ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA* SPECIES AND *CRYPTOCOCCUS NEOFORMANS* ISOLATED FROM PATIENTS AT THE KOMFO ANOKYE TEACHING HOSPITAL IN KUMASI

by

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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Abstract

Antifungal drugs are prescribed for the treatment of yeast infections at the Komfo Anokye Teaching Hospital yet the susceptibility profiles of the infecting yeasts are unknown. The aim of this study was to determine the common species of yeasts that cause infection among patients attending the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana and to determine their susceptibility profiles to antifungal agents. A total of 528 clinical samples were collected between March and June 2009 and analyzed. Samples collected from individuals consisted of 186 (35%) high vaginal swabs, 109 (21%) cerebrospinal fluid (CSF) samples, 127 (24%) urine samples and 106 (20%) sputum samples. The samples were cultured on sabouraud dextrose agar. Identification of the yeasts was performed using growth characteristics, the germ tube test and the API ID 32 C test kits. Antifungal susceptibility of yeast isolates to antifungal agents was done using ATB™ FUNGUS 3 test kits and the agar disc diffusion method. Sixty- seven (67) out of the 528 samples yielded yeasts giving yeast prevalence of 12.7%. Thirty- three (49.3%) of the isolates were *Candida albicans,* 12(17.9%) were *Candida glabrata*, 8(11.9%) *Candida tropicalis*, 4(6%) *Candida dubliniensis*, 3(4.5%) *Candida krusei*, 2(3%) *Candida sake*, 1(1.5%) *Candida guilliermondii*, 1(1.5%) *Candida parapsilosis* and 3(4.5%) *Cryptococcus neoformans*. All isolates of *Candida dubliniensis*, *Candida sake*, *Candida parapsilosis*, *Candida guilliermondii* and *Cryptococcus neoformans* were susceptible to flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole with minimum inhibitory concentrations (MICs) ranging from 0.125 to 8mg/l. All isolates of *Candida krusei* were resistant to fluconazole with MICs of ≥ 64mg/l. Seventy-nine (79) to 85 % of isolates of *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei* were susceptible to flucytosine, amphotericin B, and itraconazole with MICs ranging from 0.125 to 8mg/l. Voriconazole exhibited greatest activity against isolates of *C. albicans, C. glabrata, C. tropicalis and C. krusei* with no resistant strains identified. Voriconazole appeared to be the most effective antifungal against all the yeast isolates tested.

CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Yeasts are unicellular fungi that cause a wide range of infections commonly called yeast infections in humans. Yeast infections are categorized into two groups; superficial and systemic. Superficial yeast infections can affect different parts of the human body including the skin, mouth, digestive tract, nails, vagina and oesophagus and can become chronic (Thevissen, 2005).

Systemic yeast infections also called invasive yeast infections can occur in organs of the body such as the brain, spinal cord, eye, gall bladder, heart, lungs, kidneys and urinary bladder (Talaro & Talaro, 1996). Systemic yeast infections are seen in individuals with reduced function of the immune system. Compared to the superficial yeast infections, systemic yeast infections are characterized by lower frequencies but generally, high mortality rates of about 40 to 100% have been reported in the United States (Thevissen, 2005).

The most common yeasts that infect humans are the *Candida* species and *Cryptococcus* species (Talaro & Talaro, 1996). *Candida albicans* remains the predominant *Candida* species causing candidal infections accounting for over half of all cases in the world (Pfaller & Diekema, 2007). An increase in the prevalence of yeast infections caused by non-albicans *Candida* such as *Candida glabrata, Candida krusei, Candida tropicalis and Candida parapsilosis* have been reported in other part of the world (Pfaller & Diekema, 2007). Among the species of *Cryptococcus, Cryptococcus neoformans* is the leading cause of infection mainly in immunocompromised patients. Other *Cryptococcus* species like *Cryptococcus albidus* and

Cryptococcus laurentii also cause infections but these are rarely encountered (Kordossis *et al*., 1998).

Many epidemiological studies have investigated the prevalence of yeast infections and their etiologic agents in different parts of the world. However, the prevalence and incidence of yeast infections and their common etiologic agents among Ghanaians is unknown.

It has been reported in United States that the most common type of yeast infection is candidal vulvovaginitis (Sobel, 1992). It is estimated that at least 75% of all women suffer from at least one attack of candidal vulvovaginitis during their lifetime and nearly half of them suffer multiple episode (Saporiti *et al.*, 2001; Ferrer, 2000). In about 5 per cent of cases of candidal vulvovaginitis, the disease has a chronic course, showing frequent and refractory episodes (Ferrer, 2000). Data about recurrence of candidal vulvovaginitis at the Komfo Anokye Teaching Hospital is lacking.

The prevalence of candidal vulvovaginitis in the world is estimated in the range of 5 to 20% (Collier *et al.*, 1998). Depending on the age, locality and social economic status; the frequency of vaginal yeast isolates has been reported to be between 5 and 48.4% in women (Abu-Elteen *et al.*, 1997). A study conducted in Nigeria by Enweani *et al.* (2001) reported that the prevalence of candidal vulvovaginitis was 40.6%. There are no recent data in the literature to ascertain the prevalence of yeast infections at the Komfo Anokye Teaching Hospital in Kumasi. However, unpublished data at the microbiology laboratory at the Komfo Anokye Teaching Hospital in Kumasi, Ghana indicate that the prevalence of *Candida* species isolation from high vaginal swabs was increasing. In 2005, out of 2,686 high vaginal swabs received at the microbiology

laboratory, *Candida* species was isolated from 483 giving a prevalence of 18 %. This rose to 33% in 2008 in which 826 out of 2,491 high vaginal swabs received yielded *Candida* species.

Women who have candidal vulvovaginitis experience symptoms which include vulvar burning, thick vaginal discharge, soreness, dysuria, and itching. The itching can be either external or internal. This causes extreme discomfort and affects the wellbeing of women.

The presence of *Candida* in urine is referred to as candiduria. The majority of patients with candiduria suffer a completely benign process (Kauffman *et al.*, 2000). However, candiduria is sometimes a marker of disseminated candidiasis (Nassoura *et al.*, 1993). The prevalence of urinary *Candida* infection has been reported in some part of Ghana. A study conducted in Accra, Ghana by Ayeh-Kumi *et al.* (2007) reported that the prevalence of urinary *Candida* infection increased in both males and females from 3.5% in 2001 to 5.1% in 2003.They also reported that the observed high rate of *Candida* infection may not solely be due to *Candida albicans* but were unable to tell other species involved.

Infections caused by different species of *Cryptococcus* are called cryptococcosis. There are several researchers that have published data referring to the prevalence of cryptococcosis mainly in the United States of America, Australia, Japan and many European countries (Dangor *et al.*, 1989). Published data on the prevalence of *Cryptococcus* infections at the Komfo Anokye Teaching Hospital are scanty.

Oro-pharyngeal candidiasis is considered to be the most common opportunistic fungal disease in HIV/ AIDS patients globally, occurring in an estimated 80-95% of those with HIV disease (Priscilla *et al.*, 2002; Hodgson *et al.*, 2002). *Candida albicans* is the most common yeast

implicated in oro-pharyngeal candidiasis. Ehrahim *et al.* (2002) and Dunic *et al.* (2004) have reported prevalence levels of 52.4% and 77.7% respectively from HIV/ AIDS patients.

Since there are no vaccines currently licensed for preventing yeast infections, the only clinical recourse to combat yeast infections is the use of antifungal agents. Antifungal agents commonly used to treat candidal vulvovaginitis are topical clotrimazole, topical nystatin, fluconazole and topical ketoconazole. Other antifungal agents such as amphotericin B, voriconazole, flucytosine and capsofungin are used in treating systemic yeast infections.

The problem with the use of antifungal agents, apart from safety and cost is the development of drug resistant strains during treatment (Talaro & Talaro, 1996). The development of antifungal resistance among yeasts has been linked to misuse and inappropriate prescription of antifungal agents (Talaro & Talaro, 1996). In Ghana, antifungal drugs are sold over the counter, a practice which encourages self medication and therefore may contribute to the development and spread of antifungal resistance.

Clinical microbiology laboratories in Ghana provide a range of services in bacteriology but their services in mycology are still limited to direct microscopic examination of clinical specimens. In addition, there is no national surveillance programme to monitor antifungal resistance among yeasts and other pathogenic fungi in Ghana. As a result, no accurate information is available on the frequency and characteristics of yeasts that are responsible for yeast infections among patients that attend health facilities in the country including the Komfo Anokye Teaching Hospital in Kumasi. Moreover, information or data relating to yeasts susceptibility to antifungal agents in these facilities is scanty. Elsewhere decreased susceptibilities of several isolates of *Aspergillus, Candida* species and *Cryptococcus neoformans* to antifungal agents such as

fluconazole, itraconazole, voriconazole and amphotericin B have been reported (Marco[a] *et al.*, 1998; Moore *et al.*, 2000; Saag *et al.*, 2000).

Appropriate antifungal therapy depends upon a good knowledge of the agents causing the infections and their susceptibility patterns. The susceptibility of yeasts to antifungal agents cannot always be predicted and therefore testing individual yeast pathogens against the appropriate antifungal agents is often necessary. Antifungal susceptibility testing *in vitro* ensures that the drug that will be chosen will be active against the infecting organism and therefore provide beneficial therapeutic effect to the patient under treatment (Rex & Pfaller, 2002). Antifungal susceptibility testing also aids in drug development studies and as a means of tracking the development of antifungal resistance in epidemiologic studies (Rex & Pfaller, 2002).

The recognition of yeasts as agents of opportunistic infections, the chronic nature of diseases they cause, their unpredictable susceptibility patterns with the resultant increase of treatment failure and related costs make close monitoring of antifungal agents prudent (Alexander *et al.*, 2005; Borst *et al.*, 2005 & Spellberg *et al.*, 2006).

1.2 Statement of the problem

Antifungal drugs are sometimes prescribed for the treatment of yeast infections without confirmation of the diagnosis of yeast infection by culture. In routine clinical practice, therefore, the identities of the yeast species involved in the infections are frequently unknown. There is lack of data on susceptibility of yeasts species to antifungal agents at the Komfo Anokye Teaching Hospital. Inappropriate management of yeast infections particularly candidal vulvovaginitis may result in its progression to complicated vaginitis; enhance the development of antifungal resistance or the emergence of uncommon species.

1.3 Justification of the study

Numerous studies have examined bacterial resistance in Kumasi but less is known about the antifungal susceptibility of medically important yeasts in Kumasi. Species identification of the yeasts involved in yeast infections particularly candidal vulvovaginitis is not only an important step for a better understanding of the distribution of yeasts species in different sub-populations of patients, in particular those with recurrent infections, but the data obtained can provide a very accurate view of antifungal susceptibility rates. The emergence of resistance of yeast pathogens to antifungal agents has been reported worldwide but there are no such reports at the Komfo Anokye Teaching Hospital. Surveillance for antifungal resistance among yeast pathogens to available antifungal agents would provide physicians with information needed to prescribe proper antifungal agents for treating yeast infections. This is essential for improved patient management.

1.4 Aims/objectives

The aim of the study is:

- To determine yeast species responsible for infections among patients at the Komfo Anokye Teaching Hospital (KATH) in Kumasi and their antifungal susceptibility patterns.

The specific objectives are:

1. To isolate the yeast species that are involved in infections among patients at the Komfo Anokye Teaching Hospital.

2. To determine the susceptibility patterns of the yeasts species isolated to the commonly prescribed antifungal drugs at the Komfo Anokye Teaching Hospital.

CHAPTER TWO

LITERATURE REVIEW

Yeast infections are among the commonest fungal infections affecting humans. The severity of such yeast infections ranges from benign and transient superficial infection through more severe and chronic to systemic and potentially life–threatening diseases (Lennette *et al*., 1975).

Several factors have been cited as predisposing to yeast infections. The most important factors include the larger population of immunocompromised patients and increase in the use of broad spectrum antibacterial agents. Other factors are extremes of age, pregnancy, the use of birth control, nutritional diseases and organ transplantation (Segal *et al.*, 2006). Also factors such as higher awareness, improved and easily accessible diagnostic tools in most part of the world may have contributed to the observed increase in yeast infection in the world (Collier *et al.*, 1998).

Yeast infections are often difficult to control with the possible reason that yeasts like human cells are eukaryotic and so most of the antifungal drugs often used in treatment are toxic to both yeast and human cells (Talaro & Talaro, 1996).

The chronic nature of many diseases caused by yeasts complicates their treatment, because some of the yeasts hide in the deeper layers of the infected mucosa where they cannot be reached by the drug; thus not all the yeast cells are killed (Ghannoum & Rice, 1999). The hidden yeasts repopulate the site after antifungal medications are stopped (Ghannoum & Rice, 1999).

Antifungal susceptibility testing *in vitro* is therefore important for the selection of an appropriate antifungal agent for treatment. Antifungal activity *in vitro* is influenced by many factors which include pH and constituents of the medium, stability of the drugs and the size of the inoculum

(CLSI- M27-A, 2002). Antifungal susceptibility testing is performed when the patient is failing therapy with an antifungal agent that is known to be active against the infecting organism. Another reason is to learn of potentially efficacious alternative drugs when the pathogenic yeast is one with well known resistance to the drug of choice, and also to ascertain antifungal activity with new agents for which no substantial or previously published data base exists. It is also performed for yeasts recovered from severely immunoconmpromised host with systemic diseases in whom the yeast infection is life threatening (Ghannoum & Rice, 1999).

2.2 Structure of yeast

Yeasts are unicellular fungal cells that are encased in cell walls made up of chitin. The cells of the yeast *Cryptococcus neoformans* are surrounded by a rigid polysaccharide capsule (Prescott *et al*, 1996). Some species may become multicellular through the formation of a string of connected budding cells known as pseudohyphae or false hyphae (Kurtzman & Fell, 2006). A yeast cell has a single nucleus in the cytoplasm. Yeast cells possess membrane-bound organelles such as vacuoles, Golgi apparatus and mitochondria as well as ribosomes. They have no flagella. Generally, yeast cells are larger than bacteria and are commonly spherical to egg shaped. The sizes of yeast cells vary greatly depending on the species, typically measuring 3-4µm in diameter, although some yeasts can reach over 40µm (Walker *et al.*, 2002).

Yeasts like all other fungi lack chloroplasts and are therefore heterotrophic organisms that require preformed organic compounds as energy sources (Prescott *et al.*, 1996).

2.2 Distribution of yeasts

Yeasts are ubiquitous in the environment but are most frequently isolated from sugar-rich samples which include fruits and exudates from plants (Prescott *et al.*, 1996). Yeasts are also found on the skin surfaces and in the intestinal tracts of warm blooded animals. Yeasts belonging to the genus *Candida* are members of the normal flora of the skin, oral cavity, gastrointestinal tract and the genitals of humans (Talaro & Talaro, 1996).

Cryptococcus neoformans is a normal flora on the skin and the intestinal tracts of birds especially pigeons. *Cryptococcus neoformans* is mostly found in soil contaminated with pigeon droppings. *Cryptococcus neoformans* has also been isolated from the sap of *Eucalyptus* trees and decaying wood that form hollows in living trees (Talaro & Talaro, 1996).

2.3 Importance of yeasts

Yeasts are important to humans in both beneficial and harmful ways (Prescott *et al.*, 1996). Yeasts are essential to many processes that involve fermentation (Sreenivas *et al.*, 2004). For example yeasts are used in the making of bread, wine and beer (Sreenivas *et al.*, 2004). Yeasts such as *Saccharomyces cerevisiae* is used as nutritional supplement. It is an excellent source of protein and vitamins especially the B-complex. Some probiotic supplements use the yeast *Saccharomyces boulardii* to maintain and restore the natural flora in the large and small gastrointestinal tract after antibiotic medication (Kurugol & Koturoglu, 2005).

Yeasts are able to grow in foods with a low pH (5.0 or lower) and in the presence of sugars, organic acids and other easily metabolized carbon sources (Kurtzman, 2006). During their growth, yeasts metabolize food components and produce metabolic end products which cause the physical and chemical properties of food to change and the food is spoilt (Kurtzman, 2006).

However some species of yeasts are opportunistic pathogens where they cause infection in people with compromised immune system (Prescott *et al.,* 1996).

2.4 Nutrition and growth requirements of yeasts

Yeasts are chemoorganotrophs as they use organic compounds as a source of energy (Barnett,

1975).The main source of carbon is obtained from hexose sugars such as fructose and glucose or disaccharides such as sucrose and maltose (Barnett, 1975). Some species can metabolize pentose sugars like ribose (Barnett, 1975).Yeast species are obligate aerobes and so they require oxygen for cellular respiration (Prescott *et al.*, 1996). Some yeasts, however are facultatively anaerobic and can obtain energy by fermentation such as in the production of ethyl alcohol from glucose (Prescott *et al.*, 1996). Yeasts grow best in a slightly acidic pH environment over a temperature range of 10 to 37°C, but an optimal temperature range of 30 to 37°C (Prescott *et al.*, 1996). Common media used for the cultivation of yeast include sabouraud dextrose agar (SDA), potato dextrose agar (PDA) or potato dextrose broth and yeast peptone dextrose agar (YPD). The high dextrose content and the low pH of these media make them selective against bacteria.

2.5 Reproduction in yeasts

Yeasts have asexual and sexual reproductive cycles; however the most common mode of vegetative growth in yeast is asexual reproduction by budding or fission.

In budding the parent yeast cell forms a protuberance called a bud on its outer surface. As the bud elongates, the parent cell's nucleus divides into two and one nucleus migrates into the bud. Cell wall material is then formed between the bud and the parent cell. The bud eventually breaks

away from the parent cell (Kurtzman, 1994). Yeasts that reproduce by budding include *Cryptococcus neoformans* and the species of *Candida.*

Yeasts of the species *Schizosaccharomyces pombe* reproduce by binary fission (Balasubramanian *et al.*, 2004). During fission, the parent cell elongates and its nucleus divides into two. The two nuclei in the cytoplasm are separated by a septum that divides the elongated cell. The two daughter yeasts then dissociate after cleavage of the septum (Kurtzman, 1994).

Under high stress conditions diploid yeasts cells undergo sporulation and enter into sexual reproduction. During sexual reproduction, a variety of haploid spores are produced which mate or conjugate and form diploid cells (Neiman, 2005).

2.6 Taxonomic classification of yeasts

Yeasts are members of Kingdom Fungi. This is a large group of eukaryotic organisms that includes moulds as well as the more familiar mushrooms (Kurtzman & Piskur, 2006). Yeasts do not form a specific taxonomic or phylogenetic grouping. At present, it is estimated that only 1% of all yeasts species have been described (Kurtzman & Piskur, 2006).

The phylogenetic diversity of yeasts is shown by their placement in the divisions Ascomycota, Basidiomycota and Deuteromycota. The division Ascomycota commonly known as ascomycetes comprises the *Saccharomyces*, *Pichia*, *Endomycopsis*, *Nematospora* and *Candida*. Members of the division Basidiomycota are known as the basidiomycetes. Yeast belonging to the division basidiomycota is *Cryptococcus neoformans*. The Deuteromycota also known as Fungi Imperfecti comprises a group of fungi which includes *Rhodotorula*, *Torulopsis* and *Trichosporon.*

2.7 Spectrum of pathogens

Yeast species that cause disease in humans are mainly members of the genera *Candida* and *Cryptococcus*. The *Candida* includes about 150 different species. More than 17 different species of *Candida* have been reported to be etiologic agents of yeast infection in humans (Hazen, 1995; Pfaller & Diekema, 2004). The pathogenic yeasts of *Candida* in descending order of virulence for humans are: *Candida albicans, Candida tropicalis, Candida stellatoidea, Candida glabrata, Candida krusei, Candida parapsilosis, Candida guilliermondii, Candida viswanathii* and *Candida lusitaniae* (Hurley *et al.*, 1987). More than 90% of yeast infections due to *Candida* are attributed to the species- *Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis* and *Candida krusei.* Others are *Candida lusitaniae and Candida dubliniensis* (Pfaller & Diekema, 2004). As laboratories are better equipped to provide identification to the species level as an aid in optimizing therapy of yeast infections, more species are identified but are less commonly encountered (Pfaller & Diekema, 2004).

Some of the uncommon species of *Candida* that appear to be increasing in frequency as causes of yeast infections include *Candida guilliermondii, Candida sake, Candida rugosa, Candida inconspicua and Candida norvegensis* (Pfaller & Diekema, 2004).

There are 37 species within the genus *Cryptococcus*. However, the major human pathogen is *Cryptococcus neoformans*. Other species such as *Cryptococcus albidus* and *Cryptococcus laurentii* have been reported to cause infection rarely in humans (Krumholz, 1972; Lynch *et al.*, 1981). Within the species of *Cryptococcus neoformans*, there are three major varieties with differing biological, ecological and epidemiological characteristics. These varieties are *Cryptococcus neoformans* var *neoformans*, *Cryptococcus neoformans* var *grubii* and

Cryptococcus neoformans var *gattii* (Franzot *et al.,* 1999). *Cryptococcus neoformans* var *neoformans* consisting of the serotype D causes disease in patients with immune suppression and *Cryptococcus neoformans* var *gattii* consisting of serotypes B and C causes disease in normal hosts.

Cryptococcus neoformans var *grubii* has been introduced for serotype A strains (Franzot *et al.*, 1999). Ecologically *Cryptococcus neoformans* var *neoformans* is worldwide in distribution and is mainly isolated from soil contaminated with pigeon droppings while *Cryptococcus neoformans* var *gattii* is limited to the tropics and northern Europe where it is isolated from the *Eucalyptus* tree (Murray *et al.*, 2002). The two varieties also differ in biochemical properties such as the ability of *Cryptococcus neoformans* var *gattii* strains to use glycine as sole source of nitrogen whereas *Cryptococcus neoformans* var *neoformans* cannot (Franzot *et al.,* 1998). The var *gattii* strains are also resistant to the chemical canavanine whereas var *neoformans* strains are usually sensitive (Franzot *et al.,* 1998).

Yeast species such as *Pichia, Rhodotorula, Trichosporon* and *Saccharomyces* species have been rarely reported to cause human infections (Cassone *et al.,* 2003; Han *et al.,* 2004; Petrocheilou-Pschou *et al.*, 2001).

2.8 Virulence factors and pathogenesis of yeasts

The virulence of *Cryptococcus neoformans* is due to production of oxidase and protease enzymes, the polysaccharide capsule and the organism's ability to grow at 37°C (Kwon-Chung & Rhodes, 1986; Brueske, 1986). The polysaccharide capsule of *Cryptococcus neoformans* helps to prevent the organism from being recognized and engulfed by the white blood cells in the human body (Kwon-Chung & Rhodes, 1986; Brueske, 1986). Non- capsular mutant forms lack

pathogenicity (Kwon-Chung & Rhodes, 1986). A host site with abundant carbon dioxide concentration favours capsule bioformation in human tissues (Granger *et al.*, 1985). Infections by *Cryptococcus neoformans* occur through inhalation of the organisms which enter the lungs and then remain dormant depending on the host reaction (Neilson *et al.*, 1977). Organisms are then reactivated from previous dormant infections in the lung or lymph node when the host immune system is compromised and then spread throughout the body infecting various organs of the body (Neilson *et al.*, 1977).The clinical manifestations of cryptococcal infection can range from an asymptomatic colonization of the respiratory tract to a wide spread dissemination depending on the host immune factors. The central nervous system is commonly involved during dissemination. The basal meninges of the brain are preferentially affected causing cryptococcal meningitis (Krick, 1981).

Several factors contribute to pathogenesis of *Candida* species. These factors are the ability to bind to host tissue, the production of proteolytic enzymes particularly aspartyl proteases which facilitates tissue penetration and invasion (Collier *et al.*, 1998). Another virulence factor is the reversible transition between unicellular yeast and filamentous forms. *Candida* species are mostly found as commensals in humans and other warm-blooded animals. However, sometimes these yeasts can become pathogenic when they sprout hyphal outgrowths (Collier *et al.*, 1998).

2.9 Transmission of yeast infections

Cryptococcus neoformans is a normal flora on the skin and in the intestinal tract of birds particularly the pigeons. It is therefore shed into the environment in the dropping or faeces of pigeon. It is believed that the body temperature of birds is around 42°C which reduces the virulence of the organism and so birds are not naturally infected. Humans acquire *Cryptococcus*

neoformans from the environment when they inhale dried yeasts that are scattered into the air (Talaro & Talaro, 1996). Animals including dogs, cats, foxes, monkeys, goats and others can naturally acquire cryptococcosis; however, animal-to-person transmission has not been demonstrated (Littman & Walter, 1968).

Human-to-human transmission is also very rare (Beyt & Waltman, 1978), the only reported case has been in the setting of organ transplantation of contaminated tissue, a case in which a corneal transplant was reported to cause cryptococcal endopthalmitis in the recipient (Beyt & Waltman, 1978).

Human infections by *Candida* species are usually endogenous (Talaro & Talaro, 1996). This emerges from the fact that *Candida* species are frequently part of the normal flora of the mouth, throat, large intestine, vagina and the skin of humans. Infections are usually produced in the patients whose immune systems are compromised and in those taking broad spectrum antibiotics (Talaro & Talaro, 1996). *Candida* can be acquired from exogenous sources through the contaminated hands of health care workers, contaminated infusates and biomaterials (Khatib & Clark, 1995).

Person to person transmission of *Candida* infections is uncommon (Talaro & Talaro, 1996). It is seen primarily in oral thrush of newborns whose mothers have vaginal infections, acquiring them during birth (Talaro & Talaro, 1996). Sexual transmission from patients with vaginitis to their sexual partners has been reported (Murray *et al.*, 2002).

Table 2.1 Common yeasts implicated in human infection

Etiological agent	Probable recovery sites	Clinical implication
Candida albicans	Respiratory secretions, vagina, urine, skin, oropharynx, gastric washings, blood, stool, transtracheal aspiration, cornea, nails, cerebrospinal fluid, bone peritoneal fluid	Pulmonary infection, vaginitis, urinary tract infection, dermatitis, fungemia, mycotic keratitis,onychomycosis, meningitis, osteomyelitis, peritonitis, disseminated infection, thrush, arthritis, endocarditis
Candia glabrata	Respiratory secretions, vagina, urine, skin, oropharynx, gastric washings, blood, stool, transtracheal aspiration, bone marrow, skin (rare)	Pulmonary infection, Vaginitis, urinary tract infection, fungemia, disseminated infection, endocarditis
Candida tropicalis	Respiratory secretions, vagina, urine, skin, oropharynx, gastric washings, blood, stool, transtracheal aspiration, pleural fluid, peritoneal fluid ,cornea	Pulmonary infection, vaginitis, thrush endophthalmitis, endocarditis, arthritis, peritonitis, mycotic keratitis, fungemia
Candida parapsilosis	Respiratory secretions, urine, gastric washings, blood, vagina, oropharynx, skin, transtracheal aspiration, stool, pleural fluid, ear, nail	Endophthalmitis, endocarditis, vaginitis, mycotic keratitis, paronychial, fungemia
Candida kefyr	Respiratory secretions, vagina, urine, gastric washings, oropharynx	Vaginitis, urinary tract infection
Candida krusei	Respiratory secretions, urine, gastric washings, vagina, oropharynx, blood, skin, transtracheal aspiration, stool,	Endocarditis, vaginitis, urinary tract infection, mycotic keratitis
Candida guilliermondii	Respiratory secretions, urine, gastric washings, vagina, oropharynx, skin, bone, cornea.	endocarditis, fungemia, dermatitis, onychomycosis, mycotic keratitis, osteomyelitis, urinary tract infection

Etiological agent	Probable recovery sites	Clinical implication
Cryptococcus neoformans	Respiratory secretions, Cerebrospinal fluid, bone, blood, bone marrow, urine, skin, pleural fluid, gastric washings, transtracheal aspiration, cornea, orbit, vitreous humour	Pulmonary infection, meningitis, osteomyelitis, fungemia, disseminated infection, endocarditis, skin infection, mycotic keratitis, orbital cellulites, end ophthalmic infection
Cryptococcus albidus subsp. Albidus	Respiratory secretions, skin, gastric washings, urine, cornea	Meningitis, pulmonary infection
Cryptococcus luteolus	Respiratory secretions, skin, nose	Not commonly implicated in human infection
Cryptococcus laurentii	Respiratory secretions, cerebrospinal fluid, skin, oropharynx, stool	Not commonly implicated in human infection
Cryptococcus terreus	Respiratory secretions, skin, nose	Not commonly implicated in human infection
Rhodotorula	Respiratory secretions, urine, gastric washings, blood, vagina, oropharynx, skin, cerebrospinal fluid, stool, cornea	Fungemia ,endocarditis, Mycotic keratitis
Trichospsoron	Respiratory secretions, blood, skin oropharynx, stool	Pulmonary infection, brain abscess, disseminated infection,piedra

Note: adopted from Koneman & Roberts (1991)

2.10 Diagnosis of yeast infections

A presumptive diagnosis of *Candida* infection is made if budding yeast cells and pseudohyphae are found in specimens from localized infections during microscopic examinations of slides prepared from the specimens. Specimens are also cultured on sabouraud dextrose agar plates and incubated at room temperature (25 to 28°C) for several days during which time colonies of yeast develop. Identification of various species of yeasts is done using cultural characteristics, the germ tube test and biochemical tests.

The germ tube test is a rapid and reliable test that is used to identify *Candida albicans*. *Candida albicans* produce hyphal outgrowths called germ tubes when the isolates are suspended in serum and incubated at 37°C for about 3 hours.

A rapid diagnosis of cryptococcal meningitis can often be made by examination of an India ink preparation of cerebrospinal fluid. *Cryptococcus neoformans* appears as a single cell or budding yeast surrounded by clear halo because of the exclusion of the ink particles by the polysaccharide capsule. Serological procedures can be used to diagnose cryptococcal infection. These tests are based on the detection of antigen in clinical specimens. The latex agglutination test for the detection of cryptococcal polysaccharide antigens in cerebrospinal fluid and serum is routinely used in clinical laboratories (Murray *et al.*, 2002). The test involves the use of latex particles coated with a rabbit anticryptococcal antibody. The capsular polysaccharide present in a clinical specimen binds to the antibodies thereby agglutinating the latex particles (Murray *et al.*, 2002). The clinical specimen can be cultured on sabouraud dextrose agar and incubated at room temperature for several days. Identification of *Cryptococcus neoformans* on the sabouraud dextrose agar plates is done using biochemical tests.

Biochemical tests useful in yeast identification include assimilation of carbohydrates (the basis of API ID 32 C test), fermentation reactions, urease production and the ability to produce brown colonies on birdseed agar. A very sensitive PCR amplification technique has been developed for identifying *Candida* species directly from a clinical specimen (Murray *et al.*, 2002).

2.11 The Principles of API ID 32 C test

The API ID 32 C is a standardized system for identification of yeasts which is based on the assimilation of carbohydrates by the yeasts. The carbohydrates that are used include D-galactose, cycloheximide (actidione), D- saccharose (sucrose), N-acetyl glucosamine, lactic acid, L-arabinose, D- cellobiose, D- raffinose, D- maltose, D- trehalose, potassium 2- ketogluconate, methyl –alpha D-glucopyranoside, D- mannitol, D-lactose, inositol, D- sorbitol, D- xylose, D-ribose, glycerol, L-rhamnose, palatinose, erythritol, D- melibiose, sodium glucoronate, D –melezitose, potassium gluconate, levulinic acid (levulinate), D-glucose, L- sorbose, glucosamine, esculin and ferric citrate. These carbohydrates are incorporated into the cupules of the ID 32 C test strips. The ability of the yeast to utilize a particular carbohydrate results in the growth of the organism which produces turbidity in the cupules.

2.12 Treatment of Yeast Infections

In clinical settings, yeast infections are commonly treated with antifungal drugs. The choice of antifungal agent depends on the site of infection and the immune status of the patients. For example, the antifungal drugs commonly used to treat superficial yeast infections are topical clotrimazole, topical nystatin, fluconazole, itraconazole and topical ketoconazole (Moosa *et al.*, 2004).

Systemic yeast infections are often treated with amphotericin B alone or amphotericin B in combination with flucytosine. For example cryptococcal meningitis is often treated with amphotericin B in combination with flucytosine. Amphotericin B is active against *Cryptococcus neoformans* but exhibits relatively poor penetration into cerebrospinal fluid (Murray *et al.*, 2002). On the other hand, flucytosine has good cerebrospinal fluid penetration but the organisms rapidly develop resistance to it. Flucytosine is therefore almost always used in combination with amphotericin B to treat cryptococcal meningitis (Murray *et al.*, 2002). Alternative antifungal drugs that are used in treating severe systemic yeast infections include capsofungin or voriconazole (Murray *et al.*, 2002).

2.13 The role of diets in the management of yeast infections

Sugars assist the overgrowth of yeast, and this possibly explains the increased prevalence of yeast infections in patients with diabetes mellitus (Kidd, 2003). As many *Candida* species reside in the digestive tract, dietary changes may be effective for preventing *Candida* infection. Due to its requirement for readily-fermentable carbon sources, such as mono or dimeric sugars such as glucose and sucrose, avoiding foods that contain these nutrients in high abundance may help to prevent excessive *Candida* growth (Kidd, 2003).

2.14 The role of probiotics in the management of yeast infections

Probiotics have the potential to treat chronic *Candida* infections by eliminating the fungal form of the yeast and replenishing the gastrointestinal tract with the friendly bacteria (Fuller, 1991). For any probiotic to be effective, it has to contain organisms that survive the passage through the acidity of the stomach and exposure to bile in the small intestine, adhere to the mucosal surface of the gastrointestinal tract (Majeed *et al.*, 1998). It must also act antagonistically towards a

target pathogen and the microflora of the gut in general to establish itself. The majority of probiotics contain *Lactobacillus* species (Ghandi, 1988). *Lactobacillus sporogenes* a spore forming bacterium is very resistant to heat, acids and bile and it is very good at establishing itself in the intestinal mucosa after germination from the spores. It is a major component of the probiotic called ThreeLac which is used as supplements and helps in reestablishing the gut environment after candidiasis (Fuller, 1991; Ghandi, 1998).

2.15 Yeasts infections of the genital tract and the susceptibility patterns of vaginal yeast isolates to antifungal agents

Yeasts infections of the vagina commonly known as candidal vulvovaginitis is a common female infection primarily during the fecund period (Odds, 1988; Sobel, 1992). The prevalence of vulvovaginitis increases in groups such as pregnant or diabetic women, those using oral contraceptives or after antibiotic treatment (Sobel, 1992).

Candida albicans is the most common etiological agent of vulvovaginitis but other species such as *Candida glabrata*, *Candida krusei* and *Candida tropicalis* are also encountered (Sobel, 1997). There are many effective antifungal drugs including both topical and oral drugs that are used in treating candidal vulvovaginitis. Some of the topical antifungal drugs are clotrimazole, miconazole and nystatin. Oral alternatives include fluconazole, itraconazole and ketoconazole.

Reduced susceptibility of some vaginal yeasts isolates to some antifungal agents has been reported by Gross *et al.* (2007) in Costa Rica involving 57 yeasts isolated from high vaginal swabs. They reported that 91% of the isolates were susceptible to ketoconazole, 96.5% were susceptible to fluconazole, 98% were susceptible to itraconazole and 63% were susceptible to

miconazole. One isolate each of *Candida krusei* and *Candida glabrata* was found to be resistant to fluconazole with MIC of ≥ 64mg/l.

A study was conducted in Italy by Asticcioli *et al.* (2009) to determine the pathogen prevalence in vulvovaginal candidiasis and to evaluate the *in vitro* antifungal susceptibility of the yeasts isolated from the high vaginal swabs of women over a 6 year period (2002 to 2007). They found that of 518 yeasts isolated, 61.2% were *Candida albicans*, 28.8% were *Candida glabrata*, 2.9 % were *Candida parapsilosis*, 1.8 % each were *Candida tropicalis* and *Candida krusei* and 1.1% were *Candida guilliermondii*. Other yeasts isolated were *Saccharomyces* and *Trichosporon.* The antifungal susceptibility of the 518 yeasts to fluconazole, flucytosine, amphotericin B, ketoconazole and voriconazole reported about 90 to 95% of all the isolates to be susceptible to all the antifungal agents tested.

Ellabib & ElJariny (2001) conducted a study in Libya to determine the susceptibility of yeast isolates to amphotericin B, nystatin, ketoconazole, miconazole, clotrimazole and econazole. The yeasts were isolated from high vaginal swabs, nails, throat, hair and wound swabs. The isolated yeasts were *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida krusei*, *Candida guilliermondii* and *Candida famata*. They reported that all the isolates tested were highly susceptible to all the antifungal agents.

In India, Mohanty *et al.* (2007) determined the prevalence of *Candida* species causing candidal vulvovaginitis and tested the susceptibility of 30 *Candida* isolates to fluconazole. They reported that the prevalence of candidal vulvovaginitis was 18.5% and that 21 out of the 30 isolates tested were susceptible to fluconazole with MICs of ≤ 8µg/ml and 9 were in the intermediate category

with MICs between 16 to 32µg/ml. Of the 9 isolates found to be in the intermediate category, 7 were *Candida glabrata* and one each was *Candida albicans* and *Candida tropicalis*.

A study conducted in Brazil by Falleiros de Padua *et al.* (2003) to determine the prevalence of yeast isolation from high vaginal swabs and to determine the susceptibility of 83 yeast isolates to fluconazole, nystatin and amphotericin B reported that the prevalence of yeasts isolation from high vaginal swabs was 20.15%. They also reported that 96.4% of the yeast isolates were susceptible to fluconazole, 98.8% were susceptible to amphotericin B and 71% were susceptible to nystatin. Resistance to nystatin was not detected among the isolates but 29 % were found to be in the intermediate category.

In Italy, Arzeni *et al.* (1997) reported that the prevalence of candidal vulvovaginitis among out patients was 19% and that *Candida albicans* was the yeast species most frequently isolated from the high vaginal swabs followed by *Candida glabrata* and *Saccharomyces cerevisiae*. The antifungal susceptibility testing of the 42 yeasts isolated indicated that most of the isolates were susceptible to fluconazole, flucytosine, itraconazole, ketoconazole and miconazole. They however reported that isolates of *Candida glabrata* and *Saccharomyces cerevisiae* were less susceptible to fluconazole.

A clinical survey carried out in southern part of Nigeria to determine the distribution of genitourinary *Candida* species reported that 517 *Candida* were isolated from high vaginal swabs, endocervical swabs and urine (Okungbowa *et al.*, 2003). Yeasts isolated were *Candida glabrata, Candida albicans, Candida tropicalis, Candida guilliermondii, Candida parapsilosis and Candida stellatoidea.*

A study conducted in Brazil to determine the prevalence and antifungal susceptibility of vaginal yeasts by Galle & Gianinni (2004) reported that the prevalence of candidal vulvovaginitis was 27.6% and that 11.8%, 23.5% and 9.8% of the 69 isolates tested were resistant to fluconazole, itraconazole and amphotericin B respectively.

In Slovakia, Sojakova *et al*. (2004) tested the in vitro activities of fluconazole and itraconazole against 227 vaginal yeasts isolates and reported that 13% of the yeast strains were resistant to fluconazole with MICs of $\geq$ 64µg/ml and 18.5% were resistant to itraconazole.

2.16 Yeasts infections of the urinary tract and susceptibility patterns of urine yeast isolates to antifungal agents

The presence of yeasts species in urine is a condition known as candiduria. The significance of the presence of yeasts in urine of patients is not clearly understood (Weber *et al.*, 1992; Nucci, 2000). A common clinical problem is deciding whether candiduria represents urinary tract infections or merely bladder colonization or contamination (Akalm *et al.*, 2004). Distinguishing contamination from true infection is not easy, despite the existence of reliable diagnostic criteria for significant candiduria (Sobel, 2002).

Several predisposing factors such as use of indwelling urinary devices, diabetes mellitus, antibiotic use, immunosuppressive therapy, extremes of ages and female sex have been identified as being associated with increase of *Candida* growth in urine (Sobel, 2002).

The most frequent yeasts isolated from urine cultures are *Candida albicans* followed by *Candida glabrata* and *Candida tropicalis* (Nucci, 2000). Antifungal agents commonly used in treating

yeasts infections of the urinary tract are fluconazole and amphotericin B. Differences in antifungal susceptibilities have been reported in different countries (Tortorano *et al.*, 2004).

In Turkey, Tulumoglu *et al*. (2009) tested the antifungal susceptibility of 48 *Candida* strains isolated from urine to flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole and reported that the resistance rate to voriconazole and fluconazole were 5.45% and 3.63% respectively in *Candida albicans*. They also reported that all *Candida albicans* were susceptible to flucytosine, itraconazole and amphotericin B.

Baran *et al*. (2000) in Michigan, United States determined the susceptibilities of 80 urinary isolates of *Candida* species to fluconazole, amphotericin B and voriconazole and reported that all the isolates were susceptible to amphotericin B and voriconazole. They also reported all the isolates were susceptible to fluconazole except 5.3% of *Candida albicans* isolates which were resistant with MICs $\geq$ 64µg/ml.

In a study conducted in India to determine the antifungal susceptibility of 47 *Candida* isolates obtained from urine, sputum and high vaginal swabs, Srinivasan & Kenneth (2006) reported that 30% and 75% of all the isolates were resistant to fluconazole and itraconazole respectively. They however reported that amphotericin B and flucytosine showed high level of activity against all the isolates as no resistant strains were identified.

A study conducted in Brazil by Passos *et al*. (2005) reported that among the 153 urine samples cultured, *Candida* was recovered in 68 (44%) of them. They also reported that *Candida albicans* was the predominant yeasts species isolated from urine of 69.1% of the patients (47/68) followed *Candida glabrata* isolated in 7.4% (5/68). Other *Candida* species that were isolated in lower

percentages were *Candida kefyr, Candida parapsilosis, Candida famata, Candida guilliermondii, Candida krusei* and *Candida tropicalis.*

A study conducted by Kucukates *et al*. (2005) to determine the *in vitro* susceptibility of 27 urine yeasts isolates to fluconazole and amphotericin B in Istanbul, Turkey reported that majority of the strains had low minimum inhibitory concentration (MIC) values to both fluconazole and amphotericin B.

In a study to determine the antifungal susceptibility of 208 yeasts isolated from urine, oropharynx and cerebrospinal fluid in Turkey, Findik & Tuncer, (2002) reported that all the isolates were susceptible to amphotericin B. He however reported that 3.4% of the isolates were resistant to flucytosine and nystatin. The resistance to miconazole, econazole and ketoconazole was at 18.8%, 15.8% and 23.6% respectively.

2.17 Yeasts infections of the central nervous system and the susceptibility patterns of yeasts isolated from cerebrospinal fluid to antifungal agents

Yeasts infections of the central nervous system (CNS) are uncommon (Collier *et al.*, 1998). However, the involvement of the CNS is limited predominantly to *Cryptococcus neoformans* and *Candida albicans* (Collier *et al.*, 1998). Usually the yeasts enter the lungs where it may disseminate from the primary focus in the lungs and infect other organs such as the brain and the spinal cord. The basal meninges of the brain are preferentially affected causing thickening with subsequent invasion of the deeper brain tissues. Yeast infections of the CNS normally manifest as meningitis. The most susceptible individuals to candidal and cryptococcal meningitis are AIDS patients and infants (Collier *et al.*, 1998). Other predisposing factors to candidal and cryptococcal meningitis include corticosteroid use and organ transplant recipients. Choice of

antifungal agents for the treatment of cryptococcal and candidal meningitis depends on the site of infection and the immune status of the patients. The combination of amphotericin with flucytosine has been reported to be the best treatment option for yeast infections of the CNS.

There are now several published reports of the emergence of resistance to amphotericin B, fluconazole, flucytosine or itraconazole in *Cryptococcus neoformans* during treatment (Alves *et al.*, 2001). Most of these reports involve resistance to fluconazole emerging in the setting of meningitis in AIDS patients after long treatments or prophylaxis with fluconazole.

The antifungal susceptibilities of 1,811 clinical isolates of *Cryptococcus neoformans* obtained from 100 laboratories in Africa, Europe, Latin America, the Pacific and North America were determined by Pfaller *et al*. (2005). The study reported that resistance to amphotericin B, fluconazole and flucytosine was $\leq 1\%$ overall. They also reported that 99% of all the isolates were susceptible to voriconazole, posaconazole and ravuconazole with MICs of $\leq 1\mu$/ml.

Perkins *et al*. (2005) in Spain tested the susceptibilities of 317 *Cryptococcus neoformans* isolates obtained from cerebrospinal fluid, blood cultures, respiratory tract samples and skin of patients to amphotericin B, flucytosine, itraconazole, voriconazole and ravuconazole and reported that 5.3% (17) of the isolates were resistant to amphotericin B with MICs of $\geq$ 2mg/l. They also found that 41.3% (131), 53.3% (169), 15.8% (50) and 0.9% (3) of the isolates were resistant to flucytosine, fluconazole, itraconazole and voriconazole respectively.

A study conducted in France by Sar *et al*. (2004) to determine the susceptibility of 402 *Cryptococcus neoformans* isolates obtained from cerebrospinal fluid to amphotericin B and fluconazole reported that 14.9% of the isolates were resistant to fluconazole but all the isolates were susceptible to amphotericin B.

Zepelin *et al*. (2007) in Germany assessed the susceptibility of 561 *Candida* isolates obtained from cerebrospinal fluid and blood cultures to amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and capsofungin and reported that 3.7%, 17.6%, 0.5% and 4.5% of all the isolates were resistant to fluconazole, itraconazole, amphotericin B and flucytosine respectively. They also reported that 0.4% of the isolates were resistant to voriconazole. However, resistant of the isolates to capsofungin was not detected.

Chen *et al*. (2000) determined the antifungal susceptibility of 77 *Cryptococcus neoformans* isolated from cerebrospinal fluid in Australia and New Zealand to itraconazole, flucytosine, amphotericin B and fluconazole. They reported that all the isolates were susceptible to itraconazole with MICs $\leq$ 0.25mg/l, 98.7% of the isolates were susceptible to amphotericin B with MICs $\leq$ 1mg/l. One isolate was found to be resistant to flucytosine with MIC $\geq$ 64 mg/l and 88.3% of the isolates were found to be susceptible to fluconazole with MICs of $\leq$ 8 mg/l.

2.18 Yeasts infections of the lower respiratory system and the susceptibility patterns of yeasts isolated from sputum to antifungal agents

Yeasts infection involving the lungs or bronchial system appears predominantly in patients with underlying primary diseases (Gueteau & Darras, 1991). Yeasts infections of the respiratory tract normally manifest as candidal pneumonia and it can originate from hematogenous spread of the yeasts from the oral mucosa as part of disseminated infection or from introduction of the yeasts into the lungs through inhalation from the external environment (El-Ebiary *et al.*, 1997). Colonization of yeasts in the lower respiratory tract is common in hospitalized patients, patients with HIV/AIDS, diabetic patients, cancer patients and mechanically ventilated patients

(El-Ebiary *et al.*, 1997). *Candida* species primarily *Candida albicans, Candida glabrata* and *Candida tropicalis* as well as *Cryptococcus neoformans* are the yeasts that are mostly involved in the infection of the respiratory system (Collier *et al*, 1998). Antifungal drugs that are commonly used in treating yeast infections of the respiratory system include fluconazole, itraconazole, ketoconazole, capsofungin and amphotericin B.

Hamza *et al*. (2008) isolated 296 clinical isolates of yeasts from the oropharyngeal swabs that were taken from patients at the Muhimbili National Hospital in Dar es Salaam in Tanzania. The yeasts isolated included *Candida albicans, Candida glabrata, Candida krusei, Candida dubliniensis* and *Saccharomyces cerevisiae*. They study also determined the susceptibilities of these yeasts isolates to fluconazole, itraconazole, miconazole, clotrimazole, amphotericin B and nystatin and reported that only 15 (5%) out of the 296 isolates were resistant to fluconazole with MICs $\geq$ 64mg/l. They also reported that 25 (8.4%) of the 297 isolates were resistant to itraconazole with MICs of $\geq$1mg/l.

A study conducted by Bagg *et al*. (2005) in the United Kingdom to test the susceptibility of 270 isolates of yeasts obtained from the oral cavity to fluconazole, itraconazole and voriconazole reported that 76% (206) of the isolates were susceptible to fluconazole with MICs of 8µg/ml and 14% (39) were fully resistant to fluconazole with MICs of $\geq$64µg/ml.

The study also found that 59% (160) of the isolates were susceptible to itraconazole with MICs of $\leq$ 0.125µg/ml and 18% (49) were fully resistant to itraconazole with MICs of $\leq$ 1µg/ml. A total of 41 isolates found to be resistant to fluconazole and itraconazole included 23 *Candida glabrata*, 12 *Candida albicans*, 4 *Saccharomyces cerevisiae* and one isolate each of *Candida*

parapsilosis and *Candida tropicalis*. All the isolates were found to be susceptible to voriconazole.

A total of 137 *Candida* isolates obtained from the sputum and oropharyngeal swabs and 13 *Cryptococcus neoformans* isolated from cerebrospinal fluid were tested for their susceptibility to amphotericin B, itraconazole, voriconazole, posaconazole and capsofungin in Austria by Lass-Florl *et al*., (2008). They reported that all the isolates of *Candida* tested were susceptible to all the antifungal agents excepts for 65% of *Candida glabrata* and 35% of *Candida krusei* isolates which showed resistance to itraconazole, posaconazole and voriconazole. They also reported that all isolates of *Cryptococcus neoformans* were susceptible to all the antifungal agents except for capsofungin in which the yeasts were not inhibited.

In a study undertaken in Nigeria to determine species distribution of yeasts isolated from oro-pharynx and to analyze the in vitro susceptibility profile of the isolates to fluconazole, it was found that 58 (78.4%) out of the 74 isolates tested were susceptible to fluconazole with MICs of ≤ 8µg/ml, 7 (9.5%) were resistant with MICs of ≥ 64µg/ml (Enwuru *et al.*, 2008).

A study conducted in Kenya by Bii *et al*. (2002) to determine the antifungal susceptibility of 90 *Candida albicans* isolated from sputum and throat swabs of patients reported that 70.7% of the isolates were susceptible to amphotericin B with MICs ≤ 0.5 µg/ml and 82.9% of the isolates were susceptible to clotrimazole. They also reported that 68.3% and 90.2% of the isolates were susceptible to nystatin and flucytosine.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted at the microbiology laboratory of the Komfo Anokye Teaching Hospital (KATH) in Kumasi. KATH is a 1000 bed capacity hospital situated at Bantama a suburb of Kumasi in Ashanti Region of Ghana. Komfo Anokye Teaching Hospital is the second largest hospital in Ghana which serves the middle and the northern parts of Ghana. It receives patients with varying background with various kinds of diseases.

3.2 Study population

Clinical samples; high vaginal swabs (HVS), urine and sputum were collected from patients reporting to the microbiology laboratory with request forms. Cerebrospinal fluid (CSF) samples sent to the microbiology laboratory from the wards were also collected. High vaginal swabs were collected from patients suspected of having vagina infection. Cerebrospinal fluid samples were collected from patients suspected of having meningitis. Sputum samples were collected from patients suspected of having respiratory tract diseases and urine samples were collected from patients suspected of having urinary tract infections. A prevalence of yeasts isolation of 10.85 % estimated from the record books at the microbiology laboratory in 2007 was used in estimating the sample size. Using an estimated population of 10,000 of patients reporting to the microbiology laboratory with HVS, urine, sputum and CSF request forms and an error margin of 0.5, the sample size of 528 was estimated at 95% confidence level using EPI Info, a statistical programme.

3.3 Ethical considerations

Ethical clearance was obtained from the Committee on Human Research, Publications and Ethics (CHRPE) of the School of Medical Sciences of the Kwame Nkrumah University of Science and Technology (KNUST) and the Komfo Anokye Teaching Hospital (KATH) in Kumasi. Verbal informed consent was sought from individual patients from whom HVS, sputum and urine were collected. To do this, it was explained to the patients in languages they understood that, the isolates that would be obtained from their samples would be used in this study. Cerebrospinal fluid samples sent from the wards were processed without the consent of the patients. Samples from patients who consented were processed for yeasts at the microbiology laboratory of the KATH. High vaginal swabs, urine and sputum samples from patients who disagreed were not included in the study.

3.4 Collection of samples

Clinical samples collected were high vaginal swabs (HVS), cerebrospinal fluid (CSF), urine and sputum.

3.4.1 Collection and transport of urine

The patients were given clean dry sterile universal bottles. They were educated on how to collect the urine sample. They were asked to allow the first urine flow to pass away into the sink and then to catch the “mid stream” of the urine directly into the sterile bottle provided. After collection they were to replace the lid firmly on the bottle and then to return the specimen as soon as possible to the bacteriology laboratory.

Upon receipt at the laboratory the urine samples were labeled with the patients name, age and sex. They were also given laboratory identification numbers and the samples were processed immediately.

3.4.2 Processing of urine samples

In the laboratory, the urine sample was poured into clean, dry sterile plastic test tube and then centrifuged at a speed of 3000rpm for 5 minutes. After centrifugation, the supernatant was decanted. A little fluid remaining was used to reconstitute the sediment in the tube. An inoculating wire loop calibrated to hold 0.01ml of urine was sterilized in a Bunsen flame. The loop was allowed to cool in air. The loop was inserted vertically into the urine sediment in the test tube. The loopful of urine sediment was applied to a small area of the sabouraud dextrose agar (Oxoid, UK) plate to make a pool. The inoculating loop was then used to spread the inoculum from the pool.

A wet film was prepared by placing a drop of the urine sediment on a clean dry glass slide. The slide was then covered with clean cover slips and then examined under a microscope for yeast cells using the 10X objective lens.

The 40X objective lens was used to confirm the presence or absence of yeasts and other infectious agents.

Using a sterile inoculating loop, a loopful of the urine sediment was transferred and spread on a slide to make a thin smear. The smear was allowed to air dry. It was fixed by passing the under side rapidly through the flame of a Bunsen burner three times. The smear was allowed to cool. It was then placed on staining rack and stained by the Gram's Method. The smear was then examined under the microscope for yeast cells using the oil immersion objective lens. The

detection level for quantitative cultures used in this study was 100 CFU/ml represented by a single colony of yeast on a plate (Passos *et al.*, 2005).

3.4.3 Collection and transport of high vaginal swabs (HVS)

High vaginal swabs were taken from the patients by a trained female medical assistant at the laboratory. To do this sterile cotton wool swab was inserted carefully into the upper part of vagina and rotated gently before withdrawing. The swab was inserted into a tube from which it was taken. The tube containing the swab was labeled with the patients name, age and sex and then transported to the laboratory. In the laboratory the swabs were given laboratory identification numbers and were processed immediately.

3.4.4 Processing of high vaginal swabs

The mouth of the tube containing the swab was passed rapidly through the flame of a Bunsen burner to make the mouth area sterile. The swab was removed from the tube and then applied to a small area of sabouraud dextrose agar (SDA) (Oxoid, UK) plate to make a pool.

A few drops of normal saline was added to the swab in the tube and shaken to dislodge materials from the swab into the saline. The swab was withdrawn and a sterile inoculating loop was used to transfer and spread a small amount of the saline onto a clean dry slide to make a smear. The smear was allowed to air-dry. The dried smear was stained by Gram's method. The smear was then examined under the microscope for yeast cells using the oil immersion objective lens.

A wet film was prepared by placing a drop of the saline from the tube onto a clean dry glass slide. The slide was covered with cover slip and then examined under the microscope for yeast

cells and pseudohyphae first using the 10X objective before switching to 40X objective lens to confirm the presence or absence of yeasts.

3.4.5 Collection and transport of cerebrospinal fluid

Cerebrospinal fluid (CSF) was collected by medical officers by doing the lumbar puncture. This was done by inserting spinal needle into the subaracnoid space at the level of the lower spine. The CSF was collected into a syringe. The CSF samples were labeled with the name, age and sex of the patients and then submitted to the laboratory. Upon receipt at the laboratory, the samples were given laboratory identification numbers and then processed immediately.

3.4.6 Processing of cerebrospinal spinal fluid

A drop of CSF was released from the syringe onto SDA plate. Using a sterile inoculating loop, the CSF was streaked on the SDA plate. A wet film was prepared by releasing a drop of the sample from the syringe onto a clean dry slide. A drop of India ink was added and mixed. The slide was covered with a cover slip and then examined under a microscope for yeast cells using the 40X objective lens. Yeast cells with clear outer halos in stained background were presumptively identified as *Cryptococcus neoformans*. A drop of the sample was also released onto a clean dry slide. Using a sterile inoculating loop, the drop was uniformly spread on the slide to make a thin film. The smear was allowed to air-dry. It was heat fixed and then stained by the Gram's method. The smear was examined under a microscope for yeast cells using the oil immersion objective lens.

3.4.7 Collection and transport of sputum

The patients were provided with sterile sputum containers. They were told to open the containers only when placing the specimen into them. They were to go to open, airy place. They should take in a deep breath and then cough up sputum. They were to expectorate the sputum obtained directly into the container. The lid should be replaced tightly immediately on the container. They were to send the sample as soon as possible to the laboratory. They were told not to provide saliva. Upon receipt in the laboratory, the samples were labeled with the name, age and sex of the patients. The samples were also given laboratory identification numbers and processed immediately.

3.4.8 Processing of sputum

Sputum was processed in biological safety cabinet at the chest clinic at KATH. Sputum samples found to contain saliva were rejected. A sterile inoculating loop was inserted into the container containing the sputum to pick some sputum. The loopful of sputum was applied to a small area of the SDA (Oxoid, UK) plate to make a pool. The loop was flamed again in the flame of a Bunsen burner and then allowed to cool. The sterile loop was then used to spread the inoculum from the pool. Using a piece of clean stick, a small amount of the sputum was also transferred and spread on a clean dry slide to make thin smear. The smear was allowed to air-dry. It was heat fixed and then stained by Gram's method. The smear was then examined under a microscope for budding yeasts and pseudohyphae using the oil immersion objective lens.

3.4.9 Incubation and reading of plates

All inoculated SDA (Oxoid, UK) plates were incubated at room temperature (25-28°C) under aerobic condition for 48 hours, and then read. Plates that did not show any growth after 48 hours

were re-incubated for one week. Smears were prepared from growths that appeared on the SDA (Oxoid, UK) plates. Using a sterile inoculating loop, a distinct colony on the SDA plate was picked and emulsified in a drop of normal saline placed on a clean dry slide to make thin smear. The smear was allowed to air-dry, heat fixed and then stained by the Grams method. The smear was examined under a microscope using the oil immersion objective lens. Large Gram positive spherical cells with or without buds or pseudohyphae were suggestive of yeast cell. The isolates were stored at -70°C in 20% glycerol broth until antifungal susceptibility tests were performed.

3.5 Identification of the yeast cells isolated

All yeasts isolated from the specimens processed were identified to the species level using the germ tube test and API ID 32 C strip (bioMerieux, Marcy-l'Etoile, France).

The API ID 32 C is a commercially available test kit which is based on carbohydrate assimilation by the yeast cells.

3.5.1 The Germ tube test

Using Pasteur pipette, three drops of fresh human serum (obtained from the serology laboratory at the Komfo Anokye Teaching Hospital) were dispensed into labeled test tubes. Using a sterile inoculating loop, a colony of yeasts was transferred into the serum in the labeled test tubes. The colony was emulsified in the serum. The set up was incubated at 37°C for about 3 hours. Using a Pasteur pipette, a drop of the suspension taken from the test tube after incubation was placed on a clean dry slide. The suspension was covered with a clean cover glass. The slide was examined under a microscope for germ tubes on the yeasts using the 10X objective lens. A germ tube is a tube-like outgrowth that arises from the yeast cell. The 40X objective was used to confirm the presence or absence of germ tubes. When yeasts with germ tubes were seen, the culture was

reported as *Candida albicans* isolated. When the yeast cells do not show germ tubes, the culture was reported as yeasts isolated.

3.6 Identification of yeast isolates using the API ID 32 C test kits

3.6.1 Preparation of the inoculum

Using a sterile inoculating loop, one or several colonies from the sabouraud dextrose agar (Oxoid, UK) plates were transferred into 2ml of API® suspension medium (bioMerieux, Marcy-l'Etoile, France) as shown in Figure 3.1 below. The colonies were emulsified in the API® suspension medium to form a suspension of turbidity equivalent to 2McFarland.

The turbidity of the suspension was measured with a device called Densimat (bioMerieux, Marcy-l'Etoile, France) which accompanied the test kit. 250µl of the suspension (already prepared) was then transferred into 7ml of API C medium (bioMerieux, Marcy-l'Etoile, France) (Figure 3.2) using ATB electronic pipette (bioMerieux, Marcy-l'Etoile, France) supplied with the test kits.

Figure 3.1 API suspension medium

Figure 3.2 API C medium

3.6.2 Inoculation of the API ID 32 C test strips

The API ID 32 C test strips (bioMerieux, Marcy-l'Etoile, France) were removed from the packaging and the desiccants were discarded. Each strip was labeled with the reference number of the isolate. The API C medium (bioMerieux, Marcy-l'Etoile, France) containing the test organism was homogenized and 135µl of the suspension was dispensed into each cupule of the API ID 32 C test strip (bioMerieux, Marcy-l'Etoile, France) (Figure 3.3) using the ATB electronic pipette. The lid was placed on the test strip. The strips were placed in a card box and incubated at room temperature (25-28°C) for 24 -48 hours.

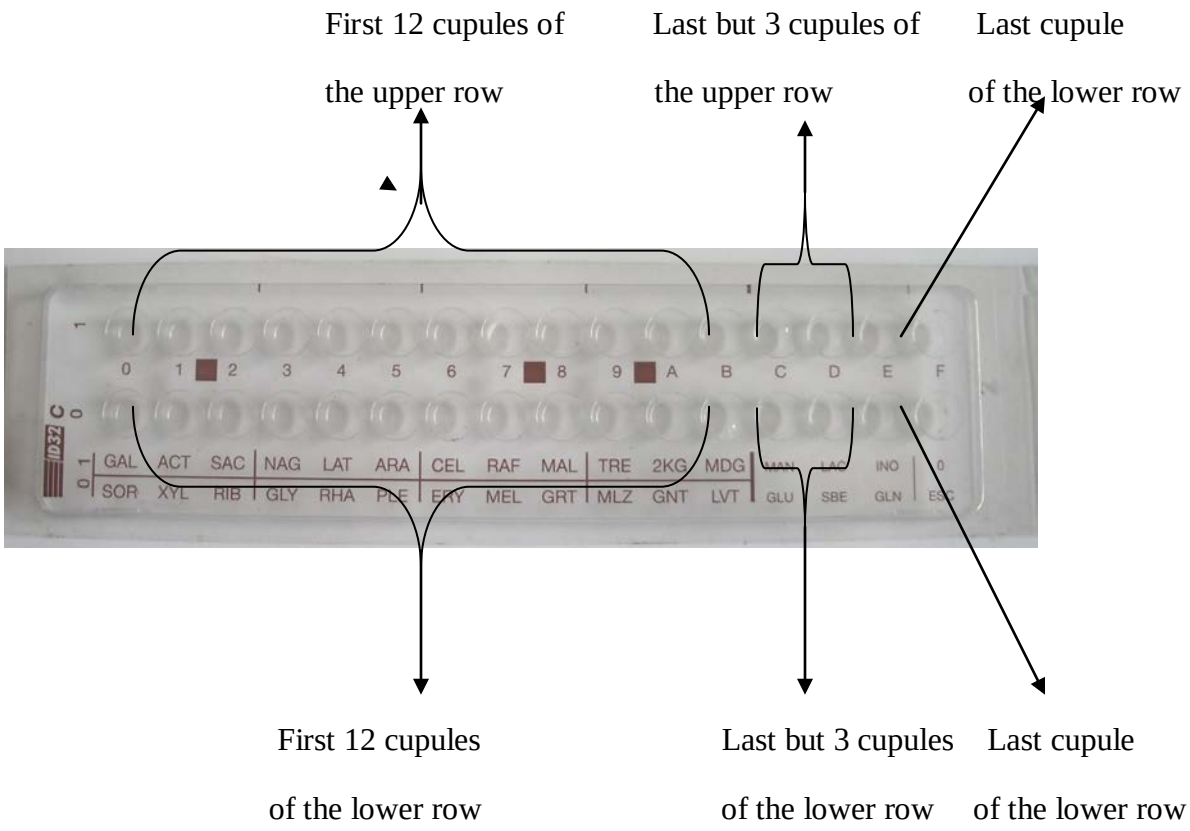


Figure 3.3 API ID 32 C test strip

- The numbers 0,1,2,3,4,5,6,7,8,9 and the letters A, B, C, D, E and F located in- between the cupules on the upper and lower rows are the labels of the cupules.

- The abbreviations GAL (D- galactose), ACT (actidione), SAC (D-saccharose), NAG (N-acetyl glucosamine), LAT (lactate), ARA (L-arabinose), CEL (D-cellobiose), RAF (D-raffinose), MAL (D-maltose), TRE (D-trehalose), 2KG (potassium 2-ketogluconate, MDG (methyl-alpha D-glucopyranoside), MAN (D-mannitol), LAC (D- lactose), INO (inositol) are the subrates that are incorporated into the cupules of the upper row.

- The abbreviations SOR(D-sorbitol), XYL (D-xylose),RIB (D-ribose), GLY(glycerol), RHA (L-rhamnose), PLE (palatinose), ERY (erythritol), MEL (D-melibiose), GRT (sodium glucoronate), MLZ (D-

melezitose), GNT (potassium gluconate), LVT (levulinate), GLU (D-glucose), SBE (L-sorbose), GLN (glucosamine), ESC (esculin) are the substrates that are incorporated in the cupules of the lower row.

3.6.3 Reading and interpretation of the API ID 32 C tests

After incubation, the test strips were removed from the card box and read visually. Each cupule on the test strip was examined carefully and any detectable growth in any cupule was recorded as positive on the result sheet (Figure 3.4). Cupules that did not show any detectable growths were assigned negative on the result sheet. On the result sheet, a set of 3 cupules was grouped together forming 10 groups that have been labeled G1 to G10 as shown in Figure 3.4. Each cupule in the group is assigned the number 1, 2 or 4. The number 1 corresponds to the first cupule in each group, 2 corresponds to the 2^{nd} or middle cupule in each group and the number 4 corresponds to the 3^{rd} or the last cupule in each group. When the 3 cupules on the test strip show detectable growths, they were assigned + + + with corresponding values 1, 2 or 4 respectively on the result sheet. The values that correspond to + + + that is 1, 2 or 4 were added and the number 7 was obtained. When the reactions were say + + - with values 1, 2 or 4 respectively, the values that correspond to the positive reactions that is 1 and 2 were added together and the number 3 was obtained. This was done until all the positive reactions in the 10 groups had been added. The numbers obtained for the 10 groups were put together to obtain a 10- digit number 7347340015 (Figure 3.4). Identification of the yeast was obtained using the apiweb™ identification software (bioMerieux, Marcy-l'Etoile, France) by manually entering the 10-digit number 7347340015 into the computer. As presented on the result sheet (Figure 3.4), isolate with which the number 7347340015 was obtained with the API ID 32 C test strip was identified as *Candida albicans.*

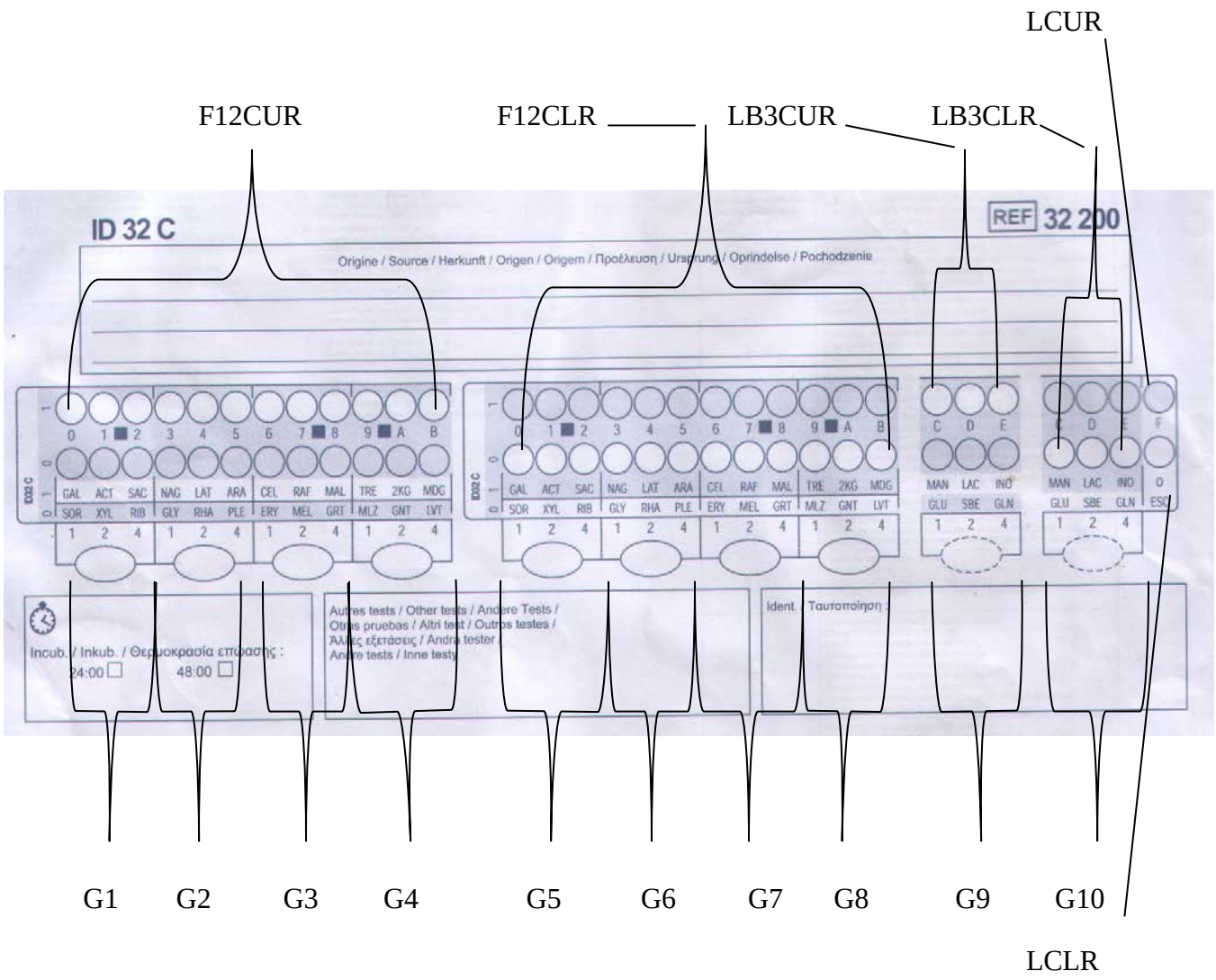


Figure 3.4 API ID 32 C result sheet

- The circles ◯ ◯ that have been labeled F12CUR in Figure 3.4 above correspond to the first 12 cupules of the upper row on the ID 32 C test strip as shown in figure 3.3.
- The circles that have been labeled F12CLR in figure 3.4 above correspond to the first 12 cupules of the lower row on the ID 32 C test strip as shown in figure 3.3.
- The circles have been labeled LB3CUR in figure 3.4 correspond to the last but 3 cupules of the upper row on the ID 32 C test strip.
- The circles that have been labeled LB3CLR in figure 3.4 correspond to the last but 3 cupules of the lower row on the ID 32 C test strip.
- The circle that has been labeled LCUR in figure 3.4 corresponds to the last cupule of the upper row on the ID 32 C test strip.
- The circle that has been labeled LCLR in figure 3.4 corresponds to the last cupule of the lower row on the ID 32 C test strip.

- The shaded circles do not correspond to any cupules on the ID 32 C test strip.

3.7 *In vitro* antifungal susceptibility testing of yeasts using the ATB Fungus 3 test kit

Antifungal susceptibility tests were performed using the ATB™ FUNGUS 3 strip (bioMerieux, Marcy-l'Etoile, France) which enables the determination of susceptibility of yeasts to antifungal agents. The ATB FUNGUS 3 strip (bioMerieux, Marcy-l'Etoile, France) consists of 16 pairs of cupules (Figure 3.7). The first pair of cupules does not contain any antifungal agent and is used as a positive growth control. The next 15 pairs of cupules contain 5 antifungal agents at increasing concentrations. The second pair of cupules contains the antifungal agent flucytosine at concentrations 4 and 16 mg/l. The concentrations of the antifungal agents on the ATB FUNGUS 3 strip (bioMerieux, Marcy-l'Etoile, France) are presented in Table 3.1. One ATB FUNGUS 3 test strip (bioMerieux, Marcy-l'Etoile, France) was used to determine the susceptibility of one yeast isolate to five antifungal agents at the concentrations shown in Table 3.1.

Table 3.1 Concentrations of the antifungal agents on the ATB FUNGUS 3 test strip

Antifungal agent	Range of concentration (mg/l)
Flucytosine	4 and 16
Amphotericin B	0.5, 1, 2, 4, 8 and 16
Fluconazole	1, 2, 4, 8, 16, 32, 64 & 128
Itraconazole	0.125, 0.25, 0.5, 1, 2 and 4
Voriconazole	0.06, 0.125, 0.25, 0.5, 1, 2, 4 and 8

Drug-free cupules

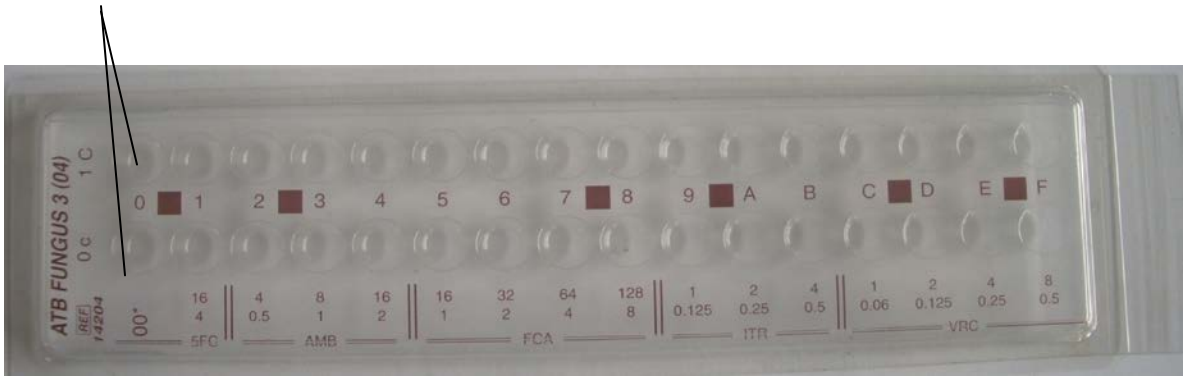


Figure 3.5 ATB FUNGUS 3 test strip

- The numbers 0,1,2,3,4,5,6,7,8,9 and the alphabet A, B, C, D, E and F located in between the cupules on the upper and lower rows are the labels of the cupules.
- The double vertical lines ‖ on the test strip separate the range of concentrations of one antifungal agent from the other.

- 5-FC represents flucytosine and the values 4 and 16 in mg/l represent the range of concentrations at which flucytosine was tested.

- AMB represents amphotericin B and the values 0.5, 1, 2, 4, 8 and 16 in mg/l represent the range of concentrations at which amphotericin B was tested.

- FCA represents fluconazole and the values 1, 2, 4, 8, 16, 32, 64 & 128 in mg/l represent the range of concentrations at which fluconazole was tested.

- ITR represents itraconazole and the values 0.125, 0.25, 1, 2 and 4 in mg/l represent the range of concentrations at which itraconazole was tested.

- VRC represents voriconazole and the values 0.06, 0.125, 0.25, 0.5, 1, 2, 4 and 8 represent the range of concentrations at which voriconazole was tested.

3.7.1 Preparation of yeast inoculum for the antifungal susceptibility tests

Prior to the antifungal susceptibility testing, the stored isolates were sub-cultured twice on sabouraud dextrose agar (Oxoid, UK) plates. This was done to ensure viability and purity of the test organisms and also to enhance optimal growth characteristics.

To prepare the sensitivity test inoculum, an inoculating loop was sterilized in Bunsen burner flame and allowed to cool. The sterile loop was used to transfer colonies of the yeast isolates from the SDA (Oxoid, UK) plates into 2ml of API® suspension medium (Figure 3.1) to form a suspension of turbidity equivalent to 2McFarland.

The turbidity of the suspension was measured with the Densimat (bioMerieux, Marcy-l'Etoile, France). A suspension of 20µl was transferred into 7 ml of ATB FUNGUS 3 medium

(bioMerieux, Marcy-l'Etoile, France) (Figure 3.6) using the ATB electronic pipette (bioMerieux, Marcy-l'Etoile, France), which was supplied with the test kit.

Figure 3.6 ATB FUNGUS medium

3.7.2 Inoculation of the ATB FUNGUS 3 test strips

ATB FUNGUS 3 test strips (bioMerieux, Marcy-l'Etoile, France) were removed from the packaging and the desiccants were discarded. Each strip was labeled with the laboratory number of the yeast isolate to be tested.

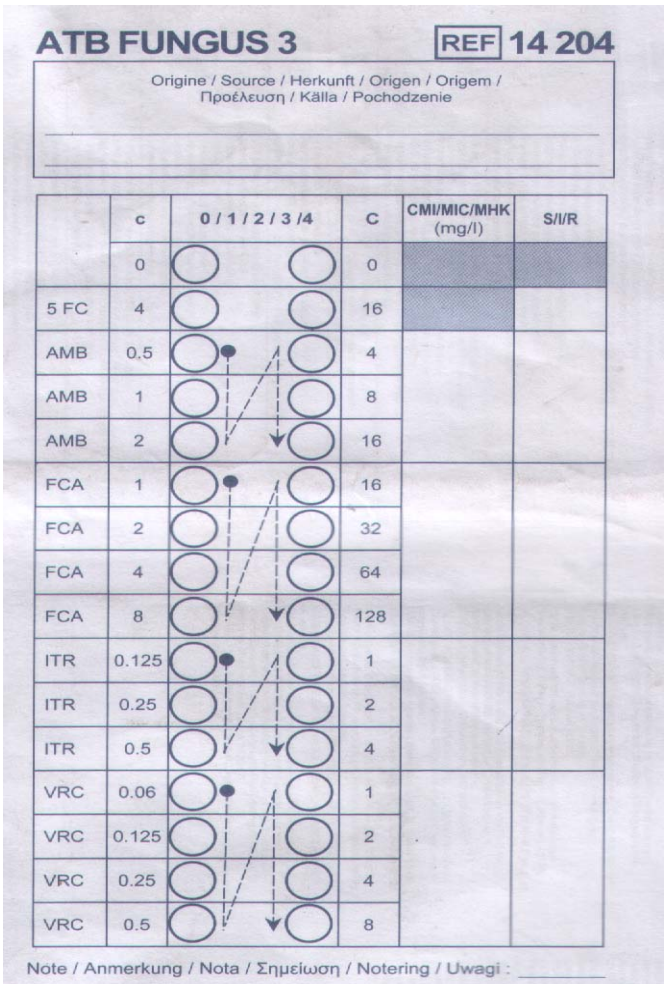
The ATB FUNGUS 3 medium (bioMerieux, Marcy-l'Etoile, France) containing the organism was homogenized and using the ATB electronic pipette (bioMerieux, Marcy-l'Etoile, France), 135µl of the suspension was dispensed into each cupule of the ATB FUNGUS 3 test strip (bioMerieux, Marcy-l'Etoile, France) (Figure 3.6). The lid was placed on the test strip and the set- up was incubated at 37°C for 24 to 48 hours. After incubation, the cupules were inspected for

growth and the results read visually. The yeast *Candida parapsilosis* NCTC 3104 was used as quality control strain in the susceptibility testing.

3.7.3 Reading of the test strips to determine the minimum inhibitory concentrations (MICs) and the susceptibility of the yeast isolates

The range of cupules for each antifungal agent was observed carefully and the degree of growth in each cupule was compared with that of the drug-free control cupules (the first pair of cupules labeled in Figure 3.6).

Beginning with the lowest concentration and working toward the highest concentration for each drug, each cupule was inspected and growth score corresponding to each cupule was recorded on the ATB FUNGUS 3 test result sheet as shown in Figure 3.8. Any cupule that did not show detectable growth was assigned the number 0 on the result sheet. Cupules that showed very weak growths compared with that of the drug-free control were assigned 1 on the result sheet. Cupules that showed prominent reduction in growth compared with that of the drug –free cupules were assigned 2.Cupules that showed slight reduction in growth compared with that of the drug-free growth control were assigned 3 on the result sheet. Cupules that did not show any reduction in growth compared with that of the drug–free control were assigned the number 4 on the result sheet (Figure 3.8).



ATB FUNGUS 3 REF 14 204

Origine / Source / Herkunft / Origen / Origem / Προέλευση / Källa / Pochodzenie

	c	0 / 1 / 2 / 3 /4	C	CMI/MIC/MHK (mg/l)	S/I/R
	0		0		
5 FC	4		16		
AMB	0.5		4		
AMB	1		8		
AMB	2		16		
FCA	1		16		
FCA	2		32		
FCA	4		64		
FCA	8		128		
ITR	0.125		1		
ITR	0.25		2		
ITR	0.5		4		
VRC	0.06		1		
VRC	0.125		2		
VRC	0.25		4		
VRC	0.5		8		

Note / Anmerkung / Nota / Σημείωση / Notering / Uwagi : _____

Figure 3.7 ATB FUNGUS 3 test result sheet

- 5-FC: flucytosine, AMB: amphotericin B, FCA: fluconazole, ITR: itraconazole, VRC: voriconazole, c : lower concentrations, C: higher concentrations; S: sensitive; I: intermediate; R: resistant

According to the manufacturers of the ATB FUNGUS 3 test kits (bioMerieux, Marcy-l'Etoile, France), the MIC for amphotericin B is the lowest concentration with a score of 0. From the result sheet presented in Figure 3.8, the MIC for amphotericin B (AMB) is 4 mg/l, which is the lowest concentration of amphotericin B with a score of 0.

The MICs for fluconazole, itraconazole and voriconazole are the lowest drug concentrations with a score of 2. From the result sheet (Figure 3.8), the MIC for fluconazole (FCA) is 2mg/l and the MIC for itraconazole (ITR) is 0.125mg/l. The MIC for voriconazole from the result sheet (Figure 3.8) is 0.5mg/l. The 2, 0.125 and 0.5mg/l are the lowest drug concentrations for fluconazole, itraconazole and voriconazole respectively with a score of 2.

The MICs for flucytosine could not be determined with the ATB FUNGUS 3 test strips because the drug was tested at two concentrations that are at 4 and 16mg/l. As a result, isolates tested against flucytosine were only classified as being susceptible, intermediate or resistant as presented in the Table 3.2.

Table 3.2 Reading and the interpretation of flucytosine test results.

Appearance of cupules at concentrations		The organism is :
4mg/l	16mg/l	
Clear	Clear	sensitive
Turbid	Clear	intermediate
Turbid	Turbid	resistant

To determine whether the isolates tested against amphotericin B, fluconazole, itraconazole and voriconazole were sensitive, intermediate or resistant, the MIC values obtained from the ATB FUNGUS 3 test strips (bioMerieux, Marcy-l'Etoile, France) were compared with the interpretive

breakpoints recommended by Clinical and Laboratory Standard Institute (CLSI, 2002) and the isolates were classified as sensitive, intermediate or resistant (Table 3.3).

Table 3.3 CLSI recommended breakpoints (in mg/l) for *Candida* species and *Cryptococcus neoformans*.

Antifungal agent	**Breakpoints for *Candida* species**			**Breakpoints for *C. neoformans***		
	sensitive	**intermediate**	**resistant**	**Sensitive**	**intermediate**	**resistant**
Amphotericin B*	ND	ND	ND	ND	ND	ND
Fluconazole	≤8	16-32	≥64	≤ 4	8	≥ 16
Itraconazole	≤0.125	0.25-0.5	≥1	ND	ND	ND
Voriconazole	≤ 1	2	≥ 4	ND	ND	ND

ND: not defined by CLSI

Notes:

- It must be stated that CLSI interpretive breakpoints for amphotericin B against *Candida* species and *Cryptococcus neoformans* are not available. However it has been suggested that isolates for which amphotericin B MICs were ≥ 2mg/l were considered resistant. Isolates with amphotericin B MICs of ≤ 1mg/l were considered to be susceptible (CLSI; M27-A, August, 2002). These were the interpretive breakpoints that were used in this study.

- Also interpretive breakpoints for itraconazole and voriconazole against *Cryptococcus neoformans* have not been formerly defined. So for itraconazole and voriconazole, a breakpoint of ≤ 1mg/l was used for *Cryptococcus neoformans* based on previous pharmacokinetic studies on *Candida* (Sheehan *et al.*, 1999; Pfaller *et al.*, 2003; Pfaller *et al.*, 2006).

3.8 Antifungal susceptibility testing using the agar diffusion method

Antifungal susceptibility testing of the yeast isolates was also assessed using the agar diffusion method. Two discs each impregnated with amphotericin B (AMB 20µg/ml) and flucytosine (FY 1µg/ml) were used. The discs were supplied by Cypress Diagnostics, Belgium. Mueller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue was used for the sensitivity testing. The addition of the glucose speeds up growth of the yeasts and the addition of the methylene blue enhances edge- definition of zones.

3.8.1 Preparation of inoculum for the agar diffusion test

Using a sterile inoculating loop, distinct colonies of yeast from the SDA plates were transferred into 2ml of normal saline in a bottle. The colonies were emulsified in the saline solution to form a suspension of turbidity equivalent to 0.5 McFarland.

3.8.2 Inoculation of the agar plates

A sterile cotton swab was dipped into the inoculum already prepared. The soaked swab was rotated firmly several times against the upper inside wall of the bottle to remove excess fluid. Using the cotton swab, the dried surface of the Mueller-Hinton agar (supplemented with glucose

and methylene blue) was inoculated with the test organism. The plates were left for 5 minutes to allow for any surface moisture to be absorbed before the drug impregnated discs were applied.

Using sterile forceps, the discs were removed from the container and then placed on the Mueller-Hinton agar. The sterile forceps was used to press down the discs to make contact with the surface of the Mueller-Hinton agar. The plates were incubated at 37°C. The yeast *Candida parapsilosis* NCTC 3104 was used as quality control strain in the susceptibility testing.

3.8.3 Reading of the plates and the measurement of diameter of zone of inhibitions

The plates were examined after 20 to 24 hours of incubation. The diameters of zones of complete inhibition were measured to the nearest whole numbers in millimeters using meter rule and recorded.

To determine whether the isolates tested against amphotericin B and flucytosine were susceptible, intermediate or resistant; the diameters of zones of inhibition obtained were compared with the standard zones interpretive breakpoints published by Cypress Diagnostics (www.diagnostics.be) and Rosco Diagnostica (www.rosco.dk) for flucytosine and amphotericin B respectively.

For flucytosine, isolates with zone diameters of ≥ 20 mm were considered susceptible;

12 to 19 mm were considered intermediate and ≤ 11 mm were considered resistant.

For amphotericin B isolates with zone diameters of ≥ 15 mm were considered susceptible;

10 to 14 mm were considered intermediate and < 10 were considered resistant.

CHAPTER FOUR

RESULTS

4.1 Culture results

During the study period of March, 2009 to June, 2009, a total of 528 clinical samples were cultured. Sixty- seven (67) samples out of the 528 tested positive for yeasts giving a prevalence rate of 12.7%. The number of samples received consisted of 186 (35%) high vaginal swabs, 127 (24%) urine samples, 109 (21%) cerebrospinal fluid samples and106 (20%) sputum samples (Table 4.1). From the total of 67 yeast isolates obtained 33 representing 49.3% were *Candida albicans,* 12 representing 17.9% were *Candida glabrata,* 8 (12%) were *Candida tropicalis,* and 4 (6%) were *Candida dubliniensis.* Other yeast species identified were *Candida krusei* 3 (4.5%), *Candida sake* 2 (3%), *Candida parapsilosis* 1 (1.5%), *Candida guilliermondii* 1 (1.5%) and *Cryptococcus neoformans* 3 (4.5%).

Of the 186 high vaginal swabs cultured, 39 yielded yeasts giving yeast prevalence in high vaginal swabs during the study period as 21%. Yeast species isolated from high vaginal swabs were *Candida albicans* 19 (48.7%), *Candida glabrata* 7(17.9%), *Candida tropicalis* 4 (10.3%) and *Candida dubliniensis* 4 (10.3%). Other species isolated were *Candida sake* 2 (5.1%), *Candida krusei* 2 (5.1%) and *Candida parapsilosis* 1(2.6%).

Among the 127 urine samples analyzed, yeasts were isolated from 14 of them giving a prevalence of 11%.Yeasts isolated from urine were *Candida albicans* 6 (42.9%), *Candida glabrata* 5 (35.7%), *Candida tropicalis* 2 (14.3%) and *Candida guilliermondii* 1 (7.1%).

The prevalence of yeast isolation from sputum was 9.4% (10/106). Of the 10 yeasts isolated from sputum, 7 (70%) were *Candida albicans*, 2 (20%) were *Candida tropicalis* and 1 (10%) was *Candida krusei*.

Four (4) yeasts were isolated from the 109 cerebrospinal fluid analyzed giving prevalence rate of 3.7%. Three (75%) were *Cryptococcus neoformans* and one (25%) was *Candida albicans.*

The study found that *Candida albicans* was the most common yeast isolated from all the samples except in cerebrospinal fluid in which *Cryptococcus neoformans* was the commonest (Table 4.2).

Candida glabrata was found in the genitourinary samples (high vaginal swabs and urine only). They were not found in sputum and cerebrospinal fluid though they were the second commonest yeast isolates.

Candida tropicalis, the third common isolate was found in high vaginal swabs, urine and sputum as opposed to *Candida krusei* which was found in high vaginal swabs and sputum but not urine.

All the 4 isolates of *Candida dubliniensis* were obtained from high vaginal swabs.

Other yeasts species were found to be few (< 4) in other samples studied (Table 4.2).

Table 4.1: Prevalence of yeasts among clinical samples

Type of sample	Number of Samples	Number negative	Number positive	Prevalence rate (%)
HVS	186.0	147.0	39.0	21.0
Urine	127.0	113.0	14.0	11.0
CSF	109.0	105.0	4.0	3.7
Sputum	106.0	96.0	10.0	9.4
Total	**528.0**	**461.0**	**67.0**	**12.7**

Table 4.2: Distribution of yeast species among clinical samples

Yeast isolate	HVS N (%)	Urine N (%)	Sputum N (%)	CSF N (%)	Total N (%)
Candida albicans	19(48.7)	6(42.9)	7(70.0)	1(25.0)	33(49.3)
Candida glabrata	7(17.9)	5(35.7)	0(0.0)	0(0.0)	12(17.9)
Candida tropicalis	4(10.3%)	2(14.3)	2(20.0)	0(0.0)	8(11.9)
Candida krusei	2(5.1)	0(0.0)	1(10.0)	0(0.0)	3(4.5)
Candida parapsilosis	1(2.6)	0(0.0)	0(0.0)	0(0.0)	1(1.5)
Candida dubliniensis	4(10.3)	0(0.0)	0(0.0)	0(0.0)	4(6.0)
Candida sake	2(5.1)	0(0.0)	0(0.0)	0(0.0)	2(3.0)
Candida guilliermondii	0(0.0)	1(7.1)	0(0.0)	0(0.0)	1(1.5)
Cryptococcus neoformans	0(0.0)	0(0.0)	0(0.0)	3 (75.0)	3(4.5)
Total	**39(100%)**	**14(100%)**	**10(100%)**	**4(100%)**	**67(100%)**

HVS: High vaginal swab; CSF: Cerebrospinal fluid; N: Number of isolates; (%): Percentage

4.2 Antifungal susceptibility tests results

Antifungal susceptibility of 67 yeast isolates to flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole was determined using the ATB FUNGUS 3 test kits. In addition, the susceptibility of the yeast isolates to amphotericin B and flucytosine was assessed using the agar diffusion method.

The results of the susceptibility tests as determined by the ATB FUNGUS 3 tests `show that 83.6% (56/67) of the isolates were susceptible to flucytosine while 10.4% (7/67) were in the intermediate category and 6% (4/67) were resistant. However, results obtained from the disc agar diffusion method show that 80.6% (54/67) of the isolates were susceptible to flucytosine with zone diameters of ≥ 20 mm, 11.9% (8/67) were in the intermediate category with zone diameters of 12 to 19 mm and 7.5% (5/67) were found to be resistant to flucytosine with zone diameters of ≤ 11 mm. Of the four isolates found to be resistant with the ATB FUNGUS 3 method, 3 were *Candida albicans* and one was *Candida krusei* (Table 4.3). One isolate of *Candida albicans* which was found to be in the intermediate category with the ATB FUNGUS 3 test was found to be resistant with the disc agar diffusion method (Table 4.4).

Against fluconazole, 71.6% (48/67) of the isolates were susceptible with MICs of ≤ 8mg/l, 23.9% (16/67) were in the intermediate category with MICs of 16- 32mg/l and 4.5% (3/ 67) were resistant with MICs ≥ 64mg/l. All the isolates found to be resistant to fluconazole were identified as *Candida krusei*. Among the 12 isolates of *Candida glabrata* tested against fluconazole 75% (9/12) were in the intermediate category. Fluconazole resistance was not detected among the 33 *Candida albicans* isolates tested.

Results from the ATB FUNGUS 3 tests showed that 77.6 % (52/67) of the isolates tested were susceptible to amphotericin B with MICs ≤ 1mg/l, 22.4% (15/67) isolates comprising 9 *Candida albicans*, 3 *Candida tropicalis*, 2 *Candida krusei* and 1 *Candida glabrata* were found to be resistant to amphotericin B with MICs ≥ 2mg/l (Table 4.3). On the other hand, results from the disc agar diffusion method indicate that 82% (55/67) of the isolates were susceptible to amphotericin B with zone diameters of ≥ 15 mm, 6.0% (4) were in the intermediate category with zone diameters of 10 to 14 mm and 12% (8/67) isolates consisting of 5 *Candida albicans*, 2 *Candida tropicalis* and 1 *Candida krusei* isolates were resistant with zones diameter of < 10mm (Table 4.4).

Findings from the study show that 70.1% (47/67) of the isolates were susceptible to itraconazole with MICs of ≤ 0.125mg/l, 22.4% (15/67) of the isolates were in the intermediate category with MICs of 0.25 to 0.5 mg/l and 7.5% (5/67) were resistant to itraconazole to itraconazole with MICs ≥ 1mg/l.

All isolates of yeasts tested were susceptible to voriconazole except for 2 isolates of *Candida albicans* which were found to be in the intermediate category with MICs of 2mg/l. (Table 4.2).

Table 4.3 Antifungal susceptibility test results as determined by the ATB FUNGUS 3 methods

Yeast isolate	No of isolates	Antifungal agents tested	Isolates sensitive		Isolates intermediate		Isolates resistant	
			(%)	MIC(mg/l)	(%)	MIC(mg/l)	(%)	MIC(mg/l)
Candida albicans	33	5-FC	78.8	-	12.1	-	9.1	-
		AMB	72.7	≤ 1	-	-	27.3	≥ 2
		FCA	81.8	≤ 8	18.2	16-32	0.0	≥ 64
		ITR	78.8	≤0.125	15.2	0.25-0.5	6.0	≥ 1
		VRC	93.9	≤ 1	6.1	=2	0.0	≥ 4
Candida glabrata	12	5-FC	91.7	-	8.3	-	0.0	-
		AMB	91.7	≤ 1	-	-	8.3	≥ 2
		FCA	25.0	≤ 8	75.0	16-32	0.0	≥ 64
		ITR	8.0	≤0.125	67.0	0.25-0.5	25.0	≥ 1
		VRC	100.0	≤ 1	0.0	=2	0.0	≥ 4
Candida tropicalis	8	5-FC	75.0	-	25.0	-	0.0	-
		AMB	62.5	≤ 1	-	-	37.5	≥ 2
		FCA	87.5	≤ 8	12.5	16-32	0.0	≥ 64
		ITR	87.5	≤0.125	12.5	0.25-0.5	0.0	≥ 1
		VRC	100	≤ 1	0.0	=2	0.0	≥ 4
Candida krusei	3	5-FC	66.7	-	0.0	-	33.3	-
		AMB	33.3	≤ 1	-	-	66.7	≥ 2
		FCA	0	≤ 8	0.0	16-32	100.0	≥ 64
		ITR	66.7	≤0.125	33.3	0.25-0.5	0.0	≥ 1
		VRC	100	≤ 1	0.0	=2	0.0	≥ 4

Key: 5-FC Flucytosine; AMB: Amphotericin B; FCA: Fluconazole; ITR: Itraconazole;

VRC: Voriconazole

Table 4.3 continued

Yeast isolate	No of isolates	Antifungal agents tested	Isolates sensitive		Isolates intermediate		Isolates resistant	
			(%)	MIC(mg/l)	(%)	MIC(mg/l)	(%)	MIC(mg/l)
Candida dubliniensis	4	5-FC	100.0	-	0.0	-	0.0	-
		AMB	100.0	≤ 1	0.0	-	0.0	≥ 2
		FCA	100.0	≤ 8	0.0	16-32	0.0	≥ 64
		ITR	100.0	≤0.125	0.0	0.25-0.5	0.0	≥ 1
		VRC	100.0	≤ 1	0.0	=2	0.0	≥ 4
Candida sake	2	5-FC	100.0	-	0.0	-	0.0	-
		AMB	100.0	≤ 1	0.0	-	0.0	≥ 2
		FCA	100.0	≤ 8	0.0	16-32	0.0	≥ 64
		ITR	100.0	≤0.125	0.0	0.25-0.5	0.0	≥ 1
		VRC	100.0	≤ 1	0.0	=2	0.0	≥ 4
Candida parapsilosis	1	5-FC	100.0	-	0.0	-	0.0	-
		AMB	100.0	≤ 1	0.0	-	0.0	≥ 2
		FCA	100.0	≤ 8	0.0	16-32	0.0	≥ 64
		ITR	100.0	≤0.125	0.0	0.25-0.5	0.0	≥ 1
		VRC	100.0	≤ 1	0.0	=2	0.0	≥ 4
Candida guilliermondii	1	5-FC	100.0	-	0.0	-	0.0	-
		AMB	100.0	≤ 1	0.0	-	0.0	≥ 2
		FCA	100.0	≤ 8	0.0	16-32	0.0	≥ 64
		ITR	100.0	≤0.125	0.0	0.25-0.5	0.0	≥ 1
		VRC	100.0	≤ 1	0.0	=2	0.0	≥ 4

Key: 5-FC Flucytosine; AMB: Amphotericin B; FCA: Fluconazole; ITR: Itraconazole;

VRC: Voriconazole

Table 4.3 continued

Yeast isolate	No of isolates	Antifungal agents tested	Isolates sensitive		Isolates intermediate		Isolates resistant	
			(%)	MIC(mg/l)	(%)	MIC(mg/l)	(%)	MIC(mg/l)
Cryptococcus neoformans	3	5-FC	100.0	-	0.0	-	0.0	-
		AMB	100.0	≤ 1	0.0	-	0.0	≥ 2
		FCA	100.0	≤ 4	0.0	16-32	0.0	≥ 64
		ITR	100.0	≤ 1	0.0	0.25-0.5	0.0	≥ 1
		VRC	100.0	≤ 1	0.0	=2	0.0	≥ 4

Key: 5-FC Flucytosine; AMB: Amphotericin B; FCA: Fluconazole; ITR: Itraconazole;

VRC: Voriconazole

Table 4.4: Antifungal susceptibility test results as determined by the disc diffusion method

Yeast isolate	No of isolates tested	Antifungal agents	Isolates sensitive		Isolates intermediate		Isolates resistant	
			(%)	Zone diameter (mm)	(%)	Zone diameter (mm)	(%)	Zone diameter (mm)
Candida albicans	33	AMB	78.8	> 15	6.0	10 to 14	15.2	< 10
		5-FC	75.8	≥ 20	12.1	12 to 19	12.1	≤ 11
Candida glabrata	12	AMB	91.7	> 15	8.3	10 to 14	0.0	< 10
		5-FC	83.3	≥ 20	16.7	12 to 19	0.0	≤ 11
Candida tropicalis	8	AMB	62.5	> 15	12.5	10 to 14	25.0	< 10
		5-FC	75.0	≥ 20	25.0	12 to 19	0.0	≤ 11
Candida krusei	3	AMB	66.7	> 15	0.0	10 to 14	33.3	< 10
		5-FC	66.7	≥ 20	0.0	12 to 19	33.3	≤ 11
Candida dubliniensis	4	AMB	100.0	> 15	0.0	10 to 14	0.0	< 10
		5-FC	100.0	≥ 20	0.0	12 to 19	0.0	≤ 11
Candida sake	2	AMB	100.0	> 15	0.0	10 to 14	0.0	< 10
		5-FC	100.0	≥ 20	0.0	12 to 19	0.0	≤ 11
Candida parapsilosis	1	AMB	100.0	> 15	0.0	10 to 14	0.0	< 10
		5-FC	100.0	≥ 20	0.0	12 to 19	0.0	≤ 11
Candida guilliermondii	1	AMB	100.0	> 15	0.0	10 to 14	0.0	< 10
		5-FC	100.0	≥ 20	0.0	12 to 19	0.0	≤ 11
Cryptococcus neoformans	3	AMB	100.0	> 15	0.0	10 to 14	0.0	< 10
		5-FC	100.0	≥ 20	0.0	12 to 19	0.0	≤ 11

Key: 5-FC Flucytosine; AMB: Amphotericin B; FCA: Fluconazole; ITR: Itraconazole;

VRC: Voriconazole

CHAPTER FIVE

DISCUSSION

This study identified the specific species of yeasts that cause infections among patients that attend the Komfo Anokye Teaching Hospital in Kumasi. The study also determined the *in vitro* susceptibility of the yeast species isolated from the various clinical samples. The antifungal agents tested include flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole. The antifungal agents used in this study were chosen on the basis of being commonly available on the local market and were among the drugs that are prescribed at the Komfo Anokye Teaching Hospital in Kumasi.

5.1 Distribution of yeasts species

Yeasts infection of the vagina is a common problem that causes significant morbidity and affects the well being of women (Asticcioli *et al*., 2009). The study showed yeast prevalence of 21% from the high vaginal swabs (HVS) within the population studied. This was lower than the 40.6% reported by Enweani *et al.* (2001) among non-contraceptive users in Nigeria. Enweani *et al.* (2001) reported that the high prevalence of vaginal colonization could be partly attributed to sexual behavioural predisposition and low personal hygiene in the society. However, this finding is consistent with findings from Gross *et al.* (2007) who reported a prevalence of 20.6% in Costa Rica. Also Mohanty *et al*. (2007) reported a prevalence of 18.5% in India while Falleiros de Padua *et al*. (2003) reported in Brazil that the prevalence of yeasts isolation from high vaginal swabs was 20.15%.

A finding from the study indicates that *Candida albicans* was the most frequent yeast isolated from HVS followed by *Candida glabrata*. This compares favourably with finding of Ellabib &

ElJariny. (2001) who reported in Libya that *Candida albicans* and *Candida glabrata* were the most common yeasts isolated from HVS taken from Libyan females. Similar results by Enweani *et al*. (2001) reported in Nigeria that *Candida albicans* was the commonest yeast isolated from HVS but reported *Candida kefyr* and *Candida stellatoidea* as the second and third most common isolates respectively. Also Asticcioli *et al*. (2009) reported in Italy that the most frequently isolated yeast from HVS was *Candida albicans* followed by *Candida glabrata* and *Candida tropicalis* results which are consistent with findings in this study. However, Okungbowa *et al*. (2003) reported in Nigeria that *Candida glabrata* was the most common yeast isolated from the genitourinary tract followed by *Candida albicans* and *Candida tropicalis*, a study in which the urinary and high vaginal swab isolates were combined.

The prevalence of candiduria detected in this study was 11%. This was higher than the 5.1% reported in 2003 in Accra, Ghana by Ayeh-Kumi *et al*. (2007) but lower than the 44.4% reported among patients in intensive care units (ICU) by Passos *et al*. (2005) in Brazil. The commonest yeast found in urine specimens during this study at the Komfo Anokye Teaching Hospital was *Candida albicans* followed by *Candida glabrata* and *Candida tropicalis*. This finding seems to be agreeable with that of Yasin *et al*. (1986) who reported in Malaysia that the commonest yeast found in urine was *Candida albicans* followed by *Candida tropicalis* and *Candida glabrata*. Also Passos *et al*. (2005) reported in Brazil that *Candida albicans* followed by *Candida glabrata* and *Candida kefyr* were the commonly isolated yeasts from urine.

During the study period it was also found that the prevalence of yeast isolation from sputum was 9.4%. This was lower than the 34.2% prevalence reported in Nigeria by Enwuru *et al*. (2008) among HIV- positive patients. Patel *et al*. (2006) reported 63% in HIV negative patients from South Africa; while Reichart *et al*. (2003) reported 48% in Thailand and Cambodia among HIV-

infected patients. In this study, it was found that *Candida albicans* was the commonest yeasts isolated from sputum giving as high as 70% prevalence, followed by *Candida tropicalis* (20.0%) and *Candida krusei* (10.0%). This does not compare with findings of Enwuru *et al*. (2008) who reported in Nigeria that the most common yeast species isolated from sputum and oropharyngeal swabs were *Candida albicans* (40.5%), *Candida tropicalis* (7.6%), *Candida krusei* (6.8%) and *Candida glabrata* (5.4%). Also Hamza *et al*. (2008) reported in Tanzania that the most frequently isolated yeast species from the sputum and oropharyngeal swabs of HIV infected patients was *Candida albicans* (84.5%) followed by *Candida glabrata* (6.8%) and *Candida krusei* (3.4%) results which compares with findings from this study. Also Patel *et al*. (2006) reported in South Africa that the most common yeast species isolated from sputum of HIV-infected patients was *Candida albicans* (78.6%) whereas non-albicans comprised 21.4% a results which also compare with findings from this study.

The prevalence of yeasts isolation from cerebrospinal fluid reported in this study was 3.6%. This finding is comparable to the 2.2% reported in New Zealand by Chen *et al*. (2000). *Cryptococcus neoformans* was the yeast commonly isolated from cerebrospinal fluid (CSF). This is consistent with reports from Castonon- Olivares *et al*. (2000) who reported in Mexico that *Cryptococcus neoformans* was the dominant yeast mostly isolated from CSF followed by *Cryptococcus albidus* and *Cryptococcus uniguttulatus*. Also Chen *et al*. (2000) reported in Australia and New Zealand that *Cryptococcus neoformans* was the most common yeast isolated from CSF.

5.2 Antifungal susceptibility testing

There is scanty data on the antifungal susceptibility of *Candida* species and *Cryptococcus neoformans* at health facilities in Ghana including the Komfo Anokye Teaching Hospital in Kumasi. This study therefore presents antifungal susceptibility data on *Candida* and *Cryptococcus neoformans* at the KATH. Monitoring antifungal resistance among yeasts is useful because apart from tracking and detection of resistance, it also gives clues to emerging threats of new resistant strains. This helps in assessing empirical treatments recommendations.

Topical clotrimazole and miconazole are the antifungal drugs that are mostly prescribed for treating candidal vulvovaginitis at the Komfo Anokye Teaching Hospital. In addition, oral alternatives such as fluconazole and itraconazole are also used in managing candidal vulvovaginitis. Fluconazole is one of the first-line antifungal drugs that are used in treating infections due to *Candida* species other than *Candida krusei* and some *Candida glabrata* isolates (Patel, 2000; Wolff *et al*., 2000). In this study it was detected that no fluconazole resistance was identified among the 19 *Candida albicans* isolated from high vaginal swabs. This finding is consistent with findings of Sobel *et al*. (2004) who reported no fluconazole resistance among the 401 *Candida albicans* recovered from women with candidal vaginitis in the United States of America (USA). Another study in USA reported fluconazole resistance to be 3.6% among *Candida albicans* isolates collected from candidal vulvovaginitis patients (Sobel *et al.,* 2003). Also Ribeiro *et al*. (2000) reported in Brazil that no fluconazole resistance was identified among 56 *Candida albicans* isolated from vagina. Although fluconazole is not the most commonly used antifungal drug for the management of vaginal candidiasis and *Candida* colonization at the Komfo Anokye Teaching Hospital, it was tested due it availability.

It was found that 9 out of 12 (75%) of *Candida glabrata* isolates were found to be in the intermediate category for fluconazole. Pfaller *et al.* (1999) reported in USA that isolates of *Candida glabrata* often generate high fluconazole MICs with as many as 15% being completely resistant. Hseuh *et al*. (2005) reported in Taiwan that among the 59 *Candida glabrata* isolates tested 16 (27%) were in the intermediate category. Also Richter *et al*. (2005) reported in a study that 57.8% of *Candida glabrata* isolates were in the intermediate category while 15.2% were resistant.

Findings from the study indicate that all the three (3) isolates of *Candida krusei* were resistant to fluconazole. This was not surprising because it is well established that *Candida krusei* is intrinsically resistant to fluconazole (Berrouane *et al.*, 1999). This was also confirmed in a study by Hamza *et al*. (2008) who reported in Tanzania that all isolates of *Candida krusei* tested were resistant to fluconazole.

Although the CLSI M27-A broth microdilution method remains the gold standard antifungal susceptibility testing method, it is not convenient to carry out on a routine basis. Alternative methods including the broth colorimetric microdilution, flow cytometry and the disc agar diffusion methods have been developed. The disc diffusion method seems to be convenient and economical for routine use. In this study the disc diffusion method was used to determine the susceptibility of 67 yeast isolates to flucytosine and amphotericin B. Analysis of result obtained with the disc diffusion method in comparison with the result obtained with the ATB FUNGUS method show that there was no significant difference in the susceptibility of the isolates to flucytosine (p-value = 0.56) and amphotericin B (p-value = 0.49). Resistance of isolates to flucytosine, itraconazole and amphotericin B ranged from 7.5% to 22.4%. This compares with the results of Araj *et al*. (1998) who reported that resistance of 70 clinical *Candida* isolates to

amphotericin B, flucytosine, itraconazole and ketoconazole existed in a range of 4 to 17%. Also a study by Asticcioli *et al.* (2009) on antifungal susceptibility of 518 yeasts to flucytosine, amphotericin B, ketoconazole and voriconazole revealed that about 5 to 10 % of the isolates were resistant to the above mentioned antifungal agents tested.

Pfaller *et al*. (2002) reported the *in vitro* activities of flucytosine against more than 8000 clinical isolates of *Candida* species at more than 200 hospitals worldwide. Resistance to flucytosine was observed in only 3% of *Candida albicans* and 1% of *Candida glabrata*. In this study it was found that 9.1% (3/33) of *Candida albicans* were resistant. Lack of information on the antifungal susceptibilities of yeasts isolates in Ghana hampers comparison with local trends.

Amphotericin B in combination with flucytosine is the drug of choice for treating cryptococcal meningitis. It was found in this study that, all the three (3) *Cryptococcus neoformans* isolated from CSF were susceptible to all the antifungal agents tested just as Sar *et al*. (2004) reported that all *Cryptococcus neoformans* strains they isolated from CSF of patients in Cambodia were susceptible to amphotericin B. Also Pfaller *et al*. (2005) determined the antifungal susceptibility of 1,811 clinical isolates of *Cryptococcus neoformans* in USA and reported resistance $\leq$ 1% to amphotericin B, fluconazole and flucytosine.

Voriconazole a derivative of fluconazole has been shown to demonstrate greater activity compared with itraconazole and higher potency than fluconazole against *Candida* species, *Cryptococcus neoformans*, *Aspergillus* species and other fungi (Kauffman & Zarins, 1998; Marco[b] *et al*., 1998; Pfaller *et al*., 1998). Findings from this study indicate that voriconazole has an excellent *in vitro* activity against all the yeasts isolates tested including the yeasts that were

found to be resistant to fluconazole such as *Candida krusei*. No isolate was found to be resistant to voriconazole though two isolates of *Candida albicans* were found to be in the intermediate category. One possible reason for the lack of voriconazole resistance detected in this study might be due to the fact voriconazole is not the frequently used at the Komfo Anokye Teaching Hospital for treating yeast infections. A study conducted in Italy by Mandras *et al*. (2009) showed that all *Candida* species that were tested in their study were susceptible to voriconazole. Similarly, Baran *et al*. (2000) reported in United States that all isolates of *Candida* tested were susceptible to voriconazole. However, Tulumoglu *et al*. (2009) reported in Turkey that the resistance rate of 48 *Candida* isolates was 5.45%.

5.3 CONCLUSION

Findings from this study show that yeast infections are common at the Komfo Anokye Teaching Hospital (KATH) with prevalence level of about 12.7%.

The prevalence of vaginal yeasts isolation was 21% among the population studied. The prevalence of yeasts isolation was 11% for urine, 9.4% for sputum and 3.6% for cerebrospinal fluid.

Candida albicans was the most commonly yeast isolated from all the clinical samples except in the cerebrospinal fluid in which *Cryptococcus neoformans* was the commonest.

The antifungal susceptibility of yeasts isolated from high vaginal swabs, urine, sputum and cerebrospinal fluids indicated that all the *Candida albicans* isolated from high vaginal swabs were susceptible to fluconazole. The overall resistance rate of the isolates to flucytosine, amphotericin B, fluconazole and itraconazole ranged from 4.5% to 22.2%. Isolates intrinsically

resistant (*Candida krusei*) and those that showed decreased susceptibility (*Candida glabrata*) to fluconazole were susceptible to voriconazole.

Since most isolates were all sensitive and the resistance levels of other isolates to the antifungal agents tested were variable, ranging from 4.5% to 22.2%, the antifungal agents tested can be relied upon for empirical treatment of yeast infections at the Komfo Anokye Teaching Hospital.

5.4 RECOMMENDATIONS

In view of the findings in this study, the following recommendations have been made:

1. Further clinical studies involving more yeast isolates need to be performed at the Komfo Anokye Teaching Hospital to confirm the findings in this study.
2. Similar studies should be carried out at other hospitals in Kumasi and Ghana as a whole to provide more information on the frequency of different species of yeasts in Ghana and to determine susceptibility patterns of the yeasts species to the common antifungal agents in Kumasi and Ghana as a whole to generate comprehensive epidemiological data.
3. Even though most of the yeasts tested were susceptible to the antifungal agents, periodic antifungal susceptibility testing should be carried out at the Komfo Anokye Teaching Hospital to monitor trends of resistance to antifungal agents among the yeasts.
4. Since *in vitro* susceptibility does not always translate into *in vivo* activity, further studies correlating *in vitro* susceptibility data with clinical efficacy of the antifungal agents should be carried out at the Komfo Anokye Teaching Hospital in Kumasi.

5. Since some species of yeasts are known to be intrinsically resistant to some antifungal agents, (example *Candida krusei* to fluconazole) there is the need to speciate *Candida* isolates so as to provide early and specific treatment remedy and avoid recurrent candidal infection.

6. This study was limited to yeasts only, studies on other fungal pathogens are recommended.

7. Routine susceptibility tests and speciation of yeasts using sugar assimilation and fermentation are expensive and time consuming and therefore more simple and rapid methods of identification and susceptibility testing may be useful.

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Appendix 1

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES
COMMITTEE ON HUMAN RESEARCH PUBLICATION AND ETHICS

Our Ref: CHRPE/KNUST/KATH/11_03_09

26th March, 2009

Mr. Patrick Feglo
Department of Clinical Microbiology
KNUST
Kumasi

Dear Sir,

EPIDEMIOLOGY OF YEAST INFECTIONS AT KOMFO ANOKYE TEACHING HOSPITAL

Your application for Ethical Committee review for the study entitled "*Epidemiology of yeast infections at Komfo Anokye Teaching Hospital.*" has been considered and approved by the Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi and the Komfo Anokye Teaching Hospital, Kumasi.

The Committee recommends that samples and/or materials taken for this study should be used for the study only. Any subsequent use of the samples for other studies will need clearance from the CHRPE.

The Committee also recommends that it should be informed of any adverse events; it would therefore expect a periodic report of your study to the committee. Its permission should be sought for any amendments to the protocol. The Committee should be informed of all publications arising from the study and copies of the same should be sent to the committee.

Also note that you are required to seek the requisite permission from any department involved in order to carry out this study.

Professor Sir J. W. Acheampong, MD, FWACP
Chairman

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Approval letter from the Committee on Human Research Publications and Ethics of the School of Medical Sciences KNUST & KATH.

Note: The title **Epidemiology of Yeast Infections at the Komfo Anokye Teaching Hospital in Kumasi** as seen in the above letter was amended to read **Antifungal Susceptibility of *Candida* species and *Cryptococcus neoformans* isolated from patients at the Komfo Anokye Teaching Hospital in Kumasi.**