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ANTIMICROBIAL DEVELOPMENT AND DRUG RESISTANCE (K CLAEYS AND A VEGA, SECTION EDITORS)

Antifungal Resistance: a Concerning Trend for the Present and Future

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Abstract

Purpose of Review The global emergence of antifungal resistance among *Candida* spp. and *Aspergillus* spp. is a growing threat to public health, driven largely by the expanding use of antifungals in both the clinical and agricultural settings. As treatment options remain limited, understanding mechanisms and risk factors for antifungal resistance is essential to retaining their clinical utility.

Recent Findings Invasive candidiasis is increasingly caused by non-*albicans Candida* species with reduced susceptibility to first-line antifungals, making empiric treatment decisions difficult. Echinocandin resistance in *C. glabrata* is increasing at some high-risk centers, and multi-drug-resistant isolates are increasingly encountered. Of large concern is the rapid and global emergence of *C. auris*, a species associated with a high propensity for developing multi-drug resistance and nosocomial transmission. Azole resistance is now becoming more common in *Aspergillus* isolates as well, with breakthrough infections occurring in patients previously managed with azoles antifungals. The appearance of azole-resistant *Aspergillus* isolates in azole-naïve patients is also concerning, given it is now accepted that this may be due to the use of non-human azole compounds in pesticides.

Summary Due to the climbing use of antifungals in both the clinical and agricultural sectors, the frequency of encounters with antifungal-resistant isolates will undoubtedly rise in parallel. Antifungal stewardship will need to become a new priority for antimicrobial stewardship programs in order to preserve our current selection of antifungal agents. Rapid diagnostics may help stewardship efforts by decreasing the time it takes to determine if an antifungal agent is indicated for a patient.

Keywords *Candida* · *Aspergillus* · Antifungal resistance · Azole · Echinocandin · Antifungal susceptibility

Introduction

Antimicrobial-resistant microorganisms, including fungi, are increasingly common. While this is a well-recognized problem, the majority of public attention and antimicrobial research and development has been focused on multi-drug-resistant bacteria, perhaps to the detriment of antifungal resistance. Mortality rates due to invasive fungal infections are often 50% or higher, particularly in the setting of antifungal resistance; therefore it is imperative to have a greater understanding on the emergence of resistance and lack of available treatment options [1, 2]. Due to their frequent empiric use in critically ill patients, routine prophylactic use, and widespread use of antifungal-based pesticides, the problem of antifungal resistance is likely to worsen significantly [3]. The worldwide emergence of *Candida auris* and resultant morbidity and mortality highlights this critical point [4]. As antifungal resistance is becoming increasingly common and relevant to clinicians, it is important to frequently review the current literature to inform the medical community of new findings and data. Therefore, we performed a comprehensive literature review to provide an update on the epidemiology and resistance mechanisms against azoles and echinocandins in *Candida* and *Aspergillus* species.

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Epidemiology of *Candida* and *Aspergillus* Resistance to Azole Antifungals

Candida Resistance

Azole antifungal agents have been a mainstay of therapy for over 50 years; therefore, it is not surprising that with such a pedigree of clinical use, we are now combating the issue of emerging resistance. *Candida albicans* has remained the most common cause of invasive candidiasis (IC) in the USA, followed by *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* [5]. Compared with the late 1990s, the frequency of IC due to non-albicans species has increased in comparison to IC due to *C. albicans* [5]. Large-scale antimicrobial surveillance studies, such as those produced by SENTRY, have found that North America has the highest rate of IC due to *C. glabrata* (24.6%), and the lowest rate of IC due to *C. albicans* (42.7%) compared with other geographical regions of the world. Furthermore, smaller surveillance studies and single-center reports have found infections due to *C. glabrata* now exceed those of *C. albicans* in some populations. The proportion of IC due to *C. albicans*/non-*albicans* is significantly different in the USA (37/63, 35/65) compared with several other regions of the world except for Latin America (38/62) and Brazil (34/66). Interestingly, the proportion is flipped in Canada (62/38), although the data is older (2003–2005) [6]. Due to these trends of increased IC due to non-*albicans* species, the emergence of resistance is of higher concern. Several surveillance population-based studies have shown increasing rates of fluconazole resistance in non-albicans species. In a Danish surveillance study, an increase in azole resistance was observed in 2012–2015 compared with 2008–2011 and 2004–2007 with a total of 60.6% of isolates being susceptible to azoles, compared with 65.2% and 68.5% respectively. In this study, the rates of non-susceptibility were mainly driven by *C. glabrata*, in which 9.1% of isolates were resistant [7]. Worldwide trends in the SENTRY program for fluconazole resistance for *C. albicans* (0.3%) has remained low in comparison to a relatively high rate of 8.1% for *C. glabrata*. Resistance rates for *C. tropicalis* (3.2%) and *C. parapsilosis* (3.9%) fall into the mid-range for resistance and differ by each region. Resistance rates have worsened over the past decade for *C. glabrata* and *C. parapsilosis*. The highest resistance rates for *C. glabrata* were observed in North America (10.6%) followed by the Asia-Pacific (6.8%), Europe (4.9%), and Latin America (2.6%). Contrasting with *C. glabrata* resistance, *C. parapsilosis* resistance to fluconazole is higher in Europe (4.6%) and Latin America (4.3%) compared with North America (3.7%) and Asia-Pacific (0.6%) [5].

Emergence of *Candida Auris*

Very recently, a non-*albicans Candida* species, *C. auris* has caused great concern in the medical community. Due to its ability to spread rapidly throughout critically ill patients and intensive care units, *C. auris* represents a new obstacle for infectious diseases practitioners and infection control specialists [8]. *C. auris* possesses several characteristics that increase its risk of becoming global threat. Multi-drug resistance is common for *C. auris* with differing resistance patterns throughout the world. In a study out of 350 isolates in India, 90% of isolates were resistant to fluconazole, 8% to amphotericin B, 2% to anidulafungin, and 2% to micafungin [9]. A separate study ($n = 54$ isolates) from Africa, Asia, and South America reported higher resistance rates with 93% resistance to fluconazole, 35% to amphotericin B, and 7% to echinocandins. Resistance to two antifungal agents was observed in 41% of isolates as well, increasing the concern regarding the tendency for *C. auris* to exhibit multi-drug-resistant properties [10]. In the USA, 90% of the reported *C. auris* isolates have been reported to have the resistance to fluconazole, 30% to amphotericin B, and 5% to echinocandins [11]. However, the prevalence of *C. auris* has been difficult to characterize until more recently. Initially, *C. auris* was rarely isolated in the SENTRY study, with 6 isolates being recorded from 2006 to 2016 and the first isolate not appearing until 2009 [5]. Contrasting with SENTRY data, a study in India isolated *C. auris* from approximately 70% of ICUs and accounted it for 5% of *Candida* bloodstream infections [12]. In a study from Kuwait, the occurrence of *C. auris* speciation from blood isolates increased from 0.5% in 2014 to 3.4% in 2017 [13]. More recently, data from the CDC have emerged that demonstrated an increase in *C. auris* clinical cases in the USA. As of June 2019, there have been 725 confirmed cases of *C. auris* in 12 states, with another 30 probable cases. In addition, patient screening efforts have uncovered another 1474 patients that were colonized with *C. auris* in ten states that have had previous confirmed cases [14]. There are currently four geographic clades that describe the genomic relationships of *C. auris*: South Asian, South African, South American, and East Asian. Isolates from the USA have shown to be related to either the South American or South Asian clades. When using conventional diagnostic identification methods (i.e., matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry), *C. auris* can be misidentified as *C. haemulonii*, which is phylogenetically related, as well as *C. catenulate*, *C. famata*, *C. guilliermondii*, *C. lusitaniae*, *C. parapsilosis*, *C. sake*, *Rhodotorula glutinis*, *Rhodotorula mucilaginosa*, and *Saccharomyces* spp. [15–24] *C. auris* outbreaks often occur in intensive care units, where multiple devices, lines, and catheters are used. This association has led many to believe that it may possess biofilm-producing characteristics [4]. It also

appears that there is a low likelihood of a community reservoir for *C. auris* and it is believed that healthcare networks are key for transmission [25•]. Combined with identification issues and its association with outbreaks in healthcare facilities, *C. auris* presents a new challenge for hospitals and providers in the changing world of antimicrobial resistance.

Aspergillus Resistance

Azole antifungal agents have been the mainstay of therapy for *Aspergillus* infections. The most common causes of infection are associated with *A. fumigatus* (90%), followed by *A. flavus*, *A. terreus*, and *A. niger* [26]. The emergence of resistance has largely been explained by the long-term use of such agents in the management infections such as chronic aspergillosis [27]. Azole resistance was first described in patients from the USA that received long-term itraconazole [28]. Further studies of *Aspergillus* isolates have shown that azole resistance was observed in the Netherlands in 1998 and 2009 due to the genetic mutations TR_{34}/L98H, and TR_{46}/Y121F/T289A respectively. Interestingly, these strains were also discovered in isolates dating back to 1998 (Italy) and 2008 (USA), although they were discovered retrospectively years later. Genetic analysis of the isolates has concluded that they likely diverged from a common ancestor [29]. Surveillance data has shown that the most common gene modification associated with resistance is TR_{34}/L98H (48.9%). It is estimated that approximately 3.2% of *A. fumigatus* isolates are resistant to one or more azole antifungal. Worldwide resistance rates for *A. fumigatus* range from 0 to 26.1%, showing that rates can differ significantly between various regions of the world. Another trend that has been observed is that azole resistance (TR_{34}/L98H or TR_{46}/Y121F/T289A) occurs more often in invasive disease (5.1%) compared with non-invasive disease. This trend may demonstrate that azole resistance does not result in a significant fitness cost for *A. fumigatus*, signifying that azole resistance due to these mutations may at least result in a similar rate of invasive disease as wild-type *A. fumigatus* [30].

Mechanisms of Azole Resistance

Candida

Azole antifungals inhibit the biosynthesis of ergosterol by binding to lanosterol 14-α-demethylase. Ergosterol is a key component of fungal cell membranes; therefore without its production, membrane stability is lost and growth cannot continue. This pathway is encoded by the gene family ERG11, which includes the genes *Cyp51A* and *Cyp51B*. Alterations to this gene family result in azole resistance. The DNA repair pathways, double-strand break repair (DSBR) and mismatch repair (MMR), have been shown to result in hyper-mutable phenotype in *C. albicans* isolates. The exact mutations, which occur in the DSBR gene *RAD50* and MMR genes *MSH2* and *PMS1*, result in fluconazole-resistant isolates. The *MSH2* mutation has also been shown to result in azole resistance and multi-drug resistance in *C. glabrata* isolates, which will be discussed later [31]. In addition to gene alteration, overexpression of ERG11 can result in azole resistance due to mutations in the UPC2 transcriptional activator. ERG3-mediated resistance, due to the production of alternative sterols, has also been reported result in resistance. The upregulation of ATP-binding cassette (ABC) drug transporters can also result in azole resistance and have been described in *C. albicans* and *C. glabrata* isolates [32]. Two common transporters, CDR1 and CDR2 (also known as PDH1), were discovered in *C. glabrata* strains that became resistant to fluconazole after treatment for oral candidiasis. CDR2 was discovered first, but it was later determined that CDR1 is likely more responsible for high-level azole resistance. Both drug transporters are expressed in response to exposure to azoles. An additional transporter, SNQ2, has also been shown to cause resistance alongside CDR1 and CDR2. When all three transporters are present in an isolate, the removal of SNQ2 results in a return of azole susceptibility; therefore, it has been concluded that SNQ2 must also play an important role in azole resistance [33]. A transporter in the major facilitator superfamily (MFS), MDR1, is also associated with *C. albicans* resistance to both azoles and human host defense mechanisms. [32, 33]. A second MFS transporter, QDR2, has also been shown to be weakly associated with resistance in *C. glabrata* isolates mainly effecting imidazoles (ketoconazole, miconazole, clotrimazole) [33].

Aspergillus

There are two main avenues as to which resistance can emerge to azoles in *Aspergillus* isolates: through the use of azole-related compounds in the environment such as during farming, and long-term azole use in humans [29]. These avenues result in various genetic mutations for *Aspergillus* isolates, mostly seen in *A. fumigatus*. The major mutations reported in *A. fumigatus* occur in *Cyp51A* and its promoter regions TR_{34}/L98H and TR_{46}/Y121F/T289A. *Cyp51A*, as mentioned earlier, is a major gene that encodes for lanosterol 14-α-demethylase, which is the target for azoles. Mutations in this gene in *A. fumigatus* typically result in an overexpression of *Cyp51A*, resulting in resistance. TR_{34}/L98H is a pan-azole-resistant mutation that is often found in azole-naïve patients. It has been hypothesized that this mutation is a result of environmental stressors such as the widespread use of pesticides that contain compounds similar to human azoles. The TR_{46}/Y121F/T289A mutation of *Cyp51A* results in voriconazole resistance. This mutation has been discovered in both clinical and environmental isolates. An additional mutation, TR_{53}, has now been reported in both a clinical and environmental isolate

which results in itraconazole and voriconazole resistance [34•]. Additional mechanisms of resistance include amino acid substitutions at Gly 54, Gly 138, Met 220, and Gly 448. These mutations are considered to be an acquired resistance in *A. fumigatus* isolates. Resistance mechanisms in *A. flavus* and *A. terreus* are rarely discussed, with case reports of several different mutations currently existing. Resistance mechanisms that are not associated with *Cyp51A* have also been reported and include ABC and MSF transporters, *HapE* modification, *Cyp51B* overexpression, and biofilm production. In contrast to many other bacterial and fungal species, drug resistance does not appear to impart a significant fitness cost in *A. fumigatus*. As such, strains harboring acquired resistance mutations are able to compete alongside wild-type isolates in both in vivo and ex vivo environments [35•]. A growing concern regarding to the mechanisms behind *Aspergillus* resistance to azoles is with its proposed relationship to agricultural pesticide use. Non-human azole antifungals are commonly used in agriculture as a way to minimize crop loss and prevent fungal plant infections. Patient to patient transfer of azole-resistant *Aspergillus* infections has not been described, therefore it is hypothesized that the presentation of resistant *Aspergillus* infection in an azole-naïve patient may be due to the inhalation of agricultural *Aspergillus* spores that were exposed to antifungal pesticide. To support this hypothesis, *Aspergillus* isolates from patients either exposed to azole therapy or environmental factors were examined. It was determined that the isolates from different patients had similar mutations in their *Cyp51A* genes (TR_{34}/L98H, G448S, and TR_{46}/Y121F/T298A). An additional study of soil samples that were treated with agricultural azoles showed that 5.8% of the samples were resistant to human azoles. With the use of whole genome sequencing, it has been shown that both clinical and environmental isolates have low genetic diversity and potentially have originated from a common ancestor genotype [28]. Further attention will likely need to be spent to further research the relationship between fungal pesticide use and the emergence of *Aspergillus* resistance to azoles.

Epidemiology of *Candida* and *Aspergillus* Resistance to Echinocandins

Candida

So far, the echinocandins (caspofungin, micafungin, and anidulafungin) are the preferred first-line therapy for invasive candidiasis; therefore, significant empiric echinocandin use exists at most institutions. They represent an extremely valuable antifungal class due to their fungicidal activity against most species of *Candida* regardless of the presence of azole and polyene resistance. But, due to their frequent use as a first-line agent, the emergence of resistance is a concern. Fortunately, it appears that resistance rates have remained low for the most common *Candida* isolates over the past 10 years: *C. albicans* (0–0.1%), *C. parapsilosis* (0–0.1%), *C. tropicalis* (0.5–0.7%), *C. glabrata* (1.7–3.5%), and *C. krusei* (0–1.7%). *C. glabrata* isolates have historically been the most resistance species of *Candida* for the echinocandins, with the highest resistance observed with anidulafungin (3.5%) and lowest with micafungin (1.7%). Peak resistance rates were observed during different time periods for each echinocandin against *Candida* spp.: anidulafungin (2012–2014): 2.8%, caspofungin (2006–2008): 6.9%, micafungin (2006–2008): 2.8%. The lowest resistance rates were observed more recently during 2015–2016 for anidulafungin and caspofungin (1.5%, 1.3%), and in 2009–2011 (1%) for micafungin. Overall resistance trends have remained stable for these isolates except for *C. tropicalis*, which demonstrated an increased resistance rate between 2015 and 2016 (1.3–2%) compared with 2006–2014 (0–1.1%). North America tends to have the highest rates of micafungin resistance compared with other regions of the world for *C. glabrata* (2.8% vs 0–0.6%), and *C. tropicalis* (1.3% vs 0–0.5%). Resistance rates for *C. albicans*, *C. parapsilosis*, and *C. krusei* were nearly identical, ranging from 0 to 0.1% [5]. Resistant *C. glabrata* isolates tended to be non-susceptible/resistant to at least two echinocandins, with 78.4% of all resistant isolates being non-susceptible to all echinocandins. Therefore, it appears that echinocandin resistance is often pan resistant. *C. glabrata* has consistently been associated with echinocandin resistance in various medical centers in the USA, with resistance rates ranging from 8% in Pittsburgh to 11% in Houston [36, 37]. In a large surveillance study conducted by the Centers for Disease Control and Prevention, it was shown that non-susceptible rates in *C. glabrata* have increased significantly over the past decade from 4.2 to 7.8% [38].

Aspergillus

The epidemiology of *aspergillus* resistance to echinocandins was rarely discussed in recent literature. *A. alliaceus*, which is genomically similar to *A. flavus*, has been shown to have high MICs to echinocandins. No additional *Aspergillus* species have shown a trend of resistance to echinocandins [39].

Mechanisms of Echinocandin Resistance

Candida

As mentioned previously, echinocandin resistance in *Candida* isolates is still rarely observed. In the SENTRY surveillance study, all *Candida* isolates with an echinocandin MIC higher than the susceptible breakpoint per CLSI were analyzed for

genetic mutations. The majority of the isolates (71%) were *C. glabrata*, all of which were found to possess a mutated *FKS* gene [5]. The *FKS* gene is responsible for encoding the putatively catalytic subunit (Fksp) of glucan synthase. Point mutations in specific regions of the gene, termed "hot spot" regions, result in the reduced sensitivity to echinocandins. These mutations, which usually occur in either *FKS1* or *FKS2* genes, can result in the reduced potency of echinocandins by 50–3000 fold. While *FKS1*-mediated echinocandin resistance occurred across all *Candida* species, *FKS2*-mediated echincoandin resistance has been exclusively observed in clinical isolates of *C. glabrata* [35•]. So far, *FKS* mutations are the only known cause of echinocandin resistance leading to breakthrough infection during treatment, and are thought to be associated with prior echinocandin exposure [36, 37]. Various *FKS* mutation genotypes have been reported, and the common mutation genotypes include S641F and S645P (*FKS1* in *C. albicans*) as well as S663P (FKS2 in *C. glabrata*). Amino acid substitutions are also responsible for resistance in *C. parapsilosis* (P649) and *C. guilliermondii* (M633/A634) [35•]. Studies have shown that echinocandin resistance due to *FKS2* is calcineurin dependent and may be reversible after administration of a calcineurin inhibitor such as tacrolimus [32]. Additionally, Suwunnakorn et al. recently demonstrated that *FKS2* and *FKS3* in *C. albicans* can act as negative regulators of *FKS1*, thereby influencing echinocandin susceptibility. This mechanism has not yet been demonstrated to result in echinocandin resistance in a clinical isolates of non-*C. glabrata* species. [40]. Prolonged exposure to echinocandins is usually the cause of *FKS* mutations, and account for an increased risk breakthrough infection compared with isolates lacking a *FKS* mutation [36]. Other resistance mechanisms, such as azole-specific gene mutations and drug transporters, do not result in echinocandin resistance [35•, 41]. In a study by Healey et al., 100% of the colonies that were resistant to echinocandins also had mutations in FKS1/2, therefore suggesting that the main explanation behind resistance are those mutations. As mentioned earlier, disruption of the MMR gene *MSH2* results in azole and multidrug-resistant strains of *C. glabrata* [31]. Alterations in *MHS2* have also been shown to result in isolates that were resistant to amphotericin B, fluconazole, voriconazole, caspofungin, and micafungin to various extents. Analysis of *MHS2* mutations in clinical isolates collected across the USA showed that the mutation was present in a significant number of susceptible strains (52%), as well as fluconazole-resistant strains (54%), and MDR-strains (62%), but a low number of echinocandin-resistant strains (20%). Further analysis of isolates that contained *MHS2* mutations showed that the presence of the mutation may promote the emergence of resistance to antifungals, namely through *FKS* mutations, instead of resulting in actual a different resistance mechanism [31]. However, in a study by Pham et al. of echinocandin-resistant isolates, only 81% of resistant isolates were found to have a *FKS* mutation, mainly *FKS2*, therefore suggesting that there are other mechanisms present in these isolates [42]. Protein kinase C (PKC), protein phosphatase calcineurin, Hsp90, and Cas5 have all been described to result in reduced echinocandin susceptibility due to mechanisms such as increased echinocandin tolerance and upregulation of chitin production [32]. For instance, Hsp90 is involved in the activation of signaling pathways that are essential for cell survival, resulting in increased antifungal tolerance. Hsp90 is most often induced due to environmental stress, such as antifungal presence [34•]. Even though there are multiple mechanisms that result in echinocandin resistance, only *FKS* mutations have been associated with worse outcomes for patients with IC. In a study by Beyda et al. of patients with candidemia, 60% of patients with a *FKS* mutation experienced treatment failure compared with 23% without *FKS* mutations [37]. Additional studies have shown that treatment failure due to *FKS* mutant *C. glabrata* isolates can range from 62 to 90%. Therefore, it is hypothesized that the presence of *FKS* mutation or elevated MICs to echinocandins may serve as a surrogate for echinocandin failure, but further research is required [36, 37].

Aspergillus

The echinocandins can be a useful class of antifungals in the management of *Aspergillus* infections, especially due to isolates with azole resistance, as cross-resistance in these isolates appears to be uncommon. Resistance mechanisms in *Aspergillus* have been less well described, due to the fact that various species have differing intrinsic susceptibility profiles. Similar to *Candida*, mutations in *FKS* have been shown to decrease echinocandin affinity for glucan synthase, resulting in a reduced susceptibility in *Aspergillus* isolates. In a study of resistant isolates, *FKS* mutations were not found, but instead higher levels of FKS gene expression. In vitro resistance to echinocandins was observed in *A. fumigatus* isolates that had induced resistance with either S6768Y or S678P substitutions in *FKS1*. This finding suggests that *FKS* mutation may contribute to echinocandin resistance in some *A. fumigatus* isolates in addition to overexpression of *FKS*. Additional explanations for echinocandin resistance in *A. fumigatus* have been described in the literature, such as increased chitin synthesis and Hsp90 mutations. The exact mechanisms behind increased chitin levels has not been determined but has been observed in *A. fumigatus* isolates with Δ*ras* mutations. Hsp90 mutation and activation has been observed in *Aspergillus* spp. isolates in a similar fashion to *Candida* spp. mentioned earlier. Resistance in *A. fumigatus* may be reversible to an extent, as inhibition of Hsp90 and calcineurin has been shown to improve echinocandin susceptibility *A. fumigatus* [34•].

Current and Future Diagnostics for Detecting Resistance

The continued development of rapid diagnostic technology and other diagnostic methods will become important to aid in the de-escalation and cessation of antifungal therapy. The use of diagnostic tests to detect resistance markers is commonly used for bacterial pathogens such as *Staphylococcus aureus*, *Enterococcus*, and Enterobacteriaecae, but has not been extensively developed or implemented for fungal pathogens. This technology is important because of the difficulty of culturing fungal pathogens. As mentioned earlier, there are multiple resistance mechanisms associated with azole resistance. Specific genetic mutations do not correlate well with MIC results; therefore, there is a need for methods to detect these underlying mechanisms and mutations. Mutations in amino acids of the ERG11 and ERG3 genes can be detected by using molecular probes, melt curve analysis, and DNA sequence analysis. Unfortunately, issues remain with these technologies due to complexity and whether or not mutations correlate well with MIC results or clinical outcomes. Another technology that has been investigated is next generation sequencing (NGS). NGS has the ability to detect multiple resistance mechanisms, included those missed by the aforementioned tests [43]. Due in part to the high costs, slow turnaround time, and need for bioinformatics expertise for interpretation, NGS is not a widely used technology at this time but is expected to become so in the future. There is concern that susceptibility testing may be unreliable for echinocandins as well. For instance, interlaboratory variability has been reported for caspofungin MICs, where some labs have reported higher MIC values resulting in a misclassification of isolates as caspofungin resistant. Due to this problem, EUCAST now recommends to not perform susceptibility testing with caspofungin, but instead with anidulafungin and micafungin as surrogate markers. Resistance to the echinocandins has been shown to accurately correlate with presence of *FKS* mutations [3, 37, 41, 44•]. Due to reliability issues with susceptibility testing, it remains exceedingly important to develop a molecular assay that is able to detect *FKS* gene mutations. DNA sequencing continues to be the most widely used method of detecting *FKS* mutations, as no such rapid diagnostic test is currently commercially available. Within the last few years, there has been an increased amount of research focused on the development of such a rapid molecular diagnostic test. In a study by Zhao et al., a molecular DNA assay for rapid genotyping of *FKS1* HS1 and *FKS2* HS1 of *C. glabrata* was evaluated. The study used blinded clinical *C. glabrata* isolates and the results of the assay were compared with microbroth dilution susceptibility testing per CLSI standards. The molecular diagnostic assay was shown to be 100% accurate in distinguishing between wild-type and *FKS1*/*FKS2* mutant *C. glabrata* strains. This test appears to be promising, as it involved a simpler design compared with previous molecular diagnostic tests and will potentially be able to be incorporated into a multiplex test [44•]. Additional tests that may help in the de-escalation and discontinuation of antifungal therapy include galactomannan, β-D-glucan, and *Aspergillus* PCR. These tests do not detect resistance, but instead detect the presence of fungal pathogens. These tests tend to have higher negative predictive values that helps with the discontinuation of empiric antifungal therapy [35•].

Clinical Implications of Emerging Antifungal Resistance and New Diagnostics

Due to the high mortality rate of invasive fungal infections, directed empiric therapy is important for proper management. The use of new diagnostic tests to determine if a fungal pathogen is resistant before traditional susceptibility testing results are finalized will help to ensure patients receive early, directed therapy. For example, the presence of genetic mutations in *Candida* species, such as *FKS1* and *FKS2*, has been shown to be an independent risk factor for treatment failure for *C. glabrata* infection [36]. Therefore, by using a rapid molecular test to determine echinocandin resistance, clinicians can be assured that an echinocandin is an appropriate choice to manage a specific case of IC. Increased work focused towards the discovery of new antifungal drug targets is ongoing. An area currently being explored is combination therapy. Preliminary data shows that azole susceptibility can be restored through the inhibition of specific resistance mutations such as PDR1 and Gal11A in *C. glabrata* isolates. The targeting of Hsp90 has also been shown to restore the activity of azoles and echinocandins [32].

Conclusion

Antifungal resistance may be discussed less frequently than antibiotic resistance, but can be equally devastating to patients. Recent work has highlighted the expanded understanding of the complexity of antifungal resistance and its link to antifungal use. Fortunately, outside of select patient populations, antifungal resistance remains relatively uncommon but must remain a consideration for all clinicians who encounter fungal infections.

Compliance with Ethical Standards

Conflict of Interest Dr. Hendrickson, Dr. Hu, and Dr. Aitken have nothing to disclose.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by either of the authors.

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