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Causal Organisms, Pathogenicity, Laboratory Diagnosis and Treatment of Candidiasis

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Abstract: A historical overview about candidiasis was provided herein. The main causal pathogens of candidiasis are *C. albicans*, *C. Tropicalis*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. lusitaniae*, *C. guilliermonndii*, *C. kefyr*, *C. utilis*, *C. inconspicua*, *C. rugosa* and *C. catenulata*. There are also few *Candida* sp., involved in human infection in only immunocompromized patients. The pathogenicity of *Candida* sp. are due to ability to adhere smooth tissue surfaces, biofilm formation and production of many virulence factors such as hydrolytic enzymes. Candidiasis is diagnosed by microscopic detection, culture media, serology and molecular fingerprints using either DNA or RNA isolated from the causal pathogen. Candidiasis is treated by many synthetic antifungal agents. The antifungal agents in development are discussed in this review study. The recent prospectives about use of natural extracts as an antifungal agents are also discussed.

Key words: Candidiasis, virulence factors, diagnosis, treatment, natural extract

INTRODUCTION

Candidiasis is a fungal disease caused by *Candida* sp. which is predominantly common in immuno compromised individuals and also a known causative agent of vaginal candidiasis in pregnant women (Shaheen and Taha, 2006; Chengo, 2012). The genus *Candida* is a taxonomic grouping that was originally used to define yeast-like organisms that were not considered to have sexual reproductive life cycle (Williams *et al*., 2011). Candida contains over 350 heterogeneous species but only minorities of these have been implicated in human disease (Williams *et al*., 2011). Among the different *Candida* sp. That cause vaginal candidiasis in pregnant women, *Candida albican*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis*, *Candida dubliniensis*, *Candida pseudotropicalis*, *Candida guillermondii* and *Candida kyfer* are commonly identified in pregnant women (Chengo, 2012; Ibrahim *et al*., 2013; Us and Cengiz, 2007; Heredia *et al*., 2006). Virulence factors of the organism and/or predisposing factors of the host determine whether the organism remains as a commensal or become pathogen and causes disease (Aslam *et al*., 2008; Alli *et al*., 2011).

Since 1970, several new antifungal agents have become available for the treatment of vulvovaginal candidiasis (Tobin, 1995). The available therapeutic agent for treatment of vulvovaginal candidiasis offers few options and includes compounds of polyenes and azoles. Azoles such as fluconazole, miconazole, itraconazole and ketoconazole and polyenes such as nystatin and some vaginal formulations containing amphotericin *B. Nystatin* in the form of a cream or vaginal suppository has been used for nearly 3 decades with a mean mycological cure rate of 75-80% (Sobel, 1993). The azoles are the treatment of choice for vulvovaginal candidiasis in many countries, however, the development of resistance to these drugs has been reported in yeasts isolated from vulvovaginal candidiasis (Ribeiro *et al*., 2001), especially in Candida other than *C. albicans* which appear to be less sensitive to the azoles (Fan and Liu, 2007; Richter *et al*., 2005). Currently, nature extracts and alum compounds inhibit *Candida* sp. The present review discussed the causal pathogens of candidiasis. Pathogenicity and virulence factors of *Candida* sp. are given. In addition, the diagnosis procedures and treatment of candidiasis are given in this review study.

HISTORICAL OVERVIEW

Candidiasis has been recognized as a clinical entity since the time of Hippocrates who described oral

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candidiasis in his treatise "Epidemics" which was published in 4th century BC (Rippon, 1988). The first research on oral candidiasis was in 1786 when Royal Society of Medicine in France, underwrote an investigation of thrush. In 1846, Lagenbeck described an organism consistent with *Candida albicans* which he cultured from the buccal mucosa of a patient with typhus. The original organism isolated by Lagenbeck was restudied by Gruby. Although Langenbeck, described the fungus in a case of oral thrush observed in a patient suffering from typhus, he misidentified it as a causative agent of the underlying disease. The correct association between oral thrush and the fungi was made only 3 years later in 1842 by Gurby who classified the microorganism as Sporotrichum. In 1846, Berg described the relationship between *Candida albicans* and thrush (Lynch, 1994). It was termed *Monilia candida* by Bonorden in 1851. In 1853, Robin renamed the organism as *Oidium albicans*, Ress in 1877 redefined it as *Saccharomyces albicans*. The dimorphic nature was noted by Grewitz in 1877. In 1877, Audrey described different morphological form of Candida. Candida is a Latin name derived from 'candidus' meaning "white". As an English name, it came into use after George Bernard Shaw's play 'Candida' (1898). In Latin 'Toga Candida' refers to the white robe worn by candidates for Roman Senate, 'albicans' also comes from the Latin word 'albicare' which means 'to whiten'. The term 'thrush' is probably derived from ancient Scandinavian, 'torsk' in the Swedish equivalent of this word (Rippon, 1988). The French word for the condition is 'le muguet' which means 'lily of the valley' (Chander, 2009). Genus *Candida* and species *C. albicans* was described by botanist Christine Marie Berkhout in her doctoral thesis at the University of Utrecht in 1923. In 1923, Berkhout clarified the taxonomy of the organism and proposed the name Candida from Latin 'Toga candida' which was accepted by 'English Botanical Congress 'at Paris in 1954. The 19th century authorities such as Trousseau and Parrot considered that 'thrush' invariably arose as a consequence of preexisting illness. During the following decades various pathological conditions were shown to be associated with yeasts. The fungus was isolated by Bennett in 1844 from the sputum of a tuberculosis patient. Wilkinson in 1849 isolated the fungi from vaginal candidiasis. Robin in 1853 isolated it from a systemic infection and Zenker from a brain infection in a debilitated patient in whom the fungus had spread haematogenously from an oral infection. In 1875, Hausemann established the possibility of infant infection during childbirth by demonstrating the analogy between the causative agent of oral and vaginal thrush. Other diseases caused by Candida were described at the beginning of 20th century. These were onychomycosis by Dubendorfer in 1904, dermatitis by Jacobi in 1907, chronic mucocutaneous candidiasis by Forbes in 1923 and cystitis by Rafin in 1910. Later in 1928, Conner described osteomyelitis in 1940 Joachim and Polayes described Endocarditis and in 1943 Suthin noted the association between pathology of endocrine system and Candida infections. Castellanni in 1912 while describing 'tea tasters cough' was probably the first to suggest the possibility that *Candida* sp. other than *C. albicans* may be involved in pathological processes.

CAUSAL PATHOGENS OF CANDIDIASIS AND THEIR CLASSIFICATION

Candidiasis is the infection caused by *Candida* species. *Candida* species are found in healthy people as normal microflora and are controlled by the immune system and beneficial bacteria (probiotics). However, if the number of probiotics are decreased, the immune system is decreased and Candida overgrowth may occure, leading to candidiasis. *Candida* species are ubiquitous yeast fungi being found on many plants and as a part of the normal flora of the mucocutaneous membranes of humans. It is present frequently in the mouth, gastrointestinal tract, respiratory tract and vagina. They are approximately 200 species of which <20 species are capable of causing disease in humans (Dalle *et al*., 2000). Formerly, *Candida* species were assigned into the Deutromycetes (Fungi imperfecti) as the anamorphic (asexual) state was only known, however an ascomycetous telemorphic (sexual) state was discovered for a number of *Candida* species but not for the major *Candida albicans*. They have diploid number of chromosomes except for some controversy regarding *Candida guilliermondii* and *Candida glabrata* (Mitchell, 1998). Members of genus *Candida* resemble each other morphologically being oval to round, Gram positive yeast cells, varying in size from oval to coccobacillus and elongated forms. On ordinary culture media they give white to creamy coloured, moist or pasty colonies (Warren and Hazen, 1999).

Systematic position of genus *Candida*: Systematic position of *Candida* species are as follows:

- Kingdom: Mycetae
- Phylum: Ascomycota
- Subphylum: Ascomycotina
- Order: Saccharomycetales
- Family: Saccharomycetaceae
- Genus: *Candida*

Segal and Elad (1998) mentioned that *Candida* species have been went through name changes, usually following the discovery of synonymy among species or the non-validity of some binomials. Some changes in nomenclature were based on molecular biological methods or analysis of iso-enzymes. The analysis of small ribosomal subunit sequences has shown that *C. albicans*, *C. parapsilosis*, *C. Tropicalis* and *C. viswanatii* form one subgroup with a more distant connection to *C. guilliermondii*. *Candida kefir* and *C. glabrata* are located in 2 different but interconnected branches, whereas *C. lusitaniae* and *C. krusei* are each on different branches.

Different species of genus *candida*: Bhavan *et al.* (2010) mentioned that *Candida albicans* is the organism most often associated with both mucosal and hematogenously disseminated infections and recently, other species such as *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis* and *Candida lusitaniae* have emerged as clinically important pathogens. This shift in species distribution has been attributed at least in part to changes in antifungal drug-prescribing practices.

***Candida albicans*:** *Candida albicans* is true opportunistic pathogen. It is the normal flora of skin, oral cavity, gastrointestinal tract, the vaginal and the urinary environments and is termed a "commensal". It is found in the environment, particularly on leaves, flowers, water and soil (Ellis, 1994; Ferreira *et al*., 2010). *Candida albicans* is a yeast-like fungus, thin-walled, microscopic morphology shows spherical to oval, 5 μm in diameter, Gram positive. It is much larger than bacteria, reproduces by budding (Webb *et al*., 1998; Kayser, 2005). It can grow at a temperature range of 20-38°C, tolerates pH in the range of 2.5-7.5 (Rinaldi, 1993). Sabouraud dextrose agar containing chloramphenicol or gentamycin favored for Candida isolation (Ellis, 1994). On cornmeal agar following 72 h incubation at 25°C, it forms abundant branched pseudohyphae and true hyphae with blastoconidia are present. The blastoconidia are formed in grape-like clusters along the length of the hyphae. Terminal chlamydoconidia may be formed with extended incubation (Sutton *et al*., 1998). One of the more well known characteristics is the ability to ferment sugars and may ferment other carbohydrates for the production of ethanol (Bhavan *et al*., 2010). There are two serotypes of *C. albicans*, named serotype A and B which are based on the differences between mannan components of the cell wall. Serotype A was found to be antigenically related to *C. Tropicalis* and serotype B was related to *C. stellotoidea* (Chander, 2009). *Candida albicans* is a dimorphic fungus, it can take two forms. The yeast form in a non-invasive and the hyphal form which can penetrate the mucosa and it is invasive. *C. albicans* exhibits a number of different morphological forms under different environmental conditions such forms include budding yeast cells (blastospores, blastoconidia), pseudohyphae, true hyphae and clamydospores and they can be used to identify *C. albicans* from different species of *Candida*. It also produces germ tubes; germ tube formation is the initial stage in the yeast-hyphal transition. However, the ability to assume various forms may be related to the pathogenicity of the organism (Molero *et al*., 1998).

***Candida glabrata*:** It is the second most prevalent *Candida* species in humans. Recently, it was demonstrated that in the elderly, *C. glabrata* has emerged as the major commensal. The prominence of it as a pathogen is of particular clinical concern because it is naturally resistant to azole drug therapy. Interestingly, it mimics in many respects the pathogenic capabilities of *C. albicans* and recent studies have demonstrated that *C. glabrata* forms pseudohyphae and non-compartmentalized tubes distinct from true hyphae in addition, it undergoes high-frequency phenotypic switching. *C. glabrata*, therefore, possesses developmental programs at least as complex as those of *C. albicans* (Srikantha *et al*., 2003). It 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. A critical distinguishing characteristic of *C. glabrata* is its haploid genome in contrast to the diploid genome of *C. albicans* and several other non-albicans *Candida* sp. (Whelan *et al*., 1984). *Candida glabrata* ferments and assimilates only glucose and trehalose (Kwon-Chung and Bennett, 1992).

***Candida tropicalis*:** It is the second most pathogenic of the *Candida* species. Unlike *C. albicans* which is a normal commensal on human mucous membranes, the detection of *C. tropicalis* is more often associated with the development of deep fungal infections, hence, *Candida tropicalis* may be more virulant than *C. albicans* (Roilides *et al*., 2003).

***Candida krusei*:** *Candida krusei* is budding yeast. *C. krusei* is an emerging fungal nosocomial pathogen primarily found in the immunocompromised and those with hematological malignancies. It was considered as normal flora in female reproductive system, it can isolate from adult stool and it can cause pericardial inflammation.

It has natural resistance to fluconazole, a standard antifungal agent and increasingly also to amphotericin B. It is most often found in patients who have had prior fluconazole exposure, sparking debate and conflicting evidence as to whether fluconazole should be used prophylactically. Mortality due to *C. krusei* fungemia is much higher than the more common *C. albicans* (Hautala *et al*., 2007; Pfaller *et al*., 2008).

***Candida parapsilosis*:** In most parts of the world *C. parapsilosisis* is the third most common cause of candidemia, especially in patients with intravenous catheters, prosthetic devices and intravenous drug use. Also, *C. parapsilosis* is one of the most common causes of candidemia in neonatal intensive care units. This species produces slime as a virulence factor enabling it to adhere to environmental surfaces and skin of hospital personnel. *C. parapsilosis* isolates can be divided into form I (arabinose positive) and form II (arabinose negative).

***Candida lusitaniae*:** Although, uncommon (1-2%), *C. lusitaniae* is of clinical importance because of intrinsic or secondary resistance acquisition to amphotericin B and is typically found in patients with haematological malignancies and patients in intensive care units.

***Candida guilliermondii*:** *Candida guilliermondii* have two varieties, *Candida guilliermondii var. guilliermondii* and *Candida guilliermondii var. carpophila*. Nosocomial *C. guilliermondii* infection was detected in a neonatal intensive care unit in heparin solution used to flush the butterfly needles and it was isolated from hands of hospital personnels. Moreover, it had been isolated as a causative agent of endocarditis in individuals using addictive drugs intravenously and in immunocompromised patients (Csank and Haynes, 2000).

***Candida dubliniensis*:** *Candida dubliniensis* is a recently described *Candida* species associated with oral colonization and infection in HIV-infected patients. More recently, it has been associated with oral carriage and infection in HIV-negative individuals and has also been recovered from a variety of specimens from non-oral sites including the vagina, the respiratory tract, urine, sputum, faeces and blood (Boyle *et al*., 2002). The close phenotypic and morphological resemblance of *C. albicans* and *C. dubliniensis* has hampered the accurate and rapid identification of the latter from clinical specimens (Kim *et al*., 2003). The majority of *C. dubliniensis* isolates studied are susceptible to commonly used azole and polyene antifungal drugs, including ketoconazole, fluconazole, itraconazole and amphotericin B. However, resistance to fluconazole has been reported in clinical isolates and studies have shown that a stable fluconazole-resistance phenotype associated with up-regulation of multidrug transporters can be generated following sequential exposure of *C. dubliniensis* isolates to increasing fluconazole concentrations *in vitro* (Fitzgerald *et al*., 2003). It is identified by germ tube and chlamydospore production by an inability to grow at 45°C and occasionally by a specific colony color on CHROM agar Candida medium.

***Candida kefyr*:** *Candida kefyr* is a cause of opportunistic infection and several cases of disseminated yeast infections. Moreover, some strains of this species are widely used as a control strain in the bioassay of antifungal agents (Barnett *et al*., 2000).

***Candida utilis*:** Elie *et al*. (1998) have mentioned that Candida utilis has been reported once in a case of candidaemia associated with catheter implantation in an HIV-infected patient. Interestingly, it has been used in industrial applications due to its ability to grow on ethanol.

***Candida inconspicua*:** *Candida inconspicua* has been proved to be nosocomially aquired by three patients with hematological malignancies; two of them had intravenous catheter-related fungaemia and the third had fungal hepatitis. This species showed resistance to fluconazole (D'Antonio *et al*., 1998).

***Candida zyelanoides*:** *Candida zyelanoides* is a weak virulent species but it can cause fungaemia in presence of indwelling devices (Hull and Johnson, 1999).

***Candida norvegensis*:** The first report of verified clinical infection with Candida norvegensis appeared at 1990 in an immunocompromised renal transplant recipient patient who was on continuous ambulatory peritoneal dialysis. It has been found to be fluconazole resistant which is an inherent property (Lockhart *et al*., 1999).

***Candida rugosa*:** *Candida rugosa* has been reported as an agent of fungaemia with an indwelling catheter. *Candida rugosa* infections has also been reported in patients suffering from burns (Abbas *et al*., 2000).

***Candida lipolytica*:** *Candida lipolytica* is a weakly virulent species and probably requires the presence of intravascular foreign body in order to cause fungaemia. This yeast has been isolated from clinical specimens as stool, sputum and traumatic ocular infections but its role as an etiological agent of candidiasis is doubtful (Shin *et al*., 2000).

***Candida catenulata*:** *Candida catenulata* is a rare agent of human disease but has been isolated from faeces, glabrous skin and skin of foot and it is also a documented agent of onychomycosis (Sutton *et al*., 1998). Other rare types are reported by Carrillo-Munoz *et al.* (1999). *Candida ciferri* is a rare agent of candidiasis but has been documented as an agent of onychomycosis. *Candida haemulonii* has been involved in at least two cases of fungaemia and is a strong candidate for superficial skin infection. *Candida pulcherrima* is a rare agent of disease and was isolated in a case of invasive disease in immunosuppressed host.

PATHOGENICITY AND VIRULENCE FACTORS OF CANDIDA

Candida expresses a variety of virulence factors that contribute to its pathogenesis for persistent infection and tissue damage of the host when immunity is debilitated (Lionakis and Netea, 2013; Williams and Lewis, 2011). Major virulence factors of Candida are its ability to adapt to a variety of habitats of the body (oropharyngeal, gastrointestinal and female genitalia), adherence to host cells, the ability to switch between the yeast form and filamentous (pseudohyphae formation), biofilm formation and production of hydrolytic enzymes such as proteinases, phospholipases, lipases and other factors play a major role in successful colonization and subsequent infection of Candida (Williams and Lewis, 2011; Lionakis and Netea, 2013; Yang, 2003; Jackson *et al*., 2009).

Ability to adapt different anatomical site: *Candida* species colonize and cause disease in different anatomical sites including skin, oral cavity and oesophagus, gastrointestinal tract, vagina and vascular system by using different virulence factors. For example, *Candida albicans* expresses potential jumonji-like transcriptional repressor for PHR1 in bloodstream or in tissue to adapt the neutral pH while it expresses RPH2 in the vagina to optimize and survive at acidic pH (Yang, 2003).

Adherence of Candida to host surfaces: The ability of Candida to adhere to host surfaces according to specific ligand-receptor interactions and nonspecific mechanisms is a prerequisite for both successful commensal carriage as well as persistence during active infection. Candida has the ability to adhere to several host cell types including epithelial, endothelial and phag-ocytic cells. Among, the different types of adhesions expressed by *Candida albicans*, agglutinin-like sequence which is consisted of several glycosylated proteins is very important for successful adhesion (Lionakis and Netea, 2013; Williams and Lewis, 2011; Yang, 2003).

Dimorfic transition (Hyphal formation): The ability to switch between the yeast form and pseudohyphal form is one of the virulence factor of *Candida* species (Yang, 2003). Among the different species of *Candida*, *Candida albicans* and *Candida dubliniensis* are associated with the generation ofhyphae (Jackson *et al*., 2009). Hyphae are believed to play an important role in tissue and biomaterial invasion, may be as a result of concentration of phospholipase at hyphal tip. Study reported that species that do form hyphae have high ability to invade tissue and are resistant to phagocytosis (Williams *et al*., 2011). In addition, germ tube formation is an important factor in the attachment of *Candida albicans*. It is induced by many different environmental factors such as mammalian serum, high temperatures (37°C) and neutral pH (Cassola *et al*., 2004).

Production of hydrolytic enzymes: Candida produces several extracellular hydrolytic enzymes including secretedaspartyl proteinases, phospholipases, lipases, phosphomonoesterase and hexosamini-dase. These enzymes have the capacity to degrade membrane structure (human proteins) and help the organism to invade the human body easily, hence are associated with tissue invasion (Williams *et al*., 2011; Yang, 2003). Secreted aspartyl proteinases are key virulence determinants of *C. albicans*. The production of proteinases activity correlate with capacity for deep organ colonization, adherence to epithelial cells, the digestion of host proteins for nutrient supply and the envasion of host defenses by degrading immunoglobulins and complement proteins and degradation of host barriers during invasion (Fallon *et al*., 1997; Chaffin *et al*., 1998; Webb *et al*., 1998; Staib *et al*., 2000). The phospholipase enzyme concentrates on the topic of mycelia form and it is contributed to invasiveness, damage of cell envelopes and evasion of host response (Chaffin *et al*., 1998; Naglik *et al*., 2003). The putative roles of extracellular lipases include digestion of lipids for nutrient acquisition, adhesion to host tissues, synergistic interactions with other enzymes, initiation of inflammatory processes by affecting immune cells, self-defense mediated by lysing competing microflora and lipolytic catalysis could directly protect *C. albicans* via degradation of antimycotic fatty acids (Gacser *et al*., 2007).

Biofilm formation: Biofilms can be defined as microbial communities or aggregation of microorganisms that are

often (but not necessarily) attached to a solid surface. Candida strains that have the ability to form biofilms are more virulent than others; this has been associated with increased expression of virulence factors as well as reduced susceptibility to antimicrobial agents (Williams and Lewis, 2011). A variety of microbial infections are caused by biofilms ranging from the common such as urinary tract infections, catheter infections, child middle-ear infections and dental plaque to more threatening infections such as endocarditis and infections of heart valves. The detachment of cells from an adherent biofilm on a catheter can give rise to a septicaemia that may respond to conventional drug therapy. However, biofilm cells are not killed by such treatment and remain as a reservoir of infection until the implant is removed (Alem and Douglas, 2004).

LABORATORY DIAGNOSIS OF CANDIDIASIS

The identification of the infectious organism to the species level has become increasingly important for several reasons. First, *Candida* species distribution has changed in the recent years and also they differ in their susceptibility to antifungal agents. Second, species-specific identification is relevant for epidemiological purposes. Third, the risk of developing deep organ involvement and the severity of clinical manifestations differs depending on the infecting species (Ellepola and Morrison, 2005).

Microscopic detiction: The specimen collected can be subjected to KOH wet mount or normal saline preparation and examined under microscope; presence of Candida is seen as yeasts cells and pseudohyphae. On gram staining Candida appears as Gram positive yeast like budding cells approximately 4-8 μm with or without pseudohyphae. (Chander, 2009).

The traditional specific test for identification of *Candida albicans* is the 'Germ tube test' in which *Candida albicans* produces hyphal outgrowths from blastospores when incubated at 37°C in serum for 2-4 h. This phenomenon is referred to as "Reynolds and Braude phenomenon" because Reynolds and Braude discovered that blood components stimulated the hyphal formation in *Candida albicans*, hence can be used for rapid test for identification of *Candida albicans*. Taschdjian first described application of this property to yeast identification in 1960 (Odds, 1988). Apart from *Candida albicans* other species also form germ tube but they are pseudohyphal and therefore have marked constriction at the junction between germ tubes and parent blastosphere, e.g., *Candida stellatoidea* and *Candida dublinensis*. So, any yeast forming germ tube in serum whose morphology appears a typical should be subjected to full battery of tests for proper species identification (Chander, 2009).

Culture detection

Conventional medium (sabouraud dextrose agar): Most popular and useful agar media used for primary isolation of pathogenic candida species are versions of peptone-glucose agar, first described by Sabouraud and hence, called as Sabouraud agar. For isolation of yeast, bacterial growth should be suppressed by addition of chloramphenicol. Cycloheximide should not be added as it suppresses the growth of certain species of candida such as *Candida glabarata*, *Candida krusei* and *Candida parapsilosis*. After incubation for 1-2 days at 37°C the colonies appear cream coloured, smooth and pasty (Chander, 2009). It is not a differential medium and colonies of different pathogenic yeast species grown on this agar can not be easily distinguished from each other. It is less effective in terms of bacterial inhibition and favors a greater development of filamentous fungi (Silva *et al*., 2004).

Chromogenic medium: The chromogenic medium incorporates substrates linked to chemical dyes in a solid medium to differentiate *Candida* species by the color and/or texture of the growth produced (Ellepola and Morrison, 2005).

CHROM agar: CHROM agar is a differential medium used for isolation and presumptive identification of clinically important *Candida* species (Murray *et al*., 2005; Klevay *et al*., 2005). It is based on the reaction between specific enzymes of different species and chromogenic substrates which results in the formation of different colored colonies. This can be used for simultaneous isolation and presumptive identification of various *Candida* species. Advantage of this media is the rapid detection of multiple species in the sample (Chander, 2009; Larone, 2002; Odds and Bernaerts, 1994; Hospenthal *et al*., 2006; Agarwal *et al*., 2011).

Candida Immuno-Diffusion (ID) selective agar: Candida ID selective agar has been developed for the identification of *C. albicans* (blue colonies) and the presumptive identification of the yeasts *C. Tropicalis*, *C. lusitaniae*, *C. kefyr* and *C. guillermondii* (pink colonies). However, Candida ID is considered to be insufficiently selective in relation to bacteria with a resulting risk of inhibition of fungal growth in specimens with polymicrobial growth and a risk of confusion between blue-stained bacterial colonies and *C. albicans* (Willinger *et al*., 2005).

BIGGY (Bismuth sulphite Glucose Glycine Yeast) agar: BIGGY agar is a selective and differential medium used for the isolation and differentiation of *Candida* species. BIGGY agar contains bismuth sulphite and growth on this medium produces brown to black colonies because of the extracellular reduction of bismuth sulphite to bismuth sulphide. The bismuth sulphite also acts as an inhibitor of bacterial growth (Yucesoy and Marol, 2003). The lower sensitivity and specificity of BIGGY agar to identify commonly isolated *Candida* species potentially limits the clinical usefulness of this agar. *Candida albicans* colonies appear brownish dark on BIGGY agar but other *Candida* sp., appear pale brownish on this medium (Badawi *et al*., 2004).

Morphological medium: The commonly used differential medium for both genus identification and speciation is the cornmeal agar plate supplemented with Tween 80 or Rice agar (Larone, 2002). Rice agar and cornmeal agar with Tween 80 are used for cultivation and differentiation of *Candida* species on the basis of mycelial characteristics. Special attention should be given to the size and shape of the pseudohyphae and the arrangement of blastoconidia along pseudohyphae. The addition of 1-2% Tween 80 to corn meal and rice agar greatly enhances the development of chlamydospores on these media (Badawi *et al*., 2004). Chlamydospore formation is a property specific to *Candida albicans*. Chlamydospores are round, thick walled spores formed directly from the differentiation of hyphae in which there is concentration of protoplasm and nutrient material. These appear to be resistant resting spores produced by the rounding up and enlargement of terminal cells of the hyphae. Cornmeal agar that stimulates Chlamydospores production has been proposed as primary isolation media as well as identification media for Candida (Chander, 2009).

Non-culture based diagnostics: There is increasing interest in the use of serological tests for the diagnosis of deep-seated invasive Candida infections. The detection of several Candida-derived molecules in the serum samples of patients has been reported to be of diagnostic value (Girmenia *et al*., 2004).

Detection of antigenic components: Na and Song (1999) developed a monoclonal-based ELISA inhibition technique to detect *C. albicans* Secreted Aspartyle Proteinases (SAPs) in serum and reported a sensitivity of 93.9% and specificity of 96% for the diagnosis of invasive candidiasis. The overall test sensitivity and specificity when testing urine specimens were 83 and 92%, respectively. The use of SAPs detection in urine could therefore provide a non-invasive means to diagnose disseminated candidiasis (Morrison *et al*., 2003). Mannan is the major cell wall mannoprotein of *C. albicans*. Mannan is cleared rapidly from the circulation, resulting in low serum concentrations, so multiple serum sampling is required for optimal detection (Sendid *et al*., 2004; White *et al*., 2005). Mannan antigenemia precedes a significant rise to its antibodies by 6-23 days. An increase in the sensitivity may be obtained by detecting the antigen in urine. Also, detection of mannan in cerebrospinal fluid could be a valuable tool for diagnosing central nervous system candidiasis. Immunohistochemical detection of mannan facilitated the diagnosis of candidal pneumonia (Ponton *et al*., 2002; Lunel *et al*., 2004).

The two predominant cytoplasmic proteins include a 47 kDa protein which is a breakdown product of a 90 kDa heat shock protein and *C. albicans* enolase. The presence of enolase in serum correlated with disseminated disease and declined following antifungal therapy. Its detection may precede positive blood cultures by several days (Ponton *et al*., 2002; Misaki *et al*., 2003).

Detection of non-antigenic metabolites: The cell walls of *Candida* species contain (1-3)-β-D-glucan as a structural component. As this polysaccharide is not found in bacteria, viruses or mammals, its presence in the circulation of patients has been used as an indicator of invasive disease (Saikia *et al*., 2001). Although, the β-D-glucan test can not identify which fungus is specifically causing an infection, results can be obtained within 2 h. Such rapidity makes it very attractive as a screening test for invasive infection (Takesue *et al*., 2004).

D-arabinitol is produced in vitro and in vivo during infections. D-arabintol is produced by some *Candida* species: *C. albicans*, *C. Tropicalis*, *C. parapsilosis* and *C. kefyr* whereas *C. glabrata* and *C. krusei* produce trace amounts (Ellepola and Morrison, 2005). Natural host serum arabinitol accumulates during renal insufficiency so that D-arabinitol levels need to be reported as a D-arabinitol/creatinine ratio to compensate for this occurrence. Elevated ratios preceded positive blood cultures in up to 50% of the cases. Moreover, serial D-arabinitol/creatinine ratios correlated with therapeutic response (Sigmundsdottir *et al*., 2000).

Detection of antibodies: The clinical usefulness of antibody detection for the diagnosis of systemic candidiasis has been limited by false negative results in immunocompromised patients who produce low or undetectable levels of antibody and by false-positive results in patients with superficial colonization (Ellepola and Morrison, 2005). IgM antibodies to

C. albicans whole cells have been detected in patient with first time candidemia and reported a 100% sensitivity and specificity. Also, IgG and IgA antibodies rise in titers have been detected in sera sequentially drawn from patients with candidemia. Elevated levels of IgE antibodies to *C. albicans* surface antigens have been detected in patients with invasive candidiasis and patient with vaginal candidiasis (Ponton *et al*., 2002). Antibodies to enolase were detected in sera from immunocompetent patients with a sensitivity of 92.5% and a specificity of 95%. In addition, antibodies to *C. albicans* enolase were also detected in patients infected by *C. parapsilosis*, *C. Tropicalis*, *C. guilliermondii* and *C. glabrata* (Ponton *et al*., 2002). Na and Song (1999) described an ELISA assay for the detection of antibodies to the SAPs of *C. albicans*. The sensitivity and specificity for this test were only 70 and 76%, respectively making it less desirable for the diagnosis of invasive candidiasis than SAPs antigen detection tests. Antimannan antibodies levels in sequential serum samples from patients with acute leukemia gave a sensitivity of 64.3% and a specificity of 97.2% in the diagnosis of invasive candidiasis. Serodiagnosis was achieved before clinical diagnosis in 67% of the patients teste (Ellepola and Morrison, 2005).

The detection of antibodies to *C. albicans* germ tube was useful in the diagnosis of invasive candidiasis in different groups of patients. The test showed an overall sensitivity of 77-89% and a specificity of 91-100%. Sera from patients at risk of developing invasive candidiasis showed antibodies to CAGT before the microbiological diagnosis was made (Ponton *et al*., 2002).

Chromatographic Methods: Microbial identification system is an automated approach to rapid identification of unknown microbial isolates. The microbial identification system consists of a gas liquid chromatograph with a flame ionization detector an auto sampler and an integrator, coupled to a computer system. The computer searches a software library of fatty acid methyl ester compositions compare the isolate's fatty acid profile with those of known species and reports the most likely species name along with the extent of correlation of the isolate's profile with a species in the data base (Missoni *et al*., 2005).

IDENTIFICATION OF CANDIDA SPECIES

Biochemical identification: Rapid identification tests such as the RapID Yeast Plus System contain conventional and chromogenic substrates and require only 4 h to complete. It is a good system for the routine identification of clinically relevant yeasts; however most of these tests are more accurate for the identification of common than uncommon yeast pathogens (Freydiere *et al*., 2001). The most convenient and popular methods for *Candida* species identification consists of strips or plates for carbohydrate assimilation and/or enzyme detection which are commercially available in a variety of different formats (Ellepola and Morrison, 2005). Their principle depends on an increase in turbidity or the production of color in each of a series of wells containing different substrates to produce a particular biochemical profile. The profile produced is interpreted using the manufacturer's reference manual. These tests give good results for the more common species of Candida (Ellepola and Morrison, 2005).

The turbidimetric systems using API 20C AUX and API ID 32C tests are relatively useful for identifying common germ tube negative *Candida* species. However, identification of less common *Candida* species is not as accurate (Mahnss *et al*., 2005; Silva and Candido, 2005). Chromogenic method using API 20C System was one of the first commercial systems to be introduced for the purpose of yeast identification and is now considered a reliable system. However, the API 20C System is still time consuming to set up and reading requires up to 72 h. of incubation and gives results that are often difficult to interpret (Campbell *et al*., 1999). The identification of *Candida* species using Auxacolor System is based on carbohydrate utilization and the growth is visualized by color change of a pH indicator. It is rapid and accurate when used to identify common yeast species from solid media (Sheppard *et al*., 1999).

Fungichrom I panel consists of 16 wells and the enzymatic activities of yeasts are detected by the visual color change of the wells. This system seems to be an appropriate for use in a clinical Microbiology Laboratory, due to its good performance with regard to sensitivity, ease of use and reading, rapidity and the cost per test (Morace *et al*., 2002).

Candifast is a simple and accurate conventional assimilation method for the rapid identification of most commonly encountered isolates of *Candida* species (Morace *et al*., 2002). The identification of the yeast is based on the susceptibility of the strain being tested to actidione and the fermentation of seven sugars which is seen by the color change of the indicator either to yellow or to fuchsia (Gundes *et al*., 2001; Badawi *et al*., 2004).

Automated biochemical systems: ID32C strip is an identification system that provides evaluation for the assimilation of 30 carbon sources and for the growth of yeasts in the presence of cyclohexemide. ID32C

have been used as a reference method because of its extensive database and accuracy. However, the interpretation of test results is difficult and requires experience (Alves *et al*., 2005).

Vitek yeast biochemical card is an automated method for the identification of clinically important yeast isolates. Vitek System is easier to use and less time consuming. It also offers early results (maximum of 48 h) and objective reading (Huang *et al*., 2001).

VITEK 2 uses fluorescence to monitor 47 metabolic reactions in the ID-YST card. The system automatically fills, seals and transfers cards into an incubator. It has the advantage of speed as results could be obtained after 15 h. A disadvantage of VITEK 2 is that cultures cannot be older than 24 h and performs less well in identification of *C. glabrata* (Massonet *et al*., 2004; Aubertine *et al*., 2006).

Molecular identification: Most nucleic acid-based systems use PCR techniques to amplify fungal DNA as the first step in the identification process. Before PCR amplification can occur, appropriate DNA targets and PCR primers must be selected. The primers that amplify the *rRNA* genes (*18S*, *28S rRNA* genes) are the most frequently used due to their universal nature and large copy number (Coignard *et al*., 2004; White *et al*., 2005).

A variety of post amplification methods have been used to utilize the variable regions within the rRNA amplicons and identify the genus or species causing infection. These include nested PCR, restriction fragment length polymorphism, PCR-enzyme-linked immunoassay, single-strand confirmation polymorphism and hybridization with specific probes and sequencing. The most promising PCR technique utilizes fluorescently labeled specific probes and real-time PCR (Borst *et al*., 2003; White *et al*., 2005).

Fluorescent *in situ* hybridization, using oligonucleotide probes directed against 18S rRNA has been used to differentiate *C. albicans* from *C. parapsilosis* in tissues of infected mice (Ellepola and Morrison, 2005).

A new Fluorescent *In Situ* Hybridization (FISH) Method using fluorescein-labeled peptide nucleic acid probes targeting 26S rRNA, detects *C. albicans* directly in smears taken from positive blood culture bottles. The Peptide Nuclic Acid (PNA) probe is added and hybridized and the smears are examined by fluorescence microscopy. The performance of the *C. albicans* PNA FISH Method as diagnostic test shows 100% sensitivity and specificity (Kempf *et al*., 2005).

Fourier transform-infrared micro spectroscopy is a whole-cell "fingerprinting" method by which microorganisms can be identified. It measures vibrations of functional groups and highly polar bonds such as O-H stretches. This analyses the vibrational features of all cell components. So, FT-IRM allows chemically based discrimination of intact microbial cells without their destruction (Natalello *et al*., 2005; Essendoubi *et al*., 2005). A wide variety of methods have been developed and adapted to detect genetic polymorphism in yeast species. Indeed, it is often recommended that more than one method be used to achieve optimal results. These methods include restriction enzyme analysis, restriction enzyme analysis and species specific DNA fingerprinting probes, oligonucleotide fingerprinting, pulsed-filed gel electrophoresis, randomly amplified polymorphic DNA analysis and multilocus enzyme electrophoresis (Leung *et al*., 2000; Bautista-Munoz *et al*., 2003; Dodgson *et al*., 2003; Sampaio *et al*., 2003).

TREATMENT OF CANDIDIASIS

Prerequists for treatment: Due to appearance of many *Candida* species resistant to one or more antifungals (Akins, 2005), it is mandatory to carry out the susceptibility of the isolated *Candida* species to antifungals. This is important to use the effective antifungal type. Additionally, Minimum Inhibition Concentration (MIC) of the fungal agent of choice should be determined and then a dose above MIC must be used for treatment (Sanglard and Bille, 2002). A variety of molecular mechanisms by which Candida can develop resistance to azole drugs have been described (Akins, 2005).

Resistance can be the result of an alteration of the target enzyme, the cytochrome P-450 lanosterol 14α-demethylase (Erg11p), either by overexpression or as a result of point mutations in the gene that encodes it ERG11 (Akins, 2005; Lepak *et al*., 2006). Failure of azole antifungal agents to accumulate inside the yeast cell is mediated by two types of multidrug efflux transporters. They include the major facilitators (encoded by multidrug resistance genes) and those belonging to the ATP-binding cassette superfamily (ABC transporters, encoded by Cerebellar degeneration-related protein 1 genes) (MacPherson *et al*., 2005; Sipos and Kuchler, 2006). Up regulation of the *CDR1* and *CDR2* genes appears to confer resistance to multiple azoles whereas upregulation of the Multidrug resistance protein1 gene alone leads to fluconazole resistance exclusively (Akins, 2005; Hiller *et al*., 2006). Resistance to azole drugs has also been associated with modifications of the ergosterol biosynthetic pathway such as defects in the sterol C5, 6-desaturation step (Pinjon *et al*., 2005).

Treatment of candidiasis by synthetic antifungal agents

Polyenes: Polyenes are fungicidal antifungal agents which act by binding to ergosterol in the fungal cell membrane, causing osmotic instability and loss of membrane integrity.

Amphotericin B (AmB) has been available for over 40 years and remains the most effective antifungal for the treatment of most systemic and visceral fungal infections in humans (Kucukates *et al*., 2005). Its usefulness is nevertheless limited by its pronounced side effects both immediate (chills, fever, nausea, headache) and delayed. In addition, because of its high affinity for biological membranes and for lipoproteins. AmB may accumulate in tissues, especially in the liver and may induce dysfunction in this organ. AmB may also induce reversible haematological alterations including normochromic anemia, thrombocytopenia and agranulocytosis (Larabi *et al*., 2004). Several strategies have been developed over the past few years in an effort to overcome the disadvantages associated with the clinical use of conventional AmB. Formulations have been developed in which this poorly water-soluble drug is associated with lipids in the form of liposomes or complexes (Gonzalez *et al*., 2004; Larabi *et al*., 2004). The lipid-based polyene products differ widely in the composition of the carrier, the polyene content, the particle size and the way that the polyene interacts with the lipid. The formulations include AmB lipid complex, AmB colloidal dispersion and liposomal AmB. All three formulations could be administered at higher doses than conventional AmB (Martino, 2004). Use of the lipid-based polyene products is appropriate in patients following treatment failure with conventional AmB or other systemic antifungals. It can be also used in patients who become intolerant to conventional AmB due to adverse reactions or those who have underlying kidney diseases or receive other nephrotoxic drug. Finally, it should be stressed that all lipid formulations of AmB are less effective than conventional AmB and this explains why higher doses of the active drug need to be given (Gonzalez *et al*., 2004; Martino, 2004; Wiley *et al*., 2005).

Nystatin (Local antifungal agents) is a polyene antifungal agent related to amphotericin B and has a similar mode of action. It can be used to treat local candidal infections of mouth and vagina. Nystatin suppresses subclinical esophageal candidiasis and gastrointestinal overgrowth of Candida. No systemic absorption occurs and there are no side effects. However, nystatin is too toxic for parenteral administration. A liposomal preparation of nystatin is currently undergoing preclinical and clinical evaluation (Sanglard and Bille, 2002).

Flucytosine: Flucytosine (FC) is a fungistatic antifungal agent which acts by inhibiting nucleic acid synthesis. The use of FC as a single agent is limited to the treatment of uncomplicated lower urinary tract and vaginal candidiasis due to the development of resistance of many fungi during monotherapy (Te Dorsthorst *et al*., 2004, Liu *et al*., 2005; Cleary *et al*., 2005).

FC is used concomitantly with other agents, mainly AmB for the treatment of systemic mycosis such as cryptococcosis, candidiasis and aspergillosis. Side effects of FC include bone marrow suppression, thrompocytopenia and abnormal liver function tests (Bennett, 2005).

Azoles: Azoles are fungistatic antifungal agents including imidazoles and triazoles. Fluconazole, itraconazole and voriconazole and the investigational posaconazole and ravuconazole are triazoles, so named because they have three nitrogens in the ring structure. This class has less impact on human hormonal synthesis and less hepatotoxic than miconazole and ketoconazole which are the widely used systemic imidazole (Bennett, 2005).

Azoles inhibit the enzyme lanosterol 14α-demethylase, produced by the *ERG11* gene in yeast. This inhibition results in a block in synthesis of ergosterol, the major sterol of the fungal cell membrane (Vermitsky and Edlind, 2004).

Miconazole: Miconazole was the first imidazole to be administered intravenously for the therapy of systemic fungal infections. However, owing to its toxicity and high relapse rates, its use has been limited to certain cases of refractory cryptococcal meningitis and coccidioidal meningitis in children.

Ketoconazole: Ketoconazole is an orally absorbed antifungal agent. It is effective in blastomycosis, histoplasmosis, chronic mucocutaneous candidiasis and esophageal candidiasis (Cleary *et al*., 2005). Side effects include itching, rash and dizziness. Hepatotoxicity is usually mild and the serum testosterone is reduced which may lead to gynecomastia in men (Bennett, 2005).

Fluconazole: Fluconazole is a very widely used systemic antifungal agent with a broad therapeutic range and little toxicity. It has demonstrated the broadest clinical efficacy for mucosal candidiasis both vaginal and oropharyngeal as well as chronic mucocutaneous candidiasis (Gupta *et al*., 2005). It is also recommended as the first choice in the treatment of invasive Candida infections in non-neutropenic patients such as solid organ transplant patients, surgical and intensive care

unit patients or those with UTIs (Cuenca-Estrella *et al*., 2005; Shan *et al*., 2006). Even in neutropenic patients, candidemia can be successfully treated with fluconazole as long as the patients are stable and the infection is not due to *Candida* species less susceptible to fluconazole such as *C. glabrata* or *C. krusei* (Shorr *et al*., 2005; Spellberg *et al*., 2006). Fluconazole concentration in serum is dependent on the dose administered, i.e., a higher dose of fluconazole leads to a higher concentration in serum and hence, the use of the susceptible-dose dependant designation (Kucukates *et al*., 2005).

Overuse of fluconazole, especially in immunocompromised patients has led to increase in colonization with less susceptible organisms and the development of resistance among usually susceptible species such as *C. albicans* (Hung *et al*., 2005).

Side effects include nausea and abdominal distress. An allergic rash may develop and is particularly common among patients infected with HIV. Rare cases of anaphylaxis, hepatic necrosis and neutropenia have been described (Clancy *et al*., 2005; Sarvikivi *et al*., 2005).

Itraconazole: Itraconazole is more active *in vitro* and *in vivo* than ketoconazole and fluconazole. It has been used successfully for the treatment of aspergillosis, candidiasis, coccidioidomycosis, blastomycosis, cryptococcosis and histoplasmosis (Pfaller *et al*., 2005; Spacek and Buchta, 2005). Except for the gastrointestinal distress from the oral solution, the toxicity of itraconazole is generally low, although life-threatening hepatotoxicity, congestive heart failure, edema, cardiac dysrhythmias and peripheral neuropathy have been reported (Conte *et al*., 2004).

New triazoles

Voriconazole: Voriconazole, a fluconazole derivative with improved antifungal activity and enhanced potency has been developed and possesses a wide spectrum of activity against yeasts, filamentous and dimorphic fungi (Magill *et al*., 2006). It has been approved for the treatment of acute invasive aspergillosis and other serious fungal infections. The *in vitro* activities of voriconazole against clinical isolates of *C. glabrata* are significantly higher than those reported for fluconazole (Barchiesi *et al*., 2004; Mallie *et al*., 2005). Voriconazole appeared to be significantly more active than fluconazole against *C. rugosa* and fluconazole-resistant species such as *C. krusei*, *C. norvegensis* and *C. inconspicua*. Voriconazole is a particularly valuable option for the treatment of fluconazole-resistant candidiasis in HIV infected patients (Linares *et al*., 2004; Pfaller *et al*., 2004). A major advantage of voriconazole over other recently approved antifungal agents used to treat systemic disease is that it can be administered orally after initial intravenous loading and administration of maintenance doses. The toxic effects of voriconazole include transient visual disturbances such as color change and blurring, hepatotoxicity and rash (Walsh *et al*., 2004; Bagg *et al*., 2005).

Posaconazole, Ravuconazole and R126638: Posaconazole and ravuconazole have potent extended-spectrum and *in vitro* activity against several commonly encountered pathogens including *Candida*, *Aspergillus*, *Cryptococcus* and *Coccidioides* species (Gupta *et al*., 2005; Vazquez *et al*., 2006).

R126638 has potent antifungal activities *in vitro* against *C. albicans* including fluconazole-resistant strains, *C. glabrata*, *C. guilliermondii* and *Cryptococcus neoformans*. However, it exhibits weak or no activity against *C. parapsilosis*, *C. krusei* and *Aspergillus* species (Bossche *et al*., 2004).

Allylamines: Terbinafine belongs to this class of antifungal agents. It targets squalene epoxidase which is critical enzyme in the biosynthesis of ergosterol. It is fungicidal against dermatophytes and filamentous fungi but is fungistatic against the majority of *Candida* species (Gupta *et al*., 2005).

Griseofulvin: Griseofulvin inhibits mitosis by interfering with microtubule function. It is active against dermatophytes and to lesser extent against filamentous fungi but without activity against yeast pathogen (Dastghaib *et al*., 2005).

Antifungal agents in development

Cyclic lipopeptides: The echinocandins including caspofungin, FK463 (Micafungin) and LY303366 (V-Echinocandin) represent the newest class of antifungal drugs. They inhibit the synthesis of 1,3-α-D glucan, a fundamental component of the fungal cell wall by the inhibition of 1,3-α-D-glucan synthase (Canton *et al*., 2005; Chandrasekar and Sobel, 2006).

They have an excellent *in vitro* fungicidal activity against fluconazole-resistant *Candida* species strains and have clinical efficacy in candidemia, esophageal candidiasis and invasive aspergillosis (Moudgal *et al*., 2005; Datry and Bart-Delabesse, 2006).

Nikkomycin Z: The nikkomycin class of antifungal agents inhibit chitin synthase in the fungal cell wall. Nikkomycin Z has demonstrated in vitro activity against *Candida* species and has been effective in the treatment of experimental blastomycosis, histoplasmosis and coccidioidal infections (Ganesan *et al*., 2004).

Pradimicins: Pradimicins, including BMS 181184 are fungicidal agent that their mode of action involves the formation of a calcium-dependent complex with the sugar moiety of manno-proteins. Pradimicins-mannoproteins complex disturb cell wall organization leading to leakage of intracellular component and cell death (Sanglard and Bille, 2002).

Sordarins: Sordarins including R-135853 are a novel class of antifungal agents that act by selectively inhibiting the protein synthesis. They exhibit potent *in vitro* activities against *C. albicans* including fluconazole-resistant strains, *C. glabrata*, *C. guilliermondii* and *Cryptococcus neoformans*. However, they exhibit weak or no activity against *C. parapsilosis*, *C. krusei* and *Aspergillus* species (Santos *et al*., 2004; Kamai *et al*., 2005).

Aureobasidins, proton ATPase inhibitors and efflux pump inhibitors: Aureobasidin A, proton ATPase inhibitors, efflux pump inhibitors are fungicidal antifungal agents. They act at the level of plasma membrane and possess activity against most Candida species (Sanglard and Bille, 2002; Monk *et al*., 2005).

Cationic antimicrobial peptides: Cationic antimicrobial peptides are peptides with a positive charge due to an excess basic amino acids compared to acidic amino acids. They are fungicidal agents and act by binding to plasma membranes and cause cell lysis (Gordon *et al*., 2005).

Mammalian peptides including defensins and salivary histadines have a different potency against *C. albicans* and Cryptococcus neoformans. Amphibian and insect peptides contain two types of antimicrobial peptides with antifungal activity; magainins and dermaseptins (Helmerhorst *et al*., 2005; Kamysz, 2005; Monk *et al*., 2005).

Treatment of candidiasis by natural extracts and harmless chemical materials: Over the last few decades, there has been an increase in the number of serious human infections in immunocompromised patients caused by fungi (Pfaller *et al*., 2006). The range of severity of these infections is a consequence of the host reaction to the metabolic products produced by fungi, the virulence of the infecting strain, the site of infection and also environmental factors (Romani, 2007). Nowadays, the increasing impact of these infections, the limitations encountered in their treatment (e.g., resistance, side-effects and high toxicity) and the rising overprescription and overuse of conventional antifungals (Perez-Parra *et al*., 2009; Ferris *et al*., 2002) all stimulate a search for alternative natural drugs. In recent years, research on aromatic plants and particularly their essential oils has attracted many investigators. Essential oils have traditionally been used for centuries for their antifungal properties (Rios and Recio, 2005). More recently, several studies have confirmed the huge potential of these natural products as antifungal agents (Bakkali *et al*., 2008; Cavaleiro *et al*., 2006; Pina-Vaz *et al*., 2004; Pinto *et al*., 2006; Zuzarte *et al*., 2009). Therefore, it is not surprising that essential oils are one of the most promising groups of natural products for the development of broad-spectrum, safer and cheaper antifungal agents. In this matter, a number of essential oils such as thyme, cress, menth, anise, lemon, olive, black cumin and clove were used (Devkatte *et al*., 2005; Saikia *et al*., 2001).

Clove oil in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other used oils (Citak *et al*., 2005). The resistance of pathogenic fungi including *Candida albicans* and non albicans species isolated from patients, against antifungal agents has increased (Citak *et al*., 2005). Based on the toxicity and low potency, combined with the increasing side effects of these drugs (Devkatte *et al*., 2005), novel fungal therapies with fewer side effects on humans like clove essential oil obtained from *Syzygium aromaticum* were used for effective management of candidiasis infections (Papadopoulou *et al*., 2005; Seneviratne *et al*., 2008). Clove represents one of the major vegetal sources of phenolic compounds as flavonoids, hidroxibenzoic acids, hidroxicinamic acids, hidroxiphenyl propens, eugenol, eugenol acetate and gallic acids. Eugenole is the main bioactive component of clove which is found in concentrations ranging from 9381.70-14650.00 mg/100 g of fresh plant material (Seneviratne *et al*., 2008). Gallic acid also found in higher concentration (783.50 mg/100 g fresh weight). However, other gallic acid derivatives as hidrolizable tannins are presented in higher concentrations (2375.8 mg/100 g) (Shan *et al*., 2005). Other phenolic acids found in clove are the caffeic, ferulic, elagic and salicylic acids. Flavonoids as kaempferol, quercetin and its derivates (glycosilated) are also found in clove in lower concentrations. Clove oil has biological activities such as antibacterial, antifungal, insecticidal and antioxidant properties and is used traditionally as a savoring agent and antimicrobial material in food (Huang *et al*., 2002; Lee and Shibamoto, 2001; Nunez *et al*., 2001). In addition, clove oil is used as an antiseptic in oral infections (Meeker and Linke, 1988; Shapiro *et al*., 1994). It was effective against *L. monocytogenes* and *S. Enteritidis* in triptone soya broth and cheese (Smith-Palmer *et al*., 2001). The high

levels of eugenol contained in clove essential oil are responsible for its strong biological and antimicrobial activities. It is well know that both eugenol and clove essential oil phenolic compounds can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of Gram negative and Gram positive bacteria as well as different types of yeast (Chaieb *et al*., 2007; Walsh *et al*., 2003). Microbial inactivation is a kinetic process in which viability of organisms exposed to biocide varies with time. The kinetics of inactivation depends on the type of microorganism, the type and concentration of biocide and environmental conditions such as temperature, pH and presence of organic matter (Weavers and Wickramanayake, 2001). Different chemicals presented as effective antimicrobial agents under ideal laboratory conditions often show significant loss of activity when exposed to organic agents such as serum, blood, proteins, etc. (Thrash and Reich, 2001). Clove is used extensively in dental care for relieving toothache, sore gums and oral ulcers. Gargling with clove oil can also aid in sore throat conditions and bad breath (ISO, 2002).

Anti-fungal activity of clove oil: Clove is also effective in reducing fungal infection such as athletes foot Mycotoxigenic fungi cause plant diseases during storage and transport which may have an effect on human health. The essential oil components investigated including eugenol showed toxic effects on the *in vitro* mycelium growth against several *Pencillium*, *Fusarium* and *Aspergillus* species and *Altarnaria altarnata*. Several other studies have confirmed the antifungal activity of eugenol against pathogens such as *A. ochraceus*, *F. graminearum* and confirmed also its activity against different candida species due to the development of antimicrobial resistance to certain drugs (ISO, 2002).

Anti-fungal activity of potassium alum solution: Alum is both a specific chemical compound and a class of chemical compounds. The specific compound is the hydrated potassium aluminium sulfate (potassium alum) with the formula $KNH4(SO_4)2{\cdot}12H_2O$. More widely, alums are double sulfate salts with the formula $AM(SO_4)2{\cdot}12H_2O$ where A is a monovalent cation such as potassium or ammonium and M is a trivalent metal ion such as aluminium or chromium (III) (Samuel and Osman, 1999). Alum occurs naturally in rocks that are located in areas where sulfide materials and potassium-bearing minerals. Alum (Aluminum potassium sulfate), the crystallized double sulfate are generally odourless, colourless crystalline solids that turn white in air.

Chemical properties of alum: Alums are useful for a range of industrial processes. They are soluble in water have a sweetish taste; react acid to litmus and crystallize in regular octahedral. When heated they liquefy and if the heating is continued, the water of crystallization is driven off, the salt froths and swells and at last an amorphous powder remains. They are astringent and acidic.

Uses of alum: Alum can be used as an astringent and antiseptic in various food preparation processes such as pickling and fermentation and as a flocculants for water purification. Between 30 and 40 ppm of alum for household wastewater, often more for industrial wastewater is added to the water so that the negatively charged colloidal particles clump together into "flocs" which then float to the top of the liquid, settle to the bottom of the liquid or can be more easily filtered from the liquid, prior to further filtration and disinfection of the water. Food and Drug Administration (FDAs) over the counter advisory panel has recommended alum as category I active ingredient in mouthwashes (Faraj, 2012). Alum is used medicinally in many subunit vaccines as an adjuvant to enhance the body's response to immunogens such vaccines include hepatitis A, hepatitis B (Doherty and Andersen, 2005). Alum is also widely used in rural areas of Nigeria for the treatment of pediatric cough (Faraj, 2012). Styptic pencils containing aluminium sulfate or potassium aluminium sulfate are used as astringent to prevent bleeding from small shaving cuts. It was used as a base in skin whiteners and treatments during the late 16th century. Alum in block form (usually potassium alum), this is according to its molecules can be used as a blood coagulant. It may be used in depilatory waxes used for the removal of body hair or applied to freshly waxed skin as a soothing agent.

Its antiperspirant and antibacterial properties (Kanlayavattanakul and Lourith, 2011; Aguilar *et al*., 1956) contribute to its traditional use as an underarm deodorant, this is due to its molecules which have a negative ionic charge, making it unable to pass through the cell wall and not absorbed. This is why these deodor-ants are safe to use and will not cause high levels of aluminum. Alum can be used also as antiyeast agent due to inhibition the growth of candida species in which its effect on the budding process (Al-Husainy, 2004).

CONCLUSION

The scientific history and actual meaning of candidiasis are given in this review. Many *Candida* sp. within family: saccharomycetaceae of order: saccharomycetales were isolated and characterized and

are known to cause vulvovaginal candidiasis in women. The hydrolytic enzymes and biofilm formation are the two important virulence factors of candidiasis. Microscopic detection, serology and molecular fingerprinting are the recent and important methods of diagnosis of candidiasis. Treatment of candidiasis needs synthetic antifungals and recently natural.

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