IDENTIFICATION AND SUSCEPTIBILITY PROFILE OF CANDIDA SPECIES AMONG DIABETIC PATIENTS IN BENIN CITY

BY

IKERU, VIVIAN ADAEZE
PG/BMS1512990

DEPARTMENT OF MEDICAL LABORATORY SCIENCE
SCHOOL OF BASIC MEDICAL SCIENCES
UNIVERSITY OF BENIN
BENIN CITY

JANUARY, 2019.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Diabetes mellitus (DM) is a clinical syndrome associated with deficiency of insulin secretion or action. It is considered one of the largest emerging threats to health in the 21st century. It is estimated that there will be 380 million persons with Diabetes mellitus in 2025 (Atkins *et al.,* 2010). Besides the classical complications of the disease, DM has been associated with reduced response of T cells, neutrophil function, and disorders of humoral immunity (Muller *et al*., 2005). Consequently, DM increases the susceptibility to infections, both the most common ones as well as those that almost always affect only people with DM (e.g. rhinocerebral mucormycosis)(Peleg *et al.,* 2007). Such infections, in addition to the repercussions associated with its infectivity, may trigger DM complications such as hypoglycemia and ketoacidosis.

Fungal infections are a major cause of morbidity and mortality in immune compromised individuals (such as diabetes mellitus) and *Candida* are among the most common pathogens in these patients (Pahwa, 2015). The prevalence of diabetes has been on the increase. Diabetes is associated with certain diseases such as candidiasis (Bader *et al*., 2015). *Candida* species are important nosocomial pathogens in critically ill patients and are associated with substantial mortality and

prolonged hospitalization in the intensive care unit (Anil *et al.,* 2012). Diabetes mellitus is one of the main risk factors of fungal infections of oral cavity, lower part of gastrointestinal tract, skin, foot, urogenital system and blood. Mycosis is a serious diagnostic and therapeutic problem and cause of mortality in diabetes. Fungal infections are also an important problem among hemodialysis patients with diabetes. *Candida* species belong to the normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract and vagina and are responsible for various clinical manifestations from mucocutaneous overgrowth to blood stream infections (Eggimann *et al.,* 2003). Although most authorities believe that patients with diabetes mellitus have an increased predisposition to *Candida* infections, some controversies still remain. *Candida* species are unusual causes of urinary tract infections in healthy individuals, but common among patients with predisposing diseases (such as diabetes mellitus). *Candida* species account for almost 10-15% nosocomial UTIs (Lundstrom *et al.,* 2001). Candiduria not properly diagnosed and treated has been a source of morbidity and mortality (Manjunath *et al*., 2011). Oksala *et* al (1990) proposed that diabetes patients are likely to develop fungal infections dues to high glucose level which in turn improves the growth of yeast in the oral cavity and urinary tract. The potential clinical importance of species level identification has been recognized as *Candida* species differ in the expression of putative virulence factors and antibiotic susceptibility (Murray *et al*.,

2005). Due to inappropriate and inaccurate identification, the emergence of non-*albicans Candida* species as potential pathogens has been underestimated, since some of these species, particularly *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, and *C. tropicalis* can be highly resistant to common antifungal drugs (Antunes *et al*., 2004). Rapid identification of yeast species guides early appropriate antifungal therapy. Yeast identification and in vitro susceptibility testing provide valuable information for antifungal treatment selection and guides early appropriate antifungal therapy and reduces chances of antifungal resistance.

1.2 Aim of Study

the study is aimed to Identify and determine the antifungal profile of *Candida* species isolated from the urine and mouth swab of diabetes mellitus patients in Benin City Nigeria.

1.3 Specific objectives

1. Determine the prevalence of Candida infection in the mouth and urinary tract of diabetes mellitus patients in Benin City.
2. Identify the species of Candida in some clinical specimens(mouth swab and urine) of diabetes mellitus patients in Benin City

3. Determine the susceptibility profile of the Candida species to antifungal agents.
4. To determine the fasting blood sugar of the subjects.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is one of a heterogeneous group of disorders of carbohydrate metabolism as a result of defects in insulin secretion, the action of insulin or a combination of both defective secretion and incorrect action (Drozdowask *et al.,* 2008). All causes of diabetes ultimately lead to hyperglycaemia (a state of elevated blood glucose) which is the hallmark of the disease (Belazi *et al*., 2005). The definition and criteria for diagnosis have evolved as more is understood about normal and abnormal glucose metabolism. In 1979, a proposed definition by organisations such as the World Health Organization (WHO) and the National Diabetes Data Group (NDDG) in the USA was based on an elevated fasting plasma glucose level or an abnormal plasma or serum glucose level following 75 grams oral glucose tolerance test (Eckhard *et al*., 2007). More recently, glucose load testing has become regarded as less indicative of diabetes and greater reliance is placed on fasting plasma glucose levels. The current diagnostic criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (sponsored by the American Diabetes Association) (Goncalves *et al.,* 2006) are:

1. Confirmed fasting plasma glucose equal to or greater than 7.0 mmol/L (126 mg/dl)

2. in the presence of symptoms of diabetes, a confirmed non-fasting plasma glucose equal to or greater than 11.1 mmol/L (200 mg/dl)
3. When considered necessary to challenge glucose metabolism with oral glucose tolerance test (OGTT), confirmed plasma glucose level after two hours equal to or greater than 11.1mmol/L (200 mg/dl). Oral glucose tolerance is affected by a number of factors unrelated to diabetes but having some effect on carbohydrate metabolism. These include ageing, stress, certain drugs, coincident diseases, low carbohydrate diet and physical activity. Therefore, if OGTTs are used to differentiate between normal and abnormal, the test conditions must take these factors into account. An OGTT should involve standard glucose dose, reasonable prior physical activity, absence of any drugs likely to affect the result and a diet including at least 200 grams of carbohydrate per day.

2.2 Classification of Diabetes Mellitus

There are several classification systems for DM by the World Health Organization (WHO) expert committee on diabetes but the current WHO classification system has been established in co-operation with the National Diabetes Group (USA) and it mainly depends on the etiology of the disease (WHO, 2002).

2.1.1 Type 1 (Insulin Dependent) Diabetes

Previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes (WHO, 1999), type 1 DM is characterized by pancreatic β-cell loss eventually producing insulin deficiency within the affected individual. This type was not addressed in the present study, and therefore will not be discussed in details. It should be noted though that, if not treated properly, patients with type 1 DM might present with diabetic ketoacidosis (DKA) (Bennett and Knowler, 2005; Masharani, 2010). The latter is marked by substantial decrease in blood insulin, as well as an abnormal increase in acidic ketone compounds (Masharani, 2010).

2.2.2 Type 2 (Non-insulin Dependent Diabetes Mellitus)

It was previously called non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes (WHO, 1999), this form of DM accounts for more than 90% of diabetics (Tripthi and Srivastava, 2006). It is most commonly seen in adults but it can occur at any age (Brownlee, 2005). It's characterized by decreased insulin sensitivity which can subsequently provoke decreased insulin secretion as a result of β-cell loss (Bennett and Knowler, 2005; Masharani, 2010). Either of which may be the predominant feature (WHO, 1999). The early stages of the disease are caused by a substantial decrease in insulin-sensitivity, inducing abnormally high levels of blood glucose. In response to high blood glucose levels, the pancreatic

beta-cells secrete correspondingly high levels of insulin. However, as the progression of the disease continues, beta-cells are lost and the secretion of insulin falls (Bennett and Knowler, 2005; Masharani, 2010). The causes of beta-cell loss are unclear, although few hypotheses exist. One of them is related to a 37-residue peptide called islet amyloid polypeptide (or amylin). It is hypothesized that the precipitation of amylin resulting in amyloid deposition might be responsible for the beta cell defect. Alternatively, increased cellular stress due to overproduction of insulin is thought to be involved in beta cell loss (Masharani, 2010). The aetiology of Type II diabetes is elusive, although it is thought to result from a combination of genetic and environmental factors (Bennett and Knowler, 2005; Masharani, 2010). People with this type of DM are not dependent on exogenous insulin, but may require it for the control of blood glucose levels if this is not achieved with diet monitoring, weight reduction, exercise and oral hypoglycaemic agents (Milchovic and Dunn-long, 2007). Obesity, which is defined as an elevated body mass index (BMI) of $\geq$30 kg/m^2, is known to increase an individual's susceptibility toward developing type II diabetes. It is estimated that over 70% of type 2 diabetics are obese (Masharani, 2010). However, the underlying mechanism is not fully clear. Obesity aggravates the insulin resistance (IR). Moreover, obese diabetics, as compared to non-obese diabetics, are at increased risk of developing macrovascular and microvascular complications (Bennett and Knowler, 2005). Fat

tissue, especially centrally-located one, is hormonally-active and can secrete high levels of adipokines which can disrupt insulin action in obese individuals (Clark, 2006; Mittal, 2007; Masharani, 2010). Among these adipokines are tumour necrosis factor-alpha (TNF-α) and resistin, which inactivate insulin receptors and decrease glucose metabolic response to insulin, respectively (Mittal, 2007; Masharani, 2010). It is also possible that genetic mutations associated with the expression or action of adipokines may contribute to an individual's predisposition toward obesity-related type-2-diabetes (Masharani, 2010).

The minimal severity of symptoms in the initial stages of type 2 diabetes can result in a delayed diagnosis (Masharani, 2010). Unlike type 1 DM, type 2 DM does not manifest diabetic ketoacidosis (DKA) episodes and exhibit relatively mild or non-existence overt symptoms, which may include a general feeling of fatigue and increased urine production (Bennett and Knowler, 2005; Masharani, 2010). Since insulin production and sensitivity is only moderately impaired in type 2 diabetics, drug-based approaches toward improving insulin production and sensitivity and regulating glucose release from the liver are common treatments. An overall decrease in obesity is a desired outcome of this approach. Sulfonylureas, metformin, and thiazolidinediones (TZDs) are typical drugs used to achieve these effects (Masharani, 2010).

In that type of DM, the circulating insulin levels may be normal or elevated. Yet, due to insulin resistance, these levels are insufficient to control blood glucose level within the normal range. Strong family history, obesity, hypertension, dyslipidemia, as well as women with a history of gestational diabetes are all risk factors for developing type 2 DM. The frequency of type 2 DM varies considerably among different racial or ethnic subgroups. For example, in the USA it was found that persons of Native American, Asian-Indian, African-American descent are at higher risk than persons of European origin (Bennett and Knowler, 2005).

2.2 Epidemiology of Diabetes

- The number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014
- The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014.
- Diabetes prevalence has been rising more rapidly in middle- and low-income countries.
- Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation.
- In 2012, an estimated 1.5 million deaths were directly caused by diabetes and another 2.2 million deaths were attributable to high blood glucose.

- Almost half of all deaths attributable to high blood glucose occur before the age of 70 years. WHO projects that diabetes will be the 7th leading cause of death in 2030.
- Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use are ways to prevent or delay the onset of type2 diabetes (WHO, 2016).

2.3 Complications of Diabetes Mellitus

2.3.1 Acute complications

This include DKA which results primarily in type 1 DM, and non-ketotic hyperosmolar state (NKHS) which is prevalent in type 2 DM. In DKA, insulin deficiency is combined with counter-regulatory hormone excess (glucagon, catecholamines, cortisol, and growth hormone); the decreased ratio of insulin to glucagon promotes gluconeogenesis, glycogenolysis, and ketone body formation in the liver and also increases free fatty acids (FFAs) and amino acids delivery from fat and muscle respectively, to the liver. Ketosis results from a marked increase in FFAs release from adipocytes due to increased lipolysis. On the other hand, insulin deficiency and inadequate fluid intake are the underlying causes of NKHS. This leads to hyperglycaemia, which induces an osmotic diuresis leading to profound intravascular volume depletion (Tripthi and Srivastava, 2006).

2.3.2 Chronic complications

This can be divided to non-vascular complications which include, gastroparesis, sexual dysfunction, and skin changes and vascular complications (both micro-and macro-vascular)

2.3.3 Microvascular Complications

Retinopathy: This is one of the most important causes of visual loss worldwide, and is the principal cause of impaired vision in patients between 25 and 74 years of age (Fraser and D'Amico, 2009). It's the most prevalent microvascular complication associated with diabetes, afflicting nearly eighty percent of individuals who have been diagnosed with diabetes for more than ten years (Ulbig and Hoops, 2000; Foweler, 2008) Clinically, retinopathy is divided into non-proliferative retinopathy (ranging in severity from mild to moderate, or severe), and proliferative retinopathy. This staging is determined by such investigations as ophthalmoscopy, and florescein angiography (Ogata *et al*., 2005) Chronic hyperglycaemia is thought to be the primary cause of retinopathy, through several possible mechanisms, (as will be mentioned below), which result in vascular changes and subsequent retinal injury and ischemia. More advanced retinal disease, including proliferative vascular changes and neovascularisation, may be mediated

by other mechanisms such as the action of insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) (Ogata *et al*., 2005).

Neuropathy: About half of diabetics have some degree of neuropathy; a progressive disorder that affects both the autonomic and peripheral nervous systems (Pinhas-Hamiel and Zeitler, 2007). Certain genetic elements may also predispose individuals to developing neuropathies (Foweler, 2008). This complication can occur from both direct hyperglycaemia that induces damage to the nerve or from neuronal ischemia leading to abnormalities of microvessels (Chen and Reaven, 19987).

Nephropathy: Diabetic nephropathy is the leading cause of kidney disease in patients starting renal replacement therapy and affects 40% of diabetic patients. The incidence of diabetic nephropathy can be as high as 25% by ten years after the initial diagnosis of diabetes and a small fraction of diabetic patients may already have nephropathy at the time of diagnosis (Gross and DeAzevedo, 2005). It increases the morbidity and mortality risks (mainly due to cardiovascular causes) (Tripthi and Srivastava, 2006). Moreover, eventual progression to chronic kidney failure may occur and necessitate dialysis or kidney transplantation. Diabetic nephropathy is defined by increased urinary albumin excretion (UAE) in the absence of other renal diseases (Gross and DeAzevedo, 2005). UAE of less than 30 mg/24 hours urine is considered normol albuminuria while micro albuminuria is

defined as UAE of 30-299 mg/24 hours, and macro albuminuria as UAE of ≥300 mg/24 hours urine (Fowler, 2008). The pathological changes to the kidney are mainly the result of hyperglycaemia resulting in high glucose uptake by microvascular endothelial cells which do not require insulin for uptake (Brownlee, 2005; Rask-Madsen *et al*., 2005). These endothelial cells begin to produce a large amount of membrane-associated glycosylated proteins and the basement membrane of the endothelial cells thickens and weakens, resulting in subsequent bleeding and insufficient supply of blood that result in the formation of mesangial nodule, and other changes. The entire process results in an initial thickening of the glomerulus and can later be diagnosed via the detection of increased serum albumin within the urine (micro albuminuria) as progressive formation of nodules destroys kidney functioning (Brownlee, 2005; Fowler, 2008). Screening for diabetic micro albuminuria may be accomplished by a 24-hour urine collection (Fowler, 2008). Alternatively, to determine the glomerular filtration rate (GFR), several other parameters are commonly adopted. Measurement of creatinine clearance is considered one of the commonly-performed tests for GFR. Nevertheless, the accurate measurement of creatinine clearance is difficult especially in outpatient since it is necessarily to obtain a complete and accurately-timed sample of urine. The usual collection time is 24 hours, which is highly dependent on patient's cooperation. An alternative to a formal measurement of

creatinine clearance is an estimated formula, the Cockroft-Gault formula, which can be reliably used to measure an estimated creatinine clearance. Plasma creatinine concentration is one of the reliable simple biochemical tests of glomerular function. Nevertheless, changes in plasma creatinine concentration can occur, independently of renal function, due to changes in muscle mass. Measurement of the plasma level of cystatin C, a low molecular weight cysteine protease inhibitor, has been insufficiently evaluated to be adopted into routine practice in place of the previously-mentioned measurements (Marshal *et al*., 2004). Several effective strategies exist to prevent the development of micro albuminuria, delay the progression to more advanced stages of nephropathy and reduce cardiovascular mortality in diabetics. Among these, are achieving the best glycaemic control, and treating both hypertension and dyslipidemia (Gross and DeAzevedo, 2005).

2.3.4 Macrovascular Complications

The primary cause of death in diabetics is cardiovascular disease (CVD). Diabetic patients exhibit both an elevated risk of myocardial infarction and coronary heart disease (Clark, 2006). Type 2 diabetics have been shown to have a greater than 200% risk of stroke and subsequent related dementias (Foweler, 2008). Type 1 diabetics are also more likely to develop coronary heart disease, specifically

ischemic heart disease, which accounts for a higher level of cardiovascular disease-related morbidity (Clark, 2006; Foweler, 2008). They are also more likely to suffer from a one- to five-fold increase in sudden death. The aetiology of this abnormality is most probably multifactorial. Factors such as myocardial ischemia from atherosclerosis, hypertension, and myocardial cell dysfunction secondary to chronic hyperglycaemia are involved. Though DM itself does not increase the levels of LDL, LDL particles in DM are atherogenic, more easily glycated, and susceptible to oxidation.

There is also strong evidence of a hypercoagulability state in DM, as manifested by increased coagulability and impaired fibrinolysis, this will be mentioned later in details. These factors are likely to further increase the risk of vascular occlusion and cardiovascular events in type 2 diabetes (Grundy *et al*., 1999; Beckman *et al*., 2002).

2.4 Candida infection

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide (Manolakaki *et al*., 2010). Many species are harmless commensals or endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease (Kourkoumpetis *et al*., 2011). *Candida albicans* is the most commonly isolated

species, and can cause infections (candidiasis or thrush) in humans and other animals. In winemaking, some species of Candida can potentially spoil wines (Fugelsang and Edwards 2010).

Many species are found in gut flora, including *C. albicans* in mammalian hosts, whereas others live as endosymbionts in insect hosts (Blackwell *et al.,* 2004). Systemic infections of the bloodstream and major organs (candidemia or invasive candidiasis), particularly in immunocompromised patients, affect over 90,000 people a year in the U.S (Enfert and Hube, 2007). When grown in a laboratory, Candida appears as large, round, white or cream (albicans means "whitish" in Latin) colonies, which emit a yeasty odour on agar plates at room temperature. *C. albicans* ferments glucose and maltose to acid and gas, sucrose to acid, and does not ferment lactose, which help to distinguish it from other *Candida* species.

Candida species are associated with human beings for quite long time as harmless commensals. They are commonly found on the mucosal surfaces of gastrointestinal and genitourinary tracts and skin of humans. However, they become opportunistic pathogens in immunologically weak and immune-compromised patients. As opportunistic pathogens, they can cause local mucosal infections and sometimes, systemic infections in which *Candida* species can spread to all major organs and colonize in these organs (Odds *et al.,* 1988). The systemic infections can be life threatening among the individuals having severely paralyzed immune system such

as AIDS patients, people undergoing chemotherapy and radiotherapy treatment for cancers, diabetes and patients undergoing organ transplants. As the number of immunocompromised patients is increasing worldwide due to change in life style and improvement in medical facilities, infections caused by Candida species have been increased dramatically in the last two decades. This has posed a serious and daunting challenge to the effective management of candidiasis and cost has been increased manifold. It is estimated that in the United States itself the excess cost due to candidemia is between $1 and $2 billion per year (Pfaller *et al.,* 2006). Among *Candida* species, *C. albicans*, which is a normal constituent of the human flora, a commensal of the skin and the gastrointestinal and genitourinary tracts, is responsible for the majority of Candida bloodstream infections (candidemia). Yet, there is an increasing incidence of infections caused by *C. glabrata* and *C. rugosa*, which could be because they are frequently less susceptible to the currently used azole antifungals (Pfaller *et al.,* 2006). Other medically important species include *C. parapsilosis*, *C. tropicalis* and *C. dubliniensis* (Enfert and Hube, 2007).

Other *Candida* species, such as *C. oleophila* have been used as biological control agents in fruit. *C. albicans*, *C. ascalaphidarum*, *C. amphixiae*, *C. Antarctica*, *C. argentea*, *C. atlantica*, *C. blattae*, *C. atmosphaerica*, *C. auris*, *C. bromeliacearum, C. carpophila, C. carvajalis* (J*ames et al., 2009), C. cerambycidarum, C. chauliodes, C. corydalis, C. dosseyi, C. dubliniensis, C.*

ergatensis, C. fructus, C. glabrata, C. fermentati, C. guilliermondii, C. haemulonii, C. humilis, C. insectamens, C. insectorum, C. intermedia, C. jeffresii, C. kefyr, C. keroseneae, C. krusei, C. lusitaniae, C. lyxosophila, C. maltosa, C. marina, C. membranifaciens, C. mogii, C. oleophila, C. oregonensis, C. parapsilosis, C. quercitrusa, C. rugosa, C. sake, C. shehatea, C. temnochilae, C. tenuis, C. theae (Chang *et al*., 2012), *C. tolerans, C. tropicalis, C. tsuchiyae, C. sinolaborantium, C. sojae, C. subhashii, C. viswanathii, C. utilis, C. ubatubensis* and *C. zemplinina*.

2.4.1 *Candida Albicans*

C. albicans is the predominant cause of invasive fungal infections (Horn et al., 2009) and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization (Almirante *et al*., 2005; Lai *et al*., 2012). Although *C. albicans* is the most prevalent species involved in invasive fungal infections, the incidence of infections due to non-albicans species is increasing. In a study with 2019 patients at major North American medical centres, a predominance of non-albicans species was observed; although *C. albicans* was the most frequently isolated species, it was followed by *C. glabrata* and other non-*C. albicans* species. This change in epidemiology could be associated with severe immunosuppression or illness, prematurity, exposure to broad-spectrum antibiotics and older patients

(Horn *et al*., 2009). In European countries, an analysis showed that more than half of the cases of candidaemia were caused by *C. albicans*, and the incidence rates for non-albicans candidaemia infections were 14 % each for *C. glabrata* and *C. parapsilosis*, 7 % for C. tropicalis and 2 % for *C. krusei* (Tortorano *et al.,* 2006). Changes in the epidemiology have also been observed in Latin American countries. In Chile, the prevalence of *C. albicans* has changed, and a progressive increase of non-albicans infection has been observed; *C. parapsilosis* was the most frequent species, followed by *C. tropicalis* and *C. glabrata*. All isolates were susceptible to amphotericin B; however, 50 % of the C. glabrata isolates were resistant to fluconazole (Ajenjo *et al*., 2011). According to the Brazilian Network Candidaemia Study*, C. albicans* accounted for 40.9 % of cases in Brazil, followed by *C. tropicalis* (20.9 %).

Common symptoms include fatigue, bloating, flatulence, anxiety, depression, vaginitis, itchy skin, impaired memory, poor concentration, and a "foggy" brain. Left untreated, *Candida albicans* overgrowth can lead to systemic infection via the bloodstream, allowing numerous disease processes to occur.

There are various treatments to reduce *Candida albicans* overgrowth, including prescription and over-the-counter antifungal medications, herbal supplements, natural remedies, healthy dietary and lifestyle changes.

Tortorano *et al.,* 2006

Figure 2.1: *Candida albican* in agar

2.4.2 *Candida tropicalis*

The second most common species, *Candida tropicalis* is believed to be responsible for up to 30% of Candida bloodstream infections (candidaemia). Overgrowth usually occurs in the gastrointestinal tract and on the skin of people with diabetes mellitus, leukemias and lymphomas, causing an array of issues including diarrhea, excessive gas, stomach cramps, and skin irritations including relentless itching, eczematous rashes and hives. *Candida tropicalis* can also cause vaginal candidiasis, symptomized by intense vaginal itching, abnormal thin watery discharge, pain

when urinating, redness and swelling of the outer genitals. Overgrowth can also cause nervous system disorders resulting in depression, anxiety, headaches and memory loss.

While *Candida tropicalis* is not as aggressive as *Candida albicans*, it's becoming more resistant to antifungal drugs such as Flucytosine, making it more difficult to treat. Fortunately there are several new generation antifungals that have been found effective when combined with a balanced treatment regimen including supplements and dietary/lifestyle modifications. Increased virulence of *C. tropicalis* isolates was observed when given orally to compromised mice, which parallels clinical observations in immune-compromised patients. Some studies showed that *C. tropicalis* is even more invasive than *C. albicans* in the human intestine, particularly in oncology patients (Wingard *et al*., 1979, Walsh and Merz, 1986). Secreted aspartyl proteinase 5 and 9 (SAP5 and SAP9) activity occurs in all Candida species, in the following order: *C. albicans*. *C. tropicalis*. *Candida kefyr*, *Candida krusei*. A few experimental studies have suggested that, following ingestion of yeast cells by phagocytic cells, SAP antigens are expressed by *C. albicans* and *C. tropicalis*, but not by *C. parapsilosis* (Rüchel *et al*., 1986., Borg and Rüchel, 1990). The aspartic proteinases secreted by *C. tropicalis* have also been demonstrated on the surface of the fungal cell walls before invading tissues during disseminated infections and invading macrophages after phagocytosis of

yeast cells (Borg and Rüchel, 1990; Borg-von Zepelin *et al*., 1998;). Some CNA species that were previously thought to be SAP negative were in fact proteolytic.

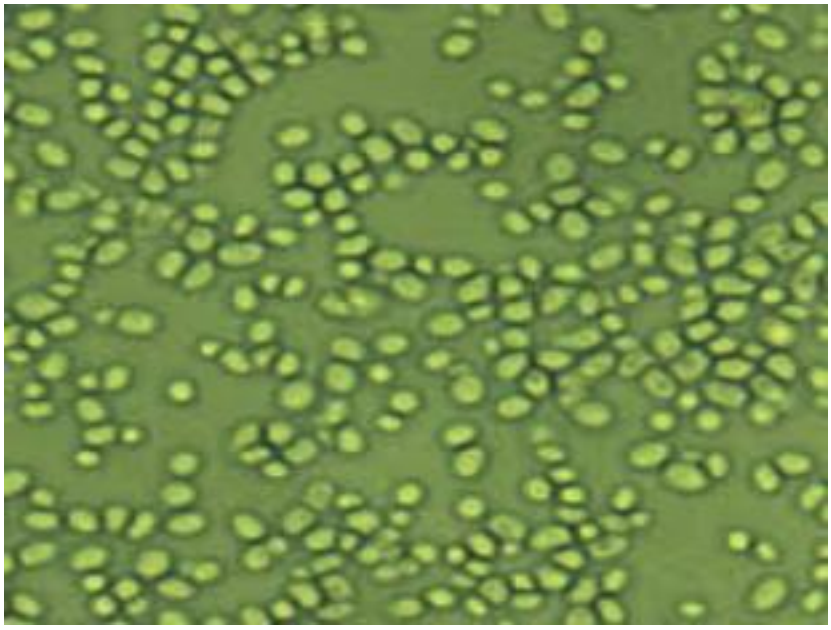
2.4.3 *Candida Glabrata*

C. glabrata, together with other *Candida* species, belongs to the class Fungi Imperfecti, the order Moniliales, and the family Cryptococcaceae (Sinnott, 1987; Kwon-Chung and Bennett. 1992). *C. glabrata* is a nondimorphic yeast that exists as small blastoconidia under all environmental conditions as a pathogen. In fact, *C. glabrata* is the only *Candida* species that does not form pseudohyphae at temperatures above 37°C. Wet-mount preparations of *C. glabrata* and *C. albicans* at similar magnifications. It is clear that *C. glabrata* blastoconidia (1 to 4 mm) are considerably smaller than *C. albicans* blastoconidia (4 to 6 mm). On Sabouraud dextrose agar, *C. glabrata* forms glistening, smooth, cream-colored colonies which are relatively indistinguishable from those of other Candida species except for their relative size, which is quite small. Two studies have implicated carriage on the hands of hospital personnel as a possible source of an outbreak (Vazquez *et al*., 1993). Thus, *C. glabrata* may be similar to *C. albicans* and other nosocomial pathogens that are acquired directly or indirectly from contaminated environmental surfaces. Previous understanding of the pathogenesis of C. glabrata colonization and infection assumed that the organisms responsible for disease were

endogenously acquired exclusively from the patients' own flora. The role of carriage by personnel in dissemination of *C. glabrata* remains to be clarified. Although *C. glabrata* is not frequently recovered from the hands of hospital personnel, transient carriage is suggested by its isolation on environmental surfaces in contact with hands (Vazquez *et al*., 1993). Perhaps more frequent culturing of the hands of personnel or the use of liquid media to recover yeasts may have improved the detection rates of *C. glabrata*. Proximity to a patient with infection or colonization increases the risk of nosocomial acquisition (Vazquez *et al*., 1993). As in earlier studies (Reagan *et al*., 1990; Vazquez *et al*., 1993), the results of longitudinal cultures showed that 75% of patients generally carried the same strain type of *C. glabrata* over time (Vazquez *et al*., 1993), with minimal strain diversity among individual patients. This finding is significantly different from the results described for the nosocomial acquisition of *C. albicans*, in which there was considerable strain diversity (Vazquez *et al*., 1993).

With the increased use of immunosuppressive agents, mucosal and systemic infections caused by *Candida glabrata* have increased significantly in recent years, according to NIH.* Estimated to be involved in 10-30 percent of yeast infections, *Candida glabrata* can cause oral thrush, which presents as creamy white, slightly raised lesions in the mouth. This can result in painful or difficulty swallowing or fever if the infection spreads beyond the esophagus. Thrush can affect anyone,

though it occurs most often in babies, toddlers, older adults, and people with immune deficiencies. Left untreated, thrush can spread to other parts of the body including the lungs and liver of immune-compromised people. Treatment modalities include various antifungal agents combined with supplements, nutritional and lifestyle modifications.



Vazquez *et al*., 1993

Figure 2.3: ***Candida glabrata***

2.4.4. *Candida Parapsilosis*

C. parapsilosis has emerged as a significant nosocomial pathogen with clinical manifestations that include endophthalmitis, endocarditis, septic arthritis, peritonitis and fungaemia, usually associated with invasive procedures or

prosthetic devices (Cantón *et al*., 2011), and with neonatal infections in the northern hemisphere, although this species is found in patients of all ages in Latin America (Almirante *et al*., 2005; Nucci *et al*., 2010). Pires *et al.*, 2011 isolated 100 strains of *C. parapsilosis* from a haemodialysis unit; using molecular analysis, 53% were found to be *C. parapsilosis* and 47% corresponded to *Candida orthopsilosis*. Tavanti *et al.*, 2005 suggested that the *C. parapsilosis* complex could be replaced by three different but related species named *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *Candida metapsilosis* on the basis of differences observed for randomly amplified polymorphic DNA and DNA sequencing of different genes. In the FUNGEMYCA study realized by Cantón *et al.*, 2011, 400 out of 1356 isolates were identified as *C. parapsilosis* sensu lato (29.5 %), and this species was isolated the second most frequently from blood after *C. albicans* in Spain. Of these 400 isolates, 364 were identified by molecular methods; *C. parapsilosis* represented 90.7% of isolates, *C. orthopsilosis* 8.2% and *C. metapsilosis* 1.1 %. Candidaemia due to *C. tropicalis* has been associated with cancer, especially in patients with leukaemia or neutropenia (Colombo *et al*., 2007). Candidaemia due to *C. glabrata* has been reported to be related to the use of fluconazole (Nucci *et al*., 2010). *Candida guilliermondii* and *Candida rugosa* were previously uncommon agents; however, the incidence of these is increasing (Pfaller *et al*., 2009).

Candida parapsilosis is believed to be involved in up to 30 percent of Candida infections, notably nail and tissue infections, and fungemia (fungal blood infection). *Candida parapsilosis* can cause severe flu-like symptoms, chronic fatigue and systemic infections, most often in immune-impaired people. Its high resistance to antimicrobial drugs is causing concern in hospitals, primarily in Europe. As with the aforementioned species of Candida, a comprehensive treatment approach is necessary to regain good health.

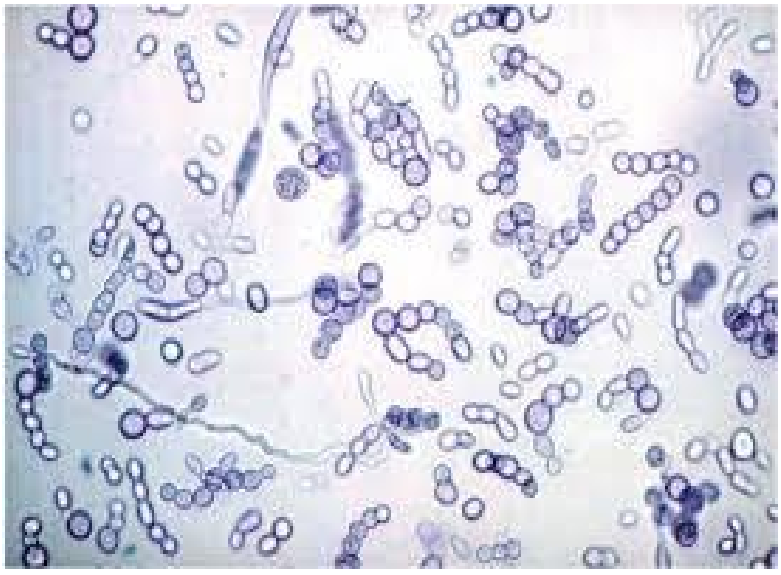

Pires *et al.*, 2011

Figure 2.4: *Candida parapsilosis*

2.3.5 *Candida krusei*

In contrast to a majority of other *Candida* spp. which is ovoid in shape, the cells of *C. krusei* are generally elongated and have the appearance of “long grain rice”. The

yeasts belonging to the genus Candida were first discovered by Langenbeck5 in 1839 from buccal aphthae in a patient with typhus, but the suggestion that C. krusei may cause disease in man was proposed by Castellani more than 75 years later. Since then, this organism has been generally recognized as a commensal in warm-blooded animals with very low pathogenicity and virulence. However, there has been a remarkable increase in the reports of *C. krusei* as a human pathogen during the last two to three decades. For instance, from 1960 onwards, > 65 articles have been published implicating *C. krusei* as an aetiological agent in human disease. Although this may be due partly to increased awareness of the organism and improvements in laboratory identification methods, there is little doubt that a true increase in the numbers of infections has occurred during this period.

A rare species, *Candida krusei* accounts for approximately 1% of candidiasis, and is usually associated with infant diarrhea and sometimes systemic candidiasis. Although it's resistant to Fluconazole, *Candida krusei* can be successfully treated with antifungal medications including Amphotericin B. in addition to a holistic regimen.

Hardy diagnostic, 2009.

Figure 2.5: ***Candida krusei***

2.3.6 *Candida lusitaniae*

Candida lusitaniae was originally isolated from the intestinal contents of warm-blooded animals. In humans, *C. lusitaniae* rarely causes opportunistic infections, although 13 cases involving various sites, including the kidneys, peritoneum, and blood stream, have been described (Guinet *et al*., 1983., Christenson *et al*., 1987; Blinkhorn *et al*., 1999). This species of Candida is of special interest because of its innate resistance to amphotericin B and its ability to develop resistance to amphotericin during therapy (Guinet *et al*., 1983., Blinkhorn *et al*., 1999). During the last 13 years after the publication of the first documented case of opportunistic infection caused by *C. lusitaniae*, 418 cases have been reported in the English literature and over 100 clinical isolations of *C. lusitaniae* have been described. 82

Of the 18 cases reported until now, 13 patients were adults, 82 were neonates, 37 and one was a teenager. Most patients with *C. lusitaniae* fungaemia were immunocompromised, usually owing to an underlying malignancy and transplantation. Not surprisingly, the use of broad-spectrum antibiotics (14 of 18 patients), the presence of an intravascular catheter (nine of 18), the use of cytotoxic or corticosteroid therapy (nine of 18), and the occurrence of granulocytopenia (five of 18) were noted frequently, while the presence of diabetes. Another rare species responsible for about 1% of Candida infections, *Candida lusitaniae* has been linked to several cases of candidemia blood infections, as well as systemic candidiasis, including sepsis and pyelonephritis, a potentially serious kidney infection. Fluconazole is an appropriate choice as first-line therapy for Candida lusitaniae, combined with dietary and lifestyle modifications. The first successful therapy of *C. lusitaniae* fungaemia was reported in 1985 before 1989, with the exception of a case of transient fungaemia not requiring therapy, all reported infections with C. lusitaniae required antifungal therapy and resistance to amphotericin B played a role in the fatal outcome of the disease. (Blinkhorn *et al*., 1999)

2.3.7 Pathogenesis of *Candida* species

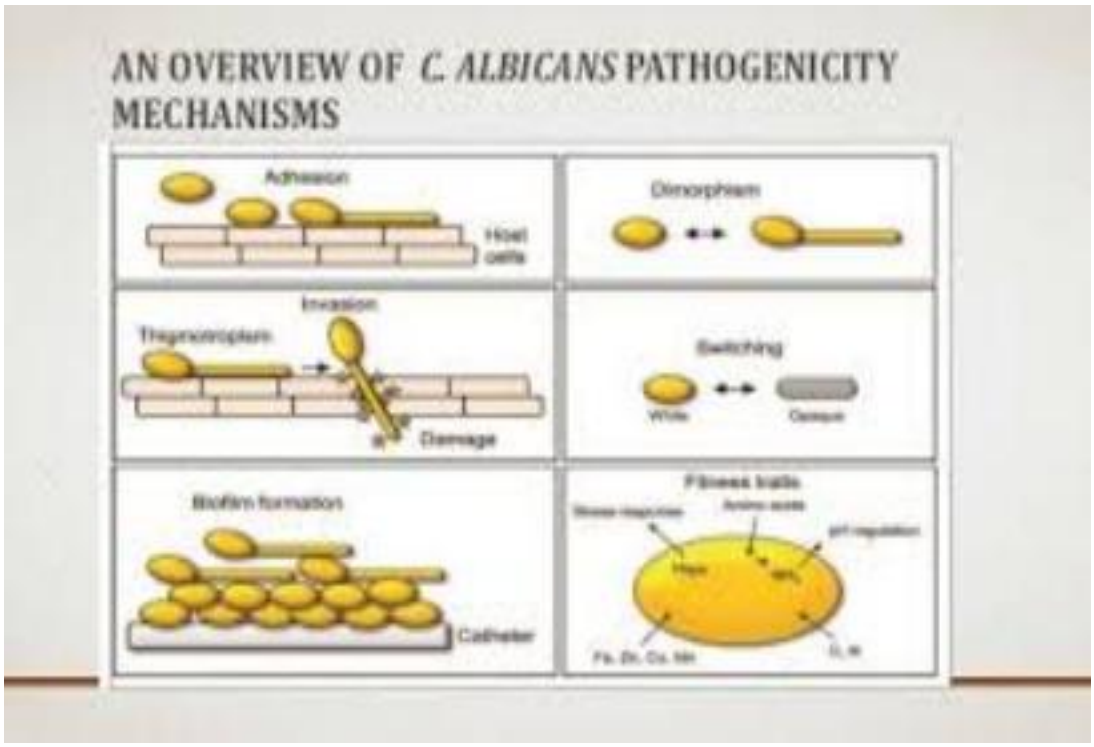
An infection caused by Candida is termed candidiasis or candidosis. Mycoses caused by these fungi show a wide spectrum of clinical presentations and can be

classified as superficial, as with cutaneous and mucosal infections, to deep, widespread and of high severity, as is the case with invasive candidiasis. According to Colombo and Guimara˜es (2003), the main transmission mechanism is through endogenous candidaemia, in which Candida species that constitute the microbiota of various anatomical sites under conditions of host weakness behave as opportunistic pathogens. Another mechanism for transmission is exogenous, and this occurs mainly through the hands of health professionals who care for patients. Also indicated in the spread of infection are health-care materials, such as contaminated catheters and intravenous solutions (Ingham *et al*., 2012). Candida species are considered important pathogens due to their versatility and ability to survive in various anatomical sites (Calderone and Fonzi, 2001). It was believed decades ago that yeasts passively participated in the process of pathogenesis in the establishment of fungal infection. Thus, organic weakness or an immunocompromised host was considered the only mechanism responsible for the establishment of opportunistic infection. Today, this concept has been modified. The current consensus is that these organisms actively participate in the pathophysiology of the disease process using mechanisms of aggression called virulence factors (Tamura *et al*., 2007). Candida species are eukaryotic opportunistic pathogens that reside on the mucosa of the gastrointestinal tract as well as the mouth, oesophagus and vagina (Kim and Sudbery, 2011; Lim *et al*.,

2012). Although this commensal organism normally colonizes mucosal surfaces in an asymptomatic manner, it can become one of the most significant causes of a disabling and lethal infection (Wisplinghoff *et al*., 2006; Vincent *et al*., 2009). In the early 1980s, fungi emerged as major causes of nosocomial infections, mainly affecting immunocompromised patients or those who were hospitalized for long periods due to serious underlying diseases (Vidigal and Svidzinski, 2009). Candida species belonging to the microbiota of healthy individuals can be found scattered in the environment. It is believed that most people usually have a single strain of Candida in different places in the body for a long period. However, some individuals have more than one strain or species at the same time, and this is commonly observed among hospitalized patients (Klotz *et al*., 2007). Moreover, the potential for Candida species to become pathogenic should be appreciated. A crucial component of this versatility is the fact that these organisms survive as commensals in diverse and distinct anatomical sites, each with its particular environmental stresses (Vidigal and Svidzinski, 2009). Although Candida species can infect different anatomical sites of the human host, evidence exists that immune protection is site-specific for each type. Moreover, cutaneous candidiasis and vaginal infections are more likely to be associated with a phagocytic response involving neutrophils and mononuclear phagocytes (Vidigal and Svidzinski, 2009). The urinary tract is the anatomical site most conducive to the development of

infections in hospitalized patients, although this remains a problem of questionable significance (Guler *et al.,* 2006; Vidigal and Svidzinski, 2009). Although most of these infections are of bacterial origin, it is estimated that at least 10% have fungi as the principal aetiological agent and that Candida species are the most frequently isolated (Sobel *et al*., 2000). Data show the isolation of Candida species in 22% of urine samples from patients admitted to intensive care units (Alvarez-Lerma *et al*., 2003). The colonization of the respiratory tract by Candida species is common in patients receiving mechanical ventilation for periods of longer than 2 days. This occurs due to haematogenous spread or pulmonary aspiration of the contents of colonies of oropharyngeal or gastric origin (Vidigal and Svidzinski, 2009). Prolonged intensive care unit stay and hospitalization are also important. Xie *et al.* (2008) evaluated the impact of invasive fungal infection in surgical patients with severe sepsis. The authors found that 28.3% of patients exhibited invasive fungal infection; *C. albicans* was most frequently isolated (58 %), followed by *C. tropicalis* (17%) and *C. glabrata* (15 %). In addition, the organ most affected by invasive fungal infection was the lung. Candida pathogenicity is facilitated by a number of virulence factors, the most important of which are those for adherence to host tissues and medical devices, biofilm formation and secretion of hydrolytic enzymes (e.g. proteases, phospholipases and haemolysins). Furthermore, although there has been extensive research to identify pathogenic factors in fungi,

particularly in *C. albicans*, relatively little is known about non-albicans species (Silva *et al*., 2011). Virulence in *C. albicans* and other pathogens includes host recognition, which enables the pathogen to bind to host cells and proteins. Additionally, degradative enzymes play a special role in virulence. Fungal invasion is facilitated more by the transition between yeast cells and filamentous growth than by yeast growth (Cullen and Sprague, 2012). The primary factor in the fungal colonization of human tissues is adherence to host surfaces; this process is controlled and induced by several cell-signalling cascades in both the fungus and the environment. In addition, *Candida* species can adhere to the surfaces of medica devices and form biofilms. The initial attachment of Candida cells is mediated by non-specific factors (hydrophobicity and electrostatic forces) and promoted by specific adhesins present on the surface of fungal cells that recognize ligands such as proteins, fibrinogen and fibronectin (Li *et al.,* 2003). The phenomenon of adhesion is exhibited by specialized surface proteins, called adhesins, that specifically bind to amino acids and sugars on the surface of other cells or promote adherence to abiotic surfaces (Verstrepen and Klis, 2006).



(Verstrepen and Klis, 2006

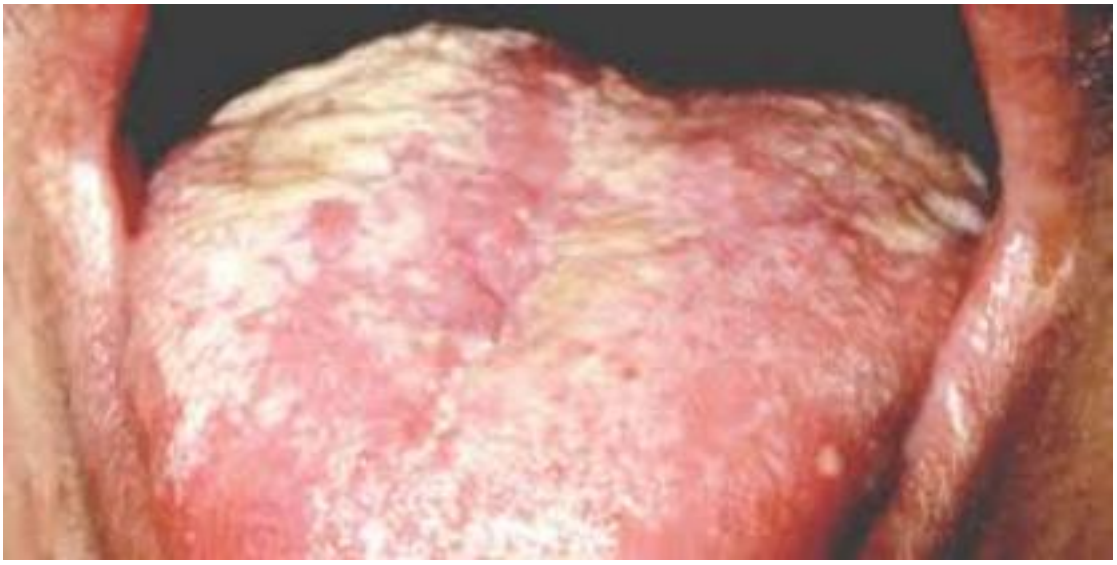
Figure 2.7: An overview of *C. albicans* pathogenicity mechanisms

2.3.8 Major types of candidiasis

Candidasis is an acute or chronic infection produced by Candida, generally limited to the skin and mucous membranes, but it could produce a serious systemic disease (Gamboa *et al*., 2012).

2.3.8.1 Mucosal candidiasis

Candida infections are restricted to non-sterile mucosal surface for example oropharyngeal and vulvuvaginal candidiasis (Terezhalmy and Huble *et al.,* 2011).



Terezhalmy and Huble *et al.,* 2011

Figure 2.8: ***Mucosal candidiasis***

2.3.8.2 Oropharyngeal candidiasis (OPC)

Oral candidiasis is one of the most common, oral mucosal infections seen in persons with HIV (Dangi *et al*., 2010). Candida is commensal organism and part of normal oral flora in about 30 to 50% of the population. There are three general factors that may lead to clinically evident oral candidiasis: immune status of host, oral mucosal environment and particular strain of *C. albicans* (hyphal form is usually associated with pathogenic infection). The appearance of OPC in HIV-positive patients heralds the onset of AIDS, corresponding to the decrease in the CD_4^+ T-lymphocyte count below 200/μL and plasma viral loads of more than 100,000 copies/ml (Reiss *et al*., 2012). However, other yeast species have been

increasingly identified, such as non- *albicans* Candida (NAC) species (*Candida glabrata, C. parapsilosis, C. krusei, C. tropicalis, C. dubliniensis, and C. guilliermondii*) (Martins *et al*., 2010).

The ability of the yeasts to overcome host clearance mechanisms and to colonize surfaces can be considered as a risk factor for oral infection. The balance between Candida colonization and candidiasis relies on the balance between pathogen characteristics (e.g. production of adhesins, secreted aspartylproteinases) and host factors

Host local predisposing conditions comprise:

i. Reduced saliva secretion,
ii. Epithelial changes and local mucosal diseases,
iii. Changes in commensal flora,
iv. High carbohydrate diet,
v. Denture wearing.

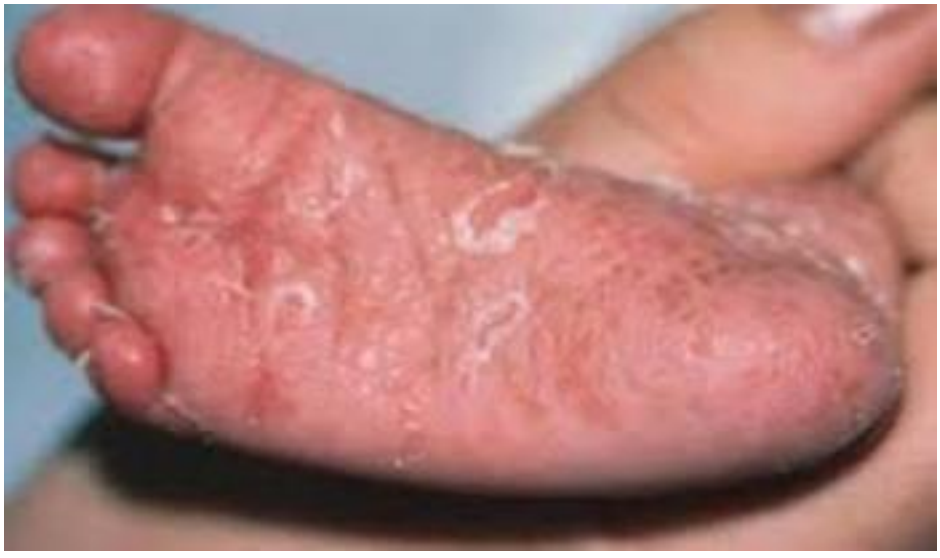
There are different types of oropharyngeal candidiasis including acute pseudomembranous, acute atrophic, chronic hyper-plastic, chronic atrophic, median rhomboid glossitis, denture stomatitis and angular cheilitis. The most discrete lesion represents conversion from benign colonization to pathological overgrowth (Akpan and Morgan, 2010).

2.3.8.3 Vulvovaginal candidiasis (VVC)

Vaginal candidiasis is the most frequent reason for gynecology consultation in primary health care services (Gonzalez *et al*., 2011). Disease is usually associated with considerable morbidity, healthcare cost, discomfort, pain and sexual functioning; however, it is seldom life threatening. The symptoms associated with VVC are eczematoid dermatitis lesions that sometimes show vesicular and grey-white psuedomembrane, vulval pruritis, burning, erythema and curd like discharge. It is a significant problem affecting 75% of women at least once during their lifetime. *C. albicans* is both the most frequent colonizer and responsible for most cases of VVC. Nevertheless, over the last decades there have been reports demonstrating an increment in the frequency of cases caused by non-*albicans* species with *C. glabrata* consistently being the leading species while other species are *C. tropicalis, C. krusei* and *C. parapsilosis.* (Ray *et al*., 2007). *C. dubliniensis* is a new species that has been recently reported from vaginal disease in the world. *C. albicans* possesses the ability to survive and proliferate in physiological extremes of pH, osmolarity, availability of nutrients and temperature. This versatility may account for the successful behavior of *C. albicans* both as a commensal colonizer of the vagina and as a pathogen.

2.3.8.4 Cutaneous candidiasis

Cutaneous candidiasis is usually secondary infection of skin and nail (body folds) in predisposed patients. It occurs as a sub-acute or chronic infection. Disease involvement may be localised or generalised to the skin or nails. The spectrum of cutaneous candidiasis includes diaper rash, intertrigo candidiasis, and Candida folliculitis, Otomycosis, Onychia and Paronychia. It usually occurs in warm, moist and creased area, such as axillary folds, inguinal or intergluteal areas. It is fairly common opportunistic disease and usually leads to maceration and trauma in skin. It is commonly found in diabetics and obese people. Other predisposing factors are antibiotic and oral contraceptives become macerated. (Xiao-dong *et at* 2008).



Xiao-dong *et at* 2008

Figure 2.10: Cutaneous candidiasis

2.3.8.5 Invasive candidiasis

Invasive infections can involve virtually any organ. *C. albicans* continue to account for the majority of invasive fungal infections, there has been a recent increase in disease due to non-albicans *Candida* species (and antifungal-resistant Candida isolates).

2.3.8.6 Systemic or disseminated candidiasis

Severe organ invasive or systemic hematogenously disseminated candidiasis is characterized by spreading of the Candida cells into almost the entire body with a tendency to create abscesses in vitally important organs, inducing their failure which leads to mortality in 50% of all cases, irrespective of administration of intensive antifungal therapy. (Pappas *et al*., 2003). Clinical signs of ongoing systemic candidiasis are hyper- and/or hypothermia, tachycardia, hypotension, high white blood cell counts, the need for vasopressor, etc. It occurs predominantly as a consequence of some invasive medical procedures, immunosuppressive therapy and aging.

Different Types of OPC with Symptoms

(i) Acute pseudomembanous candidiasis; (b) Angular cheilitis;

Pseudomembranous candidiasis is a consequence of the extended use of a

broad-spectrum antibacterial agent (Akpan and Morgan, 2010).

(a) Hyperplastic candidiasis (candidal leukoplakia) of the buccal mucosa and tongue due to heavy smoking;

(b) Candida-associated denture stomatitis;

(c) Median rhomboid glossitis (Terezhalmy and Huble, 2011).

Diabetes mellitus is a major risk factor for mycotic infections. The fungal infections are difficult diagnostic and therapeutic problems, serious cause of morbidity or mortality in diabetes. The particularly difficult problem makes up mycosis among hemodialysis patients with diabetes and in post transplant diabetes after kidney transplantation. Also, the main risk factor for fungal infections is diabetes with a pre-existing kidney graft in pancreas transplant recipients. Early diagnosis and effective treatment plays an important role in proceeding to the therapy of mycotic infections.

2.4 Diagnostic and therapeutic problems of mycosis in diabetes oral cavity

Diabetes mellitus is a predisposing factor of oral candidiasis (opportunistic infection caused by overgrowth of commensal of the mouth - Candida species e.g., the most prevalent *Candida albicans*), especially of pseudomembranous candidiasis (Akpan and Morgan 2002; Belazi *et al*., 2005). Also non-albicans

species (Pichia, Trichosporon, Geotichum) can be identified in the oral cavity of patient with poorly controlled diabetes, being prone to frequent and severe fungal infections (Manfredi *et al*., 2004; Goncalves *et al*., 2006). The poor glycemic control is associated with a high concentration of glucose in blood and saliva which can be treated as a nutrient for fungi (Manfredi *et al*., 2004). The significant role plays promoting an exaggerated inflammatory response to the periodontal microflora (Lamster *et al*., 2008). There is also a relationship between the presence of removable prostheses or cigarette smoking and higher rate of fungal infections by diabetics (Ship, 2008). Oral candidiasis include, excepting pseudomembranous candidiasis, also: median rhomboid glossitis, athrophic glossitis, denture stomatitis and angular cheilitis (Vernillo, 2003). The diagnosis includes examination of the mouth followed by culturing a swab from the affected area (labial and buccal mucosa, hard and soft palate, tongue periodontal tissues or oropharynx) and sensitivity testing (Akpan and Morgan 2002; Taylor, 2003). On the surface of oral cavity occur white patches. The tongue might be bright red. Other symptom can be burning sensation in the mouth or dysphagia (Akpan and Morgan 2002). Usually, the disease can be also asymptomatic (Drozdowska and Drzewoski, 2008). Untreated candidiasis can lead to serious, even fatal, complications or cause chronic hyperplastic candidiasis, known as candidal leukoplakia (Akpan and Morgan 2002; Sitheeque and Samaranayake, 2003). It is also worth mentioning

that periodontal infections can adversely affect glycemic control, increasing serum glucose level, in people with diabetes (Taylor, 2003). The incidence of infections can be reduced by appropriate prophylaxis. Very important is patient education, avoiding tobacco use, proper glycemic control and oral hygiene (Selwitz and Pihlstrom, 2003). Significant is rinsing the mouth with the topical antifungal, coating the whole mucosa with the medicine and holding in the mouth for a few minutes, after removing the dentures (Akpan and Morgan, 2002). The patient should soak the denture in 0.1% hypochlorite solution or chlorhexidine solution to eliminate the fungi. White vinegar (diluted 1: 20) can be used twice a week instead of it (Farah and Ashman, 2000; McCullough and Savage, 2005). The first line treatment is topical antifungal therapy polyene antibiotics: nystatin and amphotericin, eventually imidazoles. Topical therapy is advised also to patients with need of systemic treatment (intolerance or resistance to local application), because of possibility of reducing dose and duration of systemic preparations, to achieve reduction of adverse effects and drug interactions. Nystatin and amphotericin are not absorbed from the gastrointestinal tract (Akpan and Morgan, 2002). Modern medicaments and polyene antibiotics alert cell membrane permeability and azoles are inhibitors of fungal synthesizing enzymes of ergosterol (Akpan and Morgan, 2002). Recommended drugs include: nystatin, amphotericin and miconazole. First line treatment in patients without dentures is amphotericin

alone (lozenges 10 mg or suspension 100 mg/mL, four times a day after meals), or if patient is wearing dentures n amphotericin with nystatin (available as ointment (100,000 U/g, oral suspension 100,000 units/mL or pastilles (100,000 IU)) or miconazoles (oral gel 20 mg/mL, after meals). The managing takes three weeks. If the therapy is ineffective, the medications should be replaced. The lozanges of amphotericin are contraindicated in diabetics because of high sugar content. For that reason also nystatin oral rinse and clotrimazole troches should be avoided by diabetes. Alternatively, Fluconazole (100 mg/day) and itraconazole (200 mg/day) can be prescribed (Farah and Ashman, 2000; Akpan and Morgan, 2002; McCullough and Savage, 2005 Farah *et al*., 2010). In diabetic patients the modifications to insulin doses are needed for a period of oral infection to avoid infection related hyperglycemia (Vernillo, 2003). The side effects of nystatin and imidazole are: nausea, vomiting, and diarrhoea. The alternative in these cases can be clotrimazole and ketoconazole, which are applied in local form. Nowadays, the most important problem is raising drug resistance. Sensitivity test results can help in choice of the most effective treatment, however, the mostly common reason of poor response to the antibiotic is non-compliance (Akpan and Morgan, 2002; Vernillo, 2003; Farah *et al*., 2010).

2.4.2.2 Urogenital system

Candida microorganisms are responsible for vulvovaginal candidosis (VVC) (even 25% of vulvovaginal infections). Predominant yeast is *Candida albicans*, *Candida glabrata* and *Candida tropicalis* (Sobel *et al*., 2003; Urünsak *et al*., 2004). The incidence of infection affects 70-75% of women. The prevalence is higher in the sexually active, young women (Urünsak *et al*., 2004; Sobel, 2007; Ray *et al*., 2007). Women with uncontrolled, severe type 2 diabetes are more prone to be infected (Leon *et al*., 2002; Grigorious *et al*., 2006; Sobel, 2007; Ray *et al*., 2007). Antibiotic use, hyperglycemia, diabetes type and HbA1c level are recognized as a cause of VVC (; Goswami *et al*., 2000; Leon *et al*., 2002; Yildirim *et al*., 2010). Women carry candida organisms in the vagina without symptoms or signs of vaginitis, (Ferrer, 2000; Sobel, 2007) because of balance between pathological effects and vaginal defense factors to the source of infection. It may belong to intestinal reservoir, sexual transmission and vaginal relapse (Sobel, 2007). Identification of the type of infection and classification of its degree of severity can assist in the selection of appropriate therapy. Microscopic examination of vaginal secretion and culturing confirm the diagnosis. There is a possibility of use of PCR detection test. In women and adolescent girls with recurrent VVC is a glucose tolerance test recommended (Sobel, 2007; Lattif, 2011; Curran *et al*., 2011). VVC

can be asymptomatic or can clinically manifest with: acute pruritus, cottage-cheese-like vaginal discharge, vaginal soreness, irritation, vulvar burning, dyspareunia, external dysuria, slight odor, erythema and swelling of the labia and vulva (Sobel, 2007). There is the need for effective antifungal treatment with topical azoles or oral ones e.g., itraconazole, miconazole, elotrimazole, butoconazole or fluconazole (Urünsak *et al*., 2004; Sobel, 2007; Lattif, 2011). Oral drugs are more frequently associated with systemic toxicity and drug interactions (Ringdahl, 2000). The response to single dose of fluconazole is reduced by diabetics (Lattif, 2011). Important is also restricting dietary intake of sugar (Sobel, 2007). In otherwise healthy women, treatment with an antifungal in not advised (Leon *et al*., 2002; Sobel, 2007). Itraconazole has good efficacy and safety with *C. albicans* and other Candida species. Acute vulvovaginitis can be treated by using itraconazole (capsules 400 mg, single-dose: 200 mg in the morning and 200 mg in the evening) (Urünsak *et al*., 2004). In the case of limited response, the managing should be prolonged to 5-7 days. The common side effect is burning sensation (Sobel, 2007). In recurrent vulvovaginal candidosis the induction course of azole (ketoconazole 100 mg daily, clotrimazole 500 mg in suppositories, fluconazole 150 mg daily) is continued to the time, when patient is asymptomatic and culture negative. It can take 7- 14 days. The self-diagnosis and the early initiation of empiric topical therapy (500 mg clotrimazole intravaginaly). Beside of side effects

(gastrointestinal, hepatotoxicity of ketoconazole), the drug interaction with hypoglycemic agents can be essential in diabetic population (Sobel, 2007; Ringdahl, 2000). Only 33% of diabetic women with vulvovaginitis candidiasis achieve the success in treating with fluconazole (single dose 150 mg). It is associated with high prevalence of *C. glabrata* in diabetic population (Sobel *et al*., 2003; Goswami *et al*., 2006 ; Sobel, 2007). Relatively good efficacy follows boric acid (vaginal suppositories, 600 mg daily, duration: 7-14 days). It is well-tolerated. Possible adverse events are: burning sensation and vestibular erythema. The action way of boracid is unknown, but presumably it destroys the cell wall of fungi (Sobel *et al*., 2003; Ray *et al*., 2007). If there is no response to boric acid, the use of 17% flucytosine or flucytosine with amphotericin B (topical intervaginal cream, duration: 2 weeks) is successful. But the treatment should be not too long because of possibility of resistance. Intravaginal use of flucytosine don't show side effects in comparison with oral or parenteral form (gastrointestinal and bone marrow toxicity) (Sobel *et al*., 2003; Sobel, 2007). Rosenstock *et al.* reported that diabetic patients achieve better glycemic control with low rate of hypoglycemia, after adding canagliflozin (inhibitor of subtype 2 sodium-glucose transport protein, which increases urinary glucose excretion) to metformin therapy. The disadvantage of canagliflozin is higher rate of genital infections (Rosenstock *et al*., 2012). The treatment causes the higher incidence of vulvovaginal adverse events in diabetic

females (Nicolle *et al*., 2008). The other study showed that there is no influence on urinary tract infections (Nyirjesy *et al*., 2012).

2.4.2.4 Fungemia

The occurrence of the invasive candidiasis like candidemia, representing high mortality rate and the need of long treatment, has been increasing recently (Pfaller, and Diekema, 2004; Bader *et al*., 2005). It is a common medical condition in patients with immunosuppressive therapy for organ transplantation, chemotherapy for malignancy or after surgery treatment. Candidemia incidence is statistically correlated with hyperglycemia. Severe hyperglycemia is considered as important risk factor of serious consequences: increased morbidity, worst clinical course, longer hospital stay, late complications and mortality (Fraser *et al*., 1992; McNeil *et al*., 2001; Gudlaugsson *et al*., 2003, Gumbo *et al*., 2002; Marchetti *et al*., 2004 Bader *et al*., 2005;). This invasive mucosis is due to opportunistic fungal pathogens: yeasts (e.g., *Candida albicans*), molds (e.g., *Aspergillus fumigates*) and a broad list of other fungi. In diabetic population non-albicans Candida is prominent cause of candidemia. Up to 30% of all patients with candidemia are diabetic individuals (Badder *et al*., 2004; Nucci and Marr, 2005). Proliferation of fungi in deep tissues and organs is associated with disruption of this barrier due to peripheral vascular disease, neuropathy, insulin injections, surgery, or insertion of intravascular

catheter (Badder *et al*., 2004). Diagnosis depends upon clinical suspicion, isolating and identification of the infecting pathogens by culturing and histopathology (Fraser *et al*., 1992; Pfaller, and Diekema, 2004). For patients from high risk groups' prophylactic antifungal therapy and strict glycemia control are extremely important (Badder *et al*., 2004). Recommended, first-line treatment is administration of amphotericin B. Undergoing surgical debridement and eradicating therapy with alternative antifungal agents is preferred in case of nonsusceptible to standard azole or polyene therapy (Pfaller, and Diekema, 2004). *C. glabrata* infections demonstrate a high rate of fluconazole resistance. In such cases, the results can be greatly improved by using of amphotericin B or flucytosine. The most proper management for opportunistic yeast-like fungi (e.g., Trichosporon spp.) is amphotericin B, fluconazole or both of them. In infections caused by Geotrichum or Rhodotorula, amphotericin B, flucytosine or fluconazole are mainly used (Fraser *et al*., 1992; Pfaller, and Diekema, 2004). The recent study have shown the benefit from anidulafungin treatment of candidemia or invasive candidiosis in critically ill patients with fluconazole resistance (200 mg on the first day and than 100 mg daily, duration: 10-42 days, intravenous) (Ruhnke *et al*., 2012).

2.4.3 Mycosis among hemodialysis patients with diabetes: Hemodialysis patients and diabetics are both at increased risk of developing onychomycosis.

Dialysis duration and presence of diabetes mellitus are independent predisposing factors. The uremic patients with hemodialysis suffer more often from dystrophic nail changes and onychomycosis. Nail diseases in this disease affect 71.4% of patients. The most frequent occurring change is half and half nails. Absence of lunula is also common. Secondary to hypochromic anemia or chronic renal failure brittle nails are observed. The prevalence of it is also increased among immunocompromised patients like diabetics (Tercedol *et al*., 2003; Kuvandik *et al*., 2007). Patients with continuous ambulatory peritoneal dialysis with previous bacterial peritonitis and antibiotic management demonstrate an increased propensity to develop fungal peritonitis (Indhumathi *et al*., 2009). There is a pressing need to take care of hemodialysis, diabetic patient's nails and foot. Hemodialysis diabetics should be especially carefully examined for onychomycosis, because of dangerous, serious implications such as erysipelas or amputation (Kuvandik *et al*., 2007).

2.5 Laboratory Diagnosis/Identification of Candida Infection

The most reasonable approach to the diagnosis of candidiasis is to consider both symptoms, clinical and microscopical examination of smears from clinical isolates for isolation of the organism is a more sensitive method of diagnosis, (cheesbrough, 2004).

2.5.1 Direct examination of specimens

Wet mounts in a 10% to 3% potassium hydroxide solution are useful for distinguishing fungi in mucoid secretion or on skin, hair, or nails. A drop of calcoflour white stain may be added to the potassium hydroxide wet mount. The calcoflour stain fluorescence under ultraviolet light when it binds to polysaccharides in chitin in the fungal cell wall. This enhances fungal detection as the fungus wall appears bright green (Jawetz *et al*., 2000). Wet mount of the clinical specimen allows observation of the size and shape of the microorganisms, morphology of buds, the presence or absence of capsule, pseudohypae and arthroconidia (Cheesbrough, 2004).

2.5.2 Isolation of Candida from clinical specimens

Candida grows on routine mycology medium, either in sabouraud glucose (4%) agar or a chromogenic agar. Pathogenic yeast grows at 37^{o}C while saprophytic organisms grow at a lower temperature with 24hours and typical colonies become visible at 48hours.

2.5.3 Chromogenic agar: Identification of Candida species may be obtained with a chromogenic agar. CHROMagar candida allows presumptive differentiation of over 100 species of candida. The medium is based on differential release of chromogenic breakdown products from various substances as a result of enzymatic

activity (Paripokee *et al.,* 2005). When using CHROMagar the directives of the manufacture must be strictly adhered to.

2.5.4 Germ tube test (GTT)

Germ tube test is one of the most reliable tests for the identification of *Candida albicans*. Other species, such as *Candida tropicalis* and *Candida dubliniensis* also forms germ tubes, but the morphologies differ from *Candida albicans* (Cheesbrough, 2004).

2.5.5 Carbohydrate assimilation test

Identification of yeast to the species level is based on carbohydrate assimilation test. These tests measure the yeasts ability to utilize a specific carbohydrate as the sole source of carbon. A number of commercial kits (API, BBL), automated and semi-automated systems are available for testing assimilation of carbohydrate by yeast isolates (Jawetz, *et al.,* 2000)

2.5.6 Sugar fermentation test

The sugar fermentation test is used to determine if microorganism can ferment a specific carbohydrate. This method is useful in differentiating among organism groups and species; it detects the presence of acid or gas produced from carbohydrate fermentation. Basal medium containing carbohydrate such as

Glucose, Lactose, and Sucrose etc is used for this purpose. A pH indicator (such as Phenol red, Andrade's solution etc) is also added to the medium to detect the lowering of pH of the medium due to acid production. Small inverted tubes known as Durham tube is also immersed in the medium to test for the production of the gas. It is an efficient method to differentiate fungi species.

2.5.6 Cornmeal agar culture

Growth of yeast isolates on cornmeal agar can provide important information for species identification. Chlamydospores formation by Candida albicans may be observed on cornmeal agar. *Candida parapisilosis* on cornmeal agar produces multiple areas of satellite "spider colonies" along the line of streaking *Candida kefyr* and *Candida krusei* form enlongated blastoconidia in a long-in-stream arrangement when growing on cornmeal agar.

2.5.7 Rapid identification test and automated systems

In addition to biochemical test kits used for identification of multiple genera, there are kits for the identification of *Candida albicans*, *Candida krusei* and *Candida glabrata*. Examples are the *Candida albicans* screen, *Bachelorette krusei*, *Rapidec albicans*, germ tube test kit, Bacticard Candida and albicans ID. Commercial kits for diagnosis of disseminated candidiasis have been developed (Jawertz *et al*., 2000; Hazen and Howell 2007). These tests targets protein or carbohydrate antigens.

Table 2.1: Carbohydrate fermentation test for *Candida* species

Carbohydrate	*C. albicans*	*C. tropicalis*	*C. krusei*	*C. dubliniensis*	*C. parapsilosis*	*C. guilliermondii*	*C. glabrata*
Glucose	AG	AG	AG	AG	AG	AG	AG
Lactose	-	-	-	-	-	-	-
Sucrose	-	AG	-	-	-	-	
Maltose	AG	AG	-	AG	-		
Galacotose	V	AG	-	V	V	AG	-
Trihalose	V	AG	-	V	-	AG	AG

A- Acid, G – Gas and V- Variable

Table 2.2: Sugar Assimilation test for *Candida* species

Sugars	*C. albicans*	*C. tropicalis*	*C. krusei*	*C. dubliniensis*	*C. parapsilosis*	*C. guilliermondii*	*C. glabrata*
Glucose	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-
Sucrose	+	+	-	-	+	+	-
Maltose	+	-	-	+	+	+	-
Galactose	+	+	-	+	+	+	-
Mellaboise	-	-	-	-	-	+	-
Cellobiose	-	+	-	-	-	+	-
Xylose	+	+	-	-	+	+	-
Raffinose	-	-	-	-	-	+	-
trihalose	+	+	-	-	+	+	+

2.6 Treatment and prevention of Candida infections

2.6.1 Treatment

Treatment of candida infection depends on some number of factors; according to Kumamoto and Vince, (2005). These factors are;

(1) The anatomical site of the infection

(2) The patients underlying illness and immune status

(3) The patients risk factors for disease,

(4) The specific species of candida responsible for infections

(5) The susceptibility of the infecting strains to antifungal drugs

Localized cutaneous candidiasis may be treated with topical antifungal medications, such as clotrimazole, econazole, cicloprox, ketoconazole and nystatin. Candida infection of soft tissue around the nail may be treated with either oral Fluconazole or Variconazole. If an abscess is present in the soft tissue it has to be drained prior to drugs therapy (Whiteway and bachewich, 2007).

2.6.2 Prevention

Candida nosocomial infections can be prevented by the followings strategies: implementation of educational program in order to increase candidiasis awareness.

The use of alcohol and proper hand washing will reduce the risk of patients acquiring candida infection from hospital staff. Improve the placement and care of central catherters. Stress the importance of antibiotic exposure as a risk factor for candidiemia (Rex *et al*., 1995).

Experimental studies with a vaccine based on the adhesion from Candida albicans have shown increased survival of mice with disseminated candidiasis (Rex *et al*., 1995). The use of antifungal agents for the treatments of candida infections were based on predictable susceptibility, without carrying out susceptibility testing after isolation of candida species. This approach to treatment did not achieve much in the treatment of candida infection due to the development of resistance and subsequent reinfection of the patients (Sobel, 2000), this led to the introduction of antifungal susceptibility testing.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Population/Area

This study was conducted in Central Hospital Benin City, Edo State South-South Nigeria. It is within the rain forest zone of Nigeria. Samples were collected in three different hospitals within the same zone; Central Hospital Benin City, St. Philomena Hospital and Faith Mediplex Hospital in Benin City, Edo State.

3.1.1 Inclusion Criteria

Male and female diabetic patients.

3.1.2 Exclusion Criteria

Non diabetic adults and women with gestational diabetes

3.1.3 Control Group

Non diabetic males and females

3.2 Ethical Approval

Ethical approval was sought and obtained from Edo State Ministry of Health Management Board, Benin City with reference number: HM1208/266.

Informed consent was obtained from each participant prior to specimen collection.

3.3 Sample Size Determination

The sample size (N) was calculated using prevalence from previous studies done on estimated prevalence of diabetes mellitus in Lagos, Nigeria. Which was 11 % (Otekeiwebia *et al*., 2015). The sample size for this study was obtained using the formula described by Daniel *et al.,* (1999).

$$N = \frac{Z^2 P(1 - P)}{D^2}$$

N = required sample size

Z = confidence level at 95% (standard value of 1.96)

P = estimated prevalence of diabetes mellitus (11%)

D = margin of error at 5% (standard value = 0.05)

$$N = \frac{1.96^2 \text{ X } 0.11(1-0.11)}{0.05^2}$$

$$\frac{3.8416 \text{ X } 0.11(0.89)}{0.0025}$$

N = 150 minimum sample sizes

3.4 Sample Collection

A total of four hundred and thirty eight (438) consecutive clinical specimens were collected (consisting of 212 urine specimens and 226 mouth swab specimens) and processed in the Medical Microbiology Department (bacteriology Lab) of the Medical Laboratory Services of Central Hospital Benin City. Fasting blood samples were collected also to ascertain the blood glucose level of the participants as at the time of clinical specimen collection.

3.4.1 Processing of clinical specimens

All clinical specimens were processed using a modification of the standard protocol as reported by Cheesbrough (2014).

3.4.2 Urine

A loop full (0.001ml) of each freshly voided urine specimen was inoculated onto sabouraud dextrose agar containing 20µg/ml gentamicin and was incubated at 37^{o}C for 24hours. After overnight incubation the count were expressed in colony forming unit per/ml. A count of $\geq 10^5$cfu/ml was considered significant to indicate urinary tract infection. The urine specimen was thoroughly mixed and 10ml was decanted into a sterile test tube and centrifuged at 2000rmp for 5minutes the supernatant was discarded and a drop of the deposit was placed on a clean grease free slide and covered with a cover slip avoiding air bubbles, this preparation was

examined using x40 objective for the presence of pus cells, red blood cells, epithelial cells, crystals, cast and yeast cells. The presence of ≥5 yeast cells/per high field was considered significant to indicate *Candida* infection.

3.4.3 Mouth swab

Two swabs/specimens were taken for each participant. One of the specimens was for gram staining while the other was inoculated onto sabouraud dextrose agar. Containing 20µg/ml of gentamicin and incubated for 24-48 hours at 37^{o}C. Films were made from the other swabs and stained by Gram staining method. Wet preparation was made from the culture isolates to identify yeast cells.

3.4.4 Fasting blood sugar

3mls of blood was collected into fluoride oxalate containers from known diabetic patients attending clinic in the diabetics centre and diabetic clinics of these various hospitals and taken to the chemical pathology department of the medical laboratory services of central hospital for blood sugar estimations (enzymatic method).

In this method the aldehyde group of β – D glucose is oxidized by glucose oxidase to give gluconic acid and hydrogen peroxide. Addition of enzyme peroxidase and a chromogenic oxygen acceptor such as O- dianisidine result in the formation of a coloured compound that can be measured.

Material used for estimation of blood glucose.

- Micropipette 20µml and 1000µml.
- Microtips small and large.
- Dispenser 1000 variable 1 – 10ml.
- Volumetric flask 1 litre.
- Spectrophotometer Hitachi 100-10 quartz curvet.
- Magnetic stirrer c mangnet.
- Centriguge 001676
- Timer.

Reagents

- Enzymatic (GOD/ POD) Ramdox.
- Distilled water.
- Glucose reagent

Procedure;

- The blood sample was centrifuged at 3000 RPM for 5mins

	Test	Standard	Blank
Working solution	3ml	3ml	3ml
Plasma	20µl	-	-
Standard (known concentration)	-	20µl	-

Distilled	-	-	20µl

The solution was mix and allowed to stand for 15minutes at room temperature. Absorbance of test and standard was read against blank at 505nm.

Calculation:

Glucose concentration (mmol/l)= $\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}}$x Concentration of standard

Result in mmol/l x 18= mg/dl.

3.4.5 Identification of yeast isolates

Yeast colonies were identified by the colonial morphology of cream coloured pasty colonies with a distinctive yeast smell and budding cell that were observed by direct microscopy in stained or unstained preparations. Once the colonies were confirmed speciation was done by the following methods

i. Germ tube
ii. *Chlaidiospore* formation on corn meal agar
iii. Sugar fermentation
iv. Sugar assimilation
v. CHROMagar candida

3.4.5.1 Germ tube test (GTT)

This was done to differentiate *Candida albicans* from other species of yeast. The suspected yeast was lightly inoculated into 0.5ml of screened human serum and then inoculated at 37^{o}C for 3hours. A wet preparation of this mixture was examined microscopically using x40 objective lens to identify the production of germ tubes that appears as sprouting yeast cells. (cheesbrough, 2004)

3.4.5.2 Corn meal agar inoculation

Corn meal agar was used to demonstrate *chlamydiospore* formation of the yeast colonies. About 1.0mm^2 from a 2mm deep already prepared corn meal agar plate was collected and placed on a sterile grease free slide and the edges was inoculated with yeast colony under test, this was covered with a cover slip and the preparation transferred into a Petri-dish containing a glass rod and filter paper. It was left at room temperature for 72hours. The cover slip was removed and placed on a drop of lactophenol cotton blue solution this was examined microscopically using x40 objective lens to identify presence of *chlamydiospores*.

3.4.5.3 Sugar fermentation test

Sugar fermentation test was done as described by Milne (2007). It was used to detect the ability of an organism to use various sugars to generate energy. The yeast colony was inoculated into phenol red peptone water containing 1% of the various sugars; glucose, lactose, maltose, sucrose, galactose and trihalose. Only the tube containing glucose carried Durham's tube, the innoculum was incubated at 37^{o}C overnight. A change in the colour from red to yellow indicated acid production. While a space in the prefilled Durham's tube indicated gas production.

3.4.5.4 Sugar assimilation test

Sugar assimilation test was done as described by Larone (2002), briefly the yeast colony was inoculated onto carbohydrate free medium. Disc containing 20% carbohydrate solution was placed in designated areas on the medium and incubated overnight at room temperature. The yeast that assimilates a particular sugar grew around the disc. The results were interpreted as follows: *Candida albicans* assimilated glucose, maltose, sucrose, trihalose, cellibiose and galactose. *Candida tropicalis* assimilated glucose, maltose, sucrose, trehalose and xylose. *Candida krusei* assimilated glucose and xylose.

3.4.5.5 Identification by Chromogenic Agar: CHROMagar candida is a useful chromogenic method for the identification of yeast. The CHROMagar

candida was prepared according to the manufactures instructions. CHROMagar has a chromogenic substance which helps in the rapid identification of the *Candida* species based on the reactions between the specific enzymes of the different species and chromogenic substances.

The yeast colony that grew on sabouraud dextrose agar was sub cultured onto the chromogenic medium and incubated at 37^{o}C for 48hours. The colours and colonial morphology were observed at 24hours and 48hours and compared with the conventional identification chart (Paripokee *et al*., 2005). The colour distribution of the colonies were *Candida albicans* green colonies, *Candida krusie* dry flat pink colonies, *Candida tropicalis* metallic blue colonies, *Candida lusitanea* light purple colonies and *Candida parasilopsis* light pink colonies.

3.4.6 Fungal susceptibility testing

Disc fungal susceptibility testing was performed on all yeast isolates using CLSI (2009) method. Briefly, each isolates was emulsified in sterile water and the turbidity matched with 0.5 MacFaland standard (this was prepared by adding 0.5ml of 1% Barium chloride to 99.5ml of 1% sulphuric acid). Once matched, a sterile cotton swab was dipped into the suspension and excess liquid was removed by pressing the swab on the side of the test tube, the entire surface of the dried Mueller- Hinton agar (containing 2% glucose and 0.05ml of methylene blue) was

seeded by swabbing in three directions with the swab. Antifungal discs each containing 25µg Fluconazole, 10ug variconazone, 25ug amphoterincin B and 100units of nystatin was placed on the surface of the agar plate using a sterile forcepts. Each disc was pressed down to ensure its complete contact with the agar surface. The plates were incubated at 37^{o}C overnight and the zone diameter of area of inhibition was measured in millimeters. A standard chart was used to determine if the isolates were sensitive or resistant to the antifungal agents (CLSI, 2009).

3.5 Statistical Analysis

The data obtained in this study were analyzed for Mean, Standard Deviation and Standard error of Mean. T-test analysis was used to compare means of the different test groups using IBM statistical software SPSS version 20. Statistical significance was set at $P<0.05$.

CHAPTER FOUR

RESULTS

The results obtained in this study are shown in Tables 4.1-4.12 and Plates 1 –4. A total of 438 clinical specimens were processed of which 206(47.03) had *Candida* infection and 3(0.69) had mixed *Candida* infection. The prevalence of yeast infection differ significantly (P= 0.0058) among clinical specimens. The *Candida* isolates were mostly recovered from urine samples 146(68.87%). Mixed *Candida* infection was observed mostly from urine specimens 2(0.94) (Table 4.1). The prevalence of *Candida* in relation to gender is shown in table 4.2. Statistically, Gender significantly affected the prevalence of yeast infection (p=0.022) as well as the individual clinical specimens. Mouth swab Isolates from males 23(29.11%) had significantly (p= 0.003) higher prevalence of *Candida* infections than their female 37(25.17%) counterparts.

In Table 4.3, the prevalence of *Candida* infection increased from 6(33.33%) in the age group 11 – 20years to 13(40.63%) in the age group of 21-30years. Age group 31 – 40years 20(34.48) has least prevalence rate. The prevalence rose to 34(43.42%) in the age group 41-50 years and at its peak 133 (51.35%) in the age group of ≥51 years.

Mixed growth was observed only in age group of ≥51years. Age significantly (p=0.0382) affected the prevalence of *Candida* infection.

Table 4.4 shows prevalence of yeast infection in relation to fasting blood sugar range; statistically, blood sugar level significantly (P= 0.03830) affect the prevalence of yeast infection. Group with blood sugar level ≥ 200 have most Candida isolates.

Table 4.5, shows that *candida albicans* 145(70.39%) is the most prevalent specie among the isolates, followed by *Candida tropicalis* 25(12.14%) with the least been *Candida parapsilosis* 4(1.94%).

Table 4.6 shows prevalence of *Candida* species in relation to age, *Candida albicans and Candida lusitanae* were isolated from all age brackets. *Candida albicans* is most prevalent in age group except age group 11-20 *Candida lusitanae* (50.0%) is most prevalent.

Prevalence of yeast isolates in relation to clinical specimens are shown in Table 4.7 *Candida albicans* was the most prevalent yeast recovered from all the specimens. *Candida krusei* was most prevalent in mouth swab compared compared to Urine specimen. *Candida krusei* was most prevalent in males, all other yeast isolates were more prevalent in females than males (Table 4.8).

The fungal susceptibility profile of the *Candida* specie isolates recovered is shown in Table 4.8. Fluconazole was the least active antifungal agent, while the polyenes

(Amphotericin B and Nystatin) were the most active antifungal agents. Depending on the *Candida* isolate, voriconazole was as active as the polyenes. Voriconazole was more susceptible to Candida parapsilopsis than other antifungal agents used in this study.

Fluconazole resistance by the Candida isolates ranges from moderate to very high while polyenes resistance were very low and voriconazole ranges from low to moderate. (Table 4.10)

In all specimens, yeast isolates were mostly resistance to Fluconazole, (Table 11 and 12). Though, most polyene resistance was observed in isolates from mouth swab. Isolates from urine shows least resistance to all antifungals used in this study.

Table 4.1: Prevalence of *Candida* infection from various clinical specimens in Benin City, Nigeria.

Specimens	**No tested (%)**	**No infected (%)**	**No with mixed infection**
Urine	212	146(68.87)	2(0.94)
Mouth swab	226	60(26.55)	1(0.44)
Total	**438**	**206(47.03)**	**3(0.69)**

Table 4.2: Prevalence of *Candida* infection in relation to gender of participants

	Gender				
	Male		Female		
Specimen	**No tested**	**No. infected (%)**	**No tested**	**No. infected (%)**	**p-value**
Urine	75	50(66.66)	137	96(70.07)	0.0003
Mouth swab	79	25(31.65)	147	35(23.80)	0.0133
Total	**154**	**75(48.70)**	**284**	**131(46.13)**	**0.0226**

Table 4.3: Prevalence of *Candida* infection in relation to age of participants

Age (years)	No. tested	No. infected (%)	No with mixed infection (%)
11 – 20	18	6(33.33)	0(0.00)
21 – 30	24	13 (40.63)	0 (0.00)
31 – 40	58	20 (34.83)	0 (0.00)
41- 50	76	34 (44.74)	0 (0.00)
≥51	254	133(52.36)	3 (1.12)
Total	**438**	**206**	**3**

Table 4.4: Prevalence of *Candida* infection in relation to Fasting Blood Sugar Range.

FBS Range (mg/dl)	No tested	No. infected (%)
50 – 120	126	23(18.25)
121 – 200	180	83 (46.11)
≥200	132	100 (75.76)

Table 4.5: Prevalence of different *Candida* species isolated from various clinical specimens

Candida isolates	Number	Percentages
Candida albicans	145	70.39
Candida parapsilosis	4	1.94
Candida krusei	18	8.73
Candida tropicalis	25	12.14
Candida lusitanae	14	6.80

Table 4.6: Distribution of different *Candida* species in relation to age of participants

	Age(years)				
Yeast isolates	**11 – 20**	**21 – 30**	**31 – 40**	**41 – 50**	**≥51**
Candida albicans	2(33.33)	10(71.43)	15(75.0)	20(60.61)	97(72.93)
Candida parapsilosis	1(16.67)	0	0	1(3.03)	2(1.50)
Candida tropicalis	0	2(14.29)	3(15.0)	7(21.21)	14(10.52)
Candida krusei	0	1(7.14)	1(5.0)	3(9.09)	13(9.77)
Candida lusitanea	3(50.0)	1(7.14)	1(5.0)	2(6.06)	7 (5.26)

- Numbers in brackets are percentage values.

Table 4.7: Prevalence of different *Candida* isolates in relation to clinical specimens

	Specimens	
Yeast isolates	**Urine**	**Mouth swab**
Candida albicans	106(73.10)	39(26.90)
Candida parapsilosis	1(50.0)	3(50.0)
Candida tropicalis	20(80.0)	5(20.00)
Candida krusei	8(44.4)	12(55.56)
Candida lusitanea	10(71.43)	4(28.57)

- Numbers in brackets are percentage values.

Table 4.8: Prevalence of different *Candida* isolates in relation to gender of participants

	Gender	
Yeast isolates	**Male (%)**	**Female (%)**
Candida albicans	54(37.24)	91(62.76)
Candida parapsilosis	2(50.0)	2(50.0)
Candida tropicalis	7(28.0)	18(72.0)
Candida krusei	9(50.0)	9(50.0)
Candida lusitanea	6(42.86)	8(57.14)

- Numbers in brackets are percentage values.

Table 4.9: Susceptibility profile of *Candida* isolates in Benin City, Nigeria.

Yeast isolates	Antifungal agents (µg/disc)			
	FLU (25) No. (%) susceptibility	VOR (10) No. (%) susceptibility	NYS (100units) No. (%) susceptibility	Amp. B(25) No. (%) susceptibility
Candida albicans (n=145)	99 (68.28)	105 (72.41)	144 (99.31)	144 (99.31)
Candida krusei (n=18)	10 (55.56)	15 (83.33)	18 (100.0)	13 (72.22)
Candida lusitanae (n=14)	4 (28.57)	8 (57.12)	12 (85.71)	10 (71.43)
Candida tropicalis (n=25)	7 (28.00)	18(72.00)	25(100.00)	25 (100.00)
Candida parapsilosis (n=4)	2(50.00)	3(75.00)	3(75.00)	2(50.00)

Key:
FLU – Fluconazole
VOR – Voriconazole
Amp.B - Amphotericin B
NYS – Nystatin.

Table 4.10: Resistance profile of *Candida* specie isolates

Candida isolates	Antifungal agents (µg/disc)			
	FLU (25)	**VOR (10)**	**NYS (100units)**	**Amp. B(25)**
Candida albicans (n=145)	46 (31.72)	40 (27.59)	1 (0.69)	1 (0.69)
Candida krusei (n=18)	8 (44.44)	3 (16.67)	0 (0.00)	2 (11.11)
Candida lusitanae (n=14)	10(71.43)	6 (42.85)	2 (14.29)	4 (28.57)
Candida tropicalis (n=25)	15 (60.00)	7 (28.00)	0	0
Candida parapsilosis (n=4)	2(50.00)	1 (25.00)	2 (50.00)	2(50.00)

Key:

FLU- Fluconazole

VOR-Voriconazole

Amp.B- Amphotericin B

NYS - Nystatin

Table 4.11: Resistance profile of *Candida* species recovered from urine specimen.

Candida isolates	Antifungal agents (µg/disc)			
	FLU (25)	**VOR (10)**	**NYS (100units)**	**Amp. B(25)**
Candida albicans (n=46)	40	28	1	1
Candida krusei (n=8)	6	2	1	0
Candida lusitanae (n=10)	10	0	0	0
Candida tropicalis (n=12)	6	2	2	2

Key:

FLU- Fluconazole

VOR-Voriconazole

Amp.B- Amphotericin B

NYS - Nystatin

Table 4.12: Resistance profile of *Candida* species recovered from mouth swab specimen.

Candida isolates	Antifungal agents (µg/disc)			
	FLU (25)	**VOR (10)**	**NYS (100units)**	**Amp. B(25)**
Candida albicans (n=46)	6	12	0	0
Candida krusei (n=8)	2	1	0	2
Candida lusitanae (n=10)	0	6	2	4
Candida tropicalis (n=12)	9	5	0	0
Candida parapilosis(n=2)	2	3	2	2

Key:

FLU- Fluconazole

VOR-Voriconazole

Amp.B- Amphotericin B

NYS - Nystatin

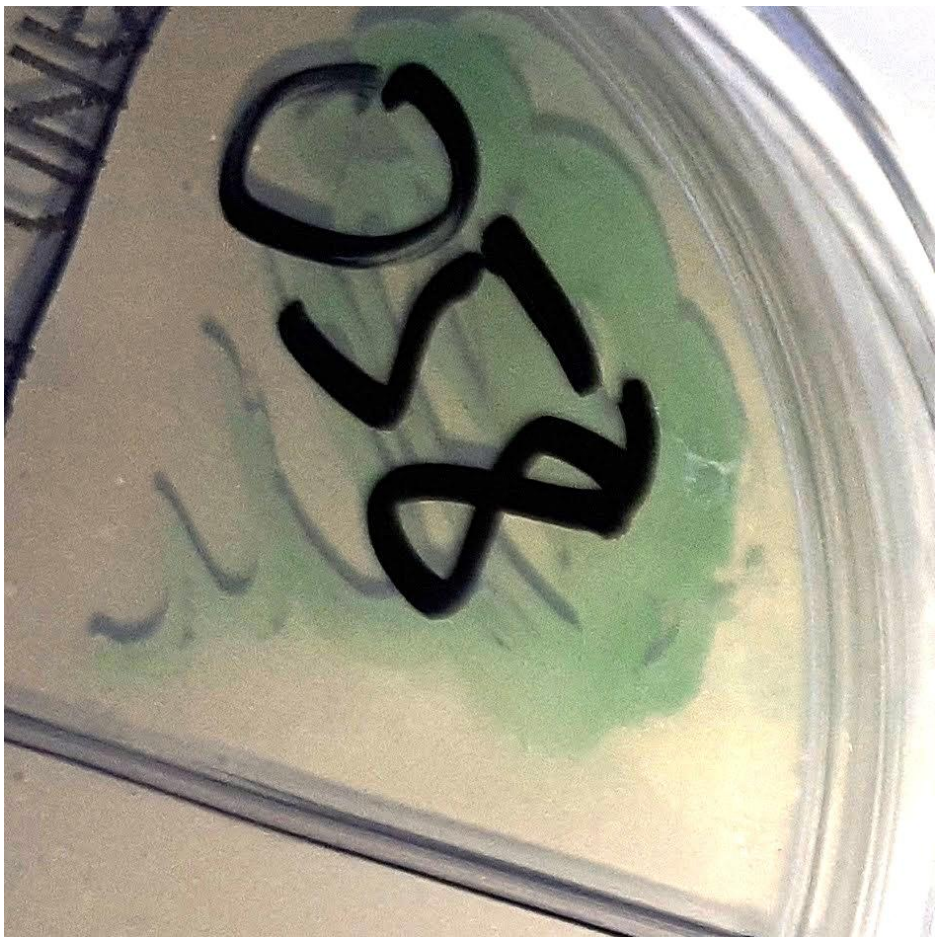


Plate 1: Candida albicans 0n CHROMagar showing green colonies.

Plate 2: Candida krusei on CHROMagar showing flat pink colonies.

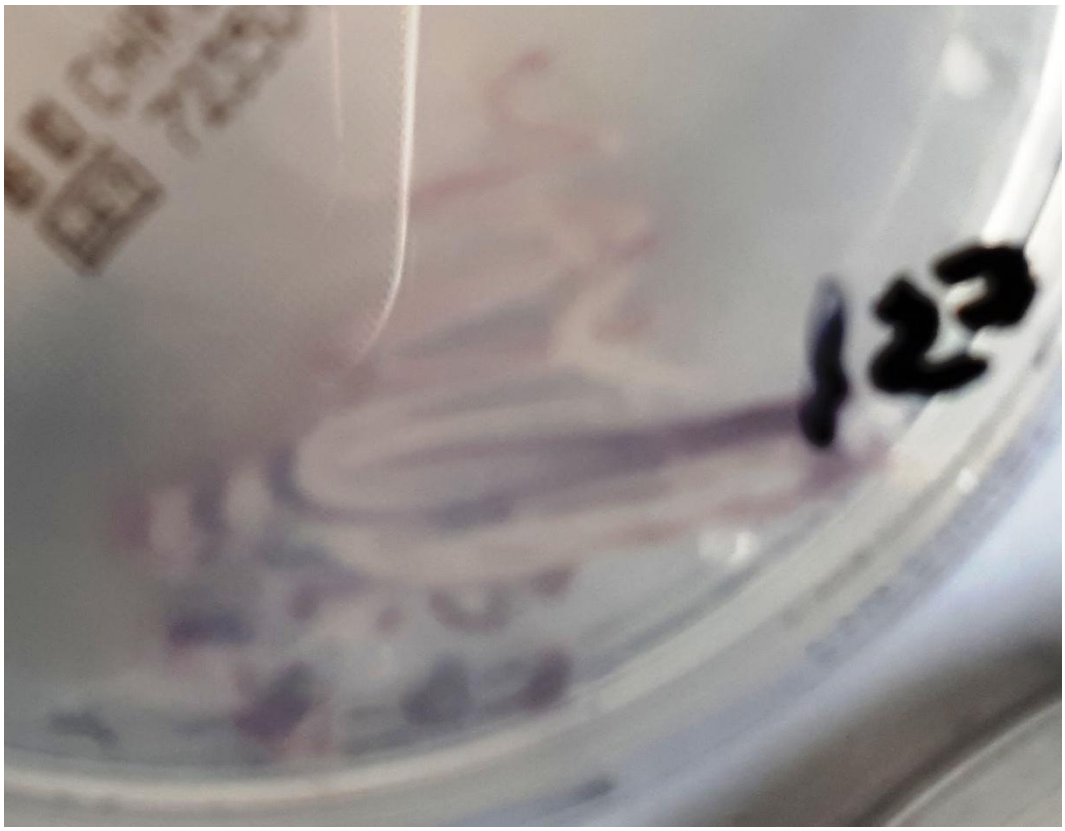

123

Plate 4: Candida lusitanea showing light purple colonies, Candida tropicalis showing metallic blue colonies on CHROMagar.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

Currently fungi infection presents a global health threat (Vandeputte *et al.,* 2012). Candida species have been implicated to be the most common causative agent of fungi infections worldwide (Saranya *et al., 2004*).

A total of 206(47.03) out of 438 clinical specimens had yeast infections. This is higher than the 30.0% reported by Krenke *et el* **(**2007). The difference can be as a result of geographical location, Krenke's study was done in HIAE, Sao Paulo, Brazil and this study was carried out in Benin City Nigeria. Frequent and indiscriminate use of antibiotics, antifungal agents, immunosuppressive conditions amongst others are risk factors for fungi infection and may also explain the high prevalence of yeast infection observed in this study.

The prevalence of yeast infections was significantly higher in isolates from urine specimens (68.87%) when compared to mouth swab specimen (26.55%) (Table1). In this study 146 out of 438 (33.33%) clinical specimens were positive for Candiduria. In a study conducted by Falahati *et al* 38 out of 305 (12.5%) positive Candiduria among diabetic patients was reported. A study by Nademi *et al* reported 4.3% (5 out of 115) of hospitalized patients. Goyal *et al*, Zarei *et al* and Padawer *et al* reported Candiduria in 2.36%, 16.5% and 19.49% of their study populations respectively. The differences among results of various studies may be as a result of study population, underlying factors, and preventive measures of patients, medications, hospitals and geographical locations.

The prevalence of yeast in males (48.70%) was higher than that of their female counterpart (46.13%), though, prevalence of yeast infection differ significantly between both genders (p=0.022) (Table 2). Nevertheless, in urine specimens, females (70.07%) had significantly higher prevalence than their female counterparts (66.66%) (p=0.0003). This is in agreement with Orrett *et al*., 2006, and Aiygoro (2007). Higher prevalence of urinary tract infection reported in females might be as a result of close proximity of the female urethral meatus to the anus, shorter urethra, and sexual intercourse (Mahajan *et al.,* 2015). This may explain the findings in this study. However, the prevalence of oral candidiasis is higher in male (31.65%) when compared with their female (23.80%) counterparts. This agrees with Javed *et al* 2009 and Paula. *et al.,* 2011 which demonstrated high prevalence of oral candidiasis in males compared to females.

The prevalence of yeast in relation to age is shown on Table 3. Age group of ≥51 (52.36%) years has significantly higher prevalence of yeast infections compared with other age groups (P=0.0382). In age group 41 – 50 (44.74%) years the prevalence is next to age group ≥ 51 years. This goes to show that diabetic patients over the age of 40years exhibit increased levels of Candida infections compared to younger patients; this is in agreement with the study conducted by Mubarak *et al.,* (2012).

In Table 4, the prevalence of yeast infection was significantly higher in patients with FBS ≥ 200 (75.76%) (P=0.03836) this is in agreement with reports by Paul *et al*, Geerling *et al*, and Falahati *et al* respectively that high plasma glucose level, poor or uncontrolled diabetes, and some other factors like aging as a correlation factor to yeast infection. However, Paula *et al.,* 2011 reported that *Candida* species recovered are the same both for diabetic and no diabetic patients. A retrospective cohort study by Bader *et al*., (2005) reported that very high blood glucose level (≥13.9mmol/l) is significant to increased mortality among hospitalized diabetic patients with Candida infection.

The prevalence of the various yeast species recovered from this study is shown in Table 5. Candida albicans is the most prevalent yeast recovered from this study. This is in unison with previous reports by Nayman *et al.,* (2011), Paula *et al.,* (2011), Mubarak *et al.,* (2012), Goyal *et al.,* (2016) among diabetic patients. Although this report of this study differs with the report of Falahati *et al*., (2016)

where *Candida glabrata* was the most isolated candida specie from diabetic patients. The disagreement between this study and Fatahati *et al*., 2016 may be due to geographic location or the nature of specimens used. This study was conducted in Benin City, Edo state, Nigeria. Using mouth swab and urine specimens whereas, Falahati *et al.,* (2016) was carried out in Tehran Iran with urine specimen only. Candida species are the major cause of yeast infections in diabetic patients. Most Candiduria cases are acquired as Nosocomial infections due to application of catheters and wide spectrum antibiotic therapy Colodner *et al*., (2008).

The age group of ≥ 51(72.93%) has the highest number of *Candida albicans* isolated in this study while age group of 11-20 (33.33%) have the least (Table 6). This agrees with Geerlings *et* al 2009 reports aging as risk factor for fungi infection in diabetic patients. However, *Candida albicans* is the most prevalent in all age group of patients studied. *Candida albicans* and *Candida lusitanae* was recovered from all age group.

Table 7 shows the various yeast isolates recovered from this study in relation to specimens. Urine specimens have the highest number of candida albican isolates, followed by candida tropicalis. This agrees with previous reports Dharwad *et al* 2001 by Achkar and Fries, 2010 and Goyal *et al* 2016, though this differs from report of Fatahati *et al* 2016 that C. glabrata was the most prevalent yeast. However, candida krusei is most prevalent in mouth swab. This agrees with Faujdar *et al*., (2016). This difference in the findings of this study with Fatahali *et al*., 2016, may be due to geographical location and type of specimen used. This study was carried out in Benin City with urine and mouth swab specimens while Fatahali *et al*., 2016 were conducted in Tehran, Iran on urine only.

The susceptibility and resistance profile of the yeast isolates recovered in this study are shown in Tables 9 and 10 respectively. The polyenes show high sensitivity against all isolates with 50% of candida parapsilosis being the least susceptible to a polyene. Susceptibility to voriconazole is moderate (57.12-83.33%). The polyenes susceptibility and resistance pattern in this study agrees with the report of Faujdar *et al*., 2016. Fluconazole resistance in this study is higher; this might be due to wide spread and indiscriminate use of Fluconazole for long time in our locality, where sales of antimicrobial agents is common and unregulated (Okeke *et al.,* 1999; Omorogie and Eghafona 2009;). Non – albicans

Candida resistance to azole (Candida tropicalis, Candida krusei, Candida listanae, and Candida parapsilosis) recorded in this study is high, this agrees with Goyal *et al*., (2016) which reported that non- albicans Candida are more resistance to azole antifungal agents with some been intrinsically resistance.

5.2 Conclusion

Generally, 47.03% of clinical specimens recovered from diabetic patients and processed in this study have yeast infection. The prevalence of yeast infection is more in females and recovered mostly from urine within age group of ≥ 51 years. *Candida albicans* was the most recovered yeast from all specimens in both male and female among all age groups. In this study polyenes were the most susceptible while Fluconazole is the least active.

5.3 Recommendation

The increasing population of Nigerians projects increase in the prevalence and incidence of diabetes mellitus given that our health care services are poorly funded. The cost of managing diabetes in Nigeria is expensive. In other to improve the management of diabetes in Nigeria, it requires collaboration between government and health sector. Government need to improve healthcare funding, make available at subsidized cost the several new drugs for management of diabetes (such as insulin analogues, glucogen, like peptide 1 analogues, amylinomimetics, inhaled insulin) not the traditional metformin, gilbenclamide and gliclazide. Preventive programs that enlighten the masses of the risk factors of diabetes mellitus should be encouraged. Affordable and compliant foods should be made available for diabetic patients by the local manufacturers and food agencies in Nigeria.

The medical laboratories should be well funded, so as to provide routine fungi speciation and fungal susceptibility testing for all yeast isolates. These will help to identify different species of Candida causing infection as well as new and emerging species. In view of the high azole resistance observed in this study, routine susceptibility test is highly recommended.

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