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SUPPLEMENT ARTICLE

Future Research Priorities in Fungal Resistance

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Improved understanding of basic mycological, pharmacological, and immunological processes has led to important advances in the diagnosis and treatment of invasive fungal infections. However, the rise of fungi that are resistant to existing antifungal agents poses a substantial threat to human health. Addressing this expanding problem is an urgent priority for the international research community. In this article, we highlight important diagnostic and therapeutic advances that address the rise of resistant fungi as well as new public health initiatives that warrant further investigation to help curb the spread of these potentially lethal organisms.

Key words. T2 magnetic resonance; resistance-associated mutations; biomarkers; qPCR; antifungal stewardship; vaccine development.

Despite the fact that more persons die from fungal infections each year than by malaria or tuberculosis, the effect of fungal infections on human health is not widely appreciated [1, 2]. The emergence of antifungal resistance to the most commonly used classes of drugs—triazoles, echinocandins, and polyenes—is an expanding public health threat, underscored by the paucity of novel antifungal compounds in preclinical or clinical development [3]. Successfully confronting antifungal resistance will require strategic investment in novel diagnostic platforms, therapeutics, and public health education, as well as enhanced approaches to chemoprophylaxis (Table 1). In this article, we identify the most urgent research priorities to address the global problem of fungal resistance, which continues to cause substantial morbidity and mortality, particularly in patients with impaired immunity.

RAPID DETECTION OF RESISTANCE

Antifungal resistance may be intrinsic or acquired and is associated with elevated minimum inhibitory concentrations, poor clinical outcomes, and breakthrough infections during antifungal treatment and prophylaxis [4]. Resistance may be encountered in the antifungal drug-exposed or drug-naive patients and is particularly challenging when it concerns mycoses with acquired resistance that cannot be predicted from the species identification itself [5]. Owing to the expanding spectrum of causative agents, fast and accurate pathogen detection systems are necessary to identify resistant organisms [6].

Several of the best tools for fungal disease diagnosis do not provide any direct resistance data, notably antigen or antibody detection, microscopy and histopathology, and all forms of imaging. Culture does, but it lacks sensitivity and speed. The development of real-time PCR assays to rapidly and reliably identify invasive fungal infections (IFIs) has been a major advance in the study and treatment of medical mycoses [7, 8]. Despite a lack of standardization regarding sample type, primer selection (panfungal, genus specific, or species specific), and methods (qualitative, quantitative, real time), nucleic acid hybridization-based techniques provide an important alternative for the species-level identification of fungal pathogens and the detection of resistant organisms [9, 10]. However, these advances should be met with a note of caution: it is unlikely that conventional PCR approaches will ever fully account for the diversity of resistance mechanisms at play in eukaryotic organisms [11, 12]. Although many promising platforms have emerged, several warrant particular attention.

PCR Detection of Resistance

Molds

Aspergillus fumigatus is the most common cause of invasive mold infection in immunocompromised patients [13]. Azole resistance in *A. fumigatus* is increasingly reported, and it is assumed that resistance is largely caused by environmental azole exposure [14]. The commercially developed PathoNostics AsperGenius species assay is a multiplex real-time PCR capable of detecting aspergillosis and genetic markers associated with azole resistance [15]. The assay is validated for testing bronchoalveolar lavage (BAL) fluids, replacing the requirement for culture to differentiate susceptible from resistant *A. fumigatus* strains [16].

The assay detects TR34, L98H, T289A, and Y121F mutations, known as resistance-associated mutations (RAMs), in *CYP51A*, a gene that encodes cytochrome p450 sterol 14α-demethylase, the target of azoles [17]. A large retrospective, multicenter study evaluated the diagnostic performance of the AsperGenius on BAL fluid and correlated the presence of these RAMs with azole

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Table 1. Research Priorities

Diagnostics	Therapeutics	Education and Prevention
PCR-based detection of resistance (acquired and intrinsic)	Functional genomics for the discovery of novel drug targets and compounds	Antifungal stewardship
Real-time qPCR to detect "mixed" infection	Combination therapy with existing compounds	Role of fungicides in antifungal resistance
High-resolution melt analysis	Pharmacokinetic studies with novel compounds	Mycotoxin studies
DNA microarray	Pharmacodynamic studies with novel compounds	Global surveillance incidence in clinical isolates
Electron-ionization mass spectrometry	Role of therapeutic drug monitoring	Global surveillance incidence in environmental isolates
MALDI-TOF	Duration of therapy for invasive mycoses	Global surveillance mechanisms
T2 magnetic resonance	Antifungal lock therapy	Role of antifungal chemoprophylaxis in clinical practice
Metagenomic shotgun sequencing	Synergy/antagonism studies with novel and existing compounds	Provider education on antifungal usage
Combination approaches with biomarkers	Immunomodulation and immunopharmacology for augmenting host response	Vaccine development

Abbreviations: MALDI-TOF, matrix-assisted laser desorption ionization time of flight; PCR, polymerase chain reaction; qPCR, quantitative PCR.

treatment failure and death in patients with hematological disease and suspected invasive aspergillosis (IA) [18].

A total of 201 patients each contributed 1 BAL sample; 88 served as positive and 113 as negative controls. PCR results were positive in 74 of 88 positive controls and azole treatment failure was observed in 6 of 8 patients with a RAM, compared with 12 of 45 patients without RAMs (P = .01). The sensitivity, specificity, positive predictive value, and negative predictive value were 84%, 80%, 76%, and 87%, respectively. The 6-week mortality rate was nearly 3 times higher in patients with RAMs (50.0% vs 18.6%; P = .07), suggesting that the assay had a good diagnostic performance on BAL fluid and that detection of RAMs was associated with poor prognosis.

This promising platform has distinct limitations. Although more than 15 *Cyp51A* RAMs have been described, only 4 appear in the current iteration of the assay, and these mutations tend to originate from the environment, not from prolonged azole treatment [19, 20]. Moreover, TR34/L98H and TR46/Y121F/T289A mutations are rarely detectable in patients with culture-negative BAL samples, and up to half of all clinical *A. fumigatus* isolates with phenotypic resistance to azoles have wild-type *cyp51A* sequences [21–23]. Other nongenotype mechanisms of resistance include increased copy number of *CYP51A*, efflux, and mutation of components of mitochondrial complex I [24, 25]. Given the potentially profound impact on patient care—early detection of RAMs can lead to prompt adaptation of the antifungal regimen—further development of this real-time multiplex PCR assay and others are needed to incorporate additional RAMs, including non-*Cyp51A* mechanisms that also confer acquired azole resistance to *A. fumigatus* and other filamentous fungi [26–28].

This proof-of-concept is encouraging. In coming years, this method may be extended to other molds that display high levels of intrinsic resistance to existing antifungal agents, including *Lomentospora prolificans* (formerly *Scedosporium prolificans*), mucormycetes, and *Fusarium* spp. to establish rapid and accurate diagnoses, particularly in high-risk patients, which may alter clinical management, prevent expensive and toxic antifungal treatments, and could potentially improve survival [29–33]. However, the lack of standardization is a major reason why PCR is not yet included in criteria for the diagnosis of most invasive mycoses [34]. It should be a research priority to design robust, prospective studies using PCR to standardize and validate promising methods to identify resistant pathogens.

Yeasts

Epidemiological cutoff values are the most sensitive means for identifying yeast strains with acquired resistance to antifungal drugs [35]. A variety of mechanisms can lead to acquired resistance of *Candida* species to azoles, including induction of the efflux pumps encoded by the *MDR* or *CDR* genes, as well as acquisition of point mutations in the gene encoding for the target enzyme *(ERG11)* [4, 36]. Acquired resistance of *Candida* species to echinocandins is typically mediated via acquisition of point mutations in the *FKS* genes encoding the major subunit of its target enzyme [11, 37]. There has been a reported increase in echinocandin-resistant *Candida glabrata* isolates at medical centers across the United States, and fluconazole insensitivity is the norm in *C. glabrata*, which limits treatment options [38]. It should be noted that fluconazole can be used at higher doses when *C. glabrata* is susceptible [39]. We anticipate that this trend will only continue.

A novel and highly accurate diagnostic platform has been developed for rapid identification of FKS mutations associated with echinocandin resistance in *C. glabrata* [40]. The assay uses allele-specific molecular beacon probes and DNA melt analysis and has the potential to overcome the deficiencies of existing in vitro susceptibility-based assays to identify echinocandin

resistance. Furthering this work to cover the entire FKS mutation spectrum would enhance its appeal as a diagnostic platform.

Drug exposure is an important factor for the emergence of resistance and the expanding use of azoles and echinocandins in prophylaxis is another crucial area for research. Although the benefits of antimicrobial prophylaxis are well documented, these interventions increase patient exposure to antifungal agents and have the potential to promote acquisition of resistance [41, 42]. Strategic investment in acquired resistance and optimal prophylactic strategies will undoubtedly shed light on mechanisms of treatment failure and may lead to more reliable prognoses and improved outcomes, particularly in high-risk patients.

Mixed Infection

The most well-known cause of opportunistic filamentous fungal infection is *A. fumigatus* [43]. However, as the population of immunocompromised patients has expanded, other filamentous fungi have altered the epidemiology of invasive mold infections and taken on increased importance in clinical practice [44, 45]. The variability of antifungal susceptibility of these organisms makes early and reliable identification crucial for effective management, particularly when there is concern for polymicrobial or "mixed" infection [46].

A real-time quantitative PCR (qPCR) assay has been developed targeting the multicopy internal transcribed spacer region of ribosomal DNA, to detect and identify genus and species of *Aspergillus*, the Mucormycetes, *Fusarium*, and *Scedosporium* directly from formalin-fixed paraffin-embedded (FFPE) tissue specimens [47, 48]. In a retrospective multicenter study, 102 FFPE tissue specimens with histopathology results were tested with this real-time qPCR assay, and molecular identification was correlated with results from histological examination [49]. The qPCR assay showed an overall sensitivity of 64% for the identification of fungi from FFPE. Among 59 qPCR-positive specimens, the identification was in agreement between PCR and histopathology in 47 specimens (80%).

However, the assay failed to detect fungal DNA in 9 samples, possibly as a result of the destruction of DNA before paraffin wax embedding. In addition, 10 samples that could not be specified histopathologically were also negative by the qPCR assay, which suggests that internal transcribed spacer sequencing should be used when direct PCR fails. The high sensitivity of this DNA extraction method, which was estimated to be 94%, suggests that this platform warrants further study, because resistant polymicrobial fungal infections are an underappreciated and potentially lethal entity in patients with impaired immunity [50, 51].

Variable Resistance

Mucormycosis is an aggressive infection associated with high morbidity and mortality rates, particularly in patients with hematological cancer [52, 53]. Data on the antifungal susceptibility of mucormycetes are limited, but in vitro studies and clinical experience indicate that fungi of the order mucormycetes have variable resistance to many of the existing antifungal agents, making early diagnosis critical for effective management [54, 55]. In contrast to IA, no such serological test is available for mucormycetes, which makes molecular methods attractive for diagnosis [10, 56].

A semiquantitative method for the specific detection of mucormycetes in tissue samples has been validated using high-resolution melt analysis (HRMA) in BAL samples from immunocompromised patients at risk of invasive fungal disease [57]. In this single-center study, only 9 of 99 BAL samples were PCR/HRMA positive, making it a very useful screening test for testing these nonsterile clinical samples. Moreover, owing to its high negative predictive value (99%), this assay represents a rapid and reliable tool for routine screening for the differential diagnosis of pulmonary infiltrates in immunocompromised patients for *Rhizopus* spp., *Rhizomucor pusillus*, *Lichtheimia corymbifera* (formerly *Absidia corymbifera)*, and *Mucor* spp. Although promising, this single-center study is limited by the low prevalence of disease in its patient cohort. Further research with animal models of mucormycosis as well as multicenter clinical trials might further elucidate the role of PCR/HRMA in clinical practice.

More recently, HRMA has also been used to evaluate mixed fungal infections. A panfungal PCR/HRMA assay has been developed to detect a broad spectrum of the most clinically important fungal pathogens including *Aspergillus* spp., *Candida* spp., and mucormycetes [6]. The high specificity (100%) and negative predictive value (94%) of the panfungal assay suggests it may be a promising screening method for patients at risk of invasive mycoses but robust, prospective studies are needed.

Expanding Role of Circulating Biomarkers

Non–culture-based methods are an appealing modality for the detection of human fungal pathogens [10]. Measurements of fungal biomarkers including (1→3)-β-D-glucan (BDG) assay and galactomannan antigen (GM) have been included as criteria in the definitions of IFI by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group [58]. However, the sensitivity and specificity of these biomarkers vary between patient populations, limiting their use and reliability in certain clinical settings [59, 60].

Recent work that suggests biomarkers in conjunction with other assays may be useful tools for a variety of aspects of patient care, including diagnosis and predicting efficacy of antifungal compounds in prophylaxis. A steroid-immunosuppressed rat model of invasive pulmonary aspergillosis was developed to examine the usefulness of GM and quantitative RT-PCR in evaluating the association between response and exposure after a high dose of prophylactic posaconazole [61]. After prophylaxis, the galactomannan index and fungal burden only decreased

in those animals infected with the most susceptible strains of *A. fumigatus*, suggesting that biomarkers might one day play a role in both risk stratification and optimization of prophylaxis strategies.

Combining non–culture-based methods seems to be an area of remarkable diagnostic promise. A 2016 study evaluated the diagnostic performance of GM and *Aspergillus* PCR by using BAL samples and blood samples obtained from 53 immunocompromised patients (16 with probable/proven IA and 37 with no evidence of IA according to the revised European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria [62]). When interpreted on their own, sensitivities of GM and *Aspergillus* PCR for detecting proven/probable IA were low in BAL samples and even lower in blood, but a sensitivity of 95% was achieved when BAL *Aspergillus* PCR, BAL GM (>1.0 optical density index [ODI]), BAL fluid culture, and serum GM (>0.5 ODI) were combined. Combination approaches warrant increased attention both for the detection of IFI, and when there is clinical suspicion of something unusual (ie, positive halo sign with nondiagnostic culture data). Persistently positive biomarkers, notably serum GM and respiratory *Aspergillus* PCR, in the face of adequate azole antifungal therapy, may be a useful indicator of azole resistance, but this has been hard to prove because cultures are so infrequently positive [63, 64].

In a multicenter prospective study, diagnostic GM, BDG, *Aspergillus* PCR, and a multifungal DNA microarray (chip) were evaluated alone or in combination in BAL and peripheral blood samples from 99 patients with hematological disease and suspected IFI [63]. Combining GM BAL with PCR in BAL samples showed convincing diagnostic potential for diagnosing IA, with sensitivity and specificity of 85% and 97%, respectively. Addition of the DNA microarray enabled detection of 2 more mucormycetes infections, suggesting that a combination of biomarkers is superior to their sole use in diagnosing IFI.

Basidiomycete yeast, such as *Cryptococcus* spp., *Rhodotorula rubra,* and *Trichosporon* spp., are occasional causes of fungemia [65]. From a diagnostic perspective they are distinctive; BDG assay results are usually negative, but results of the cryptococcal antigen test are directly positive for cryptococcosis in serum and can be positive for *Trichosporon* spp. in blood cultures [66, 67]. As pathogens, they are important to recognize rapidly because all are echinocandin resistant [68–70].

IMPROVING ACCESS TO AND USAGE OF FUNGAL DISEASE DIAGNOSTICS

A number of novel diagnostic assays are in development for the early detection of human fungal pathogens, including PCR platforms coupled with electrospray-ionization mass spectrometry or magnetic resonance imaging [10, 71]. In September 2014, the Food and Drug Administration gave marketing approval for the first direct blood test for detecting *Candida* blood stream infections (T2Candida) [72]. This is a magnetic resonance-based approach that measures how water molecules react in the presence of magnetic fields [73]. The platform allows for the lysis of fungal cells, releasing nucleic acids, then makes copies of the target nucleic acids using PCR and detects the amplified DNA in aqueous solution using magnetic resonance. The results provide partial speciation, including a combined *C. glabrata*/*Candida krusei* result, with presumed fluconazole resistance. The speed and sensitivity of T2Candida give it the potential to improve patient care—particularly by tailoring antifungal regimens in high-risk patients, which may prevent the emergence of resistance—but the reagents and instrumentation are expensive. The emergence of *Candida auris* may require reconfiguration of the assay, because this pathogen typically displays high levels of resistance to existing antifungal agents [74, 75].

Access to this and other enhanced diagnostic platforms will be crucial in the global fight to limit the emergence and spread of resistant fungal pathogens. Many clinical laboratories simply may not be able to afford the most promising platforms, which may impede efforts to identify resistant organisms, recognize clinical conditions that warrant unique attention (including cases of high fungal burden), and develop successful antifungal stewardship programs. Even in developed countries, it can be a challenge to obtain the results of GM testing in a timely fashion. Industry should be encouraged to partner with academic medical centers and to create mechanisms that facilitate access to standardized and validated diagnostics.

As novel platforms are implemented into clinical practice, research should be extended to identify barriers to accessing diagnostic services at the individual, provider, and health system levels. In addition, robust data are lacking on how clinicians exercise discretion in using new diagnostic tools for which there is no clear, high-quality evidence; such data may provide insights about how best to introduce new modalities in order to optimize their utilization [76, 77].

PREVENTING RESISTANCE

Thus far, we have focused on methods for detecting resistant fungal pathogens, but prevention of resistance is equally important. Antimicrobial stewardship has gradually matured as a key new tactic for both combating resistance and delivering healthcare savings. Most stewardship programs focus on antibacterial agents; some are specifically antifungal, and others cover all antimicrobials. *Antimicrobial stewardship* refers to coordinated interventions to monitor and direct the appropriate use of antimicrobial agents in order to minimize adverse events, limit selective pressure, and improve outcomes [78]. This involves selecting an appropriate antifungal agent and optimizing its dose and duration to treat an infection while minimizing toxicity and conditions for selection of resistant organisms [79]. Evidence from well-controlled studies examining the impact

of antifungal stewardship on emergence of resistance are limited, but available data on overuse and opportunities to optimize antifungal drug therapy suggest that these interventions are justified.

Essential elements of antifungal stewardship programs have been identified that take into account diagnostic considerations, high-risk patients, and treatment differences for various medical mycoses [80]. The most significant invasive fungal diseases that have implications for antifungal stewardship include IA and invasive candidiasis, but local epidemiology informs the choice of antifungal agents for the prevention and management of most invasive mycoses, underscoring the need for enhanced surveillance [81].

An observational prospective year-long study was conducted by an antifungal stewardship team targeting the use of echinocandins, voriconazole, and liposomal amphotericin B in a tertiary referral hospital [82]. Clinical advice was given and implemented during review of 45 micafungin prescriptions, 70 voriconazole prescriptions, and 78 cases in which liposomal amphotericin B was prescribed. This study found that a crude cost saving of approximately £180,000 in antifungal drugs was generated compared with the previous year, along with substantial improvements in patient management, suggesting that antifungal stewardship programs may be cost-effective and have a positive impact on clinical care.

Compared with antibacterial stewardship programs, antifungal stewardship is in its adolescence. However, one element found in all successful stewardship programs is provider education, either through formal presentations or informal interactions. A limiting factor is sufficient locally available expertise and authority in fungal disease management. Robust investment in research should be a priority to determine additional hospital-based interventions that truly accomplish the goals of reducing resistance and cost while improving outcomes. Ongoing whole-genome sequencing efforts and microbiome research will undoubtedly inform and enhance the capacity to recognize antifungal resistance and provide effective stewardship in the years ahead [83–85]. A recent provocative article addressed the role of better provision of fungal diagnostic testing as a means of curtailing antibacterial usage, which needs to be addressed further [86].

Important questions remain for those who pursue antifungal stewardship: (1) How does antifungal chemoprophylaxis contribute to the emergence of resistance? (2) Should a more directed approach using biomarkers be used to identify patients who will most benefit from prophylaxis? (3) What is the optimal duration of therapy for uncommon invasive mycoses? Answers will require robust investment in pharmacokinetic and pharmacodynamic studies, as well as animal studies and prospective human clinical trials.

Sequestration and Sanctuary Sites

Antifungal stewardship is not the only underappreciated mechanism for the prevention of resistance. It is increasingly recognized that fungal burden and sequestration play important roles in the formation of sanctuary sites, which can allow for emergence of resistant organisms [87]. Classic examples of the sequestration phenomenon include prostatic sequestration of *Cryptococcus neoformans* in an immunocompromised patient treated for cryptococcal meningitis and pulmonary sequestration of an aspergilloma [88, 89]. Sequestration may lead to treatment failure and relapse, which in return may lead to repeated exposure to antifungal agents, which can ultimately lead to emergence of resistance. Understanding the tissue penetration of systemically administered antifungal agents is vital to preventing treatment failure.

The distribution of antifungal drugs from the bloodstream to various tissues is highly variable, especially in sanctuary sites, such as the eye or central nervous system [90]. Moreover, there may be discordance in the shape of the concentration-time profiles for plasma and tissues, known as hysteresis [91]. Because most fungal infections are extracellular, interstitial fluid may be the closest measurable compartment to the site of infection. Although the number of novel antifungal agents in development is limited, pharmacokinetic, pharmacodynamic, and tissue penetration studies will be necessary to determine the role of these new agents in clinical practice.

Biofilms

Fungal biofilms may also serve as a reservoir for infection and promote the development of high fungal burden, which in turn may lead to resistance. Important fungal biofilm resistance mechanisms include: extracellular matrix, efflux pump activity, overexpression of drug targets, metabolic heterogeneity intrinsic to biofilms, and stress responses [92]. The degree of resistance varies with both the drug and species: *Candida albicans* and *Candida parapsilosis* biofilms are relatively resistant to fluconazole, amphotericin B, and voriconazole, whereas *A. fumigatus* biofilms are relatively resistant to itraconazole and, to some extent, caspofungin [93, 94].

Diverse fungi are capable of biofilm production, which presents a challenge in clinical practice. Biofilms have limited drug susceptibility, making device-associated infection extremely difficult to treat, and the role of source control, device removal, and surgical management in clinical practice cannot be overstated. However, it is widely appreciated that biofilmlike growth can occur during a variety of fungal infections, even when an implanted device is not present [95]. How genes and other properties among cells may contribute to the overall development and integrity of fungal biofilms is an important area of inquiry and warrants future research as do the role of combination and lock therapy in treatment.

PUBLIC HEALTH

Infection control measures play an important role in the fight against fungal resistance. These measures occur both in the

hospital and in areas that are far removed from patient care. For example, plant fungal pathogens can have devastating effects on a wide range of crops, including cereals, wheat, and grapes [96]. Azoles are an attractive drug for use in agriculture because they are stable, have a broad spectrum of activity, and are relatively inexpensive. Five azole fungicides are widely used for plant protection: propiconazole, bromuconazole, epoxiconazole, difenoconazole, and tebuconazole [97]. Until recently, the role of fungicides in the emergence of drug-resistant human fungal pathogens has been poorly understood.

Multidrug resistance caused by increased efflux activity is common in human pathogenic microbes but rarely described for plant pathogens. Limited data now suggest that there is a rising prevalence and spread of multidrug-resistant fungal populations among plant pathogens in agricultural environments, owing to the widespread use of fungicides [98]. Current data implicate the bulb industry in the Netherlands and, more recently, flower fields in Columbia [99]. Risk models based on fungal life cycles, fungicide properties, and exposure to fungicides are now being developed and refined to take into account additional traits associated with the rate of pathogen mutation [100]. Looking ahead, it will be important to understand how fungicides are used and how resistance arises to develop strategies that protect crops while limiting selective pressure for emergence of resistant fungal pathogens. It is also important that new *Aspergillus*-active antifungal drugs should be prevented from being used as fungicides through cohesive government action. It will be useful to examine governmental regulations that have similarly addressed fluoroquinolone use in livestock.

Other fungal modeling systems may also have important applications for public health. Predictive models can be a tool to develop strategies to prevent mold development and mycotoxin production, which may be relevant for patients with impaired immunity [101]. This work uses high inoculum states and builds on the work of others who have used this condition to identify resistant fungi [102]. Modeling systems will probably aid in the development of rational public health measures to curb the indiscriminate use of antifungal agents both inside and outside the hospital.

In addition to modeling, global surveillance mechanisms must be in place to monitor fungal outbreaks. The need for such a mechanism was highlighted during the 2012 fungal meningitis outbreak caused by the dematiaceous mold *Exserohilum rostratum*, which was previously an uncommon cause of disease in humans [103]. The nationwide outbreak was a reminder that, although it is often associated with immune impairment, IFI may be iatrogenic and can occur with contaminated medical supplies [103].

E. rostratum has variable susceptibility to various antifungal agents, and the breadth of the outbreak—there were 64 deaths across 20 states—underscored the need for definitive animal models for characterization of the antifungal pharmacokinetics, pharmacodynamics, molecular detection, and therapeutic monitoring to provide a scientific foundation for treating affected patients [104, 105]. Although it is no longer ongoing, the fungal meningitis outbreak revealed the difficulties and limitations associated with the diagnosis and treatment of mold infections of the central nervous system, which are often resistant to existing antifungal agents [45].

The therapeutic challenges of the outbreak reinforced the need for novel antifungal agents. The hurdles associated with antifungal drug development are well documented and are primarily due to the facts that fungi are eukaryotes and many proteins that are potential targets are also found in humans. However, functional genomics is a powerful tool in drug discovery, and the platform should be harnessed to expand the drug pipeline to treat resistant fungi. The emergence of multidrug resistant *C. auris* has served as a reminder of the need for enhanced surveillance and novel therapeutic strategies [106, 107].

CONCLUSIONS

The routine use of foreign bodies in medical practice, such as central venous catheters, prosthetic joints, and permanent pacemakers, along with successful management of immunosuppression in patients with underlying diseases ranging from cancer to inflammatory bowel disease, has contributed to the global increase in fungal infections [104, 108, 109]. In this setting, the emergence of antifungal drug resistance has become an increasing cause for concern. The development of molecular detection methods, including HRMA/PCR, microarrays, and metagenomic shotgun sequencing, has been a major advance in addressing drug resistance, but mortality rates remain high even when patients receive appropriate and timely antifungal therapy [79, 110]. Investment in research is needed to develop and enhance diagnostic platforms that ca rapidly and reliably detect resistant mycoses.

Beyond nucleic acid hybridization-based assays, other non–culture-based methods warrant further investigation. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) has been applied to both yeast and filamentous fungi for rapid and reliable diagnosis of invasive mycoses [111, 112]. This platform is based on the generation of an isolate-specific spectral profile, primarily determined by ribosomal protein content, that is compared with a database of reference spectra, yielding a list of top-matching identifications [113]. However, generating a custom reference database is not feasible for most clinical laboratories because it requires extensive in-house validation, so many laboratories rely on manufacturer-developed databases [114]. Further investment is needed both to enhance these databases and to standardize performance of MALDI-TOF for human fungal pathogens.

Novel approaches to treatment are also urgently needed. Beyond the strategies mentioned above, there is important work to do investigating the role of innate and adaptive immunity in preventing and treating resistant fungal infections. Selected targeting of signal transduction pathways, including the Toll and JAK-STAT pathways, may allow for more precise immunosuppression without significant impairment of innate host defenses against fungi [115, 116]. Moreover, improved understanding of adaptive immunity has enabled the development of several new fungal vaccines, some of which are currently being studied in human clinical trials [117–121].

In addition to early diagnosis and improved therapeutics, further research and education are needed to optimize the role of antifungals in crop protection and as antimicrobial prophylaxis in clinical practice. The significant increase in the use of antifungals in these areas has contributed to the emergence of resistant clinical isolates, particularly those resistant to triazoles and echinocandins, and further work is needed to maximize the efficacy of antifungals as fungicides and prophylactic agents [122, 123]. High-risk patients, including those with chronic chest disease and immune impairment, must be educated about the risks of various environmental exposures (eg, bark chippings, composting, remedial building work, fixing leaks at home, etc) [124–126].

We must also confront the reality that climate instability may increase the prevalence of invasive mycoses in humans [127, 128]. It has been hypothesized that this will occur via 3 mechanisms: (1) biogeographic expansion of currently pathogenic species (eg, coccidioidomycosis); (2) selection for thermotolerant species with significant pathogenic potential that are currently not pathogenic by virtue of being restricted by mammalian temperatures (eg, *Cryptococcus laurentii*); and (3) trauma (eg, necrotizing cutaneous mucormycosis after a tornado in Joplin, Missouri) [128–131].

Finally, we must invest in the development of novel agents to treat emerging and resistant fungi. The number of compounds in development is limited, but tremendous opportunities exist for drug development. These may include (1) new antifungal agents, (2) repurposed existing agents (eg, the antiparasitic miltefosine, which has antifungal activity, (3) analogues of existing antifungal agents (eg, derivatives of terbinafine), and (4) combination therapy [2, 132, 133]. Curbing the spread of resistant fungi will ultimately require sustained investment in a multifaceted approach—diagnostics, therapeutics, prevention, and education—that draws on expertise from a variety of disciplines, made accessible to all.

Notes

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