

OVERVIEW OF FUNGAL DISEASE AND MEDICAL MYCOLOGY

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I. INTRODUCTION TO THE KINGDOM MYCETEAE (FUNGI)

Mycology is a biological discipline that deals with the study of fungi. Fungi are eukaryotic organisms but are not plants or animals. Fungi are non-motile and generally multicellular in hyphal mold, mushroom forms, or unicellular in yeast forms. Fungi are incapable of producing their own food, so they get their nourishment by absorption from other sources, usually other dead or compromised eukaryotes. These absorptive heterotrophs invade the inside of their substrate or host with hyphae, digesting it externally and absorbing its nutrients. Fungi are ubiquitous decomposers and the best recyclers in nature. They have no chlorophyll, do not need light, and are not photosynthetic.

The reproduction of fungi can be either sexual or asexual. Sexual reproduction, as with other organisms, involves the fusion of two nuclei when two sex cells unite. This joining produces spores that become new organisms. Fungi reproduce asexually more commonly by fragmentation, budding, or producing spores. Separated portions of hyphae can grow new mold colonies. Somatic yeast cells form buds to reproduce. Like plants, fungi also have a cell wall but are not made of cellulose, as they are with plant cell walls. In fungi, cell walls are generally composed of the N-acetylglucosamine polymer chitin, glucans, mannans, and glycoproteins. (1)

Making use of the knowledge of fungal cell wall makeup, clinicians have implemented testing for the beta-(1,3)-d-glucan (BG) molecule as an emerging tool to assist the diagnosis of invasive fungal agents *Candida* spp., *Aspergillus* spp., and *Pneumocystis jirovecii*. (2,3) Another cell wall-molecule test is the galactomannan (GM) test for diagnosing invasive aspergillosis in patients with hematological malignancies. (4)

The Fungi Kingdom contains at least about 144,000 known species that have a wide variety of sizes and forms. (5) A simple layman categorizes them into mushrooms, molds, and yeast. The human disease-related mold and yeast fungi comprise a much smaller subgroup, estimated to be at least 300 species. (6) The most familiar fungi involved in human infections are probably those belonging to the subkingdom Dikarya, which includes mushrooms, yeast, molds, and most molds and yeasts that are human pathogens. (5) The subkingdom Dikarya is broken into two phyla, Ascomycota and Basidiomycota.

Fungi are often associated with decomposition and nutrient recycling in nature but are also famously related to some diseases worldwide. They cause massive disease to many plants and

afflict other eukaryotes as well. Panama disease of bananas is caused by the fungus *Fusarium oxysporum* f. spp. *cubense*. *Fusarium oxysporum* is a common fungus that is probably already in the soil. The fungus attacks the roots of the banana plant and affects its vascular system, so it can't properly absorb water and nutrients from the soil. It may cause the extinction of 99% of the bananas sold in the world today, which are all the same clones, the Cavendish cultivar. (7) Chytridiomycosis has been speculated to be caused by another one of these possible "Armageddon fungi" that may kill off frog species and other amphibians worldwide. As with the banana, fungus is currently the cause of the massive die-off of amphibians worldwide. Chytridiomycosis is an infectious disease in amphibians caused by the fungal chytrids *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans*. (8) It is thought to kill the frogs because their thinner skin absorbs this fungus with water in their environment. (8) Fortunately for us, this fungus has not been associated with human disease. Other fungi are, however, significantly associated with human disease. *Candida* spp., *Aspergillus* spp., *Pneumocystis* spp., and *Cryptococcus* spp. are the most common species causing serious human disease (estimated 90% of serious human fungal disease). (9) However, many other fungal agents are frequently seen in patients.

While our human immune defense mechanisms have evolved to deal with most fungal invasions successfully, there are an ever-increasing number of medically significant fungi. Some are frank pathogens and sometimes infect healthy individuals as well as the immunocompromised (*Coccidioides immitis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides* spp., *Talaromyces* (formerly *Penicillium*) *marneffei*, *Cryptococcus neoformans*, and *Cryptococcus gattii*). Still, the majority are opportunistic and attack mostly the immunocompromised host. As the number of immunocompromised patients grows, these opportunistic infections will increase. An example of a yeast that now causes human nosocomial infections in the immunocompromised is *Candida auris*. *C. auris* was first described in 2009. This multi-drug resistant yeast is already becoming a familiar and significant cause of severe nosocomial infection in many nations' immunocompromised and ICU patients. (10) The medically important fungi are found primarily in the subkingdom Dikarya which has two phyla, Ascomycota and Basidiomycota. (5)

II. MEDICAL MYCOLOGY/FUNGAL DISEASE IN HUMANS

A. The Epidemiology of Fungal Infections

Epidemiology is the foundation of our public health. It is defined as the branch of medicine that deals with the incidence, distribution, and possible control of diseases and other factors relating to human health. It is the study of the distribution and determinants of diseases or disorders within groups of people. It determines how often diseases occur in different groups of people and why. Epidemiological information is used to plan and evaluate strategies to prevent illness and as a guide to managing patients whose disease has already developed. Epidemiological research helps us understand who has a disorder or disease, why, and how it was brought to this individual or region. It establishes which diseases are endemic in an area. Epidemiological studies are crucial to developing knowledge on preventing and controlling human diseases, and more research is needed to understand fungal infections today.

Dramatic epidemiological shifts have been seen in fungal infections, with expanding geographical ranges, identifying new groups of people at risk of fungal infections, increasing

prevalence of resistant infections, and the emergence of novel multidrug-resistant patterns in pathogenic fungi. The scope of types of fungi causing human disease and the spectrum of clinical presentations associated with these infections has widened. We have just seen a newly emergent yeast, *Candida auris*, sweep the globe as a nosocomial infection since its first appearance in 2009. Many populations are susceptible to secondary fungal infections due to unforeseen epidemics like COVID-19. Many healthcare-related, environmental, and socioeconomic factors and changes have influenced these epidemiologic shifts. Some fungi seem ubiquitous and are common worldwide, like *Penicillium* and *Aspergillus*, while others prefer specific regions where they are endemic, such as *Coccidioides immitis* and *Paracoccidioides brasiliensis*. Also, see Fungal infections in the different areas of the world below.

1. The Incidence and Prevalence of Fungal Disease: Fungal infections can be fatal, and updated estimates suggest an annual incidence of 6.5 million invasive fungal infections and 3.8 million deaths, of which about 2.5 million (~68%; range 35-90%) were directly attributable to these infections. (11) The global fungal infection burden is a high total that is greater than the number of people who die from malaria, more than double the number of women who die from breast cancer, and about equal to the number who die from tuberculosis, or HIV, each year. (9)

Not all fungal infections are fatal, and they range significantly in severity. Treatable fungal skin infections, dermatophytosis, are considered the most common fungal infection of all, affecting as many as 1-2 billion people (about 20-25% of our current world population), resulting in generally mild, more superficial conditions like ringworm, skin, hair, and nail infections. However, they occasionally become invasive and severe. (12)

Aspergillus species account for ~3,000,000 severe chronic pulmonary infections, ~750,000 cases of life-threatening invasive aspergillosis, and ~4,800,000 cases of allergic bronchopulmonary aspergillosis. *Aspergillus species* are also involved in mycotoxin production and food poisoning, producing the powerful toxin aflatoxin. (9)

Candida species also cause numerous fungal infections worldwide, predominantly in immunocompromised patients. *Candida species* are estimated to colonize 24-70% of people over one year old. (13) Thus, at some time in our lives, we all likely have an incidence of colonization with a *C. species*. The most prominent member of these *Candida species* is *Candida albicans*, which, in addition to causing most of the less severe mucosal infections, also causes ~750,000 cases of serious, potentially lethal invasive candidiasis annually. (14)

There are more than one million cases of cryptococcosis, with ~650,000 deaths annually caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. (13) Cryptococcosis is one of the most common fatal fungal infections worldwide, especially in HIV-positive/AIDS patients with meningitis. (13,14)

There are ~500,000 cases of *Pneumocystis jirovecii* pneumonia annually, with many of those occurring in patients with AIDS. (14) *Pneumocystis jirovecii* pneumonia is still considered a leading cause of infection in HIV/AIDS patients. (14)

While not as numerous, there are other serious mycological infections with dimorphic fungi such as *Histoplasma capsulatum*, *Coccidioides immitis* and *Coccidioides posadasii*, *Sporothrix schenckii*, *Blastomyces dermatitidis*, *Paracoccidioides braziliensis*, and *Talaromyces* (formerly *Penicillium*) *marneffeii*, *Emergomyces* (formerly *Emmonsia*) *spp.*, and other serious non-dimorphic infections such as fungal keratitis, chromoblastomycosis, mycetoma, and mucormycosis, and many more. (14)

- 2. Geographic Distribution of Fungal Disease Agents:** Many fungal agents, such as *Candida albicans*, *Cryptococcus* species, *Pneumocystis jirovecii*, *Aspergillus fumigatus*, and mucormycosis fungi such as *Mucor* species and *Rhizopus* species, as well as most yeasts and many more fungi, have worldwide distribution in various environments.

Some fungal infections are seen more frequently in tropical areas where people may not wear shoes. People may then be exposed to fungi in puncture lesions of their feet and develop chronic infections, such as chromoblastomycosis and mycetoma. Chromoblastomycosis (chromomycosis, cladosporiosis, or Fonseca's disease) is a long-term fungal infection of the skin and subcutaneous tissue (a chronic subcutaneous mycosis). Mycetoma is a chronic infection of the skin and subcutaneous tissue that fungi and fungus-like bacteria, such as *Nocardia* *spp.* or *Actinomyces* *spp.*, can cause.

Other fungal mycoses are regional, notably the dimorphic fungi. Dimorphic fungi and their associated diseases are endemic in various regional areas. These dimorphic fungi have enhanced pathogenic potential and can attack healthy animals and humans, although they are more frequent in immunocompromised patients. They can live in a mold form in nature in the environment, especially in soil, at room temperature and can also live in a second yeast, or yeast-like form, in the tissues of humans or animals at 37° C. Hence, they have been named dimorphic (two phases at different temperatures) fungi.

The fungal infection, coccidioidomycosis, is caused by the dimorphic fungi *Coccidioides immitis* or *Coccidioides posadasii*. These are dimorphic fungi found in the desert areas of the Southwestern United States (coccidioidomycosis is also called San Joaquin Valley fever). Sporadic cases are reported from other areas, especially Mexico, Central and South America, and occasionally outside of these areas. Interestingly, there was a recent outbreak of coccidioidomycosis in central Washington State, USA, in 2010 and 2011. In this recent outbreak, soil samples from the area of the patient's likely exposure in Washington state did contain DNA of *C. immitis*. Also, they grew fifteen isolates of *C. immitis* on culture, even though this is far outside the typical endemic area for this fungus. (15) *Histoplasma capsulatum* var. *capsulatum* is another dimorphic fungus that is endemic in the United States, the Caribbean, and Central and South America, and it causes histoplasmosis. Histoplasmosis is the most common endemic fungal infection seen in humans. The central river valleys in the midwestern and south-central United States are most famously endemic for histoplasmosis. *Histoplasma* *spp.* are also found in bird and bat droppings or soil contaminated with these droppings, and these spores are inhaled. Approximately 250,000 individuals are infected annually with *Histoplasma capsulatum*. It is seen less frequently in Africa and Southeast Asia and many areas of the world in the immunocompromised, such as AIDs patients worldwide. A less common variety of this fungus species that also causes histoplasmosis, *Histoplasma capsulatum* var. *duboisii*, is usually found in Central and West Africa and Madagascar. Blastomycosis is a potentially fatal systemic and cutaneous fungal infection of humans and animals caused by a

dimorphic fungus, *Blastomyces dermatitidis*. *B. dermatitidis* is endemic mainly in moist soil and in decomposing matter such as wood and leaves in areas of the United States and Canada surrounding the Ohio and Mississippi River valleys and the Great Lakes, the midwestern, south-central, and southeastern states, and in the Saint Lawrence River. It is contracted by inhaling the spores. A dimorphic fungus causes sporotrichosis, *Sporothrix schenckii*, and it can become a potentially fatal disseminated fungal disease, particularly in the immunocompromised host, but is more frequently a subcutaneous mycosis. Infection occurs through a traumatic inoculation of soil, plants, or organic matter contaminated with the fungus. *Sporothrix schenckii* is found worldwide, especially in tropical and subtropical zones. *Talaromyces* (formerly *Penicillium*) *marneffeii*, a dimorphic fungus endemic in Thailand and South China soil causes serious systemic fungal infection. *Paracoccidioides brasiliensis*, a dimorphic fungus, causes paracoccidioidomycosis, also known as "Brazilian blastomycosis" or "South American blastomycosis." It is endemic to Central and South American areas, especially those with acidic soil. It is the most common systemic mycosis in Latin America and causes a progressive mycosis of the lungs, skin, mucous membranes, lymph nodes, and internal organs. Regionally, there are differing conditions and, thus, different concerns and epidemiological trends related to fungal infections.

North America (16, 17)

- **Yeasts:** *C. albicans* remains a substantial problem due to life-threatening invasive fungal disease, with mortality rates approaching 40%. While the number of cases of *C. albicans* overall is decreasing, the incidence of *Nakaseomyces* (formerly *Candida*) *glabrata* and *C. parapsilosis* is increasing. *C. auris* is increasingly related to outbreaks in clinical settings. About 20% of patients who acquire *Cryptococcus neoformans* infections in the U.S. now have no known immune deficiencies. Its incidence in HIV patients has decreased, but not in those without HIV. *Cryptococcus gattii* increased its range after an outbreak in Canada, which has also caused an uptick in cases in the US Pacific Northwest. *P. jirovecii* is still associated with many deaths and is increasingly seen in new immunocompromised groups of patients.
- **Molds:** Influenza and coronavirus are emerging co-infections with pulmonary aspergillosis. Azole-resistant *A. fumigatus* is increasing in prevalence. National data indicate an increase in other mold infections in hematological malignancies, immunocompromised patients, and transplant patients.
- **Dimorphs:** *Blastomyces dermatitidis* remains a problem in the United States in the midwestern, south-central, and southeastern states, particularly in areas surrounding the Ohio and Mississippi River valleys, the Great Lakes, and the Saint Lawrence River. The incidence remains constant at 1-2 cases per 100,000 population in these areas. (1) The cases of *Coccidioides spp.* have increased in recent years, reaching >15,000. Recent cases extend northward into Northern California, Washington state, and eastward into Utah. *Histoplasmosis capsulatum* is also expanding its traditional range, and cases have nearly doubled from 2001 to 2012. *Sporothrix schenckii* incidence is estimated to be two cases per 1 million people, with the highest incidence in the U.S. in southern and south-central states.

Central and South America: (18)

- **Yeasts:** The incidence of bloodstream infections with *Candida spp.* is higher than in the U.S. and Europe, and hospital-acquired infections are a common cause of morbidity and mortality. *C. albicans* is still the most common yeast isolate in Latin America. (#1) The incidence of *Nakaseomyces* (formerly *Candida*) *glabrata* is lower in South and Central America (#4) than in the U.S. *C. tropicalis* and *C. parapsilosis* are more common (#2 and #3). *C. tropicalis* is seen in neutropenic patients. *C. auris* is increasingly related to outbreaks in clinical settings. *Cryptococcus spp.* is seen in HIV-positive men, the immunosuppressed, and increasingly in patients with no known immune deficiency. *C. gattii* is endemic in tropical and subtropical areas. *P. jirovecii* is associated with immunocompromised groups of patients.
- **Molds:** Invasive fungal infections (IFIs) caused by *Aspergillus species*, *Fusarium*, *Mucormycota*, *Scedosporium*, *Trichosporon*, *Rhodotorula*, *Alternaria*, *Bipolaris*, and *Curvularia* have been described in patients with hematologic neoplasias, transplant recipients (especially bone marrow transplants), persons living with diabetes, and patients under chronic high-dose steroid treatment. In Latin America, invasive fungal infections (IFIs) have been caused by this group of pathogens, especially *Aspergillus* and the yeast *Candida albicans* (>90% of the cases). In the same group of patients, the most relevant risk factors were profound neutropenia, monocyte counts of less than 100 cells for more than four days, and elevated serum levels of reactive protein C. Agents associated with subcutaneous mycetoma are endemic in Latin America and are a constant threat in tropical and subtropical regions. Bacterial mycetoma (actinomycetoma) (a fungal-like bacteria) causes most cases of mycetoma in South and Central America.
- **Dimorphs:** *Histoplasmosis spp.* is the most prevalent disseminated mycosis in Central America. It is found in the southeastern regions of Mexico, Argentina, Brazil, Panama, Uruguay, Venezuela, Ecuador, and Colombia. South American strains usually behave more aggressively. Dermatotropism is also more common in South American *Histoplasmosis* isolates, with cutaneous involvement seen in 40-80% of patients (up to 90% in reports from Argentina and Panama), compared with <20% of U.S. patients. *Coccidioides spp.* are highly prevalent in dry regions (<600 mm of annual rainfall) with high temperatures. The species' natural habitat is 5 cm to 30 cm below the surface of alkaline soils. Mexico has three distinct endemic areas: the north (near the US border), the Pacific coast, and the central valley. In Central America, two endemic regions have been identified, the Motagua River Valley in Guatemala and the Comaya Valley in Honduras, with positive skin testing up to 42%. In South America, four countries have areas with high prevalence: in Argentina, the Sierras Pampeanas; in Colombia, the Magdalena, Guajira, and Cesar provinces; in Paraguay, the Great Chaco; and in Venezuela, the Departments of Falcon, Lara, and Zulia. An increase in incidence has recently been reported in Argentina, mainly due to population migration. *Paracoccidioides brasiliensis* is a dimorphic species that causes a South American endemic IFI. In endemic regions, the prevalence of the infection may be as high as 75%, affecting both men and women; ~1–2% will present active disease. Of all active cases, 80% are reported in Brazil, especially in the south and southeast regions. In other areas, such as north Argentina, Venezuela, and Colombia,

the incidence is much lower, at about 2.4 cases per 1,000,000 inhabitants. Sporadic cases have been reported in Mexico and Central America. *Sporothrix brasiliensis* recently emerged in South America and is associated with zoonotic transmission, spreading via animal scratches and bites.

Africa: Africa has the most significant fungal burden (19). However, it is difficult to assess in Africa due to a lack of medical laboratories that can perform medical mycology and public health facilities to track these diseases.

- **Yeasts:** *C. albicans* remains a substantial problem due to life-threatening invasive fungal disease with a high mortality rate. While the number of cases of *C. albicans* is decreasing, the incidence of other *Candida spp.* is increasing. *Cryptococcus spp.* has a high prevalence and causes a mortality rate of 15-20% among HIV-positive people in Africa. It is also growing in non-HIV-infected populations. *P. jirovecci* is still associated with many deaths and is increasingly seen in new immunocompromised groups of patients.
- **Molds:** Azole-resistant *A. fumigatus* are increasing in prevalence. Data point to an increase in other mold infections in patients with malignancies, immunocompromised, and transplant patients. Fungal agents associated with subcutaneous mycetoma and chromoblastomycosis are endemic in Africa and a constant threat in tropical and subtropical regions. *Fonsecaea spp.* (80.9%), *Cladophialophora spp.* (14.5%), and *Phialophora spp.* (1.5%) cause most cases of chromoblastomycosis in Africa. Fungal mycetoma (eumycetoma) is Africa's most common type of mycetoma. Mucormycosis is predominantly reported in North Africa.
- **Dimorphs:** Histoplasmosis in Africa has dramatically increased since the HIV/AIDS epidemic but is under-recognized. Pulmonary histoplasmosis is often misdiagnosed as tuberculosis (TB). In the last six decades, 470 cases of histoplasmosis have been reported. HIV-infected patients accounted for 38% of the cases. (20) A constant presence of *Blastomyces dermatitidis* in Africa, although at lower levels than in North America, indicates that the disease can be considered endemic to some areas of Africa. *Emergomycetes africanus* is a novel thermally dimorphic fungal pathogen that was described to cause disseminated disease among persons living with advanced HIV/AIDS in South Africa. Although the organism was initially described as an *Emmonsia*-like fungus, it is now known to belong to a new genus of thermally dimorphic fungi. (21) Madagascar and South Africa report many cases of locally acquired sporotrichosis.

The Middle East and North Africa: (22)

- **Yeasts:** Invasive candidiasis- *Candida* infections -account for approximately 70 to 90% of total IFIs, predominantly *C. albicans*. *C. auris* is increasingly related to outbreaks in clinical settings. *P. jirovecci* is still associated with many deaths and is increasingly seen in new immunocompromised groups of patients.
- **Molds:** Azole-resistant *A. fumigatus* is increasing in prevalence. Reports point to an increase in other mold infections in hematological malignancy patients,

immunocompromised patients, and transplant patients. *Rhinochlamydia mackenziei* is a dematiaceous fungus that causes high-mortality cerebral infections and is now prevalent in the Middle East. Mucormycosis is frequently reported throughout this region and is commonly associated with diabetes.

- **Dimorphs:** Dimorphic systemic fungi are not endemic, and when cases arise, they often involve travel to or migration from endemic areas. There are more cases reported in immunocompromised patients, especially in the HIV-positive population.

Europe: (23)

- **Yeasts:** *C. albicans* remains a substantial problem due to life-threatening invasive fungal disease with mortality rates of ~40%. While the overall number of cases of *C. albicans* is decreasing, the incidence of *Nakaseomyces* (formerly *Candida*) *glabrata* and *C. parapsilosis* is increasing. *C. auris* is increasingly related to outbreaks in clinical settings. *P. jirovecci* is still associated with many deaths and is increasingly seen in new immunocompromised groups of patients.
- **Molds:** Opportunistic infections like invasive aspergillosis and zygomycosis continue to be a problem, especially in immunocompromised and diabetic patients. Influenza and coronavirus are emerging co-infections with pulmonary aspergillosis. Azole-resistant *A. fumigatus* cases are increasing in prevalence. An increase in other mold infections with hematological malignancies and transplant patients has been observed.
- **Dimorphs:** Systemic dimorphs are not endemic in Europe, and when cases arise, they often involve travel to or migration from endemic areas. There are more cases reported in immunocompromised patients, especially in the HIV-positive population. Sporotrichosis is rare in Europe.

India: (24): Mycoses are frequent in India, but the medical literature lacks statistics on their incidence and prevalence. More epidemiological research and a holistic estimate of fungal disease in the Indian subcontinent are needed.

- **Yeasts:** *C. albicans* remains a substantial problem due to life-threatening invasive fungal disease with High mortality rates. While the number of cases of *C. albicans* overall is decreasing, the incidence of *Nakaseomyces* (formerly *Candida*) *glabrata* and *C. parapsilosis* is increasing. *C. auris* is increasingly related to outbreaks in clinical settings. India has one of the highest incidences of *Cryptococcus spp.* due to many persons living with HIV/AIDS (after Africa). *P. jirovecci* is still associated with many deaths and is increasingly seen in new immunocompromised groups of patients.
- **Molds:** *A. fumigatus* is an invasive disease prevalent in India. Mucorales infections are increasing significantly, as a recent study found a 2.1-fold increase in mucormycosis diagnosis. (51). Also, data point to an increase in other mold infections with malignancies, immunocompromised patients, and transplant patients. India has a high level of dermatomycoses, mostly tinea capitis in children. Agents associated with subcutaneous mycetoma are endemic in India and a constant threat in tropical and

subtropical regions. Chromoblastomycosis is not uncommon in India, and there are many case reports.

- **Dimorphs:** Dimorph cases often arise related to travel to or migration from endemic areas, but there are also endemic cases. There are more cases reported in immunocompromised patients, especially in the HIV-positive population. There is thought to be a consistent incidence of ~1.43% *Talaromyces marneffeii* cases in those with newly presenting advanced HIV disease in India. India has pockets of histoplasmosis and sporotrichosis but does not have good incidence or prevalence data for these infections. More epidemiological research is needed in India.

Asia, Pacific Islands: (25)

- **Yeasts:** *C. albicans* remains a substantial problem due to life-threatening invasive fungal disease with high mortality rates. While the number of cases of *C. albicans* is decreasing overall, the incidence of *Nakaseomyces* (formerly *Candida*) *glabrata* and *C. parapsilosis* is increasing. *C. auris* is increasingly related to outbreaks in clinical settings. Cryptococcosis has been at a high level in Asia since the HIV epidemic and is still causing many deaths. It is increasingly seen in non-HIV patients. *C. gattii* is endemic in certain areas of this region. *P. jiroveccii* is still associated with many deaths and is increasingly seen in new immunocompromised patients.
- **Molds:** Influenza and coronavirus are emerging co-infections with pulmonary aspergillosis. Azole-resistant *A. fumigatus* is increasing in prevalence. Data also point to increased mold infections with hematological malignancies and transplants. Agents associated with subcutaneous mycetoma are endemic in Asia and a constant threat in tropical and subtropical regions. Bacterial mycetoma (actinomycetoma) (fungal-like bacteria) causes most cases of mycetoma in Asian countries.
- **Dimorphs:** The most common endemic mycoses in the Asia-Pacific region are **histoplasmosis**, caused by *Histoplasma capsulatum*; penicillopsis, caused by the endemic *Talaromyces* (formerly *Penicillium*) *marneffeii*; and sporotrichosis, caused by *Sporothrix schenckii*. (25)

Australia: (26)

- **Yeasts:** *C. albicans* remains a substantial problem due to life-threatening invasive fungal disease with high mortality, as is true worldwide. *C. auris* has been isolated in over 30 countries (including Australia). Bloodstream infections are the most frequently reported infections. Infections have a crude mortality of 30-60%. The acquisition is generally healthcare-associated, and risks include underlying chronic disease, immunocompromise, and indwelling medical devices. Cryptococcosis has been at a high level since the HIV epidemic and is increasingly seen in non-HIV patients. *C. gattii* is endemic in some regions of Australia. (26)
- **Molds:** Azole-resistant *A. fumigatus* is increasing in prevalence. Data also point to an increase in other opportunistic mold infections, especially in patients with hematological malignancies, immunodeficiency, and transplant patients.

- **Dimorphs:** Dimorphs are not endemic; when cases arise, they usually involve travel to or migration from endemic areas. There are more cases reported in immunocompromised patients, especially in the HIV-positive population.
3. **Notifiable Fungal Diseases:** These are fungal infectious agents that government agencies require to be reported to public health agencies. The US public health system does collect limited data on certain fungal cases. Individual US states may also require reporting of other fungal diseases that can be systemic or cause meningitis or other fungal diseases of interest. Different nations have their own laws or regulations relating to which fungal diseases, if any, they track.
The fungal cases that are currently reportable in the US are: Coccidioidomycosis in all states. In some states, *Cryptococcus spp.*, *Histoplasmosis spp.*, and *Blastomycosis spp.* Also, fungi with unusual resistance include *Candida auris* (multidrug-resistant), some strains of other resistant yeasts, and resistant fungi such as azole-resistant *Aspergillus fumigatus*. (27)
 4. **Zoonotic Fungal Infections:** Zoonoses are infectious diseases that can be naturally transmitted between vertebrate animals and humans. Wild and domestic animals often serve as reservoirs for the infecting agent, and these infectious agents usually account for the many emerging and re-emerging infectious diseases worldwide. Fungal zoonoses include the well-known dermatophytosis, sporotrichosis, and histoplasmosis. (17) Other less-known mycoses with zoonotic potential include infections caused by *Paracoccidioides braziliensis*, *Basidiobolus ranarum*, *Conidiobolus spp.*, *Talaromyces (Penicillium) marneffeii*, *Emergomyces spp.*, and *Paracoccidioides lobi* (formerly *Lacazia lobergomycesoi*). (12,28)
 5. **Nosocomial Fungal Infections:** Healthcare-associated fungal infections have repeatedly been described in outbreaks of the last decade, often caused by the ever-present *Candida albicans* or the newly described multidrug-resistant yeast, *Candida auris*. *C. auris* has caused several severe healthcare-associated invasive fungal infection outbreaks on at least five continents. (29, 30) Other large nosocomial outbreaks were reported due to uncommon fungal species, *Exserohilum rostratum* and *Sarocladium kiliense*, that were both caused by contaminated medical products. These outbreaks were seen in immunosuppressed and intensive care unit patients. (30) Cases of rapidly fatal infections due to the fungus *Saprochaete clavata* were also reported in France within a short time in three healthcare facilities, suggesting a common source of contamination. (31) A national alert prompted the discovery of 30 cases of *S. clavata* in France over one year, including an outbreak of 18 cases over eight weeks. (31) The source of the *S. clavata* infections was never found, so issues of disease transmission remain unresolved. (30)
 6. **The Pathophysiology, Pathogenesis, and Immunity Involved in Fungal Infections:** *Fungal diseases* are most often caused by *fungi* that are common in the *environment*. *Fungi* live in nature in soil, plants and trees, many indoor surfaces, and human and animal skin. When humans encounter these environmental fungi, infections may arise from inhaling spores, direct injection of spores into tissues, or ingesting spores. Some fungal diseases are spread by person-to-person contact, such as a skin infection with ringworm, which is spread by such direct contact. Most *fungi* are not dangerous, but some types can harm health, especially the compromised or immunocompromised host. Fungal disease

can involve infectious, allergic, or toxin-associated diseases. Fungal infections are also known as mycoses.

Most of us are in contact with fungi regularly. *Aspergillus spp.*, *Penicillium spp.*, and *Rhizopus spp.* grow commonly in our refrigerator or our shower area. "Somewhere between 100 to 300 spores of a fungus called *Aspergillus species* get in our lungs every day," says Professor Neil Gow, president of the Microbiology Society. (9), and he added, "We deal with it perfectly well because our lungs are full of immune cells, which patrol around looking for these spores, and they swallow them up and kill them." (9) But for people with weakened immune systems, *Aspergillus* can cause lung disease and can kill after as little as 10-14 days, according to David Denning, professor of Infectious Diseases in Global Health from the University of Manchester. "It's fairly uncommon but still life-threatening," Denning stated. (9)

7. Fungal Infections in Special Patient Populations—Transplant Patients, Diabetic Patients, HIV Patients, Immunocompromised Patients, Etc.

- **Patients in the Intensive Care Unit:** Undoubtedly, current medical practices have extended lives for the elderly, the critically ill, and immunocompromised patients. However, these patients generally need more intensive care, medical interventions, treatments, and antibiotics. Often, they also need immunotherapy and immunosuppressive therapy. These patients usually have intravenous feeding lines, lines for medications, intubation tubes, or ports. These lines/ports involve invasive techniques that cause breaks in the normal skin protective defenses. Additionally, the plastic tubing used promotes the formation of biofilms for many fungi, especially yeasts, giving these fungi increased access around normal patient defenses directly into the patient tissues and bloodstream. Biofilms also increase the likelihood of resistance to antifungal treatment. Many of the fungal diseases in the intensive care unit, especially involving yeast, are also nosocomial infections. As mentioned, *C. auris* is a new nosocomial yeast with a drug-resistance profile, which makes it challenging to eradicate and control in the ICU.

The heavier antibiotic use in this patient population kills the normal flora in these patients, setting the stage for increased yeast infections. Increased corticosteroid use and other immunosuppressive or immunomodulation therapy often enhance fungal growth and reduce host immune defenses. These agents impair innate immunity to fungi. (32) Immune system pharmaceutical biologics such as Lemtrada® (alemtuzumab) have an increased risk of disseminated fungal disease and mortality. (32) Tumor Necrosis Factor blockers (Cimzia®, Remicade®, Humira®, and Enbrel®) can raise the risk of fungal infection. (33) As of 2008, the FDA received 240 reports of patients taking TNF blockers who developed histoplasmosis, a fungal infection that usually starts as a respiratory infection and can spread throughout the body. Of those 240 patients, 45 patients died, including at least 12 who hadn't been diagnosed with histoplasmosis right away, according to Jeffrey Siegel, MD, clinical team leader in the division of anesthesia, analgesia, and rheumatology products at the FDA's Center for Drug Evaluation and Research. This number of cases then prompted stronger warnings on these medications (33)

Epidemiological surveys suggest that infectious fungal disease is one of the most significant increasing infectious disease concerns in the intensive care unit. (34) *Candida species* (especially *albicans*) are the most common cause of infectious fungal disease in the intensive care unit, about 90% of cases. The incidence of *C. albicans* has decreased in relation to other *Candida species*. This is generally thought to be due to the introduction of azole drugs. Another *Candida spp.* that is a problem for this patient population is *C. auris*. *C. auris* is a multi-resistant species that causes nosocomial outbreaks in the intensive care unit. An additional fungal infection of concern is invasive aspergillosis. Still, other less common agents in the ICU include *Cryptococcus species*, *Pneumocystis jirovecii*, yeasts other than *Candida species*, *Fusarium species*, *Scedosporium spp.*, and the *Mucorales*.

- **Patients with Diabetes: Fungal Infections Commonly Linked to Diabetes:** Mucocutaneous candidiasis, particularly cutaneous, oral candidiasis, and vaginal candidiasis, is observed frequently in diabetic patients. Urinary tract candidiasis in diabetic patients frequently develops into systemic candidiasis or, more rarely, fungus ball formation in the kidney. (35)

Tinea pedis (fungal foot dermatophyte) and distal subungual onychomycosis (fungal nail infection) are common in diabetic patients. Romano, C. et al. conducted a study of 171 diabetic patients and 275 control patients and found Trichophyton mentagrophytes were the most common agent of this mycosis in diabetic patients. (36)

Mucormycosis, especially rhinocerebral mucormycosis, represents a severe life-threatening risk in diabetic patients. Nezafati, S. et al. conducted a ten-year study of mucormycosis in a University Hospital in Tabriz, Iran, 2007-2017, that reported 42 cases of mucormycosis during that time, with 95% of these cases of rhinocerebral form, and 90% of them occurring in diabetic patients. (37) There is a lack of worldwide reporting of fungal diseases, but this gives you an idea of the epidemiology of this type of mycosis. Results in different studies of mucormycosis vary. Roden M. et al. also reported on the epidemiology of mucormycosis in 929 patient cases. This study reported that 36% of patients with mucormycosis had either diabetes type I or type II. They also noted that common types of infection were sinusitis (39%), pulmonary (24%), and cutaneous (19%) and that diabetic patients, especially those with ketoacidosis, were at higher risk for rhinocerebral (66% of diabetic cases). Roden also reported that patients with malignancy tended to have more of the pulmonary type of infections (over half of the cases) and that 95% of these mucormycosis in malignancy cases were of hematological origin. (38)

- **Hemato-Oncology Patients:** Three primary mycoses, invasive candidiasis, invasive aspergillosis, and mucormycosis, account for most fungal infectious diseases in hematology and oncology patients. (39) Well-publicized risk factors for invasive candidiasis include neutropenia, hematological malignancy, use of broad-spectrum antibiotics, IV lines and indwelling central catheters and ports, prolonged stays in the intensive care unit, being elderly or a neonate, mucosal surface colonization with *Candida albicans*, recent gastrointestinal surgery, and renal failure.

Risk factors for developing invasive aspergillosis include hematological malignancy, corticosteroid or other immunosuppressive therapy, immunosuppression, systemic disease, extensive burns, near drowning, allogenic bone marrow transplant, graft versus host disease, cytomegalovirus reactivation, ganciclovir treatment, neutropenia, and myelosuppression.

As reported for diabetic patients above, mucormycosis represents a risk for hematological malignancy patients, who are slightly more prone to getting the pulmonary form of mucormycosis. (38) Other risk factors include diabetes, ketoacidosis, other forms of metabolic acidosis, systemic disease, immunocompromised patients, iron overload, iron chelator therapy (with deferoxamine (Desferal®), corticosteroid or other immunosuppressive therapy, trauma and burns, organ or bone marrow transplantation, neutropenia, and malignant disorders.

- **Patients with Solid Organ Transplants and Patients on Immunomodulators:** Corticosteroid (such as prednisone) use and other immunosuppressive or immunomodulation therapies are typical for long periods in transplantation and in various immune and cancer treatments. Glucocorticoids and corticosteroid receptor agonists target and repress transcriptional factors critical to immune cell proliferation, such as activating protein-1 (AP-1) and nuclear factor kappa beta (NF- κ B), and also directly enable fungal growth. Corticosteroids are thought to suppress macrophages and neutrophils and, therefore, suppress and impair innate immunity to fungal infection. (32) *Pneumocystis jiroveci* pneumonia (the most common infection reported by patients on methotrexate) and systemic fungal and fungus-like infections such as cryptococcosis, nocardiosis, aspergillosis, and histoplasmosis have been reported as side effects of methotrexate use. (40)

An ever-expanding list of biologic immunomodulators are used in immunotherapy for various immune diseases, autoimmune conditions, and cancer therapy. These are sometimes referred to as biological response modifiers, and they include immune system cytokines and chemokines and their receptors or monoclonal antibodies against these molecules. Many of these biological drugs raise the risk for infectious diseases, including fungal infectious diseases. Tumor Necrosis Factor blockers (Cimzia®, Remicade®, Humira®, and Enbrel®) are well known to increase the risk of infection, including histoplasmosis and other fungal infections. (33) Other immune system pharmaceutical biologics such as Tocilizumab (Actemra® [IL-6]; Genentech, South San Francisco, CA), Ustekinumab (Stelara® [IL-12 and IL-23]; Janssen Biotech, Horsham, PA), and canakinumab (Ilaris® [IL-1B]; Novartis Pharma, Basel, Switzerland) are all humanized monoclonal antibodies against the interleukins noted. Natalizumab (Tysabri®, Biogen, Cambridge, MA) is a recombinant human monoclonal antibody to the cell adhesion α -4-integrin that prevents the binding of leukocytes to endothelial cells. Alemtuzumab (Lemtrada®; Genzyme, a Sanofi company, Cambridge, MA) is a CD52-directed cytolytic monoclonal antibody. Rituximab (Rituxan®; Genentech, South San Francisco, CA) is a chimeric monoclonal antibody against the CD20 protein on the surface of B cells that binds and destroys B-cells. All these biologics, and more that are coming out rapidly, decrease immune protection and increase the risk of disseminated fungal disease and mortality for those taking them. (32, 41)

- **Patients with HIV/AIDS:** Fungal infections are hallmarks of and some of the most common opportunistic infections that occur during HIV infection and subsequent AIDS. HIV patients are at risk for a wide variety of fungal agents that may or may not normally cause disease. Most healthy people have innate protection against most of these agents. Because the CD4 T lymphocyte cell population of HIV/AIDS patients is depleted, often significantly, low cellular immunity makes them more susceptible to fungal infection. The incidence of fungal infections in this patient population increases as these cells decrease, the increased infections inversely mirroring any decrease in these CD4 T-cells.

HIV-infected patients have an increased number of yeast and yeast-like organism infections. *Pneumocystis jirovecii* (a yeast-like fungus) caused pneumonia remains one of the most common AIDS-defining illnesses, despite its incidence dropping more than ten-fold since anti-pneumocystis prophylaxis treatment started in 1989. Before this prophylactic treatment, 75% of HIV-infected patients in the US developed *Pneumocystis* pneumonia during their lives. (42, 43) *Candida albicans* commonly causes mucocutaneous infections such as thrush, esophageal candidiasis, and vulvovaginal candidiasis in these patients. *Cryptococcus neoformans* var. *grubi* (serotype A) causes most cases of cryptococcosis cases in patients with AIDS, with the most common presentation being meningoencephalitis. The incidence of cryptococcosis has decreased with combined anti-retroviral therapy in many industrialized nations, although there are still many deaths from this infection in Africa and Southeast Asia. Measurement of the spinal tap opening pressure is mandatory, as patient prognosis strongly correlates to the increased pressure's magnitude. (43) Magnetic resonance imaging is more sensitive in detecting meningoencephalitis than computed tomography. Pneumonia, cough, and dyspnea with hemoptysis may also occur. Skin lesions may reflect disseminated cryptococcosis. Urinary infection involvement is common in males. (43)

All of the dimorphic fungi are risks for HIV/AIDS patients, but only three are frequently found in these patients: histoplasmosis, coccidioidomycosis, and penicilliosis (caused by *Talaromyces* (formerly *Penicillium*) *marneffi*). Histoplasmosis is endemic in the United States, the Caribbean, and Central and South America. Histoplasmosis is the most common endemic fungal dimorphic infection seen in humans, and so is the most common dimorph seen in HIV/AIDS patients. The central river valleys in the midwestern and south-central United States are the most famously endemic for histoplasmosis. *Histoplasma spp.* are also found in bird, bat droppings or soil contaminated with these droppings and are seen in various pockets worldwide. Coccidioidomycosis is a great risk to HIV/AIDS patients who live in its endemic areas of the American Southwest, Mexico, and Central and South America. Extrathoracic coccidioidomycosis (*Coccidioides spp.* causing infection in sites outside of the respiratory tract) is an AIDS-defining infection. Penicilliosis is a risk for HIV/AIDS patients living in Thailand or South China. (43) *Emergomycosis spp.*, a dimorphic fungus, has been reported to be responsible for disseminated fungal disease involving HIV/AIDS patients in South Africa, although less common. (43) Invasive aspergillosis causes significant pulmonary disease and disseminated disease in HIV/AIDS patients. Many additional fungal agents have been reported to cause fungal infections in the HIV/AIDS population.

- **Neonatal Patients:** Neonates, especially those born before 28 weeks of gestation or Extremely Low Birth Weight (ELBW) neonates, have immature immune systems and impaired barrier protections from skin and mucosal membrane breaches by fungi. This makes colonization of the skin and mucous membranes more of a problem for this population as the colonizing microbes have an easier time getting access into tissues. The use of intravenous and central catheters and ports and respiratory and urinary access tubes or catheters are also risk factors for infections in neonates/ELBW neonates, just as for the elderly in intensive care units. These conditions put this population at a higher risk for mucocutaneous candidiasis (such as oral thrush or diaper rash) and more serious invasive candidiasis. Zaoutis et al. reported that neonates have as much as a three to five-fold increased risk of developing invasive candidiasis over an adult or child. (44) Thrombocytopenia and hyperglycemia are strongly associated with invasive candidiasis in neonates. (45) *Candida albicans* and *Candida parapsilosis* are the most common fungal species causing invasive fungal disease in this population (45) Other yeasts, such as *Candida glabrata* or *Candida krusei* are occasionally associated with neonatal invasive fungal disease. (45) Ideally, blood samples or maybe even CSF samples would be obtained to establish candidemia or disseminated candidiasis; however, collecting these samples, especially in premature and ELBW neonates, is difficult, and they cannot spare that much blood. Interestingly, isolation of *Candida species* from urine culture indicates invasive candidiasis in a significant number of neonates, and this finding is enough to prompt systemic evaluation and immediate initiation of antifungal therapy. (45) *Malassezia spp.* are ubiquitous yeast occasionally associated with nosocomial infections, especially involving lipid-containing parenteral infusions with a central venous catheter. (45, 46) Nosocomial cutaneous fungal infections with these molds and *Malassezia pachydermatis* (causes ear infections in dogs) via skin contact have also been reported. (46)

Although rare invasive aspergillosis fungal cases have been reported in these patients, as well as cases of Mucorales, they and other mold infections are rare. (47, 48)

- **Fungal Infections in Patients with Cystic Fibrosis, Asthma, COPD:** Cystic fibrosis patients have a genetic condition with mutations in the cystic fibrosis transmembrane regulator that cause very thick and viscous respiratory secretions. These patients have impaired mucociliary clearance and develop bronchiectasis. Although not classically immunocompromised, they are at higher risk of respiratory infection. Additionally, these patients also frequently receive either inhaled or oral corticosteroids. Their CFTR-deficient cells are less efficient at ingesting and eliminating fungal conidia, leading to increased colonization of the airway by fungi and sensitization to fungi such as *Aspergillus spp.*, *Scedosporium spp.*, *Candida spp.*, and possibly other fungi. (49) *Pseudomonas spp.* colonization or chronic infection also often precedes *Aspergillus* colonization. (49) This, in turn, stimulates T-helper (Th) type 2 responses, triggering the development of allergic bronchopulmonary aspergillosis (ABPA—mycosis with *Aspergillus spp.*) or allergic bronchopulmonary mycosis (ABPM—mycosis with any fungus), *Aspergillus* bronchitis, and aspergilloma (*Aspergillus spp.* fungus ball). If fungus spores stay in the lungs, they can grow there chronically with few symptoms. They can grow to form a mycetoma or fungus ball, a ball of mold within a body cavity such as a paranasal sinus, or an organ with a cavity such as the

lung. (39) The presence of *Pseudomonas aeruginosa* or *Burkholderia cepacia* often causes complications in the laboratory as they can overgrow and suppress any fungus present in the patient's sample without special processing. *Aspergillus* bronchitis is defined by high sputum galactomannan antigen, *Aspergillus spp.* DNA levels, and *Aspergillus spp.* serum IgG antibody.

People with asthma, COPD, and other respiratory diseases are also more susceptible to lung infection with invasive pulmonary *Aspergillus*, which can cause pneumonia-like symptoms, such as coughing up mucus, difficulty breathing, and wheezing. The mechanism of this is likely like the above, with increased thickened mucous secretions impairing the natural removal of fungal spores, followed by colonization, and then infection or allergic exacerbation. Various other fungi are occasionally associated with asthma and other chronic lung diseases. (49) *Candida species* are often considered colonizers of the respiratory tract, although a few cases of tracheobronchitis or chronic bronchitis have been associated with *Candida species*. (37) *Pneumocystis jirovecii* has been reported to cause enhanced inflammation in COPD, although its significance is unclear. (49)

B. World Health Organization Global Fungal Priorities – The Nineteen Fungal Infectious Agents with the Most Significant Public Health Impact in Different Regions Across the World

The World Health Organization has recently published (in October 2022) its first-ever Priority Fungal Pathogens List to drive needed research on fungal pathogens and their global impact. Invasive fungal diseases are not well studied and underreported despite being an emerging global health threat to millions worldwide. While many fungal infections are endemic and have a strong presence in some geographical regions, they are increasingly found in new settings with climate change and global travel increases. Other fungi seem to have a significant presence throughout most of the world, causing heavy disease levels worldwide. Some fungi, like *Candida auris*, have only made their first appearance recently (2009), yet they are already seen in hospitals worldwide. (50)

The WHO report identified and ranked the nineteen fungi with the most significant public health impact and antifungal resistance risks. They ranked these pathogens into these three priority groups:

- 1. Critical Priority:** 1) *Cryptococcus neoformans*, 2) *Candida auris*, 3) *Aspergillus fumigatus* and 4) *Candida albicans*
- 2. High Priority:** 5) *Nakaseomyces glabrata* (*Candida glabrata*), 6) *Histoplasma spp.*, 7) *Eumycetoma causative agents*, 8) *Mucorales* (formerly Zygomycota), 9) *Fusarium spp.*, 10) *Candida tropicalis spp.*, 11) *Candida parapsilosis*
- 3. Medium priority:** 12) *Scedosporium spp.*, 13) *Lomentospora prolificans* (*Scedosporium prolificans*), 14) *Coccidioides spp.*, 15) *Pichia kudriavzevii* (*Candida krusei*), 16) *Cryptococcus gattii*, 17) *Talaromyces marneffeii*, 18) *Pneumocystis jirovecii*, 19) *Paracoccidioides spp.* (50)

Different regions of the world have various patterns of these global priority fungal pathogens:

<i>Fungus</i>	<i>Regions of Prioritization for this Fungal Agent</i>
<i>Critical-priority group</i>	--
1) <i>Cryptococcus neoformans</i>	Global
2) <i>Candida auris</i>	Global
3) <i>Aspergillus fumigatus</i>	Global
4) <i>Candida albicans</i>	Global
<i>High-priority group</i>	--
5) <i>Nakaseomyces glabrata</i> (<i>Candida glabrata</i>)	Globally, it has increased in highly developed countries and among older people.
6) <i>Histoplasma spp.</i>	The Americas, Africa
7) <i>Eumycetoma causative agents</i>	Global
8) <i>Mucorales</i>	Southeast Asia
9) <i>Fusarium spp.</i>	Global
10) <i>Candida tropicalis spp.</i>	Global but increased in Latin America
11) <i>Candida parapsilosis</i>	Global but increased in Latin America and among younger populations
<i>Medium-priority group</i>	--
12) <i>Scedosporium spp.</i>	Global
13) <i>Lomentospora prolificans</i> (<i>Scedosporium prolificans</i>)	Global
14) <i>Coccidioides spp.</i>	The Americas, primarily North America
15) <i>Pichia kudriavzevii</i> (<i>Candida krusei</i>)	Global but increased in Latin America and among younger populations
16) <i>Cryptococcus gattii</i>	The Americas, Africa, Western Pacific, South-East Asia
17) <i>Talaromyces marneffeii</i>	Southeast Asia, Western Pacific
18) <i>Pneumocystis jirovecii</i>	Global
19) <i>Paracoccidioides spp.</i>	The Americas, primarily Central and South America
The above ranking from the W.H.O. is from a global perspective, but it must be remembered that the world's various regions may experience more or fewer issues than the rest of the world. For example, coccidioidomycosis and paracoccidioidomycosis might seem to be less of a concern on a global level due to case numbers globally. But, in North America, coccidioidomycosis is of greater concern, and in Central and South America, paracoccidioidomycosis is of greater importance because of regional disease burdens with these serious pathogens. (51)	

III. THE BASICS OF MEDICAL MYCOLOGY

1. Fungal Taxonomy, Classification, and Nomenclature

- Kingdom Myceteae (Fungi) (52)
- Subkingdom Dikarya (contains human pathogens)
- Phylum Ascomycota (ascomycetes)
- Phylum Basidiomycota (basidiomycetes)

- Subkingdom Fungi incertae sedis (Latin for “of uncertain position”)
- Phylum Blastocladiomycota (uni-flagellated fungi)
- Phylum Chytridiomycota (aquatic fungi)
- Phylum Cryptomycota (aquatic fungi)
- Phylum Microsporidia (opportunists in the immunocompromised)
- Phylum Mucoromycota (zygomycetes) contains human pathogens
- Phylum Nephridiophagidae (found in insects)
- Phylum Olpidiomycota (plant pathogens)
- Phylum Sanchytriomycota (new phylum)
- Phylum Zoopagomycota (new phylum)

Fungal species continue to undergo significant nomenclature changes. With recent molecular genetics methods in taxonomy, many fungal names have changed. Recently, *Candida* yeasts, in particular, have undergone several name changes. Many of these yeasts are medically significant. Of the common yeast species, *Candida glabrata*, *Candida krusei*, *Candida lusitaniae*, *Candida guilliermondii*, and *Candida rugosa* are now renamed *Nakaseomyces glabrata*, *Pichia kudriavzevii*, *Clavispora lusitaniae*, *Meyerozyma guilliermondii*, and *Diutina rugosa*, respectively. Many molds have also been renamed and reclassified. The zygomycetes are now mainly under the phylum Mucormycota. Some medical labs have adopted the practice of reporting the new vocabulary with the old name in parentheses marked “formerly known as ...” (53). These chapters will use this practice.

2. **Fungal Genetics and Genomics:** Fungal genetics is the study of the mechanisms of inherited information in fungi. Yeasts and filamentous fungi are frequently used as model organisms for genetic research in eukaryotes. These mechanisms include cell cycle regulation of cellular reproduction, chromatin structure organization, genetic regulation, and gene recombination. (54) It studies how fungi function at a genetic level, including ploidy, gene structure, and gene flow by sexual and asexual processes. Fungal genetic analysis includes gene mapping by sexual crossing, tetrad analysis, and forward genetic experimentation based on mutagenesis. Molecular genetic analysis includes gene manipulation by transformation, different gene knockout and targeting methods, and their application for forward and reverse genetic approaches to ascertain gene products and their functions.

Fungal genomics is a scientific field that explicitly concerns the genome, encompassing all of the fungi's hereditary information. This genetic information can be used to study fungal pathogenicity, antibiotic resistance, and evolution or to identify fungal infection outbreaks.

3. **Groups of Medically Important Fungi by Microscopic and Culture Appearance:** First, medically important fungi are divided into yeast, filamentous molds, and dimorphic fungi (which can exist in both yeast and mold phases). Yeasts are single-cellular eukaryotes that can reproduce asexually by budding yeast. They grow as a creamy or glabrous colony on culture media. Filamentous fungi grow in a mold form, a mat of filamentous fungal hyphae. These yeast and mold types of fungi can be differentiated under the microscope and visually on culture media.

One of the first characteristics that helps you categorize mold fungi is to look for pigment. Hyaline molds (or clear molds) have clear or only very faintly pigmented hyphae and appear light-colored on the colony's reverse side of the media. Dematiaceous fungi (phaeoid or dark-colored molds) have melanin pigment in their cell walls, giving them a dark color in their hyphae, and they appear dark brown to black on the front and reverse sides of the colony on their media. Other fungi have pigments of various colors on their tops or that diffuse into the media, but the colony reverse is not dark. Methods to differentiate molds include growth rate, texture, colonial characteristics, microscopic structures, growth on various media, and other tests.

4. Fungal Cellular and Colonial Structures and Morphology

Fungal Structures

- **Yeast with Blastoconidia:** Single round or oval cell that usually buds to form daughter cells.
- **Cigar Body- (*Sporothrix Schenkii* Yeast Form):** Forms of yeast cells of this organism resemble cigar shapes.
- **Pseudohyphae:** Elongated blastoconidia made by some yeast species, often under invasive conditions.
- **Hyphae:** septate hyphae, aseptate hyphae, coenocytic hyphae – septate refers to cross-walls. Some hyphae are septate, and those of the Mucorales (zygomycetes) are not septate (aseptate) or are coenocytic (rarely septate).
- **Conidia:** It is an asexual, non-motile spore of a fungus (mold) at the tip of a specialized hyphae.
- **Macroconidia:** A multi-celled conidium is an entire hyphal element converted into a macroconidium. This term is used only when smaller conidia are also present. Macroconidia may be thick or thin-walled, smooth or rough, club-shaped, spindle-shaped, or oval, alone or in clusters.
- **Arthroconidia:** Conidium is produced by fragmentation of the hyphal strand through the septation points. Some are made adjacent to each other (as in *Trichosporon spp.*), and others are separated by disjunct cells (disjunct cells are not arthroconidia) and are called alternating (as with *C. immitis*).
- **Chlamydospore:** A thick-walled vesicle of *Candida albicans* and some other yeasts that does not germinate or produce conidia when mature.
- **Phialide and Phialoconidia:** A phialide is a tube-shaped or vase-shaped conidiogenic cell. Phialoconidia are conidia that arise on the phialide.
- **Sporangium, Sporangiphore, and a Sporangiospore:** A sporangium is an asexual sac-like structure at the tip of a sporangiphore stalk that supports it. The sporangium contains asexual sporangiospores (produced by zygomycetes).

- ***Penicillus***: Conidiophores (stalk structures that hold conidia) that branch in multiple layers, providing a brush-like appearance.

Rhizoids-Rootlike Hyphae

- ***Coccidioides Immitis Spherule with Endospores***: A thick-walled spherical structure produced in tissue by *Coccidioides immitis*. Spherules are empty when young but filled with endospores when mature.
- ***Paracoccidioides spp***: *Mariner's wheel of multiple budding yeast*--a ring of budding yeast seen in the tissues of those infected with the dimorph *Paracoccidioides spp*.
- ***Sclerotic Bodies***: (black dot) Thick-walled cells in tissue that may be divided into horizontal and/or vertical cross septa, as observed in chromoblastomycosis.
- ***Granules***: 1-2 mm wide clump of hyphae and swollen cells in a compact mass or fungus-like bacteria growing from a necrotic center. A cement-like matrix may be present. Granules are various colors depending on the causative agent. They are observed in mycetomas.

Fungal sexual reproduction structures, if they are present, include:

- ***Basidium and Basidiospore***: A basidium is a club-like sexual mother cell from which basidiospores arise for members of the Phylum Basidiomycota.
- ***Cleistothecium, Ascus, Ascospore***: A cleistothecium is a wholly enclosed ascocarp containing asci (sexual mother cell from which ascospores arise for members of the Phylum Ascomycota).
- ***Zygosporangium and Zygospore***: A zygosporangium is a thick outer layer covering a zygospore in Phylum Mucormycota members.

IV. MEDICAL LABORATORY TESTING FOR CLINICALLY IMPORTANT FUNGI

Collect patient samples early for diagnostic testing. Consider fungi along with bacterial agents for infection. Order tests for rapid detection, confirmatory identification of the fungal agent, and susceptibility testing. Culture results can take up to 4-6 weeks to complete, and culture alone often lacks sensitivity as these organisms are sometimes difficult to cultivate. Different fungal disease agents have differing growth requirements that must be met for a successful culture. Obtaining a good quality, representative, and adequate specimen for testing is as **critical** as it is for bacterial infections. Fungal tests are very expensive and labor intensive, so don't even bother collecting and testing a poor sample that may give inadequate or misleading results; insist on getting a good sample.

Direct microscopic examination by the pathology department (histology or cytology) and direct examination by the clinical microbiology laboratory provide the best immediate information on the fungal pathogen. Antigen, serological, and molecular testing are invaluable for more rapid information about the fungal pathogen. Culturing a fungal pathogen

offers the best proof of a fungal infection and is the only way to perform susceptibility testing. See Ch.2 and 3 for the individual fungal profiles for a more complete explanation of medical lab testing for each type of fungi.

Biosafety for Medical and Lab Workers: Safety precautions must always be strictly followed in the mycology lab. There have been laboratory-associated deaths of those working with some fungal pathogens, especially dimorphs. Safety equipment is necessary to perform much of this work. A biological safety cabinet and proper ventilation are of primary concern to avoid inhaling fungal spores in growing cultures. Specimens and cultures should be handled with care and proper aseptic technique as if they contained pathogens. This is called "universal precaution". These specific instructions should be followed:

- Potential pathogens and opportunists may cause harm under unusual circumstances. You should share your information with your supervisor if you have a compromised immune system, a recent extended illness, or are pregnant.
- Organisms can infect you if they are ingested, inhaled, or come in contact with mucous membranes or any opening in the skin. If you have a cut or injury, keep it covered with bandages and keep it dry while you are working in the lab
- Wear gloves and masks when working with cultures. When your work is completed, dispose of these in the biohazard garbage. Lab coats and face shields are also required. These will be stored in the lab.
- Disinfect your work area both BEFORE and AFTER working with fungal specimens or cultures.
- Patient specimens, cultures of live microorganisms, and any material coming in contact with them must be stored appropriately or sterilized after use in the laboratory.
- Never place contaminated pipette tips (or pipettes), inoculating loops, or any other contaminated material on the benchtop. Sterilize loops before and after each use. Place contaminated pipette tips in the biohazard buckets on your bench. Place all other contaminated materials in their designated waste containers. Do not place or put anything containing live microorganisms down the sink.
- Aerosols should be avoided by using the proper technique for sterilizing the inoculating loops and mixing cultures and reagents in a way that avoids splashing.
- Cultures or reagents should always be transferred with an automatic pipettor, which will be provided. Mouth pipetting should never be employed.
- All mold work, specimen processing, or working with culture liquids should be done with gloves and a gown on, wearing a mask, and under a certified laminar flow biological safety cabinet (Class II Biological Safety Cabinet (BSCII). Cover all cultures and specimens adequately and wipe the outside of the containers with disinfectant when you are done under the hood and ready to take them out. Working in a separate room with a closed door and proper air filtering is desirable.
- Dimorphic fungi are RG-3 organisms, so extra precautions must be taken. Cultures of dimorphs are a biohazard to laboratory personnel and must be handled in a Class II Biological Safety Cabinet (BSCII).
- For mold cultures, to avoid breathing in or spreading mold spores, it is safest to use culture media in flasks with screw caps. If you use agar plates for culturing a mold or a specimen that may grow mold, tape them shut with an appropriate tape that allows oxygen in but no spores to escape so that the mold can survive. Still, if you accidentally drop the plate, it is not a biohazard to you or your coworkers.

- Always keep cultures covered or capped in proper storage racks when not in use.
- In an accidental spill involving a bacterial culture, thoroughly saturate the spill area with disinfectant, then cover with paper towels to reduce aerosols and allow the spill to sit for 10 minutes. Then carefully remove the saturated paper towels, dispose of them in the biohazard waste, and clean the area again with disinfectant. Notify your instructor about the spill. If the chemical is marked "dangerous" or "caustic," you should notify the instructor handling this type of spill.
- Immediately report all accidents, such as spills, cuts, burns, or other injuries.
- Make sure that lab benches are completely cleared (everything either thrown away or returned to the storage area) before you leave the lab.
- Clothing worn in the microbiology laboratory should be washed before being subsequently worn in a facility such as a hospital, clinic, nursing home, or area of public food preparation.
- Do not eat or drink in the lab.
- Always wash your hands before leaving the lab and whenever you think they may have come in contact with an organism or anything toxic.

V. MAKING THE DIAGNOSIS OF FUNGAL INFECTIONS

All good medical care flows from an accurate and timely diagnosis. Fungi are some of the most misdiagnosed and underdiagnosed infections in medicine. With their delayed diagnosis and their severe underlying disease, these infections are particularly debilitating and deadly. It is frustrating to see a patient, as in this chapter's case study, who does not have any fungal cultures ordered for a knee infection until they have to undergo a preventable arthrodesis (joint fusion surgery) for their joint pain and disease. This happened because the physician did not think about a fungus as the cause of the osteomyelitis and joint disease complications. If they had discovered this earlier, the patient may have avoided severe tissue damage and necrosis and the debilitating joint fusion surgery. The patient could have been treated successfully with antifungal agents much earlier rather than winding up permanently disabled. Some of the current direct examination diagnostic tools that mycologists have low sensitivity or specificity, and culture results often take weeks, further delaying the diagnosis. For the patient in the case study, direct examination of the biopsy specimen did not initially reveal the yeast characteristic of this dimorphic fungus, and this is common with these specimens, but the culture did finally grow a mold from the joint fluid in eleven days to reveal the typical white to cream color mold colonies that turned brown to black with more incubation. Radiological examination, direct immunofluorescent techniques (if available), PCR or other nucleic acid testing, and exoantigen testing can speed up the detection of fungi, greatly enhancing the sensitivity, specificity, accuracy, and timeliness of results. Exoantigen testing involves immunodiffusion testing of the mold antigens against the patient's serum antibodies and control antisera and looking for formation of antigen-antibody precipitant bands. The molecular tests are preferred for definitive diagnosis, but this older serological test is still helpful for identification. Awareness of potential fungal infections and the availability of new techniques to improve medical mycology diagnosis are both needed to give critical and timely information for the optimal care of the patient.

1. Fungal Media

Table 1: Fungal Culture Media and Their Uses

Fungal Plating Media	Use/ Interpretation
Blood culture bottles	Fungemia.
Brain-heart Infusion Agar	Primary media for the recovery of saprobic and pathogenic fungi and fungus-like bacteria.
Brain-heart Infusion Agar with Blood	Primary media for the recovery of saprobic and pathogenic fungi and fungus-like bacteria. Adding blood makes it even more nutritious and helps grow <i>H. capsulatum</i> and <i>B. dermatitidis</i> better.
Brain-heart Infusion Agar with gentamicin and or chloramphenicol (+/- Blood)	Primary media is used to recover pathogenic fungi while inhibiting the overgrowth of bacteria. Adding blood makes it even more nutritious and helps grow <i>H. capsulatum</i> and <i>B. dermatitidis</i> better.
Brain-heart Infusion Agar with gentamicin and or chloramphenicol and cycloheximide (+/- Blood)	Primary media is used to recover pathogenic fungi while inhibiting the overgrowth of bacteria and some saprobic fungi. Adding blood makes it even more nutritious and helps grow <i>H. capsulatum</i> and <i>B. dermatitidis</i> better.
Brain-heart Infusion Media Biphasic Blood culture media	Fungemia – Fungi can grow in the BHI liquid or on the BHI agar.
Dermatophyte Test Media	They are designed for the differential recovery of dermatophytes from possibly contaminated specimens. However, many dermatophytes will grow and turn the media red, so it is only recommended as a screening media.
Inhibitory Mold Agar	Primary fungal isolation agar containing chloramphenicol to inhibit bacterial overgrowth. Chloramphenicol inhibits <i>Nocardia</i> and other fungus-like bacteria and inhibits mold-to-yeast conversion.
Mycosel	It contains both chloramphenicol and cycloheximide. The former inhibits bacterial overgrowth, and cycloheximide inhibits fungus-like bacteria, yeasts, and fungal opportunists. Thus, this is an excellent primary media for dermatophytes.
Potato Flake Agar	Primary media for the recovery of saprobic and pathogenic fungi and fungus-like bacteria.
Sabhi (+/- Blood)	It is recommended for the primary isolation of respiratory specimens and is suitable for isolating <i>Histoplasma capsulatum</i> . However, it is not satisfactory for converting <i>H. capsulatum</i> mold to the yeast phase. Adding blood makes it even more nutritious and helps grow <i>H. capsulatum</i> and <i>B. dermatitidis</i> better.
Sabhi Agar with gentamicin and or chloramphenicol (+/- Blood)	Primary media is used to recover pathogenic fungi while inhibiting the overgrowth of bacteria. Adding blood makes it even more nutritious and helps grow <i>H. capsulatum</i> and <i>B. dermatitidis</i> better.
Sabhi Agar with gentamicin and or chloramphenicol and cycloheximide (+/- Blood)	Primary media is used to recover pathogenic fungi while inhibiting the overgrowth of bacteria and some saprobic fungi. Adding blood makes it even more nutritious and helps grow <i>H. capsulatum</i> and <i>B. dermatitidis</i> better.

Sabouraud Dextrose Agar (Sabdex)	It is the primary media for the recovery of saprobic and pathogenic fungi and fungus-like bacteria. It is also the classic media for colonial morphology and microscopic structures.
Sabouraud Dextrose Agar (Sabdex, SDA) Agar with gentamicin and or chloramphenicol	Primary media for the recovery of pathogenic fungi.
Yeast Extract Phosphate Agar	Primary media for isolating <i>Blastomyces dermatitidis</i> and <i>Histoplasma capsulatum</i> for contaminated respiratory specimens.
Ascospore Media (Wickersham's Malt Extract Media)	Detection of ascospores in yeast such as <i>Saccharomyces spp.</i> Enhances yeast morphology.
Cornmeal Agar with 1% Dextrose	Enhances red pigment formation in <i>Trichophyton rubrum</i> .
Cornmeal Agar with Tween 80 (+/- trypan blue) and covered with a flame-sterilized cover glass.	They are used to enhance and demonstrate chlamyospore production in <i>Candida albicans</i> under the microscope.
Czapek's Agar	For the differential identification of <i>Aspergillus spp.</i>
Kelley Agar/ Cottonseed Agar	They are used to convert <i>Blastomyces dermatitidis</i> mold to the yeast phase.
Niger Seed Agar (Caffeic Agar)	Both media grow <i>Cryptococcus species</i> and show its melanin production, turning black when present.
Nitrate Reduction Media	Detection of nitrate reduction in the confirmation of <i>Cryptococcus spp.</i> and other fungi.
Potato Dextrose Media	Enhances sporulation of molds.
Rice Extract Agar	Enhances chlamyospore formation in <i>C. albicans</i> .
Rice Media	Helpful in the identification of <i>Microsporum audouinii</i> .
Trichophyton Agars	They are used for the differential identification of the <i>Trichophyton spp.</i>
Urea agar	Detects urease activity of fungi such as <i>Cryptococcus spp.</i> , <i>Trichosporons spp.</i> , <i>Rhodotorula spp.</i> , and other fungi.
18% V-8 juice agar	Induces the conidiation of dematiaceous dermatophytes.

- 2. Patient Specimen Processing:** You must consider the specimen type, the body site, and the types of fungal pathogens that can cause infections in these sites to determine how to best process specimens for optimal results. The specimen must be collected and delivered promptly to the lab. Swiftly set up both the direct tests and the fungal cultures upon receipt of the specimen in the laboratory. Notify the physician of the direct testing results as soon as possible, and if any fungus grows, also send him a preliminary report of your findings. Fungal cultures should be held for 4-6 weeks. Table 1-2 shows the various considerations for each type of specimen submitted to the medical mycology lab. Good communication between the caregiver and the laboratory and radiology departments is crucial to obtaining reliable results and treating the patient in a timely manner to avoid tissue damage and/or death.

Table 2: Types of Mycoses based on Body Site, Signs and Symptoms, and Optimal Culture Requirements and Considerations

Body Site	Signs and Symptoms	Specimens	Potential Pathogens	Normal Flora	Culture Media Incubate Media Initially at 30°C, Except BHI, Sabhi With Blood at 37°C
Blood, cardiovascular system	Fever with or without sepsis. Radiologic evidence of a hematogenous infection spread pattern	Blood	<i>Candida spp.</i> <i>Cryptococcus neoformans</i> <i>Histoplasma capsulatum</i> Other dimorphic fungi Other non-dimorph Fungi Yeasts	None	Concentration with lysis-centrifugation using the isolator system and direct inoculation to fungal media, Sabdex, and Sabhi with blood and Anaerobic BHI with blood. This lysis method maximizes the growth rate and detection of fungi in blood cultures. Alternately, biphasic, aerobic, and anaerobic blood culture bottles can be used. (hold the culture longer (ten days) for fungal cultures than for bacterial blood cultures. (55)
Bone Marrow		Bone marrow	<i>Candida spp.</i> <i>Cryptococcus neoformans</i> <i>Histoplasma capsulatum</i> Other dimorphic fungi Other non-dimorph Fungi Yeasts	None	Directly inoculate the aspirate at the bedside to Sabdex, BHI with blood, and Anaerobic BHI with blood

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Cerebrospinal fluid	Fever, headache, stiff neck, nausea and vomiting, photophobia (sensitivity to light), altered mental status (confusion) (56)	Cerebrospinal fluid	<i>Cryptococcus species</i> <i>Candida albicans</i> Dimorphic and other fungi with disseminated disease <i>Actinomyces spp.</i> <i>Nocardia spp.</i>	None	Filter concentrate and plate 1/3 of the filter aseptically to Sabdex BHI with blood media, and Anaerobic BHI agar with blood.
Normal sterile body fluids		Peritoneal, Pleural.			Concentrate by filtering or centrifugation. Sabhi Mycosel BHI with blood Anaerobic BHI with blood
Mucocutaneous scrapings	Thickened, fragmented, hyperkeratotic nails and erythematous periungual skin. low cellular immunity	Mucocutaneous scrapings, swab	<i>Candida albicans</i> , other <i>Candida spp.</i> <i>Paracoccidioides brazilliensis</i>	Few yeast	Sabhi with blood Sabhi with gentamycin and chloramphenicol Mycosel
Skin scrapings	Discolored skin patches Red circular rash with clearer skin in the middle	Skin scrapings	<i>Superficial fungi:</i> <i>Exophiala wernedkii</i> <i>Malassezia furfur</i> <i>Fungi Invading the Skin:</i> <i>Microsporum spp.</i> <i>Trichophyton spp.</i> <i>Epidermophyton</i>	Bacterial skin flora A few yeast	Sabdex w/chloro Mycosel (DTM optional) For <i>Malassezia spp.</i> Cover agar with sterile olive oil/

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			<i>spp.</i> <i>Candida spp.</i> and more		
Hair	Black or white nodules on the hair Endothrix, Ectothrix infected hairs	Hair	<i>Superficial:</i> <i>Piedraia hortae</i> <i>Trichosporon beigelii</i> <i>Invading the hair:</i> <i>Microsporum spp.</i> <i>Trichophyton spp.</i>	Bacterial skin flora A few yeasts	Sabdex w/chloro Mycosel (DTM optional)
Nails	Thickened brittle nails, cracking and discolored	Nails	<i>Trichophyton spp.</i> <i>Epidermophyton spp.</i>	Bacterial A few yeasts	Sabdex w/chloro Mycosel (DTM optional)
Lower respiratory/ lungs	Fever and chills, cough, hemoptysis, dyspnea, chest or joint pain, headaches, and possibly skin lesions.	Sputum, bronchial washings, BAL, transtracheal aspirate, lung tissue	<i>Candida spp.</i> <i>Pneumocystis jirovecii,</i> <i>Aspergillus spp.,</i> <i>Histoplasma capsulatum</i> <i>Other dimorphs</i> <i>Other non-dimorph fungi</i>	None	Sabhi Mycosel BHI with blood, gentamycin, and chloramphenicol BHI with blood, gentamycin, and chloramphenicol and cycloheximide For transtracheal or Actinomyces, add: Anaerobic BHI with blood
Subcutaneous abscesses and mycetoma	Subcutaneous lesions, abscesses	Aspirate, tissue, skin/subcutaneous lesions, abscesses, mycetomas	<i>Candida spp.</i> <i>Histoplasma Capsulatum and other dimorphic and non-dimorph Fungi, Agents of mycetoma.</i> Sporothrix spp.	None	Sabhi Mycosel BHI with blood, gentamycin, and chloramphenicol BHI with blood Anaerobic BHI with blood

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Subcutaneous, chromoblastomycosis	Lesions, warty nodules, centripetal growth, ivory-colored scars, and plaques, sometimes over the whole limb and cauliflower limb.	Aspirate, tissue, skin and subcutaneous lesions, nodules	<i>Fonsecaea spp.</i> <i>Phialophora spp.</i> <i>Scedosporium spp.</i> <i>Exophiala spp.</i> <i>Cladosporium spp.</i> and more (57)	Skin flora that could include some yeast	Sabhi Mycosel BHI with blood, gentamycin, and chloramphenicol BHI with blood, gentamycin, and chloramphenicol and cycloheximide
Throat	White film, irritation	Throat, ear, nose, mouth	<i>Candida albicans</i> thrush in the mouth, tongue, and esophagus	Some yeast	Sabhi with gentamycin, and chloramphenicol
Nasal sinuses	Chronic congestion, pressure, facial pain/ numbness/ swelling, cough, discharge, headache, visual and mental status change, fever, dark ulcers	Nasal sinuses	<i>Mucor spp.</i> <i>Rhizopus spp.</i> Many other fungi	skin flora that could include some yeast	Sabhi Mycosel BHI with blood, gentamycin, and chloramphenicol BHI with blood, gentamycin, and chloramphenicol and cycloheximide
Kidney, UTI		Urine, surgical kidney fluid/tissue	<i>Candida albicans</i>	Kidney – none Lower UTI may have some yeast colonization	Concentrate urine by centrifugation. Sabhi Mycosel BHI with blood, gentamycin, and chloramphenicol BHI with blood, gentamycin, and chloramphenicol and cycloheximide
Genital	Vaginal, Uterine, Cervix, Urethral	Vaginal, Uterine, Cervix, urethral	<i>Candida albicans</i>	Some yeast	Sabhi Mycosel

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Wound	Varies with the agent of disease and body site, fever	Wound	<i>Candida spp.</i> <i>Cryptococcus</i> <i>Neoformans</i> <i>Aspergillus spp.</i> <i>Blastomyces dermatitidis and other dimorphic and non-dimorph fungi</i>	Superficial wounds may have skin flora that could include some yeast	Sabhi Mycosel BHI with blood, gentamycin, and chloramphenicol BHI with blood. For possible Actinomyces: Anaerobic BHI with blood
Sterile tissues, biopsies	Surgery for various reasons	Surgical tissues, biopsy	<i>Candida spp.</i> <i>Cryptococcus spp.</i> <i>Aspergillus spp.</i> <i>Blastomyces spp.</i> <i>other dimorphs and non-dimorphs</i>	None	Sabhi Mycosel BHI with blood Anaerobic BHI with blood
Eye, keratitis	Foreign body sensation, Increasing eye pain or discomfort, Sudden blurry vision, Unusual redness of the eye, Excessive tearing, discharge from the eye, Increased light sensitivity.	Corneal scraping, surgical eye biopsy, fluid sample	<i>Aspergillus species</i> <i>Candida albicans</i> <i>Fusarium species</i>	None	Sabdex
Bone and joint	Pain, heat, swelling, redness, and loss of range of motion may or may not be accompanied by	Bone and joint fluid or tissue samples	<i>Candida spp.</i> <i>Cryptococcus neoformans</i> <i>Histoplasma</i> <i>Capsulatum and other</i>	None	Sabhi Mycosel BHI with blood Anaerobic BHI with blood

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	fever. The knee is the most common joint infected.		<i>dimorphic and non-dimorph fungi</i>		
Gastrointestinal tract	Gastroenteritis, reflux disease, abdominal pain, distention, fever, history of broad-spectrum antibiotics	Gastrointestinal tract	<i>Candida albicans</i> and other fungi	<i>Candida spp.</i> other yeast	Sabhi with chloramphenicol and gentamycin Mycosel
Transplant	Signs and symptoms vary with the body site/ organ and the infecting agent. They are likely subdued due to standard immunosuppressive therapy, although there may be some typical signs of infection.	Transplant tissue	<i>Candida spp.</i> <i>Pneumocystis jirovecii</i> , <i>Aspergillus spp.</i> , <i>Histoplasma capsulatum</i> and other <i>dimorphic and non-dimorph fungi</i>	None	Sabhi Mycosel BHI with blood Anaerobic BHI with blood

- 3. Direct Testing for Fungal Agents:** Direct testing is set up at the same time as the culture. Direct Testing for fungi is valuable and rapid. Remember that, as some fungi take weeks to grow, direct examination is actually the only helpful information the physician can use quickly and is very important in mycology.

In the pathology department's cytology and histology laboratories, special stains are used to detect and identify fungi in smears of patient exudates or tissue sections by microscopy. These stains include PAS, Gomori Silver Stain, H&E, and the Fontana-Masson Stain.

Direct Preparations are made from the patient samples or from growing patient cultures in the microbiology lab to help identify the type of fungi causing infection. Light microscopy or fluorescent microscopy can be used to detect and identify specific fungi in patient specimens. India Ink preparations can help to identify or detect *Cryptococcus neoformans*. Stains such as calcofluor white can be used, which stains fungal cell walls and makes them fluoresce under a fluorescent microscope. This enhances the sensitivity of fungal detection as a larger amount of the specimen can be scanned in a shorter time. Lactophenol aniline blue is another stain that can be used to help detect fungi in direct specimen preparations and when a portion of the culture is examined. Some helpful techniques include tease mount (a small portion of the fungus is teased apart and examined for its characteristics) and cellophane (or clear scotch tape) prep, where only the very surface fungal conidia, macroconidia, and hyphae are picked up by touching the tape to the top of the fungal culture to see the natural arrangement of the conidia on the tape with LAPB on a slide under the microscope, slide culture in which the fungus is grown under a coverslip which can be examined under a microscope for fungal structures.

Table 2: Direct Specimen Preparations and Fungal Stains for Direct Specimen Smears

Fungal Stain	Fungal Element Color	Background Color
Acid-fast Stain	<i>Blastomyces spp.</i> and <i>Histoplasma spp.</i> appear red (acid-fast)	Background is blue
Calcofluor white in KOH (cover-slipped wet prep)	Fungi fluoresce apple-green or blue-white.	No fluorescence in the background.
Giemsa	Purple-blue yeast/fungus with a clear halo with a capsule	Pink-purple
Gomori Methenamine Silver (GMS)	Black	Green

Fungal Stain	Fungal Element Color	Background Color
Gram Stain	Purple/dark blue	Pink/red
Hematoxylin and Eosin (H&E) Tissue Stain (can be combined with Gomori Silver Stain)	All fungi have pink cytoplasm, blue nuclei, and no color in the cell wall. Look for classic fungal structures.	Cytoplasm and connective tissue are pink; nuclei are blue.

India Ink Prep (cover-slipped wet prep)	Yeast has a clear halo when a capsule is present	Black ink
KOH-10-20% (higher % for nails that can dissolve them) (cover-slipped wet prep)	Fungi hyphae, yeast, or other structures can be seen after KOH dissolves the tissue, nail, hair, or other specimen. The fungi are more resistant to dissolving.	Background is clear
Lactophenol Analine Blue (or Cotton Blue) Prep (LAPB) (cover-slipped wet prep)	Fungal structures are blue	Background lighter blue
Masson-Fontana	Brown	Pink, Purple
Modified Acid-fast	Nocardia is red or partially red	Blue
Papanicolaou Smear	red	Immature or intermediate cells are blue to green, and more mature or keratinized cells are red to pink.
Periodic Acid-Schiff Stain (PAS)	Magenta (pink-purple)	Green

4. Serodiagnostic Direct Detection of the Fungal Agent from the Specimen: Examining body fluids and other specimens to detect fungal antigens directly can play an essential role in indirectly diagnosing fungal disease.

Cryptococcus antigen detection by serological testing has become a standard method for diagnosing cryptococcosis (especially in spinal fluid), and these tests also play a crucial role in detecting aspergillosis and, to a lesser extent, candidiasis, depending on the underlying disease. Antibody testing is routine for many fungal diseases, including coccidioidomycosis, histoplasmosis, and many forms of aspergillosis.

Beta-D-glucan is a generic fungal antigen found in the cell walls of many fungi. Many use BDG detection to screen for the presence of fungi. Increasingly, physicians and scientists use serodiagnostic tests to diagnose and monitor fungal treatment outcomes. β -D-Glucan is an attractive antigen for testing these patients as it is found in various fungal agents, including the commonly encountered agents *Candida spp.*, *Aspergillus spp.*, and *Pneumocystis jirovecii*. (2,3)

The galactomannan (GM) serodiagnostic test is an enzyme-tagged antibody test for diagnosing invasive aspergillosis in patients, especially those with hematological malignancies. (4) Galactomannan is a polysaccharide antigen found in *Aspergillus spp.* cell walls. It may be released into the blood or body fluids in *Aspergillus spp.* infections, even in the early stages, and this antigen may be present for 1 to 8 weeks. This makes it helpful for the diagnosis of invasive pulmonary Aspergillosis. Some fluorescent-tagged or enzyme-tagged antibodies are also available for direct specimen preparation screening for fungal detection.

- 5. Molecular Direct Detection of Fungal Pathogens:** Another growing direct detection method is using molecular genetic techniques to identify the fungus present in a sample. This has solid advantages and a few disadvantages. The benefits are that it is a direct method for rapid detection and very accurate and specific identification of the fungal infectious agent.

A disadvantage is that molecular probes usually only detect one particular fungal agent, and your patient may have a different type of fungal infection than the probe you have. While you can use multiple probes, molecular testing costs more than traditional culture and identification, and multiple probes are quite costly.

- 6. Fungal Culture and Identification:** The colonial morphology, characteristics such as texture and pigment color on the front and reverse of the colony, can help you decide on a tentative grouping of your fungus. First, notice if the colony is smooth, creamy, or glabrous, like yeast or a hyphal mat-like mold. If it is mold-like, is it cottony or wooly? A characteristic of the zygomycetes is that they look cottony and fluffy, like cotton candy, and they rapidly rise to fill the culture plate or tube with this cottony mold within about 2-3 days. Or is the texture velvety, granular, or powdery? Is the topography of the colony umbonate (rounded and dome-shaped), verrucose (wrinkled), or rugose (deep radiating furrows)? What is the color of the top of the colony and the reverse of the colony? How long does the colony take to grow? Is it a rapid rate, 1-5 days, like a yeast or a zygomycete, a medium rate of 6-10 days, or a slow grower that takes 11-21 days, like some dimorphs? Please take note of these characteristics, as they are all clues to identifying your fungus. Monitor for changes in appearance over time. If a dimorph is suspected, you may need multiple subcultures to convert it to the yeast phase from the mold phase. It is generally in the yeast phase in the human body, so to convert it, you may need a warmer body-type temperature, and certain media types may also be required.

Also, be aware of the possibility of getting a plate contaminant, as mold spores often travel through the air. Is it growing where the sample was inoculated (genuinely present in the sample) or away from where it was inoculated (like a contaminant)? Fungal contaminants are common laboratory problems in microbiology.

Microscopy is critical to fungal identification. First, the fungus is recognized as yeast or mold by microscopy and appearance. Yeast are unicellular organisms with no capability or minimal capability of mycelial growth. Yeasts reproduce asexually by blastoconidia formation (budding). Then, look at the other structures. The germ tube test can help differentiate *Candida albicans*, which form germ tubes when incubated in serum or anticoagulated rabbit plasma at 37°C. If your culture is a mold, examine a portion of the mold for the type of hyphae—check if they are septate (have cross-walls) or are aseptate (have no cross-walls) or coenocytic (sparsely septate). All fungi with aseptate or coenocytic hyphae fall into the Mucorales (zygomycetes). If you then look at their sporangia, and if they have rhizoid nodes (rootlike hyphae) and the location of those rhizoids, you can identify the species of zygomycetes. For other fungi, the morphological appearance, types of conidia, macroconidia, and growth characteristics can significantly assist you in identifying your fungal isolate. Please see Figure 1-1 for a standard preliminary fungal identification flowchart.

- 7. Standardized Sensitivity Testing For Fungi:** The Clinical Laboratory Standards Institute (CLSI) has released four publications with accepted methods for standardized fungal yeast and mold susceptibility testing and to assist you in interpreting breakpoint results for clinical laboratory reporting of susceptibility or resistance. A standardized inoculum of your fungus suspension is made per recommended procedures and swabbed uniformly onto a susceptibility agar media. Mueller-Hinton agar is used. Antifungal antifungal-impregnated Kirby-Bauer disks are tamped on the media surface aseptically, and the plate is allowed to grow. After growth is present, any zone of inhibition by the antibiotic is measured to establish susceptibility and resistance to the antifungal agent.
- 8. Serological Testing for Evidence of Fungal Infections:** Examining serum and other body fluids for the presence of antibodies to fungi or the direct detection of fungal antigens can play an essential role in indirectly diagnosing fungal disease. Various serological methods are available, though the most commonly used is some form of enzyme-linked immunosorbent assay looking for patient antibodies to specific fungi, either IgG or IgM. These tests help screen populations for evidence of recent or prior fungal infections. The health department recommends obtaining an acute-phase and a convalescent-phase sample to establish rising titers to use best these tests. Skin testing is also helpful for allergic responses to fungi.
- 9. How the Clinician Should Proceed to Diagnose a Fungal Infection:** Because fungal disease is frequently missed or has delayed diagnosis, it is incumbent on both patient care and laboratory health care personnel to become more familiar with these agents and their presentation and characteristics. New and improved testing tools and techniques are needed to help detect and control these agents. MALDI-TOF mass spectrophotometry and nucleic acid testing are fast and very accurate for identifying the fungal infectious agent, and their use is encouraged and preferred in many cases because they reduce the safety risks of extensive culture work with mold pathogens. As fungal agents afflict immunocompromised and immunodeficient patients most often, and as the numbers of these types of patients are increasing, the number of fungal infections has also increased in the last decades. Communication between the laboratory and the caregivers is also helpful to the success of accurate diagnosis. The patient care provider must collect a complete history, evaluate where the patient has been and their signs and symptoms, and assess their immunological status. Because fungal cultures may take a long time for growth results, direct microscopic examination, antigen testing, serological testing, exoantigen testing, and molecular testing should also be utilized to accelerate diagnosis and patient treatment.
- 10. Diagnostic Imaging of Fungal Infections:** Fungal infections can be life-threatening, especially in the immunocompromised host. Diagnostic imaging provides a valuable rapid tool to detect and characterize fungal infections. Diagnostic imaging in patients with invasive fungal disease is multifaceted, including initial detection, evaluation for dissemination of infection beyond the primary site of disease, monitoring the response to antifungal therapy, and assessing for potential relapse. Important to note is that the radiographic appearance of infectious fungal disease in children can differ significantly from that in adults.

Invasive candidiasis can present clinically in various ways, including candidemia, disseminated candidiasis, and single-organ infection. It is helpful to have imaging details

of organs commonly affected by hematogenous (through the fungus in the bloodstream) and nonhematogenous spread patterns. *Candida* involvement of the lung is typically secondary to hematogenous dissemination. Hematogenous *Candida* meningoencephalitis (HCME) occurs because of candidemia with blood-borne dissemination to the brain. In this condition, which is more common in neonates, the diagnostic interval is too often long, the CSF findings nonspecific, the fungus difficult to culture, and the clinical course insidious. Therefore, imaging studies often provide the only evidence that central nervous system candidiasis is present. Magnetic resonance imaging also seems to be superior to computed tomography in identifying liver lesions associated with chronic disseminated candidiasis; its sensitivity and specificity are high when used appropriately. Chronic disseminated candidiasis is a persistent infection of the liver, spleen, and other tissues that can present in neutropenic patients and even after recovery from neutropenia.

In invasive Aspergillosis, the sinopulmonary tract is the most common portal of entry for aerosolized *Aspergillus* conidia. With little effective immune response, conidia can germinate into hyphae, invade pulmonary arteries, and cause pulmonary arterial thrombosis, hemorrhage, lung necrosis, and systemic dissemination. Lungs are the most frequently infected site, followed by the CNS. Diagnosing invasive aspergillosis is challenging; however, key imaging features can alert clinicians to the possible diagnosis of invasive aspergillosis. Characteristic CT (or computed tomography or CAT scan) findings of acute angioinvasive aspergillosis consist of a nodule or nodules surrounded by a halo of ground-glass appearance attenuation (“halo sign”) or pleura-based wedge-shaped areas of consolidation, a cavity, an air-crescent sign. However, these classic signs may not be as easy to spot in children and neonate imaging. (58)

11. Non-Infectious Fungal Disease: Allergies and Toxin Mediated Disease

- **Allergic Fungal Disease:** Many people develop allergies to molds. Serological and skin testing are used to detect and monitor fungal allergies. The skin test uses diluted amounts of common or suspected allergens, such as molds in the local area. During the test, these substances are applied to the skin of your arm or back with tiny punctures. If you're allergic, you develop a raised bump (hive) at the test location on your skin. A blood test called the radioallergosorbent (RAST) test can measure your immune system's response to a specific mold by measuring the quantity of specific antibodies in your bloodstream known as immunoglobulin E (IgE) antibodies to that particular fungal allergen. With the Radio-Immuno-Sorbent-Test (RIST), the IgE can be quantitatively determined. Elevated IgE-blood levels are typically found in allergies. A blood sample is sent to a medical laboratory, where it can be tested for evidence of sensitivity to specific types of mold. Some patients develop a condition known as allergic bronchopulmonary aspergillosis when exposed to specific molds such as *Aspergillus fumigatus*. Prolonged exposure to molds has the potential to trigger chronic sinusitis, a persistent inflammation of the sinus passages. Moreover, individuals with sensitive respiratory systems can experience exacerbated asthma symptoms due to mold exposure. Inhalation of mold spores can act as a catalyst for allergic rhinitis, commonly called hay fever.

Allergic fungal diseases affect the upper or lower respiratory tract or the nasal cavities, as this is where the fungal spores enter the body. Some of these allergic

fungus diseases can be severe. Fungal spores can colonize or even infect tissues in those airways. Those fungi that are not thermotolerant (live below body temperature) cannot sporulate and grow in the human airways (only colonize). In contrast, those that are thermotolerant (able to grow at body temperature) can get a foothold to infect inside these airways and be a more constant allergic irritant to the susceptible. While fungal infections often exhibit inflammatory signs and symptoms, allergic disease usually involves IgE, eosinophils, mast cells, and basophils, the features of allergic disease. It occurs in the sensitized, susceptible host. *Alternaria spp.* and *Cladosporium spp.* (not thermotolerant) are predictable from their spore levels and seem to cause short-term allergic manifestations similar to those induced by grass pollen, as they cannot establish a foothold for infection. Thermotolerant fungi such as *Aspergillus spp.* and *Penicillium spp.* can first colonize the lung, triggering allergenic stimuli and, when colonization is considerable, then invade and infect and be a more constant allergic irritant as well as an agent of invasive disease. (59) Allergies involve a host with allergic tendencies (atopic), so most allergic fungal infection likely has a genetic component. IgE sensitization to fungi is common in asthma, particularly in more severe asthmatic cases. (59)

According to the World Health Organization (WHO) estimates, 235 million people worldwide currently have asthma, and prevalence is likely to be higher, as it is often under-diagnosed and under-treated. (60) Severe asthma is thought to affect 5-20% of those with asthma, depending on the definition. Of these, about 35-50% are considered to have severe asthma with fungal sensitization (SAFS), depending on how extensively they are tested. (61) Many of the fungi that cause allergic reactions include, some alone and some collectively, are: *Alternaria alternata* (frequently associated with asthma), *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Cladosporium herbarum*, *Candida albicans*, *Trichophyton spp.*, and more. (61)

Thunderstorms are associated with increased acute asthmatic attacks due to fungal spores. During thunderstorms, they found increased airborne spores of the fungi *Didymella exitialis* and *Sporobolomyces spp.* (62) Since initial reports, many episodes of thunderstorm-related asthma have been noted in different places around the world. Increased humidity and high winds trigger increased fungal spore formation, release, and dissemination. Rapid increases of numerous fungal spores of many types in the air, such as *Alternaria spp.*, *Aspergillus species*, and many others, as well as increases in grass pollens, have all been associated with thunderstorms. Fungal spores are more highly associated with asthma than even pollen. (62) “Thunderstorm asthma” was positively correlated with a doubling of fungal spores. (62) Many of the fungi that cause allergic reactions include, some alone some collectively include *Alternaria alternata* (frequently associated with asthma), *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Cladosporium herbarum*, *Candida albicans*, *Trichophyton spp.*, and more. (62)

Occupational hypersensitivity pneumonitis is caused by a wide variety of fungal agents encountered in various work environments such as farming, maple bark work, or wood pulping millwork. Probably the best known of these is Farmer’s lung, a hypersensitivity to *Penicillium spp.*, acquired by turning or storing damp hay, opening bales for feeding livestock, or threshing moldy grain contaminated with a *Penicillium spp.* and breathing in the spores. (63) Symptoms include a flu-like illness with fever,

chills, *muscle or joint pain, headaches, chronic bronchitis, dyspnea, rales, cough, anorexia, weight loss, fatigue, lung fibrosis*, and even clubbing of the *fingers* or toes. The condition can be acute, subacute, or chronic and is caused by various environmental fungi. (59, 63)

Allergic fungal rhinosinusitis, also called eosinophilic fungal rhinosinusitis, is an allergic condition that causes nasal obstruction, loss of smell, nasal discharge, and facial pressure. Patients often have nasal polyps when they first present and if they relapse. A chronic presentation is typical, but it can also be acute. An acute can present with double vision or even vision loss, facial swelling with distorted features, and complete nasal obstruction. (59, 64) The predominant fungi responsible vary geographically but include *Aspergillus fumigatus*, *A. flavus*, *Bipolaris spicifera*, *Curvularia lunata*, *Alternaria alternata*, *Rhinocladiella mackenzei*, and other dematiaceous (brown) fungi. *Alternaria alternata* and other dematiaceous fungi predominate in the USA. (64)

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity pneumonitis reaction to *Aspergillus* and is a recognized complication of asthma and cystic fibrosis. (65, 66) *Aspergillus* sensitization can be differentiated from *Aspergillus* bronchitis by a negative galactomannan antigen test and a lack of serum IgG to *Aspergillus spp.* Patients with ABPA have highly elevated total serum immunoglobulin E levels (IgE >1,000 KIU/L) or a positive skin test specific to *Aspergillus fumigatus* and continuing respiratory symptoms. Patients may also have eosinophilia. Sputum cultures are positive for *Aspergillus* on multiple specimens in 20-60% of cases. *Aspergillus* PCR is usually positive in sputum or bronchoscopy specimens for these patients. (65, 66)

- **Fungal Toxin Illness:** Another tool for human illness for fungi is related to their potent toxins. Many are infamous for the toxicity of their poisons. While this book is not intended to be a reference for these toxic conditions or all the poisonous mushrooms, the healthcare worker should be familiar with these possible toxicities to their patients. Primarily toxin-mediated fungal disease may occur via three primary exposures: from contamination of food with a toxin-producing fungus, ingestion of poisonous mushrooms or toadstools with sufficient toxin (mycetism), or inhalation of airborne toxins from fungi growing in damp environments (“damp or sick building illness”). (67)

Food-related toxin poisoning has been familiar to humans for a long time and is caused by several fungal toxins, including ergot alkaloids, aflatoxins (*Aspergillus spp.*), ochratoxin A (*Aspergillus spp. and Penicillium spp.*), patulin (toxins made by many molds), fumonisins and other *Fusarium* mycotoxins.

There are about 100 species of mushrooms capable of poisoning humans. (Mushrooms make several important neurotoxins. (67)

12. Goals to Prevent and Manage Fungal Disease: Tools for preventing fungal infections would be beneficial to healthcare providers and the public at large. There are currently no fungal vaccine products available. As most of these infections are in the

immunocompromised and the elderly, nursing homes and hospitals should enhance infection control practices against these agents.

Prevention is a better goal than pursuing a cure, as fungi are difficult to dislodge once they start growing. There is not a single vaccine against any fungus currently. Without the option of a vaccine, hospitals must work hard to prevent patient exposure: patients can be given drugs to prevent fungal infection, hospital wards may not allow flowers or plants because of the risk of fungal spores infecting a compromised host, and hospitals also use air filtration barriers to protect their patients. However, the public must also be informed to avoid fungal exposure risks.

Hopefully, the WHO initiative report will increase recognition of the significance of fungal pathogens, spur needed research into medical mycology, and stimulate the development of more rapid diagnostic test methods. Mycology information is still not understood and fully appreciated by all members of the professional community of microbiologists and certainly not by the public. Hopefully, these little-known fungal infections will gain recognition for what they really are—significant global killers.

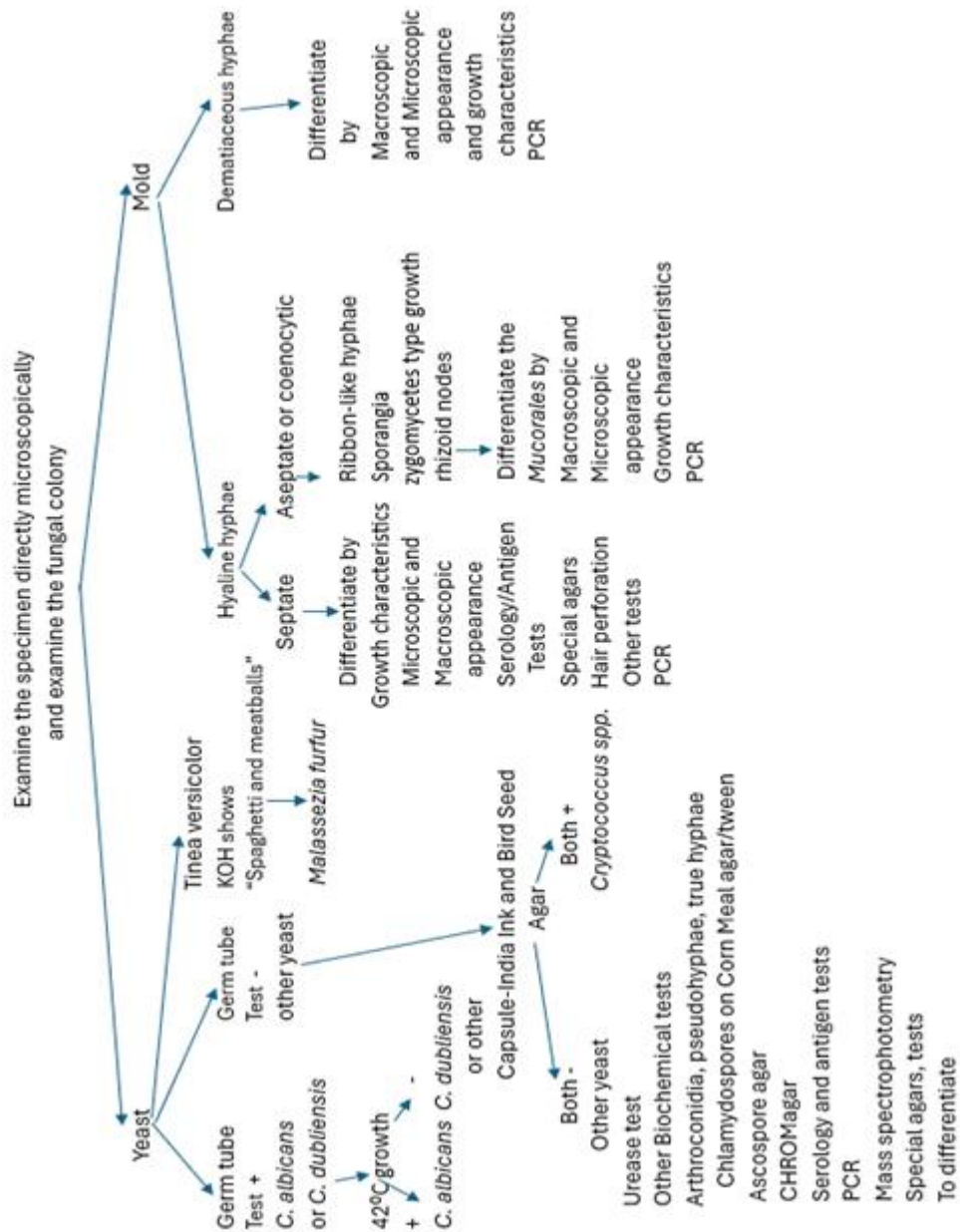


Figure 1: Preliminary Fungal Identification Flowchart

Case Study 1: Case 1 • A 46-year-old male migrant farm worker from Mexico visited a local clinic for a series of appointments related to his joint pain in September and October 2015. He underwent three different aspirations of the left olecranon bursa that had swollen and had a radiological examination of the left elbow and the right knee performed. Details of other laboratory tests done or drugs received were not available. The patient had a history of alcoholism and had been homeless for the last three years. For the previous two years, he worked as a gardener on a local Georgia farm, tending and cutting roses and other flowers. He stated that he thought that maybe his problems began when he was working in the rose gardens and adding sphagnum moss to the soil around the roses. He said he was working on his knees a lot during that time and frequently received punctures of the skin on his knees and hands from all the rose thorns. He had stiffness in the affected joints. He had a history of hypertension. His mother had rheumatoid arthritis.

On August 5, 2016, the patient presented to the emergency department with a history of gradual worsening pain and asymmetric medial swelling of the right knee. The patient had a history of progressive massive swelling of the right knee, left ankle, left wrist, and left elbow for about twelve or thirteen months. The joint swelling had started in the right knee about a year ago and, within a few weeks, began to affect the other joints. Initial examination showed marked swelling and synovial thickening of the left olecranon bursa, marked swelling of the right knee, and moderate swelling of the left wrist and left ankle. He had tenosynovial swelling of the dorsum of the left wrist, and the fourth and fifth extensor tendons seemed to be ruptured. Examination for systemic involvement was unremarkable. Laboratory tests showed a sedimentation rate of 120 mm/h and inflammatory synovial fluid with a white cell count of $54 \times 10^9/l$ (with degenerated WBCs), glucose 10 mmol/l, protein 44 g/l, and no crystals. Bacterial and mycobacterial cultures were collected and did not grow. Radiographs initially showed effusions and soft tissue swelling with no significant bony changes. A complete blood count and chemistry tests were normal or negative, including uric acid, rheumatoid factor, antinuclear antibody, and serum complement concentrations. The patient was diagnosed as having inflammatory asymmetrical oligoarthritis, possibly due to atypical gout or rheumatoid arthritis. There was no response to various nonsteroidal anti-inflammatory drugs, however. Local injection of the left olecranon bursa with methylprednisolone was performed. Radiographic images of the patient's right knee soft tissue swelling, joint effusions, and small medial tibia and femur erosions were taken.

On the return visit on September 29, 2016, proliferative synovitis of the affected joints worsened, and draining sinuses and fistulae developed on the right knee and the left ankle. A synovial biopsy specimen from the right knee showed chronic inflammatory cells with an epithelioid granuloma. On direct examination and routine bacteriological culture, the biopsy was negative for bacteria, acid-fast bacillus, and fungi. Progression of radiographic changes was noted. A synovectomy of the right knee and left ankle was performed.

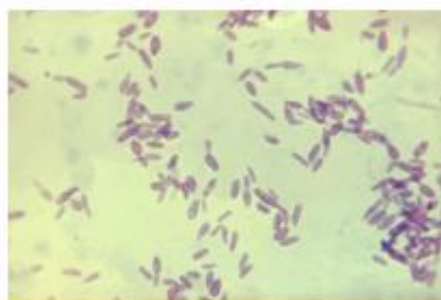
The patient again visited the hospital for knee surgery, and a fungal culture from a right knee aspirate was obtained on August 6, 2017, twelve months after the emergency room visit and twenty-four months after the start of symptoms. Advanced progression of radiographic changes was noted. Surgery was used to debride necrotic tissue in the joint bones, and the advanced joint destruction required arthrodesis (bone fusion).

Eleven days after the August 6, 2017, surgery, the patient's knee aspirate cultures grew *Sporothrix schenckii*, which eventually appeared dematiaceous (figure 2), and a diagnosis of sporotrichal arthritis was made. Treatment with intravenous Amphotericin B was begun. A subsequent review of the knee aspirate slides from August 6, 2017, showed a few cigar-shaped yeasts. (figure 3) The patient was treated with repeated courses of intravenous amphotericin B.



Case Study 1: Figure 2

Caption: This image depicted a frontal view of a Petri dish culture plate, that contained an unidentified growth medium, which was growing a single large colony of the fungal organism, *Sporothrix schenckii*, formerly known as *Sporotrichum schenckii*. Note the colony's characteristic wrinkled surface. As this colony aged further, it would continue to darken, eventually exhibiting a brown-to-black coloration. Public domain PHIL image 22310. CDC/ Dr. Lucille K. Georg, 1964



Case Study : Figure 3

Caption: Under a magnification of 970X, this photomicrograph revealed the presence of cigar-shaped yeast in the tissue-phase at 37°C of the fungal organism, *Sporothrix schenckii*, formerly known as *Sporotrichum schenckii*. Public domain PHIL image. CDC/ Dr. Lucille K. Georg, 1964.

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