undergo yeast to hyphal transformation (Moran *et al.*, 2012), which in turn may decrease its potential to invade deeper tissue.*C. parapsilosis* has emerged as a significant nosocomial pathogen with clinical manifestations that include endophthalmitis, endocarditis, septic arthritis, peritonitis andfungaemia, usually associated with invasive procedures orprosthetic devices (Canto´n *et al.*, 2011), and with neonatal infections in the northern hemisphere, although this species is found in patients of all ages in Latin America (Almirante*et al.*, 2005; Nucci *et al.*, 2010). Candidaemia due to *C. glabrata* has been reported to be related to the use of fluconazole (Nucci *et al.*, 2010). *Candida guilliermondii* and *Candida rugosa* were previously uncommon agents; however, the incidence of these is increasing (Pfaller *et al.*, 2009; Nucci *et al.*, 2010). *C. rugosa* (1.1%) has been described in the oral cavity of diabetic patients (Pires-Goncalves *et al.*, 2007).Concernis rising about the high incidence of infections caused by non-albicans

species and the emergence of antifungal resistance (Pereira *et al.,* 2010). Among the nonalbicans species, *C. tropicalis* and *C. parapsilosis* are both generally susceptible to azoles; however, *C. tropicalis* is less susceptible to fluconazole than is *C. albicans. C. glabrata* is intrinsically more resistant to antifungal agents, particularly to fluconazole (Sardi *et al.,* 2013). *C. krusei* is intrinsically resistant to fluconazole and infections caused by this species are strongly associated with prior fluconazole prophylaxis and neutropenia. *Candida lusitaniae,* which accounts for 1–2% of all candidaemias, is susceptible to azoles but has a higher intrinsic resistance to amphotericin B (Cruciani and Serpelloni, 2008).

There has been a gradual increase in the number of antifungal compounds and classes discovered since the1990s; these include polyenes, azoles, echinocandins and purine analogues. Due to the increased availability of antifungal drugs, selection has occurred with consequent resistance of these micro-organisms. Doctors retain the option of giving the drugs for prophylaxis, empiric therapy, preventive treatment or while waiting for the disease to be diagnosed, so there is a degree of excessive exposure to these agents (Rodrı´guez-Tudela *et al.*, 2007).

2.2.2 *Candida albicans* Pathogenicity Mechanisms

C. albicans can cause two major types of infectionsin humans: superficial infections, such as oral or vaginal candidiasisand life-threatening systemic infections *C. albicans* and to a lesser extent other Candida species are present in the oral cavity of up to 75% of the human population (Ruhnke, 2002). In healthy individuals this colonization generally remains benign. However, mildly immunocompromised individuals can frequently sufferfrom recalcitrant infections of the oral cavity. These oral infectionswith Candida species are termed “oral candidiasis” (OC). Such infections are predominantly caused by *C. albicans* and canaffect the oropharynx and/or the esophagus of persons with dysfunctions of the

adaptive immune system. Indeed, HIV is a major risk factor for developing OC. Further risk factors for developing OC include the wearing of dentures and extremes of age (Pappas *et al.,* 2009).

It is estimated that approximately 75% of all women suffer at least once in their lifetime from vulvovaginal candidiasis (VVC) with 40–50% experiencing at least one additional episode of infection (Sobel, 2007). Predisposing factors for VVC are less well defined than for OC and include diabetes mellitus, use of antibiotics, oral contraception, pregnancy and hormone therapy (Fidel, 2004).

Despite their frequency and associated morbidity, superficial *C. albicans* infections are non-lethal. In stark contrast, systemic candidiasis is associated with a high crude mortality rate, even with first line antifungal therapy (Perlroth *et al.,* 2004). Further risk factors include central venous catheters, which allow direct access of the fungus to the bloodstream, the application of broad-spectrum antibacterials, which enable fungal overgrowth and trauma or gastrointestinal surgery, which disruptsmucosal barriers (Spellberg *et al.*, 2012).

During both superficial and systemic infection, *C. albicans* relies on a battery of virulence factors and fitness attributes.The ability of *C. albicans* to infect such diverse host niches is supported by a wide range of virulence factors and fitness attributes.A number of attributes, including the morphological transition between yeast and hyphal forms, the expression of adhesions and invasins on the cell surface, thigmotropism, the formation of biofilms, phenotypic switching and the secretion of hydrolytic enzymes are considered virulence factors. Additionally, fitness attributes include rapid adaptation to fluctuationsin environmental pH, metabolic flexibility, powerful nutrient acquisition systems and robust stress response machineries (Nicholls *et al.,* 2011).

2.2.3 Polymorphism.

C. albicans is a polymorphic fungus that can grow either as ovoid-shaped budding yeast, as elongated ellipsoid cells with constrictions at the septa (pseudohyphae) or as parallel-walled true hyphae. Further morphologies include white and opaque cells, formed during switching, and chlamydospores, which are thick-walled spore-like structures (Sudbery *et al.*, 2004). While yeast and true hyphae are regularly observed during infection and have distinct functions, the role of pseudohyphae and switching in vivo is rather unclear and chlamydospores have not been observed in patient samples (Soll, 2009). A range of environmental cues affect *C. albicans* morphology. For example, at low pH (< 6) *C. albicans* cells predominantly grow in the yeast form, while at a high pH (> 7) hyphal growth is induced (Odds, 1988).. Indeed, a number of conditions, including starvation, the presence of serum or N-acetylglucosamine, physiological temperature and CO_2 promote the formation of hyphae (Sudbery, 2011).

Morphogenesis has also been shown to be regulated by quorumsensing, a mechanism of microbial communication (Albuquerque and Casadevall, 2012). In *C. albicans*, the main quorum sensing molecules include farnesol, tyrosol and dodecanol. Due to quorum sensing, high celldensities (> 107 cells ml-1) promote yeast growth, while low cell densities (< 107 cells ml-1) favor hyphal formation (Francois *et al.*, 2013).The transition between yeast and hyphal growth forms is termed dimorphism and it has been proposed that both growth forms are important for pathogenicity (Jacobsen *et al.*, 2012). The hyphal form has been shown to be more invasive than the yeast form. On the other hand the smaller yeast form is believed to represent the form primarily involved in dissemination. Mutants that are unable to form hyphae under in vitro conditions are generally attenuated in virulence (Lo *et al.*, 1997). However, hypha formation is linked to the expression of a subset of genes encoding virulence factors that are not involved in hyphal formation (Francois *et al.*, 2013).

2.2.4 Adhesins and Invasions

C. albicans has a specialized set of proteins (adhesins) which mediate adherence to other *C. albicans* cells to other microorganisms, to abiotic surfaces and to host cells (Garcia *et al.,* 2011) Arguably the best studied *C. albicans* adhesions are the agglutinin-like sequence (ALS) proteins which form a family consisting of eight members (Als1–7 and Als9)(Francois *et al.,* 2013). The ALS genes encode glycosylphosphatidylinositol (GPI)-linked cell surface glycoproteins. Of the eight Als proteins, the hypha associated adhesin Als3 is especially important for adhesion (Murciano *et al.,* 2012).

ALS3 gene expression is upregulated during infection of oral epithelial cells in vitro and during in vivo vaginal infection. Another important adhesin of *C. albicans* is Hwp1, which is a hypha-associated GPI-linked protein (Zordan and Cormack, 2012). Hwp1 serves asa substrate for mammalian transglutaminases and this reaction may covalently link *C. albicans* hyphae to host cells. Morphology-independent proteins can also contribute toadhesion. These include GPI-linked proteins (Eap1, Iff4 andEcm33), non-covalent wall-associated proteins (Mp65, a putativeβ-glucanase, and Phr1, a β-1,3 glucanosyl transferase), cell-surface associated proteases (Sap9 and Sap10) and the integrin-like surface protein Int1 (Naglik *et al.,* 2011).

C. albicans is a remarkable pathogen as it can utilize two different mechanisms to invade into host cells: induced endocytosis and active penetration. For induced endocytosis, the fungus expresses specialized proteins on the cell surface (invasins) that mediate binding to host ligands (such as E-cadherin on epithelialcells34 and N-cadherin on endothelial cells47), thereby triggering engulfment of the fungal cell into the host cell (Phan *et al.,* 2005). Indeed, even killed hyphae are taken up, indicating that induced endocytosisis a passive process that does not require the activities of viable fungal cells (Park *et al.,* 2005).

In contrast, active penetration is a fungal-driven process and requires viable *C. albicans* hyphae (Dalle *et al.,* 2010).

It is still unclear exactly which factors mediate this second route of invasion into host cells. Fungal adhesion and physical forces are believed to be crucial (Wachtler *et al.,* 2011). Secreted aspartic proteases (Saps) have also been proposed to contribute to active penetration. Lipases and phospholipases, on the other hand, have not been shown to contribute to this process.

2.2.5 Biofilm Formation

A further important virulence factor of *C. albicans* is its capacity to form biofilms on abiotic or bioticsurfaces. Catheters, dentures (abiotic) and mucosal cell surfaces (biotic) are the most common substrates (Fanning and Mitchell, 2012). Biofilms form in a sequential process including adherence of yeast cells to the substrate, proliferation of these yeast cells, formation of hyphal cellsin the upper part of the biofilm, accumulation of extracellular rmatrix material and, finally, dispersion of yeast cells from the biofilm complex. Mature biofilms are much more resistant to antimicrobial agents and host immune factors in comparison to planktonic cells. The factors responsible for heightened resistance include the complex architecture of biofilms, the biofilm matrix, increased expression of drug efflux pumps and metabolic plasticity (Fanning and Mitchell, 2012).

Dispersion of yeast cells from the mature biofilm has been shown to directly contribute to virulence, as dispersed cellswere more virulent in a mouse model of disseminated infection (Uppuluri *et al.,* 2010). The major heat shock protein Hsp90 was recently identified as akey regulator of dispersion in *C. albicans* biofilms. In addition, Hsp90 was also required for biofilm antifungal drug resistance (Robbins *et al.,* 2011).

Several transcription factors control biofilm formation.These include the transcription factors Bcr1, Tec1 and Efg1. (Fanning and Mitchell, 2012). The zinc-responsive transcription factor Zap1 negatively regulates β-1,3 glucan, the major component of biofilm matrix (Nobile *et al.*, 2009). Glucoamylases (Gca1 and Gca2), glucan transferases (Bgl2and Phr1) and the exo-glucanase, Xog1, are positive regulatorsof β-1,3 glucan production.55,56 While expression of GCA1 andGCA2 are controlled by Zap1, the enzymes Bgl2, Phr1 and Xog1function independently of this key negative regulator (Taff et al., 2012). Biofilms formed by mutants lacking BGL2, PHR1 or XOG1 were shownto be more susceptible to the antifungal agent, fluconazole, bothin vitro and in vivo (Taff *et al.*, 2012) Furthermore, recent studies indicate that *C. albicans* biofilms are resistant to killing by neutrophils and do not trigger production of reactive oxygen species (ROS) (Xie *et al.*, 2012) Evidence suggests that β-glucans in the extracellular matrix protect *C. albicans* from these attacks.

An important environmental cue that triggers hypha and biofilm formation in *C. albicans* is contact sensing. Upon contact with a surface, yeast cells switch to hyphal growth (Kumamoto, 2008). On certain substrates, such as agar or mucosal surfaces, these hyphae can then invade intothe substratum. Contact to solid surfaces also induces the formation of biofilms. On surfaces with particular topologies (such as the presence of ridges) directional hyphal growth (thigmotropism) may occur. Brand *et al.*, (2007) demonstrated that thigmotropism of *C. albicans* hyphae is regulated by extracellular calcium uptake through the calcium channels. Brand *et al.*, (2008) also provided evidence that *C. albicans* thigmotropism is required for full damage of epithelial cells and normal virulence in mice. Therefore, the correct sensing and response to both abiotic (biofilm formation) and biotic (invasion) surfaces is important for pathogenicity.

2.2.6 Secreted Hydrolases

Following adhesion to host cell surfaces and hyphal growth, *C. albicans* hyphae can secrete hydrolases, which have been proposed to facilitate active penetration into these cells (Wachtler *et al.,* 2012). In addition, secreted hydrolases are thoughtto enhance the efficiency of extracellular nutrient acquisition. Three different classes of secreted hydrolases are expressed by *C. albicans*: proteases, phospholipases and lipases (Francois *et al.,* 2013).The family of secreted aspartic proteases (Saps) comprises ten members, Sap1–10. Sap1–8 are secreted and released to the surrounding medium, whereas Sap9 and Sap10 remain bound to the cell surface (Albrecht *et al.,* 2006) Sap1–3 have been shown to be required for damage of reconstituted human epithelium (RHE) in vitro, and forvirulence in a mouse model of systemic infection (Schaller *et al.,* 1999). However, the relative contribution of Saps to *C. albicans* pathogenicity is controversial. However, the observed expansion of Sap-encoding genes in *C. albicans* compared with its less pathogenic relatives suggests a role for these proteases in virulence (Moran *et al.,* 2012). Indeed, the large size of the Sap family itself makes it likely that a certain degree of functional redundancy may exist.The family of phospholipases consists of four different classes (A, B, C and D). Only the five members of class B (PLB1–5) areextracellular and may contribute to pathogenicity via disruption of host membranes (Mayor *et al.,* 2005).

2.2.7 pH Sensing and Regulation

In the human host, *C. albicans* is exposed to a surrounding pH ranging from slightly alkaline to acidic (Davies, 2006). Additionally, depending on the host niche, the environmental pH can be very dynamic. Therefore, *C. albicans* must be able to adapt to changes in pH. The pH of human blood and tissues is slightly alkaline (pH 7.4), while the pH of the digestive tract ranges from very acidic (pH 2) to more alkaline (pH 8), and the pH

of the vagina is around pH 4 (Davis, 2006). Neutral to alkaline pH can cause severe stress to *C. albicans*, including malfunctioning of pH-sensitive proteins, and impaired nutrient acquisition (as aconsequence of a disrupted proton gradient)(Francois *et al.,* 2013).

Among the first proteins identified as being important for adaptation to changing pH were the two cell wall β-glycosidases Phr1 and Phr2 (Fonzi, 1999). PHR1 is expressed at neutral-alkaline pH. In contrast, PHR2 is mainly expressed at acidic pH. Correspondingly, Phr1 is required forsystemic infections, and Phr2 is essential for infections of the vagina (De Bernardis et al., 1998). *C. albicans* senses pH via the Rim101 signal transductionpathway (Davis, 2006). In this pathway, environmental pH is gauged by the plasma membrane receptors Dfg16 and Rim21. Activation of these receptors leads to induction of a signaling cascade, finally leading to activation of the major pH-responsive transcription factor Rim101 via its proteolytic cleavage. Rim101 then enters the nucleus and mediates pH-dependent responses.

C. albicans is not only able to sense and adapt to environmental pH, but can also modulate extracellular pH, actively alkalinizing its surrounding environment under nutrient starvation and, thereby, autoinducing hypha formation (Mayer *et al.,* 2012). The molecular mechanisms underlying this are beginning to be uncovered and appear to involve the uptake of amino acids and probably otheramine-containing molecules, such as polyamines, in the absence of glucose. *C. albicans* then cleaves these substrates intracellularly with the urea amidolyase Dur1, 2, and exports the resulting ammonia through the Ato (ammonia transport outward) export proteins (Vylkova *et al.,* 2011). The extrusion of ammonia leads to an alkalinization of the extracellular milieu, which in turn promotes hyphal morphogenesis.

Hyphal formation itself is considered a key virulence factor of *C. albicans* as non-filamentous mutants are attenuated in virulence.Therefore, *C. albicans* senses, adapts to

and, strikingly, also actively modulates extracellular pH. All these features contribute to its remarkable capacity to co-exist as a commensal, and to prevail as a fungal pathogen in humans. Nutrition is a central and fundamental prerequisite for survival and growth of all living organisms.

2.2.8 Metabolic Adaptation

Metabolic adaptability mediates the effective assimilation of alternative nutrients in dynamic environments (Brown *et al.*, 2012). This metabolic flexibility is particularly important for pathogenic fungi during infection of different host niches. Glycolysis, gluconeogenesis and starvation responses are all thought to contribute to host colonization and pathogenesis, but their specific contribution may be highly niche-specific and is still only partially understood (Brock, 2009). In healthy individuals *C. albicans* is predominantly found as part of the gastrointestinal microbiome. Although the concentration of nutrients in this environment can be naturally high, growth of the fungus is believed to be controlled through competition with other members of the intestinal microbial flora.

During disseminated candidiasis in susceptible individuals, *C. albicans* gains access to the bloodstream. Blood is relatively rich in glucose (6–8 mM), the preferred nutrient source of most fungi. However, phagocytic cells (macrophages and neutrophils) can efficiently phagocytose *C. albicans.* Once inside a macrophage or neutrophil, however, the nutritional environment completely changes for the fungus. Not only doesthe phagocyte produce highly reactive intermediates like ROS, reactive nitrogen species (RNS) and antimicrobial peptides(AMPs), it also restricts the availability of nutrients, thereby creating an environment of nutrient starvation (Frohner *et al.*, 2009). Prompt and efficient metabolic plasticity is therefore required for adaptation of *C. albicans* to such a hostile host milieu. Inside macrophages, the fungus initially switches from glycolysis to gluconeogenesis and a

starvation response (activation of the glyoxylate cycle). Lipids and amino acids are proposed to serve as nutrient sources within macrophages.

In addition to metabolic flexibility, the fungus has also evolved ways to escape from macrophages by inhibiting the production of antimicrobial effectors and inducing hyphal formation. Hyphae formed inside phagocytic cells can pierce through the host immune cell by mechanical forces and can permit escape (Ghosh *et al.,* 2009). During systemic candidiasis, fungal cells can disseminate to virtually every organ within the human host, each with potentially different availability of nutrients. In the liver for example, *C. albicans* has access to large quantities of glycogen, the main storage molecule of glucose. The brain has high concentrations of glucose and vitamins as potential nutrient sources. In other tissues, *C. albicans* faces relatively poor glucose concentrations and uses alternative metabolic pathways to utilize host proteins, amino acids, lipids and phospholipids. The fungus canuse secreted proteases to hydrolyse host proteins. It was recently shown that adaptation to different nutrient sources by *C. albicans* not only promotes survival and growth, but also affects virulence (Ene *et al.*, 2012).

Uptake of amino acids, and likely also polyamines, affects the virulence of *C. albicans* by allowing the fungus to autoinduce hypha formation through extracellular alkalinization (Mayer *et al.*, 2012). During infection the main nutrient sources for *C. albicans* are likely to be host-derived glucose, lipids, proteins and amino acids, depending on the anatomical niche. Besides being able to use these different nutrients individually, the ability of *C. albicans* to rapidly and dynamically respond to hostand pathogen-induced changes in micro environmental nutrient availability contributes to its success as a pathogen.

2.3 Environmental Stress Response

A robust stress response contributes to the survival and virulence of *C. albicans* by facilitating the adaptation of the fungus to changing conditions and protecting it against host-derived stresses. Phagocytic cells of the immune system produce oxidative and nitrosative stresses. pH stress occurs, for example, in the gastrointestinal and urogenital tract (Brown *et al.,* 2012). Stress-responsive regulatory pathways, as well as downstream targets, were shown to be essential not only for efficient stress adaptation, but also for full virulence of the fungus. In fact, several mutants lacking genes encoding regulators of stress response or detoxifying enzymes are attenuated in virulence (Brown *et al.,* 2012).

Cellular responses to stresses include heat shock-, osmotic-, oxidative-and nitrosative-stress responses. The heat shock response is mediated by heat shock proteinswhich act as molecular chaperones to prevent deleterious protein unfolding and aggregation (Francois *et al.,* 2013). Additionally, thermal stress leads to trehalose accumulation in *C. albicans*, which is thought to act as a "chemical chaperone" by stabilizing proteins prone to unfolding (Brown *et al.,* 2012). However, the exact function of trehalose accumulation following thermal insults remains unknown.The osmotic stress response results in intracellular accumulationof the compatible solute glycerol to counteract loss of waterdue to the outward-directed chemical gradient. Glycerol biosynthesisis mediated by the glycerol 3-phosphatase Gpp1 and theglycerol 3-phosphate dehydrogenase Gpd2 (Wachtler *et al.,* 2011).

Reactive oxygen species (ROS), such as peroxide, superoxide anions, and hydroxyl radicals, induce an oxidative stress response. Catalase Cta1 and superoxide dismutases, Sod1 andSod5, are crucial for efficient detoxification of ROS in *C. albicans* and are required for full virulence in mouse models of systemic candidiasis (Ghosh *et al.*, 2009).

Neutrophils also produce reactive nitrogen species (RNS), which induce a nitrosative stress response in phagocytosed *C. albicans* cells (Martchenko *et al.*, 2004). The major protein implicated in detoxification of RNS is the flavor hemoglobin-related protein Yhb1. In fungi, environmental signals, including stress signals, aresensed and transmitted by mitogen-activated protein (MAP) kinase pathways through sequential phosphorylation events (Monge *et al.*, 2006)

The three main MAP kinase signaling pathways in *C. albicans* are the Mkc1-, Hog1- and Cek1-MAP kinase pathway. The Mkc1 (MAP kinase from *C. albicans*) pathway is primarily involved in maintaining cellular integrity, cell wall biogenesis, invasive growth under embedded conditions and biofilm formation (Hromatka *et al.*, 2005) Mkc1 is activated upon oxidative and osmotic stress conditions.The Hog1 (High osmolarity glycerol response) pathway mediates the response to osmotic, oxidative and thermal stress, morphogenesis and cell wall formation (Monge *et al.*, 2006). Under osmotic stress, activated Hog1 leads to glycerol accumulation.The Cek1 (Candida ERK-like kinase) pathway mediates filamentation, mating and likely also adaptation to thermal stress (Hwang *et al.*, 2002).

2.4 Heat Shock Proteins

The heat shock response is a conserved reaction of living organisms to stressful conditions such as high temperature, starvation and oxidative stress. Such stresses can induce protein unfolding and nonspecific protein aggregation, ultimately leading to cell death (Francois *et al.*, 2013). In order to prevent this detrimental fate, cells produce heat shock proteins (Hsps). These specialized proteins act as chaperones and prevent protein unfolding and aggregation by binding to their clients and stabilizing them (Richter *et al.*, 2010) Six major Hsps have been identified in *C. albicans*: Hsp104, Hsp90, Hsp78, two Hsp70 proteins

(Ssa1and Ssa2) and Hsp60. HSP104 encodes a Hsp required for proper biofilm formation, and virulence in a *Caenorhabditis elegans* infection model. Hsp90 is a major Hsp in *C. albicans* and regulates drug resistance, morphogenesis, biofilm formation and virulence. HSP78 encodes an uncharacterized Hsp that is transcriptionally upregulated in response to phagocytosis by macrophages (Lorenz *et al.*, 2004). The two *C. albicans* Hsp70 family members, Ssa1 andSsa2 (stress-70 subfamily A), are expressed on the cell surface and function as receptors for antimicrobial peptides, for example Ssa2 binds histatin5 (Sun *et al.*, 2008). Expression of Hsps is mainly controlled by the transcription factor heat shock factor 1 (Hsf1). Hsf1 is phosphorylated in response to heat stress and induces transcription of Hsp-encodinggenes via binding to heat shock elements (HSEs) in their promoters.

2.4.1 Small Heat Shock Proteins

In addition to the above mentioned heat shock proteins, six small Hsps (sHsps) have also been identified in *C. albicans* (Inglis *et al.*, 2012). sHsps are low-molecular-mass chaperones that prevent protein aggregation. Upon heat, or other forms of stress, cells express sHSPs which transition from anoligomeric to a multimeric state and bind aggregated proteins.

In these chaperone-aggregate complexes, client proteins are heldready for disaggregation and refolding by other major Hsps, such as Hsp104 (Cashikar *et al.*, 2005). *C. albicans* is predicted to encode six sHsps: Hsp31, Hsp30, Hsp21, two Hsp12 proteins and Hsp10. As yet only Hsp12 andHsp21 have been investigated. Hsp12 is expressed in response to different stresses, including heat shock and oxidative stress. Importantly, Hsp21 is not found in humans. These results indicate that sHsps can act as virulence factors and might represent attractive drug targets (Inglis *et al.*, 2012).

2.5 Metal Acquisition

Trace metals are essential for the growth and survival of all living organisms including humans, animals, plants, bacteria and fungi. Among the most important metals areiron, zinc, manganese and copper, all of which are essential for the proper function of a large number of proteins and enzymes. Pathogenic microorganisms, as well as their respective hosts,have evolved elaborate mechanisms to acquire or restrict access to these metals (Hood and Skaar, 2012). To date, the most widely investigated transition metal with regard to pathogenesis is iron. *C. albicans* acquires this metal by different strategies, including a reductive system, a siderophore uptake system and a heme-iron uptake system (Almeida *et al.*, 2009). The reductive system mediates iron acquisition from host ferritin, transferring or the environment. Although *C. albicans* does not synthesize its own siderophores, the fungus uses an uptake system to steal iron from siderophores produced by other microorganisms, also known asxeno-siderophores. The only described siderophore transporterin *C. albicans* is Sit1 (Heymann *et al.*, 2002).

The heme-iron uptake system promotes iron acquisition from hemoglobin and heme proteins and is mediated by the heme-receptor gene family membersRBT5, RBT51, CSA1, CSA2 and PGA7 (RBT6) (Cleary *et al.*, 2011)*C. albicans* secretes the zinc-binding protein Pra1 (pH-regulatedantigen 1), which, analogous to siderophore-mediated iron acquisition,acts as a zincophore by binding extracellular zinc and reassociatingwith the fungal cell. Re-association of Pra1 is mediatedby the zinc transporter Zrt1 (Citiulo*et al.*, 2012). Zinc acquisition playsan important role during certain steps of infection. Copper and manganese are also essential for fungal growth; however, the mechanism by which *C. albicans* acquires these metals is currently poorly understood.

2.6 Resistance to Azole Compounds

2.6.1 Mechanism of Action of Azoles

Azoles exert their action by inhibiting the C14α demethylation of lanosterol in fungi, which interferes with the synthesis of ergosterol in the fungal cell membrane. Azoles differ in their affinities to their target, which may account for differences in their spectrum of activity (i.e., in the primary resistance profiles of various fungi) (Xiao *et al.*, 2004). In addition, variations in the structure of azoles are thought to be responsible for the cross-resistance patterns among Candida species. For example, although complete cross-resistance between the triazoles has been observed with *Candida glabrata*, no such pattern exists with *C. krusei* (Pfaller and Diekema, 2007).

2.6.2 Epidemiology of Azole Resistance

Prior to the introduction of antiretroviral therapy, there was an increase in the prevalence of fluconazole-resistant *C. albicans* among HIV-infected patients with oropharyngeal or esophageal candidiasis. Widespread use of itraconazole and fluconazole is thought to have been the major driver of azole resistance (Goldman *et al*., 2000). Up to one-third of patients with advanced AIDS in one study harbored fluconazole-resistant *C. albicans* in their oral cavities (Law *et al.,* 1994). Azole-resistant *C. albicans* is less common among patients with other diseases, such as vaginal candidiasis and candidemia (Zeina and John, 2007). In general, the rates of azole resistance among the most commonly encountered invasive Candida species remain low, with reported rates of 1.0%–2.1% in *C. albicans*, 0.4%–4.2% in *Candida parapsilosis*, and 1.4%–6.6% in *Candida tropicalis* (Pfaller *et al.*, 2004). A clear exception is *C. glabrata*, which is second to *C. albicans* in causing systemic fungal infections in the United States. According to data from the ARTEMIS Global Antifungal

Surveillance Program, the incidence of fluconazole resistance in *C. glabrata* increased from 7% in 2001 to 12% in 2004 (Zeina and John, 2007).

In addition to the changing trends in antifungal susceptibility, there has been a recent shift towards more infections in the immunocompromised host being caused by Candida species other than *C. albicans*. Several studies have initially incriminated the environmental pressure imposed by exposure to fluconazole (Price *et al.*, 1994). However, such a temporal association has not been consistently demonstrated. Other factors, such as exposure to antibacterial agents, immunosuppressive therapy, and the underlying medical condition of the host, might prove to be better predictors of the distribution of Candida species than fluconazole use.

2.6.3 Mechanisms of Azole Resistance

Four major mechanisms of resistance to azoles have been described in Candida species. More than one mechanism can be functioning in any given fungal strain with additive effects.

2.6.4 Decreased Drug Concentration

The development of active efflux pumps results in decreased drug concentrations at the site of action. Efflux pumps are encoded in Candida species by 2 gene families of transporters: the CDR genes of the ATP-binding cassette super family, and the MDR genes of the major facilitators class (Sanglard *et al.*, 1995). Up regulation of CDR1, CDR2, and MDR1 has been demonstrated in azole-resistant *C. albicans* (White, 1997). Other transporter genes have been detected in other Candida species, such as CgCDR1 and PDH1 in C. glabrata and CdCDR1 and CdMDR1 in *Candida dubliniensis*. Whereas CDR gene up-regulation confers resistance to almost all azoles, MDR-encoded efflux pumps have a narrower spectrum specific for fluconazole (Albertson *et al.*, 1996)

2.6.5 Target Site Alteration

It has been demonstrated that mutations in ERG11, the gene encoding for the target enzyme lanosterol C14α-demethylase, prevents binding of azoles to the enzymatic site. Furthermore, intrinsic resistance to fluconazole in *C. krusei* isolates has been attributed to decreased affinity of ERG11p to the drug. In excess of 80 amino acid substitutions in ERG11p have been detected (Zeina and John, 2007). Different mutations can coexist in the same gene with additive effects.

2.6.6 Up-Regulation of Target Enzyme

Some Candida isolates with reduced susceptibility to azoles have higher intracellular concentrations of ERG11p than do azole-susceptible strains (Lopez *et al,* 1998). The antifungal agent is, therefore overwhelmed, and routine therapeutic concentrations can no longer effectively inhibit ergosterol synthesis. Target enzyme up-regulation can be achieved through gene amplification, increased transcription rate, or decreased degradation of the gene product. However, this mechanism is thought to contribute little to the overall resistance burden in Candida species, because only modest increases in enzyme levels have been described (Lopez *et al.,* 1998).

2.6.7 Development of Bypass Pathways

Exposure to azole compounds results in depletion of ergosterol from the fungal membrane and accumulation of the toxic product 14α-methyl-3,6-diol, leading to growth arrest. Mutation of the ERG3 gene prevents the formation of 14α-methyl-3,6-diol from 14α-methylfecosterol (Zeina and John, 2007). Replacement of ergosterol with the latter product leads to functional membranes and negates the action of azoles on the ergosterol biosynthetic pathway. Candida strains with ERG3 mutation are also resistant to polyenes, because their cell membranes are devoid of ergosterol.

Although uncommon (with the exception of fluconazole), resistance to azole compounds among Aspergillus species is well-recognized. The first resistance mechanism described is through reduced intracellular concentration of itraconazole caused by expression of efflux pumps (Slaven *et al.,2*002). The second and more prevalent mechanism relies on modification of the 14α-sterol demethylase enzyme, which is encoded by the cyp51A and cyp51B genes. In particular, amino acid substitutions at the M220 position are associated with a resistance phenotype with elevated MICs to all azoles, whereas substitutions at G54 result in cross-resistance to itraconazole and posaconazole. Recently, a new mechanism of azole resistance in *Aspergillus fumigatus* has been described, where mutations in the promoter region of cyp51A lead to overexpression of the protein product. Continued surveillance for azole resistance and the magnitude of cross-resistance between azoles among *Aspergillus* species is warranted, especially with the increasing use of new-generation azoles for the prevention and treatment of invasive aspergillosis.

It should be additionally noted that the activity of azoles against emerging fungal pathogens, such as zygomycetes and Fusarium and Scedosporium species, is variable. Although fluconazole consistently lacks activity against these organisms, new-generation

azoles possess variable activity, highlighting the importance of relying on susceptibility testing to guide directed antifungal therapy.

2.7 Resistance to Polyenes

2.7.1 Mechanism of Action of Polyenes

Polyenes (amphotericin B deoxycholate and its lipid-associated formulations) act by inserting into the fungal membrane in close association with ergosterol. The subsequent formation of porin channels leads to loss of transmembrane potential and impaired cellular function.

2.7.2 Epidemiology of Polyene Resistance

Although resistance to amphotericin B among Candida strains remains rare, there have been recent reports of increasing MICs to amphotericin B among *C. krusei* and *C. glabrata* isolates (Tortorano *et al*., 2005). In addition, intrinsic polyene resistance is frequently noted in *Candida lusitaniae* and *Trichosporon beigelii.* However, identification of polyene-resistant isolates has been difficult to reproduce. Filamentous fungi are more likely than yeasts to have reduced susceptibility to polyenes. Among *Aspergillus* species, *Aspergillus terreus* is generally resistant to amphotericin B (Sabatelli *et al.*, 2005). Polyene resistance is increasingly encountered in other *Aspergillus* species, such as *Aspergillus flavus* and even *A. fumigatus*, which traditionally exhibits the highest susceptibility to amphotericin B.

2.7.3 Mechanisms of Polyene Resistance

Resistance breakpoints for polyenes have not been determined. Most clinicians use an MIC of $\geqslant$1.0 μg/mL to indicate resistance to amphotericin B. Defects in the ERG3 gene involved in erogosterol biosynthesis lead to accumulation of other sterols in the fungal membrance. Consequently, polyene-resistant Candida and Cryptococcus isolates have relatively low

ergosterol content, compared with that of polyene-susceptible isolates. Resistance to amphotericin B may also be mediated by increased catalase activity, with decreasing susceptibility to oxidative damage (Zeina and John, 2007).

2.8 Resistance to Echinocandins

Echinocandins inhibit the synthesis of β-1,3-d glucan, which is integral to the structure and function of the fungal cell wall. The formation of a defective cell wall leads to cell rupture in yeasts and aberrant hyphal growth in molds. Echinocandins are highly effective against Candida and Aspergillus species, but they have no activity against zygomycetes or against Cryptococcus, *Trichosporon, Scedosporium,* and *Fusarium* species. Among the *Candida* species, *C. parapsilosis* and *Candida guilliermondii* isolates have higher MIC values than do *C. albicans* isolates, although the clinical significance of this reduced in vitro susceptibility has been debated. Nonetheless, breakthrough infections with *C. parapsilosis* have been reported in patients receiving echinocandins for other indications (Cheug *et al.,* 2006). Of note, optimal detection of echinocandin resistance requires variation from the Clinical and Laboratory Standards Institute methodology.

The mechanisms of echinocandin resistance are still being investigated. In Candida species, secondary resistance is associated with point mutations in the Fks1 gene of the β-1,3-d-glucan synhase complex. Within Fks1 lies a highly conserved region where several mutations have been identified, mostly at the Ser645 position. On the other hand, the mechanism of resistance in C. neoformans is not completely understood. Possibilities include an echinocandin-resistant β-1,3-d-glucan synthase target, efflux pumps, and degradation pathways.

It has been observed that echinocandin-susceptible Candida and Aspergillus isolates have the ability to grow in vitro at concentrations exceeding the MICs of caspofungin. This

paradoxical phenomenon, which is referred to as the “eagle effect,” is strain-dependent and has been related to up-regulation of chitin synthesis in the fungal cell wall. However, its in vivo consequences have not been fully determined, and the highest treatment doses of echinocandins have not yet shown this phenomenon in humans (Clerons *et al*., 2006).

The burden of echinocandin resistance is still poorly appreciated. There have been recent reports of echinocandin resistance in patients with Candida infections (due to *C. albicans, C. glabrata, C. krusei, and C. parapsilosis*). Infections ranged from esophageal candidiasis to prosthetic valve endocarditis. In all of the described cases, resistance to echinocandins developed during therapy and was associated with treatment failure. Resistance mechanisms other than Fks1 mutations were involved in some cases (Zeina and John, 2007).

2.8 Resistance to Flucytosine

Flucytosine is a base pyrimidine analog that inhibits cellular DNA and RNA synthesis. Some yeast strains are intrinsically resistant to flucytosine because of impaired cellular uptake secondary to a mutation in cytosine permease. On the other hand, acquired resistance results from defects in flucytosine metabolism through mutations in cytosine deaminase or uracil phosphoribosyl transferase (Zeina and John, 2007). The prevalence of primary resistance to flucytosine remains low (1%–2% among Candida isolates and <2% among *C. neoformans* isolates. However, the speed at which these yeasts can develop resistance to flucytosine has prompted clinicians to use flucytosine only in combination with other antifungal agents, mainly amphotericin B.

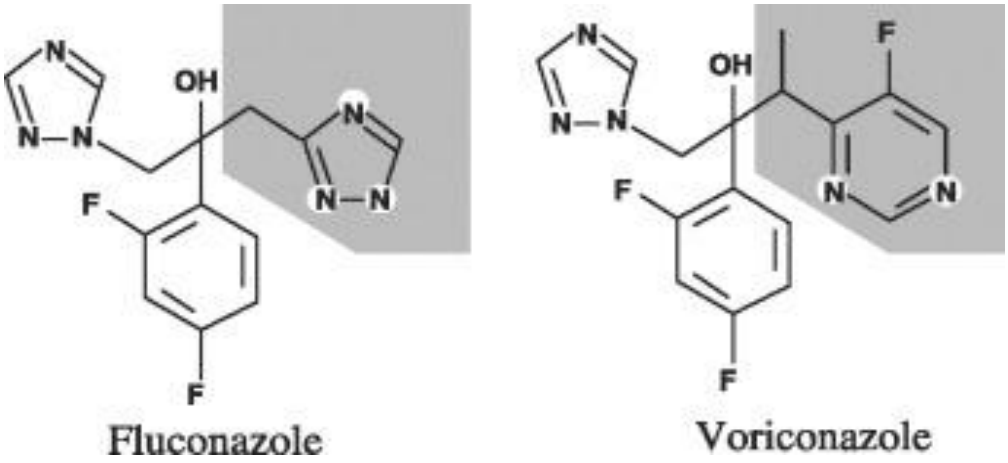

Fig 1.1 Molecular Structure of Fluconazole and Voriconazole

(www.sciencedirect.com. Assessed 1st September, 2017)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study area is Abakaliki. Abakaliki which is the capital city of the present day Ebonyi State in the southeastern Nigeria. Its coordinates are longitude 8.^{o}06'E and Latitude 6.^{o}20N (World gazetteer, 2017). It has tropical climate which is broadly divided in of two seasons. The rainy season spans between April and October and dry season covers from between November to March. Abakaliki has an average temperature of 29.6^{o}C with March being the hottest month of the year. In August the average temperature is 26.0^{o}C which is the lowest average temperature of the whole year. Humidity ranges between 50%-70% during the dry season and 60%- 90% during the rainy season (Weather Atlas, 2017). Precipitation is lowest in December with an average of 7mm. Most precipitation falls in September with an average of 291mm. Its vegetation is of the sub-savannah rain forest. The inhabitants are primarily members of the Igbo tribe. Abakaliki used to be the headquarters of the old Abakaliki zone in the old Anambra and Enugu state before the creation of Ebonyi State in 1996. Abakaliki is made up of four clans namely Ezza Ezekuna, Ngbo, Izzi and Ikwo. As at the time of this study the last known estimated population stood at 438,700. (NPC, 2015). Nigerians from other communities also reside within the State capital city. Inhabitants are predominantly farmers, though a few are civil servants and traders.

3.2 Ethical Consideration

Ethical approval for this research was granted by the Ministry of Health, Ebonyi State.

3.3 Sample population and Sample size

Sample size was determined using the formula

$n= z^2p(1-P)/d^2$ (Niang *et al.*, 2006)

Where n=sample size

P=Expected prevalence (30%; Umeh and Anaeto, 2010) (Proportion of one of 30%=0.3)

Z= the standard normal distribution at 95% confidence interval=1.96

d =precision or allowable error (5% or 0.05)

Hence n=3.84 x 0.3(0.7)/0.0025

n=1.152(0.89)/0.0025= 410

Minimum Sample size = 410

The study population comprised 565 apparently healthy females; 252 were pregnant women attending the Mile 4 hospital for antenatal care and 106 were non-pregnant women who accessed private laboratories in various parts of Abakaliki for routine check-ups. Two hundred and seven (207) were healthy female non-gravid undergraduate students of Ebonyi State University, Abakaliki. The laboratories were chosen to cover major areas of the metropolis which include, Hilltop, Waterworks, Ogoja road, Odunukwe/Nkaliki and Afikpo Road/Azuiyiokwu areas. It was confirmed that the laboratories assessed were certified to be of optimal standard by the Medical Laboratory science council of Nigeria.

Three major demographic factors considered in this study were Gravidity, Age and Trimester of pregnancy

3.4.1 Collection of Urine Samples

Each of the participants was given a sterile universal urine sample bottle and instructed on how to obtain a mid stream urine specimen. The urine specimen were received from the women, labelled with adhesive tape and transported to the Microbiology laboratory, Ebonyi State University for analysis under cold chain.

3.4.2 Collection of Vaginal Swab samples

Some of the volunteers did not consent to provide vaginal swab samples. Only two hundred and eighty-eight (288) adult females agreed to provide the vaginal swab samples. Some of the women especially the students requested to collect the vaginal swab samples by themselves. They were then given sterile swab sticks and instructed on how to obtain the swab samples. Female laboratory scientists at every laboratory visited collected the swab samples from the rest of the volunteers who consented. The vaginal swabs collected were properly labelled with adhesive tapes and transported to the laboratory under cold-chain.

3.5 Detection of *Trichomonas vaginalis* infection

3.5.1 Detection of *Trichomonas vaginalis* infection using Urine Samples

Each urine specimen collected was spun in a centrifuge at 5000rpm for 2 minutes. The test tube was tapped gently to dislodge the supernatant which was dropped on a clean grease free glass slide. The preparations were covered with cover slip and examined microscopically using the x10 and x40 objective (Cheesbrough, 2009).

3.5.2 Detection of *Trichomonas vaginalis* infection using vaginal swab samples

Each vaginal swab was placed in 10ml screw cap plastic tube containing 0.5ml of normal saline after which the swab was vigorously rotated in the saline and pressed against the side

of the tube to express as much fluid as possible. A drop of the expressed fluid was placed on glass slide with a cover slip and examined using the x10 and x40 objective lenses. Positive result is defined as the presence of one or more trichomonads with characteristic morphology and jerky motility (Cheesbrough, 2009).

3.6 Culture of Urine and Vaginal Swabs of *T. vaginalis* and *C. albicans*

Each vaginal swab specimens and the urine specimens were each cultured seperately in *Trichomonas* broth enriched with bovine serum and incubated at 37°C for 72hrs. After 72 hours, drops of each sample was placed on a clean grease free glass slide and examined for motile Trichomonads using the x10 and x40 objectives.

The *Trichomonas* broth employed for this study was selective for both *T. vaginalis* and *Candida* species. After culture in Trichomonas broth for 72 hours, samples that showed positive for budding yeast cells were further subcultured on to Sabourauds dextrose agar (SDA) plates by streaking and incubated aerobically at 37°C for 48 hours. Characteristic colonial morphology of *Candida albicans* (white to cream colony with a smooth border, pasty and moist appearance) was observed.

3.7 Identification of the Yeast Isolates

The yeast isolates were identified using the methods described by (Ochei and Kolhatkar, 2007; Cheesbrough, 2009; Chijioke *et al.*, 2016; Onianwah, 2014 and Uzoh *et al.*, 2016).The methods were germ tube test, carbohydrate fermentation, urea hydrolysis and use of fungal atlas.

a) Germ Tube Test

The method used by Ochei and Kolhatkar (2008) was adopted. Using a sterilized wire loop, each yeast isolate from SDA was suspended in 0.5ml of human serum in a test tube. The

same procedure was repeated with a known culture of *Candida albicans* (positive control) (Positive control was obtained from Microbiology unit of Federal Teaching Hospital Abakaliki).

The test tubes were incubated at 37°C for 3 hours after which drops of the yeast suspension were placed on clean grease free glass slide and examined using x10 and x40 for the presence or absence of germ tubes. *Candida albicans* produce germ tubes within 3 hours.

b) Carbohydrate Fermentation

Twenty grams (20g) of glucose was dissolved in 100mls of distilled water and placed in a water bath with temperature of 56°C for 5 minutes. The preparation was sterilized by steaming. Nine milliliters (9mls) of peptone water with Bromocresol purple indicator was pipetted in a test tube containing a Durham tube and sterilized by autoclaving at 15psi for 15 minutes at 121^{o}c and 0.5ml of the glucose solution was added to each of the peptone water tube.

Colonies of the yeast were suspended in saline and 0.2mls of the suspension were added into the glucose medium. Incubation was done out at the temperature of 27°C for up to 24 days and tubes were checked every 2-3 days for the first week for the evidence of gas in the Durham tubes and then weekly for up to 24 days.

c) Urea Hydrolysis

Each isolate was inoculated on the Christensen's urea agar slant. The slants were incubated at 30°C for 5 days. Pink or red colour of the agar indicated a positive test.*Candida albicans* is urea positive.

d) Use of Fungal Colour Atlas

Colour fungal atlas was used to match the isolates grown on Sabourauds dextrose agar. Appearance from the top and bottom of the plates were compared with those on the colour atlas. Candida Chromagar was also used.

3.8 Antifungal Drug Susceptibility

3.8.1 Disc Diffusion

Colonies of each isolate were picked up with a sterilized wire loop and emulsified in Nutirent broth and the turbidity adjusted to 0.5 McFarland standard. The Mcfarlands standard was prepared by adding 1ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) to 99ml of distilled water and dissolving 0.5g of dehydrated Barium Chloride ($Bacl_2 2H_2O$) in 50ml of distilled water in separate reaction flasks respectively. Barium chloride solution(0.6ml) was added to 99.4ml of H_2SO_4 solution in a separate test tube and the reaction mixture was well mixed to form 0.5 McFarland turbidity standard. A small portion of the turbid solution was transferred to a capped test tube similar to the test tube used for the prepapration of the test microorganisms and stored at room temperature (27^oC).

The capped solution was always used to adjust and compare the turbidity of the test organism to get a confluent growth on a culture plate when performing antibiotic susceptibility testing. Within 15 minutes of adjusting the turbidity, each isolate was plated onto a dried surface of a sterile Mueller-Hinton agar plate respectively using sterile cotton swab. Muellar Hinton agar (Oxoid Ltd) were prepared according to the manufacturers instruction. The Antimicrobial discs used for this study were commercially prepared (Oxoid Ltd) Antimicrobial disks containing 25 µg of fluconazole, 1ug of Voriconazole and 100ug of Nystatin were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure its complete contact with the agar surface. The plates were incubated at 37ºC and examined after 24 hours of incubation. The zones of inhibition were

measured in millimeter and the results were interpreted`using validated CLSI interpretive breakpoints for in vitro susceptibility testing of fluconazole as done by Iroha *et al*., (2012); Pfaller *et al.*, (2006) and Pam *et al.*, (2012).

Table 1: Interpretative Breakpoints for Different Drugs Used

Drug (Conc)	Susceptible (mm)	Resistant (mm)
Fluconazole (25µg)	≥19	≤14
Voriconazole (1µg)	≥17	≤13
Nystatin (100 µg)	≥19	≤14

Adopted from CLSI, (2007)

3.8.2 Minimum Inhibitory Concentration

The Minimum inhibitory concentration test was carried out using MIC test strips (Liofilchem, Italy). Overnight culture of Candida albcans were suspended in Sterile peptone water to obtain the Mcfarlands standard. A sterile swab stick was dipped in the broth culture and streaked on Mueller hinton agar. The plates were allowed to absorb excess moisture to ensure complete dryness of the media before the strips were placed. The strips were applied on to the agar surface with the calibrated scale facing upwards.It was ensured that the whole length of the antibiotic gradient (0.016-256 ug/ml) was in complete contact with the agar surface. The agar plates were incubated in an inverse position at 37^{O}c for 48 hours.

Table 2 Interpretative Breakpoints for Fluconazole

Antifugal Agent	S(µg/ml)	I(µg/ml)	SDD(µg/ml)	R(µg/ml)
Fluconazole	≤2	-	4	≥8

(CLSI, 2017)

KEY; S:Susceptible, I: Intermediate, SDD: Susceptible dose dependent, R: Resistant.

3.9 Molecular Studies

The molecular aspect of this study was carried out at the Biotechnology Research laboratory University of Nigeria, Nsukka.

A total of Six *C. albicans* isolates among the 82 that resisted both Fluconazole and Voriconazole were selected for ERG11 gene screening using RT- PCR. The 6 isolates were randomly selected to cover the age groups, pregnant and non pregnant participants.

a) RNA Extraction for RT-PCR

RNA extraction was carried out using Quick-RNA mini prep (Zymo Research). For each of the selected sample spiked with fluconazole, three hundred microlitres of Lysis buffer was added to 48 hours old SDA broth culture of *C. albicans* and centrifuged at 10,000rpm for 30 seconds. The clear supernatant were transferred into a spin-away filter in a 400µl collection tube and centrifuged. One volume of ethanol was added to a flow through and mixed well. The mixture was transferred to a zymo-spin IIICG column in a collection tube and centrifuged. The flow through was discarded. Four hundred microlitres of RNA prep buffer was added to the column and centrifuged followed by discarding of the flow through after which 700µl of RNA wash buffer was added to the column and centrifuged. The Flow through was discarded. Four hundred microlitres of the RNA wash buffer was added to column and centrifuged for 2 minutes to ensure complete removal of the wash buffer. The column was carefully transferred into an RNase-free tube. One hundred microlitres of DNase/RNase-free water was directly added to the column matrix and centrifuged to elute the RNA.

b) cDNA Synthesis

The cDNA synthesis was carried out using one Taq RT-PCR kit (New England Biolabs). The extracted RNA samples were mixed with primer $d(T)_{23}$ VN in sterile RNase free microfuge tubes after which they were denatured for 5 minutes at 70^{o}C. The preparations were spun briefly and put promptly on ice. To the tube containing 8ul RNA/primer/dNTP, a predesigned reaction mix and an enzyme mix were added proportionately (10µl and 2µl) as stipulated by the manufacturers and 2µl of nuclease free water was added. In the negative control tube, 10ul the reaction mix and 2µl of nuclease free water were added. The final reaction mixture (cDNA synthesis reaction) were incubated at 42^{o}C for one hour. The enzymes were finally inactivated at 80^{o}C for four minutes. The reaction was diluted with 30µl of nuclease free water and stored at -20^{o}C for PCR amplification.

c) DNA Extraction by Heat lysis

Each of the selected samples unspiked with Fluconazole, 500µl of 48 hours old culture of *C. albicans* in SDA broth was introduced into eppendorf tube after which 50µl of sterile distilled water was added and vortexed to wash the cells. Centrifugation was carried out at 12,000rpm for five minutes after which the supernatant was discarded and the sediment (cell pellets) resuspended with 50µl of sterile distilled water and heated at 99^{o}C for 10 minutes to lyse the cells and release the DNA. After heating, the cells were subjected to "Cold Shock" treatment on ice for 10 minutes. The preparation was spun at 12,000rpm for five minutes and the supernatant was transferred into fresh eppendorf tube and kept for PCR use.

d) **Polymerase Chain Reaction**

The DNA of the twelve selected *C. albicans* isolates that resisted all the azole drugs were screened by PCR method using specific primers to determine the ERG11 gene. PCR amplification was performed in volume of 25µl which consisted of 2.0µl 100ng DNA, 2.5µl of 10x Buffer (Bioline), 1.5µl of 50mM $MgCl_2$ (Bioline), 2.0µl of 2.5mM dNTPs (Bioline), and 0.2µl 500U *Taq*DNA polymerase (Bioline), 1.0µl of 10uM each of the primers and 15.05µl DEPC-treated water (Invitrogen Corporation). The PCR cycling profile used for the reaction consists of an initialstep at 96°C for 5-7 min., 35 cycles of 94°C for 35 s,58°C-60^{o}C for 1 min and 72°C for 1min., and a 10-min final extension at 72°C.

The primer pair used for this study is given below

ERG11-1CF 5'-GCAGCTTCATATGGTCAACAACC-3

ERG11-1CR 5'-TAACATTGGCAACCCCATGAG-3

(Product size:325bp)

(Strzelczyk *et al.*, 2013)

e) Gel Electrophoresis

A mixture of 1.0 g of agarose (SeaKem, Lonza, USA) and 100 ml of 1 X Tris-acetate-ethylenediaminetetraacetate (TAE; pH 8.0) buffer (Bio-Rad) was used to make up a medium size of 1 % (w/v) agarose gel. The mixture was heated up for 3 minutes in a microwave oven for the agarose to completely dissolve, then it was cooled to about 50°C and ethidium bromide (1ml) was added to stain the gel. The molten gel was casted into a gel casting tray containing combs and allowed to solidify. After about 30 minutes the gel combs were carefully removed and the gel casting tray containing the gel was placed into a

gel electrophoresis chamber or tank filled with TAE buffer (40 mM Tris, 20 mM acetic acid, and 100 mM EDTA pH 8.0). For each run, 5µl of Extend Quick-Load DNA Ladder (1kb; New England, Bio Labs) and Lambda DNA/HindIII Marker 3 (2.5kb; Thermo Fisher Scientific) was added to one of the wells to estimate the band sizes and 5 µl of negative control, comprised of Sigma water (Nuclease free water), was added to another well. The plasmid DNA and genomic DNA/PCR products, 5µl of each, were carefully loaded into the remaining wells. An electric current of 80 V; 400 mA (mini Ampere) was run through the gel for 60 minutes and gel was distained. Gels were visualized and photographed using a gel documentation system

3.9.1 Statistical Analysis

Chi square technique was used to analyze the various data obtained at 5% level of significance ($P \leq 0.05$), especially with respect to the demographic factors considered in assessing the prevalence of *T. vaginalis* and *C. albicans.*

CHAPTER FOUR

RESULTS

Results obtained from this study show that out of a total of 358 samples (including Urine and Vaginal swabs) collected from Pregnant females and 270 samples (including Urine and Vaginal swabs) collected from non-pregnant females, *Trichomonas vaginalis* was not isolated/observed in all the samples. Morphological/biochemical identification of *C. albicans* from urine and vaginal specimens of respondents are shown in Table 3.

Table 3: Morphological/Biochemical Identification of *C. albicans*from Urine and Vaginal Specimens of Respondents

Samples	Turbidity in trichomonas broth	Microscopy	Growth on SDA	Germ tube reaction	Glucose	Urease	Atlas Appearance	Organism
U_1	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_2	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_3	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_4	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_5	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_6	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_7	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_8	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_9	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{13}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{14}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{15}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{16}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{17}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{18}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{19}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{20}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{21}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{22}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{23}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{24}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{25}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

Key : OYOther yeasts +; Match -; No Match

In all 628 samples examined from both Pregnant and Non pregnant females, 82 were positive for *C. albicans.* Out of 358 samples of Pregnant females, 50 were positive and out of 270 samples of non-pregnant females, 32 were positive as presented in Table 4.

Table 4: Prevalence of *C. albicans* among Females in Abakaliki Metropolis

Group	Total No of Participants	Total number of samples examined	Number positive for *C. albicans*	% Prevalence
Pregnant	252	358	50	7.96
Non-Pregnant	313	270	32	5.09
Total	**565**	**628**	**82**	**13.05**

$\mathbf{X^2}$=0.94 at P=0.05

Out of 340 urine specimens screened, 32 (5.09%) were positive for *Candida albicans*. While out of 288 vaginal swab samples examined, 50 (7.90%) were positive for Candida albicans as shown in Table 5 *C. albicans* was isolated more from vaginal swab than urine.The statistical analyisis however showed that clinical specimen may be a factor in the prevalence of *C. albicans* as shown in the Appendix F.

Table 5: Prevalence of *Candida albicans* with Respect to Clinical Specimens Collected from females

Clinical samples	**Total number examined**	**Number positive for *C. albicans***	**% Prevalence**
Urine	340	32	5.09
VS	288	50	7.96
Total	**628**	**82**	**13.06**

$\mathbf{X^2}$=9.05 at P=0.05

In general, *Candida albicans* were observed to be more prevalent in samples of females whose age groups were 26-30 years (2.55%). It was followed by females of age groups 31-35 years (3.03%) and 20-25 years (2.55%). Women who were between the ages of 36-40 years (1.27%) were observed to be least infected with *Candida albicans* as shown in table 7.

Candida albicans were found more in samples of women whose age groups were 26-30 years. It was followed by females whose ages were between 20-25 years and 31-35 years respectively. Non-pregnant females whose ages were above 40 years were observed to be least infected by *Candida albicans.* The prevalence of *C. albicans* in non-pregnant females is shown in table 8. The prevalence of *Candida albicans* were found to be high in samples of Pregnant females between the ages of 26-30 years (2.70%), followed by females of 31-35 years (1.91%) and females whose ages were greater than 40 years (1.44%). Women whose age groups were 36-40 years were found to be least infected by *Candida albicans*.

Table 6: Prevalence of *Candida albicans* in Females with Respect to Age

Age Groups (years)	**Number of specimen Examined**	**Number positive**	**% Positive**
20-25	174	16	2.55
26-30	207	28	4.46
31-35	132	19	3.03
36-40	48	8	1.27
>40	67	11	1.75
Total	**628**	**82**	**13.06%**

$\mathbf{X^2}$=4.34 at P=0.05

Table 7: Prevalence of *Candida albicans* in *Non*-pregnant Participants with Respect to Age.

Age Groups (years)	Number of specimen Examined	Number positive	% Positive
20-25	61	8	1.27
26-30	82	11	1.75
31-35	56	7	1.11
36-40	31	4	0.63
>40	40	2	0.32
Total	**270**	**32**	**5.10%**

Table 8: Prevalence of *Candida albicans* in Pregnant Females with Respect to the Age

Age Groups (years)	**Number of specimen Examined**	**% Examined**	**Number positive**	**% Positive**
20-25	113	31.56	8	1.27
26-30	125	34.91	17	2.70
31-35	76	21.23	12	1.91
36-40	17	4.75	4	0.63
>40	27	7.54	9	1.44
Total	**358**		**50**	**7.96**

Women in their third trimesters appeared to have more *Candida albicans* infection followed by women in the second trimesters and first trimesters. The residuals were women whose trimesters of pregnancy were unknown. The distribution of the prevalence is shown in Table 9.

Table 9: Distribution of *Candida albicans* in Pregnant Participants with Respect to the Trimester of Pregnancy

Trimester of Pregnancy	**Number Examined**	**Number Positive**	**% Positive**
First	98	5	0.80
Second	110	10	1.59
Third	118	26	4.14
Residuals	32	9	1.43
Total	**358**	**50**	**7.96%**

Generally of all 82 isolates of *C. albicans* obtained from this study, there was increased resistance to the azoles drugs when compared to the non-azole drug such as nystatin as shown in Table 10. Results obtained showed that 87.80% and 69.51% of the isolates of *Candida albicans* were resistant to fluconazole and voriconazole respectively. Only 13.41% of the isolates showed resistance to the control drug, nystatin.

Out of 19 *C. albicans* isolates obtained from Urine of non-pregnant females, 13 were resistant to Fluconazole, 15 were resistant to voriconazole and 2 were resistant to Nystatin. While 6 of the isolates were susceptible to fluconazole, 4 were susceptible to voriconazole and 17 were susceptible to Nystatin. Nystatin was used as a tangential control and most of the isolates were susceptible it. The antifungal susceptibility of *C. albicans* isolated from urine of non-pregnant females at Abakaliki is shown in Table 11.

Out of 13 C. albicans isolates obtained from vaginal swab of non-pregnant females, 11 were resistant to Fluconazole, 10 were resistant to voriconazole and 2 were resistant to Nystatin. While 2 of the isolates were susceptible to fluconazole, 3 were susceptible to voriconazole and 11 were susceptible to Nystatin. The antifungal susceptibility of *C. albicans* isolated from vaginal swab of non-pregnant females at Abakaliki is shown in Table 12.

Out of 31 *C. albicans* isolates obtained from urine of pregnant females, 22 were resistant to Fluconazole, 18 were resistant to voriconazole and 1 was resistant to Nystatin. While 9 of the isolates were susceptible to fluconazole, 13 were susceptible to voriconazole and 30 were susceptible to Nystatin. The Antifungal susceptibility of *C. albicans* isolated from urine of pregnant women at Abakaliki is shown in Table 13.

Out of 19 *C. albicans* isolates obtained from vaginal swab of pregnant females, 12 were resistant to Fluconazole, 11 was resistant to voriconazole and 5 were resistant to Nystatin. While 7 of the isolates were susceptible to fluconazole, 8 were susceptible to voriconazole

and 14 were susceptible to Nystatin. The Antifungal susceptibility of *C. albicans* isolated from vaginal swab of pregnant females at Abakaliki is shown in table 14.

The MIC results shows that out of the six isolates selected for the PCR analysis that were resistant to fluconazole using disc diffusion, only one was resistant (≥8 μg/ml). The other five were susceptible. The MIC result is shown in Table 15.

Table 10: Antifungal Susceptibility of *Candida albicans* Isolated from Females at Abakaliki Metropolis

Organism	Number of isolates tested	Antifungal agents	Doses	Number (%) of isolates	
				Resistant	Sensitive
Candida albicans	82	Fluconazole	25µg	72 (87.80)	10 (12.20)
		Voriconazole	1µg	57 (69.51)	25 (30.49)
		Nystatin	100µg	11 (13.41)	61 (74.39)

Table 11: Antifungal Susceptibility of *Candida albicans* isolated from Urine of Non-Pregnant Females at Abakaliki Metropolis

Organism	Number of isolates tested	Antifungal agents	Doses	Number (%)		Total(%)
				Resistant	Sensitive	
Candida albicans	19	Fluconazole	25µg	13 (68.42)	6 (31.58)	100
		Voriconazole	1µg	15 (78.94)	4 (23.17)	100
		Nystatin	100gµ	2(10.52)	17(89.50)	100

Table 12: Antifungal Susceptibility of *Candida albicans* isolated from VS of Non-Pregnant Females at Abakaliki metropolis

Organism	Number of isolates tested	Antifungal agents	Doses	Number of isolates		Total (%)
				Resistant	Sensitive	
Candida albicans	13	Fluconazole	25µg	11(84.62)	2 (15.38)	100
		Voriconazole	1µg	10 (76.27)	3 (23.10)	100
		Nystatin	100µg	2 (15.38)	11(84.62)	100

Table 13: Antifungal Susceptibility of *Candida albicans* isolated from Urine of Pregnant Females at Abakaliki Metropolis

Organism	Number of isolates tested	Antifungal agents	Doses	Number of isolates		Total (%)
				Resistant	Sensitive	
Candida albicans	31	Fluconazole	25µg	22(70.96)	9 (29.10)	100
		Voriconazole	1µg	18(58.06)	13(41.94)	100
		Nystatin	100µg	1(3.23)	30(96.77)	100

Table 14: Antifungal Susceptibility of *Candida albicans* isolated from VS of Pregnant Females at Abakaliki Metropolis

Organism	Number of isolates tested	Antifungal agents	Doses	Number (%) of isolates		Total
				Resistant	Sensitive	
Candida albicans	19	Fluconazole	25µg	12(63.16)	7(36.84)	100
		Voriconazole	1µg	11(57.89)	8(42.11)	100
		Nystatin	100µg	5(26.32)	14(73.68)	100

Table 15: Showing the MIC values for the selected *C. albicans* isolates against Fluconazole

SAMPLE CODE	MIC VALUE (µg/ml)
S4	8
S6	2
S3	0.125
S5	0.50
S2	CI
S1	CI

KEY: CI-Complete Inhibition

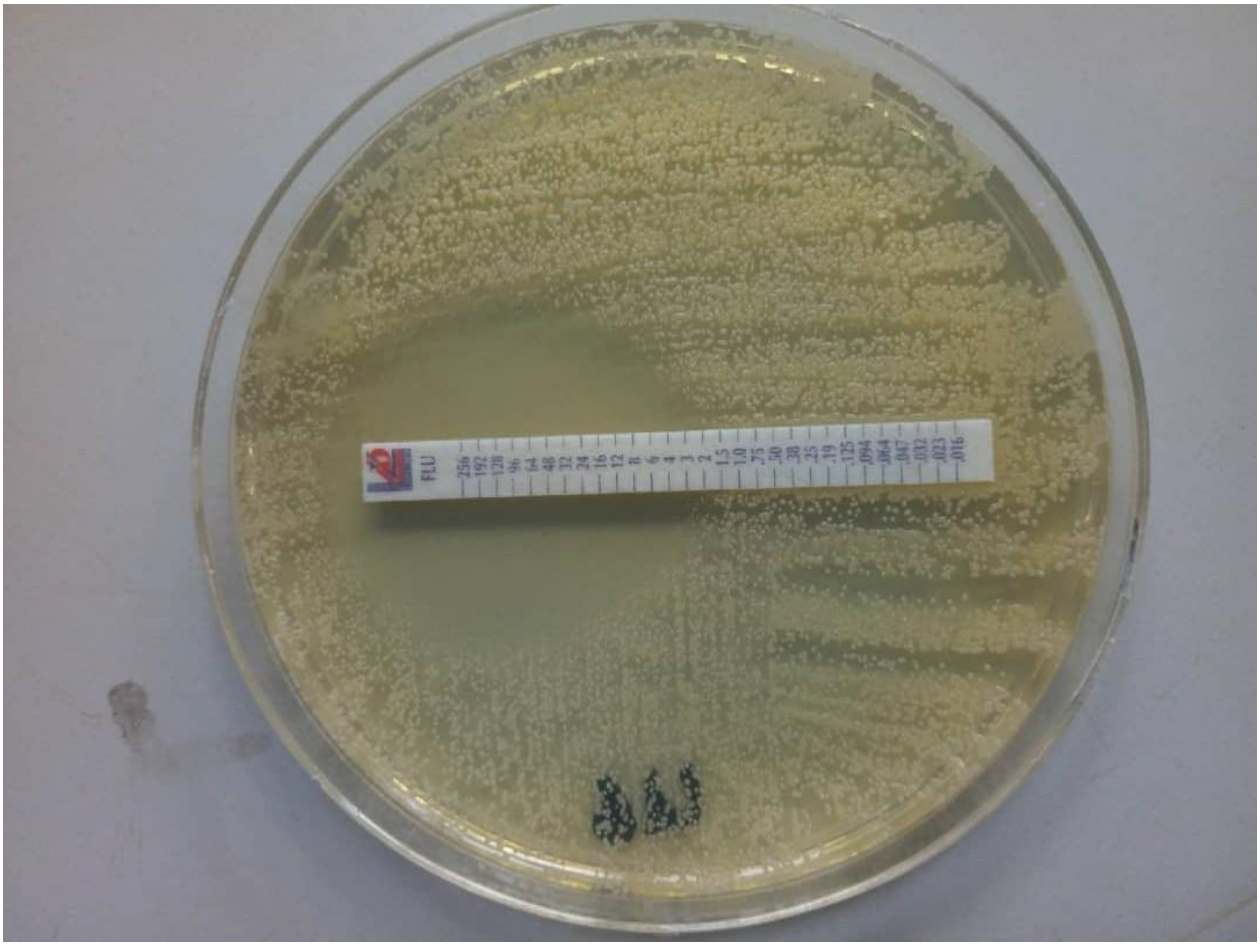

Plate 1 MIC test plate for one of the *C. albicans* isolates

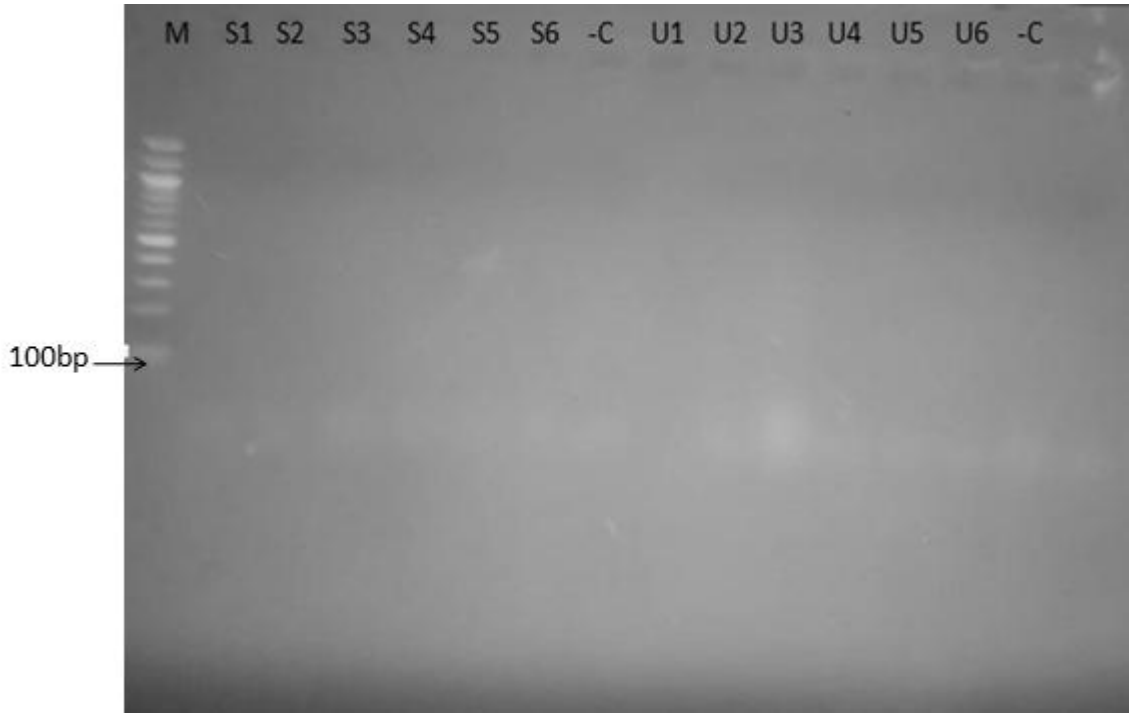

Plate 2: PCR result of six *C. albicans* isolates spiked with Fluconazole (Depicted as S) and six isolates unspiked with fluconazole (depicted as U)

CHAPTER FIVE

DISCUSSION

This study was aimed at studying the prevalence of *Trichomonas vaginalis* and azole resistant *Candida albicans* expressing ERG11 gene from genital samples of women in Abakaliki. A total of 628 urine and HVS samples were examined and yielded 0% prevalence of *Trichomonas vaginalis* within the study period. This zero prevalence is in partial agreement with various findings as regards incidence of *Trichomonas vaginalis* in various Nigerian cities. Uneke *et al* (2006) reported a prevalence of 2.8% out of 502 women examined in a study conducted in Abakaliki. A study in Onitsha carried out to check for prevalence of *T. vaginalis* in women attending various Hospitals reported an overall prevalence of 17.5% with some of the hospitals specifically presenting with 0% prevalence (Iwueze et al., 2010). Olorungbe *et al.* in 2015 reported a prevalence of 20% out of 200 women in Abeokuta, Nigeria. Another Study in Jos reported a prevalence of 4% out of 150 women using the direct wet mount method (Okojokwu *et al.*, 2015). Adeoye and Akande (2007) reported a prevalence of 3.3% out of 544 women attending Lagos state University teaching hospital. Most recently a study by Hamafyelto and Ikeh (2017) recorded a prevalence of 20.5% out of 200 women.

The prospect of finding *T. vaginalis* in culture was highly anticipated. Though no *T. vaginalis* was detected, this finding could be as a result of factors like timing and collection of the sample. Most of the samples (especially Urine) were collected on the spot and some others were brought from home after pre-information of the respondents. Vaginal swabs were collected by trained health personnel. The hygienic conditions of the respondents may have also affected the result.

Researches have been going on as regards to the best method to be used in diagnosis of *Trichomonas vaginalis* infection. Factors like exposure of specimens to light and hygienic conditions of the patient are also contributing factors to the sensitivity of the test. Prompt treatment of cases of Trichomoniasis may also be another factor as it largely decreases carrier status. Most respondents who volunteered for this study had no cases of itching.

Findings from this study showed a prevalence rate of 13% with respect to isolation of *Candida albicans* from various respondents that participated in the study. *Candida albicans* has been isolated in many studies that have looked into genitourinary candidiasis in sexually active women. Uzoh *et al* (2016) recorded a prevalence of 40% (120) out of 300 Pregnant women in Portharcourt. Their study also recorded 17% (17) out of 100 Non-pregnant women. Chijioke *et al* (2016) recorded 37.7% (107) out of 284 Asymptomatic women in a University community. A study which investigated Vulvovaginal candidiasis in female patients attending a tertiary health care institution in Abuja yielded a prevalence rate of 6.5% out of 200 volunteers for the study (Emeribe *et al.*, 2015). Most of the patients though were on antibacterial therapy and this may have had an impact on the distribution of the fungus. Another study tested vaginal swabs of 1,000 women with vaginal infections and 1,000 controls. Sixty percent of women in the infection group tested positive for vaginal candidiasis as did 120 women in the control group (Emeribe et al., 2015). *Candida albicans* was the strain of *Candida* most commonly found in cases of Candidiasis. A study by Okonkwo and Umeanaeto (2010) showed that 30% (90) of 300 women who attended some selected hospitals in Nnewi for prenatal care had vaginal candidiasis. Umar *et al*. (2017) recorded a prevalence rate of 66% (271) out of 400 symptomatic women of child bearing age attending a gyneacology clinic in Kaduna.

This study shows that *C. albicans* was isolated more from samples of pregnant women than Non pregnant women. Incidence in Pregnant women was 7.96%. Incidence in Non-pregnant

women was 5.09%. Our finding is in line with findings of Akingbade *et al* (2013) who recorded higher occurrence of vulvovaginal candidiasis in Pregnant women (31.2%) than Non-Pregnant women (17.6%). Raid *et al* (2012) also recorded a prevalence of 24% for pregnant women and 17% for non pregnant women. Pregnancy is a high-risk factor in vulvovaginal candidiasis and our present study confirms this. In pregnancy, the oestrogen level is usually high. Glycosuria is also encountered and leads to high acidity and there is usually provision of utilizable sugar which facilitates growth and multiplication of the fungus.

This study also showed that vaginal swab specimens presented with higher incidence of *C. albicans* than Urine samples. Occurrence of *C. albicans* in vaginal samples was 5.09%. In urine it was 7.96%. This is in agreement with other studies that have shown the same trend with respect to the type of specimen where *C. albicans* is isolated from. Akortha *et al* (2009) reported an 82.9% prevalence in vaginal swab samples and 17.7% incidence in urine samples of women in Benin city. Another study in Kano also reported a higher incidence in vaginal swab samples (61.3%) than urine samples (38.7%) (Tawra *et al*., 2013).

With respect to age, the age group 26-30 presented with the highest prevalence (4.46%) of *C. albicans* and the age group 36-40 presented with the lowest prevalence (1.27%). This does not agree with findings of Alo *et al.*, (2012) where the age group 36-40 had the highest distribution (33%) and the age group 20-25 had the lowest distribution (20.42%). Findings from this study agrees with findings from a study in Abuja where the highest distribution was recorded for the age group 20-30 and the least for the age group >40 (Emeribe *et al.*, 2015). Okongbuwa *et al* (2003) also presented very low prevalence for the age groups 36-45 and >46 years. Dennis *et al* (2013) reported the highest prevalence for the age group 22-26 (32.1%) followed by the age group 27-31 (28.9%). Another study in Jos recorded the highest prevalence in the age group 21-30 (43.0%) (Nwadioha *et al*., 2010).

The high prevalence in the age group 26-30 could be due to high sexual activities as this age group fall into the sexually active group of women. At this stage hormonal activities are optimally high. Pregnancy at this stage is also a contributory factor. Poor personal hygiene, use of contraceptives and drug abuse could also be contributory factors.

The low prevalence recorded in the age group 36-40 and >40 could be attributed to findings that advances in age reduces the effect of estrogen in women which leads to lower infection rate. At this stage, hormonal activities is usually very low. Hence presence of *C. albicans* becomes minimal. Hygiene could also be a contributory factor for presence of *C. albicans* in women of advanced age.

In this study, prevalence of *C. albicans* with respect to Trimester of Pregnancy was highest in women who were in their third trimester, followed by those in their second trimester and those in first. This finding disagrees with a study in Benin where women in their second trimester had the highest prevalence (68.8%) followed by those in their third trimester (30.6%) and those in first (33.3%) (Oviasogie *et al.*, 2009). Another study in India also showed that women in their second trimester recorded the highest prevalence (54%) followed by the third (30%) and first (16%). Findings from this study are in agreement with a study in Owerri where women in their third trimester had the high prevalence for *C. albicans* (47.5%). This was followed by those in their second (36.95%) and first Trimester (15.33%) respectively. Another study in Kenya also records the highest prevalence for women in their third trimester (68.09%), followed by those in their second trimester (21.28%) and those in their first (10.63%).

High rate of *Candida* infection with various stages of pregnancy observed in our study can be attributed to the fact that immunosuppression increases as pregnancy develops over time. Hence women in their second trimester of pregnancy have lower immunity than those in

their first trimester and those in their third trimester have lower immunity than those in their second trimester. In Pregnancy there is usually an increase in estrogen level and the decrease in immunity tends to decrease in the level of vaginal defense mechanisms against opportunistic infections. Use of drugs could still be another factor as the makeup of the normal flora of the vagina are usually altered when drugs are used hence leading to overgrowth of some of the normal flora especially *C. albicans*. When antibiotics are used frequently, it destroys some of the bacteria which reside in the Vagina as normal flora thereby directly having an impact on the optimal pH range of the vgaina. It is worthy of note that some bacteria in the vagina are there to play a role in pH regulation. High Sugar levels in women could still be another factor as availability of glucose influences growth of *C. albicans*. Results from routine strip test of respondents urine showed high glucose levels especially in respondents who were in their third trimester. Other analytes such as Proteins and pH were suggestive of the obvious presence of factors that support the overgrowth of *C. albicans.*

This study showed relatively high resistance patterns to the two conventional azoles used by disc diffusion method. The results show that 72% of the isolates were resistant to fluconazole and 57% were resistant to Voriconazole. Our findings totally disagreed with findings from a study in India where 91.4% of Candida species were susceptible to Fluconazole and Voriconazole (Tumkur *et al*., 2014). Efunshiile *et al* (2016) also recorded 0% resistance for Fluconazole in a study at Ogbomosho. In Ghana a study by Feglo and Narkwa recorded 97% susceptibility to Voriconazole and 71.6% susceptibility to Fluconazole.

Our observation agrees with those of Adesisji *et al.* (2011) recorded 73% resistance to Fluconazole and 61.5% resistance to Voriconazole. In their study they observed 42.3% susceptibility to Nystatin. Another study in Egpyt showed resistance of 61.2% to

Fluconazole (Mohammad *et al.,* 2017). Another study showed that a significantly higher percentage of *C. albicans* isolates had reduced susceptibility to fluconazole (14.2%) than Itraconazole (6.7%) and voriconazole (6.1%).

Findings from our study may largely be due to genetic flexibility of the isolates. Indiscriminate use of antibiotics may also be contributory factors. Selective pressure which results from relative exposure to some azole drugs may also be responsible because exposure to an antimicrobial agent precedes development of resistance to that agent. Hence the use of antifungal agents especially in pregnant women who have higher infection rates due to suppressed immunity would have created an avenue for exposure of the isolates to various azoles. In this study, Nystatin was used as a tangential control drug and the result obtained showed high susceptibility by most of the isolates. Hence it is suggested from our observations that most of our respondents must have had higher exposure to azole based drugs than Nystatin. Azole based drugs generally are the most frequently used antifungal drugs (Pam *et al.,* 2012).

The MIC test result of six isolates selected for molecular analysis showed that only one of the isolates was resistant to fluconazole. This is primarily in contrast with results obtained from the disc diffusion method where all six isolates were resistant to fluconazole. These conflicting results are in line with findings from a study by Cretella *et al*., (2016). In their study, comparison of susceptibility patterns using commercially available susceptibility testing methods performed on prevalent *Candida* species. Variability in strengths and limitations of various antifungal susceptibility test methods have been linked to inter-laboratory variability (Pappas *et al.,* 2016).

In some cases, isolates have been found to be clinically susceptible with elevated MIC values (CLSI, 2012). Hence most studies the roles that discrepant results play in differences in therapeutic outcomes remains to be determined (Andes *et al*., 2012, Pappas *et al.,* 2016)

The use of PCR to amplify DNA of 6 selected isolates that were resistant to fluconazole and voriconazole showed that there was no expression of the ERG11 gene. Our finding disagrees with results from a study in Lagos where the coding region for ERG11 in the extracted DNA of Fluconazole resistant *C. albicans* weighed 92bp (Pam *et al.,* 2012). Expression of ERG11 gene was detected in fluconazole resistant strains of *C. albicans* using Light cycler PCR in a study that looked into various resistant genes of *C. albicans* in China (Frade *et al.,* 2004). The results agree with findings by Alizadeh *et al* (2017). In their study, there was significant decrease in total cellular ergosterol content for fluconazole susceptible *C. albicans*. Another study by Arthingto-Skaggs et al (1999) showed between 72% to 100% reduction in ergosterol content after exposure of *C. albicans* to various concentrations of fluconazole.There are a lot of factors that could lead to resistance to azoles by *C. albicans*, expression of ERG11 gene is the most common mechanism (Strzelcyk *et al.,* 2013).

Fluconazole is fungistatic rather than fungicidal, so treatment provides opportunity for acquired resistance to develop in the presence of the antifngal. Fluconazole is still currently regarded as a fundamental antifungal drug. It is still opined that mechanisms of fluconazole resistance are largely unknown (Berkow and Lockhart, 2017).

Genetically different mechanisms are frequently combined to result in a stepwise development of fluconazole resistance overtime. The ERG11 gene is chiefly responsible for drug target overexpression, drug target alteration, aneuploidy and loss of heterozygosity. The ERG3 gene is responsible for bypass pathways while the efflux pump overexpression is

facilitated by the ABC (CDR1, CDR2, SNQ2, ABC1) and MFS (MDR1, TPO3) genes. The MDR1 gene is not transcribed during growth in-vitro in fluconazole susceptible *C. albicans* strains but overexpressed in many fluconazole-resistant clinical isolates resulting in reduced intracellular fluconazole accumulation. Hence it is possible that fluconazole resistance observed in this study could have been by other mechanisms not facilitated by the ERG11 gene.

5.1 Conclusion

This present study revealed the following

1. There was no prevalence of vaginal Trichomoniasis in women in Abakaliki metropolis
2. There was relatively high prevalence of Triazole resistant *Candida albicans* as demonstrated by Disc diffusion.
3. There was no expression of ERG11 gene by *C. albicans* isolated from adult females in Abakaliki.
4. Observed resistance may be due to other factors other than ERG11.

5.2 Recommendation

The high resistance recorded in this study for fluconazole which is considered the most readily available azole drug is quite alarming. The study therefore recommends a combination of azole drugs with another antifungal drug like nystatin in treatment of vulvovaginal candidiasis to lower the resistance rate. Further study should be employed in ascertaining resistance to antifungal agents as there are high chances of variability with various tests

5.3 Contribution to Knowledge

ERG11 gene was not detected in Fluconazole resistant Candida albicans isolated from Urine and Vaginal swab specimens of adult healthy females in Abakaliki, Ebonyi state.

There is high level of paucity of information on genes responsible for antifungal resistance especially in strains obtained within our immediate vicinity. Most researchers around have worked extensively on assessing antifungal susceptibility without going further to look into the causes. Studies on resistance genes in bacteria have received better attention in our locality than studies on resistance genes in Fungi. There are about five resistance genes in *Candida* and so far in Nigeria only a study by Pam *et al* (2012) has looked into the expression of ERG11.

The high resistance recorded for fluconazole which is considered the most readily available azole drug for prophylaxis is quite alarming owing to the fact that women who come down with gental infections like vulvovaginal candidiasis or vaginitis may have to resort to other antifungals or combination therapy. There is need for extensive studies on various methods used in assessing resistance profile of antifungal agents. Reoccurence of Candidiasis due to resistance posed by *C. albicans* could be as a result of poor adherence to therapeutic measures and drug abuse. Further studies should be centered on quantifying the level of expression and mutation of ERG11 gene as it can help predict the trend of resistance and help with suggestion of better antifungal regimens.

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APPENDIX

APPENDIX A RESULTS FROM MORPHOLOGICAL IDENTIFICATION OF C. ALBICANS IN URINE OF PREGNANT WOMEN
BATCH 1

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	**Atlas appearance**	**Organism**
U_1	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_2	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_3	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_4	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_5	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_6	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_7	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_8	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_9	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{10}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{11}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{12}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{13}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{14}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{15}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{16}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{17}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{18}	+ve	Yeast	+ve	+ve	+ve	-ve	+	*C.*

		seen						*albicans*
U_{19}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{20}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{21}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{22}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{23}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{24}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{25}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 2

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{26}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{27}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{28}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{29}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{30}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{31}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{32}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{33}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{34}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{35}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{36}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{37}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{38}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{39}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{40}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{41}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{42}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{43}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{44}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{45}	+ve	Yeast	+ve	-ve	-ve	-ve	-	OY

		seen						
U_{46}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{47}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{48}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{49}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{50}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 3

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{51}	+ve	Yeast seen	+ve	+ve	-ve	-ve	+	*C.albicans*
U_{52}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{53}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{54}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{55}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{56}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{57}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{58}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{59}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{60}	+ve	Yeast seen	+ve	+ve	-ve	-ve	+	*C.albicans*
U_{61}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{62}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{63}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{64}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{65}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{66}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{67}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{68}	+ve	Yeast seen	+ve	-ve	+ve	-ve	+	*C. albicans*
U_{69}	+ve	Yeast	+ve	-ve	+ve	-ve	-	OY

		seen						
U_{70}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{71}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{72}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{73}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{74}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{75}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 4

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{76}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{77}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{78}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{79}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{80}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{81}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{82}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{83}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{84}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{85}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{86}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{87}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{88}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{89}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{90}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{91}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{92}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{93}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{94}	+ve	Yeast	+ve	+ve	+ve	-ve	+	*C.*

		seen						*albicans*
U_{95}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{96}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{97}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{98}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{99}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{100}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 5

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{101}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{102}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{103}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{104}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{105}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{106}	+ve	Yeast not seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{107}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{108}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{109}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{110}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{111}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{112}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{113}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{114}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{115}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{116}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{117}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{118}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY

U_{119}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{120}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{121}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{122}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{123}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{124}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{125}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 6

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{126}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{127}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{128}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{129}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{130}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{131}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{132}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{133}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{134}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{135}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{136}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{137}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{138}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{139}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{140}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{141}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{142}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{143}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{144}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{145}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

U_{146}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{147}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{148}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{149}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{150}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 7

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{151}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{152}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{153}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{154}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{155}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{156}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{157}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{158}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{159}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{160}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{161}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{162}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{163}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{164}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{165}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{166}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{167}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{168}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{169}	+ve	Yeast	+ve	+ve	+ve	-ve	+	*C.*

		seen						*albicans*
U_{170}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{171}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{172}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{173}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{174}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{175}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 8

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{176}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{177}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{178}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{179}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{180}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{181}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{182}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{183}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{184}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{185}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

APPENDIX B RESULTS FROM MORPHOLOGICAL IDENTIFICATION OF C. ALBICANS IN URINE OF NON-PREGNANT WOMEN

BATCH 1

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_1	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_2	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_3	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_4	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_5	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_6	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_7	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_8	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_9	+ve	Yeast not seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{10}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{11}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{12}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{13}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{14}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{15}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{16}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{17}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

U_{18}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{19}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{20}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{21}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{22}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{23}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{24}	+ve	Yeast seen	+ve	-ve	+ve	-ve	-	OY
U_{25}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 2

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{26}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{27}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{28}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{29}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{30}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{31}	+ve	Yeast not seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{32}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{33}	+ve	Yeast not seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{34}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{35}	+ve	Yeast seen	+ve	+ve	+ve	-ve	-	*C. albicans*
U_{36}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{37}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{38}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{39}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{40}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{41}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{42}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{43}	+ve	Yeast	+ve	-ve	-ve	-ve	-	OY

		seen						
U_{44}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{45}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{46}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{47}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{48}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{49}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{50}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 3

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{51}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{52}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{53}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{54}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{55}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{56}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{57}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{58}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{59}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{60}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{61}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{62}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{63}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{64}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{65}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{66}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{67}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{68}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{69}	+ve	Yeast	+ve	-ve	-ve	-ve	-	OY

		seen						
U_{70}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{71}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{72}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{73}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{74}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{75}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 4

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{76}	+ve	Yeast seen	+ve	-ve	-ve	-ve	+	*C. albicans*
U_{77}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{78}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{79}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{80}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{81}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{82}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{83}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{84}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{85}	+ve	Yeast seen	+ve	-ve	-ve	-ve	+	*C. albicans* OY
U_{86}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{87}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{88}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{89}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
U_{90}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{91}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{92}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{93}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{94}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

U_{95}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{96}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{97}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{98}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{99}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{100}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 5

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{101}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{102}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{103}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{104}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{105}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{106}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{107}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{108}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{109}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{110}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{111}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{112}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{113}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{114}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{115}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{116}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{117}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{118}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{119}	+ve	Yeast	+ve	-ve	-ve	-ve	-	OY

		seen						
U_{120}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{121}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{122}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{123}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{124}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{125}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 6

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{126}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{127}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{128}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{129}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{130}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{131}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{132}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{133}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{134}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{135}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{136}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{137}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{138}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{139}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{140}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{141}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
U_{142}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{143}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{144}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{145}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{146}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{147}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{148}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{149}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{150}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 7

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
U_{51}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{52}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{53}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{54}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
U_{55}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

APPENDIX C: RESULTS FROM MORPHOLOGICAL IDENTIFICATION OF *C. ALBICANS* IN VAGINAL SWABOF PREGNANT WOMEN

BATCH 1

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_1	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_2	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_3	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_4	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_5	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_6	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_7	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_8	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_9	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{10}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{11}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{12}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{13}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{14}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{15}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{16}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{17}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{18}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

H_{19}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{20}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{21}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{22}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{23}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{24}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{25}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 2

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{26}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{27}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{28}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{29}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{30}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{31}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{32}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{33}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{34}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{35}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{36}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{37}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{38}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{39}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{40}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{41}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{42}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*

H_{43}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{44}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{45}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{46}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{47}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{48}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{49}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{50}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 3

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{51}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{52}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{53}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{54}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{55}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{56}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{57}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{58}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{59}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{60}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{61}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{62}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{63}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{64}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{65}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{66}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{67}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{68}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

H_{69}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{70}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{71}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{72}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{73}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{74}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{75}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 4

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{76}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{77}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{78}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{79}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{80}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{81}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{82}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{83}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{84}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{85}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{86}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{87}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{88}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{89}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{90}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{91}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{92}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{93}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{94}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

H_{95}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{96}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{97}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{98}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{99}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{100}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 5

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{101}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{102}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{103}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{104}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{105}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{106}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{107}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{108}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{109}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{110}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{111}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{112}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{113}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{114}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{115}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{116}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{117}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{118}	+ve	Yeast	+ve	-ve	-ve	-ve	-	OY

		seen						
H_{119}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{120}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{121}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{122}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{123}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{124}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{125}	+ve	Yeast seen	+ve	+ve	+ve	-ve	-	*C. albicans*

BATCH 6

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{126}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{127}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{128}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{129}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{130}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{131}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{132}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{133}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{134}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{135}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{136}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{137}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{138}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{139}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{140}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{141}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{142}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{143}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{144}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

H_{145}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{146}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{147}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{148}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{149}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{150}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 7

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{151}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{152}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{153}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{154}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{155}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{156}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{157}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{158}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{159}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{160}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{161}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{162}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{163}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{164}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{165}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{166}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{167}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{168}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{169}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{170}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

H_{171}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{172}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{173}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

APPENDIX D: RESULTS FROM MORPHOLOGICAL IDENTIFICATION OF C. ALBICANS IN VAGINAL SWABS OF NON-PREGNANT WOMEN

BATCH 1

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_1	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_2	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_3	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_4	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_5	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_6	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_7	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_8	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_9	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{10}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{11}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{12}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{13}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{14}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{15}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{16}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{17}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

H_{18}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{19}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{20}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{21}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{22}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{23}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{24}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{25}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 2

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{26}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{27}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{28}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{29}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{30}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{31}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{32}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{33}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{34}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{35}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{36}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{37}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{38}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{39}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{40}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{41}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{42}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{43}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{44}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

H_{45}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{46}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{47}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{48}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{49}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{50}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 3

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{51}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{52}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{53}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{54}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{55}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{56}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{57}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{58}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{59}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{60}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{61}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{62}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{63}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{64}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{65}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{66}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{67}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{68}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{69}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albican*

								s
H_{70}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{71}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{72}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{73}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{74}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{75}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 4

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{76}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{77}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{78}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{79}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{80}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{81}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{82}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{83}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{84}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{85}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{86}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{87}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{88}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{89}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{90}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{91}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{92}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{93}	+ve	Yeast	+ve	-ve	-ve	-ve	-	OY

		seen						
H_{94}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{95}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{96}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{97}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{98}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{99}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{100}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

BATCH 5

Samples	**Turbidity in trichomonas broth**	**Microscopy**	**Growth on SDA**	**Germ tube reaction**	**Glucose**	**Urease**	Atlas appearance	**Organism**
H_{101}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{102}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{103}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{104}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{105}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{106}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{107}	+ve	Yeast seen	+ve	+ve	+ve	-ve	+	*C. albicans*
H_{108}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{109}	+ve	Yeast not seen	+ve	-ve	-ve	-ve	-	OY
H_{110}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{111}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{112}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{113}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{114}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY
H_{115}	+ve	Yeast seen	+ve	-ve	-ve	-ve	-	OY

APPENDIX E: RESULTS FROM ANTIFUNGAL SUSCEPTIBILITY TEST

Sample	**Fluconazole(mm)($25\mu g$)**	**Voriconazole(mm)($1\mu g$)**	**Nystatin(mm)($100\mu g$)**
1	-(R)	-(R)	26(S)
2	26(S)	-(R)	26(S)
3	-(R)	26(S)	27(S)
4	-(R)	-(R)	26(S)
5	9(R)	8(R)	26(S)
6	-(R)	27(S)	-(R)
7	27(S)	-(R)	28(S)
8	-(R)	-(R)	27(S)
9	8(R)	8(R)	28(S)
10	-(R)	28(S)	26(S)
11	-(R)	-(R)	-(R)
12	-(R)	-(R)	28(S)
13	-(R)	26(S)	7(R)
14	-(R)	27(S)	27(S)
15	11.5(R)	27(S)	27(S)
16	26(S)	-(R)	26(S)
17	-(R)	-(R)	28(S)
18	7(R)	7(R)	28(S)
19	-(R)	26(S)	28(S)
20	-(R)	-(R)	28(S)

Sample	Fluconazole(mm)($25\mu g$)	Voriconazole(mm)($1\mu g$)	Nystatin(mm)($100\mu g$)
21	10(R)	-(R)	27(S)
22	7(R)	-(R)	27(S)
23	8(R)	-(R)	26(S)
24	10(R)	-(R)	26(S)
25	9(R)	26(S)	27(S)
26	11(R)	-(R)	27(S)
27	7(R)	26(S)	26(S)
28	7(R)	-(R)	7(R)
29	8(R)	8(R)	27(S)
30	7(R)	-(R)	27(S)
31	6(R)	27(S)	26(S)
32	11(R)	-(R)	27(S)
33	10(R)	-(R)	28(S)
34	11(R)	-(R)	27(S)
35	11(R)	27(S)	-(R)
36	8(R)	27(S)	27(S)
37	11(R)	28(S)	28(S)
38	12(R)	7(R)	27(S)
39	13(R)	-(R)	28(S)
40	12(R)	28(S)	26(S)

Sample	Fluconazole(mm)($25\mu g$)	Voriconazole(mm)($1\mu g$)	Nystatin(mm)($100\mu g$)
41	-(R)	-(R)	26(S)
42	-(R)	28(S)	26(S)
43	-(R)	-(R)	8(R)
44	-(R)	-(R)	27(S)
45	9(R)	8(R)	27(S)
46	27(S)	27(S)	28(S)
47	10(R)	-(R)	27(S)
48	-(R)	-(R)	27(S)
49	8(R)	8(R)	28(S)
50	7(R)	-(R)	7(R)
51	-(R)	-(R)	26(S)
52	28(S)	27(S)	26(S)
53	-(R)	26(S)	27(S)
54	-(R)	26(S)	27(S)
55	11(R)	-9(R)	26(S)
56	-(R)	-(R)	27(S)
57	-(R)	-(R)	28(S)
58	7(R)	7(R)	27(S)
59	-(R)	-(R)	28(S)
60	10(R)	26(S)	26(S)

Sample	Fluconazole(mm)($25\mu g$)	Voriconazole(mm)($1\mu g$)	Nystatin(mm)($100\mu g$)
61	26(S)	26(S)	26(S)
62	-(R)	-(R)	26(S)
63	-(R)	-(R)	26(S)
64	-(R)	27(S)	6(R)
65	9(R)	8(R)	27(S)
66	-(R)	-(R)	28(S)
67	27(S)	-(R)	28(S)
68	-(R)	28(S)	-(R)
69	8(R)	8(R)	27(S)
70	-(R)	-(R)	26(S)
71	-(R)	-(R)	27(S)
72	-(R)	29(S)	27(S)
73	-(R)	-(R)	27(S)
74	28(S)	-(R)	7(R)
75	11.5(R)	-9(R)	26(S)
76	-(R)	-(R)	28(S)
77	-(R)	27(S)	28(S)
78	7(R)	7(R)	27(S)
79	-(R)	-(R)	26(S)

80	-(R)	-(R)	-(R)
81	26(S)	-(R)	26(S)
82	-(R)	-(R)	27(S)

APPENDIX F

CHI SQUARE CALCULATION

Expected Values For Chi Square Calculation With Respect To Age As A Factor For Prevalence Of *C. Albicans* Infection

Age Group	**No positive (Expected value)**	**No Negative (Expected value)**	**Observed**
20-25	16(22.7)	158(151.3)	174
26-30	28(27.0)	179(180.0)	207
31-35	19(17.2)	113(114.8)	132
36-40	8(6.3)	40(41.7)	48
>40	11(8.7)	56(58.3)	67
	82	**546**	**628**

Chi Square calculated= 4.34

For Chi Square Tabulated:

Degree of Freedom = (C-1)(R-1)

C; No of Column

R; No of Row

DF= (5-1)(2-1)=4

Chi Square tabulated = 9.488at P=0.05

X^2calculated is less than X^2tabulated.

Hence Prevalence of Candidiasis may not be dependent on Age as a factor

Chi square calculation with respect to Pregnancy as a factor

$$\frac{(50-46.7)^2}{50}+\frac{(32-35.3)^2}{32}+\frac{(303-313.0)^2}{303}+\frac{(238-234.7)^2}{238}=\frac{10.89}{50}+\frac{10.89}{32}+\frac{100}{303}+\frac{10.89}{238}$$
$$=0.22+0.34+0.33+0.05=0.94$$

Expected Values For Chi Square Calculation With Respect To Pregnancy As A Factor For Prevalence Of *C. Albicans* Infection

Factor	**No positive (Expected value)**	**No Negative (Expected value)**	**Observed**
Pregnant	50(46.7)	308(313.0)	358
Non-Pregnant	32(35.3)	238(234.7)	270
Total	**82**	**546**	**628**

Chi Square calculated= 0.94

For Chi Square Tabulated:

Degree of Freedom = (C-1)(R-1)

C; No of Column

R; No of Row

DF= (2-1)(2-1)=1

Chi Square tabulated = 3.841 at P=0.05

X^2calculated is less than X^2tabulated Hence Prevalence of Candidiasis is independent of Pregnancy as a factor

Chi square calculation with respect to Clinical specimen as a factor

$$\frac{(32-44.4)^2}{32}+\frac{(308-295.6)^2}{308}+\frac{(50-37.6)^2}{50}+\frac{(288-250.4)^2}{238}=\frac{153.8}{32}+\frac{153.8}{308}+\frac{153.8}{50}+\frac{153.8}{238}$$
$$=4.80+0.50+3.10+0.65=9.05$$

Expected Values for Chi Square Calculation with Respect to Clinical Specimen as A Factor for Prevalence of *C. albicans* Infection

Specimen	**No positive (Expected value)**	**No Negative (Expected value)**	**Observed**
Urine	32(44.4)	308(295.6)	340
HVS	50(37.6)	238(250.4)	288
Total	**82**	**546**	**628**

Chi Square calculated= 9.05

For Chi Square Tabulated:

Degree of Freedom = (C-1)(R-1)

C; No of Column

R; No of Row

DF= (2-1)(2-1)=1

Chi Square tabulated = 3.841 at P=0.05

X^2calculated is greater than X^2tabulated.

Hence Prevalence

Chi square calculation with respect to Age as a factor

$$\frac{(16-22.7)^2}{16}+\frac{(158-151.3)^2}{158}+\frac{(28-27.0)^2}{50}+\frac{(179-180.0)^2}{238}+\frac{(19-17.2)^2}{19}+\frac{(113-14.8)^2}{113}$$
$$+\frac{(8-6.3)^2}{8}+\frac{(40-41.7)^2}{40}+\frac{(11-8.7)^2}{11}+\frac{(56-58.3)^2}{56}$$
$$=\frac{44.89}{16}+\frac{44.89}{158}+\frac{1}{28}+\frac{1}{179}+\frac{3.24}{19}+\frac{3.24}{113}+\frac{2.89}{8}+\frac{2.89}{40}+\frac{5.29}{11}+\frac{5.29}{56}$$
$$=2.81+0.28+0.04+0.01+0.17+0.03+0.36+0.07+0.48+0.09=4.34$$

Chi square calculation with respect to trimester of pregnancy

$$\frac{(5-13.7)^2}{5}+\frac{(93-84.3)^2}{93}+\frac{(10-16.5)^2}{10}+\frac{(100-94.6)^2}{100}+\frac{(26-16.5)^2}{26}+\frac{(92-101.5)^2}{92}$$
$$+\frac{(9-4.5)^2}{9}+\frac{(23-27.5)^2}{23}$$
$$=\frac{75.69}{5}+\frac{75.69}{93}+\frac{42.25}{10}+\frac{29.16}{100}+\frac{90.25}{26}+\frac{90.25}{92}+\frac{20.25}{9}+\frac{20.25}{23}$$
$$=15.14+0.81+4.23+0.29+3.47+0.98+2.25+0.88=8.05$$

APPENDIX G

INFORMED CONSENT

Dear Respondent,

I am Agumah Nnabuife Bernard, a Ph.D research student from the department of Applied Microbiology and Brewing, Nnamdi Azikiwe University Awka.

I am currently working on The Prevalence of *Trichomonas vaginalis* and Triazole resistant *Candida albicans* expressing ERG11 gene isolated from women in Abakaliki Metropolis.

Trichomonas vaginalis is a flagellate that causes a parasitic disease known as Trichomoniasis. *Trichomonas vaginalis* has been known to cause complications especially in pregnant women.

Candida albicans which is a normal flora of the genital tract and oral cavity causes candidiasis. They are regarded as opportunistic pathogens because they easily cause diseases especially in cases where there is a compromise in the immune system of an individual. Pregnancy is a disposing factor for Candidiasis.

Resistance to commonly used drugs for treatment of Candidiasis has been on the rise and very little has been done with finding out root causes of this resistance. ERG11 is one of the genes expressed by *C. albicans* that helps with conferring resistance traits.

I write to seek your consent as a voluntary participant in the study. If interested, please indicate by ticking the appropriate box below.

Urine and Vaginal swab specimens will be collected.

A simple questionnaire will be issued to you as well

All information as regards personal results obtained from this study will be kept strictly confidential and can be assessed by contacting the researcher (08035265293).

Do you give consent? Yes……..No……..

Thank you

QUESTIONAIRE

1 NAME (Optional):…….

2 AGE:……

3 ARE YOU PREGNANT? ……

4 WHAT TRIMESTER ARE IN? ……

5 WOULD YOU LIKE TO HAVE ASSESS TO YOUR RESULT AFTER BEEN TESTED?

YES…… NO……