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Update on Antifungal Drug Resistance

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Abstract Invasive fungal infections remain a major source of global morbidity and mortality, especially among patients with underlying immune suppression. Successful patient management requires antifungal therapy. Yet, treatment choices are restricted due to limited classes of antifungal agents and the emergence of antifungal drug resistance. In some settings, the evolution of multidrug-resistant strains insensitive to several classes of antifungal agents is a major concern. The resistance mechanisms responsible for acquired resistance are well characterized and include changes in drug target affinity and abundance, and reduction in the intracellular level of drug by biofilms and efflux pumps. The development of high-level and multidrug resistance occurs through a stepwise evolution of diverse mechanisms. The genetic factors that influence these mechanisms are emerging and they form a complex symphony of cellular interactions that enable the cell to adapt and/or overcome drug-induced stress. Drivers of resistance involve a complex blend of host and microbial factors. Understanding these mechanisms will facilitate development of better diagnostics and therapeutic strategies to overcome and prevent antifungal resistance.

Keywords Antifungal resistance · Acquired resistance · *Candida albicans* · *Candida glabrata* · *Aspergillus fumigatus* · Azoles · Echinocandins · Polyenes

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Introduction

Serious fungal infections afflict millions of patients annually resulting in more than 1,350,000 deaths [1]. The most serious fungal infections occur as a consequence of other serious health problems such as asthma, AIDS, cancer, and organ transplantation, and they all require antifungal therapy for a successful outcome. Failure to treat effectively either because of diagnostic delays or missed diagnosis often leads to death or serious illness. This recognition has resulted in a significant increase in antifungal agents use for the treatment and prevention of fungal infections. Yet, therapeutic options are limited, as the most widely used antifungal drugs comprise only a few chemical classes including azoles (fluconazole, voriconazole, posaconazole, and isavuconazole) and polyenes (amphotericin B), which modify the cell membrane, nucleic acids and protein flucytosine (5-fluorocytosine), and the cell wall echinocandins (caspofungin, anidulafungin, and micafungin). Predictably, resistant strains emerge during therapy, and it is a confounding factor for successful clinical outcome as it eliminates important antifungal classes leaving restricted treatment options. Resistance may result from selection of inherently less susceptible strains or from emergence of acquired drug resistance during therapy in otherwise susceptible strains. It is the latter that is the principal subject of this review, although many acquired mechanisms also account for naturally occurring reduced susceptibility of some species. A greater understanding of factors promoting mechanism-specific resistance is important to help overcome resistance emergence.

Epidemiology and Emergence of Multidrug Resistance

Inherent Resistance Selection

Resistance to antifungal agents remains relatively uncommon, as the vast majority of fungi retain susceptibility to commonly

used antifungal agents. In some cases, prominent resistance results from selection of less susceptible species. The azole antifungal agents are the most prominent example of drug selection for less susceptible species [2]. Numerous global epidemiological studies have documented the impact of widespread triazole use on the distribution and shift of *Candida* species toward less susceptible strains like *Candida glabrata* and *Candida krusei*. In many regions where azole use (e.g., fluconazole) is prevalent, there has been a shift away from *Candida albicans* as the predominant cause of invasive infections toward less susceptible non-*C. albicans* species [3]. *C. glabrata* has inherent reduced susceptibility to fluconazole and it is the species whose incidence has increased the most to account for a decrease in the prevalence of *C. albicans* [3, 4]. Similarly, fluconazole use is linked to emergence of the highly resistant *C. krusei* [5] and *Candida guilliermondii* [6]. In many cases, inherent resistance in *Candida* species to fluconazole also carries with resistance to more highly active triazoles like voriconazole. This is not true for *Aspergillus* and other molds that are resistant to fluconazole but susceptible to more highly active triazoles. Yet, breakthrough infections against highly active triazole drugs have been reported for *Aspergillus ustus* [7] and *Aspergillus fumigatus*-like species *Aspergillus lentulus*, which show pleiotropic resistance to multiple antifungal drugs [8, 9]. Sometimes, a susceptible species develops a prevalent variant that is the source of resistant infections. In the bacterial world, the regional and global spread of drug-resistant strains from a common progenitor is commonly observed. Such transmission is not typically observed for fungal drug resistance. A notable exception occurred with the recent emergence of a multidrug-resistant variant of *A. fumigatus* in the Netherlands [10, 11]. This highly azole-resistant strain variant was selected in the environment as a consequence of the prevalent use of agricultural azoles. The resistance mechanism unique to these isolates will be discussed later, but such resistant strains are spreading through Europe and into parts of Asia [12].

Acquired Resistance

"Acquired" refers to acquisition (or latent induction) of a resistance mechanism during therapy. It is less common but not an inconsequential event. Growing concerns have been raised about acquired antifungal drug resistance involving azole resistance in *A. fumigatus* and echinocandin resistance in *Candida* [13–15]. Azole resistance in *A. fumigatus* is widespread globally with high geographic variance since the first report of itraconazole resistance in 1997 [16]. In the Netherlands, the prevalence of resistance increased from 2 % in 2000 to 8 % in 2009 predominated by TR_{34}/L98H, a resistance mechanism which has been considered as environmentally acquired and associated with the use of agricultural fungicides [17]. While TR_{34}/L98H along with the newly emerged TR_{46}/Y121F/T289A are spreading and widely reported in many other countries [18–23], epidemiological data in the UK demonstrated a more drastic increase of resistance from 5 % in 2004 to 14 % in 2008 and 20 % in 2009 with more versatile (CYP51A and non-CYP51A mediated) underlying mechanisms, which were mainly induced by long-term azole therapy in chronic infection patients [24, 25]. Unlike azole resistance, the frequency of echinocandin resistance remains relatively low (<2–3 %) with *C. albicans* and most other *Candida* species [26–29]. However, a notable exception is *C. glabrata*, where an alarming trend of rising echinocandin resistance poses a serious clinical challenge since many isolates display azole cross-resistance [30••, 31, 32]. A recent study of *C. glabrata* bloodstream isolates documented the rising rate of echinocandin resistance from 4.9 to 12.3 % in 2001–2010 [30••]. Of note, resistance rates in *C. glabrata* varies range from ~3 % to over 10 % in recent surveillance studies, depending on the geographic region, subpopulation, and data collecting method of the study [14, 30••, 31–33] (Fig. 1). Nevertheless, rapid acquisition of resistance during therapy for *C. glabrata* infection with subsequent unfavorable outcome is worrisome.

Mechanisms of Resistance

Prominent antifungal resistance mechanisms have been detailed in recent years. The mechanisms generally involve reduced drug uptake, modification of the drug target, and/or a reduction in the cellular level of drug due to upregulation of drug efflux transporters (pumps) and biofilms, which restrict drug entry (Fig. 2). Fungi have evolved a number of genetic regulatory features that induce or promote specific resistance mechanisms.

Biofilms

Yeasts and molds readily form biofilms [34, 35], which display an organized three-dimensional structure comprised of a dense network of cells in an exopolymeric matrix of carbohydrates, proteins, and nucleic acids. Drug sequestration within the extracellular matrix is the largest determinant of the multidrug resistance phenotype of biofilms [36]. Biofilms restrict access to echinocandin drugs and they are intrinsically resistant to azoles. The mechanisms include drug sequestration and expression of drug efflux transporters [34, 35, 37–39]. Matrix production is highly regulated and is a key resistance factor for *Candida* species [40]. Biosynthesis of β-1,3-glucan by glucan synthase is critical to the biofilm resistance properties. Downstream components of the yeast PKC pathway, including *SMI1*, *RLM1*, *RHO1*, and *FKS1*, regulate β-1,3-glucan biosynthesis and biofilm matrix production [36, 41–43], as

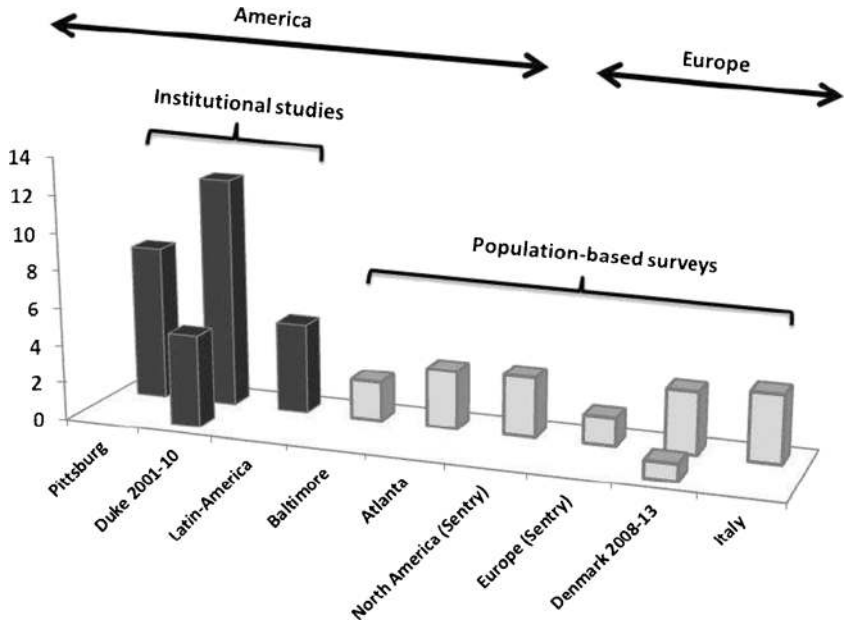

Fig. 1 Echinocandin resistance in *C. glabrata* in Europe and America. Resistance rate varies among different studies. The rate reported from institutional studies is higher than that from population-based surveys, where only the initial blood isolate is included to avoid biasing the data set. Adapted from Arendrup et al. [14]

well as other cellular components such as alcohol dehydrogenases Adh5, Csh1, and Ifd6 [44].

Drug Target Modification

Genetic modification of the drug target resulting in reduced affinity for drug is one of the most prominent mechanisms for antifungal resistance. For echinocandin drugs, target site modification is sufficient to confer resistance, as other mechanisms (e.g., drug pumps) are not associated with clinical resistance [45]. Echinocandins inhibit glucan synthase, which blocks the biosynthesis of the critical cell wall polymer (1,3)-β-D-glucan. A limited number of mutations in two highly conserved hot-spot regions of the *FKS* genes encoding glucan synthase confer resistance (Table 1). The most prominent mutations in *C. albicans* associated with clinical failures encode amino acid substitutions at *Fks1* positions Phe641 and Ser645 [46]. These target site modifications decrease the sensitivity of enzyme for drug by as much as several thousand fold [47•, 48, 49] resulting in strains that respond poorly in pharmacodynamic models [50, 51]. Related *FKS1* mutations have been found in other *Candida* species. Only in *C. glabrata*, conserved hot-spot mutations are found in both *FKS1* (Phe625, Ser629) and *FKS2* (Phe659, Ser663) with the latter occurring with twice the frequency [48].

Azoles inhibit lanosterol 14α-demethylase, which is encoded by *ERG*11 (*CYP51*A in *Aspergillus*). Triazole antifungal agents differ in affinities for their drug target, which in turn influences their spectrum of activity. Fluconazole shows the weakest interaction and displays the narrowest spectrum, as it is active against yeasts but not molds. As such, it promotes the broadest resistance. In a recent study of 63 fluconazole-resistant clinical isolates, 55 isolates carried at least one mutation in *ERG11*, representing 26 distinct amino acid substitutions [52]. In contrast, highly active triazoles (e.g., voriconazole, posaconazole) interact more strongly with the drug target, show broader activity against yeasts and molds, and reveal a narrower spectrum of resistance mutations. To date, more than 70 amino acid substitutions have been described in Erg11 (or Cyp51A) from azole-resistant clinical isolates of *C. albicans* [53–58], *A. fumigatus* [24, 59, 60], and *Cryptococcus neoformans* [61, 62]. Within the azole family, chemical diversity around a core unit facilitates differential susceptibility and also resistance cross-reactivity. For example, some mutations in *ERG11* result in fluconazole resistance only, others confer resistance to voriconazole but not posaconazole, and some display pan-resistance. Computational modeling using high-resolution structures as a template helps explain the impact of specific amino acid substitutions on drug-target interactions [63]. Recently, such modeling studies were greatly enhanced by the elucidation of a high-resolution Erg11 structure from baker's yeast [64••]. Drugs like posaconazole fill the structural space occupied by the substrate lanosterol, where they make a coordination bond with the heme iron extending from the active site to beyond the mouth of the entry channel (Fig. 3). A majority of mutations cluster in three main regions [65] with most substitutions altering the juxtaposition of drug with the heme cofactor. The structure of the active site and substrate channel helps account for the susceptibility observed for some prominent resistant mutants [64••, 66].

Finally, in *Aspergillus*, mutations in Cyp51A are sufficient to induce resistance to some or all highly active triazole drugs, while in *C. glabrata*, target site mutations in CgERG11 do not

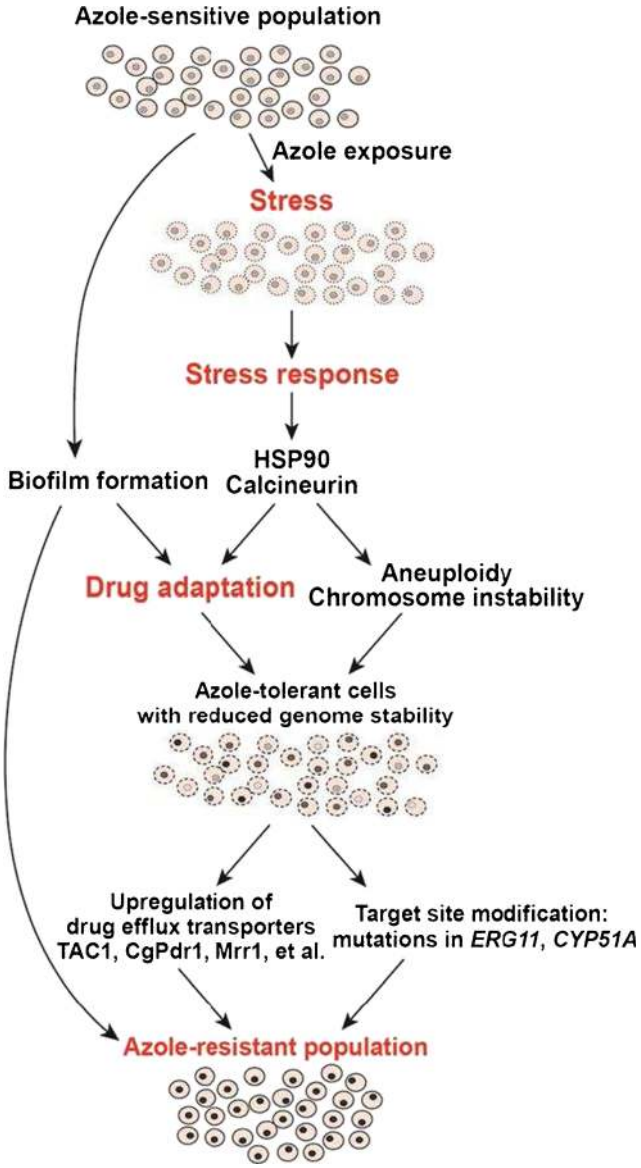

Fig. 2 Exposure to azole drugs triggers fungal stress responses that promote fungal adaptation and drug tolerance and, ultimately, emergence of stable genetic alterations that confer drug resistance. The HSP90 protein chaperone and its client, protein phosphatase calcineurin, are key stress signal transduction molecules that both upregulate pathways leading to drug tolerance and promote genome instability, increasing the likelihood of generating drug-resistant strains. Fungal biofilms, which readily form in vivo, are intrinsically resistant to azoles due to drug sequestration within the extracellular matrix and expression of drug efflux transporters

contribute to clinical resistance. In some organisms, mutations in Erg11 are but a first step toward higher-level resistance involving other resistance mechanisms such as target upregulation or overexpression of drug pumps.

Decreased Intracellular Drug Levels

As drugs need to reach their cellular targets to be effective, certain antifungals use permeases for cell entry including 5-fluorocytosine, which uses *FCA1*, *FCY2*, *FCY22*, and *FCY23* to enter *C. albicans* cells [50]. Fluconazole is believed to enter cells by an uncharacterized energy-independent facilitated diffusion mechanisms [67]. Modification of these uptake systems would confer drug resistance. Among the most common mechanisms for reducing cellular drug levels, energy-dependent drug efflux transporters recognize and extrude diverse chemical classes. Two different drug efflux systems modulate azole resistance, the ATP-binding cassette (ABC) superfamily and the major facilitator superfamily (MFS). The ATP-dependent transporters (ABC) are comprised of two transmembrane and two cytoplasmic nucleotide-binding domains, which catalyze ATP hydrolysis. Fungal genomes encode numerous ABC transporters, as they are presumed to purge the cell of toxic compounds and metabolites. *C. albicans* is predicted to contain 28 ABC proteins [68], *C. glabrata* has 18, and *A. fumigatus* and *C. neoformans* have many more [69]. Despite their prevalence, only a few contribute to antifungal resistance. The PDR class comprises the major transporters involved in azole resistance including *C. albicans CDR1* and *CDR2* [70]; CgCdr1, CgCdr2, and CgSnq2 in *C. glabrata*; and Afr1 in *C. neoformans* [71]. In *A. fumigatus*, ABC transporter genes are upregulated in response to azole exposure (*AfuMDR1* (*CDR1B)*, *AfuMDR2*, *abcA-E*) [72, 73] and in resistant clinical isolates [74–76]. MFS transporters have multiple (12 or 14) transmembrane domains and use proton-motive force to drive drug efflux. The *C. albicans* genome predicts 95 MFS transporters in 17 families [77] but only one transporter gene, *MDR1*, is associated azole resistance [78–80]. In *A. fumigatus*, *AfuMDR3* is upregulated in some itraconazole-resistant mutants [75]. It is unclear in *Aspergillus* whether induction of an ABC or MFS transporter is sufficient for resistance.

Regulation of Drug Transporters

Transcriptional regulation of ABC and MFS multidrug transporters is complex, involving *cis*- and *trans*-regulatory elements. *Cis*-acting elements regulate *CDR1*, *CDR2*, and *MDR1 in C. albicans*, with the promoters of *CDR1* and *CDR2* containing common Drug Responsive Element (DRE) sequences that are required for transcriptional upregulation [81]. *MDR1 cis*-acting elements have complex arrangements that differ depending on the inducer [82]. In *C. glabrata*, pleiotropic DREs are present in *CgCDR1*, *CgCDR2*, and *CgSNQ2* [83, 84], and help confer high level *CDR1* expression [85]. The first major *trans*-acting transcription element regulating efflux is *C. albicans* Transcriptional Activator of CDR (*TAC1*), a member of the Zn_2Cys_6 transcription factor family. Gain-of-function (GOF) mutations in *TAC1* [86•, 87] are responsible for the upregulation of *CDR1* and *CDR2* in azole-resistant isolates [88–90]. Tac1 binds to the DRE of *CDR1* and *CDR2*, likely via a consensus-binding

Table 1 Overview of Fks hot spot sequences and amino acid sequence positions resulting in echinocandin resistance

	Fks1p				Fks2p			
Species	Hot spot 1		Hot spot 2		Hot spot 1		Hot spot 2	
C. albicans	641	FLTLSLRDP	1357	DWIRRYTL				
C. dubliniensis	641	FLTLSLRDP	1357	DWIRRYTL				
C. glabrata	625	**F**LIL**S**LRDP	1340	DW**V**RRYTL	659	**F**LIL**S**LRDP	1374	DW**I**RRYTL
C. kefyr	54*	**F**LTLRLRDP	769*	DW**V**RRYTL				
C. krusei	655	FLILS**I**RDP	1364	DWIRRYTL				
C. lusitaniae	634*	FLTLSLRDP	**	DWIRRYTL				
C. tropicalis	76	FLTLSLRDP	792	DWIRRYTL				
C. parapsilosis	652	FLTLSLRD**A**	1369	DWIRRYTL				

First amino acid number is shown for each hot-spot sequence

Amino acids in bold large red letters signify most prominent resistance

Amino acids in red-brown indicate weaker resistance

Amino acid in blue is a naturally occurring polymorphism with weak resistance

Amino acids in bold indicate strong resistance

Amino acids in green indicate silent mutation, acquired or naturally occurring

Amino acids in brown indicate naturally occurring mutation of unknown impact

*Indicates amino acid position based on partial sequence, sequencing of entire gene is required

**denotes separated sequences of HS1 and HS2, thus annotation of HS2 is nonsense

motif. Similarly, another *CDR1* regulator *MRR2* is required for the basal expression of *CDR1* [91]. In *C. glabrata*, drug pump overexpression is the major mechanism responsible for azole resistance, and transcriptional activator CgPdr1 regulates expression of Cg*CDR1* and Cg*CDR2* [92]. CgPdr1 binds to the PDRE consensus in *CgCDR1* [85] and GOF mutations hyper-activate CgPdr1 upregulating ABC transporters [92–96]. Nearly 60 GOF mutations have been identified in *CgPDR1* alleles from clinical azole-resistant isolates [94]. In *C. albicans*, the Zn_2Cys_6 transcription factor Multidrug Resistance Regulator 1 (Mrr1) regulates MFS transporter gene *MDR1* [97] and at least 15 different *MRR1* GOF mutations are known [88, 98] to cause constitutive upregulation of *MDR1* [99]. Other positive regulators of MDR in *C. albicans* include Cap1 [100, 101] and Mcm1 [102, 103].

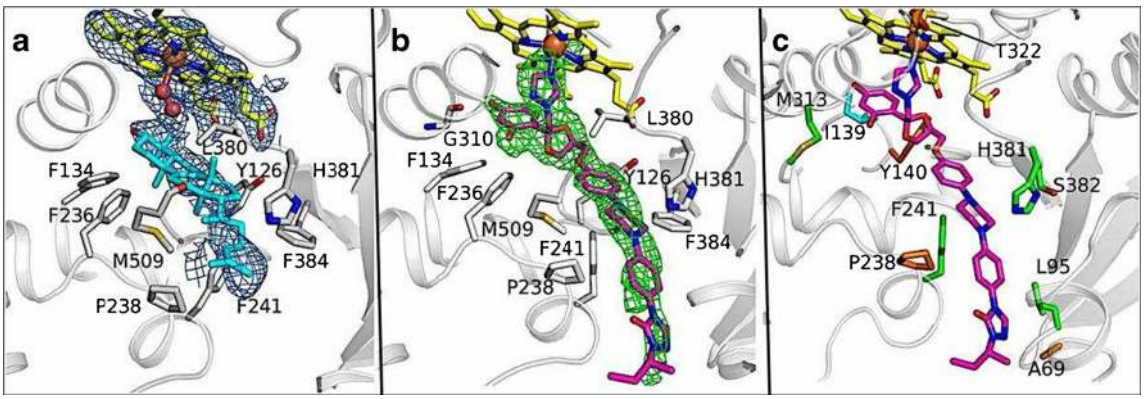

Fig. 3 Binding of lanosterol and itraconazole within active site heme region Erg11 from *S. cerevisiae*. **a** Lanosterol binding and coordination with heme shown with electron density profile. **b** Itraconazole binding to same region shown with electron density. **c** Bound itraconazole and amino acids commonly mutations to confer resistance. Adapted from Monk et al. [64••]

Chromosomal Anomalies

It is now recognized that azole resistance in *C. albicans* and other *Candida* species is associated with a variety of large-scale genomic alterations, including loss of heterozygosity (LOH) involving specific genomic regions, increased chromosomal copy number, and aneuploidies. LOH is associated with resistance factors *ERG11*, *TAC1*, and *MRR1*. It has been shown that mutations in these genes arise in a heterozygous state and are converted to homozygous form by LOH [104, 105]. Isochromosome formation is a separate and more pronounced genomic change. It increases gene copy numbers, and hence gene expression of azole resistance genes and Erg11, the azole target. Isochromosome formation on the left arm of chromosome 5i(5L) increases the copy number of *ERG11* and *TAC1* [106]. Similarly, the isochromosome variant 3i(3R) on the right arm of chromosome 3 contains *CDR1* and *MRR1* [107]. An examination of 57 clinical *C. albicans* strains, disomic or monosomic for Ch5, found that the monosomy of Ch5 caused elevated levels of cell wall chitin and repressed levels of 1,3-beta-glucan, as well as diminished membrane ergosterol. This resulted in decreased susceptibility to caspofungin and increased susceptibility to fluconazole and amphotericin B [108]. Chromosomal alterations resulting in resistance are also observed with *C. glabrata* [109]. In *C. neoformans*, azole resistance is associated with disomies of chromosomes 1 and 4, which contain *ERG11* and ABC transporter *AFR1* [110]. Hetero-resistance, observed in *C. albicans* [111], relates to subpopulations within the same clone that vary in resistance based on the frequent loss and gain of chromosomes in response to selection in *C. neoformans* [112]. *C. neoformans* is heteroresistant to azoles due to transient duplications of whole chromosomes that carry the genes for azole resistance [110]. Chromosome 1, which harbors *ERG11* and *AFR1*, encoding the azole target and an ABC transporter, respectively, is the first one to be duplicated resulting in elevated MICs; further increases in MIC result from the duplication of Chr4.

Stress Responses and Drug Adaptation

Fungi are remarkably adaptive and have numerous genetic mechanisms that help protect against cellular stresses, such as those encountered following exposure to an antifungal agent. These stress adaptation responses frequently result in elevated in vitro MICs. Typically, the increased MIC is insufficient to confer clinical resistance resulting in breakthrough infections. Rather, stress adaptation stabilizes the cell in the presence of drug and allows it to develop more profound resistance mechanisms over time that are manifested as clinical resistance (Fig. 2). As first described for azoles, Hsp90 and calcineurin are two key cellular regulators critical for orchestrating cellular responses to drug-induced stress [113, 114•]. Hsp90 is a molecular chaperone that regulates the stability and function of diverse client proteins and controls stress responses by stabilizing the protein phosphatase calcineurin [115]. Calcineurin-Crz1 signaling influences a wide range of cellular response functions including ion homeostasis and cell wall biogenesis [116]. Compromising the function of Hsp90 or calcineurin can induce fungistatic drugs to become fungicidal enhancing efficacy. Thus, inhibition of Hsp90 or calcineurin may present a strategy to enhance the efficacy of azoles against resistant fungi [117]. Hsp90 and calcineurin-Crz1 signaling also contribute to echinocandin resistance in *Candida* species [118, 119]. The cell's response to echinocandin action is highly robust, as numerous cellular responses are linked to maintaining cell wall integrity including *PKC*, calcineurin/Crz1, and *HOG* [120, 121]. Other responses such as modulation of sphingolipid biosynthesis result in a mixed phenotype involving resistance to caspofungin and hypersensitivity to micafungin [122]. Echinocandin action also results in pronounced compensatory increases in chitin synthesis, to help sustain the cell wall. Mutants with increased chitin content are less susceptible to caspofungin [120, 121, 123] and increased chitin biosynthesis has been partly invoked to account for paradoxical growth at high drug levels [124] [125]. In recent years, whole genome sequencing of serial isolates has been used to determine genetic signatures related to evolution of resistance. Whole genome sequencing of *C. glabrata* isolates before and after caspofungin treatment and breakthrough identified expected *FKS* mutations and HSP90 effects. In addition, it identified mutations in genes *MOH1*, *GPH1*, *CDC6*, and *TCB1/2*; *cdc6* mutations were independently shown to have a role in echinocandin susceptibility [119]. In total, these responses help confer drug adaption, which predispose cells for higher resistance such as the formation of a stable *FKS* mutation.

Genetic Plasticity as a Driver of Resistance

C. albicans can develop azole resistance by acquiring chromosomal disomies or segmental chromosomal duplications involving the chromosomes carrying azole target *ERG11* and drug efflux genes [106, 126]. Acquisition of multiple chromosome disomies upon azole exposure was also observed in *C. neoformans* [110]. However, appearance of significant genomic alterations is not specific to azoles, but also occurs in the presence of other types of stress. The genetic changes underlying antifungal drug resistance do not arise in a random manner, as they are promoted by varying stress inducers including antifungal drugs and host immunity. For instance, in *C. albicans*, elevated temperature and oxidative stress

promote aneuploidy and chromosome arm homozygosis [127]. In *Saccharomyces cerevisiae*, several different stresses, including oxidative, translational, and ER stress, promote chromosome loss and appearance of marked karyotype diversity [128]. Consistent with these observations, passage of *C. albicans* through the mouse promotes genome rearrangements in the fungus even in the absence of antifungal treatment, suggesting that this genetic instability is due to conditions encountered within the host [129]. Furthermore, analysis of *C. glabrata* clinical isolates indicates that this organism undergoes drastic genome rearrangements with multiple chromosomal translocations and appearance of new chromosomes [130]. Clinical isolates of *C. glabrata* have highly variable genomes [109, 126] suggesting that this species possesses mechanisms that specifically promote and/or help the cells tolerate extensive genetic changes in response to stress. Several studies suggest that an increase in the proportion of aneuploid cells happens early in response to stress [131, 132]. One factor involved in this process is HSP90, whose inhibition strongly reduces stress-induced aneuploidy and drug resistance in *C. albicans* and *S. cerevisiae* [115, 128]. Formation of aneuploidy is followed by smaller-scaled genetic changes, such as insertions, deletions, and point mutations in individual genes. What drives such changes is not well understood, although it has been shown that aneuploidy itself can promote other types of genetic alterations, possibly because it alters gene dosage of a subset of the genome, thus altering complexes involved in chromosome maintenance and DNA repair [133].

Drivers of Resistance

The development of antifungal resistance is a complex process involving the host, drug, and microbial factors, which collectively contribute to therapeutic failure. Host immune status is important as the immune system must work in concert with antifungal drugs to control an infection. Severe immune dysfunction results in patients less responsive to treatment since microbial burdens are larger and the drug must combat the infection without immune support. Surgical devices such as indwelling catheters and artificial heart valves provide surfaces for infecting fungi to establish biofilms that restrict drug access. The site of the infection contributes to clinical resistance, since it may be inaccessible to drugs. Successful therapy requires that the drug reach its microbial target with a suitable potency but this is often unknown. Blood levels of drugs may not accurately predict whether a drug reaches the primary site of infection, as it is difficult to deliver drugs at an adequate concentration to certain infected tissues and organs. Abdominal candidiasis is a high burden infection in which drug access is restricted, which leads to breakthrough infections [134]. In some cases, drugs that are highly serum protein bound, such as the echinocandins, have altered antifungal properties whereby in vitro fungicidal drugs can act as fungistatic agents in vivo [135]. In recent years, the role of environment as a driver for resistance has become prominent. As described earlier for *A. fumigatus*, triazole resistance due to two prominent modifications of Cyp51A, TR_{34}/L98H [10, 136–139], and TR_{46}/Y121F/T289A [23] arose as a consequence of azole use in the agricultural world [11]. As *Aspergillus* spores emerge from the environment, this environmentally driven resistance is spreading throughout Europe, India, and Asia [19, 140, 141]. Finally, like all anti-infectives, patient compliance is critical for effective treatment, as poor adherence to drug regimens reduce drug effectiveness, contributing to resistance. Overall, there remains a strong relationship between drug exposure and the emergence of resistance. The development of echinocandin resistance in *Candida* species typically requires prolonged drug exposure [142–145]. But it can also arise rapidly after the start of therapy [146, 147]. Horizontal transmission of resistant strains is not generally observed most likely because they carry a fitness cost. With *FKS* mutants, decreased glucan synthase activity results in less robust cells with modified cell walls [148, 49]. These *FKS* mutant strains are less virulent and compete poorly with their wild-type counterparts [48, 149, 148]. Lastly, as total drug exposure is a critical factor influencing resistance emergence, prophylaxis has emerged as a concern. Fluconazole and the echinocandins caspofungin and micafungin are excellent prophylaxis agents against invasive candidiasis because they have favorable pharmacokinetics and safety profile. However, the expanding use of antifungal prophylaxis increases patient exposure to drugs, and it is not surprising that it promotes the emergence of resistance in certain clinical settings.

Conclusion

Overall, antifungal drug resistance due to acquired mechanisms is an uncommon event, as most infecting species retain drug susceptibility. However, acquired drug resistance can be a critical factor in some settings with critically ill patients, and the emergence of significant multidrug resistance involving azoles and echinocandins in organisms such as *C. glabrata* is troubling. The mechanisms conferring drug resistance are now well defined, and ongoing studies are seeking to identify genetic factors that can influence their emergence. Fungi have evolved to respond to stress in a highly dynamic manner, ranging from specific point mutations to major chromosomal modifications that directly and indirectly influence induction of specific resistance mechanisms. There is now a strong appreciation that stress responses promote drug adaptation, which by itself does not lead to clinical failure but can

ultimately lead to development of higher-level resistance and diminished clinical response (Fig. 2). Finally, in recent years, anatomical reservoirs that restrict drug access or promote biofilm formation have been identified to be important contributors to resistance emergence in the clinic. As new molecular tools have emerged, there is now an opportunity to detect drug resistance earlier and develop therapeutic strategies to avoid or mitigate resistance.

Compliance with Ethics Guidelines

Conflict of Interest Dr. Perlin reports grants and personal fees from Astellas Pharmaceutical, grants from CIDARA, grants from Scynexis, outside the submitted work; in addition, Dr. Perlin has a patent Assays for Resistance to Echinocandin-Class Drugs issued, a patent ASSAYS FOR FUNGAL INFECTION pending, a patent NEAR INFRARED LABELS AND METHODS OF USE THEREOF pending, and a patent METHODS TO DETECT A FUNGAL CELL pending.

Dr. Shor and Dr. Zhao both declare they have no conflicts of interest to disclose.

Human and Animal Rights and Informed Consent This article contains no studies with human or animal subjects performed by the author.

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- •• Of major importance

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