

**California Association for
Medical Laboratory Technology
Distance Learning Program**

***Candida* And Its Role
In Opportunistic Mycoses**

by

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***Candida* And Its Role In Opportunistic Mycoses**

OBJECTIVES

After completing this course the participant will be able to:

1. Describe principal characteristics and classification of *Candida albicans*.
2. List *Candida* species that play a role in human disease.
3. Outline the epidemiology of *Candida* infections.
4. List human illnesses caused by *Candida* species.
5. Summarize factors that contribute to development of candidiasis.
6. Describe methods used in laboratory diagnosis of *Candida* infections.
7. Explain the nature of the immune response to *Candida*.
8. Discuss prevention of *Candida* infections.

I. INTRODUCTION

At the present time microbial infections are most frequently caused by microorganisms that constitute the resident flora of the host rather than by exogenous pathogens. Microorganisms that are generally harmless may become virulent because of changes in the host's resistance or because of an alteration in the composition of the host's microbial flora, usually caused by antibiotic therapy.

During the past 25 years fungi have emerged as a major cause of human illnesses. Infection with the yeast *Candida* is the most frequent cause of fungal disease. These yeasts are members of normal human microbial flora. They are common in the gastrointestinal and genital tracts. *Candida* species have also been isolated from the respiratory tract, mouth, skin, ear, and eye. *Candida* is a true opportunistic pathogen that under certain circumstances is able to invade tissues normally resistant to infection.

II. PRINCIPAL CHARACTERISTICS AND CLASSIFICATION OF *CANDIDA*

A. Classification

Yeasts and other fungi are classified on the basis of the sexual stage in their life cycle. However, some fungi reproduce only asexually while many other fungal species have both an asexual and a sexual state. Often only one method of reproduction can be observed under specific conditions, even among fungi that are capable of both asexual and sexual reproduction. Fungi with no demonstrable sexual phase are placed, for convenience, into a provisional taxonomic group, Fungi Imperfecti.

Once a sexual state of reproduction has been observed for a particular fungal species, the organism is assigned a separate name from the one that identifies its asexual state. This dual naming system is complex and may be confusing. For example, *Candida krusei* is *Issatchenkia orientalis* (sexual phase). Similarly, *Candida guilliermondii* has been renamed *Pichia guilliermondii* (sexual phase).

The specific type of sexual spore produced by a particular fungus determines its classification. Many species of *Candida* have been shown to form ascospores: sexual spores formed in a sac-like structure called the ascus. *Candida*, therefore, is now grouped with the ascomycetes.

The current classification of *Candida* is as follows (1):

Kingdom	Fungi
Phylum	Ascomycota
Class	Hemiascomycetes
Order	Saccharomycetales
Family	Candidaceae
Genus	<i>Candida</i>

B. Principal Characteristics of *Candida*

Yeasts that belong to the genus *Candida* are widely spread in the environment. The group is highly heterogeneous and contains close to 200 species. Many members of this group are of medical importance: 50% to 70% of yeasts isolated from human sources are *Candida* species. The most common isolate is *C. albicans*, followed by *C. tropicalis*, *C. parapsilopsis*, and *C. glabrata*. Among these isolates, *C. albicans* is most frequently associated with human disease.

1. *C. albicans* morphological forms

a. Yeast and hyphal forms:

The microscopic appearance of *C. albicans* is that of a unicellular budding yeast. The cells are generally round to oval, but may be elongated or irregular in shape. *Candida* multiplies by production of blastoconidia (buds). When blastoconidia are produced one from the other in a linear fashion without separating, a structure known as a pseudohypha is formed. The degree of elongation of pseudohyphal cells may vary but they always show a constriction at the septum which separates individual cells. By contrast, true hyphae have parallel walls at their septa. Under certain growth conditions, such as reduced oxygen tension, true septate hyphae may be formed. Thus, depending on the specific growth environment, *C. albicans* may grow as a yeast or in a hyphal form. A switch from one mode of growth to the other may occur in response to external and internal signals. This ability of growing in either the yeast or the hyphal pattern is known as dimorphism.

b. Production of chlamydospores:

Chlamydospores of *C. albicans* are distinct from the reproductive yeast or hyphal states. They are large spores with thick walls and a high lipid and carbohydrate content. These spores may be formed when the growth environment is low in oxygen, light, temperature, and nutrients. Chlamydospores may represent a resting state although their function is not precisely known.

c. Colony morphology:

Some strains of *C. albicans* are able to switch from the typical smooth, domed white to cream-colored colony morphology to colonies that are more gray and flattened. This is known as the white-opaque switch. Opaque-form cells are elongated and have a cell wall with frequent pits. Recent evidence indicates that the opaque-white transition is associated with the mating process in *C. albicans* (2).

d. The parasexual cycle in *C. albicans*:

Genetic recombination and meiosis generate diversity in progeny strains. However, *C. albicans* has a parasexual cycle which provides an alternative pathway for generating strain diversity. An elaborate mating system promotes conjugation between compatible diploid strains of *C. albicans*. The product of this mating is a tetraploid cell that undergoes reduction division in order to return to the diploid state. Progeny strains show

altered morphology on laboratory media, demonstrating that parasexual cycle may produce phenotypic variants. The process of meiosis has not been observed for *C. albicans* (3).

e. Biofilm formation

When *C. albicans* is present in its natural environment, such as the human intestinal tract, the microbial growth is closely associated with the biological surfaces present in that environment. Interaction with a solid surface alters *C. albicans* behavior and frequently leads to biofilm formation.

A biofilm is a three-dimensional community of microorganisms embedded in a matrix of polymers and attached to a surface. A well-known example of a microbial biofilm is dental plaque.

Production of biofilms by *C. albicans* proceeds in several stages. In the beginning yeast cells attach to a surface. After attachment, cells proliferate on the surface, forming microcolonies. This is followed by continued cell replication, production of hyphae, and secretion of extracellular polymers. These polymers are carbohydrates and proteins as well as unidentified components. They surround the growing cells, forming a three-dimensional matrix typical of a biofilm. A number of factors affect the content of the matrix and the morphology and organization of yeast cells. Among these factors are availability of nutrients, proportion of yeast-form cells and hyphal-form cells, concentration of glucose in nutrients that surround the biofilm (glucose favors biofilm formation) and the nature of the contact surface. One of the most troublesome characteristics of biofilms is their high level of resistance to antibiotics. The reasons for antibiotic resistance differ in early and in mature biofilms. Changes in gene expression contribute to antibiotic resistance in the early stages of biofilm formation while an alteration in membrane sterol content may be responsible for such resistance in mature biofilms (4).

Quorum sensing (communication between microbial cells via signaling molecules) also regulates biofilm formation. The quorum-sensing molecule farnesol is produced by growing *C. albicans* cells. It acts as an inhibitor of the transition from the yeast form to the hyphal state (formation of hyphae increases *Candida* virulence). Exposure of biofilms to farnesol reduces biofilm formation *in vitro*. In contrast, studies in mice with disseminated candidiasis have shown that farnesol inhibits production of cytokines, such as interferon gamma and interleukin 12. Since these cytokines are important in defense against *Candida* infections, farnesol might be considered a *Candida* virulence factor.

2. *Candida albicans* genome

The *C. albicans* genome sequencing was initiated in 1996. At the present time two complete *C. albicans* genome sequences are available: *C. albicans* strain SC 5314 (Stanford) and strain WO1 (MIT and Harvard). The *C. albicans* genome has eight chromosomes.

In 2002 a *Candida* genome database was established. This database now houses the latest genome sequence versions of *Candida* strains SC 5314 and WO1, as well as six genome sequences from species closely related to *C. albicans*.

The *C. albicans* genome is highly dynamic and shows great variability. It is characterized by structural chromosomal rearrangements and chromosomal deletions.

The sequencing of *C. albicans* genome uncovered a great deal of new information about this microorganism, including the discovery that *C. albicans* has an alternate parasexual cycle of reproduction.

III. ROLE OF *CANDIDA* IN HUMAN DISEASE

Although *Candida* species are part of the normal microbial flora, they are the most common fungal pathogens that affect humans.

A. Epidemiology of candidiasis

Candida species are generally found as commensal organisms in association with human or animal hosts. An epidemiological study found that 7.1% of infants were colonized by *Candida* on the day of birth. The rate of colonization increased to 96% after one month. The majority of adults are colonized by *Candida*, primarily in the intestinal tract although other body surfaces may also be colonized. An individual may carry a particular strain for long periods of time, but changes in the colonizing strain or the presence of unrelated strains at different sites may also occur. Apparently growth on biological surfaces is part of the natural lifestyle of *Candida*.

The environment of the host determines whether *Candida* remains a commensal or proliferates, invading tissues and causing disease. As long as the immune system is fully functional and normal microbial flora and host tissue integrity are maintained, invasion of tissues by *Candida* does not occur. Once host factors favor tissue invasion by *Candida*, either mucocutaneous or disseminated candidiasis may occur. Although the source of the infectious process appears to be endogenous, *Candida* may be acquired from the environment. Studies in hospitals have demonstrated the presence of *Candida* in the hospital environment: in foods, on counter tops, in air-conditioning vents, on floors and respirators. *Candida* had also been isolated from hospital personnel.

B. Host factors associated with candidiasis (5):

1. Age: the highest incidence of candidiasis is in infants under one year of age and in persons over 65 years of age
2. Race: the incidence of bloodstream *Candida* infections (candidemia) is at least fourfold higher in African American patients
3. Disruption of mucosal and cutaneous barriers
4. Defects in cell-mediated immunity
5. Neutrophil dysfunction
6. Metabolic disorders
7. Bone marrow transplantation
8. Solid organ transplantation
9. Use of indwelling devices, such as catheters and feeding tubes
10. Solid neoplasm
11. Recent chemotherapy or radiation therapy
12. Corticosteroids
13. Broad-spectrum antibiotics
14. Burns
15. Prolonged hospitalization
16. Premature birth
17. Hemodialysis
18. Extensive use of anti-fungal drugs

In addition to host factors listed above, occurrence of candidiasis is affected by *Candida* virulence factors.

C. *Candida* virulence factors

1. Adhesins

Candida adhesins are glycoproteins located on the surface of the cell wall. Adhesins mediate *Candida* interactions with other cells and play an important role in mating, changes in colony morphology, and in biofilm formation. Since adhesion to tissue cells is the first step in pathogenesis, *Candida* adhesins are of major medical importance.

Environmental factors may influence the process of adhesion. One of these factors is a high concentration of sugars, which enhances *Candida* adhesion to tissue cells. The presence of sucrose, glucose, and fructose in the yeast environment is particularly effective in promoting yeast adhesion to host cells. This effect of carbohydrates on yeast adhesion is of clinical importance. For instance, high concentrations of carbohydrates in the oral cavity are implicated in the development of oral candidiasis.

2. Integrin receptors

Integrin receptors are plasma membrane proteins that serve a multiplicity of functions. *Candida* has integrin proteins with antigenic and functional similarities to human complement receptors 3 and 4. It is possible that some of these fungal proteins mediate adherence of *Candida* to cells.

3. Non-specific interactions that facilitate cell adhesion

An additional method of cell adhesion is non-specific interaction between yeast surface and that of the host cell mediated by hydrophobic and electrostatic forces.

4. Proteolytic enzymes

Candida degradative enzymes protease and phospholipase are important for tissue invasion.

5. Dimorphism

The ability to form pseudohyphae plays an important role in *Candida* pathogenicity. When fungi colonize an epithelial or epidermal surface they adhere to host cells. Conversion of yeast-form cells to hyphal-form cells permits further penetration and invasion of tissues.

6. Biofilm formation

Contact with surfaces elicits formation of biofilms by *Candida*. This is of major clinical significance because of the large numbers of implanted medical devices used in modern medicine. *C. albicans*, which is generally more pathogenic than other *Candida* species, consistently produces more biofilm *in vitro* than non-*C. albicans* isolates.

IV. *CANDIDA* INFECTIONS AND CLINICAL DISEASE

Candida species cause a wide spectrum of diseases. These range from superficial mucocutaneous infections to invasive diseases involving internal organs as well as bloodstream infections. Technological advances in medicine have created new opportunities for *Candida* to gain access to the circulation and deep tissues. *Candida* species are emerging as an important cause of hospital-acquired (nosocomial) infections: 8% to 10% of all bloodstream infections acquired in the hospital are caused by *Candida* species. Critically ill patients present a prime target for candidiasis as do immunosuppressed patients and those with indwelling medical devices.

Candida infections fall into two general categories: localized infections of mucous membranes or skin, and systemic infections.

A. Mucous membrane *Candida* infections:

The most common sites of *Candida* infections of mucous membranes are 1. oral cavity and 2. vaginal canal.

1. Oral Infection

In the fourth century B.C. the oral infection, thrush, was noted and described by Hippocrates. This infection is the most common manifestation of candidiasis in humans. Among the predisposing factors are high carbohydrate concentration in the oral cavity, changes in the normal microbial flora after prolonged antibiotic therapy, low pH of the salivary secretions in the newborn, and immunosuppression. Oral candidiasis (thrush) is now recognized as a condition that defines AIDS: almost 100% of AIDS patients have this disease.

Thrush is manifested by white patches or plaques on the tongue and on the oral mucosa. These may coalesce into a membrane. Although *C. albicans* is the primary cause of thrush, a closely related species, *C. dubliniensis*, has also been implicated in this condition.

Occasionally oral candidiasis may spread to other areas of the gastrointestinal tract. Infection of the esophagus may occur. In many cases oral disease is not present when the esophagus is involved but the patient has a history of chemotherapy, treatment with broad-spectrum antibiotics or inhaled steroids, and the presence of HIV infection or malignancies. Other portions of the gastrointestinal tract may be infected with *Candida*. The gastroesophageal junction in particular is a common site for *Candida* infections, possibly due to a low pH at that site.

The respiratory tract is frequently colonized with *Candida* species, especially in hospitalized patients. Rare cases of infections of the trachea, larynx, and bronchi may occur.

2. Candidal vulvovaginitis:

Candida is a common cause of vulvovaginitis. It is estimated that 75% of women will experience a case of *Candida* vaginitis in their lifetime. *C. albicans* is the causative agent in approximately 85 to 90% of cases. The remaining cases are caused by other species of *Candida*, the most common of which are *C. glabrata* and *C. tropicalis*. Prevalence studies show that 20 to 25% of healthy, asymptomatic women have positive vaginal cultures for *C. albicans*. Factors involved in the transformation from asymptomatic colonization to symptomatic vaginitis are not fully understood. A deficiency in cell-mediated immunity may be a factor in the activation of *Candida* infection.

A number of patients diagnosed with a primary case of candidal vulvovaginitis will experience a recurrence of the disease. Recurrent episodes of vaginitis may appear as early as a few days to 3 months after cessation of apparently successful treatment. Frequently the cause for disease recurrence is not known. In some cases diabetes, hormone replacement therapy, or immunosuppression may be involved.

B. Cutaneous candidiasis:

Infections of the skin commonly occur at sites where skin areas are in close proximity, as in webs of fingers and toes. Diaper rash of newborns is also a common *Candida* infection. Hair follicles and nails may become infected with *Candida*.

Occasionally, generalized cutaneous candidiasis may occur. Metastatic skin lesions have been observed in patients with disseminated candidiasis.

C. Chronic mucocutaneous candidiasis

This is a group of *Candida* infections of the skin, hair, nails, and mucous membranes that have a protracted course. These conditions are generally associated with endocrine or immunological disorders: Addison's disease, diabetes mellitus, hypothyroidism, auto-immune diseases, and other similar conditions.

D. Systemic candidiasis:

Candida may spread from the original site of infection and enter the bloodstream. Risk factors for candidemia (bloodstream infection) include treatment with broad spectrum antibiotics, parenteral nutrition, cancer chemotherapy, immunosuppressive agents following organ transplantation, and indwelling medical devices. In hospitalized patients *Candida* species are the fourth most common cause of bloodstream infections. Mortality rates for candidemia patients are very high even when the patient is treated with anti-fungal drugs. The reported mortality rates range from 19% to 49%. Inadequate anti-fungal therapy of the patient is associated with increased risk of an adverse outcome.

E. Medical devices and candidemia:

Devices such as vascular catheters, endotracheal tubes, prostheses, urinary catheters, hemodialysis grafts, prosthetic valves, pacemakers, and ventricular assist devices represent a major risk factor for candidemia. Microbial cells grow on the surface of implanted devices forming biofilms. Organisms in biofilms are relatively refractory to antifungal drugs. Therefore, biofilm-associated infections may recur and removal of the medical device may be necessary. Central venous catheters are the most common risk factor for candidemia in patients hospitalized in intensive care units where more than half of bloodstream infections are related to catheter use. Twenty one percent of patients with urinary catheters and bloodstream infections have candidemia.

F. Disseminated *Candida* infections:

Once microorganisms enter the bloodstream they may initiate infection in any of the internal organs. *Candida* infections of the eye, kidneys, heart valves, meninges, musculoskeletal system, liver, and spleen have been reported. Many of these organ infections are due to secondary spread of *Candida* as a result of bloodstream infection. Some however are caused by medical implants or by direct infection during surgery.

V. *CANDIDA* SPECIES IMPLICATED IN CLINICAL DISEASE

C. albicans is involved in the majority of cases of systemic candidiasis. During the past 10 years, however, other species of *Candida* have been isolated with increasing frequency from patients with systemic *Candida* infections. Among non-*albicans* isolates *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* have been found most frequently.

A number of other *Candida* species have been occasionally isolated from cases of candidiasis. These species are listed below:

<i>C. krusei</i>	<i>C. norvegensis</i>
<i>C. guilliermondii</i>	<i>C. dubliniensis</i>
<i>C. lusitaniae</i>	<i>C. lipolytica</i>
<i>C. kefyr (C. pseudotropicalis)</i>	<i>C. zeylanoides</i>
<i>C. rugosa</i>	<i>C. pelliculosa</i>
<i>C. famata</i>	<i>C. nivariensis</i>
<i>C. inconspicua</i>	<i>C. bracarensis</i>

Candida glabrata has been considered a relatively non-pathogenic member of the normal flora of human mucosal tissues. With the increased use of immunosuppressive drugs *C. glabrata* infections have become more frequent. Persons infected with human immunodeficiency virus are at particular risk of infection with *C. glabrata*. This yeast differs from other *Candida* species in its nondimorphic blastoconidial morphology and a haploid genome. At the present time approximately 15-20% of cases of invasive candidiasis are caused by *C. glabrata*. Strains of *C. glabrata* are frequently resistant to antifungal drugs.

Candida krusei is known as an important fungal pathogen for patients with hematological malignancies and for transplant patients. This organism is of medical importance because of an intrinsic resistance to the antifungal drugs ketoconazole and fluconazole. *C. krusei* is also poorly susceptible to many other antifungal medications.

Candida lusitaniae is of clinical significance because it is frequently resistant to amphotericin B.

Candida parapsilosis is a common non-*albicans* species isolated from blood cultures. The frequency of bloodstream infection with this species has increased in recent years. Fortunately, candidemia due to this species is associated with a lower mortality rate than bloodstream infections caused by other *Candida* species. *Candida parapsilosis* is known for its ability to form biofilms on implanted medical devices, such as vascular catheters. This organism persists in the hospital environment and may be spread to patients by medical personnel. Strains of *C. parapsilosis* are often resistant to antifungal medications.

Candida tropicalis is an important cause of candidemia in patients with leukemia and in those who have undergone bone marrow transplantation. Some strains have inherent resistance to amphotericin B, the newer azole drugs, and to echinocandins.

Candida dubliniensis was first described in 1995. It is genetically related to *C. albicans* although the latter species is much more pathogenic. *C. dubliniensis* is associated with oral infection of diabetics and AIDS patients. Occasionally, *C. dubliniensis* had been recovered from systemic infections. This species has a reduced capacity to produce hyphae, resulting in lower levels of colonization and tissue invasion.

Candida nivariensis and *Candida bracarensis* are two recently described species of *Candida*. These species are phenotypically related to *C. glabrata* but can be differentiated from *C. glabrata* by molecular techniques. *C. nivariensis* and *C. bracarensis* have been recovered from clinical specimens.

VI. PATHOGENESIS OF *CANDIDA* INFECTIONS

Invasion of tissues by *Candida* is aided by hyphal development. The transformation of budding yeasts to hyphal growth is promoted by physical contact with surfaces and is under genetic control. When fungi colonize an epithelial or epidermal surface, they adhere to host cells and create depressions in the surface of host cells. As yeast-form cells convert to the hyphal form, the hyphae are able to penetrate into the surface of the tissue layer. The direction of hyphal growth is determined by the topography of the substratum. Hyphae are guided by ridges in the tissue layer. This behavior is known as thigmotropism. It plays an important role in the guidance of hyphal growth and in disease progression. Tissue invasion by *Candida* is facilitated by the action of degradative enzymes secreted by the pathogen and by mechanical forces exerted by the hypha.

During invasion of endothelial surfaces by *Candida* the initial entry of the pathogen into tissue cells is by endocytosis. The surface of the endothelial cells is damaged, resulting in exposure of the underlying basement membrane, which may then be invaded by hyphal-form cells. The invading pathogen exits from the endothelial cells and penetrates the surrounding tissues. This process sets the stage for a disseminated infection.

VII. IMMUNE RESPONSE IN *CANDIDA* INFECTIONS

Candida species are present as commensals in the human gastrointestinal tract and on other mucous membrane surfaces. *Candida*'s persistence in host tissues can be partly explained by the immunosuppressive properties of the *Candida* cell wall glycoproteins. The presence of this microorganism in host tissues creates a continual exposure of the host's immune system to fungal antigens. However, because of the ability of *Candida* to alternate between yeast and hyphal morphological forms, the immune response generated to antigenic determinants on the yeast form may be ineffective against the hyphal form. This complicates the ability of the immune system to provide a defense against *Candida* infections. Nevertheless, some aspects of innate and adaptive immunity are effective in controlling tissue invasion by *Candida*.

A. Role of innate immunity:

Major protection against systemic candidiasis appears to be the function of polymorphonuclear neutrophils (PMNs), macrophages, and dendritic cells. This premise is supported by the high incidence of invasive and systemic candidiasis in patients with defective neutrophils. In addition to microbicidal activity, neutrophils may exert an immunoregulatory action on T helper cells. Macrophages contribute to antifungal defense by phagocytosis and destruction of microorganisms. Dendritic cells provide a link between innate and adaptive immunity and are critical in initiating humoral and cellular immune responses. Dendritic cells phagocytose the microbial cells at the site of infection, transport them to the lymph nodes, and provide signals to local and systemic T helper cells. Different subsets of T cells may be activated and a number of cytokines produced, including interferon gamma, which is a key cytokine in controlling fungal infections.

B. Adaptive immune response:

Cell-mediated immunity is thought to be the major host defense mechanism for protection against mucosal candidiasis. There is a direct causal relationship between impaired cell-mediated immunity and incidence of chronic mucocutaneous candidiasis. Patients with immunosuppression and those with AIDS have a high incidence of mucosal candidiasis. During the cell-mediated immune response T cells of different subsets are activated and a number of cytokines are released.

C. Humoral immunity:

The role of antibody in protection against both mucosal and systemic candidiasis is controversial. The majority of patients with active mucosal *Candida* infections have normal or elevated levels of anti-*Candida* antibodies in both serum and mucosal secretions. In addition, persons with B cell deficiencies do not show increased susceptibility to candidiasis. Nevertheless, there is clinical evidence that particular types of anti-*Candida* antibodies are protective, such as antibodies to certain *Candida* proteins.

VIII. TREATMENT AND PREVENTION OF *CANDIDA* INFECTIONS

A. Treatment of *Candida* infections:

Treatment of *Candida* infections depends on a number of factors:

1. Anatomic site of the infection
2. Patient's underlying illness and immune status
3. Patient's risk factors for disease
4. Specific species of *Candida* responsible for infection
5. Susceptibility of the infecting strain to antifungal drugs

Localized cutaneous candidiasis may be treated with topical antifungal medications, such as clotrimazole, econazole, ciclopirox, miconazole, ketoconazole, and nystatin. *Candida* infection of the soft tissue around a nail may be treated with either oral fluconazole or itraconazole. If an abscess is present in the soft tissue it has to be drained prior to drug therapy.

Nail infections by *Candida* are treated with oral itraconazole. The treatment may continue for a number of months.

Oral candidiasis may be treated with topical antifungal drugs, such as nystatin and clotrimazole, or with systemic drugs such as amphotericin B or the oral azoles, fluconazole or itraconazole.

Candida infection of the esophagus requires systemic therapy, generally with fluconazole or itraconazole.

Genital *Candida* infections may be treated with either topical antifungal agents or single-dose oral fluconazole.

In *Candida* infections involving urinary catheters, the catheter needs to be removed or replaced. In non-catheterized patients with urinary infection, fluconazole is generally used.

Infections of internal organs require systemic antifungal therapy. Fluconazole or amphotericin B is generally used.

For candidemia, fluconazole has been the drug of choice. Alternative options include caspofungin acetate, anidulafungin, micafungin, voriconazole, and amphotericin B (or a liposomal preparation of amphotericin B that is less toxic to the kidneys).

Treatment of invasive candidiasis with a fungicidal monoclonal antibody preparation is under investigation.

B. Prevention of Nosocomial *Candida* Infections:

Several strategies can be used for prevention of candidal nosocomial infections. The first strategy is to implement an educational program in order to increase the compliance of hospital personnel with hand washing recommendations. The use of alcohol or chlorhexidine will decrease the risk of patients acquiring *Candida* infection from hospital staff. The second strategy is to improve the placement and care of central venous catheters. The third possibility for intervention is to stress the importance of exposure to antibiotics as a risk factor for candidemia. In addition, antifungal prophylaxis may be considered. In patients with low neutrophil blood counts administration of fluconazole has proven effective in decreasing infections due to *C. albicans*, *C. tropicalis*, and *C. parapsilosis*. Several independent studies have shown that the use of azole prophylaxis reduced the risk of invasive candidiasis by 50% to 80%.

Experimental studies with a vaccine based on an adhesin from *C. albicans* have shown increased survival of mice with disseminated candidiasis. Continued studies of *C. albicans* vaccine are in progress.

IX. LABORATORY DIAGNOSIS OF *CANDIDA* INFECTIONS

A. Direct examination of specimens

Wet mounts in a 10% to 30% potassium hydroxide solution are useful for distinguishing fungi in mucoid secretions or in the skin, hair or nails. A drop of calcofluor white stain may be added to the potassium hydroxide wet mount. The calcofluor stain fluoresces under ultraviolet light when it binds to polysaccharides in the chitin in the fungal cell wall. This enhances fungal detection as the fungus wall appears bright green. A wet mount of the clinical specimen allows observation of the size and shape of the microorganism, morphology of buds, possible presence or absence of capsules, presence of pseudohyphae, and presence of arthroconidia.

B. Isolation of *Candida* from clinical specimens

Candida grows readily on routine mycological medium. Either Sabouraud glucose (4%) agar or a chromogenic agar may be used. The temperature of incubation is important since pathogenic yeasts grow at 37°C while saprophytic organisms prefer a lower growth temperature. Growth may appear in 24 hours and typical colonies are visible in 48 hours.

C. Chromogenic agars

Presumptive identification of one or more *Candida* species may be obtained with a chromogenic agar medium. CHROMagar *Candida* (Becton Dickinson/BBL) allows presumptive differentiation of over 10 species. The medium is based on the differential release of chromogenic breakdown products from various substrates as a result of enzymatic activity. CHROMagar is useful for the presumptive identification of *C. albicans*, *C. tropicalis*, and *C. krusei*. CHROMagar *Candida* does not clearly distinguish between *C. albicans* and the closely related *C. dubliniensis*, although *C. dubliniensis* colonies usually have a darker green color. Identification of *C. dubliniensis* colonies may be confirmed with a commercial latex agglutination test, the Bichro-dubli test (Fumouze). The test consists of blue latex particles coated with a monoclonal antibody which reacts

specifically with an antigen on the surface of *C. dubliniensis*. The test is highly sensitive and specific.

When using CHROMagar, the directions of the manufacturer must be strictly followed. Additional tests are generally required for definitive identification of colonies. Recently other chromogenic culture media have been introduced.

D. Germ tube test

The germ tube test is one of the most rapid and simple tests for presumptive identification of *C. albicans*. Other species, such as *C. tropicalis* and *C. dubliniensis*, also form germ tubes, but the morphology of the germ tube formed by *C. tropicalis* differs from that formed by *C. albicans*. *C. dubliniensis* will not form germ tubes in a commercial synthetic germination medium.

E. Carbohydrate assimilation tests

Identification of yeasts to the species level is based on carbohydrate assimilation tests. These tests measure the yeasts' ability to utilize a specific carbohydrate as the sole source of carbon. At the present time a number of commercial kits (API, BBL) and automated and semiautomated systems are available for testing assimilation of carbohydrates by yeast isolates.

F. Cornmeal agar culture

Growth of yeast isolates on cornmeal agar can provide important information for species identification. Chlamyospore formation by *C. albicans* may be observed on cornmeal agar. *C. parapsilosis* on cornmeal agar produces multiple areas of satellite "spider colonies" along the streak line. *C. kefyr* and *C. krusei* form elongated blastoconidia in a "log-in-stream" arrangement when growing on cornmeal agar.

G. Rapid identification tests

In addition to biochemical test kits used for identification of multiple genera, there are kits for identification of *C. albicans*, *C. krusei*, and *C. glabrata*. Some examples are the *C. albicans* screen, Bichlorate krusei, Rapidec albicans, germ tube kit, BactiCard Candida, and Albicans ID. Commercial kits for diagnosis of disseminated candidiasis have been developed. These tests target *Candida* protein or carbohydrate antigens.

H. Nucleic acid detection tests

Molecular tests for identification of *Candida* are not routinely used in the clinical laboratory. These tests are very useful in epidemiological studies as well as for research purposes and in reference laboratories.

A commercial peptide nucleic acid fluorescent in situ hybridization kit (PNA-FISH) has been recently approved. The kit is designed to detect *C. albicans* in blood cultures by targeting specific rRNA sequences. Peptide-nucleic acid chains are used as a probe. The probe is coupled to a fluorochrome which is detectable when the probe binds to its target, the species-specific rRNA sequences.

A number of other nucleic acid detection tests are in the process of development.

SUMMARY

Candida species are yeasts that are widely distributed in the environment and are members of the normal microbial flora of the human body. These yeasts are commonly found in the human gastrointestinal and genital tracts. They may also be present in the respiratory tract, mouth, skin, ears, and eyes. The genus *Candida* is very heterogeneous and contains close to 200 species. A number of *Candida* species are capable of causing human infections. *C. albicans* is most commonly found in association with human illness.

Some species of *Candida* are able to form pseudohyphae and to grow either as yeasts or in a hyphal form. The switch from one mode of growth to the other is called dimorphism. The ability to form hyphae facilitates invasion of tissues by *Candida*. Certain species of *Candida* form biofilms on surfaces. This enables *Candida* to colonize implanted medical devices and gain access to deep tissues.

The course of *Candida* infections depends on a number of factors related to the host as well as on the virulence attributes of the pathogen. Host-related factors include metabolic disorders, disruption of mucosal and cutaneous barriers, defects in cell-mediated immunity and neutrophil function, extremes of age, chemotherapy, prolonged hospitalization, and implanted medical devices. Virulence attributes of the pathogen include cell surface receptors that play a role in attachment of *Candida* to host cells, proteolytic enzymes, dimorphism, and biofilm formation.

Candida infections may be localized or systemic. Infections of mucous membranes include thrush and vulvovaginitis. A common example of a skin infection is diaper rash of newborns. Systemic candidiasis involves the spread of *Candida* from the original site of infection to the bloodstream or internal organs. *Candida* species are the fourth most common source of bloodstream infections in hospitalized patients. *C. albicans* is responsible for most cases of systemic candidiasis but other *Candida* species are also involved.

Immune response to *Candida* involves participation of innate as well as adaptive immunity. Protection against systemic candidiasis appears to be the function of neutrophils, macrophages, and dendritic cells. Protection against mucosal candidiasis depends on a cell-mediated response to *Candida*.

Treatment of candidiasis depends on the site of infection as well as on other factors. Topical antifungal drugs are used to treat localized *Candida* infections. Other types of infections are treated with oral fluconazole, other azoles, and with amphotericin B.

Laboratory identification of *Candida* is based on a direct microscopic examination of the clinical specimen followed by culture on a suitable mycological medium. Isolated colonies are identified by various tests, including germ tube formation, growth on cornmeal agar, and carbohydrate assimilation. A number of commercial kits are available for identification of *Candida*. Nucleic acid tests are not used routinely but a commercial nucleic acid detection kit (PNA-FISH) is now available.

REFERENCES

1. Hazen KC, Howell SA. *Candida, Cryptococcus, and other Yeasts of Medical Importance*. In: Murray PR, Baron EJ, Jorgenson J, Landry M.L. *Manual of Clinical Microbiology*. 9th ed., Washington, D.C. ASM Press; 2007:1762-1788.
2. Whiteway M, Bachewich C. Morphogenesis in *Candida albicans*. *Annual Rev. Microb.* 2007;61:529-553.
3. Forche A, Alby K, Schaefer D, Johnson AD, Berman J, Bennett RJ. The Parasexual Cycle in *Candida albicans* Provides an Alternative Pathway to Meiosis for the Formation of Recombinant Strains. *PLoS Biology*, 2008;6(5):1084-1097
4. Kumamoto CA, Vences MD. Alternative *Candida albicans* Lifestyles: Growth on Surfaces. *Annual Rev. Micro.* 2005;59:113-133.
5. Hidalgo JA. Candidiasis. Available at: <http://www.emedicine.com>. December 15, 2006.

REVIEW QUESTIONS

Course #DL-986

Choose the **one** best answer:

1. *Candida* species
 - a. Never cause clinical disease
 - b. Are found only in soil
 - c. May be present in the hospital environment
 - d. Are always pathogenic

2. *Candida* are classified with
 - a. Ascomycetes, on the basis of ascospore production
 - b. Bacteria, because of their small size
 - c. Mushrooms, because *Candida* forms basidiospores (sexual spores)
 - d. Slime molds, because of hyphal formation

3. The most common *Candida* isolate from human infections is
 - a. *C. glabrata*
 - b. *C. dubliniensis*
 - c. *C. stellatoidea*
 - d. *C. albicans*

4. Dimorphism is a term which
 - a. Describes the sexual phase of reproduction in *Candida*
 - b. Describes a switch between yeast and hyphal form of growth
 - c. Does not apply to *Candida* species
 - d. Describes a pattern of growth found only in non-pathogenic fungi

5. Chlamydospores
 - a. Are resting spores formed by *C. albicans*
 - b. Are fungal reproductive spores formed during sexual reproduction
 - c. Are not produced by *Candida*
 - d. Are typically produced by spore-forming bacteria

6. Genetic diversity in *Candida* strains is generated
 - a. Through sexual reproduction and meiosis
 - b. By the parasexual cycle of reproduction
 - c. Through budding
 - d. By forming pseudohyphae

7. Biofilms are
 - a. Special culture media for fungi
 - b. Never produced by *Candida* species
 - c. Easily destroyed by antibiotics
 - d. Produced by *C. albicans* on the surface of implanted medical devices

8. Factors that affect *Candida* infections include the following:
 - a. Diets high in vegetables
 - b. Defects in cell-mediated immunity
 - c. Patient age between 20 and 40
 - d. Poor vision

9. *Candida* virulence factors include:
 - a. Blastospores
 - b. Parasexual cycle
 - c. Dimorphism
 - d. Chlamydo spores

10. *C. albicans* causes the following diseases:
 - a. Chickenpox
 - b. Ulcers
 - c. Thrush
 - d. Common cold

11. Candidemia is a
 - a. Bloodstream *Candida* infection
 - b. Skin disease
 - c. Severe rash
 - d. Form of arthritis

12. Risk factors for candidemia include:
 - a. Excess coffee consumption
 - b. A high calorie diet
 - c. Presence of indwelling medical devices
 - d. Lack of exercise

13. Systemic candidiasis
 - a. Frequently affects healthy adults
 - b. Is easily cured with antifungal drugs
 - c. Is caused only by *C. albicans*
 - d. May be caused by *C. glabrata*, *C. tropicalis*, or *C. parapsilosis*

14. Cell-mediated immunity is important in protection against
 - a. Systemic candidiasis
 - b. Mucosal candidiasis
 - c. Neither systemic nor mucosal candidiasis
 - d. Both, systemic and mucosal candidiasis

15. *Candida* infections may be successfully treated with:
 - a. Streptomycin
 - b. Fluconazole
 - c. Aspirin
 - d. Penicillin

16. Measures for prevention of hospital-acquired candidemia may include:
 - a. Treatment with antifungal drugs
 - b. Increased use of broad-spectrum antibiotics
 - c. Parenteral nutrition
 - d. Daily aspirin

17. The calcofluor white stain
 - a. Can be used in place of Gram stain
 - b. Is not suitable for examining yeast cells
 - c. Improves detection of fungi in wet mounts
 - d. Can be used for identification of *Candida* species

18. Cornmeal agar
 - a. Promotes chlamydospore formation by *C. albicans*
 - b. Is used only for identification of *C. dubliniensis*
 - c. Does not support *Candida* growth
 - d. Allows definitive identification of most *Candida* species

19. CHROMagar culture medium
 - a. Is not suitable for cultivation of *Candida*
 - b. Allows presumptive identification of several *Candida* species
 - c. Allows definitive identification of most *Candida* species
 - d. Can be used for carbohydrate assimilation tests

20. The germ tube test is
 - a. Difficult and time consuming to perform
 - b. Used for identification of *C. krusei*
 - c. Used for presumptive identification of *C. albicans*
 - d. Used to definitively identify most *Candida* species

**Course #DL-986 - Candida And Its Role In Opportunistic Mycoses
Registration/Answersheet - 2.0 CE Credit**

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Please circle the one best answer for each question.

- | | | | | | | | | | |
|-----|---|---|---|---|----|---|---|---|---|
| 1. | a | b | c | d | 11 | a | b | c | d |
| 2. | a | b | c | d | 12 | a | b | c | d |
| 3. | a | b | c | d | 13 | a | b | c | d |
| 4. | a | b | c | d | 14 | a | b | c | d |
| 5. | a | b | c | d | 15 | a | b | c | d |
| 6. | a | b | c | d | 16 | a | b | c | d |
| 7. | a | b | c | d | 17 | a | b | c | d |
| 8. | a | b | c | d | 18 | a | b | c | d |
| 9. | a | b | c | d | 19 | a | b | c | d |
| 10. | a | b | c | d | 20 | a | b | c | d |

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According to state regulations, this evaluation must be completed and returned in order to receive CE hours. Your comments help us to provide you with better continuing education materials in the distance learning format. Please circle the number that agrees with your assessment.

1. Overall, I was satisfied with the quality of this Distance Learning course.

(strongly agree) 5 4 3 2 1 (strongly disagree)

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(strongly agree) 5 4 3 2 1 (strongly disagree)

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