CHAPTER 1

REVIEW OF LITERATURE
1.1 INTRODUCTION AND HISTORICAL PERSPECTIVE

Despite the long history of medical mycology which covers a span of approximately 140 years, unlike many bacterial and viral diseases, no mycotic disease has been adequately conquered. The mycoses are among the most ubiquitous infectious diseases that plague mankind. In their varied manifestations superficial, cutaneous, subcutaneous and systemic infections, these occur in all continents and in diverse climatic zones. Fungal infections afflict all strata of society irrespective of human race, cast, creed, culture or religion. Tropical countries especially have more than their contribution of fungal diseases probably because of high temperature and socio-economic conditions. It is also true that pathogenic fungi, like most moulds and yeasts, grow best in environments that are warm and humid and where organic matter is abundant. Systemic infections caused by fungi constitute a major public health problem in many parts of the world, both in developed as well as in third world developed countries. Paradoxically, some of the mycoses are most prevalent and have a higher incidence in the most medically advanced nations.

Hippocrates in his "Epidemics" described aphthae or thrush (white patches) in debilitated patients. The term "thrush" is probably derived from ancient Scandinavian or Anglo-Saxon. "Torsk" is the Swedish equivalent of this word. The French word for the condition is "le muguet", which means "lily of the Valley". Galan described it as of common occurrence in children particularly sickly children, and the disease was noted in Repy's diary. It was recognised early as a condition of newborn in text books on pediatrics by Rosen Von Rosenstein and Underwood. The disease was so prevalent in France that the French Societe Royale de Medecine offered an award of pounds 1299 for its study in 1786. Veron postulated that it was acquired during passage through the womb. He also described the first cases of esophageal candidiasis. Berg considered the fungus to be transmitted by unhygienic conditions by feeding bottles. Debilitation was proposed by Bennett and Robin as the most important prelude to candidal infection. Bennett's work was notable for the excellent and accurate illustrations of the fungus in the lung and sputum of a patient with pneumothorax due
to "Tuberculosis". Berg\textsuperscript{9} reproduced the disease in healthy babies inoculating them with aphthous membrane material. One of them died with candidal bronchitis and pneumonia. Parrot\textsuperscript{12} noted the first pulmonary human infection. Disseminated infection involving many organ systems was recorded by Schmorl\textsuperscript{13}.

A revival of interest in systemic candidiasis and candidal endocarditis took place after 1940. The occurrence of candidiasis as a sequel to the use of antibacterial antibiotics particularly broad-spectrum antibiotics, evoked a great urge of research. The results have demonstrated the delicate ecosystem of which Candida is a member. Many fatal cases of candidiasis occurred following abrogation of this balance. At the same time, the association of candidiasis and steroid therapy, immunosuppressive drugs, cytotoxic agents, and immune defects became apparent. Recently, Candida is recognized as one of the most frequently encountered fungal opportunists and is now regarded as the commonest cause of serious fungal disease.

The classification of the organism C. albicans has been the subject of controversy since its first association with human disease. The dimorphic nature of the yeast was recognized as early as 1844 by Bennett\textsuperscript{10}, and in 1847 Robin\textsuperscript{11} placed it in the genus Oidium under the name Oidium albicans. This genus name was derived from the morphology of the egg shaped, oval yeast cell. Lodder\textsuperscript{14} lists one hundred synonyms for C. albicans in the 1970 edition of the book "yeast". Berkhout\textsuperscript{15} in 1923 erected the genus Candida to encompass asporogenous yeasts that have "few hyphae, lying flat, falling apart into longer or shorter pieces". Conidia arise by budding on hyphae or on each other. This name was accepted as a nomen conservandum by the eighth Botanical Congress at Paris in 1954.

Of all the acute or chronic human yeast infections Candida albicans is the fifth most common primary blood stream organism and seventh most common pathogen to cause nosocomial infections\textsuperscript{16,17}. Candida spp. now cause approximately 7% of all nosocomial infections. The etiological, biochemical and morphological characteristics of Candida albicans make it a unique biologically interesting microbe, and its pathological potential makes it a medically important eukaryotic microorganism. C. albicans causing candidiasis can be isolated from the gastrointestinal tracts of a wide variety of birds and
mammals. However, its association with humans is interesting from a number of viewpoints, and the exposure of humans to \textit{C. albicans} occurs early in life, during passage through the birth canal. This yeast persists as a minor and sometimes major member of the alimentary tract microbial flora for life. Normally, a balance exists between the host and its potential parasite, but when this equilibrium is disturbed by internal or external factors, the yeast multiplies wildly and its millions of cells invade and destroy vital tissues of the host.

Candidiasis is a primary or secondary infection ranging from acute, subacute and chronic to life-threatening episodes. Involvement may be localised to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs or the gastrointestinal tract, or become systemic as in case of septicemia, endocarditis and meningitis. The pathologic processes evoked are also diverse and vary from irritation and inflammation to chronic and acute suppuration or granulomatous response. Since, \textit{C. albicans} is an endogenous species, the disease represents an opportunistic infection. \textit{Candida albicans} can mimic a dermatophytic infection in the skin and nails.

Candidiasis is increasing in occurrence and severity because of advances in modern medicine and due to the indiscriminate use of antibiotics, immunosuppressive and cytotoxic therapies, immunocompromised situation, particularly in cases of granulocytopenia below 1000 elements ml$^{-1}$ and more recently in Acquired Immunodeficiency syndrome (AIDS) and AIDS Related Complex (ARC). Of the 1,40,000 cases of AIDS reported globally by the WHO from 138 countries, candidiasis due to \textit{C. albicans} was the predominant "AIDS indicative disease". Although, \textit{C. albicans} is usually encountered in most of the clinical forms of candidiasis, however, other species of \textit{Candida} that have been unequivocally demonstrated in human infections include \textit{C. tropicalis}, \textit{C. pseudotropicalis}, \textit{C. parapsilosis}, \textit{C. krusei}, \textit{C. lusitaniae}, \textit{C. stellatoidea} and \textit{C. guilliermondii}. \textit{C. tropicalis} has been reported from separate studies performed at the John Hopkin's Oncology Center, Memorial Sloankettering Cancer Center and Harper Hospital in Detroit, Michigan, U.S.A. to be the most frequently isolated fungal pathogen from patients with hematologic malignancies. \textit{C. parapsilosis} has been linked to
hyperalimentation and was the most frequent *Candida* spp. to cause intravenous drug-use-related endocarditis\(^{23}\).

*C. albicans* is a polymorphic fungus. It displays four different cellular and a variety of colony morphologies. It grows as blastospores, pseudohyphae or hyphae; it can also form chlamydospores. The blastospores are round or oval cells, 2-5 µm in diameter, with multipolar budding. Pseudohyphae consists of elongated yeast cells (Fig. 1) attached to each other; functions between pseudohyphal cells are constricted. True hyphal cells are longer than blastoconidia with perforated septa; cell junctions are not constricted. Chlamydospores are thick walled asexual spores formed by rounding up of the preexisting cells. *C. albicans* exists in two forms - yeast form and the mycelial form. Yeast to hyphal conversion occurs through an intermediate germ tube stage\(^{24}\). Yeast to hyphal transition in *C. albicans* is controlled by a number of factors, the most important being growth medium and temperature. Germ tubes are induced when blastospores are incubated at a temperature between 33 and 42°C in a medium containing aminoacids such as proline, glutamine and arginine, aminosugars such as N-acetylglucosamine (GlcNAC) and N-acetylmannosamine (MNsNAC) or ethanol. Furthermore, N-acetylhexose derivatives, such as chitin, mucin, hyaluronic acid and immobilized GlcNAC are all capable of inducing germ tubes. Since, these derivatives are not metabolized or transported into cells, it is believed that the inducer binds to a cell surface receptor and produces an intracellular signal, which primes the cell for germ tube formation\(^{25}\).

Germ tube formation is induced at 37°C. Above 33°C mycelia are formed while below that temperature pseudomycelia are formed. Divalent cations are necessary for germ-tube formation. It has been shown that magnesium ions are required for germination\(^{25}\). A pH value in the range 6-8 is critical for germ tube formation\(^{24}\). However, Pollack and Hashimoto\(^{26}\) reported that germ tubes are produced at pH value as low as 3.0, in a medium lacking glucose, suggesting that glucose, at low pH values, suppresses germ-tube formation. The mechanism of inhibition is not yet clear. *C. albicans* a commensal of the gastrointestinal tract becomes infectious as an opportunistic pathogen due to deranged host defense mechanism or due to the virulence factor of the pathogen.
1.2 PREDISPOSING FACTORS AND HOST DEFENSE MECHANISM

1.2.1 Skin and mucosal barriers. The stratified squamous epithelium normally functions as a very effective barrier against microbial invasion. Mechanical breakdown of the normal skin defenses is the most important predisposing factor for skin infections. The breakdown might occur as in burn injury or due to increased moisture and maceration of the skin. Mucosal surfaces are the initial site of infection for C. albicans and the major encounter between microbial pathogenic factors and host defenses occurs at this surface. Mucosal surfaces of the mouth and the vagina may be colonized with C. albicans in up to 80% of normal individuals and the colonization is generally increased in patient populations. The interaction between C. albicans and other commensals is the most important factor affecting the degree of colonization of mucosal surfaces. The bacterial flora prevents binding and invasion of C. albicans. Given the importance of the normal microbial flora in suppressing C. albicans colonization, antibiotic therapy thus acts as one of the predisposing factors.

1.2.2 Immune system. The immune system is normally effective in combating C. albicans. C. albicans infection is dependent on the breakdown of the host immune system. The predisposing factors in leukemic patients is possibly because of immunosuppressive therapy, in patients after surgical intervention and in AIDS patients. An analysis of the underlying defects in these patients indicated that C. albicans infection is specifically associated with defective cell-mediated immunity.

1.2.3 Inherent immunity. Polymorphonuclear neutrophils contain lysosomal granules, which process a variety of hydrolytic enzymes. These neutrophils engulf the yeast form of C. albicans. The lysosomal granules fuse with the vacuole containing C. albicans to form a phagolysosome which brings about an efficient killing of the yeast. C. albicans hyphae are also frequently encountered in tissues. However, they are too large to be phagocytozed and are probably killed by certain extracellular processes. In experimental lung infection of mice, the host
immune response is dependent on the size of the C. albicans inoculum. A large inoculum elicits neutrophil influx and a smaller inoculum elicits a soluble factor, a protein of 29 Kd, that has a direct candidacidal activity.\(^{31}\)

Macrophages (Mφ's) play a key role in cell mediated immunity, because they are involved both in initiation of responses as antigen presenting cells and in the effector phase as microbicidal cells. Mφ functions are enhanced by a process known as activation, brought about by lymphokines. The Mφ "Colony-Stimulating Factor" (CSF), which is a lymphokine, enhances the ability of Mφ's to kill C. albicans.\(^{32}\) The CSF is also believed to increase the density of mannose-binding receptors on the Mφ surface, thereby, resulting in enhanced binding and ingestion of C. albicans.

The factors that predispose C. albicans infections are low neutrophil levels and defective cell-mediated immunity. In a particular instance, T-lymphocytes from Chronic Mucocutaneous Candidiasis (CMC) patients could not proliferate in vitro, and also could not exert helper activity for B-cell antibody production.\(^{33}\) Thus, the normal anti-candida defense system would not be active to thwart C. albicans infection, leading to an inefficient invasion and progression of the disease.

1.2.4 Candida induced immunity. C. albicans also exerts a definite influence on the host immune response resulting in a debilitated candidacidal action. Mannan, a major constituent of the cell wall in C. albicans, inhibited a Candida antigen induced in vitro proliferation of normal lymphocytes and also blocked the antigen presenting ability of Mφ's. The mannan effects would result in an impaired immune response. In another study, polysaccharide fractions (containing mostly mannose and glucose residues) from C. albicans stimulated the T-cells to produce a suppressor factor, which in turn inhibits interleukin 1 and interleukin 2 production.\(^{34}\) Since, interleukins play an important role in T-lymphocyte proliferation, action of the suppressor factor would result in a poor immune system.

1.2.5 Hormone levels. Hormonal changes during pregnancy predispose women to vaginal candidiasis, particularly vaginal thrush.\(^{35}\) The presence of cornified epithelium and absence of
leukocytes, which are normally observed during oestrous phase or are due to artificial administration of oestrogen, predispose rats to vaginitis. Oestrogen administration promotes colonization by \textit{C. albicans}, but progesterone administration does not\textsuperscript{36}. Corticosteroids, progesterone and oestrogen-binding proteins have been identified in \textit{C. albicans}\textsuperscript{37}. These proteins might help \textit{C. albicans} to sense the hormonal status of the host and respond appropriately.

### 1.3 VIRULENCE FACTORS

#### 1.3.1 Morphology

Dimorphism may be implied in candidal pathogenesis. Mycelium formation has been associated with increased virulence. However, the pathogenic significance of dimorphism may be overstressed. The \textit{Candida} yeast cells can attach to endothelial cells and can resist phagocytic intracellular killing\textsuperscript{38}. Factors known to influence yeast, hyphal transition in \textit{C. albicans} include the presence of serum, bicarbonate CO\textsubscript{2} tension, cytokines (IFN-Y) and prostaglandin (E\textsubscript{2})\textsuperscript{39}.

#### 1.3.2 Adherence

Adherence of \textit{Candida} spp. to mucosal epithelial cells is an important initial step in the process of colonization and invasion. Ability to adhere to mucosal cells is a characteristic of pathogenic \textit{C. albicans} as compared to some of the less pathogenic \textit{Candida} spp. Since, increased virulence has been associated with mycelium formation, the relation between germination and adherence has been investigated. In general, \textit{C. albicans} has been found to adhere better to epithelial cells under conditions that enhance germ tube formation. Inhibition of germination has been associated with decreased adherence. However, germination may not be a pre-requisite for adherence and colonization\textsuperscript{40}. Many adherence mechanism have been reported, and factors determining adherence may differ among organs\textsuperscript{40}. Surface glycoproteins, especially mannoproteins are thought to play a role in the adherence properties of \textit{Candida} spp.\textsuperscript{41}. The putative host-cell receptors for the \textit{Candida} adhesion include fibronectin, and fibrin in thrombi. A lectin like protein that recognizes different sugar residues of epithelial cell glycoproteins has also been described on the \textit{Candida} cell surface\textsuperscript{42}. A complement
receptor like protein, CR3 binding IC3b may play a role in adherence to endothelial cells, antibodies to this protein or to IC3b blocked adherence of *C. albicans* to human and animal endothelial cells. Lipids are also involved in adherence, phospholipids, sterols and sterol esters are the major classes of lipid that blocked adherence on pretreatment of either yeast cells or target cells. Cell surface hydrophobicity seems to be important in adherence. A rough colony morphology variant with greater cell surface hydrophobicity had greater adherence capacity.

1.3.3 Secretory proteinase. In the search of virulence factors, secretory acid proteinases have gained importance. Induction by proteins leads to secretion of proteinase by *C. albicans* in culture. The proteinase are active only at low pH values (3.0-5.5). Strain specific proteinases have also been detected. Interestingly a high titre of antibody (ab) against a proteinase produced by *C. albicans* was detected in patients suffering from systemic candidiasis. A secretory proteinase defective mutant was shown to be less virulent in mice. Ross et al. have shown that for *C. albicans* virulence, secretory proteinase activity is important.

1.3.4 Other secretory hydrolytic enzymes. Production and release of phospholipases secreted by *C. albicans* have attracted considerable attention because of their possible involvement in the processes of invasion and damage to the host cells. Capacity to adhere to buccal epithelial cells and pathogenicity for mice of four *C. albicans* isolates correlated with a high phospholipase activity. Non-pathogenic yeasts and a virulent *C. albicans* strains had lower phospholipase activities. N-acetylglucosaminidase (Chitobiase) is secreted during germ tube formation by *C. albicans*. A mutant defective in production of chitobiase was less virulent compared with its parent strain.

1.3.5 Aminosugar metabolism. Aminosugars are present in mucous membranes, which are the sites of colonization by *C. albicans*. Although aminosugars are not in the free form, they are present as part of proteins (glycoproteins). Since, *C. albicans* has to survive on the mucous membrane by utilization of sugar (possibly
aminosugars) as a source of energy. Singh and Datta\textsuperscript{54} initiated studies on aminosugar metabolism in \textit{C. albicans} to gain a better understanding of the biochemical bases of candidiasis. A comparative study of utilization of N-acetylglucosamine (GlcNAC) by pathogenic and non-pathogenic strains was carried out. Non-pathogenic yeasts did not utilize GlcNAC, which suggests that the aminosugar metabolic pathway is important in pathogenesis. The aminosugar metabolic pathway is summarised in Fig. 2. Presently, it is speculative whether the capability metabolize GlcNAC is important for pathogenesis because the sugar in the free form is not available at the site of colonization. However, GLD\textsubscript{NAC} (Chitobiase), which is also induced by GlcNAC\textsuperscript{55} might release GlcNAC residues from glycoproteinase. These residues can be utilized by \textit{C. albicans} for its growth.

1.4 \textbf{CLINICAL FORMS OF DISEASE}

In a clinical approach, the pathogenic fungi could be grouped and discussed on the basis of the type of disease in their host as follows:

<table>
<thead>
<tr>
<th>Infectious Diseases</th>
<th>Allergic Diseases</th>
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<tbody>
<tr>
<td><strong>A. Mucocutaneous candidiasis</strong></td>
<td>Candidids</td>
</tr>
<tr>
<td>Oral: thrush, glossitis, stomatitis</td>
<td>Eczema</td>
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<tr>
<td>cheilitis, perleche</td>
<td>Asthma and</td>
</tr>
<tr>
<td>Vaginitis and balanitis</td>
<td>Gastritis</td>
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<tr>
<td>Bronchial and pulmonary</td>
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<tr>
<td>Alimentary esophagitis</td>
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<tr>
<td>Enteric and perianal disease</td>
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<tr>
<td>Chronic mucocutaneous</td>
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</table>
B. Cutaneous candidiasis

Intertrigious and generalized
Paronychia and onychomycosis
Diapers disease and
Candidial ganuloma

C. Systemic candidiasis

Urinary tract
Endocarditis
Meningitis
Septicemia and
Iatrogenic candidiasis

Banal mycoses of the skin and the adjacent mucous membranes caused by *Candida* species have become increasingly more widespread and constitute today one of the most frequently occurring dermatological infectious diseases. At the same time, there is a new tendency for spread of such infections to inner organs and/or to septicemia, opportunistic infections which virtually constitute new diseases. Detailed knowledge of the changing aspects of *Candida* infections forms the basis of successful prophylaxis and therapy. With respect to their pathogenicity, *Candida* spp. are divided into non-pathogenic, pathogenic and facultative pathogenic classes.\(^{56}\)

A yeast is non-pathogenic only if the host conditions are absolutely incompatible with its metabolism, i.e. if it is not able to metabolize the building materials or the excretory products of the host, or becomes in-activated by them, or cannot tolerate its environment. A yeast is pathogenic if its pathogenic potencies (pp) are more powerful than the defence factors of the non-immunised host under physiological conditions. In the case of immunisation, the resistance of the host may become the prevailing factor.

A yeast is facultatively pathogenic if its pp are weaker than the resistance of the host only to the extent that under normal conditions the host resistance prevails but, under abnormal conditions, the pp may overcome host resistance. It is
generally assumed that a facultative pathogenic yeast can only become pathogenic if the host resistance is lowered by endogenous or exogenous factors, but this is not always true. Such yeast can become parasitic in a relatively high percentage of cases also when the resistance of the host is normal. Under these circumstances, there are two possibilities either the pp of the yeast increases qualitatively or number of yeast increased quantitatively to such a degree that the defense of the host is overpowered.

By far the most important part in the facultative pathogenicity is played by those factors which cause a lowering of the resistance of the host. Those which are known to us are listed in Table 1. They may be either intrinsic or extrinsic factors

Table 1. Principal factors favouring development of *Candida* infections

<table>
<thead>
<tr>
<th>Favourable factors for <em>Candida</em> infections</th>
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<tbody>
<tr>
<td><strong>Intrinsic factors (host)</strong></td>
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<tr>
<td>Physiologic and Pathologic</td>
</tr>
<tr>
<td>Age, Pregnancy, Reticuloendo-thelial disease, Hodgkin’s disease, Sarcoidosis, Lymphoma, Aplastic anemia, Defective cellular immunity, Diabetes Endocrine diseases, AIDS/ARC Genetical disorder</td>
</tr>
<tr>
<td><strong>Extrinsic or intrinsic factors</strong></td>
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<tr>
<td>Drugs</td>
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<tr>
<td>Antibacterials, Antituberculosis drugs, Heroin, Immunosuppressors, Corticosteroids, Antimitotics, Contraceptives</td>
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<tr>
<td>Medico-surgical</td>
</tr>
<tr>
<td>Catheters, Surgery, etc.</td>
</tr>
<tr>
<td>Physical</td>
</tr>
<tr>
<td>X-rays, burns</td>
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</table>
1.5 LABORATORY DIAGNOSES

The clinical manifestations of mucosal or deeply invasive candidiasis are not specific for Candida species and can be produced by other organisms. A laboratory identification of Candida spp. is therefore essential to establishing a definitive diagnosis of candidiasis. Standard approaches to laboratory diagnosis of Candida infections depends on (a) direct inspection of freshly obtained clinical specimens for the presence of Candida spp. (b) recovery of the Candida spp. in cultures of blood, body fluids, biopsy specimens or other tissues and (c) histological identification of Candida spp. Laboratory identification of Candida to the species level has been found to carry prognostic and therapeutic significance. Laboratory methods have been improved during the past two decades to provide more rapid identification of deeply invasive candidiasis for prognosis and therapy. Nevertheless, invasive candidiasis is difficult to diagnose and is the cause of substantial morbidity and mortality in patients. Accordingly investigational non-culture-based diagnostic tests are being developed to facilitate rapid diagnosis and therapeutic monitoring of patients.

1.5.1 Conventional methods of diagnosis of invasive candidiasis.

Regardless of the advances achieved in laboratory diagnosis of invasive candidiasis, clinical evaluation remains the cornerstone of assessment of a patient with possible invasive candidiasis. Table 2 summarizes the conventional and investigative diagnostic modalities for approaching patients with possible invasive candidiasis. Recognition of the subpopulations of patients with a high risk of invasive candidiasis is essential in optimal utilization of limited laboratory resources and the appropriate design of clinical trials. Several findings on physical examination may also suggest or strongly indicate the presence of invasive candidiasis. Examples include persistent or recurrent fever despite antibacterial therapy in neutropenic patients, vitreal opacities of Candida endophthalmitis in nonneutropenic host, erythematous maculopapular cutaneous lesions associated with myalgia; and tenderness in the right or left upper quadrants consistent with hepatic or splenic candidiasis. Clinical assessment also integrates the available diagnostic
tools of blood cultures, diagnostic imaging, tissue cultures and biopsy for establishing a diagnosis of invasive candidiasis. A clinician’s clear understanding of the microbiological diagnosis of invasive candidiasis will improve the cost effective and optimal utilization of the laboratory.

**Table 2. Conventional and investigative approaches to diagnosis of candidiasis**

**Conventional approaches**

- History and physical examination
- Diagnostic imaging
- Microbiology
- Director examination of specimens
- Cultures
- Blood and other normally sterile body fluids
- Tissues
- Mucosal surfaces and
- Histopathology

**Investigational approaches**

- Immunological detection
- Amplification of *Candida* genomic sequences by Polymerase Chain Reaction (PCR)

1.5.2 Specimen collection, transportation and storage. Appropriate methods for collection, transport and storage of clinical specimens are necessary for the detection of *Candida*
spp. Specimens from mucosal or cutaneous surfaces are collected under aseptic conditions. Specimen containers are properly prelabelled before collection to avoid confusion of mixing specimens. All specimens are analysed promptly in the laboratory for mycological investigations with a view to impact optimally on patient care. The storage of the specimen at 4°C may preserve the viability of Candida spp., but not necessary for other fungal organisms.

1.5.3 Direct examination of specimens. Direct examination of a specimen may provide information about the relative amount of Candida spp. present and about the morphological features of the organism such as the presence of blastoconidia (budding yeast forms), pseudohyphae and hyphae.

A direct examination of specimens provides a rapid diagnosis of Candida spp. This may enable the initiation of antifungal therapy. Direct examination of material from mucosal infections, e.g., scrapings from an oropharyngeal lesion, brushings from an esophageal plaque, swab from a vaginal discharge or aliquot of urine will disclose rapidly whether Candida spp. are involved in the invasive process. Direct examination of urine may promptly identify Candida spp. as the cause of a urinary tract infection or as the cause of renal candidiasis. The significance of candiduria, however, is controversial and must be interpreted in the clinical context of patient risk factors and specimen acquisition. In another example, inspection of homogenised liver biopsy tissue for fungal element may rapidly establish a diagnosis of hepatic candidiasis particularly when cultures of biopsy specimens from this disease have a low yield. Mere presence of Candida spp. in oropharyngeal and vaginal secretions are not sufficient to establish a diagnosis of mucosal Candida infection. A direct examination may provide a semiquantitative estimate of the amount of Candida spp. and establish the presence of neutrophils in non-neutropenic patients. Thus, direct examination should be coupled with culture of suspected candidiasis whenever possible.

1.5.4 Histopathology. The usual tissue response to Candida spp. is an acute inflammatory reaction, with polymorphonuclear leukocytes predominating causing microabscesses. In sections
stained with hematoxylin and eosin (H&E), pale-blue yeast-like bodies with pseudohyphae can be seen between pus cells. With appropriate special stains, such as periodic acid-Schiff (PAS) and Gomori’s silver methenamine (GMS), oval blastospores with thin walls are clearly visible measuring 3 to 6 µm, pseudohyphae are usually present. When the organisms are growing on a surface, abundant true hyphae are present and usually interposed between them are clusters of oval blastospores.

Most pathogenic fungi can be identified, at generic level, by histological examination of specimens. Tissues stained with H & E stain used in combination with special histochemical techniques such as PAS, GMS and Grindley stain can help distinguishing between different types of fungi, but there is a need for more specific differentiation. This is especially true in examining tissues of immunosuppressed patients. Several techniques have been demonstrated to aid in the histopathological identification of fungi. These include fungal autofluorescence, calcofluor white, 2-hydroxystilbamide isethionate, blakophor BA 267 percent and diethanol. In general, these methods are more rapid but somewhat less sensitive or specific than conventional fungal stains.

Direct immunofluorescence (IF) staining of fungi in formalin-fixed, paraffin-embedded tissue sections is a rapid, relatively inexpensive, and extremely helpful procedure for confirming a presumptive histologic diagnosis of a mycosis, especially when atypical forms of a fungus are seen.

The histopathological differential diagnosis of fungal infections in immunosuppressed patients is based mainly on the recognition of the characteristic morphologic features of the fungi with the use of histochemical stains. The other important feature is the tissue reaction to the pathogenic organism. However, this tissue reaction depends on the host’s immunologic status which may be altered by the disease or by the therapy. The pathologists who deal with these infections must become familiar with the many therapeutic and supportive measures used in the management of the immunosuppressed patient that may alter the morphologic manifestations of the infections process. In addition, the pathologists must know the specific procedures for handling various types of tissue specimens in order to provide a rapid and accurate diagnosis.
1.5.5 Clinical laboratory identification of Candida species. Identification of Candida spp. is conducted to the species level. The identification of different Candida spp. carries both prognostic and therapeutic significance.

1.5.5.1 Microscopic characters

1.5.5.1(i) Germ tube test. The 2 hours germ tube test permits the distinction between C. albicans (germ-tube positive) and non-albicans Candida spp. (germ tube-negative). The test is performed by suspending a small portion of a colony into rabbit plasma, bovine serum albumin, fetal calf serum, sheep serum, or into a defined medium such as cell culture medium. The suspension is incubated at 37°C for 2 hr. Over inoculation of the test broth may lead to suppression of germination and negative result in detection of C. albicans. The germ tube of C. albicans is distinguishable from the blastoconidial germination of organisms such as C. neoformans. Germ tubes arise as direct parallel cell wall extensions from the yeast. Germination of non-albicans Candida spp. is characterized by a constriction between the growing hyphal cell wall structure and the yeast form. Parallel controls of known C. albicans, C. tropicalis and C. glabrata are used during the test to guide identification of key structures.

1.5.5.1(ii) Chlamydospore test. C. albicans may also be identified by terminal chlamydospore formation on cornmeal agar with tween-80. The microscopic features of other Candida spp. can be distinguished on this medium by the arrangement of blastoconidia and the morphological features of pseudohyphae and hyphae. Other yeasts, such as C. neoformans and C. glabrata, produce only yeast forms and not pseudohyphae or hyphae in this medium. C. neoformans appears as uniformly round polysaccharide blastospores with considerable variation in diameter. The capsule may not be well developed in the agar at the early stage. Table 3 summarizes the differential diagnostic features on cornmeal agar of different yeast isolates.
<table>
<thead>
<tr>
<th>Candida species</th>
<th>Morphological features</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>Pseudohyphae with clusters of round blastoconidia at junctions and septa; true hyphae may be present, terminal thick-walled chlamydospores</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>Blastoconidia are distributed singly or in small clusters along the entire length of the pseudohyphae; true hyphae may be present</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>Overall pattern of curved hyphae conveys a &quot;sage-brush&quot; pattern, true hyphae may be present</td>
</tr>
<tr>
<td>C. krusei</td>
<td>Overall pattern of pseudohyphae and elongated blastoconidia convey a &quot;crossed sticks&quot; pattern</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>Masse features resembling both C. tropicalis and C. parapsilosis with curved hyphae</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>Clusters of small blastoconidia at junctions of short pseudohyphae</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>Small oval to round single-budding nonencapsulated blastoconidia; no pseudohyphae or hyphae are present</td>
</tr>
</tbody>
</table>

* Morphological features of Candida spp. on corn meal agar are not sufficient in the clinical laboratory as the sole source of diagnosis of Candida spp. and are used in conjunction with biochemical and other methods.

* The germ tube test provides a rapid means of determination of C. albicans. C. stellatoidea is morphologically identical and also generates germ tubes. As a distinguishing feature, C. stellatoidea does not assimilate sucrose.
1.5.5.2 Biochemical determinations. Biochemical identification of *Candida* spp. is based on carbohydrate assimilation or carbohydrate fermentation patterns. Assimilation is the ability of an organism (measured by growth) to use sugars (carbon compound) as the sole energy source in the presence of oxygen while fermentation is the ability of an organism to use a compound (sugar) as the sole source of energy in the absence of oxygen (measured by gas production and change in pH). The classical carbohydrate assimilation test of Wickerham and Burton\(^70\) is time tested but labour intensive.

1.5.5.3 Investigational methods of nonculture diagnosis of invasive candidiasis

1.5.5.3(i) Serological tests. Immunologic tests have been proved useful for the diagnosis of candidiasis. Although, positive cultures provide the strongest proof for a fungal infection, cultural methods have several limitations. First, they are generally negative in patients with mild infections. Second, the significance of positive cultures for *Candida* may be difficult to assess, since these organisms may represent colonizers of mucosal or skin surfaces, rather than true pathogens. Serologic tests have proved useful in identifying patients with negative cultures, obviating the need for invasive diagnostic procedures, and providing rapid diagnostic clues on which to base therapy. A number of methods for rapid serologic diagnosis of significant *Candida* infection have been investigated. Classical tests for antibodies to *Candida* antigens have been widely studied, although both sensitivity and specificity have been only suboptimal\(^71\). In recent years, the use of quantitative assays of antibody to specific surface and non-surface antigens has been carried out\(^72\). Unfortunately, the antigenic efficacy of these techniques remains controversial. In this regard, measurement of *Candida* antigens or metabolites has been attempted, since this approach does not depend on a host response.

The use of antibody detection methods is limited on patients who are likely to have intact immune response. Various serological tests\(^73\) that are considered practical include latex agglutination (LA), immunodiffusion (ID), and counter
immunoelectrophoresis (CIE). These reactions show nearly equivalent sensitivities (detection rate) of about 90%, whereas, specificities are higher in ID and CIE than in LA. The CIE test yields results essentially similar to those of the ID test, but much faster.

Mannan, a polysaccharide comprising mainly the Candida cell wall has been detected in the serum by several methods. Kaufman and Reiss demonstrated the detection of mannan antigenemia by double-antibody sandwich enzyme-linked immunosorbent assay (EIA, ELISA). 65-75% sensitivity of detection and a specificity of 100% was noted in human cancer patients. Since, mannan is not a normal serum constituent, concentrations greater than 2 ng/ml give precise evidence of an infection. The sensitivity limit for mannanemia as detected by EIA is 1 ng/ml. The EIA inhibition test can be considered practical, since the enzyme-labelled anti-human immunoglobulin antibody is available commercially, although its sensitivity is lower than that of the double-antibody sandwich EIA.

Recently, a LA test for the detection of circulating Candida protein antigens was introduced commercially. Using this Candida detection system (CAND-TEC), Fung et al. differentiated between C. albicans colonization and disease. With a 1:8 or greater titer as a criterion for dissemination, the sensitivity of the CAND-TEC system was 71%, with a specificity of 88%. On the other hand, Mathews et al. isolated immunodominant 47-Kd antigen from sera from patients. Mathews and Burnie introduced a new dot immunobinding system. The use of the 47-Kd antigen-specific antibody probe increased both the sensitivity and specificity of the assay system, and earlier detection of systemic candidiasis was made possible. The rate of detection of systemic C. albicans infections in neutropenic patients was 77% in the dot immunobinding assay and 29% with the LA test.

1.5.5.3(ii) DNA based diagnostic test

1.5.5.3 (iia) DNA fingerprinting of C. albicans strains. DNA fingerprinting represents a powerful tool for epidemiological studies of C. albicans. DNA isolated from each Candida isolate is digested with a specific endonuclease, separated by gel electrophoresis, transferred to a membrane and hybridized with
the labelled nucleotide or a probe. The pattern obtained is seen on the autoradiogram. This system was recently employed in an analysis of commensalism in 17 body locations of 52 healthy females. Candida was cloned from one or more sites 73% of test individuals. By fingerprinting, it was demonstrated that isolates from different body locations of the same individual were either identical, unrelated, or similar but not non-identical.

1.5.5.3(iib) Amplification of Candida DNA by polymerase chain reaction (PCR). Detection of circulating Candida genome is possible by DNA amplification by PCR. Recent studies of the amplification of the genome of lanosterol C 14-demethylase as a fungus-specific genome was found useful for detection of Candida species in clinical sample. Likewise, Holmes and colleagues used cytochrome P450 gene, as a probe to identify C. albicans in clinical samples. Because this gene is present at a single copy locus in C. albicans, it was necessary to use PCR amplification of the target DNA. Similar type of work has been reported by different workers also. Improvement in PCR methodology may lead to increased sensitivity and specificity. Several laboratories are actively pursuing the development of PCR-based systems for detection of invasive candidiasis and other mycoses especially in immunocompromised patients.

1.6 THERAPY OF CANDIDIASIS

Candida spp. are capable of causing superficial infections that may be treated with topical preparations while disseminated systemic infections require systemic therapy. Candida spp are eukaryotic cells, like humans cells and are not prokaryotic like bacteria, hence, it is not surprising that antifungal therapy against candidiasis is often associated with substantially more toxicity than antibacterial agents. Candida organisms do contain cellular constituents such as glucans, mannans, and glycoproteins etc. that are uniquely different from those of mammalian cells. It might be anticipated that antifungal agents could be designed against the metabolic pathways producing these unique substances and, thus, minimizing toxicity to human cells. But this has not yet proved to be easy to accomplish. A variety
of clinical regimens exists for systemic candidiasis\textsuperscript{84} viz., polyenes: amphotericin B, nystatin, hamycin; imidazoles: ketoconazole, miconazole, clotrimizole, fluconazole; and pyrimidine: 5-fluocytosine etc. Since, these drugs have several limitations on account of their mechanisms of action that are directed against sterols in the cell membrane or against enzymes involved in the nucleic acid synthesis, as a consequence, none of these antifungals are ideal for management of disease due to adverse effects in therapy. Thus, there is a need to evaluate better anticandidal compounds (derived from natural or synthetic sources) which are devoid of such limitations. To overcome such problems we have made efforts to evaluating some product(s) which are discussed in the preceding chapters.
REFERENCES


Figure 1. Microphotograph showing *C. albicans* blastospores (budding yeast, 300 x, Ph)
Figure 2. Aminosugar metabolism in *C. albicans*:

[A] N-acetyl-glucosamine permease,
[B] N-acetylglucosamine kinase,
[C] N-acetylglucosamine-6-phosphate deacetylase,
[D] glucosamine-6-phosphate deaminase,
[E] N-acetylmanno-samine-2-epimerase,
[F] general sugar permease.

*C. albicans* strains seem to vary in their capacity for
*N*-acetyl-*mannosamine* transport. (From Datta et al. 53)