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Review

Invasive fungal pathogens from the tropical and temperate areas – a challenge in pathology and diagnosis

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Abstract

Fungi play a vital role in ensuring a physiological balance in the surrounding environments, interacting closely with humans, plants, and animals. While most of the time their contribution is beneficial, occasionally, they can become harmful, especially in patients with weakened immune systems. The work at hand aims to present the most common fungal pathogens involved in invasive infections, focusing on fungi that are present in the tropical and temperate areas of the world. While in the former, due to the humid climate, most fungal infections are caused by dimorphic fungi such as *Coccidioides* spp., *Blastomyces* spp., *Histoplasma* spp., *Emergomyces* spp. and *Paracoccidioides* spp., in the latter, after *Candida* spp., the most frequent fungi that are involved in disseminated mycosis are *Aspergillus* spp., *Fusarium* spp. and species from the order Mucorales. Nowadays, the etiology, severity, and number of cases of fungal diseases are starting to rise significantly. There are no exact reasons reported for this increase, but several factors are thought to be incriminated: the expansion of the range of medical conditions that constitute risk factors for developing the disease, an improvement in the available diagnostic methods, the commodity offered by modern traveling services associated with the lack of an available vaccine against fungal infections, as well as climatic influences. All the above-mentioned aspects consequently caused infections that used to be endemic to be spread worldwide. Therefore, it is of critical importance to understand the epidemiology, clinical manifestations of fungi induced diseases, virulence factors, and diagnosis for each of those pathogens.

Key words: Tropical fungi; temperate area fungi; diagnosis; invasive pathogens.

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Introduction

Fungi can be found everywhere in the environment and play a huge role in interacting with humans, plants, and animals to ensure a physiological balance. Even though most of the time those interactions are beneficial, occasionally fungi can cause life-threatening diseases, especially in the presence of different risk factors such as those related to the host, including chronic obstructive pulmonary disease, asthma, chronic bronchitis, bronchiectasis, cystic fibrosis, uncontrolled diabetes mellitus, hematological malignancies, transplant recipients and environmental factors such as construction or renovation of medical facilities or contamination of air systems [1]. Nowadays, a slight increase in the number of invasive fungal infections was noted due to the immunosuppression caused by the infection with the SARS-CoV-2 virus, even in patients who had no previous comorbidities. Even though they are not that frequent, they can lead to a complicated evolution of the disease and further cause a severe increase in the mortality rates [2].

Besides their direct effect on human health, molds also play a big part in causing food spoilage, both during the pre-and post-harvesting processes, but also during plant growth by producing their secondary metabolites, the most discussed being mycotoxins [3].

It is estimated that there are around 12 million fungal species on our planet, but only several hundred of them can cause disease in humans, and even fewer can infect healthy individuals [4].

Fungal infections are one of the hardest-to-manage diseases known to humankind. To produce infection in humans, fungi need to fulfill four basic conditions [4]:

- tolerance towards high temperatures (37 °C or above);
- the ability to invade the human host to reach the tissue that it will parasitize, either by penetrating it or by circumventing through the airborne cells;
- the lysis and digestion of the human tissue;
- the ability to withstand the immune system of the human host.

Due to the huge climatic variations and other external environmental factors that affect their dissemination (e.g. freezing, sunlight, and desiccation), different fungal species are more or less common in different areas of the world [4].

The tropical area is considered the zone around the Earth that spreads between two geographic parallels, the Tropic of Cancer to the north and the Tropic of Capricorn to the south, comprising countries such as Central and South America, Equatorial Africa, Southern Asia, Northern Australia and the islands of the Caribbean and Southeast Asia. Several important climatic characteristics are the high temperatures (always above 25 °C) and the high humidity (above 50%, with an average of 66%) caused by the frequent rainfalls, which are ideal for fungi to grow and spread [5].

Fungal infections in humans are more commonly found in the tropics and subtropics mostly due to the warm and humid climates, which offer them a better environment for dissemination. Certain species can be found anywhere in the world and some fungi are specific to certain endemic climatic areas, that can reach people from any corner of the world due to the recent increase in traveling and migration [5].

The temperate area includes, based on the Köppen climate classification, countries or states (in the case of US) in which the mean temperature is above -3 °C (26.6 °F) and below 18 °C (64.4 °F). This includes around 10 European countries (Albania, Belgium, France, Greece, Italy, Netherlands, North Macedonia, Portugal, Spain, and United Kingdom) as well as some parts of Asia, Africa and North and South America [6].

In European countries, invasive fungal diseases are becoming more and more researched. If in the past, infections with *Candida* spp. used to be the most feared fungal threat, nowadays, emerging molds like *Aspergillus* spp., the zygomycetes or *Fusarium* spp. are slowly gaining ground. The life-expectancy raised as medical care improved in the past years, and along with it, the number of people who present risk factors increased, thus becoming potential hosts for different types of fungal infections. Risk factors for developing invasive fungal diseases include immunosuppressive agents, antineoplastic agents, broad spectrum antibiotics, grafts, prosthetic devices, excess of food intake, patients who undergo hematopoietic stem cells or organ transplantation, patients with hematological malignancies, HIV/AIDS, or who are admitted to the ICU, surgery departments or burn patients [2].

This review aims to highlight the high variability of the fungal strains and their requirements that make them potentially pathogenic for humans, as well as the methods used for diagnosing them.

Table 1. Classification of fungal species from the tropical and temperate areas.

	Genus	Important species	Disease
***Dimorphic fungi (tropical area)* [1]**	*Blastomyces*	*Blastomyces dermatitidis* *Blastomyces gilchristii* *Blastomyces helicus* *Blastomyces percus*	*Blastomycosis*
	Coccidioides	*Coccidioides immittis* *Coccidioides posadasii*	*Coccidioidomycosis*
	Histoplasma	*Histoplasma capsulatum (var capsulatum and var duboisii)*	*Histoplasmosis (Darling's disease)*
	Paracoccidioides	*Paracoccidioides lutzii* *Paracoccidioides brasiliensis*	*Paracoccidioidomycosis (Lutz-Splendore-Almeida disease or South American blastomycosis)*
	Emergomyces	*Emergomyces pasteurianus* *Emergomyces africanus* *Emergomyces canadensis* *Emergomyces orientalis* *Emergomyces europaeus*	*Emergomycosis*
***Molds (temperate area)* [2]**	*Aspergillus*	*Aspergillus fumigatus* *Aspergillus flavus* *Aspergillus terreus* *Aspergillus niger*	*Aspergillosis*
	Fusarium	*Fusarium solani* *Fusarium oxysporum* *Fusarium verticillioidis* *Fusarium moniliforme*	*Fusariosis*
	Actinomucor	*Actinomucor elegans*	*Mucormycosis*
	Mucor	*Mucor amphibiorum* *Mucor circinelloides* *Mucor ellipsoideus*	
	Mycotypha	*Mycotypha microspora*	
	Rhizomucor	*Rhizomucor parasiticus*	
	Rhizopus	*Rhizopus nigricans*	

Mycology and epidemiology

The main causes of systemic mycoses in tropical areas are dimorphic fungi, which are characterized by the two different shapes they can take: most commonly, they grow in a mycelial form in the soil or at environmental temperature (around 22 °C), and as yeasts or spherules at body temperature or in the incubator (at 37 °C) [7]. The most frequent endemic systemic mycoses are coccidioidomycosis, histoplasmosis, paracoccidioidomycosis, blastomycosis, and, slowly gaining ground, emergomycosis. The dimorphic fungi represent the main cause of this disease, with their classification presented in Table 1. In Europe, these mycoses occur mostly in travelers who return from tropical areas, aid workers, archaeologists, and immigrants [8], thus being considered "imported" cases. In contrast, in the tropical area, each dimorphic fungus has its specific endemic region, based on the climatic conditions that allow them to develop and spread further, causing the disease (Table 2).

In temperate areas, the mold-related invasive fungal infections that have the biggest impact on the healthcare system are aspergillosis, fusariosis, and mucormycosis (Table 1). The epidemiology of these molds is particular, as all three genera are ubiquitary, and therefore the chance to contact them is unavoidable. *Aspergillus* spp. and *Fusarium* spp. are both found in water, food, air, and soil [9]. In contrast, the fungi from the order *Mucorales* are facultative parasites, regularly originating from soil, compost piles, animal fecal matter, decaying organic matter, agricultural debris, and can therefore be considered opportunistic pathogens for plants, animals, and humans [10]. For *Aspergillus* spp., climatic differences regarding temperature and humidity play a very important role in the incidence of the disease, while in *Fusarium* spp., the affinity for marine or river environments makes it more common in the northern temperate regions [9]. Mucormycosis is also dependent upon climatic conditions, studies showing a strong connection between seasonal variations and an increase in the number of cases of mucormycosis during August to November [10].

Virulence factors

In the pathogenesis of fungi, two processes are involved [13]:

- Survival and growth of the microorganism that produces the infection;
- The damage caused to the host disrupts the homeostasis of the organism and further manifests as symptoms of the disease.

To become pathogenic, microorganisms possess a series of virulence factors, which can be classified into immune evasion strategies and adhesion and invasion factors [13].

Immune evasion strategies

Immune evasion strategies are methods used by microorganisms to avoid or escape the immune

Table 2. Epidemiology of dimorphic fungi.

Genus	Area	Environment			Dissemination
		Temperature	Moisture	Factors that facilitate dissemination	
Blastomyces spp [1]	North America, Africa, Sporadic cases: Central and South America, Mexico, India and Middle East	20 °C to 40 °C	moist, acidic soils associated with decaying organic matter that are close to lakes and rivers	Farming, agriculture, fishing, camping and outdoor professions	- Inhalation of spores or conidia - Open wound contact with the conidia or spores
Coccidioides spp [3]	American continental areas	0 °C to 45 °C	semi-deserted areas with very dry conditions and low precipitations	dust storms, earthquakes, droughts and earth excavation	- mycelia lay latent in the soils - they become arthroconidia - they are then spread by the wind
Histoplasma spp. [1, 3]	Africa, United States, Latin America	20 °C to 30 °C	High humidity, chalky soil	gardening, caving, mining, construction, farming	- Inhalation of spores or conidia - Open wound contact with the conidia or spores
Paracoccidioides spp. [4]	Latin America	mild temperatures	humid regions, with medium to high pluviosity	soil, decaying plants, water and other organic substrates	- Inhalation of spores or conidia - Open wound contact with the conidia or spores
Emergomyces spp. [5]	Asia, Africa, Europe, North America	mild temperatures	humid regions	Heavily populated endemic areas (only people with highly impaired cellular immunity develop the disease)	- Inhalation of spores or conidia

response. Microbes implement these strategies in a variety of ways, commonly by concealing their immunogenic surface structures (covering them with capsules or host molecules such as pigments), or by other mechanisms used to escape phagocytosis. For example, *Blastomyces dermatitidis* secretes, besides its main virulence factor *BAD1*, a dipeptidyl-peptidase IVA (DppIVA) which modulates host immunity. DppIVA is a serine protease that cleaves and inactivates GM-CSF, a cytokine that normally would activate macrophages and neutrophils to kill fungi [14].

Temperature-based dimorphic fungi can change the chemical composition of their cell wall distinctly, depending on the phase in which they stand at a certain moment. For *Paracoccidioides* spp. and *Histoplasma* spp., the cell wall is composed of β-1,3-glucan in the mycelial phase, while in the yeast phase, this polysaccharide is substituted with α-1,3-glucan, offering the fungus an increased virulence. Furthermore, the α-1,3-glucan masks the β-1,3-glucan component of the cell wall, making it unrecognizable by the phagocytic cells, therefore avoiding phagocytosis [15]. The β glucan is recognized by the Dectin-1 receptor of macrophages and triggers the formation of reactive oxygen species and proinflammatory cytokines, aiding the inflammatory process, being part of the molecules that modulate the activity of the immune system [16].

As in the case of dimorphic fungi, *Aspergillus* spp. has a cell wall constituted from a polysaccharide-based three-dimensional dynamic structure that is constantly changing. As the main defense line of the fungus against hostile environmental stress, its main purpose is to ensure the physical protection and structural identity of the cell. Also, it is to be noted that its components are usually the main targets of the immune system of the host. The major polysaccharides are similar: α(1,3)-glucans, which are synthesized due to three main genes *ags1*, *ags2* and *ags3*, and the β(1,3)-glucans, synthesized due to the *fks1* gene [17]. β-glucan is present in almost all fungi and has been used as a diagnostic tool for invasive mycosis, as its levels correlate well with those of galactomannan (the primary exoantigen that is released during tissue invasion) in patients with invasive aspergillosis [17].

Furthermore, there are several factors related to the surface structures of *Aspergillus* spp. that interact and activate the immune system. The main attribute of this fungus is its ability to escape the immune system by interfering with the complement cascade [17]. An important gene involved in this virulence factor is the *alb1* gene, which is required for the pigmentation of the conidial surface, similar to the melanin pigment of dimorphic fungi, and has been shown to increase the virulence and limit C3 complement deposition and neutrophil activation [18]. Furthermore, *Aspergillus fumigatus* can bind factor H, factor H-like protein 1 (FHL-1), and C4BP on their surface to down-regulate complement cascade and produce a soluble complement-inhibitory factor [18].

Another cell wall constituent with a role in fungal virulence is the production of melanin or melanin-like pigments by species such as *Coccidioides* spp., *Cryptococcus neoformans*, *Aspergillus* spp., *Sporotrix schenckii*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* and *Blastomyces dermatidis* [19]. Melanins are negatively charged, hydrophobic pigments with a high molecular weight. They are formed through the oxidative polymerization of phenolic or indolic compounds. There is little information about the exact localization of melanin in fungi that are pathogenic to humans, but it has been described both intracellularly and extracellularly [15]. The main role of this dark pigment located in the cell wall is to protect the fungi from external environmental factors such as extreme temperatures, UV light, toxic metals, peptides, and antifungal drugs. The melanization process decreases the fungal susceptibility to amphotericin B and caspofungin and was proven to have anti-inflammatory effects [20]. Cell melanization also greatly contributes to the protection of the fungal strains against nitrogen- or oxygen radicals mediated injury and enhances its resistance to H_2O_2 and hypochlorite, by free radical scavenging and electron transfer properties [20].

Another protein with a role in virulence that is related to the cell wall is *YPS3*, being found in *Histoplasma capsulatum* and usually released in the culture media. This factor is only produced by the yeast form, being expressed differently in relation with their virulence (higher levels are found in more virulent strains). The exact role of this molecule is yet to be determined [21]. Virulent strains make use of their ability to modulate the immune system by calcium-binding protein, hydroxamate, or heat shock proteins. *Histoplasma* growth is inhibited in calcium-free environments; therefore, the yeast form secretes a calcium-binding protein during the multiplication phase, to ensure the necessary calcium required for its survival. Also in *Histoplasma* spp., a newly characterized virulence factor is hydroxamate, important for the intracellular growth [16]. Heat shock proteins (HSP) are expressed to ensure the virulence of *Paracoccidioides* spp. and *Histoplasma* spp. They are ubiquitously present in the cells and are generally upregulated as a response to

various stressful situations. HSP are usually released following a sudden temperature increase, ensuring the dimorphic transition of the fungus. Another important gene in this process is SCONC, which is involved in sulfur metabolism, influencing the virulence of the strains [15].

One of the most important traits of *Aspergillus* spp. is its thermophilicity, which consists of the fungal ability to grow better at 40-41 °C (fever temperature) than at 25 °C. However, no specific pathway controlling this virulence factor has been linked to its growth in human hyperthermic conditions [22]. There are four genes that have been studied in relation to the thermotolerance of the fungus. The *thtA*, *cgrA,* and *afpmt1* genes are required for fungal growth at 48 °C, 37 °C, and 37 °C respectively, but none of them are involved in the fungal virulence. Cells exposed to non-lethal high temperatures become slowly resistant to heat by producing some compounds named heat shock proteins (HSPs) [17]. There are approximately 50 documented HSPs in the genome of *Aspergillus fumigatus*, but most of them have been identified as molecular chaperones associated with stress resistance, rather than heat [22]. Out of them, *HSP30/HSP42* and *HSP90* showed the highest increase in expression during the heat shock response by *Aspergillus fumigatus*. Of the dimorphic fungi, *Histoplasma* spp. has two specific heat shock proteins: *HSP60* and *HSP82*. Heat shock protein 60 (*HSP60*) has multiple important roles: in chaperoning intracellular proteins, in the supervision of appropriate protein folding and as an essential surface molecule involved in mediating the recognition and phagocytosis of the yeast by macrophages [23]. Heat shock protein 82 (*HSP82*) is an essential factor for normal growth, but is also involved in the response to cellular stress (heat and oxidative stress). Low levels of *HSP82* decrease the fungal virulence in macrophages and severely impairs its ability to infect the lungs [24].

Coccidioides spp. strains produce enzymes as virulence factors during the infection. For instance, when the fungi meet an acidic pH, they produce urease in order to decrease the pH and further stimulate their multiplication and invasion, exacerbating the tissue damage. Urease is found in the cytoplasm, vesicles and central vacuole of the spherules, being released during endosporulation, acting as a hydrolyzation agent (divide urea into two ammonium molecules) [25]. The production of this enzyme is not strictly limited to *Coccidioides* species; other pathogens, such as *Aspergillus* spp., *Emergomyces* spp., *Candida* spp., *Cryptococcus* spp., *Rhodotorula* spp., *Trichosporon* spp., but also bacteria such as *Helicobacter pylori*, are able to produce urease, but its role as a pathogenicity factor was only proved in *Coccidioides* and *Cryptococcus* spp. [25].

Another virulence factor, present in *Aspergillus* spp., *Fusarium* spp., and many other fungi is the ability to produce mycotoxins. This chemically diverse group of organic substances with low molecular weight are secondary fungal metabolites produced in the hyphae during their growth and can be released in the environment either directly or following the hyphal damage/death. Mycotoxins are usually produced by the fungi to protect themselves from the competitors in their ecosystem, but can also contribute to the pathogenesis in the infected host. They affect different processes such as protein, DNA or RNA synthesis or can alter the cell membranes, which cause death or impairment of cellular functions [17].

Virulence factors that have a role in adhesion and modulation of the immune system

Adhesion and invasion factors are proteins or other molecules that mediate the attachment to host cells. These factors are fundamental in the pathogen-host interactions, which consist of surface structures that facilitate adherence to the cell host [26]. Usually, fungi do this through the filamentous growth that penetrates the tissues. Tissue colonization typically occurs due to proteins named adhesins, ligands encoded by the pathogen, and a cell receptor (e.g., secreted cell products, extracellular matrix proteins like collagen type I and IV, fibronectin, fibrinogen, laminin). Additionally, other factors such as aldolase proteins of 1,6-bisphosphate and enolase are further used by the fungus to penetrate the deeper tissues. Virulence factors can have a role strictly related to the adhesion of the fungus or they can have a complementary role in virulence through immune modulation [15].

BAD1, formerly known as WI-1, is a multipurpose protein that serves as an adhesion molecule and immune defense protein. It is mostly known as a virulence factor belonging to *Blastomyces dermatitidis* and *Blastomyces gilchristii*. The main role of *BAD1* is in the attachment of the yeast form of the fungus to the cellular surface, a process which also involves other components such as chitin, heparan-sulfate and complement receptors [27]. The *BAD1*-intermediated attachment of yeast cells to the cellular wall results in the inhibition of TNF-α production by macrophages and neutrophils, which further weakens the immune response by suppressing the activation of CD4+ T lymphocytes and decreasing the levels of other cytokines such as IL-17 and IFN-γ [28].

In most cases of infection with dimorphic fungi, the arthroconidia initiate infection through inhalation by the host, where they further undergo a thermally induced switch to their pathogenic phase. As the fungi begin the multiplication process, in the initial stages of the pulmonary infection, hundreds of endospores begin to differentiate inside the large spherule. Furthermore, the spherule sheds a membranous layer formed from polysaccharides, lipids, and proteins called the spherule outer wall (SOW). In the case of coccidioidal infection, the SOW glycoproteins (SOWgp) are responsible for the human immune response to infection [19]. Moreover, it has been suggested that SOWgp stimulates a proliferative response of immune CD4+ T cells in vitro, directing Th2 cells to secrete cytokines that stimulate B lymphocytes to produce antibodies [19]. Other studies performed to detect the role of SOWgp during infection showed that it is also involved in the fungal adhesion process, as it can bind to the mammalian extracellular matrix (ECM) components such as laminin, fibronectin and collagen [19].

The most important virulence factor possessed by *Paracoccidioides* spp. is a 43kDa protein (gp43). Gp43 contains different regions that allow interactions with laminin, collagen, and fibronectin, facilitating the fungal adhesion to the cell. It is usually secreted during active infection and it contains some epitopes that are capable of triggering a cellular immune response in patients, causing lymphocytes to produce IFN-γ and stimulating the formation of granulomas that contain yeast cells. Gp43 can be used as a serological marker for infection [29].

Clinical manifestations

As it can be seen from Table 3, the majority of dimorphic fungi primarily affect the lungs and the respiratory tract, with secondary disseminations to other organs such as the skin, mucous membranes, gastrointestinal tract, genitourinary tract, central nervous system, eyes, and the osteoarticular system. None of those fungi require immunosuppression as a risk factor, therefore the infection can appear both in healthy individuals (who develop asymptomatic forms of the disease) and in people with chronic diseases (who usually have fulminant manifestations in the target organs and are at higher risk of developing chronic forms of infection). The only exception is emergomycosis, where immunosuppression is the main factor that leads to disseminated forms of the disease. Frequently, this mycosis is associated with HIV infection, but other risk factors such as neutropenia, hematological malignancies, use of immunosuppressive drugs, or solid organ transplantation can be present [12].

Acute forms of the disease usually manifest with flu-like symptoms (e.g., dry or productive cough, fever, chills, malaise, and pleuritic pain), because the respiratory system is the main target organ of the disease. In rare cases, the skin can be the primary foci [31].

Respiratory infections are usually acquired through the inhalation of spores of the mold phase of the microorganism, which transform into a yeast form once they reach the lungs [31]. Primary cutaneous forms are usually due to the traumatic inoculation of the skin and

Table 3. Clinical manifestations – the affected organ systems.

		Target organ								**Associated with SARS-CoV-2 infection [6]**	**Chronic forms**
	Species	**Lungs**	**Skin**	**Mucous membranes**	**Gastrointestinal tract**	**Genitourinary tract**	**Central nervous system**	**Osteoarticular system**	**Eyes**		
Dimorphic fungi (tropical area)	*Blastomyces* spp. [7]	++++	+++	+++	-	+++	+++	+++	-	No	Yes
	Coccidioides spp. [1,3]	++++	+	-	-	-	+	+	-	Yes	Yes
	Histoplasma spp. [8]	++++	++	++	-	-	-	-	-	Yes	Yes
	Paracoccidioides spp. [9]	++++	+	+++	+	-	++	-	-	No	Yes
	Emergomyces spp. [5]	++++	++++	++++	+	-	-	-	-	No	Rare
Molds (temperate areas)	*Aspergillus* spp. [10, 11]	++++	-	+	-	-	+	+	+	Yes	Yes
	Fusarium spp. [12]	+	++++	++	-	-	+	++	++++	Yes	Yes
	Species from order Mucorales [13]	+++	++++	++++	++	-	++++	+	+	Yes	Yes

++++ extremely frequent involvement; +++ moderate involvement; ++ low involvement; + rarely involved; - no involvement.

are associated with work exposure in medical personnel [36].

Regarding the secondary foci, the lesions are characteristic for each target organ:

- *Skin*: superficial ulcers with granular appearance and hemorrhagic points (mulberrylike stomatitis) – in the case of paracoccidioidomycosis and nodular or verrucous aspects, abscesses, ulcers or retracting scars – in the case of coccidioidomycosis;
- *Central nervous system*: headache, gait abnormalities, focal neurological defects, seizures, mental status changes, focal neurological defects and sometimes signs similar to meningitis; as the disease spreads, it can cause symptoms comparable to a space-replacing mass - increased intracranial pressure, headache, impaired vision, nausea and vomiting, blood pressure changes – in the case of blastomycosis [37];
- *Mucosa*: symptoms such as dysphonia, when the laryngeal mucosa is involved – in paracoccidioidomycosis [33];
- *Gastrointestinal tract*: abdominal pain, nausea, vomiting, constipation or diarrhea, fever and anorexia – in paracocidioidomycosis [33].

Chronic forms usually manifest as fever, weight loss and night sweats and the persistence of symptoms for more than 3 months [38].

Infections with *Aspergillus* spp. and *zygomycetes* can be acquired by inhaling the conidia or by ingesting them through contaminated food, water or other environmental sources (Table 3). The former, once it reaches the lungs, germinates into hyphae in an uncontrolled manner, spreading fast towards a potentially invasive form of the mold [34]. The latter colonizes the respiratory or the gastrointestinal system, waiting for the favorable ground of the host in order to cause infection [10]. In contrast, *Fusarium* spp. rarely causes lung infections, therefore its portal of entry is different based on the organ where the infection appears. The symptoms of the disease are also highly dependent upon the immune system of the host [35]. In immunocompetent people, it is the most common etiological agent of superficial skin infections such as keratitis and onychomycosis, but it can affect other organs causing various infections such as peritonitis in patients receiving dialysis, thrombophlebitis, arthritis, osteomyelitis, endophthalmitis, fungemia, sinusitis and pneumonia; in severely immunocompromised patients, locally invasive or disseminated infections are more frequent and are usually associated with positive blood cultures [35].

Lung infections in aspergillosis include asthma, allergic bronchopulmonary aspergillosis, allergic sinusitis (developed in healthy people who have an atopic field), aspergilloma, chronic cavitary pulmonary aspergillosis and invasive aspergillosis [34]. Other organs that can be affected include sinus cavities (aspergillar rhinosinusitis), eyes (ocular aspergillosis), bones (aspergillar osteomyelitis) and central nervous system. Symptoms are usually specific to the affected organ and can easily spread to the surrounding structures [9].

Based on the organ that is affected, fungi from the Mucorales order can cause several types of diseases: rhinocerebral mucormycosis, pulmonary mucormycosis, cutaneous mucormycosis, gastrointestinal mucormycosis, disseminated mucormycosis and mucormycosis with uncommon presentations such as renal disease [10].

Clinical manifestations vary, from the pneumonia-like symptoms that are resistant to antibiotic treatment in aspergillosis and mucormycosis (chronic cough, hemoptysis, shortness of breath, tiredness, chest pain and fever) [10] to those of the superficial infections caused by *Fusarium* spp. such as onychomycosis (skin and nail lesions), keratitis (ocular lesions) and peritonitis in patients receiving dialysis (symptoms similar to bacterial peritonitis - diffuse abdominal pain, cloudy dialysate, fever) [39].

Nowadays, acquiring invasive mold infections is slowly becoming a complication that is feared in people who suffer from COVID-19 [2]. Unfortunately, *Aspergillus* spp. became the most common fungal pathogen that complicates the evolution of this disease [40]. Moreover, this fungal infection is found not only in severe or critical cases of the disease, but even in asymptomatic, mild or moderate forms. The fungal species commonly associated with COVID-19 are *Aspergillus fumigatus,* followed by *Aspergillus flavus*. Sadly, the outcome of the disease is poor in most of the cases, even though antifungal treatment is administered [40].

Diagnostic

The diagnostic is based on several criteria: anamnesis (living or history of traveling in endemic areas), histopathology, direct microscopical examination straight from the pathological product, cultivation, microscopical examination from the culture, immunological diagnostic, serological testing or molecular diagnostic [9]. In disseminated infections, blood cultures are indicated [32].

Direct microscopy from the biological sample

For laboratory diagnostic, microscopic examinations from sputum or bronchial lavage (pulmonary involvement) or pus and secretions (skin involvement) can be used – either directly from the lesion using potassium hydroxide (KOH) 10-20% or calcofluor white or after cultivation of the fungi [8]. When performed directly from the pathological sample, this examination can aid in acquiring a presumptive diagnosis, as culture usually takes a longer time to grow and molecular diagnostic is not always available [35].

In dimorphic fungi, the typical finding is yeast cells with different aspects: for example in blastomycosis, single budding cells are pathognomonic, while in paracoccidioidomycosis, multiple budding cells are characteristic [26,36]. In histoplasmosis, the yeast cells can be seen as round shapes inside the macrophages, while in coccidioidomycosis, spherules with endospores are noticed [41].

In filamentous fungi, early fungal identification is very important in order to be able to assure a prompt and targeted treatment, especially in cutaneous mucormycosis. This can be achieved by performing a KOH mount or calcofluor white staining directly from the tissue and performing a microscopical examination, where non-septate hyaline hyphae with irregular branching at right angles can be seen. Fungal cultures on potato dextrose agar are positive in only 50% of the cases [42].

Cultivation

Cultivation is another key component of the diagnostic methods. Different culture media are available: heart brain-infusion, potato dextrose agar, potato flakes media, or Sabouraud dextrose agar. Also, the common agar media used in bacteriology such as blood agar or chocolate agar are suitable for cultivation [43].

For dimorphic fungi, culturing the pathogen is extremely dangerous and should be saved for specialized laboratories. If the plates are incubated at 25 °C to 30 °C for up to 6 weeks, fluffy colonies should appear (characteristic of the mycelial phase of the fungus), while incubation at 37 °C will lead to the growth of colonies characteristic of the yeast phase [41]. If the mycelial phase of these fungi is incubated at 37 °C, yeast-like growth of the colonies will occur, only proving the characteristic dimorphic aspect of the fungus and not the species. This procedure takes a long time, therefore it is not used as a diagnostic technique anymore [41]. In contrast, even though all dimorphic fungi grow as white fluffy colonies, the mold forms can be differentiated based on their shape, color, and growth time. *Histoplasma* spp. appears as dry colonies, angel-hair-like, which turn pigmented as they age, while *Blastomyces* spp. turn grayish with time [8,36,41]. *Paracoccidioides* spp. is atypical, as it grows as glabrous leathery, flat colonies which can vary to a wrinkled, folded, floccose form, with a brownish to velvety, white, pink, and beige color [8]. *Emergomyces* spp. differentiates from the others as well, as it has yellowish, white to tan colonies, with an initial glabrous aspect, becoming powdery with time, slightly raised and furrowed, reaching 2.5-3.5 cm in diameter in 2 weeks [12].

For molds, the temperatures used for cultivation can vary: for *Aspergillus* spp. and *Fusarium* spp. between 30 °C to 37 °C, and in the case of *zygomycetes,* between 25 °C and 30 °C. The plates should be kept in the incubator for up to 72 hours [44]. Identification of the level of species can be done based on the macroscopical and microscopical examinations, which are great tools in aiding a preliminary diagnosis [44].

The macroscopical aspect of the *Aspergillus* spp. culture is not a very accurate diagnostic tool, as the representatives of this genus have a variety of colors, from white to dark blue-green colonies to yellow-brown or yellow-greenish. In this case, the suspicion for genus identification is raised by the microscopical examination, and for species, a molecular diagnostic technique such as PCR is required. The culture for *zygomycetes* stands out from the others due to its invasive and fast growth. The colonies are white-gray, fluffy with a wooly aspect [45].

The cultures can be further studied by microscopic examinations.

Microscopical examinations from culture

In the case of dimorphic fungi, if microscopical examination is performed from the yeast culture, budding yeast cells can be seen. A specific aspect can be noted for *Paracoccidioides* spp.: a globose large, central cell which has around it several narrow-necked budding yeasts, which resemble a "pilot well", a "floating mine" or if the mother cell has only two budding cells, a "Mickey Mouse" head [11]. For the mycelial phase, lactophenol-cotton-blue staining can help for a presumptive diagnostic, based on the differentiation between species. Lactophenol-cotton-blue microscopic examination of *Blastomyces dermatitidis* shows, in the mold phase, conidiophores that emerge at a right angle and have a single terminal conidia with a piriform to ovoid shape – "lollipop" aspect [36,37]. In the case of *Coccidioides* spp., the microscopic examination from the culture usually

reveals septate hyphae and barrel-shaped arthroconidia [8,43], while in *Paracoccidioides* spp., different types of conidia are present: chlamydoconidia, conidia and arthroconidia (the infectious form) [11]. *Histoplasma* spp. has a typical aspect of pear-shaped microconidia and thick-walled, spiny macroconidia [41]. *Emergomyces* spp. appears in lactophenol-cotton-blue as slender conidiophores arising at right angles from hyaline hyphae with thin walls, slightly swollen at the tip, with short, secondary conidiophores bearing "florets" of solitary single-celled subspherical conidia [12].

For *Aspergillus* spp., microscopy is characteristic, showing the septate hyphae which branch at an acute angle and the aspergillar head. A presumptive differentiation between species of the genus can be made based on the aspect of the head, that can have uniseriate or biseriate conidia, but a certain species identification can be done only based on molecular diagnostic using PCR or MALDI-TOF. For *Fusarium spp.*, the same septate hyphae branching at an acute angle aspect can be seen. But what is characteristic is the presence of micro- and macroconidia. Macroconidia are hyaline, multicellular clusters of cells that have the shape of a banana or gondola and are supported by a foot cell at the base. Microconidia are hyaline, unicellular and have an ovoid to cylindrical shape. If microconidia are present, it is important to determine the shape, number of cells and mode of cell formation [46]. In contrast, species from order *Mucorales* are easy to differentiate, due to the lack of septae on the hyphae [42].

Complementary tests

Histopathological examinations usually consist of microscopical examinations from bioptic lung tissue in Periodic acid-Schiff staining and Gomori methenamine silver, the typical findings including granulomas and intracellular budding yeast cells.

Immunological examinations such as latex agglutination or enzyme-linked immunosorbent assay (ELISA) can be used in endemic regions, but have a high rate of false positive results [8,32]. Serological examinations such as ELISA and double immunodiffusion can be performed to detect antibodies. Sometimes, cross-reactivity is possible, such as in the case of *Blastomyces* spp. and *Histoplasma* spp., therefore they should be mostly used for screening in endemic areas and not for diagnostic [47]. For *Emergomyces* spp., there are no specific antibodies, but cross-reactivity has been noted in *Histoplasma* galactomannan enzyme immunoassay [12].

When the culture shows atypical colonies, molecular diagnostic or experimental inoculation in laboratory animals can be further used to identify the species [8,43]. Even nowadays, there is still a lot of research going on for a faster and more accurate method of diagnostic than microscopy or culture. Lately, molecular diagnostic has been taking ground, as it can be seen from Table 4. Primers are starting to become available, and more and more studies regarding PCR diagnostic of different fungal species directly from samples are being published, making this a promising option in the future [35]. For example, in emergomycosis, even though routine diagnostic based on the morphological characteristics is still used in some laboratories, those features may overlap with other fungal genera, therefore requiring molecular diagnostic such as PCR and sequencing for confirmation. However, an accurate diagnostic depends on several factors such as proper specimen collection, the fungal load and primer design (pan-fungal or species specific). At this moment, no commercial kits for fungal identification are available. Basically, fungal identification consists of DNA extraction, followed by PCR and sequencing of the *ITS* and *LSU* regions, using the primers mentioned in Table 4. Also, some fungi can be detected by PCR, by following specific genes for the species.

Unfortunately, at the moment, there are still no validated PCR methods for detection of filamentous or dimorphic fungi from patient samples, even though it is a cheaper, faster and more accurate method [35].

Antigen detection can be used as an alternative diagnostic method. Some examples of antigenic testing include histoplasmin skin test, paracoccidin intradermal test, or blastomycin skin test [8]. *Blastomyces* spp. antigens can be detected in the urine or serum. The test can be performed in both pulmonary and extrapulmonary forms of the disease, but it has its limitations, such as the very high degree of cross-reactivity with *Histoplasma* spp. antigens. Therefore, a negative antigen test in a symptomatic person from an endemic area cannot exclude the diagnosis [48].

Galactomannan is a polysaccharidic component of the fungal wall of *Aspergillus* spp., which is released by the hyphae as they invade the lungs. It has been suggested that the level of galactomannan in the blood is proportional to the fungal load; therefore, it can be used more as a prognostic marker than as a diagnostic criteria. The detection method for galactomannan is ELISA, with good sensitivity and specificity [34]. False positives (patients receiving piperacillin-tazobactam and amoxicillin-clavulanate, histoplasmosis,

blastomycosis) and false negatives (hematologic malignancies, patients who undergo antifungal therapy) have been reported. Galactomannan is also present in the wall of *Histoplasma* spp. and it can cross-react with the cell wall components of *Emergomyces* spp. [12].

Antifungal susceptibility testing

Antifungal susceptibility testing (AFST) has the most value when the fungal infection is invasive, when acquired drug resistance is suspected, or when the treatment does not seem to show the expected results. For all those situations, knowing the exact *in vitro* susceptibility is of great aid. There are four main methods of performing AFST: broth microdilution (following the CLSI or EUCAST standards), disk diffusion, gradient diffusion strips and agar screening for *Aspergillus* resistance [58]. In the case of dimorphic fungi, the broth microdilution technique can be used to determine the minimum inhibitory concentration (MIC). Even though there are no standardized breakpoints available, studies proved that adjusting the dosage of the antifungal drug according to the MIC, lead to a better therapeutic outcome [59].

Table 4. Diagnostic aspects of mycoses.

	Genus	Microscopy (from pathological product)	Cultivation		Microscopy (from culture)		Molecular diagnosis
			Yeast	Mold	Yeast	Mold	
Dimorphic fungi (tropical area)	*Blastomyces* spp.	single budding yeast cells [7]	butter appearance [7]	white fluffy colonies, become grayish with time [7]	yeast cells with double refractile walls budding in one direction [14]	conidiophores that emerge at a right angle with a single terminal piriform conidia – “lollipop” aspect [14]	**BD1 + BD2** [15] Fw: 5′-GCGATGGTAAGGCAGTTT-3′ Rv: 5′-ACCTCTCTTTGCTGGGAAAA-3′
	Coccidioides spp.	spherules with endospores [1]	butter appearance [1]	white, dry colonies, angel-hair like, turning pigmented as they age [3]	budding yeast cells [3]	septate hyphae and barrel-shaped arthroconidia [3]	**Coi9** [16] Fw:5′-ACGGTGTAATCCCGATACA-3′ Rv:5′-GGTCTGAATGATCTGACGCA-3′
	Histoplasma spp.	ovoid cells inside the macrophages [17]	butter appearance [17]	white, fluffy mycelia [17]	budding yeast cells [17]	pear-shaped microconidia and thick-walled, spiny macroconidia [17]	**M protein** [18] *Msp*1 Fw: 5′-ACAAGAGACGACGGTAGCTTCACG-3′ Rv: 5′- GCG TTG GGG ATC AAG CGA TGA GCC-3′ *Msp*2 Fw: 5′- CGGGCCGCG TTTAACAGCGCC-3′ Rv: 5′-ACCAGCGGCCATAAGGACGTC-3′ **H antigen** [19] Hc2 + Hc3 Fw: 5-GCGGGGTTGGCTCTGCTCT-3′ Rv: 5-TTGGAAACCCCGGGCTTG-3′ **100kDa protein** [20] HcIII + HcIV Fw: 5-GAGATCTAGTCG CGGCCAGGTTCA-3′ Rv: 5-AGGAGAGAACTG TATCGGTGGCTTG-3′
	Paracoccidioides spp.	multiple budding aspect [4]	butter appearance [4]	hyphae [4]	mother cell surrounded by several blastoconidia - can resemble a “pilot well”, a “floating mine” or “Mickey Mouse” head) [4]	chlamydoconidia, conidia and arthroconidia (the infectious form) [4]	**MG2 (1)** [21] Fw: 5′-GGGATTCCCTAGGCAAACACTTGTGTGA-3′ Rv: 5′-GTGCAGTTATCCACAAGCCATATATTC-3′ **MG2 (2)** [21] Fw: 5′-GGAGATGATCTGACGTTAGTACGTGATG-3′ Rv: 5′-ATGCTAATTTATGTCATTCCGCGTCTG-3′
	Emergomyces spp.	intracytoplasmic narrow-based budding yeasts, measuring 2–5 µm in size [5]	butter appearance	yellowish white to tan, glabrous becoming powdery, slightly raised [5]	oval yeast cells with narrow based budding [5]	slender conidiophores arising at right angles from thin-walled hyaline hyphae [5]	**ITS5** [5] 5′-GGAAGTAAAAGTCGTAACAAGG-3′ **ITS4** [5] 5′-TCCTCCGCTTATTGATATGC-3′ **LR0R** [5] 5′-ACCCGCTGAACTTAAGC-3′
Filamentous fungi (temperate area)	*Aspergillus* spp.	hyphae [22]	-	***Aspergillus fumigatus*** dark blue-green colonies, sometimes with a red pigment; ***Aspergillus flavus*** yellow-brown color, sometimes brown; ***Aspergillus niger*** black surface of the colony; ***Aspergillus terreus*** yellow-greenish colonies [22]	-	*Hyphae:* wide, septate, hyaline, acute angle tree- or fan-like branching hyphae *Conidial head:* Uniseriate or biseriate, columnar conidia in chains or detached and dispersed [22]	**Pan-Asp** [23] Fw: 5′-GTGGAGTGATTTGTCTGCTTAATTG-3′ Rv: 5′-TCTAAGGGCATCACAGACCTGTT-3′ ***Aspergillus fumigatus*** [23] Fw: 5′-GCCCGCCGTTTCGAC Rv: 5′-CCGTTGTTGAAAGTTTTAACTGATTAC-3′ ***Aspergillus flavus*** [23] Fw: 5′-CGAGTGTAGGGTTCCTAGCGA-3′ Rv: 5′-CCGGCGGCCATGAAT-3′ ***Aspergillus niger*** [23] Fw: 5′-GCCGGAGACCCCAACAC-3′ Rv: 5′-TGTTGAAAGTTTTAACTGATTGCATT-3′ ***Aspergillus terreus*** [23] Fw: 5′-CATTACCGAGTGCGGGTCTTTA-3′ Rv: 5′-CCCGCCGAAGCAACAAG-3′
	Fusarium spp.	hyphae [12]	-	fast-growing white, lavender, pink, salmon or grey colonies with a cottony surface [12]	-	Hyphae: hyaline filaments, septate and typically branch at acute or right angles Macroconidia: hyaline, multicellular clusters of cells Microconidia: hyaline, unicellular and have an ovoid to cylindrical shape [12]	***Fusarium*** spp. [24] Fw: 5′-ATGGGTAAGGARGACAAGAC-3′ Rv: 5′-GGARGTACCAGTSATCATGTT-3′ **Fa** [24] Fw: 5′-TCGTCATCGGCCACGTCGACTCT-3′ **Ra** Rv: 5′-CAATGACGGTGACATAGTAGCG-3′
	Species from order Mucorales	hyphae [13]	-	White-grey color with a wooly aspect of the colonies; fast growing, invasive culture [13]	-	Hyphae: wide, aseptate, non-radiating, 90º angle branching; folds in the hyphae may look similar to septae [13]	[25] Fw: 5′-ATT ACC ATG AGC AAA TCA GA-3′ Rv: 5′-TCC GTC AAT TCC TTT AAG TTT C-3′

Broth microdilution is performed similarly to the technique used for yeasts: in a 96-well microdilution plate, a culture medium (containing L-glutamine) and an antifungal agent (reconstituted from analytical-grade powder) are mixed in different concentrations. The fungi that are tested have to be subcultured at least twice on antimicrobial-free media, and spores from approximately five colonies of at least 1 mm diameter have to be suspended in sterile saline, vortexed, and adjusted using a spectrophotometer to 0.5 McFarland units, which will be considered the stock solution. Of this, 1: 100 and 1: 20 dilutions are prepared in RPMI 1640 culture medium. Each suspension is then added to plates, followed by incubation at 35 °C for 46 to 50 hours, except Mucorales, for which 24 hours of incubation is sufficient. Afterward, the MIC is interpreted by finding the first well that presents 100% inhibition for azoles and amphotericin B, which prevents the germination of mold conidia [58].

There are many sources of variation in the detection of MIC for antifungal drugs: the growth medium, inoculum concentration, pH, the solvent used to prepare the stock solution, and even the procedures. A particular case is that of *Aspergillus* spp., where multiple studies suggest the addition of a nonionic surfactant such as Tween 20 to the fungal colonies, before transferring them to the sterile saline tube for broth microdilution. The role of this substance is to facilitate the dispersion of the conidia in the saline. The exact benefit of Tween 20 is still not completely known, as it interacts with both the microorganism and the antifungal drugs, potentially affecting the final MIC result. Gomez-Lopez *et al.* [60] followed the effect of different concentrations of Tween 20 (0.5%, 3%, and 5%) on the MIC of different antifungal drugs, and proved that higher Tween 20 concentrations can facilitate the preparation of the inoculum, but can result in higher MIC values.

Disk diffusion can also be performed using standardized disks with known concentrations of antifungal drugs. The method has its limitations, as it cannot detect the MIC, and the results for filamentous fungi are not reliable, as they rarely correlate with the results obtained by broth microdilution. On the other hand, the gradient diffusion strips proved to be a good method of detecting antifungal resistance in molds, especially in the case of triazoles and amphotericin B [58].

Agar screening for *Aspergillus* resistance was recently developed by EUCAST in order to detect azole resistance. It requires using a four-sector agar screening plate containing itraconazole 4 µg/mL, voriconazole 2 µg/mL, posaconazole 0.5 µg/mL, and a drug-free sector. *Aspergillus* conidia are inoculated on the plate and incubated for 48 hours. Growth in any sector besides the control suggests antifungal resistance and requires further confirmation by the broth microdilution [58].

Conclusions

Invasive fungal infections are becoming a more and more feared health threat, as they are hard to diagnose and treat, and are usually found in patients with associated diseases.

In tropical countries, the most feared fungal pathogens are dimorphic fungi. They used to be restricted to certain areas of the globe where they had suitable climatic conditions for spreading and causing infections. Nowadays, due to an increase in traveling and migration and due to the lack of vaccines, it is becoming imperative for health practitioners from all around the globe to be able to diagnose such infections. The common diagnostic methods, including microscopical examinations, the dimorphism of the fungus, or antigen detection are becoming obsolete, as nowadays, a lot of ongoing research targets a faster and more accurate diagnostic, based on PCR identification directly from the pathological sample.

In the temperate area, behind the well-known yeasts, the feared molds silently hide. They have lately become slightly more frequent due to the altered immune status associated with HIV, diabetes, hematological disorders, or even with the current SARS-CoV-2 virus. Diagnosing such infections solely on the clinical symptoms of the patient is difficult, requiring fungal culture, followed by a preliminary identification based on the macroscopical and microscopical findings, and completed with MALDI-TOF or PCR for species identification.

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Authors' Contributions

Conceptualization, A.C., A.M.; investigation, A.C., A.D.M., L.R.C. and A.M.; writing—original draft preparation, A.C.; writing—review and editing, A.M., A.D.M., R.L.C.; visualization, A.D.M.; supervision, A.M. All authors have read and agreed to the published version of the manuscript.

References

1. Garbee DD, Pierce SS, Manning J (2017) Opportunistic fungal infections in critical care units. Crit Care Nurs Clin North Am 29: 67–79. doi: 10.1016/j.cnc.2016.09.011

2. Cighir A, Mare AD, Cighir T, Coșeriu RL, Vintilă C, Man A (2023) Filamentous fungi infections: yet another victim of COVID-19? Life 13: 546. doi: 10.3390/life13020546
3. Man A, Mare A, Toma F, Curticapean A, Santacroce L (2016) Health threats from contamination of spices commercialized in romania: risks of fungal and bacterial infections. Endocr Metab Immune Disord Drug Targets 16: 197–204
4. Köhler JR, Casadevall A, Perfect J (2015) The spectrum of fungi that infects humans. Cold Spring Harb Perspect Med 5: a019273. doi: 10.1101/cshperspect.a019273
5. Hsu LY, Wijaya L, Shu-Ting Ng E, Gotuzzo E (2012) Tropical fungal infections. Infect Dis Clin North Am 26: 497–512. doi: 10.1016/j.idc.2012.02.004
6. D'Amico F, Marmiere M, Righetti B, Scquizzato T, Zangrillo A, Puglisi R, Landoni G (2022) COVID-19 seasonality in temperate countries. Environ Res 206: 112614. doi: 10.1016/j.envres.2021.112614
7. Anstead GM, Bicanic T, Arathoon E, Graybill JR (2011) Histoplasmosis, blastomycosis, coccidioidomycosis, and cryptococcosis. in: Tropical infectious diseases: principles, pathogens and practice. Elsevier, pp 573–581
8. Bonifaz A, Vázquez-González D, Perusquía-Ortiz AM (2011) Endemic systemic mycoses: coccidioidomycosis, histoplasmosis, paracoccidioidomycosis and blastomycosis: Academy. JDDG J Dtsch Dermatol Ges 9: 705–715. doi: 10.1111/j.1610-0387.2011.07731.x
9. Cadena J, Thompson GR, Patterson TF (2016) Invasive Aspergillosis. Infect Dis Clin North Am 30: 125–142. doi: 10.1016/j.idc.2015.10.015
10. Hassan MIA, Voigt K (2019) Pathogenicity patterns of mucormycosis: epidemiology, interaction with immune cells and virulence factors. Med Mycol 57: S245–S256. doi: 10.1093/mmy/myz011
11. Queiroz-Telles F vio de, Peçanha Pietrobom PM, Rosa Júnior M, Baptista R de M, Peçanha PM (2020) New insights on pulmonary paracoccidioidomycosis. Semin Respir Crit Care Med 41: 053–068. doi: 10.1055/s-0039-3400544
12. Samaddar A, Sharma A (2021) Emergomycosis, an emerging systemic mycosis in immunocompromised patients: current trends and future prospects. Front Med 8: 670731. doi: 10.3389/fmed.2021.670731
13. Brunke S, Mogavero S, Kasper L, Hube B (2016) Virulence factors in fungal pathogens of man. Curr Opin Microbiol 32: 89–95. doi: 10.1016/j.mib.2016.05.010
14. Sterkel AK, Lorenzini JL, Fites JS, Subramanian Vignesh K, Sullivan TD, Wuthrich M, Brandhorst T, Hernandez-Santos N, Deepe GS, Klein BS (2016) Fungal mimicry of a mammalian aminopeptidase disables innate immunity and promotes pathogenicity. Cell Host Microbe 19: 361–374. doi: 10.1016/j.chom.2016.02.001
15. Santos LA, Grisolia JC, Burger E, de Araujo Paula FB, Dias ALT, Malaquias LCC (2020) Virulence factors of Paracoccidioides brasiliensis as therapeutic targets: a review. Antonie Van Leeuwenhoek 113: 593–604. doi: 10.1007/s10482-019-01382-5
16. Mihu MR, Nosanchuk JD (2011) Histoplasma virulence and host responses. Int J Microbiol 2012: e268123. doi: 10.1155/2012/268123
17. Abad A, Victoria Fernández-Molina J, Bikandi J, Ramírez A, Margareto J, Sendino J, Luis Hernando F, Pontón J, Garaizar J, Rementeria A (2010) What makes Aspergillus fumigatus a successful pathogen? Genes and molecules involved in invasive aspergillosis. Rev Iberoam Micol 27: 155–182. doi: 10.1016/j.riam.2010.10.003
18. Tsai HF, Chang YC, Washburn RG, Wheeler MH, Kwon-Chung KJ (1998) The developmentally regulated alb1 gene of Aspergillus fumigatus: its role in modulation of conidial morphology and virulence. J Bacteriol 180: 3031–3038. doi: 10.1128/JB.180.12.3031-3038.1998
19. Hernandez H, Erives VH, Martinez LR (2019) Coccidioidomycosis: Epidemiology, Fungal Pathogenesis, and Therapeutic Development. Curr Trop Med Rep 6: 132–144. doi: 10.1007/s40475-019-00184-z
20. van Duin D, Casadevall A, Nosanchuk JD (2002) Melanization of cryptococcus neoformans and histoplasma capsulatum reduces their susceptibilities to amphotericin b and caspofungin. Antimicrob Agents Chemother 46: 3394–3400. doi: 10.1128/AAC.46.11.3394-3400.2002
21. Bohse ML, Woods JP (2007) RNA interference-mediated silencing of the YPS3 gene of Histoplasma capsulatum reveals virulence defects. Infect Immun 75: 2811–2817. doi: 10.1128/IAI.00304-07
22. Latgé J-P, Chamilos G (2019) Aspergillus fumigatus and Aspergillosis in 2019. Clin Microbiol Rev 33: e00140-18. doi: 10.1128/CMR.00140-18
23. Long KH, Gomez FJ, Morris RE, Newman SL (2003) Identification of heat shock protein 60 as the ligand on histoplasma capsulatum that mediates binding to cd18 receptors on human macrophages. J Immunol 170: 487–494. doi: 10.4049/jimmunol.170.1.487
24. Edwards JA, Zemska O, Rappleye CA (2011) Discovery of a role for hsp82 in histoplasma virulence through a quantitative screen for macrophage lethality. Infect Immun 79: 3348–3357. doi: 10.1128/IAI.05124-11
25. Mirbod-Donovan F, Schaller R, Hung C-Y, Xue J, Reichard U, Cole GT (2006) Urease produced by Coccidioides posadasii contributes to the virulence of this respiratory pathogen. Infect Immun 74: 504–515. doi: 10.1128/IAI.74.1.504-515.2006
26. de Oliveira HC, Assato PA, Marcos CM, Scorzoni L, de Paula E Silva ACA, Da Silva JDF, Singulani J de L, Alarcon KM, Fusco-Almeida AM, Mendes-Giannini MJS (2015) Paracoccidioides-host Interaction: An Overview on Recent Advances in the Paracoccidioidomycosis. Front Microbiol 6:
27. Gauthier GM (2017) Fungal dimorphism and virulence: molecular mechanisms for temperature adaptation, immune evasion, and in vivo survival. Mediators Inflamm 2017: e8491383. doi: 10.1155/2017/8491383
28. Finkel-Jimenez B, Wüthrich M, Klein BS (2002) BAD1, an Essential virulence factor of blastomyces dermatitidis, suppresses host TNF-α production through TGF-β-dependent and -independent mechanisms. J Immunol 168: 5746–5755. doi: 10.4049/jimmunol.168.11.5746
29. Cardim-Pires TR, Sant'Anna R, Foguel D (2021) Peptides derived from gp43, the most antigenic protein from Paracoccidioides brasiliensis, form amyloid fibrils in vitro: implications for vaccine development. Sci Rep 11: 23440. doi: 10.1038/s41598-021-02898-5
30. Pemán J, Ruiz-Gaitán A, García-Vidal C, Salavert M, Ramírez P, Puchades F, García-Hita M, Alastruey-Izquierdo A, Quindós G (2020) Fungal co-infection in COVID-19 patients: Should we be concerned? Rev Iberoam Micol 37: 41–46. doi: 10.1016/j.riam.2020.07.001
31. Castillo CG, Kauffman CA, Miceli MH (2016) Blastomycosis. Infect Dis Clin North Am 30: 247–264. doi: 10.1016/j.idc.2015.10.002

32. McKinsey DS, McKinsey JP (2011) Pulmonary histoplasmosis. Semin Respir Crit Care Med 32: 735–744. doi: 10.1055/s-0031-1295721
33. Ramos-e-Silva M, Saraiva L do ES (2008) Paracoccidioidomycosis. Dermatol Clin 26: 257–269. doi: 10.1016/j.det.2007.11.005
34. Barnes PD, Marr KA (2006) Aspergillosis: spectrum of disease, diagnosis, and treatment. Infect Dis Clin North Am 20: 545–561. doi: 10.1016/j.idc.2006.06.001
35. Nucci F, Nouér S, Capone D, Anaissie E, Nucci M (2015) Fusariosis. Semin Respir Crit Care Med 36: 706–714
36. López-Martínez R, Méndéz-Tovar LJ (2012) Blastomycosis. Clin Dermatol 30: 565–572. doi: 10.1016/j.clindermatol.2012.01.002
37. Schwartz IS, Kauffman CA (2020) Blastomycosis. Semin Respir Crit Care Med 41: 031–041. doi: 10.1055/s-0039-3400281
38. Wheat LJ, Azar MM, Bahr NC, Spec A, Relich RF, Hage C (2016) Histoplasmosis. Infect Dis Clin North Am 30: 207–227. doi: 10.1016/j.idc.2015.10.009
39. Gaur S, Rajgopal A, Ashbee R (2010) A successfully treated case of peritonitis due to Fusarium dimerum. J Infect 61: 86–88. doi: 10.1016/j.jinf.2010.03.020
40. Lai C-C, Yu W-L (2021) COVID-19 associated with pulmonary aspergillosis: A literature review. J Microbiol Immunol Infect 54: 46–53. doi: 10.1016/j.jmii.2020.09.004
41. Azar MM, Loyd JL, Relich RF, Wheat LJ, Hage CA (2020) Current concepts in the epidemiology, diagnosis, and management of histoplasmosis syndromes. Semin Respir Crit Care Med 41: 013–030. doi: 10.1055/s-0039-1698429
42. Castrejón-Pérez AD, Welsh EC, Miranda I, Ocampo-Candiani J, Welsh O (2017) Cutaneous mucormycosis. An Bras Dermatol 92: 304–311. doi: 10.1590/abd1806-4841.20176614
43. Welsh O, Vera-Cabrera L, Rendon A, Gonzalez G, Bonifaz A (2012) Coccidioidomycosis. Clin Dermatol 30: 573–591. doi: 10.1016/j.clindermatol.2012.01.003
44. Lass-Flörl C (2019) How to make a fast diagnosis in invasive aspergillosis. Med Mycol 57: S155–S160. doi: 10.1093/mmy/myy103
45. McClenny N (2005) Laboratory detection and identification of Aspergillus species by microscopic observation and culture: the traditional approach. Med Mycol 43: S125–S128. doi: 10.1080/13693780500052222
46. Cighir A, Mare AD, Vultur F, Cighir T, Pop SD, Horvath K, Man A (2023) Fusarium spp. in human disease: exploring the boundaries between commensalism and pathogenesis. Life 13: 1440. doi: 10.3390/life13071440
47. Bradsher R (2008) Pulmonary blastomycosis. Semin Respir Crit Care Med 29: 174–181. doi: 10.1055/s-2008-1063856
48. Bariola J, Vyas K (2011) Pulmonary blastomycosis. Semin Respir Crit Care Med 32: 745–753. doi: 10.1055/s-0031-1295722
49. Sidamonidze K, Peck MK, Perez M, Baumgardner D, Smith G, Chaturvedi V, Chaturvedi S (2012) Real-Time PCR assay for identification of blastomyces dermatitidis in culture and in tissue. J Clin Microbiol 50: 1783–1786. doi: 10.1128/JCM.00310-12
50. Umeyama T, Sano A, Kamei K, Niimi M, Nishimura K, Uehara Y (2006) Novel approach to designing primers for identification and distinction of the human pathogenic fungi *Coccidioides immitis* and *Coccidioides posadasii* by PCR amplification. J Clin Microbiol 44: 1859–1862. doi: 10.1128/JCM.44.5.1859-1862.2006
51. Guedes HL de M, Guimarães AJ, Muniz M de M, Pizzini CV, Hamilton AJ, Peralta JM, Deepe, Jr. GS, Zancopé-Oliveira RM (2003) PCR assay for identification of histoplasma capsulatum based on the nucleotide sequence of the M antigen. J Clin Microbiol 41: 535–539. doi: 10.1128/JCM.41.2.535-539.2003
52. Bracca A, Tosello ME, Girardini JE, Amigot SL, Gomez C, Serra E (2003) Molecular detection of histoplasma capsulatum var. capsulatum in human clinical samples. J Clin Microbiol 41: 1753–1755. doi: 10.1128/JCM.41.4.1753-1755.2003
53. Bialek R, Feucht A, Aepinus C, Just-Nübling G, Robertson VJ, Knobloch J, Hohle R (2002) Evaluation of two nested pcr assays for detection of histoplasma capsulatum dna in human tissue. J Clin Microbiol 40: 1644–1647. doi: 10.1128/JCM.40.5.1644-1647.2002
54. San-Blas G, Niño-Vega G, Barreto L, Hebeler-Barbosa F, Bagagli E, Olivero de Briceño R, Mendes RP (2005) Primers for clinical detection of paracoccidioides brasiliensis. J Clin Microbiol 43: 4255–4257. doi: 10.1128/JCM.43.8.4255-4257.2005
55. Walsh T, Wissel M, Grantham K, Petraitiene R, Petraitis V, Kasai M, Francesconi A, Cotton M, Hughes J, Greene L, Bacher J, Manna P, Salomoni M, Kleiboeker S, Reddy S (2011) Molecular detection and species-specific identification of medically important aspergillus species by real-time PCR in experimental invasive pulmonary aspergillosis. J Clin Microbiol 49: 4150–7. doi: 10.1128/JCM.00570-11
56. Karlsson I, Edel-Hermann V, Gautheron N, Durling MB, Kolseth A-K, Steinberg C, Persson P, Friberg H (2016) Genus-specific primers for study of fusarium communities in field samples. Appl Environ Microbiol 82: 491–501. doi: 10.1128/AEM.02748-15
57. Bialek R, Konrad F, Kern J, Aepinus C, Cecenas L, Gonzalez GM, Just-Nübling G, Willinger B, Presterl E, Lass-Flörl C, Rickerts V (2005) PCR based identification and discrimination of agents of mucormycosis and aspergillosis in paraffin wax embedded tissue. J Clin Pathol 58: 1180–1184. doi: 10.1136/jcp.2004.024703
58. Berkow EL, Lockhart SR, Ostrosky-Zeichner L (2020) Antifungal susceptibility testing: current approaches. Clin Microbiol Rev 33: e00069-19. doi: 10.1128/CMR.00069-19
59. Dukik K, Al-Hatmi AMS, Curfs-Breuker I, Faro D, de Hoog S, Meis JF (2017) Antifungal susceptibility of emerging dimorphic pathogens in the family Ajellomycetaceae. Antimicrob Agents Chemother 62: e01886-17. doi: 10.1128/AAC.01886-17
60. Gomez-Lopez A, Aberkane A, Petrikkou E, Mellado E, Rodriguez-Tudela JL, Cuenca-Estrella M (2005) Analysis of the influence of tween concentration, inoculum size, assay medium, and reading time on susceptibility testing of *Aspergillus spp*. J Clin Microbiol 43: 1251–1255. doi: 10.1128/JCM.43.3.1251-1255.2005

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