Resistance to Antifungal Agents: Mechanisms and Clinical Impact

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Despite advances in preventive, diagnostic, and therapeutic interventions, invasive fungal infections cause significant morbidity and mortality in immunocompromised patients. The burden of antifungal resistance in such high-risk patients is becoming a major concern. A better understanding of the mechanisms and clinical impact of antifungal resistance is essential to the prompt and efficient treatment of patients with invasive mycoses and to improving the outcome of such infections. Although recent guidelines have attempted to standardize antifungal susceptibility testing, limitations still exist as a result of the incomplete correlation between in vitro susceptibility and clinical response to treatment. Four major mechanisms of resistance to azoles have been identified, all of which rely on altered gene expression. Mechanisms responsible for polyene and echinocandin resistance are less well understood. In addition to discussing the molecular mechanisms of antifungal resistance, this article elaborates on the concept of clinical resistance, which is critical to the understanding of treatment failure in patients with invasive fungal infections.

Invasive fungal infections constitute a significant burden in patients with impaired immunity [1, 2]. The spectrum of fungal pathogens causing infections in immunocompromised hosts is growing [3]. However, the available therapeutic options are limited, particularly for pathogens that are resistant to ≥1 class of antifungals (table 1) [4–24].

We review the molecular mechanisms that underlie in vitro resistance of yeasts and molds to various classes of antifungals. We also discuss the causes and implications of clinical antifungal resistance and offer directives regarding the optimal approach to treatment failure in fungal infections.

DEFINITIONS

Microbiological resistance refers to nonsusceptibility of a fungus to an antifungal agent by in vitro susceptibility testing, in which the MIC of the drug exceeds the susceptibility breakpoint for that organism. Microbiological resistance can be primary (intrinsic) or secondary (acquired). Primary resistance is found naturally among certain fungi without prior exposure to the drug and emphasizes the importance of identification of fungal species from clinical specimens. Examples include resistance of Candida krusei to fluconazole and of Cryptococcus neoformans to echinocandins. Secondary resistance develops among previously susceptible strains after exposure to the antifungal agent and is usually dependent on altered gene expression. The development of fluconazole resistance among Candida albicans and C. neoformans strains illustrates this type of resistance [25, 26].

Clinical resistance is defined as the failure to eradicate a fungal infection despite the administration of an antifungal agent with in vitro activity against the organism. Such failures can be attributed to a combination of factors related to the host, the antifungal agent, or the pathogen. Although clinical resistance cannot always be predicted, it highlights the importance of individualizing treatment strategies on the basis of the clinical situation.

ANTIFUNGAL SUSCEPTIBILITY TESTING

Until recently, the techniques for antifungal susceptibility testing were not standardized. The Clinical and Laboratory Standards Institute published reference methods for susceptibility testing of yeasts [27] and filamentous fungi [28]. These guidelines have created a standard for comparison of clinical data. The European Committee on Antibiotic Susceptibility Testing
has also published guidelines for testing Candida and Aspergillus isolates [29–31]. However, clinicians are still faced with the challenge of how to interpret the results of in vitro antifungal susceptibility testing. MIC values do not always directly associate with response to antifungal therapy [32]. Although in vitro resistance predicts treatment failure in patients with HIV infection with oropharyngeal or esophageal candidiasis [33], no such correlation has been replicated in other settings [34]. The discordance between in vivo and in vitro data is illustrated by the “90–60 rule,” which maintains that infections due to susceptible strains respond to appropriate therapy in >90% of cases, whereas infections due to resistant strains respond in ~60% of cases [32].

Another limitation of MIC determination is that MIC levels are not always the most optimal measure of resistance. Some reports have suggested that the minimum effective concentration, defined as the drug concentration at which morphological alterations of hyphal cells are detected, might be a more appropriate end point than MIC for testing the susceptibility of filamentous fungi to echinocandins [35]. Furthermore, with mold infections, antifungal exposure detects activity against conidia rather than activity against the more clinically relevant hyphal structures.

RESISTANCE TO AZOLE COMPOUNDS

Mechanism of Action of Azoles

Azoles exert their action by inhibiting the C14α-demethylation of lanosterol in fungi, which interferes with the synthesis of ergosterol in the fungal cell membrane. Azoles differ in their affinities to their target, which may account for differences in their spectrum of activity (i.e., in the primary resistance profiles of various fungi) [36]. In addition, variations in the structure of azoles are thought to be responsible for the cross-resistance patterns among Candida species [37–41]. For example, although complete cross-resistance between the triazoles has been observed with Candida glabrata, no such pattern exists with C. krusei [42].

Epidemiology of Azole Resistance

Prior to the introduction of antiretroviral therapy, there was an increase in the prevalence of fluconazole-resistant C. albicans among HIV-infected patients with oropharyngeal or esophageal candidiasis. Widespread use of itraconazole and fluconazole is thought to have been the major driver of azole resistance [43]. Up to one-third of patients with advanced AIDS in one study harbored fluconazole-resistant C. albicans in their oral cavities [44]. Azole-resistant C. albicans is less common among patients with other diseases, such as vaginal candidiasis [45] and candidemia [46]. In general, the rates of azole resistance among the most commonly encountered invasive Candida species remain low, with reported rates of 1.0%–2.1% in C. albicans, 0.4%–4.2% in Candida parapsilosis, and 1.4%–6.6% in Candida tropicalis [47, 48]. A clear exception is C. glabrata, which is second to C. albicans in causing systemic fungal infections in the United States [47]. According to data from the ARTEMIS Global Antifungal Surveillance Program, the incidence of fluconazole resistance in C. glabrata increased from 7% in 2001 to 12% in 2004 [49]. In addition to the changing trends in antifungal susceptibility, there has been a recent shift towards more infections in the immunocompromised host being caused by Candida species other than C. albicans [50, 51]. Several studies have initially incriminated the environmental pressure

Table 1. Antifungal drug susceptibility of selected drug-resistant fungi.

<table>
<thead>
<tr>
<th>Species</th>
<th>ICZ</th>
<th>VCZ</th>
<th>PCZ</th>
<th>AMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus lentulus</td>
<td>0.5–1 (8)</td>
<td>1–2 (8)</td>
<td>NA</td>
<td>1–2 (8)</td>
</tr>
<tr>
<td>Aspergillus ustus</td>
<td>1 to &gt;8 (10)</td>
<td>4–8 (10)</td>
<td>2 (1)</td>
<td>0.25–8 (10)</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>0.03–2 (30)</td>
<td>0.25–4 (30)</td>
<td>0.06–0.25 (8)</td>
<td>0.12–16 (30)</td>
</tr>
<tr>
<td>Scedosporium apiospermum</td>
<td>0.03–2 (30)</td>
<td>0.03 to 0.5 (30)</td>
<td>0.125–1 (3)</td>
<td>0.5 to &gt;16 (30)</td>
</tr>
<tr>
<td>Scedosporium prolificans</td>
<td>2 to &gt;32 (55)</td>
<td>0.5–8 (55)</td>
<td>2 to &gt;8 (55)</td>
<td>1 to &gt;16 (55)</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>&gt;8 (15)</td>
<td>1–4 (10)</td>
<td>&gt;8 (6)</td>
<td>0.25–8 (15)</td>
</tr>
<tr>
<td>Paeilomyces lilacinus</td>
<td>1 to &gt;8 (3)</td>
<td>0.2–1 (3)</td>
<td>0.12–0.5 (3)</td>
<td>&gt;8 (3)</td>
</tr>
<tr>
<td>Scopulariopsis brevicaulis</td>
<td>&gt;8 (25)</td>
<td>&gt;8 (25)</td>
<td>&gt;8 (25)</td>
<td>8 to &gt;16 (25)</td>
</tr>
<tr>
<td>Zygomycetes</td>
<td>0.03–32 (51)</td>
<td>2–64 (51)</td>
<td>0.06–2 (36)</td>
<td>0.03–2 (51)</td>
</tr>
<tr>
<td>Trichosporon asahii</td>
<td>0.03–8 (15)</td>
<td>0.015–8 (15)</td>
<td>0.12–1 (5)</td>
<td>0.5–16 (15)</td>
</tr>
<tr>
<td>Geotrichum capitatum</td>
<td>0.03–0.5 (23)</td>
<td>0.03–0.5 (23)</td>
<td>NA</td>
<td>0.06–0.25 (23)</td>
</tr>
<tr>
<td>Cladophialophora bantiana</td>
<td>≤0.03 to 0.25 (10)</td>
<td>≤0.03 to 1 (10)</td>
<td>≤0.03 to 0.06 (5)</td>
<td>0.25–0.5 (10)</td>
</tr>
</tbody>
</table>

NOTE. Data are compiled from [4–24]. AMB, amphotericin B; CAS, caspofungin; ICZ, itraconazole; NA, not available; PCZ, posaconazole; VCZ, voriconazole.
imposed by exposure to fluconazole [52, 53]. However, such a
temporal association has not been consistently demonstrated
[54, 55]. Other factors, such as exposure to antibacterial agents,
immunosuppressive therapy, and the underlying medical con-
tdition of the host, might prove to be better predictors of the
distribution of Candida species than fluconazole use [56, 57].

Mechanisms of Azole Resistance

Four major mechanisms of resistance to azoles have been de-
scribed in Candida species. More than 1 mechanism can be function-
ing in any given fungal strain with additive effects.

Decreased drug concentration. The development of active
eflux pumps results in decreased drug concentrations at the
site of action. Efflux pumps are encoded in Candida species by
2 genes families of transporters: the CDR genes of the ATP-
binding cassette super family, and the MDR genes of the major
facilitators class [58, 59]. Up regulation of CDR1, CDR2, and
MDR1 has been demonstrated in azole-resistant C. albicans [60, 61]. Other transporter genes have been detected in other Can-
dida species, such as CdCDR1 and PDH1 in C. glabrata and
CdMDR1 and CdMDR1 in Candida dublinensis [62]. Whereas
CDR gene up-regulation confers resistance to almost all azoles,
MDR-encoded efflux pumps have a narrower spectrum specific
for fluconazole.

Target site alteration. It has been demonstrated that mu-
tations in ERG11, the gene encoding for the target enzyme
lanosterol C14α-demethylase, prevents binding of azoles to the
enzymatic site [63]. Furthermore, intrinsic resistance to flu-
conazole in C. krusei isolates has been attributed to decreased
affinity of ERG11p to the drug [64]. In excess of 80 amino
acid substitutions in ERG11p have been detected [65]. Different
mutations can coexist in the same gene with additive effects.

Up-regulation of target enzyme. Some Candida isolates
with reduced susceptibility to azoles have higher intracellular
concentrations of ERG11p than do azole-susceptible strains
[66]. The antifungal agent is, therefore, overwhelmed, and rou-
tine therapeutic concentrations can no longer effectively inhibit
ergosterol synthesis. Target enzyme up-regulation can be
achieved through gene amplification, increased transcription
rate, or decreased degradation of the gene product. However,
this mechanism is thought to contribute little to the overall
resistance burden in Candida species, because only modest in-
creases in enzyme levels have been described.

Development of bypass pathways. Exposure toazole com-
pounds results in depletion of ergosterol from the fungal
membrane and accumulation of the toxic product 14α-methyl-
3,6-diol, leading to growth arrest. Mutation of the ERG3 gene
prevents the formation of 14α-methyl-3,6-diol from 14α-meth-
yldecoesterol [67]. Replacement of ergosterol with the latter
product leads to functional membranes and negates the action
of azoles on the ergosterol biosynthetic pathway. Candida
strains with ERG3 mutation are also resistant to polyenes, be-
cause their cell membranes are devoid of ergosterol.

Although uncommon (with the exception of fluconazole),
resistance to azole compounds among Aspergillus species is
well-recognized. The first resistance mechanism described is
through reduced intracellular concentration of itraconazole
caused by expression of efflux pumps [68]. The second and
more prevalent mechanism relies on modification of the 14α-
sterol demethylase enzyme, which is encoded by the cyp51A
and cyp51B genes [69, 70]. In particular, amino acid substi-
tutions at the M220 position are associated with a resistance
phenotype with elevated MICs to all azoles, whereas substitu-
tions at G54 result in cross-resistance to itraconazole and
posaconazole. Recently, a new mechanism of azole resistance in
Aspergillus fumigatus has been described, where mutations in
the promoter region of cyp51A lead to overexpression of the
protein product [71]. Continued surveillance for azole resis-
tance and the magnitude of cross-resistance between azoles
among Aspergillus species is warranted, especially with the in-
creasing use of new-generation azoles for the prevention and
treatment of invasive aspergillosis.

It should be additionally noted that the activity of azoles
against emerging fungal pathogens, such as zygomycetes and
Fusarium and Scedosporium species, is variable. Although flu-
conazole consistently lacks activity against these organisms,
new-generation azoles possess variable activity, highlighting the
importance of relying on susceptibility testing to guide directed
antifungal therapy.

RESISTANCE TO POLYENES

Mechanism of action of polyenes. Polyenes (amphotericin B
deoxycholate and its lipid-associated formulations) act by in-
serting into the fungal membrane in close association with
ergosterol. The subsequent formation of porin channels leads
to loss of transmembrane potential and impaired cellular
function.

Epidemiology of polyene resistance. Although resistance to
amphotericin B among Candida strains remains rare [72], there
have been recent reports of increasing MICs to amphotericin
B among C. krusei and C. glabrata isolates [73]. In addition,
intrinsic polyene resistance is frequently noted in Candida lu-
sitaniae [74] and Trichosporon beigelii [75]. However, identi-
fication of polyene-resistant isolates has been difficult to re-
produce [76, 77]. Filamentous fungi are more likely than yeasts
to have reduced susceptibility to polyenes. Among Aspergillus
species, Aspergillus terreus is generally resistant to amphotericin
B [78]. Polyene resistance is increasingly encountered in other
Aspergillus species, such as Aspergillus flavus and even A. fu-
migatus, which traditionally exhibits the highest susceptibility
to amphotericin B [78, 79]. According to the SENTRY pro-
gram, the prevalence of polyene resistance among Aspergillus species

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has increased remarkably, with only 11.5% of A. fumigatus isolates inhibited at \( \leq 1 \mu g/mL \) [79]. However, large longitudinal studies are lacking. In addition, rare Aspergillus species, such as Aspergillus ustus or Aspergillus lentulus, which are relatively resistant to most antifungal agents, have been reported to cause invasive disease [17, 80]. Other molds, such as Scedosporium apiospermum, Scedosporium prolificans, and Fusarium species, are typically resistant to amphotericin B [78].

Mechanisms of polyene resistance. Resistance breakpoints for polyenes have not been determined. Most clinicians use an MIC of \( \geq 1.0 \mu g/mL \) to indicate resistance to amphotericin B. Defects in the ERG3 gene involved in ergosterol biosynthesis lead to accumulation of other sterols in the fungal membrane. Consequently, polyene-resistant Candida and Cryptococcus isolates have relatively low ergosterol content, compared with that of polyene-susceptible isolates [81]. Resistance to amphotericin B may also be mediated by increased catalase activity, with decreasing susceptibility to oxidative damage [82].

RESISTANCE TO ECHINOCANDINS

Echinocandins inhibit the synthesis of \( \beta-1,3-D \text{-glucan}, \) which is integral to the structure and function of the fungal cell wall. The formation of a defective cell wall leads to cell rupture in yeasts and aberrant hyphal growth in molds. Echinocandins are highly effective against Candida and Aspergillus species, but they have no activity against yzogomycetes or against Cryptococcus, Trichosporon, Scedosporium, and Fusarium species [12]. Among the Candida species, C. parapsilosis and Candida guilliermondii isolates have higher MIC values than do C. albicans isolates, although the clinical significance of this reduced in vitro susceptibility has been debated [83]. Nonetheless, breakthrough infections with C. parapsilosis have been reported in patients receiving echinocandins for other indications [84]. Of note, optimal detection of echinocandin resistance requires variation of the Clinical and Laboratory Standards Institute methodology [85].

The mechanisms of echinocandin resistance are still being investigated. In Candida species, secondary resistance is associated with point mutations in the Fks1 gene of the \( \beta-1,3-D \text{-glucan} \) synthase complex [86]. Within Fks1 lies a highly conserved region where several mutations have been identified, mostly at the Ser645 position. On the other hand, the mechanism of resistance in C. neoformans is not completely understood. Possibilities include an echinocandin-resistant \( \beta-1,3-D \text{-glucan} \) synthase target, efflux pumps, and degradation pathways [87].

It has been observed that echinocandin-susceptible Candida and Aspergillus isolates have the ability to grow in vitro at concentrations exceeding the MICs of caspofungin [88]. This paradoxical phenomenon, which is referred to as the “eagle effect,” is strain-dependent and has been related to up-regulation of chitin synthesis in the fungal cell wall [89]. However, its in vivo consequences have not been fully determined, and the highest treatment doses of echinocandins have not yet shown this phenomenon in humans [90].

The burden of echinocandin resistance is still poorly appreciated. There have been recent reports of echinocandin resistance in patients with Candida infections (due to C. albicans, C. glabrata, C. krusei, and C. parapsilosis) [91–94]. Infections ranged from esophageal candidiasis to prosthetic valve endocarditis. In all of the described cases, resistance to echinocandins developed during therapy and was associated with treatment failure. Resistance mechanisms other than Fks1 mutations were involved in some cases [94].

RESISTANCE TO FLUCYTOSONE

Flucytosine is a base pyrimidine analog that inhibits cellular DNA and RNA synthesis. Some yeast strains are intrinsically resistant to flucytosine because of impaired cellular uptake secondary to a mutation in cytosine permease. On the other hand, acquired resistance results from defects in flucytosine metabolism through mutations in cytosine deaminase or uracil phosphoribosyl transferase. The prevalence of primary resistance to flucytosine remains low (1%–2% among Candida isolates [53] and <2% among C. neoformans isolates [95]). However, the speed at which these yeasts can develop resistance to flucytosine has prompted clinicians to use flucytosine only in combination with other antifungal agents, mainly amphotericin B [96].

CLINICAL RESISTANCE

Recent therapeutic trials in invasive mycoses have found that overall (empirical and directed) treatment success rates have ranged from 32% to 74% [83, 97, 98], indicating that in vitro susceptibility testing alone is not sufficient to predict clinical success. The majority of patients with invasive mycoses experience treatment failure because of clinical resistance, which is a concept critical to the outcome of a fungal infection. Clinical resistance can occur under several circumstances (table 2).

First, an incorrect diagnosis can be categorized into 2 areas: (1) with the use of empirical and preemptive strategies, treatment failure may relate to another diagnosis or multiple pathogens; (2) in patients receiving cytotoxic therapy, monoclonal antibodies, antiretroviral therapy, and other immune modulating therapies, the immune reconstitution inflammatory syndrome can dominate the clinical response to antifungal therapy. Because it is associated with prominent signs and symptoms of inflammation, immune reconstitution inflammatory syndrome can be confused with failure to control fungal growth [99].

Second, the net state of immunosuppression is often so negative that antifungals cannot overcome the severe immunodeficiencies [100]. For instance, marrow failure and prolonged...
Table 2. Principal factors determining antifungal clinical resistance.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrong diagnosis</td>
<td>Weak diagnostics and/or IRIS</td>
</tr>
<tr>
<td>Net state of immunosuppression</td>
<td>Improvement in immunity of host is essential</td>
</tr>
<tr>
<td>High burden of fungus at initiation of treatment</td>
<td>Earlier treatment intervention improves outcome</td>
</tr>
<tr>
<td>Strain acquisition of increased virulence</td>
<td>Probably less of a problem than host factors but can be measured</td>
</tr>
<tr>
<td>Pharmacokinetics and/or pharmacodynamics</td>
<td>Drug toxicity, drug–drug interaction, drug levels</td>
</tr>
<tr>
<td>Site of infection</td>
<td>Drug penetration, tissue necrosis, foreign body</td>
</tr>
<tr>
<td>Length of treatment and/or compliance</td>
<td>Precision is not certain; patient and clinician may lose focus on long-term drug administration</td>
</tr>
<tr>
<td>Underlying disease</td>
<td>Final arbitrator in most invasive mycoses</td>
</tr>
</tbody>
</table>

NOTE. IRIS, immune reconstitution inflammatory syndrome.

neutropenia are difficult to overcome in the treatment of invasive aspergillosis, and specific immune modulators have not been convincingly shown to improve host immunity [101, 102].

Third, recent data support that early treatment of fungal infection with a lower burden of organisms reduces the number of treatment failures [103–105]. In some patients, the burden of the fungus is too great for antifungals to help recovery.

Fourth, some fungal strains have been found to possess more virulent characteristics than other strains, and infection with such strains might lead to worse prognosis. For example, an outbreak of Cryptococcus gattii infections in Vancouver, Canada, was linked to the creation of a more virulent cryptococcal strain through a recombination event in nature [106]. Most patients were immunocompetent individuals who resided in or had recently traveled to Vancouver [107]. In contrast, infections with the relatively less virulent C. parapsilosis can be often successfully treated with echinocandins despite reduced in vitro susceptibility [108].

Fifth, when using a polypharmacy approach to care, toxicities from polyenes (nephrotoxicity) and azoles (hepatitis) can be a cause of treatment failure [83, 97]. Furthermore, drug-drug interactions can contribute to morbidity and mortality [109, 110]. High fluconosine levels have always been a cause for concern regarding toxicity. As for azole compounds, the increasing use of serum drug levels has begun to emphasize the correlation between direct drug exposure and outcome [111–114]. For example, a drug-resistant infection could result from poor drug absorption or genetic differences in the metabolism of antifungals. Therefore, it is important to be cognizant of the importance of adequate dosing, especially in the less-studied pediatric population.

Sixth, the site of infection can have a major influence on drug resistance. For example, the good penetration of fluconazole into the CSF (≥70% of serum concentration [115]) makes it a better choice for treating meningitis than itraconazole (the rate of recrudescent among patients with cryptococcal meningitis after initial response to itraconazole is 20% [116]). Voriconazole, with its antifungal activity and CNS penetration, has become a first-line choice for certain CNS mold infections [117]. When the site of infection is necrotic with poor blood supply, a debulking surgery is essential to overcome antifungal treatment resistance [118]. Finally, the ability of fungi to form biofilms on foreign bodies is a primary reason for clinical failure. The echinocandins and lipid formulations of amphotericin B can impact fungal growth in biofilms better than amphotericin B and azoles [119]. However, numerous trials involving candidemia have shown that treatment failure and mortality are high among patients with catheter-related infections without removal of the catheter [120–122].

Seventh, a suboptimal length of treatment often predicts failure. Rigorously studied appropriate lengths of therapy are lacking for most mycoses. Moreover, compliance is a potential problem for success with prolonged courses of therapy.

Finally, the biggest obstruction to success is the underlying disease, which is the barometer that measures clinical success and failure. It is necessary to either prevent fungal infections in high-risk patients or successfully control the underlying disease when mycoses occur.

CONCLUSIONS

Antifungal drug resistance is a prominent feature in the management of invasive mycoses, and its epidemiological characteristics continue to evolve. Fortunately, unlike bacteria, there are no described drug resistance plasmids or transposons to amplify antifungal resistance. A principal factor in patients with serious underlying diseases is clinical resistance. This term covers less mechanistic understandings but can lead to an unfavorable outcome, and clinicians must approach patients who are experiencing treatment failure with the 8 factors of clinical resistance in mind.

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