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Review Paper

FACTORS EXACERBATING IMMUNODEFICIENCY AND ANTIFUNGAL RESISTANCE IN THE TREATMENT OF FUNGAL INFECTIONS

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ABSTRACT

Pathogenic fungi with very low inherent virulence are now increasingly causing life-threatening infections in a large number of immunocompromised individuals. Incidence of these infections has paralleled the emergence of acquired immune deficiency syndrome and other macro disruptive procedures that result in lowered resistance of the host to fungal infections. Innate resistance to antifungal agents is becoming a major problem in the management of these diseases. Majority of these fungal infections are caused by Candida species, with Candida albicans mostly implicated in bloodstream infections. Both Candida and Aspergillus infections account for about 90% of all nosocomial fungal infections while Cryptococcus neoformans has become a major opportunistic pathogen in immunocompromised individuals. Zygomycetes, Trichosporon beigelii, Blastoschizomyces capitatus, Dematiaceous fungi and species of Fusarium, Scedosporium, and Acremonium have been recognized as sources of deep fungal infections with intrinsic resistance to available antifungal drugs. Generally, primary antifungal resistance is intrinsic while secondary antifungal resistance can develop in response to drug exposure. Amphotericin B, Flucytosine, Azoles, and Echinocandins are four classes of antifungals now available for treatment of invasive mycoses. Despite marked advances in antifungal therapy, infections caused by opportunistic fungi continue to be associated with high morbidity, mortality, and poor prognosis due to combined effects of drug-resistant strains, lack of robust clinical treatment evaluations, and severe underlying diseases in patients.

Keywords: Fungal infections, immunodeficiency, antimicrobial treatment, antifungal resistance

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INTRODUCTION

Infections by fungi, also known as mycoses, are important causes of morbidity and mortality in humans. Some of these human infections are endemic, and are caused usually by fungal spores in the environment. There are also some fungal infections that are opportunistic because the causative agents cause mild or no disease in healthy individuals but may infect and cause severe disease in those that are immunodeficient (Eliopoulos et al., 2002). Fungal spores have the potential to reach lung tissue through the nostril and produce disease. In the immunocompromised host, many fungi, including those typically considered nonpathogenic, have the potential to cause serious morbidity and mortality. Over the

past decades the advent of the human immunodeficiency virus (HIV) pandemic and the increasing use of immunosuppressive drugs for serious medical conditions have dramatically increased the number of persons who are severely immunocompromised (Lortholary , 1997). In addition, the range and diversity of fungi that cause disease have broadened. Although *Candida* and *Aspergillus* species are the most frequent cause of invasive mycoses in immunocompromised persons, infections due to previously uncommon hyaline and dermatiaceous filamentous fungi are being reported with increasing frequency (Romani, 2008). This is significant because, despite marked advances in antifungal therapy, infections caused by opportunistic fungi continue to be associated with high morbidity and mortality and poor patient outcomes (Romani, 2008). This results from a combination of drug-resistant strains, lack of robust clinical studies evaluating treatments, and severe underlying diseases in the patient (Romani, 2008).

The principal mediators of innate immunity against fungi are neutrophils and macrophages, and patients with neutropenia are extremely susceptible to opportunistic fungal infections (Brown, 2011). Phagocytes and dendritic cells sense fungal organisms by TLRs and lectin-like receptors called dectins. Neutrophils liberate fungicidal substances, such as reactive oxygen species and lysosomal enzymes, and phagocytose fungi for intracellular killing (Brown, 2011). Many extracellular fungi elicit strong TH17 responses, which are driven in part by the activation of dendritic cells by fungal products binding to the dectin receptor and resulting production of TH17- inducing cytokines (IL-6, IL-23) from the dendritic cells. The TH17 cells stimulate inflammation, and the recruited neutrophils and monocytes destroy the fungi. Candida infections often start at mucosal surfaces, and cell-mediated immunity is believed to prevent spread of the fungi into tissues. The TH1 responses are protective in intracellular fungal infections, such as histoplasmosis, but these responses may elicit granulomatous inflammation, which is an important cause of host tissue injury in these infections though fungi also elicit specific antibody responses that are of protective value (Romani, 2008).

FUNGAL INFECTIONS

Fungal infections under review include candidiasis, cryptococosis, pneumocystosis, aspergillosis, and zygomycosis.

CANDIDIASIS

Candidiasis is caused by infection with species of the genus Candida, predominantly with *Candida albican*s. Candida species are ubiquitous fungi that represent the most common fungal pathogens that affect humans. The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for Candida species to invade tissues normally resistant to invasion. Candida species are true opportunistic pathogens that exploit recent technological advances to gain access to the circulation and deep tissues (Pappas et al., 2009). Increased prevalence of local and systemic disease caused by these yeasts has resulted in numerous new clinical syndromes, the expression of which depends primarily on the immune status of the host (Mayer et al., 2013). Candida species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to invasive illnesses whose clinical manifestations may be acute, subacute or chronic to episodic (Mayer et al., 2013). Manifestation of these infections may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicemia, endocarditis and meningitis (Mayer et al., 2013). In healthy individuals, Candida infections are usually due to impaired epithelial barrier functions and occur in all age groups, but are most common in the newborn and the elderly, and usually remain superficial and respond readily to treatment (Talapko et al., 2021). Systemic candidiasis is usually seen in

patients with cell-mediated immune deficiency, and those receiving aggressive cancer treatment, immunosuppression, or transplantation therapy (Talapko et al., 2021). The management of serious and life-threatening invasive candidiasis remains severely hampered by delays in diagnosis and the lack of reliable diagnostic methods that allow detection of both fungaemia and tissue invasion by Candida species (Maródi et al.,2007)

The first step in the development of a candidal infection is colonization of the mucocutaneous surfaces. All of the factors outlined here are associated with increased colonization rates, the routes of candidal invasion include (1) disruption of a colonized surface (skin or mucosa), allowing the organisms access to the bloodstream, and (2) persorption via the gastrointestinal wall, which may occur following massive colonization with large numbers of organisms that pass directly into the bloodstream (Guery et al., 2009). Candida species are the most common cause of fungal infection in immunocompromised persons. Oropharyngeal colonization is found in 30-55% of healthy young adults, and Candida species may be detected in 40-65% of normal fecal microbiota (de Repentigny et al., 2004; Alexander and Pfaller, 2006). Candida species are yeast like fungi that can form pseudohyphae and some species can develop true hyphae, as *Candida albicans* do. For the most part, Candida species are confined to human and animal reservoirs; however, they are frequently recovered from the hospital environment, including on foods, countertops, air-conditioning vents, floors, respirators, and medical personnel. They are also normal commensals of diseased skin and mucosal membranes of the gastrointestinal, genitourinary, and respiratory tracts (Pappas et al., 2009; Sobel et al., 2007; Malani et al., 2007).
Candida species also contain their own set of well-recognized but not well-characterized virulence factors that may contribute to their ability to cause infection. The main virulence factors include the following: surface molecules that permit adherence of the organism to other structures (e.g., human cells, extracellular matrix, and prosthetic devices. Acid proteases and phospholipases that involve penetration and damage of cell envelopes and ability to convert to a hyphal form (phenotypic switching) (Yang, 2003).

Over 200 species of Candida exist in nature, so far, only a few species have been associated with diseases in humans. The medically significant Candida species include the following:

i. *C albicans*, the most common species identified (50-60%)
ii. *Candida glabrata* (previously known as Torulopsis glabrata) (15-20%)
iii. *C. parapsilosis* (10-20%)
iv. *Candida tropicalis* (6-12%)
v. *Candida krusei* (1-3%)
vi. *Candida kefyr* (< 5%)
vii. *Candida guilliermondi* (< 5%)
viii. *Candida lusitaniae* (< 5%)
ix. *Candida dubliniensis*, primarily recovered from patients infected with HIV (Vazquez et al., 2003).

Candida glabrata and *C. albicans* account for approximately 70-80% of Candida species recovered from patients with candidaemia or invasive candidiasis. *Candida glabrata* has recently become very important because of its increasing incidence worldwide, its association with fluconazole resistance in up to 20% of clinical specimens, and its overall decreased susceptibility to other azoles and polyenes. *Candida krusei* is important because of its intrinsic resistance to ketoconazole and fluconazole (Diflucan); it is also less susceptible to all other antifungals, including itraconazole (Sporanox) and amphotericin B (Guinea et al., 2006; Ghannoum and Rice 1999).

Another important Candida species is *C lusitaniae.* Although not as common as other Candida species, *C lusitaniae* is of clinical significance because it may be intrinsically

resistant to amphotericin B, although it remains susceptible to azoles and echinocandins. *C. parapsilosis* is also an important species to consider in hospitalized patients. It is especially common in infections associated with vascular catheters prosthetic devices. Additionally, *in vitro* analyses have shown that echinocandins have a higher minimum inhibitory concentration (MIC) against *C parapsilosis* than other Candida species but the clinical relevance of this *in vitro* finding has yet to be determined (Eiland et al., 2008). *C tropicalis* has frequently been considered an important cause of candidemia in patients with cancer (leukemia) and in those who have undergone bone marrow transplantation.

Diagnosis of *Candida* diseases

The diagnosis of almost any form of *Candida* disease requires an integration of clinical, epidemiological, and laboratory findings. Unfortunately, results from the routine laboratory studies are often nonspecific and not very helpful. Clinicians are required to act definitively and early based on a high index of suspicion (Alexander et al., 2006). In the past, many patients with life-threatening candidiasis died without receiving antifungal therapy (Alexander et al., 2006). Systemic candidiasis should be suspected in patients with persistent leukocytosis and either persistent neutropenia or other risk factors and who remain febrile despite broad-spectrum antibiotic therapy. To be effective, antifungal therapy should be provided early and empirically in such high-risk patients. Cultures of nonsterile sites, although not useful for establishing a diagnosis may demonstrate high degrees of candidal colonization. Always consider positive culture results from sterile sites to be significant and evidence of infection (Alexander et al., 2006).

Candida albicans and *C. dubliniensis* can be identified morphologically via germ-tube formation (hyphae are produced from yeast cells after 2-3 h of incubation) or biochemical assays. CHROMagar Candida allows for the presumptive identification of several Candida species by using color reactions in specialized media that demonstrate different colony colors depending on the species of Candida. API20C and API32C are biochemical assays that allow for the identification of the different Candida species with more precision. These assays evaluate the assimilation of numerous carbon substrates and generate profiles used in the identification of different fungal species (Pincus et al., 2007).

In vitro susceptibility testing for *Candida* species is now standardized using the Clinical Laboratory Standards Institute (CLSI) microbroth dilution (CLSI M27-A2, 2002) or the disk diffusion (CLSI M44-P, 2003) methodology. This was formerly known as the National Committee for Clinical Laboratory Standards (NCCLS) microbroth dilution.

Treatment of *Candida* infections

The treatments used to manage *Candida* infections vary substantially and are based on the anatomic location of the infection, the patients' underlying disease and immune status, the patients' risk factors for infection, the specific species of *Candida* responsible for infection, and, in some cases, the susceptibility of the Candida species to specific antifungal drugs. There have been significant changes in the management of candidiasis in the last few years, particularly related to the appropriate use of echinocandins and expanded-spectrum azoles for candidemia, other forms of invasive candidiasis, and mucosal candidiasis (Kett et al., 2011). These latest recommendations include the echinocandins caspofungin, micafungin, and anidulafungin, along with voriconazole and posaconazole, as well as lipid formulations of amphotericin B in various situations. Fluconazole is still considered a first-line agent in non neutropenic patients with candidemia or

suspected invasive candidiasis (Pappas et al., 2004).

CRYPTOCOCOSIS

Cryptococcus neoformans is encapsulated yeast, and since its discovery, researchers (Srikanta et al., 2014), have identified the diverse spectrum of host responses to cryptococcal infection. The responses range from a harmless colonization of the airways and asymptomatic infection in laboratory workers to meningitis or disseminated disease. Although virulence in animals and, possibly, humans varies among strains of cryptococci, virulence probably plays a relatively small role in the outcome of an infection. The crucial factor is the immune status of the host; the importance of host immunity to the development of cryptococcosis is the single most critical feature of this infection from diagnosis to prognosis (Chayakulkeeree et al., 2006; Heitman et al., 2011)

A major pathological principle in understanding of cryptococcosis is that many individuals are infected with this yeast but their immune system controls the infection with minor and insignificant symptoms. However, like tuberculosis, the yeast can persist in tissue in a dormant state for long periods of time to then reactivate and produce disease during an immunosuppressive state. Furthermore, this reactivation scenario has been supported by recent observations with HIV infection progression to low CD4 counts (50-100 CD4 cells/µl) and this immunosuppressive lymphopenia directly linked with higher risk of cryptococcosis as the reduction of cell-mediated immune cells occurs (Crowe et al., 1991). There are a series of well-known risk factors associated with cryptococcal disease. The two highest risk factors are HIV infection and corticosteroid use. The corticosteroid use as a risk factor incorporates most of the transplant recipients and particularly, the solid organ transplants with their long term corticosteroid exposure and relatively high daily doses (>20mg/day of prednisone) (Husain, 2001) Among the other risk factors that require some discussion are the lymphomas/chronic leukemias and the connective tissue diseases in which most of these cases are aided by corticosteroid usage. Diabetes continues to be present in a large number of patients with cryptococcosis (Pappas et al., 2001). It should be noted, however, that not all patients with cryptococcosis have an underlying disease. In fact, if HIV- infected patients are excluded, approximately 20-30% of patients with disseminated cryptococcosis will present with no apparent underlying diseases or known risk factors (Pappas et al., 2001).

Cryptococcus neoformans has become a major human pathogen and a common infection in certain immunocompromised hosts (Sungkanuparph et al., 2009). Cryptococcosis, the disease resulting from infection with *C. neoformans*, varies from a localized skin lesion or asymptomatic colonization of the respiratory tree to a widely disseminated life-threatening infection, which may infect all organs of the body. However, *C. neoformans* has a special propensity for invading the central nervous system, and cryptococcal meningoencephalitis is the primary clinical presentation for the life-threatening stage of this infection (Sungkanuparph et al., 2009). Although the genus Cryptococcus contains more than 50 species, only *C neoformans* and *Cryptococcus gattii* are considered principal pathogens in humans. Previously, *C neoformans* was defined as having two varieties—var neoformans and var gattii. However, based on the elucidation of the genomic sequences, *C neoformans* and *C gattii* are now considered two distinct species. These two species have 5 serotypes based on antigenic specificity of the capsular polysaccharide; these include serotypes A, D, and AD (*C. neoformans*) and serotypes B and C (*C. gattii*). *C neoformans* is the most common species in temperate climates throughout the world and is found in aged pigeon droppings. Until recently, *C gattii*

was found principally in tropical and subtropical climates (D'Souza et al., 2011).

Worldwide, *C. neoformans* serotype A causes most cryptococcal infections in immunocompromised patients, including patients infected with HIV. For unknown reasons, *C. gattii* rarely infects persons with HIV infection and other immunosuppressed patients. Patients infected with *C. gattii* are usually immunocompetent, respond slowly to treatment, and are at risk for developing intracerebral mass lesions (e.g., cryptococcomas). Naturally occurring cryptococcosis occurs in both animals and humans, but neither animal-to-human transmission nor person-to-person respiratory transmission via the respiratory route has been documented. Transmission via organ transplantation has been reported when infected donor organs were used. *Cryptococcus neoformans* causes the vast majority of cryptococcal infections in immunosuppressed hosts, including patients with AIDS, whereas *C. gattii* causes 70%-80% of cryptococcal infections among immunocompetent hosts (Mirza et al., 2003).

PNEUMOCYSTOSIS

Pneumocystis organisms are commonly found in the lungs of healthy individuals. Most children are believed to have been exposed to the organism by age 3 or 4 years, and its occurrence is worldwide (Slogrove et al., 2010). *Pneumocystis carinii* pneumonia (PCP), the causative organism which has been renamed *Pneumocystis jiroveci*, is the most common opportunistic infection in persons with HIV infection. Pneumocystis first came to attention as a cause of interstitial pneumonia in severely malnourished and premature infants during World War II in Central and Eastern Europe. *P. jiroveci* is now one of many organisms known to cause life-threatening opportunistic infections in patients with advanced HIV infection worldwide. While officially classified as a fungal pneumonia, PCP does not respond to antifungal treatment. Although a histopathologic demonstration of the organism is required for a definitive diagnosis, treatment should not be delayed (Hui et al., 2006).

Disease occurs when both cellular immunity and humoral immunity are defective. Once inhaled, the trophic form of Pneumocystis organisms attach to the alveoli. Multiple host-immune defects allow for uncontrolled replication of Pneumocystis organisms and development of illness. Activated alveolar macrophages without CD4+ cells are unable to eradicate Pneumocystis organisms. Increased alveolar-capillary permeability is visible on electron microscopy. Infection with *P jiroveci* causes PCP. Persons with HIV infection whose CD4+ cells fall below 200/µL and who are not receiving PCP prophylaxis, persons with primary immune deficiencies including hypo-gammaglobulinemia and severe combined immunodeficiency (SCID), persons receiving long-term immunosuppressive regimens for connective-tissue disorders, vasculitides, or solid-organ transplantation (e.g., heart, lung, liver, kidney), persons with hematologic and nonhematologic malignancies, including solid tumors and lymphomas, and persons with severe malnutrition are at risk for PCP (Otieno-Odhiambo et al., 2019)

Before the widespread use of prophylaxis for PCP, the frequency of Pneumocystis infection in lung transplant patients alone was as high as 88% but now, with the routine use of prophylaxis, PCP is very rare in solid-organ transplant patients and has significantly decreased in patients infected with HIV (Wang et al., 2012). Prior to the widespread use of highly active antiretroviral therapy (HAART), PCP occurred in 70-80% of patients with HIV infection but the frequency of PCP is decreasing with the use of PCP prophylaxis and HAART. However, PCP is still the most common opportunistic infection in patients with HIV infection who are more prone to PCP recurrence than patients not infected with HIV (Abouya et

al., 1992). In developing regions of the world, the prevalence of PCP was once thought to be much lower, but studies (Abouya et al., 1992) have shown that the lower reported incidence is likely a failure to accurately diagnose PCP. An accurate diagnosis requires access to modern medical care, which is not available worldwide (Abouya et al., 1992). The frequency of documented Pneumocystis infection (Murray et al., 2005) is increasing in Africa, with Pneumocystis organisms found in up to 80% of infants with pneumonia who have HIV infection. In sub-Saharan Africa, tuberculosis is a common co-infection in persons with PCP (Murray et al., 2005).

ASPERGILOSIS

Aspergilosis is disease condition caused by Aspergillus species which are ubiquitous molds that are found in organic matter. Although more than 100 species have been identified, the majority of human illness is caused by *Aspergillus fumigatus and Aspergillus niger* and, less frequently, by *Aspergillus flavus* and *Aspergillus clavatus* (Latgé, 1999). The transmission of fungal spores to the human host is via inhalation (Bennett, 1995). Aspergillus causes a spectrum of disease, from colonization to hypersensitivity reactions to chronic necrotizing infections to rapidly progressive angioinvasion, often resulting in death. Rarely found in individuals who are immunocompetent, invasive Aspergillus infection almost always occurs in patients who are immunosuppressed by virtue of underlying lung disease, immunosuppressive drug therapy, or immunodeficiency (Virnig et al., 2007). Aspergillus primarily affects the lungs, causing four main syndromes, including allergic bronchopulmonary aspergillosis (ABPA), chronic necrotizing Aspergillus pneumonia (or chronic necrotizing pulmonary aspergillosis (CNPA), aspergilloma, and invasive aspergillosis (Jack and Bajaj 2022). However, in patients who are severely immunocompromised, Aspergillus may hematogenously disseminate beyond the lung, potentially causing endophthalmitis, endocarditis, and abscesses in the myocardium, kidney, liver, spleen, soft tissue, and bone (Virnig et al., 2007). Aspergillus is second to Candida species as a cause of fungal endocarditis, and Aspergillus-related endocarditis and wound infections occur in the context of cardiac surgery (Virnig et al., 2007). Allergic bronchopulmonary aspergillosis is a hypersensitivity reaction to *A. fumigatus* colonization of the tracheobronchial tree and occurs in conjunction with asthma and cystic fibrosis (CF). Also, allergic fungal sinusitis may occur alone or with ABPA (Virnig et al., 2007). Bronchocentric granulomatosis and malt worker's lung are 2 hypersensitivity lung diseases that are caused by Aspergillus species, but they are rare (Ader et al., 2009).

Invasive aspergillosis is a rapidly progressive, often fatal infection, associated with significant mortality, with a rate of 30-95% that occurs in patients who are severely immunosuppressed, including those who are profoundly neutropenic; have received bone marrow or solid organ transplants; and patients with advanced AIDS or chronic granulomatous disease. This infectious process is characterized by invasion of blood vessels, resulting in multifocal infiltrates, which are often wedge-shaped, pleural-based, and cavitary; and dissemination to other organs, particularly the central nervous system, may occur (Naidich et al., 1997). Although many species of Aspergillus have been isolated in nature, .*A. fumigatus* is the most common cause of infection in humans. *A. flavus* and *A. niger* are less common; this likely relates to the ability of A. fumigatus, but not most other Aspergillus species, to grow at normal human body temperature (Latgé, 1999).

Human host defense against the inhaled spores begins with the mucous layer and the ciliary action in the respiratory tract. Macrophages and neutrophils encompass, engulf, and eradicate the fungus. However, many species of Aspergillus produce toxic metabolites that inhibit macrophage and

neutrophil phagocytosis (Dutta et al., 2017) though corticosteroids also impair macrophage and neutrophil function. Underlying immunosuppression (e.g., HIV disease, chronic granulomatous disease, pharmacologic immunosuppression) also contributes directly to neutrophil dysfunction or decreased numbers of neutrophils (Dutta et al., 2017). In individuals who are immunosuppressed, vascular invasion is much more common and may lead to infarction, hemorrhage, and necrosis of lung tissue. Persons with CNPA typically have granuloma formation and alveolar consolidation. Hyphae may be observed within the granulomata (Magill et al., 2008)

Selection of therapy also needs to consider the certainty of the diagnosis. Voriconazole, itraconazole, the investigational azoles with anti-mould activity, and amphotericin B all possess a reasonably broad-spectrum of activity against Aspergillus and the related hyaline moulds spp (Herbrecht et al., 2002; Marr et al., 2004). Their activity does, however, vary for the agents of zygomycosis, with posaconazole being the azole with the most reliable activity against this class of fungi while the echinocandin glucan synthesis inhibitors (caspofungin, Micafungin (FK463) and anidulafungin) possess a narrower spectrum of activity and should only be used if the infection is known to be due to Aspergillus spp (Herbrecht et al., 2002; Marr et al., 2004)

ZYGOMYCOSIS

Zygomycosis is an infection caused by fungi in the orders Mucorales and Entomophthorales. The Mucorales order contains two families: Mucoraceae and Cunninghamellaceae. The Zygomycetes have emerged as common causes of invasive fungal infections (Kontoyiannis et al., 2000).The pathogens that cause zygomycosis are commonly found in the environment, on fruit, on bread, and in soil, and are common components of decaying organic debris. These organisms are ubiquitous and generally saprophytic, rarely causing disease in immunocompetent hosts, but they are the third-most-common cause of invasive fungal infection in immunocompromised patients, especially stem cell transplant recipients and patients with underlying hematologic malignancies (Rippon et al 1998). Fungi are ubiquitous in the natural world, often found in association with plants, mammals, and insects. Accordingly, humans are continually exposed to multiple genera of fungi via various routes, including the respiratory and gastrointestinal routes, which allow the possibility of colonization. Depending on the interaction between host mucosal defense mechanisms and fungal virulence factors, colonization may be transient or persistent, or local disease may ensue (Greenberg et al. 2004)

Zygomycosis caused by *R. arrhizus* is acute and rapidly fatal despite early diagnosis and treatment. These organisms have a particular predilection for invading major blood vessels, with ensuing ischemia, necrosis, and infarction of adjacent tissues, resulting in the production of black pus (Petrikkos et al., 2003). Persons at particular risk include those with granulocytopenia and acidosis; and for unknown reasons, the Zygomycetes have a propensity for patients with acidosis, particularly those with diabetes. They also infect patients with acidosis secondary to renal insufficiency, diarrhea, and aspirin intake (Petrikkos et al., 2003). Patients who are receiving glucocorticoids or deferoxamine and those who have undergone splenectomy also are at risk (Petrikkos et al., 2003). The overall mortality rate associated with zygomycosis is approximately 50% and has remained at this level for the past 50 years (Roden et al., 2005). *Rhinocerebral zygomycosis* carries a high mortality rate of approximately 85% because by the time zygomycosis is suspected and diagnosed, it has frequently spread diffusely and caused extensive tissue destruction. However, the risk of mortality varies depending on the characteristics of the host, the type of infection, the site of infection, and the use of surgical intervention (Petrikkos et al., 2003).

In general, antifungal therapy and surgical management independently decrease the likelihood of death (Ibrahim et al., 2003). Zygomycosis manifests as a spectrum of diseases, depending on the portal of entry and the predisposing risk factors of the patient. The 5 major clinical forms include rhinocerebral zygomycosis, pulmonary zygomycosis, abdominopelvic and gastric (gastrointestinal) zygomycosis, primary cutaneous zygomycosis, and disseminated zygomycosis (Ibrahim et al., 2003).

Most persons who develop zygomycosis are immunocompromised, although 15-20% of patients have no evidence of any underlying condition at the time of the diagnosis. Thus, sporadic cases in immunocompetent hosts are not uncommon (Roden et al., 2005). The most common risk factors include the following: Stem cell transplantation, Poorly controlled diabetes mellitus, either type 1 or type 2, Hematologic malignancy (e.g., leukemias, lymphomas), Solid organ transplants, Steroid use, Metabolic acidosis, Deferoxamine therapy, Severe and prolonged neutropenia, Intravenous drug use, Renal failure, Peritoneal dialysis, Burns and rarely Penetrating trauma (Ibrahim et al., 2003; Kauffman, 2004).

ANTIFUNGAL RESISTANCE

Historically, treatment of fungal infections has relied heavily on just four classes of antifungal drugs: the polyenes, azoles, echinocandins and the pyrimidine analogue 5-flucytosine (Robbins et al., 2017). However, fungi respond evasively to chemical attack, and treatment failure which is a common occurrence can be attributable to factors such as; underlying host immune defects, antifungal and fungal characteristics such as diverse cell morphologies, antifungal tolerance and antifungal resistance. Resistance to antifungal drugs is emerging concern worldwide (Fisher et al., 2018). Also noticed, include novel resistant variants of previously susceptible pathogens, for example, the ubiquitous mould Aspergillus fumigatus as well as entirely new emerging species that are resistant to multiple antifungal drugs as observed in the yeast Candida auris (Verweij et al., 2020; Rhodes et al., 2019). The increasing public health burden of both of these pathogens is now officially recognized with the listing on the urgent antimicrobial resistance (AMR) threat list of the US CDC (CDC, 2019).

Formally, AMR programmes excluded antifungals because fungi have been widely neglected as a threat to public health (Fisher et al., 2020; Rodrigues et al., 2020). The widespread use of broad-spectrum antibacterial antibiotics like β-lactams, cephalosporins, carbapenems, quinolones and macrolides have profoundly impacted bacterial communities by purging susceptible genotypes in favour of those harbouring polymorphisms and genes conferring resistance, the fittest examples of which can go on to become globally widespread (Baker et al.,2018) . It is known that all pathogenic fungi can acquire resistance through adaptation to drug selection pressure (Fisher et al., 2018); and antifungal resistance is usually acquired due to changes that directly or indirectly affect the drug–target interaction. Drug resistance may arise via genetic changes to the target binding site as in the case of mutation of the genes encoding lanosterol demethylase for azoles or β-glucan synthase for echinocandins, via overexpression of amount of target available and/or by altering the effective drug concentration (via elevated drug efflux activity for intracellular drugs such as azoles, or inhibition of prodrug activation for flucytosine (Fisher et al., 2018; Edlind et al., 2010).

In contrast to antifungal resistance, antifungal tolerance refers to the ability of drug-susceptible cells to grow at drug concentrations above the minimum inhibitory concentration (MIC) and involves a wide range of general stress response and/or epigenetic pathways (Berman and Krysan, 2020). Tolerance is most evident with fungi static drugs, and has been

measured and characterized most extensively in isolates treated with fluconazole. Acquisition and emergence of antifungal drug resistance is fundamentally an evolutionary response to the selective pressure exerted by the drug. The likelihood of resistance emerging due to genetic changes is governed by the size of the population exposed to the selective pressure, the rate of cell doubling, the number of different pathways (physiological mechanisms and genetic changes) that confer resistance, and the fitness costs associated with each of them (Berman and Krysan, 2020).

Importantly, antifungal drug resistance may originate in the host or in the environment. On one hand, in vivo resistance evolves de novo in individuals during antifungal therapy and causes treatment failure for a spectrum of pathogenic fungi spanning moulds and yeasts (Ballard et al., 2018; Shields, et al., 2012). This is highly relevant for diverse Candida yeasts that are leading cause of nosocomial bloodstream infections and show widespread emergence of resistance to antifungals (Steinmann et al., 2015; Pristov et al., 2019). For instance, emergence of azole resistance in C. albicans during prolonged fluconazole therapy for oral candidiasis in individuals infected with HIV was well documented (Johnson et al., 1995). This phenomenon is not restricted to azole antifungals as progressive loss of echinocandin activity has also been reported during prolonged caspofungin therapy for C. albicans esophagitis (Laverdiere et al., 2006). Environmental resistance on the other hand can emerge due to prior exposure of human pathogenic fungi to fungicides in nature (Verweij et al., 2020). The environmental pressure of fungicides drives the evolution of resistance against all major classes of fungicides, including benzimidazoles, anilinopyrimidines, strobilurins, succinate dehydrogenase inhibitors and the sterol demethylation inhibitors (DMIs) including azoles (Fisher et al., 2018).

Prevalence of Azole resistance

A 3-year longitudinal study of bloodstream Candida infections in North America and Latin America that included episodes caused by C. albicans (54%), C. glabrata (16%), C. parapsilosis (15%), C. tropicalis (8%), C. krusei (1.6%), and other Candida species (4.6%) showed that resistance to triazoles is still not usually a frequent event. Globally, <2.5% and <9% of the Candida species isolates analyzed were resistant to fluconazole and itraconazole, respectively but C. albicans and C. dubliniensis are the 2 species most susceptible to the currently available antifungal azoles in-vitro (Pfaller et al., 2000; Pfaller et al., 1999). Candida lusitaniae, C. parapsilosis, and C. guilliermondii are generally susceptible to azole agents in-vitro but C. glabrata is frequently less susceptible, as is C. krusei, which is intrinsically resistant to fluconazole in-vitro (Lyon et al., 2010). Candida glabrata isolates generally exhibit bimodal susceptibility to azoles, with some isolates demonstrating azole resistance (MIC, >64 μg/mL), whereas others are significantly more susceptible. Similarly, some strains of C. tropicalis exhibit azole resistance but MIC90 for this species indicates general susceptibility to azoles, and in-vitro resistance appears often as a result of its strong tendency to produce trailing growth (Lyon et al., 2010). Other studies (Pfaller et al., 2000) have highlighted important geographic variations in the distribution of Candida species and differences in the prevalence of resistance.

Patterns of antifungal susceptibility of Candida species that cause bloodstream infection

Evidence links empirical or prophylactic use of antifungal agents and selection for yeasts other than C. albicans that exhibit decreased susceptibility to these agents. Examples include the emergence of C. krusei and C. glabrata infections in patients receiving fluconazole prophylaxis (Wingard, 1995; Wingard et al., 1993; Wingard et al., 1991). For C. albicans, the development of

secondary resistance to fluconazole treatment has been most commonly encountered in HIV-infected patients with oropharyngeal candidiasis (OPC) who are receiving prolonged treatment with fluconazole, affecting up to 21% of these patients (Sangeorzan et al., 1994; Kirkpatrick et al., 1999). Resistance to itraconazole is also increasingly reported among these patients, mainly in Candida isolates that also exhibit decreased susceptibility to fluconazole in vitro (Kirkpatrick et al., 1999). The emergence of antifungal-resistant C. albicans fungemia has been reported in bone marrow transplant recipients being administered long-term fluconazole prophylaxis (Marr et al., 1997). Although the benefit of fluconazole use in decreasing the incidence of fungemia has been demonstrated for these patients, it is important to consider that, if breakthrough yeast infections do occur, they may be resistant to azole therapy. However, the number of cases of fluconazole-resistant candidemia caused by C. albicans in patients with cancer who are receiving azole prophylaxis still remains small (Marr et al., 1997; Slavin et al., 1995; Nolte et al., 1997; Mori et al., 1997).

MOLECULAR MECHANISMS OF AZOLE RESISTANCE

Secondary resistance to fluconazole in C. albicans isolates recovered from HIV-infected patients with OPC has been shown to be a multifactorial process. Resistance can be the result of an alteration of the target enzyme, the cytochrome P-450 lanosterol 14α-demethylase, either by overexpression or by point mutations in its encoding gene (ERG11). The former creates the need of a higher intracellular azole concentration to complex all of the enzyme molecules present in the cells, and the latter leads to amino acid substitutions that result in a decreased affinity for azole derivatives (Perea et al., 2001). A second major mechanism is the failure of azole antifungal agents to accumulate inside the yeast cell as a consequence of enhanced drug efflux. This mechanism is mediated by 2 types of multidrug efflux transporters, the major facilitators (encoded by MDR genes) and those belonging to the ATP-binding cassette superfamily (ABC transporters, encoded by CDR genes). Up regulation of the CDR genes appears to confer resistance to multiple azoles, whereas up regulation of the MDR1 gene alone leads to fluconazole resistance exclusively (Prasad et al., 1995; Sanglard et al., 1995; Sanglard et al., 1997; Sanglard et al., 1998; White, 1997; Lopez-Ribot et al., 1998; Lopez-Ribot et al., 1999; Perea et al., 2001). Up regulation of CDR1 has been also associated with the development of fluconazole resistance in C. albicans that caused disseminated infection in a patient who underwent marrow transplantation (Marr et al., 1998). The mechanism of fluconazole resistance in C. glabrata and C. dubliniensis isolates recovered from HIV-infected patients with OPC who receive fluconazole treatment involves up regulation of multidrug efflux transporter genes CgCDR1, CdCDR1, and CdMDR1 (Sanglard et al., 1999; Moran et al., 1998; Perea et al., 2002). The primary resistance of C. krusei to azole antifungals (fluconazole and itraconazole) appears to be mediated through reduced susceptibility of the target enzyme, lanosterol 14α-demethylase, to inhibition by this drug (Orozco et al.,1998) although markedly improved susceptibility to the newer generation of triazole antifungals, such as voriconazole, has been demonstrated (Orozco et al.,1998).

AMPHOTERICIN B RESISTANCE AND DEVELOPMENT OF RESISTANCE DURING TREATMENT

During the past several years, invasive infections due to amphotericin B-resistant Candida isolates have been increasingly reported in association with the use of this antifungal agent (Nguyen, et al., 1996; Dick et al., 1985; Fan-Havard et al., 1991; Powderly et al ., 1988; Dick et al.,1980; Kovacicova et al., 2001). Many, but not all C. lusitaniae and some C. guilliermondii isolates demonstrate primary resistance to amphotericin B, which is in contrast to other Candida species (C. albicans, C. tropicalis, C. parapsilosis, and C. dubliniensis) that are

usually quite susceptible to amphotericin B (Eliopoulos et al., 2002). Secondary resistance to amphotericin B also appears to be an uncommon development. There have been reports of some cases of disseminated infections due to C. glabrata, C. krusei, and C. albicans isolates that developed amphotericin B resistance during treatment. The mechanism of amphotericin B resistance appears to be an alteration or a decrease in the amount of ergosterol in the cell membrane (Safe et al., 1997). An in-vitro reversible switching of C. lusitaniae was demonstrated (Yoon et al., 1999), which may be responsible for frequent in vitro resistance of that species to amphotericin B and could have an impact on the selection of antifungal therapies that could result in antifungal resistance.

FLUCYTOSINE RESISTANCE

Flucytosine has in-vitro activity against many isolates of Candida species, but it is not commonly used because of drug toxicities as well as the frequent development of resistance when used as a single agent (Pfaller et al., 2002). Primary resistance to flucytosine is a common phenomenon in Candida species; almost 10% of clinical isolates are intrinsically resistant (MIC for resistance, ⩾32 μg/mL; MIC for intermediate resistance, 8–16 μg/mL; MIC for susceptibility, ⩽4 μg/mL). In addition, the infections in up to 30% of the patients who receive monotherapy with flucytosine develop secondary resistance (Pfaller et al., 2002). The highest rates of primary resistance are found in C. albicans serotype B, C. glabrata, C. krusei, and C. guilliermondii and is usually the result of a defect in cytosine deaminase while secondary resistance in C. albicans is primarily due to a decrease in the activity of the uracil phosphoribosyl transferase (Barchiesi et al., 2000; Whelan, 1987).

NECESSITY FOR AUGMENTATION AND STANDARDIZATION OF ANTIFUNGAL SUSCEPTIBILITY TESTING

Contrary to the situation for antimicrobial agents, antifungal susceptibility testing has not been used as a guide for antifungal therapy in the past because of the lack of standardization of testing procedure but in 1997, a reproducible standardized antifungal susceptibility testing method for yeasts (M27-A) was developed (Pfaller et al., 2002). Since that development, efforts have been focused on the determination of interpretative breakpoints — MICs that predict clinical response to antifungal treatment and the establishment of in-vitro – in-vivo correlation. Breakpoints for MICs of fluconazole and itraconazole determined by the National Committee for Clinical and Laboratory Standards (NCCLS) methodology has been established (Rex et al., 1997; Pfaller et al., 2006). Yeast isolates are classified as susceptible to fluconazole and itraconazole if the MICs are ⩽8μg/mL and ⩽0.125μg/mL, respectively. Similarly, the resistance breakpoints for fluconazole and itraconazole were defined as ⩾64 μg/mL and ⩾1μg/mL, respectively. This new breakpoint emphasizes the importance of attaining a significant level of drug in the blood and tissue for isolates with higher MICs, and if the MIC of the isolate falls in this category, dosages of 400 mg per day or more of fluconazole and itraconazole concentrations in plasma of ⩾0.5μg/mL are needed for optimal response (Rex et al., 1997).

ANTIFUNGAL RESISTANCE AND ITS IMPLICATION IN THERAPY

It is important to remember during the selection of antifungal drugs for the treatment of serious yeast infections that in general, the susceptibility of Candida species can be predicted on the basis of the specific yeast species. For this reason, careful mycological identification at the species level for all Candida isolates recovered is imperative for the empirical selection of antifungal agents. Because of limitations of in-vitro susceptibility testing, routine antifungal susceptibility testing of all clinical specimens may not be possible but due to geographical and institutional variation in antifungal susceptibility patterns, it is important to periodically determine the

distribution of Candida species and susceptibility patterns in every institution, especially when antifungal prophylaxis is a common practice (Lyon et al., 2010; Eliopoulos et al., 2002). Antifungal susceptibility testing is also recommended for patients with life-threatening Candida infections and HIV-infected patients with OPC who do not respond to treatment (Rex et al., 1997; Rex et al., 2001).

There was high likelihood of resistance to the early triazole antifungals, so infection due to C. krusei should not be treated with fluconazole or itraconazole (Ghannoum and Rice 1999). Excellent in-vitro susceptibility to the newer azoles, particularly voriconazole, has been demonstrated, suggesting that the newer azoles may become preferred therapy for this often multidrug-resistant pathogen (Pfaller et al ., 2002). In case of infections due to C. glabrata, dose-dependent responses may be observed. For HIV-infected patients with OPC caused by C. albicans isolates that have developed resistance to fluconazole, itraconazole retains activity, and the infection may still respond to itraconazole therapy, although higher doses might be required to achieve cure (Eliopoulos et al.,2002). Therapeutic options are more limited if there is cross-resistance to other antifungal azoles, for example, in azole-resistant C. albicans or C. glabrata, higher MICs of other azole antifungals are likely, although the better susceptibility of the newer azoles (i.e., voriconazole, posaconazole, and ravuconazole) may be clinically preferred choice (Pfaller et al., 2002). Intravenously administered amphotericin B has been one of the few effective alternative agents for treatment of infection with antifungal-resistant yeasts but even in these infections, higher MICs are likely with C. glabrata, which may require a higher dose of amphotericin B (0.7–1.0 mg/kg q.d., compared with 0.5 mg/kg q.d. for antifungal-susceptible C. albicans (Eliopoulos et al., 2002). The lipid-associated amphotericin B agents offer less toxicity but no clear advantages for the treatment of yeast infections (Eliopoulos et al., 2002). New antifungal compounds, such as broad-spectrum triazoles and echinocandins, are being developed and may be used against fluconazole-resistant OPC (Keating et al., 2001; Sheehan et al., 1999) and to treat serious Candida infections. Patients with C. lusitaniae, C. guilliermondii, or C. glabrata infections should be carefully monitored during therapy because of the risk of development secondary resistance. These patients will likely require higher dosages of antifungals, including high dosages of amphotericin B (approaching or exceeding 1 mg/kg q.d.), especially in profoundly immunosuppressed patients, and Flucytosine should never be used as a single agent for treatment of serious Candida infection because of the frequent development of resistance (Coleman et al,. 1998; Rex et al., 2000).

CLINICAL IMPLICATIONS OF ANTIFUNGAL RESISTANCE

Antifungal therapy for invasive aspergillosis is not guided by in-vitro susceptibility test results because of the lack of studies correlating the MICs of amphotericin and azoles with the clinical outcome of the patients. As previously mentioned (Lamoth et al., 2020; Mosquera et al., 2001), the high mortality rate among patients with invasive aspergillosis probably has more relation to other factors, such as immunosuppression and delay in the diagnosis, than to the development of resistance to antifungal treatment, which, until now, has appeared to occur infrequently. As in case of Candida species, the most useful information for the selection of the antifungal treatment comes from the complete identification of the isolate to the species level. The development of azole resistance observed in some A. fumigatus strains should urge a thorough study of the molecular mechanisms of resistance, because most of the newer drugs being developed for the treatment of aspergillosis, such as voriconazole, posaconazole, and ravuconazole, are azole-

based drugs (Sheehan et al., 1999). However, the clinical impact of resistance in Aspergillus species, to date, has been confined to A. terreus (Beardsley et al., 2018; Shishodia et al., 2019) which often demonstrates higher MICs of not only amphotericin B but also the azole antifungals, including the newer azole agents, agents that have been successfully used to treat some patients with infections due to that organism.

ASPERGILLOSIS

The frequency of invasive aspergillosis has increased during recent years, surpassing candidiasis as the fungal infection most frequently detected after death, and invasive aspergillosis is associated with a high mortality rate >85% (Alexander and Pfaller 2006). *Aspergillus fumigatus* is the most common species to cause Aspergillus infection worldwide, accounting for ∼90% of the cases, followed by *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus niger*. Aspergillosis is frequently seen in patients with leukemia, bone marrow transplant recipients, solid-organ transplant recipients, and, to a lesser extent, patients with late-stage or end-stage AIDS (Patterson et al., 2000; Denning, 1998).

Although treatment failure is common in patients with invasive aspergillosis, the correlation with resistance to antifungal treatment is difficult to establish but factors such as the immune status of the patient and delay in diagnosis may contribute to the poor response to treatment (Patterson et al., 2016). The lack of reliable methods for in vitro testing has also hindered the detection of drug-resistant strains of *Aspergillus* species, therefore, the true rate of antifungal resistance is unknown. A proposed method (M38-P) to standardize the in-vitro antifungal susceptibility testing for molds was established, and improvements to these guidelines are being developed (NCCLS, 1998; Espinel-Ingroff et al., 2001). Large-scale prospective studies to compare antifungal susceptibility and clinical outcomes are very difficult to conduct because the incidence of infections with most opportunistic mold pathogens is low; for this reason, minimal clinical data ((Rex et al., 2001; Rex et al., 2002) exist to support the relevance of susceptibility testing in-vitro of filamentous fungi. Experience with diverse antifungal susceptibility testing methodology indicates that the amphotericin B susceptibility of *Aspergillus* species varies according to species, although such variation has thus far been limited. Most isolates of *A. fumigatus* have a low MIC of amphotericin B (⩽1μg/mL) (Verweij et al., 1998; Chryssanthou, 1997). However, A. terreus has high MIC of amphotericin B (>2 μg/mL), and infections due to this organism seem to be refractory to amphotericin B therapy (Dannaoui et al.,1999; Dannaoui et al., 2000).

Like many other molds, *Aspergillus* species are intrinsically resistant to fluconazole. Most isolates of *A. fumigatus* appear to be susceptible to itraconazole, low MICs (⩽1 μg/mL) (Verweij et al., 1998; Chryssanthou, 1997). Though, a limited number of *A. fumigatus* isolates that demonstrated in vitro resistance to itraconazole were reported (Chryssanthou, 1997; Dannaoui et al., 2001), in some of these strains, the resistance detected in-vitro has been confirmed in-vivo in animal models, which suggests that this resistance may have clinical significance (Denning et al., 1997; Dannaoui et al., 1999); particularly because susceptibility to newer azoles with Aspergillus activity, such as voriconazole and posaconazole, may be observed. In some cases (Eliopoulos et al., 2002), cross-resistance between itraconazole and posaconazole was demonstrated while susceptibility to voriconazole was maintained, although the significance or likelihood of that association has not been established in a large study of isolates. In addition, these newer azoles may also demonstrate fungicidal activity against *Aspergillus* species, although the clinical relevance of that finding has not been demonstrated. There have been no reported

isolates with clinical resistance caused by decreased susceptibility to caspofungin, a glucan-synthesis inhibitor, which has shown clinical efficacy for the treatment of aspergillosis (Keating et al., 2001).

CRYPTOCOCCAL DISEASE

The overall incidence of cryptococcal, caused by *Cryptococcus neoformans*, has increased as a result of the AIDS pandemic, cancer chemotherapy, and immunosuppression for organ transplant recipients. Before the advent of HAART, among patients with AIDS in the United States, cryptococcosis was the defining illness in 5% of the patients, with an overall prevalence of 5%–10% (Pappas, 2013). Infections are rarely completely cured in patients with AIDS without return of immune function, and certain immunosuppressed patients with neoplastic disease are subject to even more rapid mortality or treatment failure. The mortality rate for treated cryptococcal meningitis may be as high as 25%–30% in high-risk patients, although improved outcomes associated with aggressive antifungal management have been reported (Warkentien et al., 2010) . Resistance to therapy can be caused by a variety of factors, including the following: underlying disease of the patient; secondary complications, such as hydrocephalus, drug intolerance, poor compliance with therapy, and pharmacokinetic factors; and the development of either primary or secondary drug resistance (Warkentien et al., 2010).

Initially, when flucytosine was used as single agent for the treatment of cryptococcal meningitis, primary resistance to the treatment was uncommon. However, the frequent development of secondary resistance during treatment (up to 57% of cases) precluded its use as a single agent (Whelan, 1987). Studies (Brandt et al., 2001) have shown that primary flucytosine resistance is uncommon, and was reported in 14 of 732 isolates tested in the United States since the 1990s. With regard to azole drugs, there has been an increasing number of relapses of cryptococcal meningitis in patients with AIDS associated with the development of secondary resistance to fluconazole caused by *C. neoformans* isolates that showed decreased susceptibility in vitro (Casadevall et al., 1993; Paugam et al., 1994) although the use of HAART will substantially reduce the likelihood of relapse, which would obviously reduce the likelihood of developing resistance. No case of primary resistance to amphotericin B has been clearly documented, although sporadic reports have reported the development of secondary resistance (Witt et al., 1996; Aller et al., 2000). However, resistance may be important in determining the outcome in selected patients, such as those who received amphotericin B monotherapy and those with uncontrolled underlying immunosuppression (Witt et al., 1996). The echinocandins appear to offer little, if any, activity against *Cryptococcus* species, possibly because of the composition of its fungal cell wall (Whelan, 1987).

The molecular mechanism of secondary flucytosine resistance is primarily due to a single mutation event in uridine-5-monophosphate pyrophosphorylase, uracil phosphoribosyl transferase, or cytosine permease–desaminase (Whelan, 1987). Studies of azole-resistant *C. neoformans* isolates have shown that the secondary resistance is associated with changes in the affinity of the target enzyme, the cytochrome P-450 lanosterol 14α-demethylase, and decreases in the cellular content of the azole due to the overexpression of MDR efflux pumps (Lamb et al., 1995; Joseph-Horne et al., 1995). Secondary resistance to amphotericin B has been associated with defects in sterol biosynthetic pathway, such as alterations in the sterol δ8→7 isomerase enzyme (Joseph-Horne et al., 1995; Kelly et al., 1994).

It was clear throughout the development of the M27 methodology that this approach was suboptimal, because of the slow growth rate of fungi, requiring 72 h of incubation, and

because some strains did not even grow. For that reason, detection of susceptibility and resistance to both fluconazole and amphotericin B appears to require a modification of the reference testing method. The number of studies that have established the value of susceptibility as a predictor of clinical response in patients with *C. neoformans* infections is still limited (Casadevall et al., 1993; Paugam et al., 1994; Birley et al., 1995; Armengou et al., 1996), so interpretative breakpoints for this pathogen have not yet been established. However, it is clear that antifungal drug resistance does occur, whether it is primary or secondary but because of the aforementioned limitations of in-vitro susceptibility testing, routine antifungal susceptibility testing of all clinical specimens is not recommended (Rex et al., 1997; Rex et al., 2001).

FUTURE HOPE AND DIRECTIONS

Since 1998, there has been a great improvement in the standardization of antifungal susceptibility tests for yeast and filamentous fungi (NCCLS, 1998). Much research needs to be performed to correlate in-vitro values with the clinical outcome of the patients with systemic mycoses. In addition, the search for new antifungal drugs with new targets should be a priority because of the intrinsic resistance or the development of resistance during treatment to the available antifungal drugs shown by some of these fungi (Perea et al., 1999). Antifungal drug resistance can be characterized as microbiological or clinical. Microbiological resistance is defined as the non-susceptibility of a fungal pathogen to an antifungal agent as determined by in-vitro susceptibility testing and compared with other isolates of the same species, and can be further categorized as intrinsic or acquired. Intrinsic resistance is found naturally among certain fungal strains without prior exposure to drugs but acquired resistance develops in previously susceptible fungal strains following drug exposure and can often occur as a result of altered gene expression (Dick et al., 1985). In contrast, clinical resistance refers to the persistence of a fungal infection despite treatment with adequate therapy. Although microbiological resistance can contribute to the development of clinical resistance, other factors may also be involved, such as impaired immune function, underlying disease, reduced drug bioavailability and increased drug metabolism (Fan-Havard et al., 1991).

ANTIFUNGAL RESISTANCE AND TREATMENT OPTIONS

The Infectious Diseases Society of America guidelines (Pappas et al., 2016) currently favour echinocandins as first-line treatment for systemic candidiasis in patients with moderate-to-severe infection and in those with prior exposure to azoles, with fluconazole reserved for the treatment of patients with less severe infection. The European Society of Clinical Microbiology and Infectious Diseases (Auzinger et al., 2015; Perlin, 2014) recommend echinocandins as first-line treatment for all patients with systemic candidiasis (Powderly et al., 1988). Resistance to the polyene antifungals (e.g., amphotericin B) remains relatively uncommon among Candida isolates (Dick et al., 1985; Dick et al., 1980). Candida species exhibit varying degrees of susceptibility to the most commonly used antifungal drugs. For instance while *C. kruseiis* intrinsically resistant to fluconazole, with a global resistance rate of 78.3%, *C. glabrata* is plays reduced dose-dependent susceptibility in comparison to other Candida species and has a global resistance rate of 15.7% (Lyon et al., 2010). Primary resistance to fluconazole is rare for *C. albicans* (1.4%), *C. parapsilosis* (3.6%) and *C. tropicalis* (4.1%) (Mori et al., 1997). In contrast, the echinocandins exhibit potent antifungal activity against most Candida species, with the exception of *C. parapsilosis*, for which higher minimum inhibitory concentrations (MICs) have been reported (Kovacicova et al., 2001; Safe et al., 1977; Barchiesi et al., 2000; Pfaller et al., 2002). An increasing number of rarer

Candida species have been reported to be intrinsically less susceptible to the azoles (*C. ciferrii, C. guilliermondii, C. inconspicua, C. humicola, C. lambica, C. lipolytica, C. norvegensis, C. palmioleophila, C. rugosaand C. valida*) and echinocandins (C. fermentati and C. guilliermondii) (Mori et al., 1997; Walmsley et al., 2001; Nguyen et al., 1998). Acquired resistance is generally less common than intrinsic resistance; however, various studies suggest that it is beginning to emerge in some countries. In a study conducted at two tertiary-care cancer centres in Boston, MA, USA, Oxman and colleagues between 2001 and 2004 reported that 19 % of their Candida infections involved either fluconazole-resistant strains or strains with reduced susceptibility. *Candida albicans, C. tropicalisand, C. parapsilosis* that are generally considered to be susceptible to fluconazole, accounted for 36% of isolates with reduced susceptibility and 48% of resistant isolates (Sanglard et al., 1998).

Challenges to therapeutic management of drug-resistant fungal infections

Challenges to a therapeutic manage of drug resistant fungal infections today include the lack of access to sensitive and specific diagnostic tests, the lack of clinically calibrated antifungal susceptibility testing and a limited repertoire of antifungal drugs. Furthermore, the diversity of the fungal kingdom ensures a limitless reservoir of new pathogens, alongside endless occurrence of variants of old ones, that readily adapt and evolve when exposed to antifungal chemicals. The sheer ecological breadth of fungal species, with their unique and varied ecological trophisms, in rapidly changing environments means that human health will always be confronted with the complex ecology of fungal communities, whether commensal or environmental. Similarly, the simultaneous need to control fungal disease in agricultural environments and the clinic means that integrated responses take these needs into consideration. Pathogenic fungi are widely spread both actively and passively, which puts individual in immunodeficient state at elevated risk of morbidity and mortality. Therefore, tackling antifungal resistance both in the clinic and in the field requires a coordinated global response.

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