Antifungal Resistance in Pathogenic Fungi

Sofía Perea and Thomas F. Patterson

Department of Medicine, Division of Infectious Diseases, The University of Texas Health Science Center at San Antonio, and Audie Murphy Division, South Texas Veterans Health Care System, San Antonio, Texas

Pathogenic fungi are the cause of life-threatening infections in an increasing number of immunocompromised patients. The intrinsic resistance to antifungal therapy observed in some genera, along with the development of resistance during treatment in others, is becoming a major problem in the management of these diseases. We reviewed the epidemiology of the most common systemic fungal infections for which antifungal resistance is a potential problem, the mechanisms of antifungal resistance, the correlation between in vitro susceptibility testing and clinical outcome, and the clinical implications of antifungal resistance.

Several factors have contributed to the increase of life-threatening systemic fungal infections in recent years. Among them is the expansion of severely ill and/or immunocompromised patient population, including HIV-infected patients, patients with cancer who have chemotherapy-induced neutropenia, and transplant recipients who are receiving immunosuppressive therapy. Other factors that have also contributed are the frequent use of more-invasive medical procedures, treatment with broad-spectrum antibiotics and glucocorticoids, receipt of parenteral nutrition, and receipt of peritoneal dialysis or hemodialysis [1]. The majority of nosocomial fungal infections are caused by Candida species, with Candida albicans being the most common etiological agent of fungal bloodstream infections. Together with Candida infections, Aspergillus infections, which mostly affect granulocytopenic and other immunocompromised patients, account for 90% of all nosocomial fungal infection [2]. Cryptococcus neoformans has become also a major opportunistic fungal pathogen in immunocompromised individuals, mainly HIV-infected patients and those receiving immunosuppressive treatment for cancer, organ transplantation, and other serious medical conditions [3]. Several other less common fungi, many of which are intrinsically resistant to the available antifungal drugs, are becoming increasingly recognized as sources of deep fungal infections, such as Zygomycetes (Rhizopus arrhizus, Absidia corymbifera, and Rhizomucor pusillus), Fusarium species, Trichosporon beigelii, Blastoschizomyces capitatus, Scedosporium species, Acremonium species, and dematiaceous fungi [2].

The antifungal drugs currently available for the treatment of invasive mycoses can be divided in 4 different classes on the basis of their mechanisms of action: (1) alteration of membrane function (amphotericin B); (2) inhibition of DNA or RNA synthesis (flucytosine); (3) inhibition of ergosterol biosynthesis (azoles [fluconazole, itraconazole, and the newer agents voriconazole, posaconazole, and ravuconazole]); and (4) inhibition of glucan synthesis (echinocandins [caspofungin, micafungin, and anidulafungin]).

Clinical resistance is classically defined as persistence or progression of an infection despite the administration of appropriate antimicrobial treatment. The prediction of the clinical outcome for a patient with a mycotic infection is often a difficult question—and one in which many factors intervene. The antifungal susceptibility of the fungal isolate is only one of the elements that contribute to clinical resistance; other factors include the pharmacokinetics of the antifungal drug used, host factors, the site of infection, and the fungal pathogen itself. In general, fungi can be intrinsically resistant to antifungal drugs (primary resistance) or can develop resistance in response to exposure to the drug during treatment (secondary resistance).

CANDIDA INFECTIONS

Epidemiology. The rates of candidemia are increasing worldwide; candidemia now represents the fourth most common
nosocomial bloodstream infection in the United States [4]. Systemic Candida infections are associated with a high mortality rate (38%) and prolonged hospital stay. Presently, C. albicans accounts for ~50%–60% of all nosocomial Candida infections, although a noticeable shift toward Candida species other than C. albicans has been observed, which is important because of intrinsic or acquired antifungal resistance in several of these species. These non-albicans species of Candida yeasts include, in order of typical decreasing frequency, Candida glabrata, Candida parapsilosis, Candida tropicalis, and Candida krusei, among others [5].

Prevalence of azole resistance and the development of resistance during treatment. A recent 3-year longitudinal surveillance study of bloodstream Candida infections in North America and Latin America that included episodes caused by C. albicans (54%), C. glabrata (16%), C. parapsilosis (15%), C. tropicalis (8%), C. krusei (1.6%), and other Candida species (4.6%) showed that resistance to triazoles is still not usually a frequent event. Globally, <2.5% and <9% of the Candida species isolates analyzed were resistant to fluconazole and itraconazole, respectively [4]. C. albicans and Candida dubliniensis are the 2 species most susceptible to the currently available antifungal azoles in vitro [4, 6]. C. parapsilosis, Candida lusitaniae, and Candida guilliermondii are generally susceptible toazole agents in vitro. C. glabrata is frequently less susceptible, as is C. krusei, which is intrinsically resistant to fluconazole in vitro.

C. glabrata isolates generally exhibit bimodal susceptibility to azoles, with some isolates demonstrating frank azole resistance (MIC, >64 μg/mL), whereas others are significantly more susceptible. Similarly, some strains of C. tropicalis also exhibit azole resistance, although, generally, the MIC₉₀ for this species indicates general susceptibility to azoles, and often in vitro resistance appears as a result of its strong tendency to produce trailing growth. Recent studies have highlighted important geographic variations in the distribution of Candida species and differences in the prevalence of resistance [4, 7]. Table 1 summarizes the characteristics of Candida species and the general patterns of susceptibility to antifungal agents.

Table 1. Characteristics of Candida species that cause bloodstream infection and patterns of antifungal susceptibility.

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Source</th>
<th>Predisposing factor(s)</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Flucytosine</th>
<th>Amphotericin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>Endogenous (gastrointestinal tract), exogenous</td>
<td>Neutropenia, mucosal damage, presence of vascular catheter</td>
<td>S</td>
<td>S</td>
<td>S to R²</td>
<td>S</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>Endogenous</td>
<td>Solid tumor, abdominal surgery, prior use of azoles</td>
<td>S-DD to R</td>
<td>S-DD to R</td>
<td>S</td>
<td>S to I</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>Exogenous, endogenous</td>
<td>Presence of vascular catheter, hyperalimentation, presence of prosthetic heart valve</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>Endogenous</td>
<td>Neutropenia, mucosal damage</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C. krusei</td>
<td>Endogenous</td>
<td>Neutropenia, receipt of fluconazole prophylaxis</td>
<td>R</td>
<td>S-DD to R</td>
<td>I to R</td>
<td>S to I</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>Endogenous, exogenous</td>
<td>Neutropenia, hematologic malignancy</td>
<td>S</td>
<td>S</td>
<td>S to R</td>
<td>S</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>Endogenous</td>
<td>Neutropenia</td>
<td>S</td>
<td>S</td>
<td>S to R</td>
<td>S</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>Endogenous</td>
<td>Neutropenia, mucosal damage, HIV infection</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

**NOTE.** Data are from [8] and [9]. I, intermediate resistance; R, resistant; S, susceptible; S-DD, susceptible, dose dependent.

* Serotype B.
Table 2. Molecular mechanisms involved in the development of secondary resistance to antifungals in pathogenic fungi.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Molecular mechanisms involved in the development of secondary resistance, by antifungal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluconazole</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Overexpression/mutations of target enzyme, lanosterol 14α-demethylase; overexpression of efflux pumps (CDR and MDR genes)</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Overexpression of efflux pumps (CgCDR genes)</td>
</tr>
<tr>
<td>Candida dubliniensis</td>
<td>Fluconazole: overexpression of efflux pumps (CdCDR and CdMDR genes)</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>Primary resistance to fluconazole</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>—</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>Fluconazole: alterations in target enzyme, lanosterol 14α-demethylase; overexpression of efflux pumps (MDR genes)</td>
</tr>
</tbody>
</table>

— Defects in sterol biosynthetic pathway (alterations in sterol Δ7-24 isomerase enzyme)

Cryptococcus neoformans (C. neoformans) and Candida dubliniensis use of this antifungal agent [33–38]. Many—but not all—C. albicans and some C. guilliermondii isolates demonstrate primary resistance to amphotericin B, which is in contrast to other Candida species (C. albicans, C. tropicalis, C. parapsilosis, and C. dubliniensis) that are usually quite susceptible to amphotericin B. Secondary resistance to amphotericin B also appears to be an uncommon development. There have been reports of some cases of disseminated infections due to C. glabrata, C. krusei, and C. albicans isolates that developed amphotericin B resistance during treatment [24]. The mechanism of amphotericin B resistance appears to be an alteration or a decrease in the amount of ergosterol in the cell membrane [39]. Recently, Yoon et al. [40] demonstrated in vitro reversible switching of C. lusitaniae, which may be responsible for frequent in vitro resistance of that species to amphotericin B and could have an impact on the selection of antifungal therapies that could result in antifungal resistance.

Prevalence of fluconazole resistance and the development of resistance during treatment. Fluconazole has in vitro activity against many isolates of Candida species, but it is not commonly used because of drug toxicities as well as the frequent development of resistance when used as a single agent. Primary resistance to fluconazole is a common phenomenon in Candida species; almost 10% of clinical isolates are intrinsically resistant (MIC for resistance, >32 µg/mL; MIC for intermediate resistance, 8–16 µg/mL; MIC for susceptibility, ≤4 µg/mL). In addition, the infections in up to 30% of the patients who receive monotherapy with fluconazole develop secondary resistance. Primary resistance is usually the result of a defect in cytosine deaminase. The highest rates of primary resistance are found in C. albicans serotype B, C. glabrata, C. krusei, and C. guilliermondii. Secondary resistance in C. albicans is primarily due to a decrease in the activity of the uracil phosphoribosyl transferase [24, 41, 42].

When to test for antifungal susceptibility. Unlike the sit-
uation for antimicrobial agents, antifungal susceptibility testing has not been used as a guide for antifungal therapy in the past because of the lack of standardization of testing methodology. It was not until 1997 that a reproducible, standardized antifungal susceptibility testing method for yeasts was developed (M27-A) [43]. Since then, efforts have been focused on the determination of interpretative breakpoints—MICs that predict clinical response to antifungal treatment and the establishment of in vitro–in vivo correlation. Breakpoints for MICs of fluconazole and itraconazole determined by the National Committee for Clinical and Laboratory Standards (NCCLS) methodology have been established (Table 3): yeast isolates are classified as susceptible to fluconazole and itraconazole if the MICs are ≤8 μg/mL and ≤0.125 μg/mL, respectively. Similarly, the resistance breakpoints for fluconazole and itraconazole were defined as ≥64 μg/mL and ≥1 μg/mL, respectively. A novel category, “susceptible, dose dependent,” was also established. This new breakpoint emphasizes the importance of attaining a significant level of drug in the blood and tissue for isolates with higher MICs. If the MIC of the isolate falls in this category, dosages of 400 mg per day or more of fluconazole and itraconazole concentrations in plasma of ≥0.5 μg/mL are needed for optimal response [8].

Susceptibility data are best supported for patients with OPC and C. albicans infection and are limited for infections due to yeasts other than C. albicans and for invasive infections [44]. For amphotericin B, NCCLS methodology generates a narrow MIC range, limiting its ability to identify isolates likely to cause therapeutic failure [45]. Clinically useful amphotericin B susceptibility breakpoints continue to be the subject of ongoing studies. The echinocandins, such as caspofungin, which was initially approved only for the treatment of refractory Aspergillus infections, have excellent in vivo and in vitro activity against Candida species, although the correlation between the susceptibility of yeasts to this new class of drugs and clinical outcome is not established, so susceptibility testing against these agents is not generally recommended.

**Clinical implications of antifungal resistance: selecting an agent.** An important feature to remember during the selection of antifungal drugs for the treatment of serious yeast infections is that, generally, the susceptibility of Candida species can be predicted on the basis of the specific yeast species. For that reason, careful mycological identification at the species level for all Candida isolates recovered from sterile sites is imperative for the empirical selection of antifungal agents. Because of the aforementioned limitations of in vitro susceptibility testing, routine antifungal susceptibility testing of all clinical specimens is not recommended. However, because of the geographical and institutional variation in antifungal susceptibility patterns, it is important to periodically determine the distri-

### Table 3. Interpretive breakpoints for Candida species.

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>Susceptible</th>
<th>Susceptible, dose dependent</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flucunazole</td>
<td>≤8</td>
<td>16–32</td>
<td>≥64</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>≤0.125</td>
<td>0.25–0.5</td>
<td>≥1</td>
</tr>
</tbody>
</table>

**NOTE.** Data are from [8]. Candida krusei is assumed to be intrinsically resistant to fluconazole and should not be tested.

Distribution of Candida species and susceptibility patterns in every institution, especially when antifungal prophylaxis is a common practice. Antifungal susceptibility testing is also recommended for patients with life-threatening Candida infections and HIV-infected patients with OPC who do not respond to treatment [8, 46].

Serious infection due to C. krusei should not be treated with fluconazole or itraconazole because of the high likelihood of resistance to the early triazole antifungals. However, excellent in vitro susceptibility to the newer azoles, particularly voriconazole, has been demonstrated, suggesting that the newer azoles may become preferred therapy for this often multidrug-resistant pathogen [43]. For infections due to C. glabrata, dose-dependent responses may be observed. However, in patients who develop breakthrough infections while receiving lower doses of azole therapy, and, in patients with neutropenia or severe immunosuppression, alternative agents are preferred. For HIV-infected patients with OPC caused by C. albicans isolates that have developed resistance to fluconazole, itraconazole retains activity, and the infection may still respond to itraconazole therapy, although higher doses might be required to achieve cure.

Therapeutic options are more limited when there is cross-resistance to other antifungal azoles. For example, for azole-resistant C. albicans or C. glabrata, higher MICs of other azole antifungals are likely, although the better susceptibility of the newer azoles (i.e., voriconazole, posaconazole, ravuconazole) may be clinically relevant [43]. Intravenously administered amphotericin B has been one of the few effective alternative agents for treatment of infection with antifungal-resistant yeasts, but even in these infections, higher MICs are likely with C. glabrata, which likely will require a higher dose of amphotericin B (0.7–1.0 mg/kg q.d., compared with 0.5 mg/kg q.d. for antifungal-susceptible C. albicans). The lipid-associated amphotericin B agents offer less toxicity but no clear advantages for the treatment of yeast infections. New antifungal compounds, such as broad-spectrum triazoles and echinocandins, are being developed and may be used against fluconazole-resistant OPC [47, 48] and to treat serious Candida infections. Patients with C. lusitaniae, C. guilliermondii, or C. glabrata infections should be carefully monitored during therapy because of the risk of...
Aspergillus fumigatus is the most common species to cause infection worldwide, accounting for approximately 85% of invasive aspergillosis. This fungus is the most common cause of invasive aspergillosis following bone marrow transplantation, and it frequently causes infections in patients with leukemia, bone marrow transplant recipients, and organ transplant recipients, as well as patients with advanced AIDS [49, 50]. The high mortality rate of invasive aspergillosis exceeds 85% in patients with advanced AIDS [50, 51], particularly because susceptibility to newer azoles with Aspergillus activity, such as voriconazole and posaconazole, may be observed in some cases. Cross-resistance between itraconazole and posaconazole was demonstrated while susceptibility to voriconazole was maintained, although the significance or likelihood of that association has not been established in a large study of isolates. In addition, these newer azoles may also demonstrate fungicidal activity against Aspergillus species, although the clinical relevance of that finding has not been demonstrated. To date, there have been no reported isolates with clinical resistance caused by decreased susceptibility to caspofungin, a glucan-synthesis inhibitor, which has shown clinical efficacy for the treatment of aspergillosis [47].

Aspergillus Infections

Epidemiology. The frequency of invasive aspergillosis has increased during recent years, surpassing candidiasis as the fungal infection most frequently detected after death, and invasive aspergillosis is associated with a high mortality rate (>85%). Aspergillus fumigatus is the most common species to cause Aspergillus infection worldwide, accounting for ∼90% of the cases, followed by Aspergillus flavus, Aspergillus terreus, and Aspergillus niger. This disease is frequently seen in patients with leukemia, bone marrow transplant recipients, solid-organ transplant recipients, and, to a lesser extent, patients with late-stage or end-stage AIDS [50, 51].

Prevalence of antifungal resistance and the development of resistance during treatment. Although treatment failure is common in patients with invasive aspergillosis, the correlation with resistance to antifungal treatment is difficult to establish. Other factors, such as the immune status of the patient and delay in diagnosis, may contribute to the poor response to treatment. The lack of reliable methods for in vitro testing has also hindered the detection of drug-resistant strains of Aspergillus species. Therefore, the true rate of antifungal resistance is unknown. Recently, a proposed method (M38-P) to standardize the in vitro antifungal susceptibility testing for molds was established, and improvements to these guidelines are being developed [52, 53]. Because the incidence of infections with most opportunistic mold pathogens is low, large-scale prospective studies to compare antifungal susceptibility and clinical outcomes are very difficult to conduct. For this reason, minimal clinical data exist to support the relevance of susceptibility testing in vitro of filamentous fungi. To date, experience with diverse antifungal susceptibility testing methodology indicates that the amphotericin B susceptibility of Aspergillus species varies according to species, although such variation has thus far been limited. Most isolates of Aspergillus fumigatus have a low MIC of amphotericin B (<1 µg/mL) [54, 55]. However, Aspergillus terreus has a high MIC of amphotericin B (>2 µg/mL), and infections due to this organism seem to be refractory to amphotericin B therapy [56, 57].

Like many other molds, Aspergillus species are intrinsically resistant to fluconazole. Most isolates of Aspergillus fumigatus appear to be susceptible to itraconazole, low MICs (<1 µg/mL) [54, 55]. Recently, a limited number of A. fumigatus isolates that demonstrated in vitro resistance to itraconazole were reported [55–58]. For some of these strains, the resistance detected in vitro has been confirmed in vivo in animal models, which suggests that this resistance may have clinical significance [59, 60], particularly because susceptibility to newer azoles with Aspergillus activity, such as voriconazole and posaconazole, may be observed. In some cases, cross-resistance between itraconazole and posaconazole was demonstrated while susceptibility to voriconazole was maintained, although the significance or likelihood of that association has not been established in a large study of isolates. In addition, these newer azoles may also demonstrate fungicidal activity against Aspergillus species, although the clinical relevance of that finding has not been demonstrated. To date, there have been no reported isolates with clinical resistance caused by decreased susceptibility to caspofungin, a glucan-synthesis inhibitor, which has shown clinical efficacy for the treatment of aspergillosis [47].

Molecular mechanisms ofazole resistance. Only a single report documents the molecular mechanisms of itraconazole resistance in A. fumigatus. Two distinct mechanisms of resistance were identified among the isolates obtained from 2 patients, and these mechanisms involved alterations in the target enzyme, the cytochrome P-450 lanosterol 14α-demethylase, causing reduced binding of the target enzyme to itraconazole and increased drug efflux, leading to a reduced intracellular concentration of itraconazole (table 2) [56].

When to test for antifungal susceptibility and clinical implications of antifungal resistance. Antifungal therapy for invasive aspergillosis should not be guided by in vitro susceptibility test results because of the lack of studies correlating the MICs of amphotericin and azoles with the clinical outcome of the patients. As previously discussed, the high mortality rate among patients with invasive aspergillosis probably has more relation to other factors, such as immunosuppression and delay in the diagnosis, than to the development of resistance to antifungal treatment, which, until now, has appeared to occur infrequently. As in case of Candida species, the most useful information for the selection of the antifungal treatment comes from the complete identification of the isolate to the species level. The development of azole resistance observed in some A. fumigatus strains should urge a thorough study of the molecular mechanisms of resistance, because most of the newer drugs being developed for the treatment of aspergillosis, such as voriconazole, posaconazole, and ravuconazole, areazole-based drugs [48]. However, the clinical impact of resistance in Aspergillus species, to date, has been confined to A. terreus, which often demonstrates higher MICs of not only amphotericin B but also the azole antifungals, including the newer azole...
agents—agents that have been successfully used to treat some patients with infections due to that organism.

**CRYPTOCOCCUS NEOFORMANS INFECTIONS**

**Epidemiology.** The overall incidence of cryptococcal disease has increased during the past 2 decades as a result of the AIDS epidemic, cancer chemotherapy, and immunosuppression for organ transplant recipients. Before the advent of HAART, among patients with AIDS in the United States, cryptococcosis was the defining illness in 5% of the patients, with an overall prevalence of 5%–10%. Infections are rarely completely cured in patients with AIDS without return of immune function, and certain immunosuppressed patients with neoplastic disease are subject to even more rapid mortality or treatment failure. The mortality rate for treated cryptococcal meningitis may be as high as 25%–30% in high-risk patients, although improved outcomes associated with aggressive antifungal management have been reported. Resistance to therapy can be caused by a variety of factors, including the following: underlying disease of the patient; secondary complications, such as hydrocephalus, drug intolerance, poor compliance with therapy, and pharmacokinetic factors; and the development of either primary or secondary drug resistance.

**Prevalence of antifungal resistance and the development of resistance during treatment.** Initially, when flucytosine was used as single agent for the treatment of cryptococcal meningitis, primary resistance to the treatment was uncommon. However, the frequent development of secondary resistance during treatment (up to 57% of cases) precluded its use as a single agent [42]. Recent studies have shown that primary flucytosine resistance is uncommon: it was reported in 14 of 732 isolates tested in the United States since the 1990s [61]. With regard to azole drugs, there has been an increasing number of relapses of cryptococcal meningitis in patients with AIDS associated with the development of secondary resistance to fluconazole caused by C. neoformans isolates that showed decreased susceptibility in vitro [62–63], although the use of HAART will substantially reduce the likelihood of relapse, which would obviously reduce the likelihood of developing resistance.

No case of primary resistance to amphotericin B has been clearly documented, although sporadic reports have reported the development of secondary resistance [66, 67]. However, resistance may be important in determining the outcome in selected patients, such as those who received amphotericin B monotherapy and those with uncontrolled underlying immunosuppression [66]. The echinocandins appear to offer little, if any, activity against Cryptococcus species, possibly because of the composition of its fungal cell wall [47].

**Molecular mechanisms of antifungal resistance.** The molecular mechanism of secondary flucytosine resistance is primarily due to a single mutation event in uridine-5-monophosphate pyrophosphorylase, uracil phosphoribosyl transferase, or cytosine permease–desaminase [42]. Studies of azole-resistant C. neoformans isolates have shown that the secondary resistance is associated with changes in the affinity of the target enzyme, the cytochrome P-450 lanosterol 14α-demethylase, and decreases in the cellular content of theazole due to the overexpression of MDR efflux pumps [68, 69]. Secondary resistance to amphotericin B has been associated with defects in sterol biosynthetic pathway, such as alterations in the sterol Δ^{7-2} isomerase enzyme (table 2) [69, 70].

**When to test for antifungal susceptibility and the clinical implications of antifungal resistance.** It was clear throughout the development of the M27 methodology that this approach was suboptimal, because of the slow growth rate of fungi, requiring 72 h of incubation, and because some strains did not even grow. For that reason, detection of susceptibility and resistance to both fluconazole and amphotericin B appears to require a modification of the reference testing method. The number of studies that have established the value of susceptibility as a predictor of clinical response in patients with C. neoformans infections is still limited [62–65]. For that reason, interpretative breakpoints for this pathogen have not yet been established. However, it is clear that antifungal drug resistance does occur, whether it is primary or secondary. Because of the aforementioned limitations of in vitro susceptibility testing, routine antifungal susceptibility testing of all clinical specimens is not recommended [8, 46].

**FUTURE DIRECTIONS**

Since 1998, we have witnessed a great improvement in the standardization of antifungal susceptibility tests for yeast and filamentous fungi [71]. Much research needs to be performed to correlate in vitro values with the clinical outcome of the patients with systemic mycoses. In addition, the search for new antifungal drugs with new targets should be a priority because of the intrinsic resistance or the development of resistance during treatment to the available antifungal drugs shown by some of these fungi [72].

**References**

4. Pfaffer MA, Jones RN, Doern GV, et al. bloodstream infections due to Candida species: SENTRY antimicrobial surveillance program in North


