

MEDICINE
UPDATE



CONTINUING MEDICAL EDUCATION
ANTIFUNGALS

Program Information

CME objectives

1. To identify briefly the clinical presentations of different types of superficial and deep fungal infections
2. To broadly understand the currently recommended diagnostic and treatment approach for fungal infections.

Target participants

Physicians

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Method of participation in CME

- Enroll for the program by filling enrollment form
- You will receive the program module containing complete CME with post - test questions
- Study all parts of the educational activity
- Complete the online questions and submit your answers
- A Medicine Update CME Certificate will be issued to participants upon completing the post - test with a score of 60% or better.

CME activity

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CONTINUING MEDICAL EDUCATION

ANTIFUNGALS

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SECTION 1

Fungal Infections

An Overview

THE WORLD OF FUNGI

Fungi are ubiquitous organisms, with approximately 1.5 million different species present on the Earth; however, only about 300 of these fungi have medical importance and are known to cause fungal diseases.¹ These eukaryotic microorganisms are heterotrophic and essentially aerobic with limited anaerobic capabilities. There are two basic forms of fungi – yeasts and molds – in which most of these organisms exist; nonetheless, some organisms can exhibit a combination of both the forms, a phenomenon known as dimorphism;^{2,3} herein, yeasts are single-celled (unicellular) forms, which reproduce by budding, whereas molds are filamentous and occur in long filaments known as hyphae, which grow by apical extension. Dimorphic fungi exist as molds in the environment and convert to parasitic yeast in the human body - they grow as yeasts or spherules in vivo, and in vitro at 37°C, but as molds at 25°C. Besides temperature, dimorphism is regulated by several other factors, such as CO₂ concentration, pH, and the levels of cysteine or other sulfhydryl-containing compounds, dependent on the dimorphic fungus.² Common dimorphic fungi of medical importance, known to cause invasive fungal infections, include *Blastomyces dermatitidis*, *Histoplasma capsulatum*, and *Coccidioides immitis*.^{4,5}

INTRODUCTION TO FUNGAL DISEASES

Fungal diseases or infections have been known to affect a large proportion of the population, and are a significant cause of morbidity and mortality.⁶ The spectrum of fungal infections is extensive (Table 1),^{4,5} resulting in a wide variety of diseases, which range in severity from mild superficial infections to life-threatening invasive

infections, involving different underlying organs. Herein, the hosts' immunity status is a significant factor that seems to play key role in deciding the infection and its clinical presentation.⁷ While mild superficial infections mostly affect the large proportion of otherwise healthy population; in contrast, life-threatening invasive diseases are usually limited mostly to the vulnerable immunosuppressed and/or immunocompromized patients.⁶ In general, the most frequent fungal diseases, including infections of mucosal surfaces and integument, tend to be chronic or relapsing in nature; whereas invasive fungal diseases are progressive and often result in death or disability if not promptly recognized and treated.

CLASSIFICATION AND COMMON PRESENTATIONS: CURRENT SPECTRUM OF FUNGAL INFECTIONS

There are different clinical nomenclatures used to describe fungal infections, essentially based on the following criteria: (i) site of the infection; (ii) route of acquisition of the pathogen; or (iii) the type of virulence exhibited by the fungus.⁸ Usually, most of the pathogenic fungi are classified by tissue or organ levels that are the main sites of colonization. These infections can thus present clinically in following general manners - superficial, cutaneous, subcutaneous, or deep (invasive) infections, dependent on the type and degree of tissue involvement and hosts' response to pathogen.^{2,8,9} When classified according to route of acquisition, a fungal infection may be designated as exogenous (airborne, cutaneous or percutaneous route of entry) or endogenous (acquired from colonization or reactivation of a fungus from a latent infection) in origin.⁸ Finally,

Table 1: Major medically important fungi

Group	Disease	Etiological agent
Molds Black fungi	Chromoblastomycosis	<i>Cladosporium carriponii</i>
		<i>Fonsecaea pedrosoi</i>
	Phaeohyphomycosis	<i>Exophiala jeanselmei</i>
		<i>Wangiella dermatitidis</i>
		<i>Xylohypha bantiana</i>
Dermatophytes	Tinea capitis	<i>Microsporum canis</i>
		<i>Trichophyton tonsurans</i>
	Tinea corporis	<i>Microsporum gypseum</i>
		<i>Trichophyton mentagrophytes</i>
		<i>Trichophyton rubrum</i>
	Tinea cruris	<i>Epidermophyton floccosum</i>
	Tinea pedis	<i>Trichophyton mentagrophytes</i>
		<i>Trichophyton rubrum</i>
Dimorphic	Blastomycosis	<i>Blastomyces dermatitidis</i>
	Coccidioidomycosis	<i>Coccidioides immitis</i>
	Histoplasmosis	<i>Histoplasma capsulatum</i>
	Paracoccidioidomycosis	<i>Paracoccidioides brasiliensis</i>
	Sporotrichosis	<i>Sporothrix schenckii</i>
Opportunistic infections	Aspergillosis	<i>Aspergillus flavus</i>
		<i>Aspergillus fumigatus</i>
	Mycetoma	<i>Madurella mycetomatis</i>
	Zygomycosis	<i>Absidia corymbifera</i>
		<i>Rhizomucor pusillus</i>
		<i>Rhizopus arrhizus</i>
Yeasts	Candidiasis	<i>Candida albicans</i>
		<i>Candida tropicalis</i>
	Cryptococcosis	<i>Cryptococcus neoformans</i>
	Pityriasis versicolor	<i>Malassezia furfur</i>

Source: McGinnis MR, Tyring SK. Introduction to Mycology. Medical Microbiology. 4th edition. Baron S (ed.). Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Available online at: <https://www.ncbi.nlm.nih.gov/books/NBK8125/> [Accessed on 9/5/2017].

fungal infections may also be classified according to the virulence of causative organisms, as primary or as opportunistic infections. A primary pathogen may cause infection in normal immunocompetent hosts; whereas, an opportunistic pathogen mostly causes disease in individuals with compromised host defense mechanisms.

Superficial infections

Superficial infections are categorized such given the fact that they are limited to the stratum corneum layer. Broadly, following fungal infections and their etiological agent are included in this category: black piedra (*Piedraia hortae*), white piedra [*Trichosporon beigelii* (*T. asahii*)], pityriasis versicolor (*Malassezia furfur*), and tinea nigra [*Hortaea werneckii* (*Phaeoannellomyces werneckii*)].⁸⁻¹²

Superficial fungal infections rarely elicit an immune response from the host (except occasionally by *M. furfur* infections).¹³ In fact, *M. furfur* and *T. asahii* were also implicated as opportunistic agents of disease, especially in immunosuppressed or otherwise debilitated patients, who usually get infected through indwelling catheters or intravenous (i.v) lines.¹³⁻¹⁵

Cutaneous (Dermatophyte) infections

Cutaneous fungal infections are caused by fungi that colonize the skin, hair, and nails on the living host (dermatophytes) and possess greater invasive properties than those causing superficial infections, yet they are keratinophilic and limited to the keratinized tissues;¹⁶⁻¹⁸ besides, there is tissue inflammation, which can be elicited by the organism or its products. Trauma is considered to play an important role in dermatophytes infection.¹³

Broadly, cutaneous fungal infections may be classified as dermatophytoses, which are caused by agents of the genera *Epidermophyton*, *Microsporum*, and *Trichophyton*, or as dermatomycoses, which are cutaneous infections attributable to other fungi, the most common of which are *Candida* spp. Usually, dermatophytoses are clinically characterized by the anatomic site-specificity according to genera.⁸ For instance, *Microsporum* spp. infect hair and skin, but do not involve nails, whereas *Epidermophyton floccosum* infects only skin and nails, but does not infect hair shafts and follicles. *Trichophyton* spp. may infect hair, skin, and nails.¹⁹ Dermatophytic infections are especially predominant in developing countries like India where hot climate and humid weather is favorable to the acquisition and maintenance of disease.¹⁶

Subcutaneous infections

There are three general types of subcutaneous fungal infections: chromoblastomycosis, mycetoma, and sporotrichosis,⁹ all of which appear to be caused by traumatic inoculation of the etiological fungi into the subcutaneous tissue.^{2,8} Although most of the fungi implicated in this category exist in a hyphal morphology, the agents causing chromoblastomycosis and sporotrichosis are exceptions.^{8,13}

Chromoblastomycosis is characterized by skin lesions, which can present clinically in five different forms: nodular, tumoral lesions, verrucous, plaque and cicatricial; on histological examination, characteristic dark-colored, thick-walled, muriform cells i.e. sclerotic cells (Medlar bodies) are observed, which is a histopathological criterion for the diagnosis.²⁰ In contrast, mycetoma represents a progressive subcutaneous granulomatous infection, which is destructive to the adjacent subcutaneous tissue, muscle, and bone. Mycetoma is characterized by presence of draining sinus tracts from which small but grossly visible pigmented grains are extruded, which are essentially microcolonies of the fungi causing the infection.^{8,21-23}

There are only few fungi that are known to cause chromoblastomycosis and mycetoma. Chromoblastomycosis usually results from a traumatic injury and inoculation of microorganism from specific group of dematiaceous fungi (usually *Fonsecaea pedrosoi*, *Phialophora verrucosa*, *Cladophialophora carrionii*).²⁰ In contrast, mycetoma has more diverse causes, and can be classified as eumycotic and actinomycotic mycetoma. Both eumycetoma and actinomycetoma present as a progressive, subcutaneous swelling, although actinomycetoma has a more rapid course.²⁴ Common agent for eumycotic mycetoma is *Pseudallescheria boydii*, and that of actinomycotic mycetoma is *Nocardia brasiliensis*. Many of the fungi causing mycetoma are dematiaceous (melanized; pigmented brown to black) fungi.^{8,25} These fungi may also produce deep (invasive) infection characterized by presence of dematiaceous hyphal and/or yeast-like cells in tissue; though deep infections because of dematiaceous fungi are termed phaeohyphomycosis.⁸

Sporotrichosis is the next class of subcutaneous fungal infections, which occurs due to the dimorphic *Sporothrix schenckii*, generally by traumatic inoculation of soil, plants, and organic matter contaminated with the fungus.²⁶ The infection usually spreads along cutaneous

lymphatic channels of the extremity involved.⁸ Clinical manifestations of sporotrichosis vary dependent on several factors, such as inoculum load, immune status of the host, virulence of the inoculated strain, and depth of traumatic inoculation.^{13,26}

Deep (invasive) infections

The increased incidence of invasive fungal infections in the past two decades has been overwhelming.²⁷ Deep fungal infections can involve different organ systems, though the most common portals of entry for causative pathogens are the respiratory tract, gastrointestinal (GI) tract, and blood vessels.⁸ It is considered that advances in medical technology, including organ transplantation, cancer chemotherapy, widespread use of indwelling i.v. catheters, and increased use of broad-spectrum antibiotics and corticosteroids has contributed to an increase in invasive fungal infections, which occur most frequently in immunocompromized patients and are associated with significant morbidity and mortality.^{3,28-30}

Primary vs. opportunistic fungal infections

Deep fungal infections can be caused by primary pathogenic and opportunistic fungal pathogens. As mentioned earlier, primary pathogenic fungi can establish infection in a normal host; whereas, opportunistic pathogens require some degree of immunocompromise in order to establish an infection.⁸ Major primary invasive fungal pathogens include *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, and *Paracoccidioides brasiliensis*, whereas opportunistic fungal pathogens include *Cryptococcus neoformans*, *Candida* spp., *Aspergillus* spp., *Penicillium marneffei*, the Zygomycetes, *Trichosporon* spp., and *Fusarium* spp.^{8,31,32}

Primary fungal infections

Most of the cases of primary deep fungal infections are asymptomatic or clinically mild infections occurring in normal individuals living or traveling in endemic areas;¹³ however, patients exposed to a high inoculum or those with altered host defenses may suffer life-threatening progression or reactivation of latent foci of infection.⁸ Amongst these primary pathogens, *H. capsulatum*, *B. dermatitidis*, *P. brasiliensis*, *C. immitis*, and *P. marneffei* are dimorphic, changing from a mycelial to a unicellular morphology in vivo, except *C. immitis* that forms spherules in the lungs (parasitic phase).¹³

Most cases of coccidioidomycosis are mild infections.

However, some patients have severe symptomatic pulmonary infections, and ~1% develop disseminated disease, which can involve the skin, joints, bones, central nervous system (CNS), or other organs.³³ Typically, coccidioides meningitis is a life-threatening infection requiring chronic treatment.⁸

Histoplasmosis is a primary pulmonary infection resulting from inhalation of conidia of *H. capsulatum* which convert in vivo into the budding yeast form. The extent of disease depends on number of conidia inhaled and the function of host's cellular immune system; varying from mild pneumonitis to severe acute respiratory distress syndrome (ARDS).³⁴ Dissemination to hilar and mediastinal lymph nodes, spleen, liver, bone marrow, and brain may be life-threatening in immunocompromized patients.^{8,35}

Similar to histoplasmosis, blastomycosis is a primary pulmonary infection resulting from inhalation of conidia from the mycelial phase of *Blastomyces dermatitidis*, which convert in vivo to the parasitic yeast phase. The most common sites of clinical disease are the lungs and skin; bone, genitourinary, and CNS manifestations follow in decreasing order of frequency.³⁶

Opportunistic fungal infections: Often difficult to treat

A change in systemic fungal infections is being witnessed, both globally and in India, wherein systemic infection due to species under *Candida*, *Aspergillus* and *Zygomycetes* is widely prevalent in nosocomial setting.²⁷ Especially, in the past few decades, opportunistic fungal infections have emerged as important causes of morbidity and mortality in patients with severe underlying illnesses.³⁷ Species of *Candida* and *Aspergillus* are the most common causes of invasive fungal infections, but other yeasts and filamentous fungi are also emerging as significant pathogens.^{38,39}

Candidiasis

Candidiasis is the most common opportunistic fungal infection,⁴⁰ and can be caused by several *Candida* species, including *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*;⁴¹ nevertheless, *C. albicans* is the most prevalent species involved in invasive fungal infections.^{8,42} Candidiasis may occur as superficial or deep. While the former may involve epidermal and mucosal surfaces; invasive infections include bloodstream infections (candidemia) and/or invasive candidiasis, with major portals of entry being

alimentary tract and i.v. catheters.⁸ Invasive candidiasis is associated with high morbidity and mortality.⁴³

Aspergillosis

The most common molds that cause clinical infections are the *Aspergillus* species; common species of Aspergillosis include *Aspergillus fumigatus*, *A. flavus*, *A. terreus*, and *A. niger*.³ Amongst these agents, *A. fumigatus* is by far the most important agent, largely responsible for the increased incidence of invasive aspergillosis in the immunocompromized patient population.^{41,44} Invasive aspergillosis most frequently involves the lungs and paranasal sinuses, since main portal of entry is the respiratory tract; however, injuries to skin may also introduce the organism into susceptible hosts.⁴¹ Further, the fungus may disseminate to involve other vital organs.⁸

Zygomycosis

Zygomycosis (also known as mucormycosis) is a rare but emerging invasive fungal infection caused by Zygomycetes, which are filamentous fungi with a worldwide distribution.⁴⁵ Zygomycetes encompasses two orders - the Mucorales and the Entomophthorales. Members of Entomophthorales class are associated with chronic cutaneous and subcutaneous infections, which are limited to the tropics and rarely disseminate to internal organs. In contrast, Mucorales includes several species involved in rhinocerebral, pulmonary, cutaneous, GI disease, and is characterized by a tendency to disseminate, especially in immunocompromized patients. Portals of entry of Zygomycetes can include the skin, lungs, and GI tract; though in immunocompetent patients, trauma is the most common predisposing factor leading to zygomycosis.⁴⁶ Zygomycetes have a characteristic tendency to invade blood vessels and to cause thrombosis-processes, which result in subsequent necrosis of involved tissues, causing invasive infections.⁸

Cryptococcosis

Cryptococcosis is an opportunistic fungal infection caused by *Cryptococcus neoformans*, an encapsulated yeast. Invariably, the lung is the portal of entry and initial site of infection for *C. neoformans*, but in immunosuppressed patients all areas of the body can be infected; CNS involvement is the most severe complication. As such, the infection most frequently causes pneumonia and/or meningitis, and remains highly lethal disease of human immunodeficiency virus (HIV) positive individuals

especially in the developing world. In India, an increased incidence of cryptococcosis is reported from all centers with the emergence of acquired immunodeficiency syndrome (AIDS).^{3,8,27,47}

Phaeohyphomycosis

Phaeohyphomycosis is an uncommon infection, but has become increasingly recognized in a wide variety of clinical syndromes.^{48,49} The disease is caused by brown to black pigmented fungi of the cutaneous, superficial, and deep tissues, especially brain, and occurs as a life-threatening condition in various immunocompromized states.^{8,50}

Hyalohyphomycosis

Hyalohyphomycosis is an opportunistic fungal infection caused by saprophytic fungi with hyaline hyphal elements. The term is essentially a counterpart to phaeohyphomycosis wherein pathogens develop septate brown-walled hyphae in tissue. In contrast, hyalohyphomycosis is caused by non-dematiaceous molds or yeasts in which the tissue form is colorless (hyaline) septate fungal hyphae, branched or unbranched, with no pigment within the walls.^{51,52} The members of this group are extremely heterogeneous and include Acremonium, Arthrobotrys kalrae, Chrysosporium, Fusarium, Paecilomyces, Penicillium marneffei, Scopulariopsis, and Trichoderma.^{8,22}

EPIDEMIOLOGY AND BURDEN OF FUNGAL INFECTIONS: FOCUS ON INVASIVE PATHOGENS

Despite the significant impact of fungi on human health, precise estimates of the rates of fungal diseases are generally lacking.⁶ Nonetheless, while superficial fungal infections are considered to be widespread, with an estimated worldwide prevalence of 20%–25%,⁵³ concurrently, it seems increasingly evident that the range of patients at risk for invasive fungal infections continues to expand to encompass those with acquired immunodeficiencies, those immunosuppressed due to cancer chemotherapy and organ transplantation, and those undergoing major surgical procedures. These patients are more likely than healthy people to develop opportunistic and invasive fungal infections.⁵⁴ Furthermore, as the population at risk expands, so does the spectrum of opportunistic fungi infecting these patients. As such, opportunistic fungal pathogens are becoming increasingly important in medical microbiology. This

is especially concerning considering the fact that many of the deeply invasive fungal infections are difficult to diagnose early and often difficult to treat effectively, resulting in high morbidity and mortality.^{8,55-62}

PATHOGENESIS OF FUNGAL INFECTIONS

Although a great deal of information is available concerning the bacterial pathogenesis and its molecular basis, there is little information about the mechanisms of fungal pathogenesis.¹³ *Per se*, fungal pathogenesis is intricate and involves a complex interplay of many fungal and host factors, including the enzymes, such as keratinase, phospholipases, lipases and proteinases, the presence of capsule (in *C. neoformans*), the ability to grow at 37°C, dimorphism, and other factors such as melanin production, superoxide dismutase, rapid growth and affinity to the blood stream, and toxin production.^{13,63} Thus, fungi often develop both virulence mechanisms (e.g., capsule and ability to grow at 37°C) and morphologic forms (e.g., yeasts, hyphae, spherules, and sclerotic bodies) in order to facilitate their multiplication within the host.¹³ Nevertheless, among these several mechanisms facilitating their colonization *in vivo*, their ability to grow at 37°C and dimorphism perhaps appears most important.^{2,13,41}

TAKE HOME POINTS

- ◆ Fungal infections can present clinically in following general manners - superficial, cutaneous, subcutaneous, or deep (invasive) infections, dependent on the type and degree of tissue involvement and hosts' response to pathogen.
- ◆ Advances in medical technology, including organ transplantation, cancer chemotherapy, widespread use of indwelling i.v. catheters, and increased use of broad-spectrum antibiotics and corticosteroids has contributed to an increase in invasive fungal infections.
- ◆ Deep fungal infections can be caused by primary pathogenic and opportunistic fungal pathogens, and are associated with significant morbidity and mortality. Species of *Candida* and *Aspergillus* are the most common causes of invasive fungal infections.

SECTION 2

Management of fungal infections

Diagnosis & treatment

DIAGNOSTIC METHODS FOR FUNGAL INFECTIONS

The prompt diagnosis of fungal infections remains a challenge, especially in the immunocompromized hosts.⁶⁴ Clinically, symptoms and signs of fungal infections are non-specific,⁶⁵ and patients may have many comorbid diseases, rendering difficulty for invasive diagnostic procedures. Besides, simple colonization is often difficult to distinguish from active infections, and blood cultures are commonly negative. This seems especially concerning for invasive fungal infections since conventional microbiologic and histopathologic approaches often do not detect invasive fungal infection until late in the course of disease.⁶⁶ Therefore, confirmatory diagnosis of fungal infections often requires more informative laboratory techniques.

Broadly, a laboratory diagnosis of fungal infections can be made via direct or indirect methods (Table 2,3). The direct methods include direct inspection, microscopy, histological examination and culture identification; whereas indirect methods use several non-culture based tests to confirm fungal infections without actual growth of pathogens. They compose of Wood's lamp examination, intra-dermal skin test, breath test, image studies, metabolite assays, serologic examinations and molecular techniques to detect fungal antigens or host antibodies.^{2,67}

Usually, in many cases, clinical presentation is often the first criteria that raise suspicion of a fungal infection. Subsequently, microscopic examination of skin scrapings,

a vaginal discharge or bronchoscopic washings might reveal dermatophytes, candidiasis or histoplasmosis, respectively. Often, the success of such laboratory confirmation of clinically diagnosed fungal infection relies on proper collection of specimens for microscopic examination and culture.⁶⁸ Direct microscopic examination of a potassium hydroxide (KOH) mounted preparation is the most simple and important test for diagnosing superficial fungal infection and dematiaceous fungal infection,^{69,70} whereas a chest x-ray would be useful in detecting most pulmonary fungal infections. In other cases, a biopsy may be required as is the use of special stains to visualize the responsible fungi.²

Often, direct microscopic examination and culture of appropriate specimens are used in conjunction for a mycological diagnosis;^{69,71,72} Nonetheless, before a specific fungus can be confirmed as the cause of a disease, it must be isolated from serial specimens and fungal elements morphologically consistent with the isolate must be observed in tissues taken from the lesion. Such identification by histopathology and culture remains the cornerstone of fungal diagnosis.^{13,73} However, conventional assays still take 24 to 72 hours to complete. In fact, since fungi take a long time to grow, it is suggested that culture should be incubated for at least 30 days before giving a negative report.⁷⁴

Non-culture methods

As mentioned above, although culture remains gold standard to make fungal diagnosis, it is time-

Table 2: Direct methods in diagnosis of fungal infections

Direct microscopy	<ul style="list-style-type: none"> It is possible to visualize fungal elements only when they are very abundant in clinical samples. Vigorous fungal stains can increase the microscopic yield; however, false-negative rates are common.
Smear	<ul style="list-style-type: none"> Microscopic identification of fungal elements directly in clinical samples using 10% potassium hydroxide (KOH) - the most practical and rapid screening method. Direct ink preparation - useful in detecting <i>C. neoformans</i> in cerebrospinal fluid (CSF).
Cytology	<ul style="list-style-type: none"> Most fungi may be visualized during cytologic preparations via Papanicolaou and Giemsa or Wright stains; however, the fungal wall is not highlighted with either stain. KOH exam and Calcofluor white stain could highlight fungal elements in bright field or phase contrast microscopy and fluorescence microscope, respectively.
Tissue slides	<ul style="list-style-type: none"> Histological examination should always start with hematoxylin and eosin (H&E) stain, wherein all fungi show pink cytoplasm and blue nuclei. Periodic Acid Schiff (PAS) stain - features fungal walls as pink to red purple since they contain glycogen. Fontana-Masson stain - reveals melanin in fungal walls but also stains normal epidermal melanins. Absence of melanin in hyphae of filamentous fungi distinguishes dematiaceous from other filamentous fungi; for instance, it is useful to differentiate <i>Cryptococcus</i> spp. from <i>Candida</i> spp. in the tissue sections.
Histopathology	Selected yeast or yeast-like organisms
	<p><i>Candida</i> spp.</p> <ul style="list-style-type: none"> Present as small yeasts (3-5 µm) with intermingled pseudohyphae. <i>C. glabrata</i> is the predominant <i>Candida</i> spp. that does not produce filaments. Histological examination of specimens is important to determine invasion of tissues and vessels
	<p><i>Cryptococcus</i> spp.</p> <ul style="list-style-type: none"> Appear as thick-encapsulated narrow-based budding yeasts (5-10 µm) Capsule is highlighted negatively with India Ink, stained with Alcian blue or Mucicarmine stain and Fontana-Masson stain
	<p>Endemic dimorphic fungi (<i>Histoplasma</i> spp., <i>B. dermatitidis</i>, <i>C. immitis</i>, <i>P. brasiliensis</i> and <i>P. marneffei</i>)</p> <ul style="list-style-type: none"> <i>Histoplasma capsulatum var. capsulatum</i> - present as small intracellular narrow based budding yeasts (2-4 µm), which cluster within histiocytes and sometimes within neutrophils. <i>Sporothrix schenckii</i> - appear as round, oval or cigar shaped yeasts (≥2-6 µm) with narrow base or tube-like budding and surrounding asteroid body in most cases.
	Selected filamentous fungi
	<p><i>Aspergillus</i> spp.</p> <ul style="list-style-type: none"> Usually described as thin (3-12 µm), septate, acute-angle (45°) or dichotomous branching hyphae.
	<p><i>Fusarium</i> spp., <i>Scedosporium</i> spp., <i>Trichoderma</i> spp. and <i>Paecilomyces</i> spp.</p> <ul style="list-style-type: none"> Non-pigmented, septated hyphae with acute angle branching similar to Aspergillosis spp. <i>Fusarium</i> spp. - may show prominently constricted hyphae with varicosities and intercalated chlamydoconidia.
	<p><i>Zygomycete</i> spp.</p> <ul style="list-style-type: none"> Present as non-pigmented, thin-walled, pauci-septate ribbon like hyphae (5-20 µm) with acute-angle branching. Important diagnostic features include identification of fungal elements invading the blood vessels.
	<p>Dematiaceous fungi; (including <i>Madurella</i> spp., <i>Fonsecacea</i> spp., <i>Cladophialophora</i> spp., <i>Exophiala</i> spp., <i>Curvularia</i> spp., and <i>Bipolaris</i> spp.)</p> <ul style="list-style-type: none"> Show pigmented irregular hyphae and yeast-like structures both with septations and "copper pennies" or "sclerotic bodies" in chromoblastomycosis. Fontana-Masson staining is required to demonstrate melanins. Hyphae tend to be thin (width 2 - 6 µm), but may be swollen with prominent septa, which show constrictions

Histopathology	Mycetomas	<ul style="list-style-type: none"> Characterized by production of grains. Unlike dematiaceous fungi, which produce black grains, other fungi (usually <i>Scedosporium</i> spp. and <i>Acremonium</i> spp.) or bacteria produce white grains.
	Dermatophytes	<ul style="list-style-type: none"> Characteristic hyphae in keratin layer. <i>Malassezia</i> spp. show typical spaghetti and meatball appearance.
Culture (gold standard of the diagnosis)	Microbiological isolation and identification of the fungal pathogen	
	Fungemia	<ul style="list-style-type: none"> Require at least 24-48 hrs for simple genus-specific identification, an additional 24-72 hrs for species determination, several days for final result. Serial blood cultures should be collected during infective and/or febrile episode in order to improve the detection rate of fungemia.
	Culture methods	<ul style="list-style-type: none"> If a polymicrobial infection or commensal bacterial flora is collected, selective media is recommended such as Sabouraud agar containing antibiotics with chloramphenicol and gentamicin, for further fungal identifications. Growth requirements for fungi often differ from those for bacteria, most notably with regard to optimal growth temperature and media. For instance, most yeast grow best at 37°C, whereas filamentous fungi often grow best at room (27-30°C) temperatures. Therefore, cultures must be incubated both at 30°C and 37°C. Most yeasts grow in 2 to 3 days, while dimorphic fungi may take as long as 3 to 6 weeks. Newer blood culture methods seem to have shorter recovery time and higher recovery rate than broth culture, thereby resulting in improved fungal detection.

Sources: 1. Laboratory techniques used in the diagnosis of mycosis. Lee CH (ed). OMICS Group eBooks, 2015. USA. Available online at: <https://www.eSciencecentral.org/ebooks/laboratory-techniques-used/pdf/laboratory-techniques-used-in-the-diagnosis-of-mycosis.pdf> [Accessed on 12/5/2017]. 2. Shahid M, Ahmad I, Malik A, Jahan N, Tripathi T. Laboratory Diagnosis of Fungal Infections: An Overview. Chapter Combating Fungal Infections, pp 173-211. Springer Berlin Heidelberg, 2010. Available online at: https://link.springer.com/chapter/10.1007%2F978-3-642-12173-9_9#page-1 [Accessed on 17.05.2017].

consuming and has low sensitivity. Besides, a definitive identification of species based on morphological and biochemical characteristics of cultured fungi requires highly experienced and well trained laboratory personnel. Considering these limitations, there has been considerable interest in developing non-culture approaches, which may provide another avenue to further improve fungal diagnostics.^{66,74,75} A variety of non-culture methods, including antibody and antigen-based serological assays, metabolite detection and molecular identification, have been evaluated and are expected to provide important diagnostic clues.⁶⁴ Serological assays include glucan in a wide range of fungal pathogens, Galactomannan (GM) antigen testing in invasive aspergillosis, mannan antibodies and antigenemia in candidiasis.^{67,76} Molecular methods include the detection of different fungal species grown in culture and sample, using Polymerase Chain Reaction (PCR) products by gel or capillary electrophoresis, restriction fragment length polymorphism analysis, single-strand conformational polymorphism, Southern blot hybridization assays with oligonucleotide probes, PCR-Enzyme Immunoassays (EIAs), real-time PCR assays, microarray technology or

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry. These non-culture methods could help facilitate early diagnosis of fungal infections.⁶⁷ In fact, in some cases, results from molecular biology methods down to the species level may be available as early as 24 hours.⁷⁷ For instance, MALDI-TOF mass spectrometry enables species-specific identification of yeast isolates within an hour.⁷⁶

TREATMENT OF FUNGAL INFECTIONS

Control of fungal infections includes both prevention and treatment. Prevention mainly encompasses avoidance of risk factors such as environments and conditions conducive to fungal growth. For instance, in hospital settings, maintenance of a “spore-free” environment can reduce the incidence of nosocomial fungal infections.² For individual patients, clinical outcomes with fungal infections are indeed better when the appropriate (antifungal) treatment is initiated on time.^{63,67} Nevertheless, the management could be dependent on not only the specific pathogen but also the host disease status. For instance, in case of immunocompromized patients, management of underlying disease also may

Table 3: Indirect methods in diagnosis of fungal infections

Wood's lamp examination	<ul style="list-style-type: none"> Can be used to diagnose superficial dermatophyte infections, zoophilic ectothrix organisms <i>Microsporum canis</i> and <i>Microsporum audouinii</i>, which fluoresce blue-green, pityriasis versicolor caused by <i>Malassezia furfur</i>, which fluoresces yellow to orange. Most superficial fungi however do not fluoresce upon Wood's lamp emission, limiting its utility 												
Intra-dermal skin test	<ul style="list-style-type: none"> Skin tests by injection of specific fungal antigen into skin Histoplasmin test, paracoccidioidin test, coccidioidin test and sporotrichin test are used to diagnose exposure to <i>H. capsulatum</i>, <i>P. brasiliensis</i>, <i>C. immitis</i> and <i>S. schenckii</i> respectively. The tests are performed by injecting 0.1 ml antigen into the superficial dermal layer on the forearm, and recording the reactions 48 hours after inoculations; considered positive when a diameter of induration is >5 mm. Cross reaction may occur between histoplasmin test and <i>B. dermatitidis</i> and paracoccidioidin test between <i>H. capsulatum</i>. 												
Imaging studies	<ul style="list-style-type: none"> Conventional x-ray examination may not be able to detect clearly diffuse micro-nodular lesions found in invasive fungal infections. Ultrasonography, high-resolution computerized tomography (CT) and magnetic resonance imaging (MRI) may help to obtain early detection or diagnosis of fungal infections. 												
Blood examinations	Include metabolite assays and antibody or antigen detections.												
	<table border="1"> <tr> <td>Metabolite assay</td> <td> <ul style="list-style-type: none"> D-arabinitol, D/L arabinitol ratio and mannose by gas-liquid chromatography - used for detection of invasive candidiasis. The level is not high in uninfected or colonized patients. However, it may elevate in non-fungus infected patients with abnormal renal function. Arabinitol/creatinine ratios may be more accurate in diagnosing Candida infections, though reported sensitivity is low. </td> </tr> <tr> <td colspan="2">Serological tests (detection of antibodies or antigens related to specific fungal infections)</td> </tr> <tr> <td>Pan-Fungal Antigen: (1,3)-B-D-Glucan (BDG)</td> <td> <ul style="list-style-type: none"> A fungal wall component, detectable in blood during invasive fungal infections. Can be used in detecting infections caused in species of <i>Aspergillus</i>, <i>Candida</i>, <i>Fusarium</i>, <i>Trichosporon</i>, <i>Saccharomyces</i>, <i>Acremonium</i>, <i>Penicillium</i>, <i>Cephalosporium</i>, <i>C. immitis</i>, <i>H. capsulatum</i>, <i>S. schenckii</i> and <i>Pneumocystis jirovecii</i> Normal individuals usually have BDG concentration <10 pg/mL in blood; patients with invasive fungal infections show concentration >20 pg/mL. </td> </tr> <tr> <td>Galactomannan (GM) antigen</td> <td> <ul style="list-style-type: none"> A cell wall component of <i>Aspergillus</i> spp. Appears in body fluids (bronchoalveolar lavage, serum, CSF and urine) of patients with invasive aspergillosis in very early phase of infection. </td> </tr> <tr> <td>Cell wall mannoprotein (mannan) and heat-labile antigen</td> <td> <ul style="list-style-type: none"> Markers for invasive candidiasis. </td> </tr> <tr> <td>Cryptococcus capsular polysaccharide</td> <td> <ul style="list-style-type: none"> Detection of capsular polysaccharide by latex agglutination - most useful method for rapid diagnosis of cryptococcal meningitis; positive in ~90% of cases with specificity >95% </td> </tr> </table>	Metabolite assay	<ul style="list-style-type: none"> D-arabinitol, D/L arabinitol ratio and mannose by gas-liquid chromatography - used for detection of invasive candidiasis. The level is not high in uninfected or colonized patients. However, it may elevate in non-fungus infected patients with abnormal renal function. Arabinitol/creatinine ratios may be more accurate in diagnosing Candida infections, though reported sensitivity is low. 	Serological tests (detection of antibodies or antigens related to specific fungal infections)		Pan-Fungal Antigen: (1,3)-B-D-Glucan (BDG)	<ul style="list-style-type: none"> A fungal wall component, detectable in blood during invasive fungal infections. Can be used in detecting infections caused in species of <i>Aspergillus</i>, <i>Candida</i>, <i>Fusarium</i>, <i>Trichosporon</i>, <i>Saccharomyces</i>, <i>Acremonium</i>, <i>Penicillium</i>, <i>Cephalosporium</i>, <i>C. immitis</i>, <i>H. capsulatum</i>, <i>S. schenckii</i> and <i>Pneumocystis jirovecii</i> Normal individuals usually have BDG concentration <10 pg/mL in blood; patients with invasive fungal infections show concentration >20 pg/mL. 	Galactomannan (GM) antigen	<ul style="list-style-type: none"> A cell wall component of <i>Aspergillus</i> spp. Appears in body fluids (bronchoalveolar lavage, serum, CSF and urine) of patients with invasive aspergillosis in very early phase of infection. 	Cell wall mannoprotein (mannan) and heat-labile antigen	<ul style="list-style-type: none"> Markers for invasive candidiasis. 	Cryptococcus capsular polysaccharide	<ul style="list-style-type: none"> Detection of capsular polysaccharide by latex agglutination - most useful method for rapid diagnosis of cryptococcal meningitis; positive in ~90% of cases with specificity >95%
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Source: Laboratory techniques used in the diagnosis of mycosis. Lee CH (ed). OMICS Group eBooks, 2015. USA. Available online at: <https://www.esciencecentral.org/ebooks/laboratory-techniques-used/pdf/laboratory-techniques-used-in-the-diagnosis-of-mycosis.pdf> [Accessed on 12/5/2017].

help in reducing the incidence of fungal infections. In other situations like AIDS, cancer patients receiving chemotherapy or organ transplant recipients, prophylaxis with antifungal agents may be required to alleviate the risk of opportunistic invasive fungal infections.²

Antifungal agents

The development of antifungal drugs has lagged behind that of antibacterial agents possibly as a predictable consequence to the cellular structure of organisms involved. Bacteria are prokaryotic and hence offer

numerous structural and metabolic targets, which differ from those of the human host. In contrast, fungi are eukaryotes, and consequently most agents toxic to fungi are also toxic to the host.⁷⁹ Moreover, because fungi generally grow slowly, often in different forms, they are more difficult to quantify than bacteria.

This difficulty complicates the experiments designed to evaluate the *in vitro* or *in vivo* properties of a potential antifungal agent. Nevertheless, despite these limitations, several advances have been made in understanding and developing the antifungal agents.⁷⁹ Yet, it is important to note that despite pharmacotherapeutic advances, serious fungal infections remain difficult to treat, and resistance to available drugs is emerging. It therefore appears prudent that use of the currently available main antifungals like azoles in combination with other antifungals with different mechanisms of action will likely provide enhanced efficacy in treatment of wide array of fungal infections, especially invasive fungal infections, which are associated with high rates of

mortality if not readily diagnosed and treated; Table 4 depicts several currently available antifungal agents for the therapy of systemic fungal infections.⁷⁸ In individual patients, several key elements in selecting an appropriate antifungal agent include the type of patient (immunocompetent or immunocompromized), severity of immunosuppression, history of prolonged exposure to antifungal drugs, and knowledge of the genera and species of the infecting pathogen and its susceptibility pattern.³⁸

Ergosterol in fungal cell membrane: Major antifungal target

Antifungal agents are designed to selectively eliminate the fungal pathogens with minimal toxicity to the hosts.⁷⁹ Herein, whether used for prophylaxis or treatment, many antifungal drugs take advantage of the fact that the sterol in fungal cell membrane is ergosterol instead of cholesterol as in humans; several antifungal agents,

Table 4: Current antifungal agents available for therapy of systemic fungal infections

Antifungal spectrum	AMB	5FC	FLU	ITR	VOR	POS	CAS	MIC
<i>Candida albicans</i>	++	++	++	++	++	++	++	++
<i>Candida glabrata</i>	++	++	+	+	++	++	+	+
<i>Candida parapsilosis</i>	++	++	++	++	++	++	++	++
<i>Candida tropicalis</i>	++	++	++	++	++	++	++	++
<i>Candida krusei</i>	++	+	-	+	++	++	++	++
<i>Candida lusitaniae</i>	-	++	++	++	++	++	++	++
<i>Aspergillus fumigatus</i>	++	-	-	+	++	++	+	+
<i>Cryptococcus neoformans</i>	++	++	++	++	++	++	-	-
Mucorales	++	-	-	-	-	-	-	-
Fusarium spp.	+	-	-	+	++	++	-	-
Scedosporium spp.	+	-	-	+	+	+	-	-
<i>Blastomyces dermatitidis</i>	++	-	+	++	++	++	-	-
<i>Coccidioides immitis</i>	++	-	++	++	++	++	-	-
<i>Histoplasma capsulatum</i>	++	-	+	++	++	++	-	-
Class Administration	Polyene Intravenous	Pyrimidine Oral			Azole Oral/Intravenous		Echinocandins Intravenous	

5FC, flucytosine; AMB, amphotericin B; CAS, caspofungin; FLU, fluconazole; ITR, itraconazole; MIC, micafungin; POS, posaconazole; VOR, voriconazole.

Source: Souza AC, Amaral AC. Antifungal Therapy for Systemic Mycosis and the Nanobiotechnology Era: Improving Efficacy, Biodistribution and Toxicity. *Front Microbiol.* 2017 Mar 7;8:336.

including azoles, allylamine and morpholine antifungal drugs, interfere with the synthesis of ergosterol.

The first step in the synthesis of both ergosterol and cholesterol is demethylation of lanosterol. Cytochrome P450 catalyzes the 14- α -demethylation of lanosterol, an essential step in the synthesis of ergosterol. The azole antifungals interfere with cytochrome P450-dependent 14- α -demethylase, which inhibits the formation of ergosterol, resulting in changes in plasma membrane permeability and inhibition of growth.⁴ The allylamine class, represented by terbinafine and naftifine, function by inhibiting the early steps of fungal ergosterol biosynthesis by targeting the enzyme squalene epoxidase, encoded by ERG1.⁸⁰ Polyene antifungal agents (such as amphotericin B) bind to ergosterol to form complexes, which permit rapid leakage of the cellular potassium, other ions, and small molecules;⁸¹ the loss of potassium results in inhibition of glycolysis and respiration.⁴ Recent evidence though suggests that polyenes also cause oxidative damage, which may contribute to their antifungal activity.⁸² Other antifungal agents inhibit mitosis (e.g. griseofulvin) or DNA and RNA synthesis (e.g. 5-fluorocytosine).²

Polyene antifungals

The polyene antifungal agents (nystatin, amphotericin B, and pimaricin)⁸³ are so named because of alternating conjugated double bonds that constitute a part of their macrolide ring structure. These drugs are all products of *Streptomyces* species, and interact with sterols in cell membranes (ergosterol in fungal cells; cholesterol in human cells) to form channels through the membrane, through which small molecules leak from the inside of fungal cells to the outside.⁷⁹

Nystatin was the first successful antifungal antibiotic to be developed, and is still in general use. It is representative of the polyene antifungal agents developed later.⁷⁹ The drug has a broad-spectrum antifungal activity, but this therapeutic promise is offset by restricted topical use and host toxicity.⁸⁴ Its use is hence limited to topical use, where it has activity against yeasts such as the *Candida* species. Pimaricin (natamycin), another polyene, is used topically to treat superficial mycotic infections of the eye. It is active against both yeasts and moulds.⁷⁹

Amphotericin B - discovered in 1956 - has remained a mainstay antifungal agent for treatment of life-threatening fungal infections and for most other fungal infections, with possible exception of the dermatophytes.^{79,85} Its broad spectrum of activity includes several medically

important pathogens such as *C. immitis*, *H. capsulatum*, *B. dermatitidis*, *P. brasiliensis*, *C. neoformans* var. *neoformans*, and *S. schenckii*.^{79,81} It is an important drug in treating most opportunistic fungal infections caused by fungi such as *Candida* spp., *C. neoformans*, *Aspergillus* spp., and the Zygomycetes. Nevertheless, resistance to amphotericin B is notable for *Pseudallescheria boydii*, *Fusarium* spp., *Trichosporon* spp., certain isolates of *Candida lusitaniae* and *C. guilliermondii*.⁷⁹ In addition, there are few other important limitations to the use of this drug. Amphotericin B must be administered i.v. and is associated with numerous side effects, which range from phlebitis at the infusion site and chills to renal toxicity; these in fact may be severe and limit clinical use of the conventional formulation.^{79,81} However, newer lipid formulations (amphotericin B lipid complex and liposomal formulation) have been developed, which promise improved tolerability.⁸⁶

Azole antifungals

The azole antifungal agents have 5-membered organic rings, which contain either 2 or 3 nitrogen molecules, and are accordingly classified as imidazoles and triazoles, respectively,^{87,88} which share similar mechanism of action and currently are the most rapidly expanding group of antifungal compounds.⁸² Imidazoles have a 2-nitrogen azole ring, are predominantly used topically, and have been replaced for systemic administration by the triazoles, which have 3-nitrogens in the azole ring.⁸¹ Triazoles - introduced almost 30 years ago - have a more favorable pharmacokinetic profile than imidazoles and do not significantly inhibit human sterol synthesis, which contributes to an improved tolerability profile.

The clinically useful imidazoles include clotrimazole, miconazole, and ketoconazole.⁷⁹ The latter - ketoconazole - in fact set the stage for orally administered antifungal azoles. It can be administered both orally and topically and has a range of activity including infections caused by *H. capsulatum* and *B. dermatitidis*, for which it is often used in non-immunocompromized patients.⁷⁹ The drug is also active against mucosal candidiasis and a variety of cutaneous fungal infections, including dermatophyte infections, pityriasis versicolor, and cutaneous candidiasis. However, it is not indicated for the treatment of aspergillosis or of systemic infections caused by yeasts. Other currently available topical imidazoles include econazole, oxiconazole, isoconazole, bifonazole, sertaconazole, tioconazole, butoconazole, eberconazole, and luliconazole.⁸⁹

With the exception of ketoconazole, use of the imidazoles is limited mainly to treatment of superficial fungal infections. In contrast, the triazoles have a broad range of applications in the treatment of both superficial and systemic fungal infections.⁹⁰ Examples of triazoles antifungal drugs include fluconazole, itraconazole, voriconazole, and posaconazole.⁸¹ Fluconazole and itraconazole were the first azoles in clinical practice, and in fact have become the standard for azoles, replacing amphotericin B for managing certain forms of the systemic fungal infections.^{79,87} These agents exert a fungistatic effect by dose-dependent inhibition of CYP-dependent 14α-demethylase, which is necessary for conversion of lanosterol to ergosterol that *per se* is essential for fungal cell membrane structure and function. Both of these agents thus exhibit a broad spectrum of activity and are frequently used for treatment of fungal infections in different settings (Table 5, 6).^{79,88}

Specifically, fluconazole is routinely used to treat candidemia in non-neutropenic hosts, and has also gained acceptance for use in cryptococcosis and selected forms of coccidioidomycosis.⁷⁹ It remains a valuable low-cost choice for the treatment of various fungal infections, including candidiasis and cryptococcosis.⁸⁷ The drug is well-tolerated, has relatively few drug interactions, excellent bioavailability, attaining good

Table 6: Indications of fluconazole and itraconazole

Azole	Indications
Fluconazole	<ul style="list-style-type: none"> Vaginal, oropharyngeal, and esophageal candidiasis Cryptococcal meningitis Prophylaxis to decrease the incidence of candidiasis in patients undergoing bone marrow transplant who receive cytotoxic chemotherapy and/or radiation Pulmonary and extrapulmonary blastomycosis Histoplasmosis, including chronic cavitary pulmonary disease and disseminated, non-meningeal histoplasmosis Aspergillosis in patients refractory to, or intolerant of, amphotericin B therapy
Itraconazole	<ul style="list-style-type: none"> Non-immunocompromized patients: treatment of onychomycosis of toenail, with or without fingernail involvement, or of the fingernail alone, due to dermatophytes (<i>Tinea unguium</i>) Oropharyngeal and esophageal candidiasis Empiric therapy of febrile neutropenic patients with suspected fungal infections

Source: Gavarkar PS, Adnaik RS, Mohite SK. An overview of azole antifungals. *IJPSR*. 2013;4(II):4083-4089.

Table 5: Spectrum of activity of common azole antifungals

Fluconazole	<ul style="list-style-type: none"> In-vitro activity generally considered fungistatic Very active against <i>Candida</i> species including <i>C. albicans</i>, <i>C. parapsilosis</i>, <i>C. tropicalis</i>, and <i>C. lusitaniae</i> Also has activity against <i>C. neoformans</i> and <i>Coccidioides immitis</i>
Itraconazole	<ul style="list-style-type: none"> Fungicidal activity against filamentous fungi and some strains of <i>C. neoformans</i>; generally fungistatic against many yeasts Moderately to very active against most medically important fluconazole-susceptible and -resistant <i>Candida</i> species (except <i>C. glabrata</i>) Modest activity against <i>C. neoformans</i> Excellent in-vitro activity against common dimorphic or endemic fungi including <i>C. immitis</i>, <i>H. capsulatum</i>, <i>B. dermatitidis</i>, and <i>S. schenckii</i>. Good activity against many <i>Aspergillus</i> spp.

Source: Gavarkar PS, Adnaik RS, Mohite SK. An overview of azole antifungals. *IJPSR*. 2013;4(II):4083-4089.

concentrations in different organic fluids, including in the CSF.⁸¹ Itraconazole is another key azole, and exhibits significant activity against *Aspergillus* and the endemic fungi.⁸⁷ It has proven to be effective for histoplasmosis, blastomycosis, sporotrichosis, coccidioidomycosis, consolidation treatment for cryptococcosis, and certain forms of aspergillosis. Together, it demonstrates consistent in vitro activity against the group fungi causing phaeohyphomycosis, and should be considered the drug of choice for most such situations, given the greater clinical experience associated with its use.⁴⁸ The concentrations of itraconazole in the liver, lungs, and bones surpass the serum level.⁸¹

Fluconazole can be administered either orally, or i.v.⁷⁹ Likewise, itraconazole is available for oral use, and an i.v. formulation seems a significant addition directed at improved bioavailability.⁹¹ All azoles have serum half-lives long enough to make treatment with one or two daily doses possible.⁸¹

Voriconazole is useful in treatment of invasive aspergillosis in severely immunocompromized patients, but its use is compromised by unpredictable nonlinear

pharmacokinetics with extensive interpatient and intrapatient variation in serum levels, notable drug interactions, and relatively significant adverse events. Therapeutic drug monitoring is essential when using this agent.⁹² Posaconazole, a fluorinated triazole, has potent in vitro activity against a broad range of fungi and molds, including *Aspergillus*, *Candida*, *Cryptococcus*, filamentous fungi, and endemic mycoses including coccidioidomycosis, histoplasmosis, and blastomycosis, and, apparently, optimal tolerability profile. The drug has a significant role for the prophylaxis of invasive fungal infections in severely immunocompromized patients, and is currently used predominantly for prophylaxis and salvage therapy of fungal infections in adults.⁹³ However, multiple daily dosing, need for fatty foods for absorption, and limited experience with newer formulations restrict its use to selected populations.

Other antifungals

Besides the above described major classes of antifungals, various other drugs have been used or tested for different fungal infections. Griseofulvin is an antifungal antibiotic produced by *Penicillium griseofulvum*, which is active in vitro against most dermatophytes, and has been extensively used in chronic infections caused by these fungi (e.g., nail infections with *T. rubrum*) since it is orally administered and presumably incorporated into actively growing tissue owing to its capacity to concentrate in the keratinous layer of the epidermis.⁹⁴ The drug inhibits mitosis in fungi and is used in such instances, but it has been challenged by some newer azole antifungal agents.⁷⁹

Two other classes of antifungal agents represented additions to the topical treatment of dermatomycoses. The two allylamines (naftifine and terbinafine) inhibit ergosterol synthesis at the level of squalene epoxidase [squalene epoxidase, an enzyme, together with (2,3)-oxidosqualene cyclase, is responsible for the cyclization of squalene to lanosterol]; one morpholene derivative (amorolfine) causes inhibition at subsequent step in the ergosterol pathway, and exhibit both fungistatic and fungicidal activity in vitro.^{79,82,95,96}

Contrary to the situation with antibacterial agents, few antimetabolites are available for use against the fungi. The typical example is 5-fluorocytosine, a fluorinated analog of cytosine that inhibits both RNA and DNA synthesis via intrafungal formation of two metabolites: 5-fluorouridine triphosphate, which inhibits RNA processing, and 5-fluorodeoxyuridine monophosphate, which inhibits thymidylate synthetase and hence the

formation of deoxythymidine triphosphate needed for DNA synthesis.^{79,97} However, like other antimetabolites, emergence of drug resistance is a problem, and hence 5-fluorocytosine is seldom used alone. Though, in combination with amphotericin B, it is an important treatment for cryptococcal meningitis and is effective against a number of other fungal infections, including some caused by the dematiaceous fungi and perhaps even by *C. albicans*.⁷⁹

Echinocandins – caspofungin, micafungin, anidulafungin – represent a novel category of antifungal agents.^{78,98} They are semi-synthetic lipopeptides with a chemical structure of cyclic hexapeptides connected to a lateral chain of fatty acid. These drugs target the cell walls, inhibiting the enzyme linked to the synthesis of beta-(1,3)-D-glucan. Echinocandins are effective against various yeasts and filamentous fungi in vitro; however in experimental infections only its activity on *Candida* and *Aspergillus* species is relevant. For the latter agent, it is only fungistatic.⁸¹ Furthermore, an exclusive i.v. administration and restricted spectrum of effect limit clinical use of echinocandins to more severe infections caused by *Candida* and *Aspergillus*. Since echinocandins target β-glucan, which is not present in mammalian cell, they present minimal side effects in humans.

In addition, several other agents have demonstrated antifungal properties by possibly interfering with the cell membrane. Restricticin, restricticinol and lanomycin are reported to act by inhibiting C-14 α-demethylase. Likewise, zaragozic acid has been found to inhibit squalene synthase; while several pyrimidinium compounds have been reported to have antifungal activity by acting as squalene cyclase inhibitors.⁸²

TAKE HOME POINTS

- ◆ The prompt and confirmatory diagnosis of fungal infections remains a challenge, especially in the immunocompromized hosts, and often requires more informative laboratory techniques.
- ◆ Often, direct microscopic examination and culture - the gold standard to make fungal diagnosis - of appropriate specimens are used in conjunction for a mycological diagnosis.
- ◆ Clinical outcomes with fungal infections are better when the appropriate (antifungal) treatment is initiated on time.

- ◆ The azole antifungal agents include imidazoles and triazoles, and currently are the most rapidly expanding group of antifungal compounds.
- ◆ Triazoles have a more favorable pharmacokinetic profile than imidazoles. In fact, fluconazole and itraconazole have become the standard for azoles, replacing amphotericin B for managing certain forms of the systemic fungal infections.
- ◆ Specifically, fluconazole is routinely used to treat candidemia in non-neutropenic hosts, and has also gained acceptance for use in cryptococcosis and selected forms of coccidioidomycosis.
- ◆ Likewise, itraconazole exhibits significant activity against *Aspergillus* and the endemic fungi, and has proven to be effective for histoplasmosis, blastomycosis, sporotrichosis, coccidioidomycosis, consolidation treatment for cryptococcosis, and certain forms of aspergillosis.
- ◆ In addition, several other agents have demonstrated antifungal properties, and tested for different fungal infections. For instance, echinocandins represents a novel category of antifungals agents.

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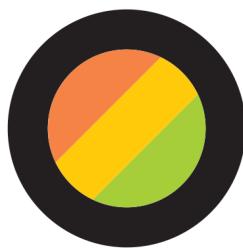
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