Clinical utility of antifungal susceptibility testing

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Invasive fungal diseases cause significant morbidity and mortality, in particular affecting immunocompromised patients. Resistant organisms are of increasing importance, yet there are many notable differences in the ability to both perform and interpret antifungal susceptibility testing compared with bacteria. In this review, we will highlight the strengths and limitations of resistance data of pathogenic yeasts and moulds that may be used to guide treatment and predict clinical outcomes.

Introduction

The development of novel monoclonal antibodies, transplant techniques and other immunomodulatory treatments has revolutionized the field of medicine. Consequently, infections by organisms that take advantage of the immunosuppressed host, such as invasive fungal disease (IFD), have increased as a cause of morbidity and mortality.1,2 Fungi occupy three places on the CDC’s 2019 Antibiotic Resistance Threats Report—Candida auris, drug-resistant Candida and azole-resistant Aspergillus fumigatus. Antifungal resistance testing data are not always clear and cannot be readily translated into a prediction for or against clinical success. While in vitro susceptibility has the potential to predict clinical success, this decreases with increasing patient and infection complexity and resistance is even less likely to be correlated with predictive failure.3,4 In the following sections we discuss the performance of susceptibility testing in fungi and provide a foundation for assisting the clinician with interpreting these data.

Methods of antifungal resistance testing

Similar to bacterial infections, obtaining in vitro antifungal susceptibility testing for pathogenic fungi has become important for guiding effective treatment.5 The identification of fungi (especially moulds) requires complex macro- and microscopic examination by skilled laboratory scientists, and relatively few companies produce either manual or automated antifungal testing products. Furthermore, although there are many bacteria that have standardized breakpoint values for determining susceptibility or resistance, relatively few fungi have assigned breakpoints, and those that do may only have breakpoints for a small subset of antifungal compounds. The following section will review testing methodology and definitions as well as outline limitations to the current practice of antifungal susceptibility testing. Figure 1 from an antifungal susceptibility testing review paper by Dr Nathan Wiederhold shows a variety of examples of these testing methodologies.5

Standardized methods for broth macro- and microdilution testing are available from CLSI and EUCAST for yeast and filamentous fungi.6,7 The yeast standards include how to test pathogenic yeasts such as Candida spp. and Cryptococcus spp. whereas the filamentous fungi standards are intended for those fungi that cause invasive and cutaneous infections. Preparation of yeast for testing is quite simple—a dilution of colony growth directly from the plates is created to meet a specific turbidity measurement.6,7 Filamentous fungi, however, require culturing to first induce conidial induction followed by removal of the conidia and adjustment to specific optical densities or conidial concentrations (tasks often outside of the ability of most clinical microbiology laboratories).6,9 Results are read at variable intervals based on the species of yeast or mould, ranging from 24 to 96 h. Interpretation of testing results may be difficult; yeasts are read to the first well with approximately 50% reduction in growth (compared with the control) for the azoles, echinocandins and flucytosine, whereas amphotericin B is read to the first well with no discernible growth. Interpretation of results for filamentous fungi are more tenuous, with some agents judged at 50% inhibition while others are read at 80% or 100% inhibition.

Some antifungal compounds are available for testing through gradient diffusion or disc diffusion methods.10,11 An advantage of disc and gradient diffusion methods is the greater accessibility to clinical laboratories, resulting in a faster turnaround time as testing can be performed in house rather than sent to a reference laboratory. Furthermore, gradient diffusion testing presents a simpler method for determining inhibitory values. Disadvantages include not all antifungal agents being available (or regulatory-body approved) to test by these methods in every country. Disc diffusion interpretive breakpoints are only available for some Candida spp.

Both broth microdilution and gradient diffusion testing allow for the determination of minimum inhibitory concentrations (MICs), the lowest concentrations (mg/L) in which the agent causes a specified reduction in visible growth of a microorganism. Importantly, the MIC value is different from the minimum effective concentration (MEC), or the lowest...
Figure 1. Examples of antifungal susceptibility testing. Examples of various phenotypic susceptibility results for yeasts and moulds. (a) shows the results of broth microdilution susceptibility testing per the Clinical and Laboratory Standards Institute methodology for fluconazole against Candida albicans, voriconazole against Aspergillus fumigatus, and amphotericin B against Purpureocillium lilacinum. (b) shows susceptibility results as measured by the YeastOne colorimetric assay against Candida species. (c) shows susceptibility results as measured by gradient diffusion for amphotericin B against A. fumigatus, isavuconazole against Cryptococcus neoformans, and caspofungin against Candida glabrata. (d) shows the minimum effective concentration (MEC) results for micafungin against an Aspergillus nidulans and an unidentified mould isolate. All testing was performed in the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio. Red boxes in (a), (b) and (d) indicate minimum inhibitory concentration (MIC)/MEC values. Figure and legend reused with permission from: Wiederhold NP. Antifungal susceptibility testing: a primer for clinicians. Open Forum Infect Dis 2021; 8: ofab444; https://doi.org/10.1093/ofid/ofab444 (CC BY-NC-ND 4.0). Part labels have been re-lettered and spellings in the legend have been changed to UK English in line with JAC-AMR style.
concentration in which the agent leads to growth of small, compact, hyphal forms as compared with the growth seen in the no-drug control. MEC is only used for the echinocandin class of drugs in filamentous fungi. These values can then be interpreted using standardized breakpoints to determine whether an organism is susceptible, susceptible dose-dependent, intermediate or resistant to the tested compound. Of note, both CLSI and EUCAST publish breakpoints for in vitro antifungal susceptibility testing. Differences between these standards are evident when comparing the species with breakpoints, agents with breakpoints, and actual breakpoint values. For example, CLSI regards *Candida albicans* to be susceptible to fluconazole when in vitro MIC values are $\leq 2$ mg/L, susceptible dose-dependent at $4$ mg/L, and resistant at concentrations $\geq 8$ mg/L whereas EUCAST has determined breakpoints to be at $\leq 2$ mg/L and $\geq 4$ mg/L for susceptible and resistant, respectively. For the filamentous fungi, CLSI only has breakpoints for voriconazole and *A. fumigatus* whereas EUCAST has developed breakpoints for five *Aspergillus* species against multiple azoles and amphotericin B.

For organisms that do not have MIC or MEC breakpoint values established, both CLSI and EUCAST have developed epidemiological cut-off values (ECVs). An ECV is the highest MIC or MEC value within the WT population that designates whether a given isolate tests as WT or as non-WT against the tested agent. In comparison to MIC or MEC breakpoints, which incorporate pharmacokinetics, laboratory and clinical data, ECVs are based solely on *in vitro* laboratory testing of large numbers of isolates and should not be used to predict the clinical outcome of the agent. Instead, the ECV is meant to show whether an isolate displays WT characteristics (an MIC or MEC at or below the ECV) or whether it has acquired a resistance mechanism and is now non-WT (an MIC or MEC above the ECV). For example, *C. albicans* does not have clinical breakpoints for amphotericin B. Instead, *C. albicans* has an ECV of $2$ mg/L, suggesting that any isolate testing greater than this value has acquired a resistance mechanism to amphotericin B and treatment with this drug may not be effective. Although this appears straightforward, some ECVs may not be as intuitive. For instance, a mould may have an ECV for an antifungal agent at a very high MIC/MEC, such as $128$ mg/L. However, this value simply means that isolates testing at values below the ECV (such as $64$ mg/L) are considered WT even though the antifungal agent may have no effect in vivo. As such, discussions between the laboratory, infectious diseases pharmacist and infectious diseases physician are essential to determine the utility of an ECV in each clinical scenario.

**Candida**

*Candida* species are the fungal pathogens for which antifungal susceptibility testing can be an accurate predictor of treatment outcome. The availability of commercial assays with good agreement with broth microdilution reference methods are available for in-house testing, but these may not be available in every hospital and could still require sending out to reference labs. *Candida* spp. are among the 10 most common causes of bloodstream infections. *C. albicans* causes the majority of pathogenic human disease worldwide; in North America this has fallen to a plurality rather than a majority as non-*albicans* species have increased in frequency, particularly *Candida glabrata*, as the second most common species. These same SENTRY data show significant worldwide variability. *C. albicans* as the most common species in every region, however the Asia-Pacific region shows roughly even amounts of *C. glabrata* (renamed to *Nakaseomyces glabrata*), *Candida parapsilosis* and *Candida tropicalis*; Europe has higher rates of *C. albicans* than other regions with even rates of *C. glabrata* and *C. parapsilosis*; and the Latin American region has higher rates of *C. parapsilosis* and *C. tropicalis* with comparatively little *C. glabrata*. Identification of the species can inform risk of resistance in combination with knowledge of local prevalence, but shifts over time have made this increasingly imperfect, emphasizing the importance of accessible testing. Echinocandins represent the mainstay of empirical treatment with fluconazole serving the role of targeted therapy when patients improve and species and/or resistance information is available.

**Flucanazole resistance**

Flucanazole resistance in *Candida* species can be mediated via multiple different mechanisms, however the majority focus on the 14α-demethylase gene that is key in the production of ergosterol, usually leading to resistance across theazole class. Other methods such as change in sterol composition and efflux pumps also occur.

Resistance to flucanazole is uncommon, ranging from less than 1% to over 10% depending upon species, time period and geography. With respect to *C. albicans*, flucanazole resistance remains rare, occurring in less than 1% of isolates in both CDC and SENTRY data. *C. tropicalis* and *C. parapsilosis* demonstrate more flucanazole resistance that is also more variable, with CDC data suggesting a rise in *C. parapsilosis* from 4.4% to 14% over time while SENTRY data ranges from 2.5%–5.5%; *C. tropicalis* is more consistent between the two ranging from 1.7%–7.9% with the CDC and 2.0%–4.9% with SENTRY data. With these baseline resistance rates in mind, the IDSA Candida treatment guidelines allow for starting with empirical flucanazole instead of an echinocandin in haemodynamically stable patients with low rates of resistance, further emphasizing the need to know the local ecology as well as individual patient risk factors. *C. glabrata* and *Candida krusei* (renamed to *Pichia kudriavzevii*) are the predominant species found to have flucanazole resistance. As *C. krusei* is innately resistant to flucanazole due to reduced susceptibility to inhibition of its 14α-demethylase enzyme, flucanazole resistance in *C. glabrata* has remained relatively stable with both CDC and SENTRY data consistent with each other ranging from 5%–10% of isolates with resistance. As *C. auris* continues to spread throughout the globe, increasingly encountered in multiple countries, it’s important to note that near universal resistance to flucanazole has been observed caused by varying mutations in ERG11 depending on the geographic clade. In practice, risks for both non-*albicans* candidaemia and flucanazole resistance itself should be considered if contemplating empirical azole therapy or if an echinocandin is not available. Multiple studies have shown prior antifungal exposure to be a risk factor for the development of both flucanazole resistance as well as non-*albicans* species, with one study also showing certain antibacterial agents could increase the risk for flucanazole resistance. High rates of non-*albicans* candidaemia and candidaemia have also been identified in patients with malignancy or other immunosuppressive conditions including transplantation. Currently established breakpoints for *Candida* have shown a consistent correlation with outcome prediction in candidaemia, invasive candidiasis and mucosal/oropharyngeal candidiasis.

**Echinocandin resistance**

Echinocandins function via the inhibition of 1,3-β-glucan synthase, disrupting the cell wall formation. *In vitro* studies have shown a high frequency of elevated echinocandin MICs in *C. parapsilosis*, but this has not been associated with treatment failure. Resistance is primarily driven by point mutations in the genes encoding the fks1 and fks2 subunits of the glucan synthase enzyme. Echinocandin resistance leading to treatment failure was first established in *C. albicans* in 2004 emerging during caspofungin treatment for azole-resistant oesophageal candidiasis. The following years saw increased reports of clinical failure, and the CLSI breakpoints that were initially established in 2007 had to be revised in 2009 to much lower MIC cut-offs. With the establishment of these updated interpretation guidelines, cut-offs are specific to both the species and echinocandin, having previously begun as a single breakpoint applied to all species and consistent across all echinocandins. The most common species to exhibit echinocandin resistance and subsequent treatment failure is *C. glabrata* with frequency ranging from 1.3%–8.2% depending on the time period and epidemiological survey.
with resistance only rarely identified in other species. Treatment failure can also be seen in the presence of mutations in these genes without phenotypic resistance.Individual centres have reported rates in excess of 10%. Prior exposure to an echinocandin is the most consistent risk for either phenotypic resistance or presence of an FKS mutation. It should be noted that caspofungin is a less reliable agent for assessing for resistance compared with micafungin and anidulafungin, and both micafungin and anidulafungin can be used as surrogates across the spectrum of echinocandins. Combined with the knowledge that higher rates of caspofungin are noted that caspofungin is a less reliable agent for assessing for resistance compared with micafungin and anidulafungin, and both micafungin and anidulafungin can be used as surrogates across the spectrum of echinocandins. Combined with the knowledge that higher rates of caspofungin are also seen in the presence of mutations in these genes without Cl. glabrata occur in the immunocompromised, our most at-risk patient population is then the subject to the greatest risk of encountering resistance. The majority of C. auris isolates are found to have in vitro susceptibility to echinocandins although resistance is well documented and varies by geographic region. Clinicians must have a high degree of suspicion when patients fail to respond to an echinocandin and a low threshold to ensure susceptibility testing is undertaken.

**Cryptococcus**

Antifungal resistance among Cryptococcus species is incompletely understood, and there is disagreement as to the clinical relevance of elevated MICs of selected agents, in particular the azole antifungals. Moreover, there are no currently accepted clinical breakpoints for individual antifungal agents among either of the two major cryptococcal species, Cryptococcus neoformans and Cryptococcus gattii. Most of our knowledge pertaining to antifungal resistance in Cryptococcus is based on in vitro data, as there are few clinical correlates that correspond with higher MICs. In the absence of clinical breakpoints, ECVs have been defined for Cryptococcus.

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**Aspergillus**

Aspergillus fungi cause a wide range of human disease: allergic conditions such as allergic bronchopulmonary aspergillosis and sinusitis; colonization or semi-invasive infection of cavitary lung lesions, such as aspergilloma or chronic pulmonary aspergillosis; and invasive disease, which typically occurs among immunocompromised patients, patients in the ICU, or those with recent viral pneumonia. Clinical outcomes, especially for invasive aspergillosis (IA), have improved over the past several decades. An important component associated with improved clinical outcomes was the development of the triazole antifungal drugs (i.e. fluconazole, voriconazole, posaconazole and isavuconazole). These drugs have proven to be safe and effective and are used routinely in the prevention and treatment of various forms of aspergillosis.

Although antifungal resistance among Aspergillus is uncommon, there has been an emergence of azole resistance. In early reports, A. fumigatus azole resistance was identified among patients who were treated with prolonged courses of itraconazole. This suggested that chronic exposure to azoles, perhaps at subtherapeutic levels, was important to induce resistance. However, azole resistance was also detected among patients without clinical exposure to azole antifungals. The resistance mechanisms identified in these patient isolates were found to be present in environmental isolates, suggesting a second method of resistance induction, whereby fungi in the environment are exposed to azole compounds (fungicides in agriculture) that are similar to triazoles. Indeed, azole fungicides are used throughout the world to prevent and treat fungal infections of crops and use has increased in multiple countries over the past decade.

Among resistant isolates, the most common resistance mechanisms are point mutations in the Cyp51A gene. Cyp51A encodes 14α-demethylase, a target of azoles in the pathway of ergosterol synthesis. Among environmental isolates, resistance is often mediated by tandem repeats of base pairs within the promoter region of Cyp51A, which leads to increased gene expression. Prevalent mutations include TR34/L98H and TR46/Y121F/T289A and these often result in multi-azole resistance.
Determining the prevalence of Aspergillus resistance is challenging. First, as discussed previously, susceptibility testing has limitations and breakpoints for resistance are not available for all drugs.5 Most microbiology laboratories do not perform in-house antifungal susceptibility testing for aspergilli. Second, most cases of invasive aspergillosis are diagnosed using fungal biomarkers (galactomannan, β-D-glucan) and cultures may not be available to perform susceptibility testing. To date, non-culture-based molecular testing that identifies Aspergillus resistance genes is not widely employed.79

There have been numerous reports of resistance among Aspergillus isolates, with azole resistance dating back to the 1990s.67–70 Resistance is now widespread in the world and an emerging problem in the last two decades. Among European countries, resistance appears to be highest, and has been seen in up to 25% of isolates in some areas, such as the Netherlands.80,81 Outside of Europe, resistance appears to be less common, but it has been reported in the USA, Japan, China, Iran, India, Taiwan, Brazil, Peru, Mexico, Australia and Argentina.68,70,82–86

Pre-clinical data in animal models using Aspergillus isolates with increased MICs of azoles demonstrates decreased efficacy.87,88 It is important to note that many factors, including resistance, underlying disease or comorbidities, disease burden and immune suppression, among others, may contribute to outcomes in patients with aspergillosis. There are several human retrospective studies that highlight mortality and clinical responses among patients with azole-resistant aspergillosis.81,89–91 Early studies showed that in general, patients with azole-resistant aspergillosis have increased mortality (>50%), although not all studies were well-controlled.81,93

A recent retrospective, multicentre study evaluated mortality among 196 patients with IA, 37 (19%) of whom had a voriconazole-resistant infection.92 When compared with voriconazole-susceptible cases, voriconazole resistance was associated with an increase in overall mortality by 21% at Day 42 and 25% at Day 90. Moreover, mortality among patients who received appropriate initial therapy was lower than in those who did not, despite patients who switched to appropriate therapy later in the study, suggesting that early institution of appropriate therapy impacts clinical outcomes.92–94 Heo and colleagues95 investigated in vitro susceptibilities of Aspergillus to triazoles and outcomes in a large tertiary-care cancer centre from 1999–2015. Non-WT MICs were identified in 37 (13%) isolates. In contrast, there was no correlation of MICs with 42 day mortality in patients with invasive pulmonary aspergillosis, respective of antifungal therapy. Andes and colleagues96 evaluated clinical outcomes by MIC values for isavuconazole and voriconazole from a recent clinical trial.94 They concluded that for Aspergillus species with high MICs (>16 mg/L), isavuconazole and voriconazole may have reduced efficacy; however, this was not apparent for aspergilli with lower MICs (<16 mg/L).

On the basis of available data, it is important to understand local epidemiology of azole-resistant aspergillus, but as mentioned previously, an important limitation is lack of routine susceptibility testing for Aspergillus at many centres and lack of global surveillance systems.95,96 As early appropriate treatment may impact patient outcomes, resistance has resulted in a re-evaluation of management strategies at some centres. Some experts recommend alternative antifungal therapy to azoles when resistance among Aspergillus isolates is above a certain threshold.96 For example, for patients with suspected IA in an area with resistance >10%, empirical therapy with a triazole plus an echinocandin or a lipid preparation of amphotericin B should be considered.96,98–100

In summary, azole resistance among Aspergillus species, primarily A. fumigatus, is emerging worldwide, and likely related to use of fungicides in agriculture. Although data on MICs and clinical correlation are limited, there is concern for decreased azole efficacy and increased patient mortality in patients with azole-resistant aspergillosis. To improve patient care, coordinated global surveillance, increased availability of antifungal susceptibility testing and dialogue with the agriculture industry are needed.

Other moulds

Resistance among non-Aspergillus moulds is poorly understood, in particular in the context of the ability to predict clinical success in failure. This is related to the complexity of the patient population at highest risk for invasive mould disease, as it is rarely found outside of a variety of immunocompromised patient populations. Immune function of the patient figures heavily into treatment success or failure. Additionally, given this lack of clear evidence for a baseline understanding, it’s difficult to describe any potential changes in the epidemiology of resistance in these pathogens.

It is difficult to both obtain and interpret antifungal susceptibility testing for non-Aspergillus moulds; therefore this section will focus on three of the more common genera encountered, their innate resistance patterns based on clinical practice and epidemiological surveys, and the importance of early identification to allow for optimal antifungal therapy.

Mucorales

The importance of Mucorales as a devastating pathogen has been highlighted by the high rates of complication in the setting of the COVID-19 pandemic.97 The most common syndromes caused by Mucorales are rhino-orbital-cerebral, pulmonary, and cutaneous disease, and there should be a high degree of suspicion in rapidly progressive, necrotic presentations of these conditions, in particular in the immunocompromised patient. Adjunctive assays for possible fungal infection (1,3-β-D-glucan, Aspergillus galactomannan) are expected to be negative in these patients. The diagnosis is made via a combination of the clinical syndrome, histopathology and fungal culture of the involved site.

Treatment of choice begins with amphotericin B preparations and surgical debridement of the involved tissue. Therapeutic options when ready for an oral transition are posaconazole and isavuconazole. A study of over 800 isolates collected over 52 months by Badali et al. showed amphotericin B has the most reliable and best in vitro activity, albeit less Cunninghamamelia. Comparing the two triazole options, posaconazole was more active than isavuconazole, but significant variability was seen across both agents. Resistance in this instance is intrinsic and specific to the various genus and species. At minimum, species level identification should be obtained prior to considering a transition to oral step-down therapy to ensure the highest potential activity.

Fusarium

Fusarium can cause a variety of superficial and invasive syndromes. It is a common pathogen of keratitis and onychomycosis in the immunocompetent patient population.99,100 In the immunosuppressed the most common syndrome is disseminated disease in the setting of prolonged neutropenia.101 Syndromes that can be encountered before and after dissemination include cutaneous, pulmonary and sinus disease. Among invasive moulds, Fusarium has the unique feature of frequently identified fungiacta, with blood cultures positive in nearly half of patients.101

When evaluating potential cut-off values for the definition of reduced susceptibility, significant variation is noted across three primary species complexes based around Fusarium solani, Fusarium oxysporum, and Fusarium verticillioides, although this does not encompass all pathogenic species.102–104 In spite of this variability and high rates of resistance to the most commonly used agents (amphotericin B, mould-active triazoles), cure is feasible and in vitro resistance is not consistently predictive of clinical outcomes; resolution of neutropenia was associated with a greater likelihood of clinical success.104,105 This discrepancy emphasizes the need to devise a means for prospective trials aimed at these rare moulds to achieve better data-driven treatment approaches. A typical approach would be to start with amphotericin B, voriconazole, or a combination of the two, while awaiting species identification and taking into account the presence of mould-active prophylaxis in the case of breakthrough infections.105,106
Lomentospora

Lomentospora prolificans (formerly Scedosporium prolificans) is primarily a pathogen of the immunocompromised, though local infections, especially in the setting of contaminated trauma, can be seen in the immunocompetent.107–109 Pneumonia is the most common invasive infection, but it is also known to cause keratitis, CNS infection, skin and soft tissue infection, osteomyelitis and disseminated disease (including endocarditis). Currently available antifungals demonstrate poor in vitro activity against L. prolificans.110,111 There are some in vitro data to suggest the addition of terbinafine to an azole could have improved activity via synergy.112,113 A FungiScope registry study showed higher rates of survival and treatment success with a combination of voriconazole with terbinafine compared with monotherapy regimens.114

Conclusions and future directions

Antifungal resistance and its clinical implications are incomplete ly understood. While there are some areas such as the management of Candida that can show correlation between susceptibility, resistance, and treatment outcomes, these data are still underwhelming for many other invasive fungal infections. This is likely owed in no small part to the underlying conditions of patients who are prone to developing these infectious syndromes, as many can be a part of polymicrobial infections or in the setting of significant immunocompromising conditions. For most invasive fungal infections with culture growth of the causative organism, we suggest routinely obtaining antifungal susceptibility testing, with Cryptococcus being the lone exception as detailed above. However, it must be stressed that in vitro susceptibility testing should be viewed as one data point used to best guide treatment for an individual patient and not seen as infallible.

Hope for the future exists in the form of investigational agents. While the rate of new antifungals has lagged the number of new antibacterial agents, there are multiple important agents offering novel mechanisms of action for pathogenic yeasts and moulds.115 There are two agents, fosmanogepix and ibrexafungerp, that have been shown to retain activity against azole- and echinocandin-resistant strains of Candida.116–118 Olorofim and fosmanogepix both show a higher degree of in vitro activity compared with currently approved agents for Lomentospora as well as other moulds.119–124 Both agents are in Phase 2 clinical trials investigating clinical efficacy against these pathogens, including Fusarium and Aspergillus; in the case of fosmanogepix, Mucorales is also included (ClinicalTrials.gov identifiers: NCT03583164 and NCT04240886).

Transparency declarations

T.P.M. reports research contracts with Pfizer, Amplyx (now acquired by Pfizer), F2G, T2 Biosystems, Scynexis and Cidara; consulting with T2 Biosystems; and speaking honoraria with GenMark Dx. P.M.L. reports leadership roles (unpaid) with American Society for Microbiology Maryland Branch and Fungal Diagnostics Laboratory Consortium. J.W.B. reports consulting with Pfizer. P.G.P. reports research contracts with Scynexis, Cidara, Mayne and Astellas and consulting fees with F2G and Cidara.

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