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REVIEW

New pathogens, new tricks: emerging, drug-resistant fungal pathogens and future prospects for antifungal therapeutics

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Fungal pathogens are a growing threat to public health. As human immunodeficiency becomes increasingly common, fungal infections are becoming more prevalent. The use of antifungal agents for prophylaxis and treatment of fungal infections has favored the emergence of previously rare or unidentified species of drug-resistant fungal pathogens, including several *Candida* and *Cryptococcus* species, as well as mold pathogens. As these new and increasingly drug-resistant fungal pathogens continue to emerge, novel strategies for rapid identification and treatment are necessary to combat these life-threatening infections.

Keywords: fungal pathogens; emerging pathogens; antifungal drug resistance

Introduction

Fungal pathogens are emerging as critically important threats to global health. Recent estimates indicate that more than 300 million people are affected by serious fungal diseases worldwide, resulting in 1.6 million deaths annually[1,2]—similar to the number of deaths caused by tuberculosis.[2,3] Fungal pathogens are a highly diverse group of infectious agents, including yeast, or yeast-like species such as *Candida* and *Cryptococcus* species, and molds, such as *Aspergillus* species. These pathogens are responsible for a range of diseases, with variable prevalence and clinical outcomes. Nearly, one quarter of the world's population (~1.7 billion people) is estimated to have superficial skin, hair, and nail fungal infections caused primarily by dermatophyte fungi.[2–4] Mucosal fungal infections of the oral and genital tracts are also exceedingly common, with ~75% of women experiencing at least one vulvovaginal fungal infection caused by *Candida* species during their lifetime.[3,5] *Aspergillus* species are responsible for ~3 million cases of chronic lung disease, and are a substantial cause of fungal-associated asthma, affecting ~10 million people.[2,3] While significantly less common, invasive fungal diseases caused by *Cryptococcus*, *Candida*, *Aspergillus*, and *Pneumocystis* species are associated with exceedingly high mortality rates, ranging from 30% to 90%, depending on the fungal pathogen and patient group.[6,7] These species are currently the most common cause of fungal diseases; however, many new and emerging fungal pathogens are being identified, and are poised to significantly threaten human health.

Emerging pathogens may include newly identified species, as well as species that are dramatically increasing in prevalence (e.g., as a result of a growing population of vulnerable hosts) or spreading to new geographic niches.[8,9] Here, we define emerging fungal pathogens as recently identified species in clinical settings, and species undergoing a population expansion, change of ecological niche, or a geographic spread. However, for completeness and comprehensiveness, within our definition of "emerging," we also consider pathogenic fungi with

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a history of human and plant pathogenesis that presently demonstrate increased rates of occurrence attributed to a growing population of vulnerable hosts and to improved detection methods, awareness, and surveillance, including *Fusarium* spp., *Scedosporium* spp., and *Mucormycetes*. Overall, the focus of our review is to comprehensively address emerging fungal pathogens associated with high rates of antifungal drug resistance and to present novel strategies to combat drug resistance.

Several factors contribute to the escalating emergence of new human fungal pathogens in the modern era. Fungi are commonly opportunistic pathogens—exploiting susceptibilities presented by a host with a compromised immune system, an altered microbiome, or breached physical barriers, to cause infection. Specifically, the growing prevalence of fungal infections is directly associated with ever-increasing rates of medical vulnerabilities, including (1) immunosuppression, which is becoming more common as a result of HIV/AIDS,[10,11] hematologic cancers and cancer chemotherapeutics,[12,13] the use of immunosuppressive agents for organ and bone marrow transplantation,[14,15] and an aging population;[16,17] (2) pervasive dysbiosis of the human microbiome associated with exposure to broad-spectrum antimicrobials;[18,19] and (3) breached barriers linked to the frequent use of modern medical devices such as ventilators, stents, and catheters.[20,21] Aside from medical factors leading to expanding pervasiveness of fungal pathogens, environmental fluctuations such as global climate change are contributing to geographic spread of many fungal crop pathogens[22] and may be associated with the emergence of newly pathogenic fungal species.[23] Of particular concern is the emergence of previously unidentified or rare fungal pathogens with high rates of antifungal drug resistance, such as the recent discovery of *Candida auris*,[24] and the emergence of rare but highly drug-resistant molds. These emerging pathogens are likely selected due to the mounting clinical administration of antifungal drugs for both prophylaxis and disease treatment.[25]

In this review, we discuss emerging fungal pathogens that pose a growing threat to human health. In particular, we highlight the emergence of drug-resistant fungal pathogens—including less common *Candida* and *Cryptococcus* species and emerging filamentous fungal pathogens—and the difficulties associated with treating their associated infections. Initially, we present an overview of existing antifungals and clinically relevant mechanisms of drug resistance, and then discuss specific emerging drug-resistant pathogens. Further, we discuss future prospects for the development of innovative antifungal drugs, new treatment regimens, and novel approaches to sensitize drug-resistant fungal infections.

Antifungal agents and drug resistance

Given the growing importance of fungal diseases, novel treatment options are in high demand. As eukaryotic fungal cells share a close evolutionary relationship with their human hosts, antifungal drug development presents a unique challenge for the pharmaceutical industry. Specifically, antifungal agents must target the eukaryotic fungal cell while ensuring limited damage to human cellular function during treatment. In general, antifungal agents are classified as fungistatic drugs that inhibit fungal cell growth, or fungicidal drugs that lead to fungal cell death. These classifications are important for defining mechanisms of drug action and understanding treatment options and outcomes. Presently, four classes of antifungal therapies are routinely used in monotherapy or in combination: polyenes, azoles, pyrimidine analogs, and echinocandins.[26] Although these classes of antifungal agents are often active against many infections, limitations associated with off-target host toxicity, limited fungicidal activity, drug–drug interactions, prolonged treatment courses, and the emergence of drug resistance can significantly impede their applicability and efficacy.

Antimicrobial resistance is a growing threat for bacteria as well as fungal infections and is one of the most critical global health concerns faced today. For fungal infections, rates of resistance vary by geography, species, and available treatment options; however, the expansion of drug-resistant fungal species is universal. In general, intrinsic and acquired mechanisms of resistance of fungal pathogens are associated with decreasing the effective drug concentration, altering the drug target, or diverting antifungal toxicity by metabolic modification.[27,28] Here, we present each class of antifungal agents along with their mechanisms of action and occurrence of resistance, as well as an overview of laboratory methods to quantify antifungal drug resistance.

Polyenes

Polyenes were the first antifungal drugs available for clinical use and are considered to be dose-dependent fungicidal agents with broad-spectrum activity.[29,30] They are macrocyclic organic molecules derived from natural products that interact and disrupt ergosterol-containing fungal cell membranes through pore formation. Specifically, exposure to polyenes results in the leakage of cytoplasmic contents and oxidative damage, leading to fungal cell death.[31] First-generation amphotericin B, which is active against most systemic infections caused by yeasts and filamentous fungi, binds ergosterol to form an extramembranous fungicidal sterol sponge for the destabilization of membrane function following intravenous administration.[32] Additionally, nystatin and natamycin (administered as topical agents due to low gut absorption and high toxicity) are used for the treatment of mucocutaneous candidiasis and fungal keratosis.[33] Polyenes also interact with cholesterol-containing membranes, albeit with a lower affinity than to ergosterols, resulting in high host toxicity.[34] For polyenes, resistance is often intrinsic and only observed in fungal species with nonsusceptible or mildly affected ergosterol membranes. However, prolonged exposure to polyenes can lead to the emergence of clinical resistance.[35] To combat challenges associated with drug efficacy, toxicity, and resistance, second- and third-generation polyenes focus on the applicability of lipid formulations to reduce toxicity. Specifically, for amphotericin B, structural alterations enabling the preferential formation of monomers[36] may improve penetrability to specific body compartments and reduce membrane toxicity.[37] Future generations may involve oral formulations,[38] nanoparticles,[39] and polysaccharide conjugation.[40]

Azoles

Azoles are the most common antifungal drugs in clinical use today. They exert broad-spectrum activity through inhibition of lanosterol 14α-demethylase (encoded by *ERG11/CYP51*), a cytochrome P450-dependent enzyme that converts lanosterol to ergosterol, a primary target in the fungal membrane that is absent in the host cell membrane.[41] First-generation azoles, including the imidazoles (clotrimazole, miconazole, and ketoconazole), displayed high host toxicity, severe side effects, and numerous off-target interactions.[42] These were replaced with second-generation triazoles, such as fluconazole, which is active against several pathogenic yeast species, including *Candida* spp., *Cryptococcus neoformans*, and *Histoplasma*, but lacks activity against molds,[43,44] and itraconazole, which shows broader spectrum activity against yeasts as well as *Aspergillus* species.[45] Though widely employed, azoles show variable fungicidal activity against fungal pathogens. While several azole drugs display fungicidal activity against *Aspergillus* species,[46] they generally act in a concentration-independent fungistatic manner against yeast pathogens, including *Candida* and *Cryptococcus* species.[29,30] To overcome certain limitations of efficacy, third-generation azoles with extended-spectrum activity, including voriconazole, posaconazole, efinaconazole, and isavuconazole, have been developed through extensive structural modifications for improved activity, safety, formulations, and pharmacokinetic properties.[47]

The fungistatic nature of the azoles against common yeast pathogens can impose a strong selection pressure on surviving populations to evolve resistance to these antifungal agents.[48,49] Additionally, increased azole resistance associated with fungistatic versus fungicidal activity of the triazoles can limit their effectiveness against emerging fungal pathogens.[50] For azoles, intrinsic or acquired resistance is possible through genetic mutations to *ERG11/CYP51*, which prevents blockage of catalytic activity by the drug, as well as target gene amplification to overwhelm the inhibitory capacity of the drug.[51] Target mutations and amplifications are often associated with cross-resistance between certain azoles (i.e., fluconazole and voriconazole).[52–55] Another mechanism of azole resistance involves amplification or induction of efflux pumps for removal of azoles from the fungal cells, resulting in decreased drug efficacy.[56] Efflux-related resistance has also been associated with cross-resistance between azoles and amphotericin B.[57] Other resistance mutations, such as C-5,6-desaturase mutations that block the synthesis of ergosterol and lead to the accumulation of an alternate sterol in the fungal membrane, are associated with cross-resistance to amphotericin B, along with azole resistance.[58,59]

Pyrimidine analogs

Pyrimidine analogs, including 5-flucytosine, exert their activity through conversion by cytosine

deaminase into the toxic compound 5-fluorouracil, which interferes with RNA and DNA biosynthesis in the fungal cell.[60] Flucytosine is primarily used to treat pathogenic yeasts, and has little activity against most filamentous fungi. Host toxicity is less severe than other antifungals due to little or no cytosine deaminase activity in mammalian cells; however, there are still side effects associated with flucytosine treatment. Development of antifungal drug resistance to flucytosine is rapid and common. Specifically, a point mutation in *FUR1* encoding uracil phosphoribosyltransferase leads to complete resistance to flucytosine and 5-fluorouracil in fungi.[61] Moreover, a second point mutation in the cytosine deaminase-encoding genes *FCY1* and *FCY2* results in flucytosine resistance in many *Candida* strains.[62] Therefore, flucytosine is rarely used in monotherapy, but rather in combinatory therapeutic approaches, such as in combination with amphotericin B for the treatment of cryptococcosis.[63] Indeed, amphotericin B and flucytosine have been shown to act synergistically; the combination of these drugs is significantly more effective than either amphotericin B or flucytosine monotherapy for reducing fungal burden in murine models of cryptococcosis.[64,65]

Echinocandins

Echinocandins, a group of lipopeptides derived from natural products and the newest class of antifungal agents, target the fungal cell wall. This is achieved by noncompetitively inhibiting the plasma membrane-bound β-(1,3)-D-glucan synthase enzyme complex associated with synthesis of the structural polymer β-(1,3)-D-glucan, causing osmotic instability and fungal cell death.[66] Caspofungin, micafungin, and anidulafungin represent the three first-line echinocandins with broad-spectrum fungicidal activity against yeasts, specifically used to treat invasive systemic fungal infections including nosocomial candidemia and invasive candidiasis.[43] However, echinocandins are not active against many important fungal pathogens, including *Cryptococcus* and *Fusarium* species.[67] Interestingly, echinocandins display opposite fungicidal activity compared with azole drugs: they are generally fungicidal against pathogenic yeasts such as *Candida* species, but fungistatic against *Aspergillus* species.[67]

The echinocandins display good safety profiles, limited interactions with other drugs, and minimal host toxicity due to the absence of the enzyme in mammalian cells. Additionally, these drugs generally do not display cross-resistance to other existing antifungal agents, and are therefore broadly effective against azole-resistant pathogens.[68] For echinocandin resistance, mutations in the gene encoding the echinocandin target (β-(1,3)-D-glucan synthase (*FKS1*)) are by far the most common mechanisms of drug resistance.[66] These mutations, which typically occur in certain hot-spot regions of the gene *FKS1*, usually impart cross-resistance to all of the echinocandin drugs.[68]

Measuring antifungal drug resistance

Antifungal drug resistance profiles for fungal pathogens are established using specific assays and protocols. Resistance is measured as the reduction of growth of fungal cells in the presence of the drug *in vitro*, compared with cells grown in the absence of drug. Such measurements can be established experimentally with antifungal drug gradients in liquid or on solid agar media.[28,69,70] In order to establish unified, reproducible methods of measuring antifungal susceptibility between laboratories, internationally recognized standard testing methods have been established from two agencies: the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing.[71] In both cases, susceptibility measurements rely on broth microdilution testing along a drug concentration gradient in order to establish a minimum inhibitory concentration (MIC)—the lowest concentration of an antimicrobial that will inhibit fungal growth. A fungal strain is deemed drug resistant if its MIC value falls above clinical breakpoints that have been previously established for specific antifungals and specific fungal pathogens. In some cases, all strains of a given fungal species are not susceptible to a particular antifungal (without having been previously exposed to that drug) and these species are considered to be intrinsically resistant to that drug.[28]

There are certain issues that arise while determining antifungal susceptibility of fungal pathogens—and particularly, of emerging pathogens. Susceptibility testing relies on the use of established breakpoints, and if these have not been determined for a newly emerging pathogen, it can

be difficult to infer how *in vitro* susceptibility will reflect clinical *in vivo* drug resistance. Even for well-studied pathogens, drug resistance defined by *in vitro* analysis is often not sufficient to explain clinical outcomes.[72] Additionally, antifungal drug tolerance—defined as a pathogen's ability to withstand killing at drug concentrations above the MIC[73]—is a less well understood biological phenomenon, likely attributed to an epigenetic, rather than genetic, mechanism that facilitates fungal survival in the presence of drug.[73]

Emerging threats of drug-resistant yeast fungal pathogens

Yeast and yeast-like species are a diverse group of fungal organisms that grow as single cells and divide through budding or binary fission. Some yeast species, such as *Candida albicans*, are considered dimorphic (or polymorphic) as they grow in both yeast morphology as well as elongated filamentous cells.[74] Yeast pathogens are among the most pervasive and deadly to humans, including *Candida* and *Cryptococcus* species. The emergence of rare or previously unidentified species of *Candida* and *Cryptococcus* is becoming more frequent and poses a challenge to human health.

Emerging drug-resistant Candida *pathogens*

Candida species can cause a range of human diseases, from superficial mucosal infection of the oral and urogenital tracts to life-threatening invasive infection in immunocompromised individuals.[74,75] *Candida* species are the fourth leading cause of hospital-acquired bloodstream infections and the most common fungal species isolated from medical device-associated biofilm infections.[7,76] The most commonly encountered of these yeast species is *C. albicans*, which accounts for more than half of all cases of invasive candidiasis.[75] However, despite the prevalence of *C. albicans*, newly emerging non-*albicans Candida* species are increasingly being documented in clinical settings.[77] Worryingly, many of these emerging *Candida* pathogens, including *Candida glabrata, Candida krusei*, *Candida lusitaniae*, and the very newly emerging pathogen *C. auris*, are associated with greater resistance to antifungal therapeutics[78,79] and are a growing threat to public health.

Among non-*albicans Candida* pathogens, *C. glabrata* is the most common and of growing clinical concern among elderly patients, HIV positive individuals, and those receiving solid organ transplants (SOTs) and bone marrow transplants.[75,80] *C. glabrata* is the second most common cause of invasive candidiasis, representing 15–30% of clinically isolated pathogens.[75,80–82] It is intrinsically less susceptible to fluconazole and amphotericin B;[77,83,84] ∼10–15% of clinical isolates are resistant to fluconazole and voriconazole, and strains are likely to exhibit cross-resistance to multiple azole drugs.[77] Most clinical azole resistance in *C. glabrata* has been associated with upregulation of ATP-binding cassette (ABC) transporters, such as Cdr1 and Cdr2.[85,86] As a result of higher rates of innate and acquired azole resistance, *C. glabrata* is often found to cause infection in patients exposed to fluconazole as prophylaxis or for treatment of other fungal infections.[77,87–89] Notably, while most *Candida* species have relatively low frequency of resistance to echinocandins,[90–92] *C. glabrata* isolates have unusually high rates of resistance, ranging from less than 5% in 2001, to 12% in 2010,[93] often associated with mutations in the drug target *FKS* genes.[92] Of particular concern is the high frequency of multidrug-resistant *C. glabrata* isolates with reduced susceptibility to both azoles and echinocandins. One study indicates that 36% of echinocandin-resistant *C. glabrata* strains are also resistant to fluconazole,[94] limiting treatment options for this increasingly prevalent fungal pathogen. A recent study suggested that a prevalent "mutator" genotype—routinely identified among *C. glabrata* clinical isolates—may promote multidrug resistance.[95] Such mutator strains have mutations rendering the fungus defective in mismatch repair, leading to high rates of genetic mutations and a high propensity to develop multidrug resistance.[95]

Less frequently encountered emerging *Candida* pathogens, including *C. krusei*, *C. lusitaniae*, and *C. haemulonii*, also have intrinsic antifungal drug resistance profiles that hinder successful antifungal treatment. Similar to *C. glabrata*, *C. krusei* is intrinsically resistant to fluconazole, mainly due to a diminished sensitivity of the target enzyme CYP51 to fluconazole inhibition.[96] As a result, *C. krusei* is frequently associated with infection in patients receiving prophylactic or therapeutic fluconazole treatment.[97,98] Additionally, *C. lusitaniae* is generally susceptible to azoles, but is associated with intrinsic resistance to amphotericin B.[99] Moreover,

isolates of both *C. krusei* and *C. lusitaniae* have also been identified as multidrug resistant to azoles and echinocandins.[100,101] Finally, *C. haemulonii* isolates are frequently found to be resistant to both amphotericin B and azoles,[102–104] and azole-resistant *Candida parapsilosis* clinical isolates are increasingly being identified.[105,106]

Candida auris

The appearance of newly emerging drug-resistant *Candida* species is ongoing. *C. auris* is the most recent and worrisome example of a previously unidentified pathogen that is highly refractory to existing antifungal treatment. Initially identified in Japan in 2009,[107] *C. auris* infections simultaneously and seemingly independently emerged in hospital settings in distinct geographical locations,[24,108] and have now been identified across five continents.[24,109] In some geographical regions, *C. auris* is becoming quite prevalent. For example, in India, a multicenter study of intensive care units found *C. auris* in 19 out of 27 units, accounting for ∼5% of all candidemia cases.[110] Inaccuracies associated with the diagnosis of *C. auris* have resulted in misidentification of this pathogen and difficulties in quantifying the prevalence of these infections,[111,112] highlighting the need for accurate diagnostic tests. Although *C. auris* does not seem to form hyphae or pseudohyphae, some isolates display a cellular aggregation phenotype and are able to form biofilms.[113] *C. auris* also harbors virulence factors, including phospholipase activity and secreted proteinase activity.[114]

C. auris strains are frequently resistant to antifungal drugs, although currently there are no antifungal clinical breakpoints reported for this pathogen. A global study of *C. auris* isolates was conducted from 54 patients across three continents from 2012 to 2015.[108] This analysis found that an overwhelming majority of *C. auris* isolates are resistant to fluconazole (∼93% of isolates[108]), and approximately half of the tested isolates are also resistant to voriconazole.[108] Additionally, 35% of *C. auris* isolates are resistant to amphotericin B, and 7% to echinocandins.[108] Multidrug resistance is common in *C. auris*, where 41% of strains are resistant to at least two classes of antifungals and 4% are resistant to all antifungal agents.[108] While *C. auris* research is still in its infancy, studies suggest that elevated drug resistance is due to a large number of genomically encoded efflux pumps,[115,116] enhanced efflux pump activity,[117] and mutations in the ergosterol biosynthesis pathway.[108]

Emerging drug-resistant Cryptococcus *pathogens*

Cryptococcus species are responsible for causing cryptococcosis, an important, primarily opportunistic fungal infection common among patients with HIV/AIDS. Infection begins with inhalation of desiccated yeast cells or spores from the environment, causing primary infection in the alveolar macrophages of the lungs, followed by dissemination to the central nervous system, leading to fungal meningitis, as well as bloodstream infections.[118] Cryptococcal meningitis is one of the most important HIV/AIDS-related infections, responsible for 15% of AIDS-related deaths.[119] *C. neoformans* and *Cryptococcus gattii* are responsible for an overwhelming majority of cryptococcal disease.[118] Specifically, the emergence of *C. gattii* since 1999 in the Pacific Northwest of the United States and on the west coast of Vancouver Island, Canada, has identified the species as a significant human pathogen causing infection in immunocompetent individuals.[120] While *C. gattii* is more susceptible to amphotericin B and flucytosine compared with *C. neoformans*, it is less susceptible to fluconazole and other azole drugs;[121,122] however, mechanisms of resistance have not been defined.[123] *Cryptococcus* species are not susceptible to the echinocandin class of antifungals;[67,74] therefore, additional antifungal drug resistance severely limits treatment options. Additionally, mismatch-repair defective mutator strains have been identified for *Cryptococcus* species and are associated with high rates of mutations and antifungal drug resistance.[124–126]

Aside from *C. neoformans*, the majority of *Cryptococcus* species were historically believed to be avirulent in humans. However, in recent years, less common non-*neoformans Cryptococcus* species have been increasingly identified as opportunistic human pathogens, causing infection primarily in patients with impaired cellular immunity due to hematological cancer, neutropenia, use of immunosuppressive drugs, or HIV/AIDS.[127] The rise in incidence of these pathogens may be due to improvements and availability of diagnostic tools. The most commonly identified emerging cryptococcal species associated with immunocompromised hosts are *Cryptococcus laurentii* and

Cryptococcus albidus, which account for 80% of non-*neoformans* and non-*gattii Cryptococcus* infections.[127,128] Susceptibility testing has revealed that these species are not susceptible to flucytosine, and 50% of *C. laurentii* and 80% of *C. albidus* have an elevated MIC to fluconazole ($\geq$16 mg/L),[78] although the mechanisms of resistance have not been conclusively identified. This mirrors the trend observed among emerging non-*albicans Candida* species with high rates of fluconazole resistance and suggests that widespread use of this antifungal may be responsible for the new emergence of diverse, fluconazole-resistant fungal pathogens. The majority of *Cryptococcus* isolates remain sensitive to amphotericin B, which is used to successfully treat these infections—but a trend toward emerging *Cryptococcus* species with expanded drug-resistance profiles results in a scarcity of therapeutic options.

Emerging threats of drug-resistant filamentous fungal pathogens

Filamentous fungi or molds are ubiquitous within the environment and grow by apical extension of their filaments, or hyphae, with or without septation, leading to the formation of mycelia. The occurrence of invasive filamentous fungal infections has risen over the past few decades and these infections are often associated with high rates of morbidity and mortality. Moreover, in recent years, the increasing emergence of pathogenic fungi, including *Aspergillus* spp., Mucormycetes, *Fusarium* spp., and *Scedosporium* spp., that display intrinsic or acquired mechanisms of drug resistance demands our attention.

Emerging drug-resistant Aspergillus *pathogens*

Aspergillus species are saprotrophic fungi commonly found in soil and decaying organic material within the environment. *Aspergillus fumigatus* is the most common *Aspergillus* spp., causing invasive aspergillosis (IA) in immunocompromised individuals, particularly patients undergoing chemotherapy treatment, hematopoietic stem cell transplantations (HSCTs), SOTs, immunosuppressive drug treatment, or patients with HIV/AIDS.[129] Other species capable of causing disease include *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus clavatus*, and *Aspergillus nidulans*.[130] Annually, *Aspergillus* causes over 200,000 life-threatening infections, with mortality rates ranging from 30% to 95%.[131] Aside from IA infections, the occurrence of allergic bronchopulmonary aspergillosis affects 4.8 million people worldwide, with an estimated 400,000 with chronic pulmonary aspergillosis.[132,133] While *A. fumigatus* itself is not a new pathogen, the emergence of azole resistance in this species is relatively recent and concerning. Since azole-resistant strains were first identified in 1997, the rate of resistance has risen rapidly to account for ~5–6% of *A. fumigatus* isolates.[134,135] There is evidence to suggest that mounting rates of azole resistance are linked to the exposure of *A. fumigatus* to azole-based agricultural fungicides in the environment and the resultant selection for resistant strains.[136–139]

Given the clinical relevance of *Aspergillus* spp., the emergence of opportunistic *A. terreus*, the third leading cause of global IA, is of particular concern. For example, *A. terreus* presents a growing problem among patients with hematological malignancies (HMs),[140,141] as it causes severe infections and has a propensity to disseminate, resulting in systemic patient infections. Due to high rates of *in vitro* and *in vivo* resistance to antifungal agents,[142,143] including intrinsic resistance to amphotericin B,[144] *A. terreus* infections are associated with very poor patient outcomes.[143,145] Recently, evaluation of the mechanism of resistance to amphotericin B determined that ergosterol content plays a minor role in intrinsic resistance and that the occurrence of higher catalase production in resistant isolates may block oxidative damage caused during antifungal treatment.[146] In addition, reduced azole susceptibility, attributed to mutations in the target Cyp51 protein that are associated with acquired resistance,[147] has been reported in *A. terreus* infections in Spain, the United States, and Denmark.[131,148,149] Alternatively, posaconazole appears promising for *A. terreus* treatment based on its efficacy in animal models and clinical studies.[150]

Emerging drug-resistant Mucormycetes *pathogens*

Mucormycetes (formerly Zygomycetes) infections are fungal infections caused by pathogenic orders Mucorales and Entomophthorales. Here, we focus on Mucorales, the most common emerging cause of non-*Aspergillus* mold infections in humans, with reported increases in incidence across various

geographical regions.[151–153] Mucorales represent fast-growing, common saprobes that cause mucormycosis by invasion of blood vessels and tissue necrosis. Mucormycosis occurs primarily in immunocompromised patients and results in mortality rates of 40–70% despite antifungal treatments.[154] Among Mucorales, *Rhizopus* spp. are the most commonly identified in human infection, along with *Mucor*, *Rhizomucor*, *Lichtheimia*, and *Cunninghamella* spp., which are particularly aggressive in HSCT recipients, with a mortality rate of 80%.[155]

Treatment of infection is often challenging due to delayed diagnosis, acute disease progression, and intrinsic resistance to many currently available antifungal agents.[156] Treatment strategies often involve surgery at the pathogen-infected body sites, as well as antifungal therapies. Susceptibility to drug treatments varies significantly among Mucorales, and microenvironmental factors at the site of fungal growth, such as tissue hypoxia, may affect antifungal drug efficacy.[157,158] Mucorales are intrinsically resistant to fluconazole and highly resistant to voriconazole,[159,160] and, as a result, are commonly associated with infections of HSCT recipients following prophylactic voriconazole treatment.[161] Posaconazole and isavuconazole generally demonstrate significant anti-Mucorales activity and are routinely used for treatment of mucormycosis;[162] however, *Cunninghamella bertholletiae* is highly resistant to posaconazole treatment. Additionally, *in vitro* testing suggests that flucytosine and echinocandins have little activity against Mucorales.[160,163,164] Morbidity and mortality rates for mucormycosis remain high, most likely attributed to poor clinical drug efficacy of monotherapies, demonstrating the need for novel treatment strategies.

Emerging drug-resistant Fusarium *pathogens*

Fusarium species are a large genus of environmentally ubiquitous hyphomycete fungi. *Fusarium* are well-known plant pathogens but are also classified as emerging, opportunistic human pathogens. Specifically, *Fusarium* spp. represent the second most common category of non-*Aspergillus* molds responsible for human infections.[165] Approximately 50% of reported infections are due to the most pathogenic species, *Fusarium solani*, followed by 20% of infections attributed to *Fusarium oxysporum*.[166] Primarily acquired from the environment, *Fusarium* spp. cause keratitis and onychomycosis among immunocompetent hosts, and respiratory and disseminated infections in immunocompromised individuals due to the weakened defenses of pulmonary alveolar macrophages and neutrophils.[167] The occurrence of fusariosis, endemic in tropical and subtropical countries, is associated with high mortality and morbidity rates of >80% in patients with prolonged and severe neutropenia, HMs, and HSCT recipients.[168,169]

Intrinsic multidrug resistance to a broad range of antifungals, along with species- and isolate-dependent resistance, is characteristic of *Fusarium* species. For example, second-generation broad-spectrum triazoles (fluconazole, itraconazole, voriconazole, and posaconazole), flucytosine, and echinocandins (caspofungin, anidulafungin, and micafungin) have limited activity in almost all *Fusarium* spp., and cross-resistance has been observed among echinocandins.[170,171] Although most *Fusarium* species have reduced susceptibility to these diverse antifungals, *F. solani* is considered the most highly resistant to antifungal drugs.[172,173] While the basis for *Fusarium* species' near-universal intrinsic drug resistance profile is not fully understood, intrinsic echinocandin resistance may be attributed to mutations occurring in the gene for the Fks1 target protein.[174]

Beyond intrinsic resistance, secondary or acquired resistance to azoles develops among previously susceptible strains of *Fusarium* after exposure to an antifungal agent. The mechanism of resistance is usually dependent on altered expression of CYP51, consisting of three CYP51 paralogues (CYP51A, -B, and -C).[175,176] Owing to high levels of resistance, monotherapy for *Fusarium* infections is associated with poor patient prognosis. However, synergistic antifungal interactions between drug combinations, including natamycin and voriconazole, and amphotericin B with caspofungin, rifampin, 5-flucytosine, or voriconazole, hold promise for more effective treatment of these infections.[177,178] Taken together, emerging *Fusarium* species pose a major challenge for medicine and agriculture alike, with very limited treatment options available for patients and crops.

Emerging drug-resistant Scedosporium *pathogens*

Scedosporium, including *Scedosporium apiospermum* and the related fungal species *Lomentospora*

prolificans (previously *Scedosporium prolificans*), are hyphomycete fungi of significant emerging medical importance. Although rare human pathogens, the incidence of *Scedosporium* infections has been rising significantly.[179] Globally, infection is acquired through inhalation of contaminated plant and soil residues and occurs in both immunocompetent and immunocompromised individuals.[180] Risk factors associated with infection include chronic obstructive or suppurative lung disease (including cystic fibrosis (CF)), HM, SOT or HSCTs, corticosteroid use, neutropenia, and diabetes mellitus.[181,182] *L. prolificans* is the second most abundant filamentous fungi colonizing the respiratory tract of CF patients after *Aspergillus* species.[183,184] Infection by *Scedosporium* species can cause a broad range of diseases, including localized skin and soft tissue infections, pneumonia, meningitis, and disseminated, multiorgan infection in immunocompromised hosts. For immunocompromised hosts, particularly those with neutropenia, the risk of *Scedosporium* infection dissemination is high, and associated mortality rates are >80%.[185]

Scedosporium species are among fungi with the highest degrees of resistance to antifungal compounds, and the majority of *Scedosporium* isolates have multidrug-resistance profiles.[186,187] *L. prolificans* in particular is one of the most highly drug-resistant fungal organisms identified, with high rates of resistance to polyenes,[188] azoles (including posaconazole and itraconazole),[188,189] and echinocandin antifungals.[190] *S. apiospermum* is also resistant to polyenes, echinocandins, and some azoles, but is susceptible to voriconazole and posaconazole.[191] While the mechanisms of *Scedosporium* resistance remain to be fully elucidated, echinocandin resistance has been largely attributed to hot spot mutations in the gene for the target protein Fks1, which render the fungi intrinsically resistant to echinocandin treatment.[190] Currently, the first-line treatment for *Scedosporium*-associated infection is voriconazole, as resistance against this compound is rare.

Future prospects for emerging fungal disease: diagnosis and treatment

Given the prevalence of intrinsic drug resistance among emerging fungal pathogens, it is critical that these less common species are correctly identified, as misidentification can lead to inappropriate therapeutic treatment and poor patient prognosis. This is of particular concern in resource-limited hospital environments, where species-level pathogen diagnostics may not be readily available. Furthermore, since fluconazole is by far the most readily available and inexpensive antifungal, the ability to acquire more expensive and more effective alternatives is limited in developing countries—presenting an additional hurdle to treating these emerging pathogens, many of which are intrinsically resistant to this ubiquitous antifungal. Difficulties associated with diagnosis and treatment of emerging pathogens may explain why these infections are associated with significantly longer hospital stays and higher inpatient costs compared with other infections.[28,192–194]

Future outlook for diagnosis of emerging fungal disease

Accurate identification of newly emerging fungal species is critical. In some cases, fungal pathogens classified as emerging are not in fact new pathogens but have been historically misclassified or mistaken for a more common species. For instance, a retrospective review of *Candida* isolates found a misidentified strain of *C. auris* from 1996[195]—13 years before *C. auris* was first identified as an emerging fungal pathogen. Additionally, intrinsic drug-resistance associated with many emerging fungi means that accurate, species-level diagnosis is crucial to ensure that proper treatment is administered. Most current fungal diagnostic techniques, such as culturing strains, microscopy-based staining, or antigen-based tests are time-consuming, may not provide species-level detection specificity, and/or require specialized and costly equipment and reagents. Therefore, novel tools for the rapid and accurate detection of fungal pathogens are imperative, and, given the prevalence of fungal infections in resource-limited environments, low-cost and low-tech diagnostic tools would significantly improve detection of these organisms in the developing world.[196]

Identification of fungal species based on genetic signatures provides accurate detection of pathogens and distinguishes between closely related species based on unique genomic identifiers. PCR-based diagnostics are often used for fungal

identification,[197] but require the use of specialized instrumentation. However, new, rapid, low-cost PCR-based technologies[198–200] may improve global access to these diagnostic techniques. New technologies are focusing on whole-genome sequencing for pathogen detection.[201] For example, portable genomic sequencers, such as the minION from Oxford Nanopore Technologies,[202] have been successfully used for rapid and relatively low-cost next-generation sequencing for the identification of viral[203–205] and bacterial[206] diseases in resource-limited settings, and could similarly be employed for the diagnosis of fungal disease. Whole-genome sequencing has the additional benefit of detecting not only a particular species, but also any potential drug-resistance mutations. Additionally, cutting-edge technologies for nucleic acid detection, including paper-based synthetic gene networks[207,208] and CRISPR-based diagnostic platforms,[209] are rapid, inexpensive, and sensitive methods for infectious disease detection that could be applied to fungal pathogens.

In addition to novel genomic technologies, other newer diagnostics focus on biomarkers of fungal infection. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has been successfully exploited for species-level fungal pathogen identification based on unique proteomic signatures.[210,211] This approach has been particularly useful in the identification of rare and commonly misidentified fungal species, including unusual *Aspergillus* pathogens,[212,213] and the identification of antifungal drug-resistant *Candida* strains.[214,215] Moreover, quantitative bottom-up proteomic analysis combined with selected reaction monitoring has discovered secreted cryptococcal proteins in infected tissue samples, suggesting the utility of such techniques for detection of diagnostic biomarkers of fungal infection.[216] Finally, compelling recent work has exploited engineered *Saccharomyces cerevisiae* as a biosensor capable of detecting fungal pathogen-derived peptides in order to develop a low-cost and nontechnical fungal diagnostic.[217]

New antifungal drugs for emerging infections

New antifungal drugs and treatment strategies are urgently needed to combat ever-emerging and increasingly drug-resistant fungal infections,[26,218,219] and several novel promising treatment strategies are currently being researched and developed (Table 1). For example, improved versions of existing antifungals, such as potent new azoles for candidiasis (VT-1161),[220] cryptococcosis (VT-1129),[221] and coccidioidomycosis (VT-1598, whose development has recently been fast-tracked by the Food and Drug Administration),[222] and CD101, a novel echinocandin with an improved safety profile,[223–225] are under investigation or development. Another important new antifungal, ASP2397, displaying potent fungicidal activity against several mold species, disrupts intracellular fungal biochemical machinery through uptake by a siderophore iron transporter.[226] In addition, several existing pharmaceutical agents are being repurposed as antifungals, such as the use of the selective serotonin reuptake inhibitor sertraline as an adjuvant to potentiate azole activity in cryptococcal infections,[227] which is currently in phase III clinical trials,[26] and the anticancer drug bortezomib, which interferes with virulence factor production and disease caused by *C. neoformans*.[228] Finally, new antifungal drugs with novel targets are also under development, such as APX001 (currently completed phase II clinical trials), a prodrug targeting glycosylphosphatidylinositol synthesis that has broad-spectrum activity against diverse yeast and mold pathogens, including highly drug-resistant emerging pathogens such as *Scedosporium* species.[229–231]

In addition to these antifungals currently in development, ongoing research is continually identifying new compounds with antifungal activity or new fungal factors that may serve as targets for antifungal therapy. Recent screens of bio-active molecules identified drugs with antifungal activity[232–235] that may serve as promising leads for antifungal drug development. Other work has focused on identifying and characterizing prospective antifungal drug targets, including key fungal stress response factors, such as the molecular chaperone Hsp90 (see Refs. 236–238), and the protein phosphatase calcineurin.[236,239–241] Antifungal drug target identification has also benefited from computational methods used to compare human and fungal proteomes, in order to identify fungal-specific domains with potential to bind small molecules.[242] These avenues lay the groundwork for future antifungal drug development pipelines.

Table 1. Emerging fungal pathogens: mechanisms of antifungal drug resistance profiles and future therapeutic prospects

	Pathogen	Resistance profile	Future therapeutic prospects	Reference
Candida species	*Candida glabrata*	Decreased intrinsic susceptibility to fluconazole, amphotericin B	New azoles (i.e., VT-1161); new echinocandins (i.e., CD101)	Garvey *et al.*,[220] Ong *et al.*,[223] Pfaller *et al.*[225]
		High rates of acquired resistance to fluconazole, voriconazole, and echinocandins	New antifungal APX001 targeting GPI synthesis	Watanabe *et al.*,[229] Hata *et al.*[230]
	Candida krusei	Intrinsic resistance to fluconazole	Targeting fungal stress response	Cowen *et al.*,[236] Juvvadi *et al.*[239]
	Candida lusitaniae	Intrinsic resistance to amphotericin B	Computational methods for antifungal drug target discovery	Barrera *et al.*[242]
	Candida haemulonii	High rates of resistances to azoles and amphotericin B	New antifungal adjuvants	Pfaller *et al.*,[254] Fiori *et al.*,[255] Liu *et al.*,[257] Shekhar-Guturja *et al.*,[258] Spitzer *et al.*,[259] Robbins *et al.*[260]
	Candida parapsilosis	Increasing resistance to azoles	Cytokine therapy as antifungal adjuvants	Delsing *et al.*[270]
	Candida auris	High rates of resistance to fluconazole, voriconazole, and amphotericin B	Monoclonal antibody therapy	Pachl *et al.*,[238] Bugli *et al.*[277]
		Very high prevalence of multidrug-resistant strains (including strains resistant to all existing antifungals)	Novel techniques in immuno-modulation	Zhao *et al.*[281]
			Fungal vaccine	Xin *et al.*,[290] Xin *et al.*[291]
			Targeting fungal virulence	Zhang *et al.*,[294] Romo *et al.*,[295] Vila *et al.*,[296] Fazly *et al.*,[297] Shareck and Belhumeur,[298] Murzyn *et al.*,[300] Mayer and Kronstad[301]
			Targeting mechanisms of antifungal drug resistance	Niimi *et al.*,[302] Niimi *et al.*,[303] Lee *et al.*,[304] Keniya *et al.*,[305] Holmes *et al.*,[306] Nishikawa *et al.*[307]
Cryptococcus species	*Cryptococcus gattii*	Resistance to azoles	New azoles (i.e., VT-1129)	Lockhart *et al.*[221]
	Cryptococcus laurentii	Intrinsic resistance to flucytosine	SSRI sertraline as azole adjuvant	Rhein *et al.*[227]
		High rates of resistance to fluconazole	New antifungal APX001 targeting GPI synthesis	Schell *et al.*[231]
	Cryptococcus albidus	Intrinsic resistance to flucytosine	Targeting fungal stress response	Juvvadi *et al.*[239]
		High rates of resistance to fluconazole	Computational methods for antifungal drug target discovery	Barrera *et al.*[242]
			New antifungal adjuvants	Pfaller *et al.*,[254] Shekhar-Guturja *et al.*,[258] Spitzer *et al.*,[259] Robbins *et al.*,[260] Brown *et al.*[262]
			Cytokine therapy as antifungal adjuvants	Pappas *et al.*,[268] Jarvis *et al.*[272]
			Monoclonal antibody therapy	Larsen *et al.*,[274] Rachini *et al.*[276]
			Fungal vaccine	Chow and Casadevall,[286] Upadhya *et al.*,[287] Mor *et al.*,[288] Wormley *et al.*[289]
Mold pathogens	*Aspergillus terreus*	Intrinsic resistance to amphotericin B	New echinocandins (i.e., CD101)	Pfaller *et al.*[225]
		Acquired azole resistance	New antifungal APX001 targeting GPI synthesis	Hata *et al.*[230]
	Mucorales species	Intrinsic resistance to fluconazole, highly resistant to voriconazole	New antifungal ASP2397	Nakamura *et al.*[226]
		Resistant to flucytosine and echinocandins	Targeting fungal stress response	Cowen *et al.*,[236] Juvvadi *et al.*,[239] Nambu *et al.*[241]
	Fusarium species	High rates of resistance to azoles, echinocandins, and flucytosine	Computational methods for antifungal drug target discovery	Barrera *et al.*[242]
	Scedosporium species	High rates of resistance to azoles, echinocandins, and polyenes	New antifungal adjuvants	Pfaller *et al.*,[225] Shekhar-Guturja *et al.*,[258] Robbins *et al.*[260]
			Cytokine therapy as antifungal adjuvants	Shao *et al.*,[269] Delsing *et al.*,[270] Armstrong-James *et al.*,[271] Goldman *et al.*[273]
			Monoclonal antibody therapy	Rollin-Pinheiro *et al.*[278]
			Novel techniques in immuno-modulation	Kumaresan *et al.*[280]

Prospects for combination antifungal drug therapy

Combination drug therapy is another promising therapeutic strategy for the treatment of fungal infections, as well as many other infectious and noncommunicable diseases.[243–245] Combination therapies can improve the effectiveness of drug treatment and may limit or prevent the emergence of drug resistance,[246–250] making this strategy of particular interest for emerging and highly drug-resistant fungal pathogens. Combination therapy has been proposed as a treatment option

for highly drug-resistant fungal pathogens, including *Fusarium* and *Scedosporium* species, where treatment with combinations of existing antifungals has shown promising results as effective therapeutics,[177,178,251] as well as combination therapy between antifungals and antiparasitics.[252]

In addition to combinations of existing antifungals, many new promising antifungal adjuvants have been described and are in various stages of clinical development, including: histone deacetylase inhibitors, which synergize with azoles and echinocandins;[253,254] iron and calcium homeostasis inhibitors,[255–257] which enhance azole activity; and beauvericin, which enhances the efficacy of azoles and blocks the emergence of antifungal resistance.[258] Additionally, several compelling novel screening platforms have identified new molecules that act to enhance the activity of existing antifungals[259–261] and may serve as a template for future screening efforts to identify antifungal adjuvants.

In addition to drug screening, an alternative strategy for target identification in combination therapy is to use chemical-genomic approaches or genetic interaction analysis. Chemical-genomic screening has been successfully used to identify fungal genetic mutants with altered susceptibility to chemical perturbations, and further exploitation of this chemogenomic information represents a powerful tool to predict antifungal drug synergies in *Cryptococcus*.[262] Genetic interaction analysis can also be utilized to identify drug target synergies by identifying synthetic lethal interactions,[263] where simultaneous deletion of perturbation of two genes results in lethality to the cell.[264] Innovative tools for genetic interaction and synthetic lethal screening in fungal pathogens[265] may facilitate such identification of fungal targets for combination antifungal therapy.

New alternatives for antifungal drug therapy

Given the prevalence of antifungal drug resistance among emerging fungal pathogens, unconventional therapeutic strategies may be essential for treating these infections. One alternative treatment strategy for fungal diseases aims to manipulate the host immune system to better combat infection. This includes cell-based therapies focused on replenishing immune cells[266,267] and the use of cytokines as adjunctive antifungal therapy,[268–272] which has shown promising results for the treatment of invasive fungal infections in organ transplant patients,[271] for HIV/AIDS-associated *Cryptococcus* infections,[268,272] and for highly drug-resistant *Scedosporium* infection.[273] Additionally, there is growing interest in the use of monoclonal antibodies to target fungal pathogens[238,274–278] or for use in radioimmunotherapy.[279] Other innovative approaches to immune-based antifungal therapy include a bioengineering strategy to genetically modify T cells to redirect their specificity toward *Aspergillus*,[280] and a targeted kinase approach in mammalian cells that negatively regulates the host antifungal immune response in order to boost innate immunity against fungal infection.[281] Finally, there has been substantial interest in the development of antifungal vaccines.[282–285] There is mounting evidence for the efficacy of antifungal vaccines in preventing infection in animal models,[286–291] and a vaccine for recurrent vulvovaginal candidiasis has completed phase II clinical trials.

Another new paradigm for antimicrobial therapy is targeting virulence factors.[292,293] This strategy may be of particular interest for treating emerging drug-resistant fungi, as antivirulence therapeutics are predicted to impose less selection pressure toward drug resistance. Several recent studies have identified molecules that reduce virulence of *Candida* pathogens by preventing fungal adhesion, cellular morphogenesis, and/or biofilm formation.[294–298] Other factors targeting fungal virulence have also been identified through interactions with competing microbial organisms. The probiotic yeast *Saccharomyces boulardii* inhibits *C. albicans* filamentation, adhesion, and biofilm formation,[299,300] and a *Bacillus* bacterial species is able to inhibit virulence factor production and biofilm formation in both *C. neoformans* and *C. albicans*,[301] suggesting that investigating natural products from other microbial organisms may be a promising strategy to identify novel ways to target fungal virulence.

Finally, targeting antifungal resistance mechanisms holds potential as a powerful strategy to eliminate drug-resistant emerging fungal pathogens. Targeting drug efflux pumps has been the primary tool employed to overcome antifungal drug resistance. Inhibitors of ABC[258,302–304] and major facilitator superfamily[305] transporters, as well as broad-spectrum efflux pump inhibitors,[306]

have been identified, with the ability to reverse antifungal drug resistance in fungal pathogens, including drug-resistant *C. glabrata* species. Recent work exploited a high-throughput screening platform to identify a small-molecule inhibitor of a critical protein–protein interaction between the Pdr1 efflux pump in *C. glabrata* and the Mediator complex.[307] This new inhibitor blocks efflux pump activity and successfully reduced *C. glabrata* resistance to azole antifungals in *in vitro* and *in vivo* models of fungal infection,[307] demonstrating a compelling and innovative strategy to desensitize drug-resistant fungal pathogens.

Conclusion

Pathogenic fungi are a leading cause of human mortality, particularly among an ever-increasing population of immunocompromised individuals. As newly identified fungal pathogens continue to emerge and previously rare pathogens increase in both prevalence and resistance to antifungal agents, combating these infections is becoming a pressing challenge for global health. Despite the significant burden of fungal infections, fungal diseases have been generally neglected by many public health initiatives[308,309] and require our renewed attention. The development of innovative diagnostics and therapeutics for emerging drug-resistant fungal pathogens holds promise for the future of treating fungal disease, and must continue to be emphasized.

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Competing interests

The authors declare no competing interests.

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