Antifungal Drug Resistance in *Candida albicans*

Fungal infections and drug resistance are on the rise for this near-ubiquitous commensal organism

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Although a potential pathogen, *Candida albicans* is present as a commensal organism in many, if not most, healthy individuals, where it may be found on the skin and in the oral cavity, gastrointestinal tract, and vagina. When something perturbs the balance between the host and this yeast, notably when the host’s immune system becomes compromised or when the microenvironment shifts to favor the growth of the yeast, this commensal organism readily becomes pathogenic. For example, when the immune systems of HIV-infected individuals begin to deteriorate, they frequently develop oral candidiasis. This condition also often develops among individuals with normal immune systems who are taking antibiotics because such drugs eliminate the normal, local bacteria and allow *C. albicans* to grow in this microenvironment. Similarly, changes in hormone levels that affect the microenvironment of the vagina lead certain women to experience frequent episodes of vaginal candidiasis.

During the past 15 years, fungal infections have become an increasingly important cause of disease among both immunocompetent and immunocompromised individuals. For example, besides candidiasis, cryptococcosis and aspergillosis cause major problems within the HIV-infected population. Aspergillosis is also increasingly common throughout the United States and in other developed countries among other immunocompromised individuals. Meanwhile, several other fungal diseases, including blastomycosis, histoplasmosis, and coccidioidomycosis, are each recognized as endemic in specific regions of the United States.

Despite these increased numbers and diversity of fungal infections and a growing awareness of them, therapeutic options for treating such infections are relatively limited. Moreover, although several antifungal drugs are currently under development and testing, most individuals with fungal infections are treated with either azole or polyene antifungal drugs—typically for prolonged periods, but not always with clinical success. Not surprisingly, azole-resistant strains of fungal pathogens, particularly *C. albicans*, are frequently isolated from HIV-infected patients and are emerging among patients in other immunocompromised population groups, such as bone marrow transplant recipients. Published reports also indicate a sporadic incidence of polyene-resistant strains of *C. albicans* and *Cryptococcus neoformans*.

**Mechanisms of Action of the Azole and Polyene Antifungal Drugs**

Currently, the most widely used drug for treating candidiasis is fluconazole (Diflucan), a triazole drug that can be administered orally and is effective against oral, vaginal, and invasive *C. albicans*. In ordinarily administered doses, the azoles are fungistatic rather than fungicidal, meaning they inhibit the growth of but do not kill yeast cells. Other members of theazole family of antifungal drugs, several of which are administered only as topical agents or show poorer bioavailability than fluconazole, include itraconazole, ketoconazole, clotrimazole, and miconazole. Although members of the polyene class of antifungal drugs, such as amphotericin
B and nystatin, are fungicidal, they also are considered more toxic than the azoles.

Both major classes of the commonly used antifungal drugs, the azoles and polyenes, target ergosterol in the fungal plasma membrane. Azole drugs inhibit a key enzyme in the biosynthetic pathway for ergosterol, the major sterol component of the fungal plasma membrane and a chemical relative of cholesterol, which is a component of mammalian plasma membranes. The target yeast enzyme, lanosterol 14α-demethylase (14DM), is a cytochrome P-450 enzyme containing a heme cofactor in the catalytic site, to which the azole drugs bind. When azoles are present, this enzyme produces less of its normal ergosterol end product. Consequently, sterol precursors are introduced into the plasma membrane, thereby disrupting several of its functions and reducing the effectiveness of several membrane-associated enzymes. Azole drugs bind preferentially to the fungal rather than the comparable mammalian enzyme, meaning there is little if any disruption of host cell membranes.

Amphotericin B and other polyene drugs are amphiphatic molecules that bind preferentially to membranes containing ergosterol. In yeast cells, these drugs form pores in the plasma membrane, causing essential cytosolic components to leak from the cells. Amphotericin B can also interact with mammalian membranes, which can lead to toxicity, especially in the kidneys.

Whether the polyenes and azoles act synergistically, additively, or perhaps even antagonistically when administered together is not fully understood. In any case, because the biochemical target for both classes of drug is the yeast cell membrane, resistance to drugs from one class of antifungals sometimes may elicit resistance to drugs of the other class. Surely, new classes of antifungal drugs with entirely different modes of action would be of great clinical value.

**Clinical Components of Azole Resistance**

*C. albicans* manifests resistance to antifungal drugs at two (if not more) distinct levels, cellularly and clinically. Clinical resistance is defined as any case of candidiasis that is not resolved following standard doses of an otherwise appropriate antifungal drug. By definition, cellular resistance is independent of the host. Thus, it involves strains of *C. albicans* that are less susceptible in vitro to otherwise inhibitory doses of standard antifungal drugs. Cellular resistance can be a major cause of clinical resistance.

Ten years ago, clinical antifungal drug resistance was a rare occurrence in cases of infection with *C. albicans*, and it occurred mainly among patients with chronic mucocutaneous candidiasis who were receiving long-term ketoconazole therapy. During the AIDS epidemic, however, recurrent oral and esophageal candidiasis has become common among the HIV-infected population. Oral fluconazole was introduced seven or eight years ago as a means for treating such patients, and, because it was nearly universally effective and produced few side effects, it quickly became the drug of choice.

During the last few years, however, clinical resistance to fluconazole has become an increasing problem, particularly in the HIV-infected population. Clinically resistant candidiasis is now found globally in the HIV-infected population, suggesting that the emergence of azole resistance may not be attributable merely to the spread of a few resistant strains of *C. albicans*. One estimate in a study from Great Britain suggests that 33% of AIDS patients harbor strains of *C. albicans* that are resistant to fluconazole. From this and other studies, there is no indication that *C. albicans* is resistant only to fluconazole. Indeed, many fluconazole-resistant strains are also resistant to several other azoles.

Inconsistencies among laboratory tests complicated early efforts to detect cellular antifungal drug resistance. To overcome this problem, a collaboration of several laboratories has developed a reference method for antifungal susceptibility testing. This method, published by the National Committee for Clinical Laboratory Standards (NCCLS), enables investigators to determine minimum inhibitory concentrations (MICs) for yeast clinical isolates and then reliably compare those values to MICs obtained in other labora-
tories. The new standardized method for determining MICs in yeasts greatly reduces variations in observed antifungal susceptibilities among laboratories testing fungal isolates, and it also provides a relatively good correlation between in vitro MICs and treatment failure for oral-pharyngeal candidiasis. However, these correlations are typically not so close for deep-tissue or disseminated candidiasis.

Nonetheless, correlations between in vitro MICs for antifungal agents and clinical outcome are being extensively evaluated for the first time. Although there are exceptions, strains with low MICs can usually be successfully treated with fluconazole, whereas strains with high MICs cannot. Correlations between MIC values and clinical success in treating fungal infections with fluconazole are comparable in reliability to those for treating bacterial infections with antibiotics.

Several factors contribute to the development of clinical resistance, some of which reflect resistance at the cellular level, whereas others do not (Table 1). Among all these factors, an individual's total cumulative dose of fluconazole—whether administered to treat diagnosed infections or for prophylaxis—appears to be the single most important risk factor so far for the emergence ofazole resistance. Other factors that contribute to resistance in *C. albicans* are familiar from studies of antibiotic resistance in bacterial pathogens. For example, low, intermittently administered doses of azole are associated with the appearance of resistance. Similarly, several years ago HIV-infected patients were being treated prophylactically with low doses of fluconazole for oral candidiasis, a practice that also appears to have contributed to the emergence of cellular resistance. Currently, individuals who develop cryptococcal meningitis are routinely treated on a long-term basis with azole drugs to prevent recurrences. Whether this practice encourages the development of azole-resistant *Candida* or *Cryptococcus* strains remains to be seen.

At the cellular level, at least two distinct factors contribute to the development of resistance to the azole drugs. First, these drugs are fungistatic—they do not kill the yeasts but only inhibit their growth. Second, the type of *C. albicans* typically residing on the mucosal surfaces of any individual is not easily eliminated or replaced with another strain. Therefore, these fungistatic drugs exert pressure for resistant mutations that focuses very narrowly on the resistant *C. albicans* strain.

In any random sample of yeast isolates from different patients, the MICs range from highly susceptible (<0.25 μg ml⁻¹) to highly resistant (>64 μg ml⁻¹). Several observations suggest that azole resistance develops from susceptible strains with MICs that are higher than average (4 to 8 μg ml⁻¹). This phenomenon may reflect the fact that strains that are slightly less susceptible to azoles will be less inhibited than other strains when the drug is present, encouraging selection for still higher levels of resistance.

Several factors that contribute to clinical resistance to azoles are not directly related to measured MICs of *C. albicans* strains (Table 1). First and foremost, the underlying state of the infected individual's immune system has profound effects on clinical outcome when treating fungal infections. Azole-resistant candidiasis occurs frequently among AIDS patients whose immune systems are particularly debilitated. This correlation appears to be independent of the individual's cumulative dose of fluconazole.

Other medical complications contribute to antifungal drug resistance. For instance, AIDS patients are prone to xerostomia, or dry mouth, reflecting a reduced flow of saliva that, in turn, reduces the amount of fluconazole that reaches the oral cavity to treat local candidiasis. Other drugs may affect this condition. For example, because antidepressant drugs can cause xerostomia, they may also increase the necessary dose of fluconazole needed to treat oral infections.

Other sorts of drug interactions affect clinical resistance. For instance, drugs that alter the gastrointestinal tract affect absorption of fluconazole and, hence, the dose that may be needed to treat oral candidiasis. Finally, noncompliance with prescribed antifungal therapy has a major effect on drug resistance in two ways. If the patient does not take the prescribed drugs, the oral

### Table 1. Clinical Components of Antifungal Resistance

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<td>Drug/drug interactions</td>
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candidiasis will appear to be resistant. Moreover, noncompliance amounts to self-directed intermittent dosing, which itself encourages selection of azole-resistant *C. albicans* strains.

**Cellular Mechanisms of Azole Resistance**

Several factors account for cellular resistance to antifungal agents, defined specifically as resulting in MIC values of greater than 64 μg/ml for fluconazole (Table 2). One common yet frequently overlooked factor is that some strains of *C. albicans* have intrinsically high MICs. Within a large selection of clinical isolates, some strains are highly susceptible whereas others are highly resistant to fluconazole, even among isolates that presumably were never exposed to antifungal drugs.

Intrinsically high MICs occur in two fungal species that are closely related to *C. albicans*, namely *Candida glabrata* and *Candida krusei*. Clinical isolates of these two species ordinarily have relatively high MICs to fluconazole. According to recent surveys, in HIV-infected patients and other immunocompromised populations that have been exposed to fluconazole, these two species are causing infections more and more often. Typically, the initial oral fungal isolate from such patients will be a susceptible strain of *C. albicans*, but soon after azole therapy clears that problem, the susceptible *C. albicans* will be replaced with an intrinsically resistant *C. glabrata* or *C. krusei* strain.

*C. albicans* strains usually persist for long periods as commensals. Only rarely in the absence of azole drug treatment are such strains replaced with other strains, whereas strain replacement occurs as often as 40% of the time when antifungal drug resistance enters the picture. However, the source of new fungal strains has seldom been documented, and usually new strains have been traced to those carried by a sexual partner. Presumably, a resistant strain is transferred from a patient with a history of azole therapy to a sexual partner who has not received antifungal therapy.

Azole therapy can select for mutant strains of *C. albicans* that are less susceptible to these drugs than is the parent. These mutations may persist in the strain even in the absence of selective pressure by the drug; once a strain has been genetically altered, it will remain azole resistant even if the drug pressure is removed (Fig. 1). Alternatively, azole resistance sometimes is transient—apparent when fluconazole is present but gone soon after the drug is removed. This transient or epigenetic resistance is most likely the result of an increase or decrease in expression of a gene important for drug resistance. Epigenetic expression is particularly difficult to study in a clinical setting since the trait disappears as soon as the clinical isolate is removed from the patient.

**Molecular Mechanisms of Azole Resistance**

How azoles enter susceptible fungal cells is not known, although their relative hydrophobicity may facilitate entry by passive diffusion. Once inside cells, azoles interact with the 14DM enzyme in the ergosterol biosynthetic pathway, allowing precursors to be incorporated into newly synthesized regions of plasma membrane. Not all of theazole that enters the cell remains there, because two low-level active efflux systems, the ABC transporters and the major facilitators, pump free drug from the cell.
In a prototype azole-resistant cell, several mechanisms contribute to its lower susceptibility to azole drugs (Table 3 and Figure 1). Presumably, azoles enter resistant cells just as they ordinarily enter susceptible cells. However, if the sterol composition of the cell membrane is altered, perhaps the usual uptake process also becomes altered. Thus, for example, changes to other genes in the ergosterol pathway may also affect a cell’s susceptibility to azoles. Although such changes have been identified biochemically in resistant strains, the specific genetic changes have not.

Once the antifungal molecules enter a cell, their interaction with the target enzyme, 14DM, can be modified in at least two ways. First, certain point mutations in the gene for 14DM (ERG16) make the enzyme less sensitive to azole drugs. For at least one such specific mutation, the mutation appears to have been copied to both alleles, creating a strain with inherently higher resistance than others. In addition to point mutations, enzyme overexpression, which leads to more of the azole target molecule per cell, necessitates higher doses of drug to achieve inhibitory effects comparable to those seen in susceptible cells. Although overexpression of 14DM through gene amplification has been documented in a highly resistant strain of *C. glabrata*, it has not yet been shown in *C. albicans*.

Efflux mechanisms, which reduce the cytoplasmic concentration of drugs and other small molecules, are a major factor affecting a cell’s susceptibility to azoles. Dominique Sanglard at the Centre Hospitalier Universitaire Vaudois in Lausanne, Switzerland, has been investigating two classes of efflux systems, the ABC transporters and major facilitators, in *C. albicans*. ABC transporters are associated with drug resistance in a wide variety of organisms besides yeasts, including mammals. The genes of the ABC transporters encode several components, including a transmembrane pore composed of several segments and two ATP-binding cassettes (ABC), which are situated on the cytosolic side of the membrane and provide metabolic energy for the pump.

The major facilitators are also associated with drug resistance, but so far seem to be active
mainly in prokaryotic systems. The genes of this system encode a transmembrane pore composed of several segments. Instead of an ABC system for metabolic energy, the major facilitators use membrane potential or cotransport. The genes encoding several distinct ABC transporters have been cloned from *C. albicans*, including a series of CDR genes (for *Candida* drug resistance), while only one major facilitator (MDR1, for multidrug resistance) from *C. albicans* has been identified so far. Both CDR and MDR1 genes are overexpressed in azole-resistant isolates of *C. albicans*. Moreover, when these genes are deleted in such strains, the cells become hypersensitive to azole drugs. The CDR proteins serve as efflux pumps for most azole drugs, whereas the MDR1 protein appears to prefer fluconazole, although it can facilitate transport of unrelated nonazole drugs.

Although each of these factors contributes to azole resistance in one or another strain of *C. albicans*, independent clinical isolates rarely, if ever, exhibit all of the changes that have been identified so far, and still other mechanisms undoubtedly have yet to be identified. Nonetheless, azole-resistant isolates that have been evaluated manifest a subset of these mechanisms. For example, within one series of 17 isolates in which resistance developed gradually, at least five genetic alterations contribute to the final resistant phenotype. These changes include overexpression of three genes (MDR1, CDR1, and ERG16) and a point mutation and gene conversion in ERG16. Which of these mechanisms are the most common in azole drug resistance is not known.

**Clinical Implications and Future Directions**

While there are no comprehensive clinical data, azole dosages likely influence the development of drug resistance. On the basis of what is known about antibiotic resistance in bacterial pathogens, low doses of drugs administered over long periods, either continuously or intermittently, likely accelerate the development of resistance, whereas high doses of drug administered long enough to resolve the infection would likely avoid the development of resistance. Whether these principles apply to the development of clinical resistance to azole drugs remains to be determined.

Because so many molecular mechanisms can lead to azole resistance at the cellular level, devising a simple molecular test for it seems unlikely. Nonetheless, information about these mechanisms has profound implications for the development of antifungal drugs, especially for those meant for treating fluconazole-resistant *C. albicans*. For example, structurally modified versions of azole drugs may well be substrates for the ABC transporters or other efflux pumps, suggesting that cells may quickly overcome their susceptibility to such "new" antifungal agents. Even if such new azoles are not ejected from cells by characterized pumps, the genomes of *C. albicans* and related yeasts may encode the components of more than 50 efflux pumps, according to current estimates, any one of which could be overexpressed or modified under azole drug selection pressure to help in removing such chemically modified drugs from cells.

When azole resistance modifies the sterol components of the plasma membrane, the cell might also become increasingly resistant to polyenes such as amphotericin B. Modifications in the ergosterol pathway could reduce ergosterol levels in the plasma membrane, resulting in fungal strains with reduced susceptibility to amphotericin B. Since the azoles and polyenes are currently the main antifungal drugs used to treat both mucosal and disseminated forms of candidiasis, developing resistance to both types of drug would have serious medical consequences.

Given the significance of efflux pumps in azole resistance and the wide variety of compounds that can be substrates for these cellular pumps, investigators developing new antifungal compounds, such as the pneumocandins, will need to test them for hydrophobicity and other chemical properties that could influence their ability to serve as substrates for these pumps. Even if these new drugs have entirely unrelated modes of action, their intracellular concentrations may still be reduced by the overexpression of an efflux pump. Conversely, the development of an effective inhibitor of these efflux pumps might aug-
ment azole activity or perhaps prove an effective antifungal agent on its own. However, drugs such as verapamil that inhibit ABC transporters in mammalian systems have not proved effective when tested in fluconazole-resistant *C. albicans*. The yeast cell wall may prevent such drugs from reaching appropriate sites where they can act on fungal efflux components.

Meanwhile, at the clinical level, the judicious use of available azoles and polyenes could at least reduce the development of drug resistance among some fungal pathogens. Thus, if dosing of new antifungal drugs were more carefully controlled from the outset of their clinical use and if new compounds were monitored for their ability to act as substrates for efflux pumps, their medically useful lifetimes might be extended. Moreover, the best way to avoid development of resistance to antifungal agents may entail treating infected individuals with drug combinations—simultaneously administering two or more drugs, each of which has a separate mode of action.

A more detailed and comprehensive review of antifungal resistance has been submitted to *Clinical Microbiological Reviews*.

**ACKNOWLEDGMENTS**

I thank Marty Thorning for her help and patience during the preparation of the color figure. I thank many colleagues working on antifungal drug resistance for their contributions to the field, their stimulating and creative discussions, and their advice. I thank Scott Filler, Kieren Marr, Christine Morrison, Tom Patterson, Jo-Anne van Burik, and members of my laboratory and SBI for their comments on the manuscript.

My work in antifungal resistance is supported by National Institutes of Health NIDR grant RO1 DE-11367.

**SUGGESTED READING**


