Azole Antifungal Drug Cross-Resistance: Mechanisms, Epidemiology, and Clinical Significance
Michael A Pfaller and Daniel J Diekema

Amphotericin B Resistance: Epidemiology, Mechanisms, and Clinical Relevance
Stephanie A Knechtel and Michael E Klepser

Echinocandin Antifungal Drug Resistance
Thomas R Rogers, Elizabeth M Johnson, and Carol Munro
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Dear Colleagues,


As with the previous issues, this issue contains review articles from leading specialists in the field. These have been peer-reviewed for quality and CME/CPE-accredited to provide an ongoing educational resource. Three such leading articles are included, and all discuss drug resistance in invasive fungal infections.

The widespread use of fluconazole and other azole antifungal agents in the treatment and prophylaxis of candidiasis has led to concerns over drug resistance, either by the selection of intrinsically resistant species or by the development of secondary resistance. Michael Pfaller and Daniel Diekema (University of Iowa Carver College of Medicine, Iowa City, IA, USA) provide a thorough overview of the evidence for, and the extent of, cross-resistance among a variety of fungal species. The authors also go on to consider the possible mechanisms, epidemiology, and clinical importance of such cross-resistance.

Following this, Stephanie Knechtel and Michael Klepser (Ferris State University College of Pharmacy, Kalamazoo, MI, USA) review the mechanisms, epidemiology, and clinical relevance of amphotericin B resistance. Although resistance to amphotericin B has apparently been slow to develop, despite several decades of use, resistance has been difficult to detect owing to the limitations associated with the available techniques. The authors caution against drawing conclusions regarding trends in polyene resistance due to the fact that the current methods for detecting decreased susceptibility to these agents are often inadequate.

The third and final leading article of this issue, Thomas Rogers and colleagues (Trinity College Dublin, St James's Hospital, Dublin, Ireland) discuss resistance to echinocandins, a relatively new class of antifungal drugs. As with any new antifungal therapy, the concern is the development of reduced susceptibility or resistance following widespread exposure to the drug in high-risk patient populations leading to failure of therapy. The authors report that only a small number of case studies have documented this happening, which is encouraging, but suggest that prospective surveillance is warranted.

The Clinical Review section provides a thorough analysis from J Peter Donnelly (Radboud University, Nijmegen, The Netherlands), Zeina Kanafani (Duke University Medical Center, Durham, NC, USA), and Jörg Janne Vehreschild (Klinikum der Universitaet, Koeln, Germany) of recently published trials, case reports and reviews. The issue concludes with a report by Eric Dannaoui (Institut Pasteur, Paris, France) from the European Congress for Clinical Microbiology and Infectious Diseases (ECCMID) / 25th International Congress of Chemotherapy (ICC) Munich, Germany, March 31–April 03, 2007.

We hope you find this issue of The Journal of Invasive Fungal Infections an educational and valuable tool. We welcome your feedback regarding the material presented as well as your suggestions for future topics to be covered.

John R Perfect
Editor-in-Chief
Resistance to antimicrobial agents is an issue of concern worldwide, with important implications for morbidity, mortality, and the costs of healthcare. Although much attention has been focused on antibacterial resistance [1,2], innate or acquired resistance to antifungal agents is now recognized among pathogenic fungi [3–5]. This recognition has stimulated the development of new antifungal agents as well as intensive efforts to define the molecular mechanisms of resistance to various agents [3–12]. The extensive use of fluconazole has been coupled with increasing reports of fluconazole resistance [3,9,11,13,14], and some of the most elegant investigations of antifungal resistance mechanisms have involved the azole class of antifungal agents and Candida spp [3,5,8,12].

Treatment failures associated with the development of resistance have been observed following the use of both fluconazole and itraconazole for the management of candidiasis and other opportunistic mycoses [13,15,16]. Prior to the introduction of highly active antiretroviral therapy (HAART), resistance was associated with relapses of oropharyngeal candidiasis (OPC) in AIDS patients [3,13,15,17], but azole resistance has also been observed in other settings and for other fungi [3,16,18–20].

The broad use of azole antifungal agents in both prophylaxis and treatment of opportunistic mycoses has been associated with shifts in the causative agents of these infections [11,18,21,22]. The emergence of both yeasts and molds with reduced susceptibility to azoles has raised...
the specter of cross-resistance among both new and established agents of this class [11,18,22].

The new extended-spectrum triazoles (posaconazole, ravuconazole, and voriconazole) exhibit greater potency and spectrum than either fluconazole or itraconazole [6]. The activity of these broad-spectrum triazoles extends to some fluconazole-resistant strains of Candida as well as to molds and endemic pathogens [6,23–25]. These agents share a common mechanism of action with fluconazole and itraconazole and, in many instances, are affected by the same resistance mechanisms [3,5,8,9]. Given the shared mechanisms of action and resistance within the azole class, concerns regarding the development of cross-resistance are understandable.

Cross-resistance was first seen between fluconazole, itraconazole, and ketoconazole in AIDS patients with refractory mucosal candidiasis [12]. This was first appreciated clinically and was subsequently confirmed by in vitro studies [3,12]. Although the extended-spectrum triazoles have been shown to be efficacious in both primary and salvage therapy of invasive candidiasis and other fungal infections [23–29], several investigators have urged caution regarding their use in heavily azole-exposed patients due to the potential for cross-resistance, especially with fluconazole-resistant strains of Candida glabrata [30–33].

In vitro studies have clearly demonstrated the existence of cross-resistance between fluconazole and all of the new triazoles among Candida spp [34–36]. Considerably less information is available for the non-candidal yeasts [22,37]. Although azole-resistance is rare among Aspergillus spp, cross-resistance between some, but not all, azoles has been well documented [19]. The clinical impact of azole cross-resistance is only likely to become apparent in very complicated clinical situations involving seriously ill, immunocompromised patients with extensive exposure to antifungal agents. Recent case reports and case series demonstrating the clinical relevance of cross-resistance in invasive candidiasis are instructive and provide very real examples of the potential for cross-resistance between fluconazole and itraconazole and the newer generation of azoles such as voriconazole and posaconazole [30,32,33,38–40]. Extension of this concept to infections with other fungal pathogens seems reasonable but clinical data are lacking. In this review, we will summarize the evidence for, and the extent of, cross-resistance among species of Candida, the opportunistic non-candidal yeasts, and the filamentous fungi. The possible mechanisms, epidemiology, and clinical importance of this resistance issue will also be addressed.

Mechanisms of resistance to azole antifungal agents

The azoles all act by inhibiting the fungal cytochrome P450-dependent enzyme lanosterol 14α-demethylase, which is encoded by the gene ERG11. This enzyme converts lanosterol to ergosterol, and its inhibition disrupts membrane synthesis in the fungal cell [10]. Among the triazoles, posaconazole and itraconazole differ structurally from fluconazole and voriconazole due, in part, to the presence of a long hydrophobic side chain that provides more extensive contact between posaconazole and itraconazole and the target enzyme than that seen with the more compact structures of fluconazole and voriconazole [41]. It has been postulated that the more extensive binding of posaconazole and itraconazole to the target enzymes of C albicans and Aspergillus fumigatus serves to stabilize the binding of these drugs to the target, making them less susceptible to the effect of point mutations in the ERG11 gene [41,42].

The mechanism of azole resistance in Candida has been extensively investigated for fluconazole and C albicans (Table 1) [5,8,9,12]. Although similar mechanisms have been shown to be associated with resistance to other triazoles and among other species of Candida (Table 1) [43–51], the level of understanding is scant compared to that of fluconazole and C albicans.

Resistance can arise from a modification in the quality or quantity of the target enzyme, reduced access of the drug to the target, or some combination of these mechanisms (Table 1) [5,7–9,12]. In the first instance, point mutations in ERG11 leads to an altered target enzyme with decreased affinity for azoles. It has been suggested, based on molecular modeling studies, that certain mutations near the heme site of the C albicans enzyme result in significant levels of resistance to fluconazole and voriconazole but have less effect on the susceptibility of the organisms to posaconazole and itraconazole, presumably due to the additional contacts with the target afforded by the long side chains of the latter drugs [41]. In a series of isolates of C albicans from an AIDS patient with refractory OPC, Li et al. demonstrated that isolates resistant to fluconazole and voriconazole, but susceptible to itraconazole and posaconazole, all shared the same five missense mutations in ERG11 that specifically reduced binding of fluconazole and voriconazole to the target enzyme [52]. Notably, the three isolates in this isogenic series that were obtained during the course of posaconazole therapy had all acquired an additional mutation leading to the disruption of the binding of the posaconazole and itraconazole side chain within the hydrophobic channel of the enzyme [52]. These three isolates exhibited resistance to posaconazole and itraconazole as well as to fluconazole and voriconazole.

In addition to the mutations in ERG11, overexpression of the gene results in the production of high concentrations of target enzyme, creating the need for higher intracellular fluconazole concentrations to inhibit all of the enzyme present in the cell [8,12]. Loss of allelic variation in the
The major facilitators (encoded by the \( \text{ERG11} \) promoter) may result in a resistant strain that is homozygous for the gene mutation [53].

The second major mechanism of azole resistance in \( \text{C albicans} \) involves active efflux of fluconazole out of the cell through the activation of two types of multidrug efflux transporters: the major facilitators (encoded by \( \text{MDR} \) genes) and those of the ATP-binding cassette superfamily (encoded by \( \text{CDR} \) genes) (Table 1) [5,7–9,12]. Uprogulation of \( \text{MDR1} \) genes leads to elevated fluconazole (and to a lesser extent voriconazole) minimum inhibitory concentrations (MICs), whereas upregulation of \( \text{CDR} \) genes leads to resistance to multiple azoles including voriconazole, posaconazole, and ravuconazole [8,12,44,52,54–57]. Evidence that these mechanisms (overexpression/mutation of target enzyme, \( \text{MDR} \) and \( \text{CDR} \) efflux pumps) may act individually, sequentially, and in concert has been derived by studying serial isolates of \( \text{C albicans} \) from AIDS patients with OPC [17,42,52,53,58,59], as well as from patients with invasive disease [54,60–62]. Although high-level azole resistance in \( \text{C albicans} \) usually develops gradually as a result of sequential alterations due to continuous pressure exerted by the drug [63], rapid, transient azole resistance (fluconazole, ketoconazole, itraconazole) was associated with increased expression of \( \text{CDR} \) encoded efflux pumps in a series of isolates of \( \text{C albicans} \) obtained from a patient who developed disseminated infection despite receipt of fluconazole [54,60]. Recently, Andes and colleagues [64,65] considered the impact of fluconazole dosing regimens and pharmacodynamics on resistance development in \( \text{C albicans} \) and found that regimens that produced prolonged sub-MIC concentrations were associated with resistance development. The emergence of the resistant phenotype was associated with increased expression of \( \text{CDR1} \) and \( \text{CDR2} \) encoded efflux pumps but not \( \text{MDR1} \) encoded pumps or \( \text{ERG11} \) [64,65]. Likewise, \( \text{ERG11} \)

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Posaconazole</th>
<th>Voriconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Candida glabrata} )</td>
<td>Overexpression of ( \text{CgCDR1} ) and ( \text{CgSNQ2} ) encoded efflux pump. Overexpression/mutations of target enzyme, lanosterol 14(\alpha)-demethylase. Respiratory deficiency due to mutations in mitochondrial DNA (increased ( \text{CgCDR1} ) and ( \text{CgCDR2} ) expression).</td>
<td>Overexpression of ( \text{CgCDR1} ) and ( \text{CgSNQ2} ) encoded efflux pump. Overexpression/mutations of target enzyme. Respiratory deficient mutants (increased ( \text{CgCDR1} ) and ( \text{CgCDR2} ) expression).</td>
<td>Overexpression of ( \text{CgCDR1} ) and ( \text{CgSNQ2} ) encoded efflux pumps.</td>
<td>Overexpression of ( \text{CgCDR1} ) and ( \text{CgSNQ2} ) encoded efflux pump. Respiratory deficient mutants (increased ( \text{CgCDR1} ) and ( \text{CgCDR2} ) expression).</td>
</tr>
<tr>
<td>( \text{Candida tropicalis} )</td>
<td>Overexpression/mutation of ( \text{CtERG11} ) encoded target enzyme, lanosterol 14(\alpha)-demethylase. Over-expression of ( \text{CtMDR1} ) encoded efflux pump.</td>
<td>Overexpression of ( \text{CtERG11} ) encoded target enzyme.</td>
<td>N/A</td>
<td>Overexpression/mutation of ( \text{CtERG11} ) encoded target enzyme.</td>
</tr>
<tr>
<td>( \text{Candida dubliniensis} )</td>
<td>Overexpression of ( \text{CdCDR1} ) and ( \text{CdMDR1} ) encoded efflux pump.</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>( \text{Candida krusei} )</td>
<td>Decreased susceptibility of target enzyme to inhibition by fluconazole.</td>
<td>Reduced drug accumulation.</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>( \text{Cryptococcus neoformans} )</td>
<td>Alterations in target enzyme. Overexpression of ( \text{MDR} ) encoded efflux pump.</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>( \text{Histoplasma capsulatum} )</td>
<td>Mutation in target enzyme.</td>
<td>N/A</td>
<td>N/A</td>
<td>Mutation in target enzyme.</td>
</tr>
<tr>
<td>( \text{Aspergillus fumigatus} )</td>
<td>Overexpression/mutations in target enzyme. Decreased drug accumulation.</td>
<td>Mutation in target enzyme.</td>
<td>N/A</td>
<td>Mutation in target enzyme.</td>
</tr>
</tbody>
</table>

N/A: data not available.
mutations were not responsible for fluconazole resistance in the isolates studied.

It is now well established that the primary mechanism of resistance to fluconazole in C. glabrata involves upregulation of the CgCDR1 and CgCDR2 genes, resulting in resistance to multiple azoles (Table 1) [43–45,49,50,66]. Indeed, Borst et al. observed the rapid development ofazole drug resistance (fluconazole, itraconazole, and voriconazole) following in vitro exposure to fluconazole among C. glabrata isolates that had never previously been exposed to azole antifungal agents [44]. The resistance was stable and was associated with increased expression of CgCDR1 and CgCDR2, but not CgERG11. Likewise, Sanguinetti et al. performed a careful analysis of azole resistance profiles of clinical isolates of C. glabrata and found that the majority of the isolates were resistant to multiple azoles (fluconazole, itraconazole, ketoconazole, and voriconazole) and that the resistance phenotypes were strongly associated with upregulation of the ATP-binding cassette (ABC) efflux transporters CgCDR1, CgCDR2, and CgSNQ2 [50]. Interestingly, they too found no alteration or overproduction of CgERG11 among 20 different azole-resistant isolates. They concluded that cross-resistance is a very common feature inazole-resistant C. glabrata isolates, especially in those that are capable of expressing multiple mechanisms of resistance [50].

Others have shown that petite (small, slow-growing) colony mutants of C. glabrata may appear upon exposure to fluconazole and that these azole-resistant subpopulations contain a respiratory deficiency due to mutations in mitochondrial DNA [45]. Further investigation demonstrated increased drug efflux associated with increased expression of CgCDR1 and CgCDR2 genes encoding efflux pumps [45]. These authors did not find any alteration or overexpression of CgERG11 in the petite mutants [45]. Similar findings have been reported by Sanglard et al. [67] in a study of high-frequency acquisition of azole resistance in C. glabrata. The pathogenicity of these petite mutants remains to be defined, but they may play a role in infection similar to the small colony variants of staphylococci that are associated with persistent, antibiotic-resistant and relapsing infections [68–70].

Although it has been suggested that alteration or overexpression of CgERG11 is not involved in azole-resistance of C. glabrata [50], both Marichal et al. [47] and Redding et al. [49] have reported an increase in lanosterol 14α-demethylase in fluconazole-resistant isolates of C. glabrata. Marichal et al. [47] found an increase in the amount of CYP51 (ERG11) mRNA transcript that was due to a duplication of the entire chromosome containing the gene, whereas Redding et al. [49] found upregulation of CgERG11 along with that of CgCDR1 and CgCDR2 in a fluconazole-resistant strain of C. glabrata from a patient with OPC undergoing treatment with fluconazole. Taken together, the literature indicates that although efflux is a major mechanism ofazole resistance in C. glabrata, the development of resistance in this species is a highly varied process involving multiple molecular mechanisms [49].

Relatively little information is available regarding the mechanisms ofazole resistance in other species of Candida. C. dubliniensis isolates recovered from HIV-infected patients with mucosal candidiasis who received fluconazole treatment were shown to exhibit upregulation of multidrug efflux transporter genes (CdCDR1 and CdMDR1) [71,72]. Fluconazole resistance in C. tropicalis has been associated with both a point mutation in, and over-expression of, CIErg11 [51], whereas the primary resistance of C. krusei to fluconazole appears to be mediated through reduced sensitivity of the target enzyme to inhibition by fluconazole [48]. Venkateswarlu et al. reported that itraconazole resistance in C. krusei was mediated by reduced drug accumulation [73], although other investigators have failed to demonstrate a role of efflux in isolates of C. krusei [46]. Neither of these mechanisms appears to have an effect on the activity of the newer triazoles (voriconazole, posaconazole, or ravuconazole) against C. krusei.

Resistance to the triazole antifungal agents appears to be relatively uncommon among clinical isolates of Aspergillus spp [19,74]. Virtually all of the studies concerning mechanisms of resistance to azoles in Aspergillus have been performed in A. fumigatus and they have uniformly shown that resistance was associated with modification of the 14α-sterol demethylase target enzyme (CYP51), specifically mutations in the gene cyp51A [74–80]. Importantly, different mutations appear to result in resistance to posaconazole and itraconazole versus voriconazole and ravuconazole [41,78,79]. Cross-resistance to itraconazole and posaconazole has been associated with amino acid substitutions at glycine 54 (G54) [78,81,82], whereas cross-resistance to voriconazole and ravuconazole has been associated with amino acid substitutions at G448 [41,79]. It has been postulated, based on molecular modeling studies, that a substitution at G54 in the A' helix of AF-CYP51A confers resistance to posaconazole and itraconazole by perturbing the binding of the long side chain in the hydrophobic channel (channel 2) of the enzyme [41]. Given that voriconazole and ravuconazole lack a long side chain, substitutions at G54 would be predicted to have no effect on the binding of these compact triazoles to the target. Conversely, substitution near the heme cofactor (e.g. G448) would disturb the binding of voriconazole and ravuconazole to a greater extent than that of posaconazole and itraconazole due to the stabilizing influence of the side chain present in the latter two agents [41].

Recently, a third pattern ofazole resistance was reported in A. fumigatus [78,81,82]. This new pattern is characterized by high MICs for itraconazole, voriconazole, ravuconazole,
and posaconazole. The majority of strains with this phenotype harbor amino acid substitutions at methionine 220 (M220) [79]. The role of the M220 substitutions in multi-azole resistance was confirmed by Mellado et al. who introduced mutated cyp51A genes into an A fumigatus wild-type strain with resulting resistance to all triazole agents [79]. They also detected M220 substitutions in five clinical isolates of A fumigatus that exhibited the multi-azole-resistant phenotype. Thus, it appears that the mechanisms in which the various mutations in AF-CYP51A impact the susceptibility to specific azoles reflect differences in the ways the azoles interact with the target protein.

An additional mechanism of resistance to azoles in A fumigatus is reduced intracellular accumulation of the drugs. This was first demonstrated in laboratory-derived itraconazole-resistant mutants of A fumigatus where the reduced accumulation of itraconazole was postulated to be due to either diminished permeability or to defects in an energy-dependent uptake process [83]. More recently, Nascimento et al. found that in addition to a mutation at G54 in the AF-CYP51A target, itraconazole-resistant isolates of A fumigatus also exhibited high-level expression of two genes, Afu-MDR3 and Afu-MDR4, which encode for drug efflux pumps [82]. As with other fungi, it appears that multiple resistance mechanisms in A fumigatus are involved in resistance to the triazole antifungal agents.

Finally, secondary resistance to azoles has also been studied in isolates of Cryptococcus neoformans [9,84–86] and Histoplasma capsulatum [87]. The C neoformans isolates that have developed secondary resistance to fluconazole have been shown to have an altered target enzyme as well as overexpression of MDR efflux pumps [84–86]. Wheat et al. described the emergence of resistance to fluconazole and voriconazole but not to posaconazole and ravuconazole, in paired pre- and post-treatment isolates of H capsulatum obtained from patients with AIDS who failed fluconazole [87]. Comparison of the CYP51 Ap (14 α-demethylase) amino acid sequences from a fluconazole-susceptible pretreatment isolate and a post-treatment isolate exhibiting reduced susceptibility to both fluconazole and voriconazole identified a point mutation in the active site of the CYP51 protein from the post-treatment isolate. It was presumed that the mutation was responsible for the reduced susceptibility to fluconazole and voriconazole, analogous to that seen in C albicans [41].

**Antifungal susceptibility testing**

Antifungal susceptibility testing *in vitro* is assuming an increasing role in antifungal drug selection, as an aid in drug development studies, and as a means of tracking the emergence of antifungal resistance in epidemiological studies [14,88–90]. The Clinical and Laboratory Standards Institute (CLSI) Subcommittee for Antifungal Testing has developed standardized broth microdilution (BMD) [91] and disk diffusion [92] methods for *in vitro* susceptibility testing of Candida spp and other yeasts, as well as BMD methods for filamentous fungi [93]. These methods are reproducible and accurate and provide clinically useful information that is comparable to that of antibacterial testing [90,94–96]. Interpretive breakpoints for fluconazole, itraconazole, and voriconazole have been developed by considering data relating the MICs to known resistance mechanisms, the MIC (and zone diameter) distribution profiles, pharmacokinetic and pharmacodynamic parameters, and the relationship between *in vitro* activity (MIC or zone diameter) and clinical outcomes, as determined by available clinical efficacy studies [90,95–98]. Although interpretive breakpoints have not been established for posaconazole and ravuconazole, the CLSI Subcommittee has come to a consensus on standardized methods for these agents, and it is expected that interpretive breakpoints will be established in the near future [34,99]. For the purposes of this discussion we have applied the MIC breakpoints established for voriconazole to both posaconazole and ravuconazole (susceptible [S], ≤1 μg/mL, susceptible dose dependent [SDD], 2 μg/mL, and resistant [R], ≥4 μg/mL).

One of the important byproducts of the standardization process has been the ability to conduct surveillance of resistance to antifungal agents by uniform methods [34,35,37,89,99–101]. Meaningful large-scale surveys of antifungal susceptibility and resistance conducted over time would not be possible without a standardized BMD or disk diffusion method for performing the *in vitro* studies. Studies of trends in resistance to commonly used antifungal agents such as fluconazole [37,101,102] and itraconazole [103,104] and comparative analyses of licensed and newly introduced agents against Candida, other yeasts, and filamentous fungi [4,22,35–37,99,100,102,105–110] have provided large amounts of useful data and have been greatly facilitated by standardized testing methods.

**Epidemiology and clinical significance of azole cross-resistance**

*Candida* and candidiasis

*Species distribution*

Although >100 species of Candida have been described, only a few species have been implicated in human infection [111–113]. C albicans is the species most commonly recovered from clinical material and generally is responsible for >90% of mucosal infections and 50–70% of episodes of candidemia [112–115].

More than 95% of invasive infections due to *Candida* spp are attributed to five species (Table 2): *C albicans*, *C glabrata*, *C parapsilosis*, *C tropicalis*, and *C krusei* [101,111–113,116].
Beyond these five species, the list of reported azoles on antifungals [15,119–122]. However, the overall effect of (BSI) with increasing patient age [100,101,113,116,117], Candida albicans participating in the ARTEMIS surveillance study [102,113]. 6–9 years among 134 sentinel surveillance sites in 40 countries (overall decrease of 10–11%) was noted over the past a decreasing trend in the proportion of infections [11,14,37,113,115]. Globally, through the world from 47% in Latin America to 73% causing candidemia, the frequency of occurrence varies in the species level as an aid in optimizing therapy of candidal infections [11,14,37,113,115].

The species distribution of Candida bloodstream infection isolates by geographic region*.

<table>
<thead>
<tr>
<th>Region</th>
<th>Sites (n)</th>
<th>Isolates (n)</th>
<th>CA</th>
<th>CG</th>
<th>CP</th>
<th>CT</th>
<th>CK</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia-Pacific</td>
<td>17</td>
<td>441</td>
<td>73.5</td>
<td>10.2</td>
<td>8.4</td>
<td>3.9</td>
<td>3.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Europe</td>
<td>40</td>
<td>775</td>
<td>57.6</td>
<td>12.9</td>
<td>14.1</td>
<td>7.5</td>
<td>3.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Latin America</td>
<td>18</td>
<td>560</td>
<td>46.6</td>
<td>7.5</td>
<td>17.1</td>
<td>21.3</td>
<td>3.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Canada</td>
<td>8</td>
<td>623</td>
<td>58.9</td>
<td>20.1</td>
<td>10.3</td>
<td>5.9</td>
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<td>USA</td>
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<td>3683</td>
<td>54.4</td>
<td>18.3</td>
<td>13.2</td>
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<td>2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Total</td>
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<td>6082</td>
<td>55.9</td>
<td>16.2</td>
<td>13.1</td>
<td>9.6</td>
<td>2.5</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*Data compiled from [101]. CA: Candida albicans; CG: Candida glabrata; CK: Candida krusei; CP: Candida parapsilosis; CT: Candida tropicalis.

Most notable is the very low frequency of *C. glabrata* as a cause of BSI in Latin America, where only 4.0–7.5% of Candida BSIs are attributed to this species (Table 2).

Despite these findings, the association with fluconazole use and increased isolation of *C. glabrata* is well known [119,120,142–144]. The evidence for the association is strongest for cancer centers and less so for individual nonspecialty hospitals [123,125,128,136,137,141,145]. Recent studies by Lin et al. [145] and Malani et al. [128] did not find exposure to fluconazole to be predictive of *C. glabrata* BSI. Malani et al. found that older adults (>60 years) not only had an increased risk of fungemia due to *C. glabrata* but also appeared to have an increased risk of dying from the event [128]. They reported that the most common risk factors for *C. glabrata* BSI were use of broad-spectrum antibiotics, use of central venous catheters, receipt of parenteral nutrition, and stay in an ICU [128]. Lin et al. found that the use of piperacillin–tazobactam and vancomycin was significantly associated with nosocomial BSI due to *C. glabrata* (and *C. krusei*) even after adjusting for clinical risk factors and other antimicrobial uses [145]. Taken together, these results appear to contradict the widely held assumption that prior exposure to fluconazole is the single most important predisposing factor for subsequent *C. glabrata* BSI [123,143,145]. Patient age, exposure to specific antibacterial agents, and severity of underlying disease may be more important than fluconazole exposure in promoting *C. glabrata* candidemia [113].

In contrast to the situation in the US, in other countries *C. parapsilosis* and *C. tropicalis*, not *C. glabrata*, are the most common non-*albicans* Candida species causing BSI (Table 2). *C. parapsilosis* is an exogenous pathogen that is notorious for its ability to form biofilms on catheters and other implanted devices [21,146–150], for nosocomial spread by hand carriage, and for persistence in the hospital environment [147,149–151]. It is also well known for causing infections in infants and neonates [151–155]. Given the exogenous
Table 3. Fluconazole susceptibility and resistance among Candida spp over 14 years (1992–2005)*.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (91 495)</td>
<td>S</td>
<td>97.6</td>
<td>98.2</td>
<td>97.7</td>
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<tr>
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<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
<td>0.4</td>
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<td>1.5</td>
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<tr>
<td></td>
<td>R</td>
<td>8.3</td>
<td>18.3</td>
<td>14.7</td>
<td>16.9</td>
<td>14.3</td>
<td>15.2</td>
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<tr>
<td>C. parapsilosis (9871)</td>
<td>S</td>
<td>98.6</td>
<td>91.6</td>
<td>93.9</td>
<td>93.5</td>
<td>94.1</td>
<td>92.9</td>
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</tr>
<tr>
<td></td>
<td>SDD</td>
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<td>4.2</td>
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<td>2.6</td>
<td>2.9</td>
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</tr>
<tr>
<td></td>
<td>R</td>
<td>0.4</td>
<td>4.2</td>
<td>3.9</td>
<td>3.1</td>
<td>3.3</td>
<td>4.2</td>
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<tr>
<td>C. tropicalis (11 014)</td>
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<td>92.6</td>
<td>88.0</td>
<td>87.7</td>
<td>91.9</td>
<td>92.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SDD</td>
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<td>4.4</td>
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<td>7.3</td>
<td>4.6</td>
<td>4.2</td>
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<tr>
<td></td>
<td>R</td>
<td>1.4</td>
<td>3.0</td>
<td>6.6</td>
<td>5.0</td>
<td>3.5</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>

*Data compiled from [101] and [37]. MIC: minimum inhibitory concentration; R: resistant (MIC ≥ 64 μg/mL, zone diameter ≥ 14 mm); S: susceptible (MIC ≤ 14 μg/mL, zone diameter ≤ 19 mm); SDD: susceptible dose dependent (MIC 16–32 μg/mL, zone diameter 15–18 mm).

The origin of C parapsilosis, BSI due to this species should be preventable by careful attention to good infection control techniques, including hand hygiene and appropriate catheter placement and care [150]. Although antifungal prophylaxis with fluconazole has proven effective in preventing candidemia due to C parapsilosis in the neonatal setting [156], a recent example of the emergence of fluconazole resistance in a strain endemic to a Finnish neonatal ICU underscores the importance of good infection control practices, rather than antifungal prophylaxis, in preventing infection with C parapsilosis [151].

C. tropicalis is an important fungal pathogen in patients with neutropenia and those with hematological malignancies [119,120,157,158]. In the US, fluconazole prophylaxis has decreased the frequency of BSI due to C tropicalis [120]; indeed, the lack of fluconazole prophylaxis was shown to be a predictor of C tropicalis fungemia in patients with hematological malignancies [119,157]. C tropicalis is generally the fourth most common species of Candida causing BSI in most geographic settings (Table 2); however, it ranks second in Latin America (21.3%), superceding both C glabrata and C parapsilosis [37,101]. As many as 60–80% of neutropenic patients who are colonized with C tropicalis eventually develop invasive infection with this species, underscoring the important role of fluconazole prophylaxis in this patient population [157,159,160].

Like C tropicalis, C krusei is an important pathogen among patients with hematological malignancies and among blood and marrow transplant (BMT) recipients [119,120,158,161]. C krusei accounts for 2–4% of all Candida BSIs (Table 2), although higher frequencies have been reported for cancer patients in Europe [162] and the US [119,120,161]. Although C krusei has clearly emerged among those BMT recipients receiving fluconazole prophylaxis, exposure to this agent alone cannot explain the reported increase in infections caused by this species. An increase in the prevalence of C krusei infections predated the use of fluconazole in some institutions [158,163,164], and in others it has not changed despite long-term exposure to fluconazole [123]. Whereas Abi-Said et al. found that infections with C krusei were strongly associated with fluconazole prophylaxis among neutropenic patients at a Texas cancer center [119], Lin et al. found this not to be the case in a less-specialized tertiary care setting in Chicago [145]. The latter authors found that patient exposure to piperacillin-tazobactam and vancomycin was more important than exposure to fluconazole in promoting C krusei BSI.

**Extent of fluconazole resistance**

Fluconazole has been the focus of resistance surveillance efforts since its introduction in the early 1990s [37,101,110,113]. Among >129 000 clinical isolates of Candida comprising the top four species tested by CLSI methods between 1992 and 2005 (Table 3) [37,101], resistance in isolates of C albicans (1.0–2.1%), C parapsilosis (0.4–4.2%), and C tropicalis (1.4–6.6%) has remained infrequent worldwide (isolates obtained from >130 institutions in 40 different countries). A slight increase in the frequency of resistance was noted for both C parapsilosis and C tropicalis when comparing results for isolates collected during the time period 1992–2000 versus those collected during 2001–2005, although frequency of resistance over the latter time period did not show a continued trend toward increasing resistance. A similar increase in resistance was observed for C glabrata, with sustained high rates of resistance (14.3–18.3%) over the past 5 years. Given its prominence as a cause of IC in many settings, C glabrata remains a focus for concern regarding
fluconazole resistance [22,101,113]. Resistance to fluconazole among BSI isolates of C glabrata also varies according to the geographic region and from institution-to-institution [101,113]. Isolates from North America show the highest resistance rates (18–20%) and isolates from the Asia-Pacific and Latin American regions are less resistant to fluconazole (10–13%) [37,113].

Activity of itraconazole and the extended-spectrum triazoles

The extended-spectrum triazoles (posaconazole, ravuconazole, and voriconazole) all have significantly greater potency than either fluconazole or itraconazole against Candida spp, including some species such as C krusei and C guilliermondii with reduced susceptibility to fluconazole (Table 4) [34–37, 102,105,109,110,113,165]. There are now several large multicenter surveys that have been conducted with CLSI – or very similar (EUCAST: European Committee for Antimicrobial Susceptibility Testing) – methods to provide a direct comparison of in vitro activities of the extended-spectrum triazoles against clinical isolates of Candida spp [36,105,110,113,165]. The data shown in Table 4 provide a comparison of results obtained from the Global Surveillance Program survey conducted by the University of Iowa, Iowa City, IA, USA [34–37,99,102,109] for the three extended-spectrum triazoles and itraconazole. These results, encompassing several thousand clinically significant isolates of Candida spp, clearly document the excellent, and comparable, potencies of posaconazole, ravuconazole, and voriconazole. Although itraconazole has similar activity to the other triazoles against C albicans, it is considerably less active against the other species. The results show MIC₉₀ values of ≤1 μg/mL with 97–100% susceptibility, for all three extended spectrum triazoles and all species, with

Table 4. Comparative in vitro susceptibility of >10 000 clinical isolates of Candida spp to itraconazole, posaconazole, ravuconazole, and voriconazole determined by Clinical and Laboratory Standards Institute broth microdilution methods*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Antifungal agent Tested (n)</th>
<th>MIC (μg/mL)**</th>
<th>50%</th>
<th>90%</th>
<th>S</th>
<th>% R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C albicans</td>
<td>Itraconazole 7647 0.007–8+</td>
<td>0.06</td>
<td>0.12</td>
<td>96.1</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole 5827 0.007–2</td>
<td>0.015</td>
<td>0.06</td>
<td>99.9</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ravuconazole 7521 0.007–8+</td>
<td>0.007</td>
<td>0.015</td>
<td>99.8</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole 5826 0.007–4</td>
<td>0.007</td>
<td>0.015</td>
<td>99.9</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>C glabrata</td>
<td>Itraconazole 1929 0.03–8+</td>
<td>1.0</td>
<td>2.0</td>
<td>2.3</td>
<td>59.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole 1517 0.03–8+</td>
<td>1.0</td>
<td>2.0</td>
<td>79.6</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>0.25</td>
<td>1.0</td>
<td>90.3</td>
<td>7.8</td>
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<tr>
<td></td>
<td>Voriconazole 1516 0.007–8</td>
<td>0.25</td>
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<td>91.1</td>
<td>5.7</td>
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<tr>
<td>C parapsilosis</td>
<td>Itraconazole 1508 0.15–2</td>
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<td>0.5</td>
<td>47.8</td>
<td>3.1</td>
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<td></td>
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<tr>
<td></td>
<td>Ravuconazole 1485 0.007–1</td>
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<td>100.0</td>
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<td>Voriconazole 1541 0.007–8</td>
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<td>0.06</td>
<td>99.6</td>
<td>0.1</td>
<td></td>
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<tr>
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<td>53.0</td>
<td>3.1</td>
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<tr>
<td></td>
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<td>0.12</td>
<td>99.9</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ravuconazole 1185 0.007–8+</td>
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<td>0.12</td>
<td>98.4</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole 1197 0.007–8</td>
<td>0.03</td>
<td>0.06</td>
<td>99.8</td>
<td>&lt;0.1</td>
<td></td>
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<tr>
<td>C krusei</td>
<td>Itraconazole 306 0.12–4</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>56.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole 305 0.03–4</td>
<td>0.25</td>
<td>1.0</td>
<td>99.0</td>
<td>0.3</td>
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<tr>
<td></td>
<td>Ravuconazole 302 0.03–2</td>
<td>0.5</td>
<td>0.5</td>
<td>99.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole 305 0.007–4</td>
<td>0.25</td>
<td>0.5</td>
<td>99.7</td>
<td>0.3</td>
<td></td>
</tr>
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<td>C guilliermondii</td>
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<td>1.0</td>
<td>8.2</td>
<td>35.3</td>
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</tr>
<tr>
<td></td>
<td>Posaconazole 138 0.015–2</td>
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<td>0.5</td>
<td>97.1</td>
<td>0.0</td>
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<tr>
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<td>1</td>
<td>97.1</td>
<td>2.9</td>
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</tr>
<tr>
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<td>Voriconazole 138 0.005–2</td>
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<td>0.12</td>
<td>99.3</td>
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<td>C lusitaniae</td>
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<td>0.25</td>
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<td>Posaconazole 129 0.015–1</td>
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<td>Voriconazole 134 0.007–8</td>
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<td>C dubliniensis</td>
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<td>0.25</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole 103 0.015–8+</td>
<td>0.03</td>
<td>0.06</td>
<td>98.1</td>
<td>1.9</td>
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<tr>
<td></td>
<td>Ravuconazole 103 0.007–8+</td>
<td>0.007</td>
<td>0.03</td>
<td>98.0</td>
<td>2.0</td>
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<tr>
<td></td>
<td>Voriconazole 103 0.007–8+</td>
<td>0.007</td>
<td>0.03</td>
<td>98.1</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

*Data compiled from [34–37,99,102,109]. **50%, MIC encompassing 50% of all isolates tested; 90%, MIC encompassing 90% of all isolates tested. MIC: minimum inhibitory concentration; R: resistant at MIC breakpoint for itraconazole (≤1 μg/mL) and all other agents (≤4 μg/mL); S: susceptible at MIC breakpoints for itraconazole (≥0.12 μg/mL) and all other agents (≥1 μg/mL).
respectively, at an MIC of

are susceptible to itraconazole and posaconazole,

isolates testing as SDD to fluconazole, 76.4% and 83.2%
of

available triazoles is clearly evident, especially among species

Mechanistically, the potential for cross-resistance among the

Cross-resistance among the triazoles

incremented affinity (versus that of fluconazole) for the

susceptible to the three newer triazoles, reflecting their

Notably, more than 99% of

at the voriconazole susceptible breakpoint of

Table 5. In vitro activity of triazole antifungal agents against clinical isolates of Candida species stratified by fluconazole susceptibility*.

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>Fluconazole susceptibility category (n)</th>
<th>0.007</th>
<th>0.015</th>
<th>0.03</th>
<th>0.06</th>
<th>Cumulative % at MIC (μg/mL) of:</th>
</tr>
</thead>
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<td>Itraconazole</td>
<td>All (9276)</td>
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<td>8.1</td>
<td>29.3</td>
<td>51.8</td>
<td>70.5</td>
</tr>
<tr>
<td></td>
<td>S (8353)</td>
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<td>9.0</td>
<td>32.5</td>
<td>57.5</td>
<td>78.1</td>
</tr>
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<td></td>
<td>SDD (707)</td>
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<td>0.8</td>
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<td>R (216)</td>
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<td>22.7</td>
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<td>61.1</td>
</tr>
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<td>All (10 807)</td>
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<td>31.5</td>
<td>54.3</td>
<td>70.9</td>
<td>80.2</td>
</tr>
<tr>
<td></td>
<td>S (9667)</td>
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<td>36.3</td>
<td>60.6</td>
<td>79.2</td>
<td>89.1</td>
</tr>
<tr>
<td></td>
<td>SDD (868)</td>
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<td>0.6</td>
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<td></td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>R (272)</td>
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<td></td>
<td>2.2</td>
</tr>
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<td>Ravuconazole</td>
<td>All (12 796)</td>
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<td>65.1</td>
<td>74.3</td>
<td>80.1</td>
<td>85.5</td>
</tr>
<tr>
<td></td>
<td>S (11 666)</td>
<td>55.8</td>
<td>71.4</td>
<td>81.4</td>
<td>87.6</td>
<td>93.0</td>
</tr>
<tr>
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<td>SDD (766)</td>
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<td>3.7</td>
<td></td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td>R (364)</td>
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<td>1.4</td>
<td></td>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>All (13 338)</td>
<td>46.3</td>
<td>63.5</td>
<td>72.8</td>
<td>80.9</td>
<td>87.4</td>
</tr>
<tr>
<td></td>
<td>S (12 087)</td>
<td>51.1</td>
<td>70.1</td>
<td>80.3</td>
<td>89.1</td>
<td>95.9</td>
</tr>
<tr>
<td></td>
<td>SDD (855)</td>
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<td></td>
<td></td>
<td></td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>R (396)</td>
<td>0.8</td>
<td>1.5</td>
<td>8.8</td>
<td>33.8</td>
<td>48.5</td>
</tr>
</tbody>
</table>

*Data compiled from Pfaller et al. [34–37,99,102,103,109]. MIC: minimum inhibitory concentration; R: resistant, MIC ≥8 μg/mL; S: susceptible, MIC 1–8 μg/mL; SDD: susceptible dose dependent, MIC 16–32 μg/mL.

the exception of C glabrata. More than 90% of C glabrata
isolates were susceptible to ravuconazole and voriconazole
at the voriconazole susceptible breakpoint of ≤1 μg/mL,
whereas only 79.6% were susceptible to posaconazole.
Notably, more than 99% of C krusei isolates appear
susceptible to the three newer triazoles, reflecting their
increased affinity (versus that of fluconazole) for the C krusei
14α-demethylase target enzyme.

Cross-resistance among the triazoles

Mechanistically, the potential for cross-resistance among the
available triazoles is clearly evident, especially among species of Candida capable of overexpression of CDR efflux pumps, and to a lesser extent those with overexpression/mutations in the target enzyme [8,12]. Although posaconazole, ravuconazole, and voriconazole have all been shown to be active against isolates of Candida with decreased susceptibility to fluconazole, cross-resistance has been demonstrated by in vitro studies [34–36,105,165].

An analysis of cross-resistance between fluconazole and the four other triazoles is shown in Table 5. It is immediately apparent that >99% of all fluconazole-susceptible isolates are susceptible to the four comparators at an MIC of ≤1 μg/mL, the S breakpoint for voriconazole. Among the isolates testing as SDD to fluconazole, 76.4% and 83.2% are susceptible to itraconazole and posaconazole, respectively, at an MIC of ≤1 μg/mL whereas >95% of the fluconazole-SDD isolates are susceptible to ravuconazole and voriconazole at the same S breakpoint. Between 46% and 49% of fluconazole-R isolates are inhibited by ≤1 μg/mL of all four agents, substantiating previous claims for activity against fluconazole non-susceptible isolates [6,23,29,166], yet also demonstrating a significant fall-off in activity of all agents with increasing fluconazole resistance.

The complexity of the cross-resistance issue is seen when the activity of itraconazole and the extended-spectrum triazoles is determined against individual fluconazole-resistant species of Candida (Table 6). First of all, essentially complete cross-resistance between fluconazole and itraconazole is seen with C krusei whereas all three extended-spectrum triazoles are active against this intrinsically fluconazole-resistant species. Likewise, the lack of complete cross-resistance is also apparent for C albicans and C parapsilosis where 50–100% of fluconazole-resistant isolates remain susceptible to posaconazole (100%), ravuconazole (64.3–100%), and voriconazole (51.0–62.5%). Finally, none of the triazoles exhibit any meaningful activity against fluconazole-resistant isolates of C glabrata or C tropicalis. Thus, among the two most common fluconazole-resistant species, essentially complete cross-resistance between the triazoles is seen with C glabrata and a complete lack of cross-resistance is seen with C krusei. These findings are entirely consistent with the respective mechanisms of azole resistance reported for these two species (Table 1).

In the course of large in vitro surveys of candidiasis, it has become apparent that decreased susceptibility to fluconazole, on the order of that seen with the well-known resistant species C glabrata and C krusei, is seen among some less common species of Candida (Table 7) [37,101,102,113]. Substantial cross-resistance between fluconazole and voriconazole is apparent for many of these
AZOLE ANTIFUNGAL DRUG CROSS-RESISTANCE

THE JOURNAL OF INVASIVE FUNGAL INFECTIONS VOL 1 NO 3 2007 83

less common species such as *C. rugosa*, *C. famata*, and *C. lipolytica*, whereas voriconazole activity is retained against the highly fluconazole-resistant species, *C. inconspicua* and *C. norvegensis* (Table 7). The latter two species behave similarly to *C. krusei*, whereas the remaining species are more similar to *C. glabrata* in their cross-resistance profile. Given the uncommon frequency of these species clinically, it is not surprising that little is known of their mechanisms of resistance or the activity of the other azoles in vitro.

Further analysis of cross-resistance between itraconazole, posaconazole, and voriconazole was reported by Sabatelli et al. [36]. Consistent with the data presented in Tables 5–7, isolates with elevated MICs to one azole were generally less susceptible to all azoles (Table 8).

**Clinical significance of cross-resistance**

Thus far, both *in vitro* susceptibility data and consideration of mechanisms ofazole resistance support the plausibility of clinically significant cross-resistance among the azoles. The data are strongest for *C. glabrata* but other species of *Candida*, including *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, are clearly capable of developing a multi-azole-resistant phenotype. Both voriconazole [23,25] and posaconazole [29] have demonstrated clinical efficacy in the treatment of

Table 6. *In vitro* susceptibility of fluconazole-resistant isolates of *Candida* spp to four extended-spectrum triazoles*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Antifungal agent</th>
<th>Total tested (n)</th>
<th>Fluconazole resistant** (n)</th>
<th>% of resistant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S</td>
<td>SDD</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>Itraconazole</td>
<td>3895</td>
<td>4</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>5827</td>
<td>8</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Ravuconazole</td>
<td>7521</td>
<td>42</td>
<td>64.3</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>7725</td>
<td>49</td>
<td>51.0</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>Itraconazole</td>
<td>1054</td>
<td>88</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>1517</td>
<td>145</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Ravuconazole</td>
<td>1869</td>
<td>164</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>1966</td>
<td>181</td>
<td>16.6</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>Itraconazole</td>
<td>1028</td>
<td>8</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>1542</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Ravuconazole</td>
<td>1485</td>
<td>7</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>1623</td>
<td>8</td>
<td>62.5</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>Itraconazole</td>
<td>829</td>
<td>3</td>
<td>66.6</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>1198</td>
<td>2</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>Ravuconazole</td>
<td>1185</td>
<td>16</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>1253</td>
<td>19</td>
<td>10.5</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>Itraconazole</td>
<td>206</td>
<td>70</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>305</td>
<td>103</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td>Ravuconazole</td>
<td>302</td>
<td>122</td>
<td>98.4</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>312</td>
<td>126</td>
<td>98.4</td>
</tr>
</tbody>
</table>

*Data compiled from [34–37,99,102,103,109]. **Fluconazole MIC ≥ 64 μg/mL. MIC: minimum inhibitory concentration; R: resistant, itraconazole (≥ 1 μg/mL), all others (≥ 4 μg/mL); S: susceptible, itraconazole (≤ 0.12 μg/mL), all others (≤ 1 μg/mL); SDD: susceptible dose dependent, itraconazole (0.25–0.5 μg/mL), all others (2 μg/mL).*

Table 7. *In vitro* susceptibility of uncommon fluconazole-resistant species of *Candida* to voriconazole*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total tested (n)</th>
<th>Fluconazole resistant (n)</th>
<th>% of resistant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>SDD</td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td>1088</td>
<td>113</td>
<td>47.8</td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td>835</td>
<td>36</td>
<td>52.8</td>
</tr>
<tr>
<td><em>C. kefyr</em></td>
<td>698</td>
<td>23</td>
<td>65.2</td>
</tr>
<tr>
<td><em>C. rugosa</em></td>
<td>502</td>
<td>230</td>
<td>26.5</td>
</tr>
<tr>
<td><em>C. famata</em></td>
<td>462</td>
<td>47</td>
<td>31.9</td>
</tr>
<tr>
<td><em>C. inconspicua</em></td>
<td>354</td>
<td>186</td>
<td>83.9</td>
</tr>
<tr>
<td><em>C. norvegensis</em></td>
<td>171</td>
<td>63</td>
<td>85.7</td>
</tr>
<tr>
<td><em>C. lipolytica</em></td>
<td>97</td>
<td>30</td>
<td>30.0</td>
</tr>
</tbody>
</table>

*Data compiled from [37]. MIC: minimum inhibitory concentration; R: resistant (MIC ≥4 μg/mL, zone diameter ≤13 mm); S: susceptible (MIC ≤1 μg/mL, zone diameter ≥17 mm); SDD: susceptible dose dependent (MIC 2 μg/mL, zone diameter 14–16 mm).*
fluconazole-refractory candidial infections, although the lowest response rate to voriconazole as salvage treatment was observed with C glabrata (38%) [23]. Likewise, a significantly lower response rate to voriconazole primary therapy, along with a significantly higher voriconazole MIC, was observed among patients with C glabrata fungemia versus fungemia due to other species of Candida [26,95]. It was hypothesized that the low response rate of C glabrata may be related to the inherent ability of this organism to develop resistance during azole therapy [23]. Despite these findings, it has been stated that further clinical experience in the treatment of disease caused by Candida strains with documented fluconazole or itraconazole resistance is required before defining the significance of azole cross-resistance and the role of voriconazole (or posaconazole) in this setting [11,23,29].

Clinically significant resistance to voriconazole [167,168] and to posaconazole [52] has been seen in C albicans isolates with pre-existing fluconazole resistance when these agents were used to treat AIDS patients with OPC. In these reports, resistance to multiple azoles was documented by in vitro testing, and in one report, multiple mutations in the ERG11 gene were demonstrated [52]. The accumulated experience in the treatment of OPC in AIDS patients indicates that azole cross-resistance, as defined by in vitro susceptibility testing, is indeed clinically important but complete multi-azole cross-resistance occurs only after prolonged exposure to an azole antifungal agent, in the face of severe and unrelenting immunosuppression. When multi-azole cross-resistance does occur, it is due to several mechanisms of resistance acting in concert.

Additional evidence supporting the clinical relevance of azole cross-resistance has been documented in case reports and case series of deep-seated invasive candidiasis (candidemia, liver abscess, endocarditis, esophagitis) where clinically significant microbial resistance to voriconazole has been reported among immunocompromised patients (e.g., ICU, stem cell transplant, hematological malignancy patients) with a high level of azole (i.e., fluconazole before voriconazole) exposure (Table 9). Although most of the resistant isolates in these series were C glabrata, a number of C albicans, C tropicalis, and C parapsilosis isolates were also found to have reduced susceptibility or resistance to both fluconazole and voriconazole, and to other azoles including itraconazole, ravuconazole, and posaconazole [30,32,33,38–40]. Furthermore, in two reports, both multi-azole and multi-echinocandin resistance was documented in patients refractory to both classes of antifungal agents [38,39]. These last two reports serve to underscore the unusual, complex, and refractory nature of these infections to any and all antifungal agents. In every instance, the patients were highly immunocompromised and/or had prosthetic material that could not be removed, and were exposed to several classes of antifungal agents in addition to azoles, over a long period of time. The duration of exposure coupled with a high organism burden provides an ideal setting for the emergence of a multi-azole- (multi-drug-) resistant phenotype. In one report of C glabrata fungemia in which multi-azole (fluconazole, ketoconazole, itraconazole, voriconazole) resistance appeared after fluconazole treatment, the patient’s initial, azole-naïve, isolate showed minimal expression of the CgCDR1 and CgCDR2 genes, whereas subsequent isolates showed overexpression of both genes, suggesting a secondary acquisition of resistance [40].

Thus, decreased susceptibility to fluconazole may precede, or even predict, decreased susceptibility to
Voriconazole, posaconazole, and ravuconazole, especially with *C. glabrata* [30,39,40]. Diligent monitoring of candidal infections in highly compromised patients must be continued despite voriconazole or posaconazole coverage, particularly when the use of these agents is preceded by prolonged fluconazole exposure [30,32,33,40]. These concerns have led Magill et al. to suggest reflexive fluconazole susceptibility testing of patients with initial blood isolates of *Candida* as a means of identifying those who may not respond optimally to either fluconazole or voriconazole (or posaconazole) therapy [32]. This support is suggested by our recent study of azole cross-resistance where fluconazole MICs of ≤32 μg/mL predict susceptibility, and MICs of ≥64 μg/mL predict resistance of *Candida* spp to voriconazole with an absolute categorical agreement of 97% (0.1% very major [false–susceptible] errors and 1.4% major [false–resistant] errors) [35]. Similar results have been shown for the comparison of fluconazole with either posaconazole [99] or ravuconazole [34].

**Non-Candida yeasts**

Although uncommon, the number and types of non-candidal yeasts isolated from clinical specimens has increased in recent years [18,22,37,102,169]. *Cryptococcus neoformans* clearly predominates, but infections with *Saccharomyces, Trichosporon, Rhodotorula, Blastoschizomyces*, and *Pichia* spp also merit consideration (Table 10) [37,169]. These organisms have all been shown to cause serious, often disseminated, infections in immunocompromised individuals and are associated with a high crude mortality rate [169]. Furthermore, all demonstrate some antifungal resistance including decreased susceptibility to azoles, variable susceptibility to echinocandins, and a variable response to amphotericin B [22,37,169]. In this regard, prompt identification is essential for optimization of antifungal therapy.

As seen in Table 10, all of these opportunistic yeasts are less susceptible to fluconazole when compared with the majority of *Candida* spp. Indeed, reports of breakthrough infections during prophylaxis with fluconazole have been...
described for most of these organisms [169]. The emergence of resistance to fluconazole in the setting of cryptococcal meningitis in AIDS patients after long-term exposure to fluconazole is well described [170–172]. Aside from Rhodotorula spp, these organisms all exhibit susceptibility to voriconazole (Table 10). Posaconazole has also been shown to be comparable to voriconazole in terms of \textit{in vitro} activity against these yeast-like fungi [105]. Interestingly, although \textit{Rhodotorula} spp are generally considered to be resistant to the azoles [105,106,173], among the extended-spectrum triazoles, ravuconazole MICs have been shown to be about four-fold lower than voriconazole or posaconazole MICs (MIC\textsubscript{50}/MIC\textsubscript{90}, 0.25/1 μg/mL vs. 2/4 μg/mL, respectively) [106].

Unfortunately, as with \textit{Candida} spp, fluconazole-resistant isolates of these non-candidal yeasts also exhibit decreased susceptibility to voriconazole (Table 11) [37]. Given their intrinsic resistance to the echinocandins and their variable response to amphotericin B, these yeasts may pose considerable problems in the future [22,37,106,169,174]. Whether these organisms also exhibit cross-resistance to posaconazole is currently unknown. With the exception of \textit{C neoformans}, there is little or no information regarding mechanisms of resistance or the clinical importance of azole cross-resistance among these opportunistic yeasts. Clinical success was observed in 14 of 29 (48%) subjects with amphotericin B-and/or azole-refractory cryptococcal meningitis treated with posaconazole [175]. Among 12 patients with failure to improve on a primary regimen that included fluconazole, five (42%) had a complete or partial response to posaconazole [175]. Although these data may speak to a lack of complete azole cross-resistance with \textit{C neoformans}, the absence of any \textit{in vitro} susceptibility data and the select group of seriously ill subjects included in this study precludes any meaningful conclusions on this matter. The role, if any, of voriconazole in the management of cryptococcosis is even less well studied [11]. Less than 39% of patients had a complete or partial response to voriconazole in a small salvage therapy study [25].

\textbf{Aspergillus and other molds}

As noted previously, azole resistance among \textit{Aspergillus} spp is uncommon. Denning and colleagues have shown a

---

\textbf{Table 10.} \textit{In vitro} susceptibility of opportunistic non-\textit{Candida} yeasts to fluconazole and voriconazole*.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antifungal agent</th>
<th>Total tested (n)</th>
<th>% by category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>\textit{Cryptococcus neoformans}</td>
<td>Fluconazole</td>
<td>2230</td>
<td>78.0</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>2209</td>
<td>97.1</td>
</tr>
<tr>
<td>\textit{Saccharomyces cerevisiae}</td>
<td>Fluconazole</td>
<td>709</td>
<td>88.6</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>697</td>
<td>95.1</td>
</tr>
<tr>
<td>\textit{Trichosporon spp}</td>
<td>Fluconazole</td>
<td>688</td>
<td>83.9</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>665</td>
<td>93.2</td>
</tr>
<tr>
<td>\textit{Rhodotorula spp}</td>
<td>Fluconazole</td>
<td>286</td>
<td>39.9</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>285</td>
<td>51.2</td>
</tr>
<tr>
<td>\textit{Blastoschizomyces capitatus}</td>
<td>Fluconazole</td>
<td>86</td>
<td>81.4</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>86</td>
<td>91.9</td>
</tr>
<tr>
<td>\textit{Pichia spp}</td>
<td>Fluconazole</td>
<td>109</td>
<td>84.4</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>107</td>
<td>97.2</td>
</tr>
</tbody>
</table>

*Data compiled from [37]. MIC: minimum inhibitory concentration; R: resistant (fluconazole, MIC ≥ 64 μg/mL [≤ 14 mm], voriconazole, MIC ≥ 4 μg/mL [≤ 11 mm]; S: susceptible (fluconazole, MIC ≤ 8 μg/mL [≥ 19 mm]; voriconazole, MIC ≤ 1 μg/mL [≥ 17 mm]); SDD: susceptible dose dependent (fluconazole, MIC 16–32 μg/mL [15–18 mm]; voriconazole, MIC 2 μg/mL [14–16 mm]).

\textbf{Table 11.} \textit{In vitro} susceptibility of fluconazole-resistant opportunistic non-\textit{Candida} yeasts to voriconazole*.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total tested (n)</th>
<th>% by category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>\textit{Cryptococcus neoformans}</td>
<td>228</td>
<td>79.8</td>
</tr>
<tr>
<td>\textit{Saccharomyces cerevisiae}</td>
<td>43</td>
<td>34.9</td>
</tr>
<tr>
<td>\textit{Trichosporon spp}</td>
<td>62</td>
<td>45.2</td>
</tr>
<tr>
<td>\textit{Rhodotorula spp}</td>
<td>158</td>
<td>18.4</td>
</tr>
<tr>
<td>\textit{Blastoschizomyces capitatus}</td>
<td>10</td>
<td>60.0</td>
</tr>
<tr>
<td>\textit{Pichia spp}</td>
<td>9</td>
<td>88.9</td>
</tr>
</tbody>
</table>

*Data compiled from [37]. MIC: minimum inhibitory concentration; R: resistant (voriconazole, MIC ≥ 4 μg/mL [≤ 13 mm]); S: susceptible (voriconazole, MIC ≤ 1 μg/mL [≥ 17 mm]); SDD: susceptible dose dependent (voriconazole, MIC 2 μg/mL [14–16 mm]).
maximum itraconazole resistance rate of 4.2% in *Aspergillus* spp and 2.1% in *A. fumigatus* [19,176]. These findings are supported by those of Verweij et al. [104] and Pfaller et al. [103]. Clinical and *in vivo* resistance to itraconazole [74,177] and elevated voriconazole MICs have been described for *A. fumigatus* clinical isolates [178], and clear advances have been made in defining the mechanisms of azole resistance in this species [19,41,74–80,82,83]. However, no study has investigated azole cross-resistance in more than a few clinical *Aspergillus* isolates [19].

A large *in vitro* survey that included 6423 clinical isolates and 10 species of *Aspergillus* demonstrated superior potency of both posaconazole and voriconazole over that of itraconazole (Table 12); however, no analysis of cross-resistance was provided [36]. In a study of 338 Spanish clinical isolates of *Aspergillus* spp (13 species), Gomez-Lopez et al. demonstrated comparable *in vitro* activity for both itraconazole and voriconazole [179]. Among the 12 isolates for which the the itraconazole MIC was >4 μg/mL, the voriconazole MIC was >2 μg/mL for eight isolates (range 2–8 μg/mL), suggesting some degree of cross-resistance. Likewise, Cuenca-Estrella et al. compared the activities of itraconazole, posaconazole, and voriconazole against 697 isolates of *Aspergillus* spp (seven species) and found these azoles to be moderately active, with posaconazole showing slightly greater potency (geometric mean [GM] MIC 0.1 μg/mL) than either itraconazole (GM MIC 0.33 μg/mL) or voriconazole (GM MIC 0.48 μg/mL) [105]. Among nine itraconazole-resistant isolates, posaconazole was active against five (55%) isolates. Cross-resistance involving all three azoles was observed for isolates of *A. fumigatus*, *A. niger*, *A. nidulans*, and other *Aspergillus* spp. Finally, Mosquera and Denning studied 11 isolates of *A. fumigatus* defined as resistant to itraconazole (MIC >4 μg/mL) *in vitro* [19]. They found that elevated itraconazole MICs were uniformly associated with elevations in posaconazole MICs of four- to 256-fold, whereas elevated itraconazole MICs were not usually associated with elevated MICs of ravuconazole or voriconazole. The susceptibility pattern of the isolates against voriconazole and ravuconazole was similar, suggesting similar modes of action and mechanisms of resistance. These findings suggest considerable heterogeneity in azole susceptibility among isolates of *A. fumigatus* [19]. Although posaconazole is consistently more active than itraconazole, the susceptibility patterns of these two azoles are similar, as are those of voriconazole and ravuconazole. These relationships may be expected from structural considerations as discussed previously, and are supported by molecular modeling studies and current knowledge of mechanisms of resistance [41,77–79]. Unfortunately, the clinical implications of these different profiles are, as yet, unknown. At this time, given the fact that individual variations of MICs are not entirely predictable, *in vitro* susceptibility testing of clinical isolates to each of the triazoles is likely to be useful and will allow one to select the most active agent [19].

Among the various species of *Aspergillus* causing clinical infection, there are two that merit attention due to resistance to multiple antifungal agents *in vitro*: *A. lentulus* [180,181] and *A. ustus* [182–186]. *A. ustus* has been implicated in breakthrough disseminated infection in HSCT recipients receiving amphotericin B, itraconazole, voriconazole, or caspofungin and exhibits *in vitro* resistance to itraconazole and voriconazole as well as amphotericin B and caspofungin [182–186]. *A. lentulus* is a recently recognized species that morphologically resembles *A. fumigatus* but has a resistance

### Table 12. Comparative *in vitro* activities of extended-spectrum triazoles against filamentous fungi.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Antifungal agent</th>
<th>Total tested (n)</th>
<th>50%*</th>
<th>90%*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em> spp</td>
<td>Itraconazole</td>
<td>1423**</td>
<td>0.5</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td></td>
<td>0.12</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td></td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Zygomycetes</em></td>
<td>Itraconazole</td>
<td>86**</td>
<td>1.0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td></td>
<td>0.5</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td></td>
<td>16</td>
<td>128</td>
</tr>
<tr>
<td><em>Fusarium</em> spp</td>
<td>Itraconazole</td>
<td>67**</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td></td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td></td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td><em>Scedosporium</em> prolificans</td>
<td>Itraconazole</td>
<td>55*</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td></td>
<td>&gt;8.0</td>
<td>&gt;8.0</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td></td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td><em>Scedosporium</em> apiospermum</td>
<td>Itraconazole</td>
<td>13*</td>
<td>0.5</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td></td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td></td>
<td>0.25</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*50% and 90%, minimum inhibitory concentrations encompassing 50% and 90% of isolates tested, respectively. **Data compiled from [36]. †Data compiled from [198].
posaconazole may be useful in the treatment of amphotericin experience, however, indicates that both voriconazole and in vitro appears to be active against most of the zygomycetes, both posaconazole stands apart from voriconazole in that it due to any of the zygomycetes [31,187,188]. Conversely, that this agent lacks any clinical utility in treating infections decreased susceptibility to itraconazole but it is quite clear that mechanism of resistance to voriconazole but it is quite clear that this agent lacks any clinical utility in treating infections due to any of the zygomycetes [31,187,188]. Conversely, posaconazole stands apart from voriconazole in that it appears to be active against most of the zygomycetes, both in vitro and in vivo [22,36,105,189]. A recent case series of 91 patients with zygomycosis suggests that posaconazole is promising as an oral treatment alternative for patients with zygomycosis who cannot tolerate or do not respond to intravenous amphotericin B products (with 60% of patients experiencing a complete or partial response) [189].

Among the new triazoles, only modest activity is seen in vitro against isolates of Fusarium spp [36]. Clinical experience, however, indicates that both voriconazole and posaconazole may be useful in the treatment of amphotericin B-refractory fusariosis [25,27,28,190,191]. There have been no studies of cross-resistance involving Fusarium and it appears that in vitro susceptibility results may underestimate the utility of these agents as salvage therapy [36,105].

Within the genus Scedosporium, S apiospermum (teleomorph, Pseudallescheria boydii) and S prolificans represent two important antifungal-resistant opportunistic fungal pathogens. S apiospermum is a well-known cause of mycetoma and may cause deep-seated infections (e.g. central nervous system [CNS] abscesses and disseminated infection) in BMT recipients and other neutropenic, immunosuppressed individuals [192–194]. The extended-spectrum triazoles are active in vitro against S apiospermum, and both posaconazole and voriconazole have successfully been used for the treatment of infections caused by this organism [36,105,175,193,197–200]. Voriconazole is generally the most active of the triazoles against this species, followed by posaconazole and itraconazole [198]. Meletiadis et al. examined in vitro cross-resistance among miconazole, itraconazole, albaconazole, voriconazole, and posaconazole for 13 clinical S apiospermum isolates and found a significant correlation (cross-resistance) among all azoles, except posaconazole [198]. They interpreted this to mean that the mechanism of action or the mechanism of resistance for posaconazole might be different from those for the other azoles. However, there are no mechanistic or clinical studies addressing these potential differences in activity and voriconazole is generally recommended as the treatment of choice for infections with S apiospermum [112,192,197].

S prolificans causes bone and soft tissue infections in immunocompromised individuals and deeply invasive and disseminated infections in immunocompromised patients [169]. S prolificans is considered resistant to virtually all of the systemically active antifungal agents, including the extended-spectrum triazoles (Table 12). There are no in vitro or clinical studies that address cross-resistance; however, synergy between triazoles and terbinafine have been demonstrated in vitro [198], and both localized and disseminated infection due to S prolificans has successfully been treated with a combination of voriconazole and terbinafine, in addition to surgical debridement [201,202]. Surgical resection remains the only definitive therapy for infections caused by S prolificans [169].

Summary and Conclusions

Cross-resistance within a given class of antimicrobial agents is well known and of proven clinical importance in the treatment of bacterial infections. One of the best examples is the cross-resistance seen for the β-lactam agents in organisms that produce β-lactamase or those with altered penicillin-binding proteins. There are now sufficient in vitro, mechanistic, and clinical data to make the same statements for theazole antifungal agents and some, but not all, fungi. The data are most compelling for Candida spp where in vitro cross-resistance has been demonstrated and is well supported by mechanisms of resistance studies. Considerations of molecular structure and the interaction of the different triazoles with the target enzyme would predict cross-resistance between fluconazole and voriconazole on the one hand, and between itraconazole and posaconazole on the other. These relationships have held true for certain strains of C albicans with specific target enzyme mutations; however, a more common pattern seen with Candida, especially C glabrata, is one of multi-azole resistance due to the over-expression of CDR efflux pumps. The clinical relevance of such cross-resistance is now readily apparent in case reports and case series of critically ill patients with deep seated Candida infections where fluconazole prophylaxis or therapy has preceded voriconazole therapy. A similar set of data suggests that cross-resistance maybe be relevant for treatment of aspergillosis. The in vitro cross-resistance between itraconazole, posaconazole, voriconazole, and ravuconazole is now well described for A fumigatus and the primary mechanisms of resistance involve point mutations in the target enzyme. Depending on the number and location of the mutations one may see one of three different resistance patterns:
AZOLE ANTFUNGAL DRUG CROSS-RESISTANCE

- Resistance to itraconazole and posaconazole, but not voriconazole or ravuconazole.
- Resistance to voriconazole and ravuconazole, but not itraconazole and posaconazole.
- Resistance to all four azoles.

The clinical impact of these different profiles is unknown; however, the current recommendations indicate that in vitro susceptibility testing may be useful to elucidate the different patterns and to select the most active agent.

It appears, based on in vitro studies, that cross-resistance may be important in other opportunistic yeasts and molds. The fact that these uncommon pathogens often exhibit resistance to echinocandins and polyenes make the issue of azole resistance all the more important to understand. Unfortunately, studies of mechanisms and clinical importance of cross-resistance are lacking. Irrespective of the pathogen, the issue of cross-resistance is most likely to be considered in a seriously compromised individual with a deep-seated fungal infection and extensive exposure to either fluconazole or itraconazole. Assuming the infecting agent can be isolated, the way forward is to identify it to the species level and to strongly consider antifungal susceptibility testing to identify the resistance profile. For Candida, one may use fluconazole as a surrogate marker for resistance to voriconazole, posaconazole, or ravuconazole, but for other fungi, it may be prudent to obtain results for all of the triazoles. Clearly, this is an important issue that must be understood in order to provide optimal therapy for infected patients. It certainly merits continued investigation.

Acknowledgments
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Disclosures
Dr Pfaffer has received research support from, and serves as a consultant for, Astellas, Merck, Pfizer, and Schering-Plough.

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Dr Pfaffer has received research support from, and serves as a consultant for, Astellas, Merck, Pfizer, and Schering-Plough.

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1997; Antimicrob Agents Chemother
1996; Antimicrob Agents Chemother


Amphotericin B Resistance: Epidemiology, Mechanisms, and Clinical Relevance

Stephanie A Knechtel and Michael E Klepser
Ferris State University College of Pharmacy, Kalamazoo, MI, USA

Amphotericin B, a polyene antifungal, was once considered the gold standard for the management of a number of fungal infections. Despite several decades of use, resistance to amphotericin B has been seemingly slow to develop. Although innate resistance has been described among some Candida species and various molds including several Aspergillus species, reports of secondary resistance emerging while on therapy appear to be relatively scarce. Several mechanisms of polyene resistance have been described, including those mediated by mutations in genes encoding for various enzymes involved in the ergosterol biosynthetic pathway. Such mutations generally result in the synthesis of a fungal cell membrane that possesses altered sterol composition and is thus less susceptible to the fungicidal activity of the polyenes. With respect to amphotericin B, resistance has been difficult to detect owing to the limitations associated with conducting in vitro susceptibility tests. Furthermore, the narrow range of minimum inhibitory concentrations generally observed and the absence of meaningful susceptibility breakpoints make the clinical relevance of amphotericin B resistance difficult to assess. Currently, polyene resistance among the majority of clinically encountered fungal pathogens appears to be low; however, the validity of this perception remains somewhat unclear. J Invasive Fungal Infect 2007;1(3):93–8.

Epidemiology
Although “resistance” to amphotericin B among commonly encountered fungi continues to be rare, reports of isolates exhibiting elevated minimum inhibitory concentrations (MICs)
to amphotericin B have become increasingly common [1].
Owing to the lack of a definitive susceptibility breakpoint
and variability associated with in vitro testing methods, it is
impossible to report rates of resistance to amphotericin B.
However, if an MIC of 1 μg/mL is used as an anchor of
normality, one may examine the dispersion of MICs around
this point as a gauge for relative susceptibility. Using this
approach, conclusions regarding the general activity of
amphotericin B appear to support the notion that the drug
is highly active against Candida species (Table 1) [1–4].
However, this observation is tainted by the fact that most
reports summarizing Candida susceptibility patterns are
weighted heavily with data on Candida albicans. Closer
inspection of the data reveals a troubling trend suggestive of
diminishing susceptibility to amphotericin B among several
Candida species, including Candida krusei and Candida
glabrata [1–7]. Increased MICs to amphotericin B have also
been described for other, less frequently encountered,
Candida species such as Candida lusitaniae, Candida
guilliermondii, and Candida rugosa [1,2,5,7]. In contrast,
rates of susceptibility among isolates of C neoformans have
remained stable over the previous few decades, allowing
amphotericin B to remain a frequently utilized treatment
option for cryptococcal infections [8].

In contrast to the relative susceptibility of Candida
species to available antifungals, molds frequently exhibit
reduced susceptibility to many agents, including
amothepticin B. Aspergillus species are the most commonly
encountered molds in the clinical setting. Historically,
susceptibility data have revealed that as an aggregate,
75–90% of Aspergillus species are inhibited at a
concentration of amphotericin B of ≤1 μg/mL [9,10].
However, as susceptibility data from specific species are
examined, intraspecies variability with respect to
amothepticin B MIC distributions become apparent (Table 1).
Specifically, Aspergillus terreus commonly exhibits
amothepticin B MICs >1 μg/mL [9–11]. In fact, Sutton and
colleagues reported that <2% of the 101 A terreus isolates
examined were inhibited by amphotericin B at a
concentration of ≤1 μg/mL [11]. In addition to A terreus,
A flavus and A nidulans have also been reported to be less
susceptible to amphotericin B than other Aspergillus species
[9,10,12]. Of particular concern however, are data from a
recent study of 52 isolates of Aspergillus fumigatus that
noted a dramatic drop in the percent of isolates inhibited by
amothepticin B at concentrations ≤1 μg/mL [3]. In that
study, the authors reported that <12% of the isolates tested
were inhibited at this threshold. These findings are in stark
contrast with earlier studies that reported 90–98% of
A fumigatus isolates to be inhibited at amphotericin B
concentrations of ≤1 μg/mL [9,10].

### Table 1. In vitro susceptibility of various yeasts and
filamentous fungi to amphotericin B*.

<table>
<thead>
<tr>
<th>Organism (number tested)</th>
<th>Amphotericin B MIC (μg/mL)</th>
<th>50%</th>
<th>90%</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans (2728)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.016–4</td>
<td></td>
</tr>
<tr>
<td>C parapsilosis (666)</td>
<td>1</td>
<td>2</td>
<td>0.25–16</td>
<td></td>
</tr>
<tr>
<td>C glabrata (722)</td>
<td>1</td>
<td>2</td>
<td>0.03–16</td>
<td></td>
</tr>
<tr>
<td>C tropicalis (528)</td>
<td>1</td>
<td>2</td>
<td>0.06–32</td>
<td></td>
</tr>
<tr>
<td>C krusei (143)</td>
<td>4</td>
<td>8</td>
<td>0.03–16</td>
<td></td>
</tr>
<tr>
<td>C lusitaniae (54)</td>
<td>0.25</td>
<td>1</td>
<td>0.06–16</td>
<td></td>
</tr>
<tr>
<td>C guilliermondii (102)</td>
<td>0.25</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C dubliniensis (164)</td>
<td>0.5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C rugosa (13)</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus species:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A fumigatus (1119)</td>
<td>0.5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A niger (101)</td>
<td>0.125</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A flavus (89)</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A terreus (22)</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A nidulans (20)</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium species (67)</td>
<td>8</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucor species (18)</td>
<td>0.25</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizopus species (32)</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scedosporium prolificans (80)</td>
<td>13</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scedosporium apiospernum (26)</td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data compiled from [1,6,7,10]; MIC: minimum inhibitory concentration.

With respect to molds other than Aspergillus species,
data regarding susceptibility to amphotericin B are relatively
scant and suggestive of a high degree of variability among
species. However, available data suggest that molds such as
Fusarium species, Scedosporium species, and various
zygomycetes exhibit high (2–32 μg/mL) amphotericin B
MICs [9,10]. However, despite elevated MICs, amphotericin
B may remain a therapeutic alternative in the treatment of
some of these pathogens.

### Mechanisms of Resistance

Despite the relative infrequency with which polyene resistance
is observed, a number of investigators have examined the
mechanisms accounting for this resistance, with most data
focusing on amphotericin B. Several species have demonstrated
varying degrees of innate resistance to the polyenes, including
yeasts, such as C lusitaniae, C guilliermondii, and Tricorporon
species, as well as a variety of molds such as A terreus and
Scedosporium and Fusarium species [13,14]. Although
observed rarely, the development of secondary resistance
to the polyenes is also of concern, particularly among immunocompromised patients that may require long-term antifungal prophylaxis and/or treatment. The exact mechanism of action of the polyenes has not been definitively characterized. While several mechanisms have been proposed, for example theories involving the role of oxidative cell damage, the fungicidal activity of the agents is widely believed to occur once drug binds to ergosterol in the fungal cell membrane and induces the formation of pores in the phospholipid bilayer [15]. This action results in altered membrane permeability and cell death [15]. Given this mode of action, proposed mechanisms of resistance to the polyenes typically involve the production of a cell membrane possessing altered sterol composition [15]. Mutations that lead to the production of unusual sterol types, alter the stereochemistry of membrane ergosterol, and/or decrease the overall quantity of ergosterol in the cell membrane may result in diminished polyene binding capacity and thus lead to decreased efficacy [16].

Polyene resistance has been best defined among Candida species, and is commonly attributed to a defect in the ergosterol biosynthetic pathway. Nolte and colleagues described resistance among C albicans isolated from two patients with leukemia [17]. Both patients developed fungemia with strains that were resistant to fluconazole and amphotericin B. Upon evaluation, the authors discovered that these strains likely possessed altered activity of Δ5,6-sterol desaturase. This mutation led to the increased production of abnormal membrane sterols, including 3β-ergosta-7,22-dienol, and a decreased production of ergosterol [17]. These findings are consistent with those of Kelly and colleagues who documented the same mutation in polyene-resistant strains of C albicans isolated from a patient with AIDS [18]. Inactivation of the Δ5,6-sterol desaturase enzyme occurs secondary to a mutation of the ERG3 gene [19]. This form of ERG3-mediated resistance may also affect azole antifungal activity [19]. Cross-resistance between the azoles and polyenes has also been noted among Cryptococcus neoformans; however, some data suggest that a resistance mechanism other than alterations in ergosterol synthesis may be responsible [20].

Another alteration in the ergosterol biosynthetic pathway, this time involving the ERG11 gene, may also yield polyene resistance among C albicans [21]. In the presence of amphotericin B in vitro, Sanglard and colleagues noted the development of a mutant with defects in the ERG11 gene [22]. The significance of this mutation is unclear, since models examining ERG11 mutants of Saccharomyces cerevisiae suggest that in order to be viable in aerobic conditions, an additional mutation in ERG3 must also be present [23]. The expression of a variety of additional genes may be affected following exposure to amphotericin B; however, the relevance of these mutations has not been well established [21].

Isolates of C lusitaniae may possess innate resistance to the polyenes, or may rapidly develop secondary resistance. Yong and colleagues evaluated several resistant isolates of C lusitaniae and noted increased levels of ERG6 transcription as well as reduced expression of ERG3 [23]. Following an evaluation of the membrane sterol and fatty acid composition of several clinical isolates of C lusitaniae, Peyron and colleagues noted a defect in the Δ8→7 isomerase among amphotericin B-resistant strains [25]. Of importance is the ability of isolates of C lusitaniae to exhibit phenotypic switching in the presence of environmental stressors such as exposure to amphotericin B [25–27]. McClenny and colleagues reported a patient who received amphotericin B therapy for C lusitaniae fungemia [26]. Although in vitro testing demonstrated initial susceptibility of this isolate to amphotericin B, the isolate was noted to exhibit a four-fold increase in MIC following 7 weeks of therapy. The authors also observed that resistant isolates were associated with an accompanying change in colony morphology [26]. In vitro data have also demonstrated the ability of C lusitaniae isolates that were once fully susceptible to undergo phenotypic switching, which alters not only their cellular morphology, but also results in diminished susceptibility to amphotericin B [28]. Additionally, isolates possessing the aptitude for phenotypic switching have also demonstrated the capability to switch back to the susceptible phenotype at a much less frequent rate [28].

Among other Candida spp., mechanisms of resistance again typically appear to correlate with a defect in ergosterol synthesis. A mutation in the ERG6 gene among C glabrata leading to decreased membrane ergosterol and increased sterol intermediates has been associated with reduced susceptibility to the polyenes [29]. An analysis of C krusei, C parakrusei, and C tropicalis revealed that resistant strains had a decrease in the quantity of membrane sterols when compared with wild-type strains [30].

In addition to alterations in ergosterol synthesis, oxidative changes within the fungal cell may also play a role in polyene resistance among Candida species. Following exposure to erythromycin, isolates of C albicans demonstrated decreased susceptibility to amphotericin B, which likely occurred secondary to a decrease in aerobic respiration [31]. Because ergosterol synthesis is oxygen-dependant, alterations in cellular oxidative processes may lead to decreased ergosterol production and subsequent resistance to amphotericin B [31,32]. In contrast, among azole-resistant isolates of C glabrata with deficiencies in cellular respiration, increased susceptibility to the polyenes has been documented [33].
This may result from mutations that actually lead to an increase in free ergosterol content among these isolates [33]. Further evaluation of the role of oxidative processes in polyene resistance is required in order to better characterize the implications of these changes among Candida species.

Decreased susceptibility to the polyenes has also been described among the filamentous fungi. However, specific resistance mechanisms among molds have not been well studied, and theories regarding the mechanism of resistance among species such as Aspergillus are largely derived from studies evaluating yeasts such as S cerevisiae, C neoformans, and C albicans [34]. As such, the most commonly proposed resistance mechanism involves alterations in ergosterol synthesis via mutations in the ERG3 gene [34]. Additionally, some data suggest that Aspergillus spp. may exhibit decreased susceptibility to the polyenes via the production of reducing enzymes that impair the oxidative activity of amphotericin B on the cell membrane [34]. This mechanism of resistance may also be expressed among some Candida spp. [34,35].

Whether complete cross-resistance exists between nystatin and amphotericin B is also unclear at this time. In theory, because of the similarities of these two agents with respect to their mechanisms of action, one would intuitively suspect relatively complete cross-resistance. However, Hapala and colleagues noted a lack of cross-resistance among S cerevisiae [36]. Resistance to nystatin was attributed to alterations in cell membrane sterols whereas resistance to amphotericin B occurred secondary to abnormal composition of the fungal cell wall [36].

An additional method by which fungi, namely Candida spp., may achieve sustained growth in the presence of antifungal therapy is the formation of biofilms. These biofilms, composed primarily of carbohydrate, protein, hexosamine, phosphorus and uronic acid, may serve to protect Candida colonies growing on indwelling devices and prosthetics from antifungal exposure [37]. Notably, the penetration of amphotericin B through the biofilm matrix is significantly impaired [37–39]. However, Kuhn and colleagues utilized a bioprosthetic model to evaluate the susceptibility of Candida biofilms to a variety of antifungals, and these investigators found that the lipid formulations of amphotericin B may achieve appreciable activity against Candida biofilms [40]. The clinical relevance of these in vitro findings has not been well defined.

In vitro susceptibility testing

Perhaps the greatest challenge associated with tracking polyene resistance is the lack of a uniform and meaningful means with which to conduct in vitro susceptibility testing. The National Committee for Clinical Laboratory Standards (NCCLS), now Clinical and Laboratory Standards Institute (CLSI), first developed and approved testing methods for antifungal susceptibility testing of yeasts in 1997 (NCCLS M27-A). This document represented the culmination of over 15 years of research by experts in medical mycology. Despite this success, it was readily apparent that the approved broth dilution techniques were woefully inadequate for the reliable determination of amphotericin B MICs [41]. The primary limitation noted with the broth microdilution methods was the apparent lack of discriminatory power afforded. Following the M27-A methodology, MICs determined for Candida species exhibit a narrow distribution pattern, with clustering of MICs around a value of 0.5–1 μg/mL [41]. This observation has made it virtually impossible to establish a correlation between amphotericin B MICs and clinical outcomes [41].

Several investigators have suggested various modifications to the approved testing standards or have proposed different methods for determining amphotericin B MICs altogether [41–45]. Common modifications that have been suggested for the broth-based methods include the use of an alternate growth media that provides a wider dispersion of amphotericin B MICs or determination of the minimum lethal concentration [41–45]. Some investigators have even proposed abandoning broth-based amphotericin B susceptibility testing entirely in favor of agar-based methods such as Etest® (AB BIODISK, Solna, Sweden) [45]. Unfortunately, despite the ability to generate a broader MIC distribution profile with these alternative techniques, correlation between amphotericin B MICs and clinical outcomes among Candida species has not been consistently demonstrated [44,45]. In a recently published study, Park and colleagues determined the MIC of amphotericin B for 107 Candida isolates using five different methods including broth microdilution with several media and Etest [45]. Clinical outcomes data following treatment with amphotericin B were available for each of the study isolates and correlation with MICs determined by each method was attempted. Despite the ability to broaden the range of MICs, the authors were not able to correlate susceptibility data (generated with any of the methods utilized) to treatment failure or success. This led the investigators to reiterate the conclusion that treatment failure in candidiasis are often due to factors other than elevated MICs of the organisms [45].

Unfortunately, many of the same limitations noted for in vitro susceptibility testing of yeasts have also been encountered with testing of filamentous fungi. Furthermore, in vitro testing of molds can be complicated by the different growth characteristics exhibited by these fungi with different media (i.e. agar versus broth). Although standardized methods for broth dilution antifungal susceptibility testing of filamentous fungi were approved in 2002, few data have been published.
been published that have correlated amphotericin B MICs with clinical outcome (CLSI M38-A). Owing to the relative infrequency with which various filamentous fungi are encountered and the difficulty associated with the diagnosis and isolation of these pathogens, the limited data that are available regarding amphotericin B MICs and outcome involve Aspergillus species. Lass-Flörl and colleagues retrospectively examined the microbial risk factors associated with treatment failure among a cohort of 29 bone marrow transplant patients infected with Aspergillus species who were treated with amphotericin B [46]. Twelve, nine, and eight patients were infected with A flavus, A terreus, and A fumigatus, respectively. Of the 29 isolates tested, 23 (79%) exhibited amphotericin B MICs \( \geq 2 \mu g/mL \) (A flavus eight of 12; A terreus nine of nine; A fumigatus six of eight). It was noted that the degree of susceptibility to amphotericin B was the only factor that correlated with clinical outcome. Irrespective of the species, all six patients infected with isolates exhibiting amphotericin B MICs \(< 2 \mu g/mL \) survived, whereas 22 of 23 patients from whom isolates with MICs \( \geq 2 \mu g/mL \) expired [46]. In a more recent study, Liorakis and colleagues were unable to detect a correlation between amphotericin B MIC and clinical outcome [47]. The initial patient population in this study consisted of 116 patients infected with Aspergillus species. However, the authors were only able to utilize 18 patients in their final analysis. Patient exclusion occurred secondary to a variety of complicating factors including use of multiple antifungals, isolation of more than one fungal species, and inadequate treatment duration. As a result, the final sample size was reduced significantly and most likely was not sufficient to allow the detection of a statistically significant difference [47]. However, this study exemplifies the complexity of the patient population likely to be infected with Aspergillus species and highlights the multiple factors that will likely impact treatment outcome. It is important to note that differences in the in vitro susceptibility testing methods used in the two aforementioned studies may have contributed the disparate results.

An additional obstacle with respect accurate in vitro susceptibility testing for amphotericin B relates to the various lipid formulations that are currently available. While these formulations offer a less toxic and equally effective therapeutic alternative to the deoxycholate salt of amphotericin B in vivo, they may have appreciably diminished efficacy when examined in vitro. Swenson and colleagues observed in vitro resistance to amphotericin B lipid complex (ABLC) among C albicans mutants lacking phospholipases [48]. These investigators speculated that absence of phospholipase activity in vitro was responsible for the increases in the MICs for these mutants, owing to the inadequate release of amphotericin B from the lipid complex. However, due to the endogenous production of phospholipases, sustained efficacy of ABLC was observed for the C albicans mutants, and for several Aspergillus species, in vivo [48]. Additional studies also confirm the perception that in vitro testing of amphotericin B lipid products may yield variable results [49–51]. At present, it is difficult to characterize the clinical relevance of in vitro susceptibility testing for the amphotericin B lipid products given the disparity between laboratory findings and in vivo efficacy.

**Conclusion**

The development of immunomodulating agents and the advancement of medical technologies have helped to extend the life of patients with a variety of previously uniformly fatal health conditions. However, as is often the case, gain is not without consequence, and many of these therapies have contributed to the development of a subset of patients highly susceptible to infection with a variety of fungal pathogens. It is this group of patients that may experience frequent and repeated antifungal exposure and in whom the development of resistance is of particular concern. Owing to its broad spectrum of activity and our clinical familiarity, amphotericin B is commonly utilized among patients with invasive fungal infections. Despite frequency of use, the expression of amphotericin B resistance appears relatively rare at this time. However, drawing such conclusions regarding trends in polyeine resistance must be done cautiously and with the realization that our current methods for detecting decreased susceptibility to these agents are unfortunately laden with inadequacies. In spite of the considerable effort afforded to establishing methods of detecting polyeine resistance that are reproducible and clinically meaningful, this objective has not yet been achieved. In addition, given the availability of less toxic therapeutic alternatives our comfort with and willingness to use amphotericin B may continue to erode.

**Disclosures**

The authors have no relevant financial relationships to disclose.

**References**

10. Sabatelli F, Patel R, Mann PA et al.
Echinocandin Antifungal Drug Resistance

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The recent introduction of the echinocandins to clinical practice represents a welcome addition to the antifungal armamentarium. This new class of drugs with their novel mode of antifungal action have several advantages over existing agents. They are most active against the principal invasive fungal pathogens Candida and Aspergillus spp, they have low toxicity for patients, and they have potential value in combination therapy. Antimicrobial drug resistance is always a concern as it is a dynamic biological process. In view of the limited experience gained so far in using the echinocandins, especially caspofungin, it is too early to be sure whether or not this will be a significant issue. Research into in vitro susceptibility determination has established reliable methods, although there are no agreed breakpoints that can be applied to correlating these with outcome of therapy. While certain fungi are inherently resistant to the echinocandins, a greater concern is the potential for failure of echinocandin therapy due to development of reduced susceptibility or resistance following widespread exposure to the drug in high-risk patient populations, as has been seen with fluconazole. The fact that only a small number of case reports have documented this is encouraging, but prospective surveillance is warranted.


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candidemia, and invasive candidiasis, and has received approval for use by the FDA for these indications [8]. Its mode of action and antifungal spectrum of activity is similar to the other echinocandins and includes good activity in vitro against Aspergillus spp, although clinical efficacy data in invasive aspergillosis are relatively limited.

Aminocandin is a more recent addition to the class and is in the early stages of development [9].

Determining echinocandin susceptibility in vitro

It is highly desirable to have reliable methods for determination of fungal susceptibility in vitro to systemically administered antifungals. This will allow reliable predictive breakpoints to be developed that correlate with, or at least help predict, clinical activity.

Yeasts

Antifungal agents of the echinocandin class have presented a challenge in terms of optimization of in vitro susceptibility testing. Early testing suggested that although the microtiter broth microdilution method M-27A [10], standardized by the Clinical and Laboratory Standards Institute (CLSI) for other classes of antifungal agents, would be suitable for echinocandins, some modification may be necessary [11]. In an extensive interlaboratory comparison of susceptibility testing with caspofungin against Candida and Aspergillus spp, considerable variation was observed [12]. The conditions producing the greatest level of interlaboratory agreement for testing yeast isolates were incubation in Roswell Park Memorial Institute Medium (RPMI) 1640 at 35°C with endpoint determination after 24 h and the minimum inhibitory concentrations (MIC) defined as a “proliferative growth reduction” (≥50% inhibition relative to control). Such conditions have now been widely validated and produce reproducible results that are able to detect phenotypic resistance in isolates with mutation in the fungal FSK1 gene, which display reduced susceptibility to the echinocandins in both in vitro and animal models [12,13]. In addition, disc diffusion and Etest methods have shown to be suitable for determining the activity of echinocandins against yeast isolates producing easy-to-read, sharp zones of inhibition [14,15].

Given their shared mechanism of action it is not surprising that all three available echinocandin agents demonstrate similar potency. Scatterplots of micafungin or anidulafungin MICs versus caspofungin MICs show high levels of correlation (R=0.83) [13]. In vitro time-kill assays have also demonstrated similar concentration-dependent fungicidal activity against most Candida spp for caspofungin, micafungin, and anidulafungin [16–19]. Interestingly, it has been observed that caspofungin is not fungicidal for isolates of C parapsilosis or C guilliermondii [20].

Global surveillance involving susceptibility studies on thousands of Candida isolates has been undertaken to examine geographical and temporal trends in susceptibility to caspofungin. These have been summarized by Pfaller and Diekema [13]. The data demonstrate that >99% of isolates tested in each year since the introduction of caspofungin for clinical treatment (2001–4) have MICs of ≤1.0 mg/L. This does not differ from the susceptibility profiles encountered in the years prior to its introduction (1992–2000), indicating little problem with innate or emerging resistance in this genus.

Breakpoints for the echinocandin class of agents have not been established and in vitro-in vivo analysis is hampered by the dearth of resistant isolates. A paper by Kartsonis and colleagues failed to establish any relationship between baseline caspofungin MICs and clinical outcome with isolates from both mucosal and invasive Candida infections [21]. Notably, the data set included just three isolates with reduced susceptibility to caspofungin (MIC ≥4.0 mg/L) from patients subsequently treated with the drug. An interesting in vitro finding is reduced susceptibility of strains of C parapsilosis to the echinocandins [22]. Caspofungin appears to be as effective in vivo as amphotericin B in the treatment of candidemia due to this species [22], although the reduced virulence of C parapsilosis compared with that of C albicans may also be a factor. This suggests that although breakpoints for susceptibility to the echinocandins have yet to be established, it is important that the normal ranges of the majority of Candida spp should be encompassed in the proposed susceptible range. A caspofungin MIC of ≤2.0 mg/L, a blood concentration that is easily achievable in vivo under normal dosing, would encompass 99.7% of all clinical isolates of Candida spp without bisecting any species group [11]. At this time it is difficult to predict what caspofungin MIC is likely to indicate drug resistance and predict failure of therapy.

Molds

Due to their effect on cell wall synthesis in elongating hyphae, the echinocandins often do not produce a clear-cut endpoint in broth-based susceptibility tests with filamentous fungi. For this reason, a new interpretative endpoint had to be described for the activity of the echinocandin class of agents against molds. Therefore, instead of achieving an MIC, the activity marker that is looked for is the minimum effective concentration (MEC) [23]. This is a microscopic observation in which the form of growth changes from a mat of hyphae in the absence of an echinocandin, to small, discrete micro-colonies with truncated, swollen, distorted ends to the hyphae in the presence of inhibitory concentrations, which correlates with activity against 1,3-β-D-glucan synthase. Viability staining of caspofungin-treated A fumigatus reveals that the older subapical areas of the hyphae with
preformed wall material remain viable, while the apical areas of hyphae undergoing active new cell wall synthesis are killed [24]. Support for this endpoint determinant came from studies of murine coccidioidomycosis, which found caspofungin to be equally effective in treating infections with two strains that both had MECs of 0.125 mg/L but corresponding MICs of 8.0 mg/L and 64 mg/L [25].

A similar effect to that seen in broth dilution tests has been observed in disc tests of caspofungin against Aspergillus spp. Small intrazonal colonies demonstrated short and stubby branches and a star-like morphology appearing within a wider zone of inhibition [26]. If subcultures from the intrazonal colonies are re-tested, an identical pattern is observed to initial testing, thus excluding the possibility of heterogeneous resistance. Similar trailing endpoints are observed with the Etest for caspofungin against Aspergillus species. However, if background colonies within the zones are ignored, there is good correlation with MEC endpoints derived by testing in the CLSI M38-A format [27,28].

Results with strains of Aspergillus and Fusarium tested by broth dilution suggest that MEC is a stable in vitro measurement for determining activity of caspofungin against molds [29]. Results obtained by broth dilution correlate well with disc diffusion tests. Arikan et al. examined two itraconazole-resistant A fumigatus isolates and found no evidence of cross-resistance to caspofungin, a predictable finding given their distinctive modes of action [26]. Support for the MEC endpoint for assessing activity against Aspergillus spp also came from an extensive study of interlaboratory reproducibility of susceptibility testing with caspofungin [12].

Inherent resistance
In contrast to the good in vitro activity of the echinocandins against Candida and Aspergillus spp, they exhibit little activity against other yeast genera, such as Cryptococcus, Rhodotorula, and Trichosporon spp, molds of the genus Fusarium, and the zygomycetes. These demonstrate either innate resistance to this class of agents, as they possess insufficient target for inhibition, or mutated forms of the target [26,30–32].

Acquired “resistance” to echinocandins in Candida species
Following exposure of C albicans to caspofungin in vitro it is possible to select mutants that have reduced susceptibility to the drug [33]. This appears to be due to point mutations within the FKS1 gene, which encodes the polytopic membrane protein Fks1, a subunit of the target 1,3-β-D-glucan synthase enzyme complex. Mutations can be either homozygous or heterozygous as this diploid organism has two alleles of the gene. Differences in levels of resistance may be evident depending on which in vitro susceptibility method is used. Such mutants show reduced susceptibility to caspofungin treatment in a murine model of disseminated candidiasis [33].

Park et al. investigated five Candida isolates with reduced susceptibility that had been recovered from three patients who were part of a clinical trial that included caspofungin-treated patients [33]. Four C albicans isolates showed amino acid changes at Ser645 of CaFks1. These isolates similarly had reduced response to caspofungin in the murine model and further study indicated a homozgyous mutation in the FKS1 gene. Multilocus sequence typing of the two mutants and two wild-type, fully echinocandin-sensitive, isolates showed that they were genetically indistinguishable. This provides evidence to suggest that the reduced susceptibility had arisen in an initially susceptible strain. Other drug–protein interactions in the glucan synthase complex may also account for the inhibitory action of the echinocandins and could be relevant to the evolution of drug resistance.

Most of the mutations detected by Park et al. were between positions 641 and 648 of the Fks1 protein, and most common among these was the mutation in Ser645 [33]. Such Fks1 mutations have now been identified in a number of fungal species (see below) and are concentrated in two “hot spot” regions – the first spanning amino acid residues 641–649, and the second residues 1345–1365, numbered according to C albicans Fks1 [33–35]. These sequences map onto loops in the predicted protein structure that extend into the cytoplasm. One example is a C krusei strain with reduced echinocandin susceptibility that was found to harbor a substitution at Arg1361 of Fks1p [33].

Using the standard CLSI protocol with RPMI 1640, Katiyar et al. examined the Fks1 sequence of C albicans strain 20464 that had caspofungin MICs of 1 mg/L and 2 mg/L at 24 h and 48 h, respectively [34]. Sequencing of a polymerase chain reaction amplicon spanning the mutational hot-spot region revealed a homozygous mutation that altered both copies of the gene to encode tyrosine at position 641 instead of phenylalanine. In the same study, a mutation at the identical position of the Fks2 1,3-β-D-glucan synthase subunit was identified in a C glabrata isolate with reduced susceptibility. Transformation of the mutated Fks2 sequence into a susceptible C glabrata isolate rendered that isolate less susceptible to caspofungin with a two-fold increase in MIC after 24 h. In addition, residue 641 is a tyrosine in the unique Fks1 homologs of intrinsically less susceptible phytopathogenic molds: F solani [32], F graminearum, F verticillioides, Magnaporthe grisea, and in Neurospora crassa, and C guilliermondii [34]. This suggests that these point mutations may contribute to reduced echinocandin susceptibility in a wide range of fungal species.

Clinical echinocandin resistance has not yet been documented among Aspergillus species. However, an
A *fumigatus* isolate with reduced echinocandin susceptibility (16-fold higher MEC compared with wild-type cells) has been engineered in the laboratory by creating the analogous S678Y mutation in the *Afk51* gene [36]. In a parallel approach, Gardiner et al. isolated spontaneous mutants by regenerating spheroplasts in the presence of 10 mg/L of caspofungin [36]. These mutants displayed markedly reduced sensitivity to caspofungin on solid plates, the Fks1 sequence was unaltered, and both glucan synthase activity and the enzyme’s sensitivity to caspofungin were comparable with wild-type. The mechanism of reduced susceptibility was further investigated by mini-array analysis, which highlighted a number of genes whose homologues have been implicated in cell wall biosynthesis, signaling pathways, and drug transport in *Saccharomyces cerevisiae* [36].

**Genes regulated in response to caspofungin treatment**

The ATP-binding cassette transporter Cdr2p mediates azole drug resistance. Its potential role in caspofungin resistance has been investigated in *C. albicans* [37,38]. The CDR1 and *CDR2* transporter genes were upregulated in clinical isolates with reduced azole susceptibility and the same isolates displayed reduced susceptibility to caspofungin by agar sensitivity assays. Over-expressing *C. albicans* Cdr2p in *S. cerevisiae* or *C. albicans* conferred caspofungin resistance upon drug-sensitive isolates [37]. However, a later study that over-expressed CaCDR1, CaCDR2, and CaMDR1 in *S. cerevisiae* measured no significant changes in caspofungin and micafungin susceptibility by liquid microdilution assays and only slight increases in susceptibility with agar plate sensitivity assays [38].

To date, there is no firm evidence of a significant impact of azole resistance mediated by CDR pumps on echinocandin resistance in clinical isolates of *Candida* spp.

A number of genome-wide studies in *S. cerevisiae* have highlighted groups of genes that have a role in the response to caspofungin and these include genes associated with cell wall, membrane, vacuole, and transport functions, as well as cell signaling [39,40]. Over-expression of the Golgi protein Sbe2 resulted in decreased caspofungin susceptibility [41]. Furthermore, deletion of two vacuole-protein sorting genes of *C. albicans*, VPS28 and VPS32, conferred increased caspofungin susceptibility [42]. Therefore, there are a number of examples where alterations in gene expression can modify caspofungin susceptibility *in vitro*.

**The paradoxical effect of caspofungin**

Although reports of clinical isolates with reduced echinocandin susceptibility remain rare, a phenomenon termed “the paradoxical effect of caspofungin” has been observed. This is seen where caspofungin has decreased activity against *C. albicans* at concentrations well above the typical MIC [42–45]. Low concentrations of caspofungin around the MIC inhibited growth, but at supra-MIC levels (12.5 mg/L caspofungin) 16% of clinical isolates (n=24) were able to grow [43]. This phenomenon was not associated with the hot-spot mutations described above and there was no strong evidence that known azole resistance mechanisms were involved [44]. However, the paradoxical effect could not be demonstrated reproducibly in a murine model of systemic candidiasis [46]. Caspofungin was efficacious *in vivo* against three of the four clinical isolates that demonstrated *in vitro* paradoxical growth. The remaining isolate survived better at a 20 mg/kg caspofungin regimen than one at 5 mg/kg, although this could not be reproduced in subsequent experiments [46].

The ability of *C. albicans* to grow at very high concentrations of caspofungin may be due to activation of a cell wall repair mechanism. The fungal cell wall is under dynamic regulation. Compensatory mechanisms have been identified that are activated by cell wall perturbations and upregulate cell wall genes to remodel the cell wall and, thus, maintain cell integrity [47]. In many cases the output of these salvage pathways is increased chitin formation and a higher degree of cross-linking of cell wall proteins to chitin [47]. Stevens et al. were able to show that one clinical isolate that exhibited the paradoxical growth phenotype had significantly elevated cell wall chitin levels when grown in the presence of 12.5 mg/L caspofungin [48]. In addition, wild-type *C. albicans* cells have elevated chitin when treated with sub-MIC concentrations of caspofungin (Monro, Walker and Gow, unpublished). The protein kinase C (PKC) cell integrity and calcineurin signaling pathways play regulatory roles in cell wall biosynthesis and maintenance, and have recently been implicated in the regulation of chitin synthesis [49].

In *S. cerevisiae*, global approaches have implicated components of these pathways in the response to caspofungin [39,40]. Furthermore, Reinoso-Martín et al. showed that Slt2/Mkc1, the mitogen-activated protein kinase of the protein kinase C pathway, was phosphorylated in response to caspofungin; providing evidence of activation of the pathway [50]. In *C. albicans*, MKC1 mRNA levels were increased in a caspofungin dose-dependent manner and the paradoxical effect was diminished in the *mkc1/mkc1* null mutant [45]. Addition of the calcineurin pathway inhibitor, cyclosporine, also blocked paradoxical growth [45].

**Clinical reports of failure of echinocandin therapy and breakthrough fungal infections**

There is a small but increasing number of reports of failure of echinocandin therapy of *Candida* infections in different
clinical settings. Some of these cases are accompanied by in vitro susceptibility data and molecular investigation of recovered isolates (Table 1).

There are three reported cases of Candida esophagitis treatment failure or post-treatment relapse [51–53]. These were caused by C albicans where the underlying disease was HIV infection; a common feature was multiple antifungal drug treatments for relapsing or refractory esophagitis. One patient was treated with several courses of caspofungin [53], and another initially with caspofungin and later with micafungin [52]. In two of the three reports, genetic typing and MIC data showed that the original and later isolates were indistinguishable and that the strain had developed reduced susceptibility to either caspofungin or all three echinocandins. Mutations in the previously recognized hot-spot regions of the FKS1 gene were documented.

Hakki and colleagues reported on a case of C krusei bloodstream infection in a patient being treated for acute myeloid leukemia [54]. Despite 17 days of caspofungin (50 mg/day), the patient developed signs of fungal endophthalmitis, presumably due to C krusei. There was accompanying severe oropharyngeal candidiasis, which yielded a further C krusei isolate. A four-fold increase in MIC value was recorded when the original blood isolate was tested against the later throat isolate; these were shown by genetic fingerprinting to be indistinguishable from each other. Initial investigation failed to show a mutation in the FKS1 region, as might have been predicted from the earlier reports relating to C albicans. However, more recent interrogation of the resistant strain showed that it contained a heterozygous mutation in the hot-spot 1 region of FKS1.

In another report, a patient presented with candidemia due to C parapsilosis 7 months after aortic valve replacement [56]. The patient was initially treated with amphotericin B and flucytosine, and changed to caspofungin 50 mg/day plus fluconazole for 6 weeks. The clinical diagnosis was prosthetic valve endocarditis. This regimen resulted in clinical improvement and clearance of the Candida from his blood cultures. However, 3 months later, he again presented with C parapsilosis candidemia. The recovered strain from this episode was shown to have significantly elevated MICs compared with the original isolate, both to caspofungin (2 to >16 mg/L) and micafungin (8 to >16 mg/L), and interestingly also to fluconazole (1 to >64 mg/L). However, it did not show elevated MICs to anidulafungin.

**Table 1. Clinical and laboratory data on a selection of reported cases of echinocandin therapy failure.**

<table>
<thead>
<tr>
<th>Clinical setting [ref]</th>
<th>Pathogen</th>
<th>Echinocandin therapy and duration</th>
<th>Outcome and resistance data</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azole refractory esophagitis in an AIDS patient [52]</td>
<td>C albicans</td>
<td>Initially caspofungin then micafungin for total of 10 months</td>
<td>Clinical failure: Two mutations in Fks1</td>
<td>Two mutations in Fks1 MLST of pre- and post-treatment isolates confirmed a single strain was involved</td>
</tr>
<tr>
<td>Recurrent oesophagitis in an AIDS patient [53]</td>
<td>C albicans</td>
<td>Caspofungin: two courses with dose escalation</td>
<td>Clinical failure: Only one isolate tested for MIC 8 mg/L</td>
<td>Homozygous mutation in hot-spot 1 region of Fks1</td>
</tr>
<tr>
<td>Candidemia in a patient with acute and myeloid leukemia neutropenia [54,55]</td>
<td>C krusei</td>
<td>Caspofungin for 17 days</td>
<td>Developed endophthalmitis and severe oropharyngeal candidiasis while receiving caspofungin. MICs of throat isolate versus blood isolate showed ≥4-fold increase to all three echinocandins</td>
<td>Heterozygous mutation in hot-spot 1 region of Fks1</td>
</tr>
<tr>
<td>Prosthetic aortic valve endocarditis [56]</td>
<td>C parapsilosis</td>
<td>Caspofungin + fluconazole for 6 weeks</td>
<td>Subsequent relapse: Increase in caspofungin, MIC from 2 to &gt;16 mg/L; micafungin 8 to &gt;16 mg/L</td>
<td>Strain also became fluconazole-resistant</td>
</tr>
<tr>
<td>Intra-abdominal sepsis, candidemia becoming disseminated infection [59]</td>
<td>C krusei</td>
<td>Caspofungin for 15 days</td>
<td>Infection progressed</td>
<td>Pre-treatment blood isolate caspofungin MIC 2 mg/L</td>
</tr>
<tr>
<td>Candidemia in a patient following gall bladder removal [60]</td>
<td>C glabrata</td>
<td>Caspofungin for 136 days</td>
<td>Increase in caspofungin MIC from &lt;1 to &gt;8 mg/L</td>
<td>One isolate had intermediate susceptibility to amphotericin B in a mouse model (MIC&gt;1 mg/L)</td>
</tr>
</tbody>
</table>

MIC: minimum inhibitory concentration; MLST: multilocus sequence type.
In an earlier brief report, a patient suffered multiple episodes of \textit{C. albicans} prosthetic aortic valve endocarditis that was complicated at an early stage by cerebral abscesses presumed to be fungal [57]. The original isolate was highly sensitive to caspofungin. There was no evidence presented to suggest reduced susceptibility of the \textit{C. albicans}, in fact, the authors argued that failure to control the infection was due to poor penetration of caspofungin into the brain. However, it also failed to eradicate the heart valve infection, which may be because of poor penetration of the drug into vegetations on a prosthetic valve. It has been shown experimentally that caspofungin and micafungin have considerably elevated MICs against \textit{C. parapsilosis} growing in biofilms [58]. Therefore, it is worth speculating that echinocandin penetration and antifungal action in \textit{Candida} biofilms could be a factor accounting for clinical failure of therapy in some cases of prosthetic valve endocarditis. Pelletier et al. report on a case of intra-abdominal \textit{C. krusei} infection that progressed to a disseminated infection with cerebral involvement while the patient was receiving caspofungin 50 mg daily [59]. The MIC of the strain was 2 mg/L and the authors suggest that at this level, the drug may not be effective for infections in “difficult” body sites, such as the brain.

A critically ill patient with \textit{C. glabrata} fungemia was reported by Krogh-Madsen et al. to have isolates that were both amphotericin B and caspofungin resistant [60]. The patient was admitted to intensive care due to complications arising from a gall bladder operation and was administered amphotericin B on days 6–46 and caspofungin on days 9–144 (50 mg daily). A series of consecutive \textit{C. glabrata} isolates were recovered; four (numbered A–D) were selected for further investigation. Susceptibility testing by Etest indicated that all four isolates were resistant to amphotericin B (≥21 mg/L) while microdilution methods indicated that isolates A and C were susceptible to caspofungin (MIC ≤1 mg/L). However, isolates B and D were “resistant” (MIC ≥8 mg/L). An arbitrarily primed (AP) PCR study suggested the four isolates to be clonal. When three of the isolates were tested in a murine model of infection, amphotericin B was effective against isolate C, while isolate D was shown to have intermediate susceptibility. In the animal model, caspofungin was effective against isolates A and C, demonstrating good agreement with the susceptibility testing results.

Breakthrough fungal infections due to \textit{Trichosporon} spp in patients receiving echinocandin therapy have been reported from a Japanese hospital [61]. Four such cases were identified over a 3-year period among patients being treated for hematological malignancies. Although this phenomenon was reported earlier in a US bone marrow transplant recipient [62], it does not appear to be a predictable consequence of exposure to echinocandin therapy. For example, in a large study of micafungin prophylaxis for fungal infections in hematopoietic stem cell transplant recipients, there were no documented case of breakthrough trichosporonosis in the micafungin arm of the study [63].

**Conclusion**

In the short time since they became available for clinical use, the echinocandins have established themselves as valuable agents for treatment of candidiasis and aspergillosis. Most experience to date has been with caspofungin for which \textit{in vitro} susceptibility test methods have become established and been validated. These will facilitate ongoing surveillance of drug susceptibility patterns for the different echinocandins against the range of currently susceptible fungi. As yet, there are no indicative breakpoints to aid clinical decision making. However, it is encouraging that, so far, there have only been a few cases of clinical failure of echinocandin therapy, in some of which comparison between pre- and post-treatment isolates has confirmed the genetic basis of resistance. Other reasons for failure may be poor penetration into biofilms, or privileged sites such as the central nervous system.

**Disclosures**

Prof. Rogers has served on advisory boards for Gilead, MSD, Pfizer, and Schering-Plough. He has also received honoraria from Gilead, MSD, and Pfizer. Dr Johnson has served on advisory boards for, and received honoraria for participating in postgraduate educational meetings from, Gilead, MSD, Pfizer, Schering-Plough, and Zeneus, and received financial lab support from Gilead, Pfizer, and Schering-Plough.

Dr Munro has participated on Gilead advisory boards and received honoraria from Gilead for participating in educational meetings.

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CLINICAL REVIEWS
Commentary and Analysis on Recent Key Papers

Clinical reviews were prepared by J Peter Donnelly, Zeina Kanafani, and Jörg Janne Vehreschild

ASPERGILLOSIS

Pathogenesis of *Aspergillus fumigatus* and the kinetics of galactomannan in an *in vitro* model of early invasive pulmonary aspergillosis: implications for antifungal therapy
Hope WW, Kruhlak MJ, Lyman CA et al.

An *in vitro* model of the alveolus allowed the relationship between the pathogenesis of *Aspergillus fumigatus*, the immunological effectors, and antifungal drug exposure to be explored. Simulation showed that a dose of ≥0.6 mg/kg of amphotericin B was necessary to achieve sufficient drug exposure, and that the drug was only effective when combined with macrophages.

Although the detection of *Aspergillus* galactomannan is widely used for screening and diagnosis, little is known about the kinetics of early disease, the impact of immune effectors, and the influence of antifungal therapy. *Aspergillus* species readily release galactomannan during growth under optimal conditions, but there are a variety of circumstances which would lead to low antigen levels [1]. For instance, less antigen might be released from the hyphal growing tip when glucose is in short supply, since galactomannan may be consumed as a carbon source. Autolysis induced by a lack of oxygen and antifungal therapy might cause a sudden release of galactomannan, and then nothing more as the fungus dies. It is also unclear whether the hyphae have to penetrate the pulmonary capillaries before antigen is released into the circulation, as in angioinvasive disease, or whether galactomannan freely diffuses from the lung through the endothelial lining.

In order to answer these questions, Hope et al. designed an *in vitro* model of the human alveolus. The model consisted of a chamber with two compartments separated by a porous membrane with a layer of human pulmonary artery endothelial cells on the underside and human alveolar cells on the upper side. To recreate natural circumstances, serum was present in the endothelial compartment but not in the alveolar compartment. Furthermore, the alveolar cell line used in the model secreted surfactants, and the addition of macrophages increased the likeness of the model to normal lungs. The alveolar cells in the upper chamber were exposed to *Aspergillus* spores, which germinated and penetrated into the epithelial chamber 14–16 h after inoculation, which nonetheless remained sterile. This was not reflected in a neutropenic rabbit model, in which there was a long delay before galactomannan was detected in serum, and serum levels never reached those found in bronchoalveolar lavage, indicating that the airways and vasculature should be considered as separate compartments.

Exposure to ≤2 mg/L amphotericin B or macrophages failed to inhibit growth or the release of galactomannan in the chamber, whereas a combination of the two did have an effect. Monte Carlo simulation showed that at least 0.6 mg/L amphotericin B was required to achieve adequate exposure. Hence, the release of galactomannan is a reflection of the invasion of *Aspergillus* into the blood vessels. The model provided insights into not only the pathogenesis of invasive pulmonary aspergillosis but also its diagnosis, since release of galactomannan into the blood stream coincided with fungal invasion, adding support to findings in the clinical setting [2]. The target attainment rates associated with 0.6–1 mg/L amphotericin B also concur with clinical practice [3]. Given the mode of action of amphotericin B, it remains to be seen whether or not the same observations will be made when this model is used to explore other antifungal agents, such as the triazoles and echinocandins.


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Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign

An analysis of computed tomography scans of patients with invasive pulmonary aspergillosis who had participated in a randomized trial of voriconazole versus amphotericin B showed that 61% had a halo sign. This was associated with a better response and improved survival rate.

The diagnosis of invasive pulmonary fungal disease has been markedly improved with the availability of computed tomography (CT). Indeed, imaging by CT chest scan forms the cornerstone of the European Organisation for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definition of invasive pulmonary aspergillosis (IPA), which recognizes three types of imaging findings associated with invasive pulmonary fungal disease [1]:

- Halo signs.
- Air crescent signs.
- Cavitary lesion.

The utility of the halo sign as an early indicator of IPA has been contentious. In the present study, the authors re-examined this controversy, and also investigated whether or not a halo sign could indicate a better response to therapy.

CT scans were analyzed from 235 patients who had participated in the previous study by Herbrecht et al. [2], and who had been classified as having either probable or definite IPA. All of the patients had ≥1 lesion at baseline. A nodule of ≥1 cm in diameter was found in 222 patients (94%), and was surrounded by a perimeter of ground-glass opacity, thereby meeting the criteria of a halo sign, in 143 cases (61%). In contrast, a cavitary lesion was found in 48 of the patients (20%), and an air-crescent sign in 24 cases (10%). Importantly, a nodular lesion without a halo sign was found in 79 cases, indicating that this should be considered a specific sign of IPA in the neutropenic setting.

At 12 weeks, 75 of the 143 patients (52%) with a baseline halo sign responded to treatment, compared with 23 of the 79 patients (29%) with other imaging findings. Furthermore, the survival rate was higher in the patients with a halo sign compared with the patients without this CT finding (71% vs. 53%).

The authors concluded that the halo sign was not just a marker of IPA, but also indicated a better outcome, regardless of neutropenic status or underlying condition.

In an accompanying commentary, Vandewoude and Vogelaers agreed that the findings confirmed the utility of the halo sign. However, they observed that this was to be expected, given the fact that the halo sign is a key EORTC/MSG criterion for defining IPA [3]. Moreover, recipients of allogeneic hematopoietic stem cell transplants who developed a halo sign were diagnosed as having probable IPA in the present study. That said, it is clear that the presence of a nodular or cavitary lesion on the pulmonary CT scan of neutropenic patients provides a very helpful clue to the diagnosis of IPA [3]. Indeed, the revised EORTC/MSG definitions of invasive fungal disease have placed imaging center-stage in defining associated clinical features [4].

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Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial

This study evaluated the efficacy of posaconazole versus conventional salvage therapy (primarily amphotericin B or itraconazole) for invasive aspergillosis refractory to first-line treatment. The response rate was higher in the posaconazole group than in the control group (42% vs. 26%). Posaconazole also appeared to provide a survival benefit.

Invasive mold infections are a major concern in immunocompromised patients. In addition to difficulties in making a definitive diagnosis and determining susceptibility to antifungal agents, clinicians are faced with significant therapeutic challenges. Success rates for the initial treatment of invasive aspergillosis usually range from 40–60% [1]. Patients who are refractory to first-line treatment regimens, thereby requiring salvage therapy, often have lower response rates.

Compared with amphotericin B, posaconazole exhibits more potent activity against Aspergillus spp and better in vitro activity than voriconazole and itraconazole against Aspergillus fumigatus [2]. Recently published trials have demonstrated that posaconazole is useful in preventing invasive fungal infections in neutropenic patients [3] and in patients...
with graft-versus-host disease [4]. Its role in the treatment of documented mold infections is still to be determined.

The current open-label, multicenter study included 193 patients with invasive aspergillosis who were refractory to or intolerant of first-line treatment. Patients received either posaconazole (n=107; 800 mg/day) or conventional salvage therapy (n=86; primarily amphotericin B or itraconazole) for up to 372 days. At the end of therapy, the response rate was higher in the posaconazole group than the control group (42% vs. 26%, odds ratio 4.06, 95% confidence interval 1.50–11.04; p=0.006) as was the cumulative survival rate (38% vs. 22%; p=0.0003 for difference between Kaplan–Meier survival curves).

The authors acknowledge that the most reliable data are generated from randomized controlled trials. However, they were able to address several potential sources of bias by carefully selecting an external control group and having a blinded assessment of outcome for all patients by a panel of experts. Although the current study design does not allow testing for superiority, posaconazole appears to be more efficacious than conventional therapy with amphotericin B and itraconazole. Whether similarly promising results would be achieved if posaconazole was compared with the more modern therapeutic agents, such as voriconazole or caspofungin, remains to be determined.


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Invasive pulmonary aspergillosis in patients with decompensated cirrhosis: case series

Prodanovic H, Cracco C, Massard J et al.


These authors argue that cases of “probable” aspergillosis preceded by liver cirrhosis can be assumed based on clinical features and mycological findings alone. However, since none of the cases described by the authors of the present paper had host factors that met the criteria of the European Organisation for Research and Treatment of Cancer/Mycoses Study Group definitions, there is a clear need to define them for non-neutropenic patients in the intensive care unit.

Invasive fungal diseases are no longer confined to patients considered to be severely immunosuppressed, such as those being treated for hematological malignancies and recipients of hematopoietic stem cell and organ transplants. Furthermore, the boundaries between invasive disease and local infection are not always clear. This situation is illustrated by the three cases described by the authors of the present paper.

All three patients suffered from liver failure, developed sepsis requiring admission to the intensive care unit (ICU), and were given steroid treatment (for a relatively short time).

In addition, all three had some kind of pulmonary damage, and bronchoalveolar lavage detected *Aspergillus* in two of the cases and galactomannan in one case. Computed tomography scans were not done for practical reasons and, although the patients could not be considered to be neutropenic, all three were lymphopenic to some degree. Therefore, due to the absence of host factors, all three cases could be interpreted as having “probable” aspergillosis on the basis of clinical features and mycological evidence alone. All three patients died, despite treatment with voriconazole, probably because the disease had advanced too far for the therapy to be effective. Unfortunately, autopsies were not undertaken.

The authors highlight the fact that the current European Organisation for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions only apply to patients being treated for cancer and recipients of a hematopoietic stem cell transplant [1]. However, this has been addressed by a Consensus Group of the EORTC/MSG and led to revised definitions that will extend their scope to other patient populations [2]. Notably, patients in the ICU were excluded because appropriate host factors have yet to be defined. The authors of the current article argue that decompensated liver cirrhosis may be considered a host factor of invasive aspergillosis, although they did acknowledge that this factor must be put into perspective by other potential risk factors. They also address the need for diagnosis, including obtaining samples for mycological tests using bronchoscopy, and suggest that imaging may contribute to diagnosis. However, the question remains as to which form or forms of aspergillosis the cases described in the article represent. There was no apparent evidence for invasive disease in any of the three patients [3], although evidence that *Aspergillus* was involved in an infective process in some way is compelling. Clearly, there is much research to be done in this area, and the authors have provided food for thought.


Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality


This article provides an analysis of the outcomes of stem cell transplant recipients with proven or probable invasive aspergillosis (IA). Rates for all-cause mortality and mortality attributable to IA showed a consistent and significant decrease over the duration of the study. Voriconazole therapy was associated with fewer IA-related deaths.

Until recently, studies have reported a trend of rising incidence of invasive aspergillosis (IA) among stem cell transplant recipients. The increasing use of voriconazole, among other factors, has prompted a shift in the epidemiology of invasive fungal infections towards other filamentous mold infections such as zygomycosis and scedosporiosis [1]. However, IA remains a serious infection that is associated with high rates of morbidity and mortality. In the present article, Upton and colleagues studied IA-related mortality in a large cohort of stem cell transplant recipients with proven or probable IA diagnosed between 1 January 1990 and 31 December 2004.

The incidence of IA appeared to have decreased over the last 3 years of the study period (from 23.5% in 1999–2001 to 13.6% in 2002–2004), which is commensurate with findings in the published literature. No information is provided on the incidence of other mold infections, as patients who had mixed infections were excluded from the analysis. The results demonstrated that mortality due to IA decreased and that the probability of survival, which is inversely proportional to all-cause mortality, improved over the study period. These are very important findings that reflect the significant advances in diagnostic and therapeutic measures in patients at high risk of invasive fungal infections.

The large number of patients enrolled in the study and the prospective nature of the database are major strengths of this analysis and increase the reliability of the data provided by the authors. It would have been interesting to study the incidence of and mortality associated with cytomegalovirus (CMV) disease in this cohort of patients with IA. The immunomodulatory properties of CMV alter the host’s susceptibility to invasive fungal infections and may have influenced the response to therapy and mortality rate seen in these patients.


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Detection of Coccidioides species in clinical specimens by real-time PCR


This article reports on a real-time polymerase chain reaction method for detection of Coccidioides spp in respiratory specimens, fresh tissue, and paraffin-embedded tissue samples. The method showed a high sensitivity and specificity in fresh clinical specimens, and the authors conclude that it may be used as a means of rapid diagnosis, obviating the need to grow the organism in culture.

Coccidioides spp are fungi endemic to certain parts of North and South America. They are known to cause infections of the upper respiratory tract that may progress to disseminated disease. Diagnosis includes serological tests, culture, and histopathology. However, serological tests may fail to identify these species at early stages of disease and in immunocompromised patients, and it may take several days to grow organisms in culture.

Real-time polymerase chain reaction (PCR) is a rapid means of detecting numerous pathogens in a variety of clinical samples. False-positive findings caused by cross-reactivity and contamination are a common drawback of PCR techniques. Binnicker et al. used the ITS2 region of Coccidioides for amplification via LightCycler PCR (Roche Applied Sciences, Indianapolis, IN, USA). They conducted a number of tests for evaluation. Forty culture samples of Coccidioides spp were correctly identified by PCR. A total of 266 respiratory specimens were examined by culture and PCR. There were no false-negative findings with the PCR, but four samples showed positive results that did not show growth in culture. Still, in three of these four cases, the patients later developed signs and symptoms of coccidioidomycosis. The authors believe that this might indicate a higher sensitivity of the PCR compared with the culture. Analysis of 66 fresh tissue specimens resulted in one false-positive and one false-negative result. Sensitivity was lower (73.4%) when using paraffin-embedded tissue (n=148). No difference was seen between Coccidioides immitis and C posadasii.

In summary, Binnicker et al. present a very promising new means of diagnosing coccidioidomycosis. Future studies will need to demonstrate that these good results can be obtained in other centers and regions, where other contaminants and cross-reactions may be involved.

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**CANDIDIASIS**

**Posaconazole for the treatment of azole-refractory oropharyngeal and esophageal candidiasis in subjects with HIV infection**

Skiest DJ, Vazquez JA, Anstead GM et al.


This study compared two doses of posaconazole for the treatment of HIV patients with azole-refractory oropharyngeal and/or esophageal candidiasis. The overall response rates were similar for the two regimens. Posaconazole was well tolerated and was effective against *Candida* isolates that were resistant to fluconazole, itraconazole, or both.

Oropharyngeal and esophageal candidiasis is a common AIDS-defining illness in patients with HIV infection; >90% of patients with HIV will eventually develop oropharyngeal candidiasis. Several studies have shown a decrease in the incidence of esophageal candidiasis since the advent of highly active antiretroviral therapy. According to EuroSIDA, a pan-European longitudinal, prospective observational study, there was a 32% decrease in the incidence of esophageal candidiasis.

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**Coccidioides immitis fungemia: clinical features and survival in 33 adult patients**

Rempe S, Sachdev MS, Bhakta R et al.

*Heart Lung 2007;36:64–71.*

Acute respiratory distress syndrome developed in 25 (76%) of 33 cases of fungemia caused by *Coccidioides immitis*; 22 (66%) died in hospital (median survival 7 days), and only 15 (45%) had received antifungal therapy during the index admission. The prognosis for fungemia due to *C immitis* was dismal.

Coccidioidomycosis is an invasive fungal disease caused by the dimorphic fungus *Coccidioides immitis*. The fungus lives in soil in the arid areas of the Southwestern US, northern Mexico, and the upper part of Central America, where the disease is endemic. Infection is contracted by inhalation of the arthroconidia. Coccidioidomycosis mainly affects otherwise-healthy immunocompetent hosts, presents as a pulmonary disease, and is usually self-limiting. However, when the disease develops in immunocompromised patients, such as patients with AIDS and organ transplant recipients, it can disseminate via the blood stream to other organs and systems, including the skin, bones, joints, lymph nodes, adrenal glands, and central nervous system, and can prove fatal.

In this study, 33 patients with fungemia due to *C immitis* were seen between 1990 and 2002 in a 550-bed hospital in Phoenix, AZ, USA. All but four patients had HIV infection and had not received adequate antiretroviral therapy. The CD4+ lymphocyte counts of 17 patients were <100 cells/mm$^3$. Diffuse pulmonary infiltrates indicating acute respiratory distress syndrome were seen in 25 cases (76%). Six patients had evidence of disseminated disease. Survival rates were dismal: 22 patients (67%) died within a median of 1 week, two patients (6%) were lost to follow-up, and only seven patients (21%) survived >28 days. Only 15 patients (45%) had received antifungal therapy (six received fluconazole, three received amphotericin B, and six received both).

The authors were surprised at the low incidence of fungemia due to *C immitis* in the study center (only 33 adults between 1990 and 2002), as this was already an AIDS-defining disease during the period of study. However, this observation may have been related to the number of patients with low CD4+ lymphocyte counts (indicative of active HIV infection) analyzed in the study. Of the 29 patients with concurrent coccidioidomycosis and HIV, 23 had CD4+ counts of <200 cells/mm$^3$, and the authors speculate that *C immitis* fungemia is associated with marked immunosuppression in patients with HIV. It is also surprising that the mortality rate was >2-fold greater than that associated with patients with bacteremia and HIV infection.

However, these results are not that much different from those reported by Ampel et al. 20 years ago, before the advent of highly active antiretroviral therapy [1]. The high mortality rate may simply be due to the protracted delay in detecting fungemia, as it can take up to 4 days to detect infection when the number of circulating colony forming units is low [2]. Notably, the high death rate was barely influenced by the measures taken to combat septic shock, which reinforces the importance of early detection since outcome is determined by the duration of illness and by the extent of pulmonary involvement before diagnosis [3]. The authors suggest that antifungal therapy, given empirically, might be the best option for a good outcome when fungemia due to *C immitis* is suspected. The best therapeutic choices include amphotericin B, fluconazole, and itraconazole, although voriconazole, caspofungin, or micafungin therapy may be of benefit [4].

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**References**


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candidiasis between 1995 and 2004 [1]. On the other hand, infections caused by non-albicans Candida spp are now more common. Such infections are more difficult to eradicate with azole compounds and have a higher recurrence rate than those caused by C albicans [2].

In the present study, Skiest and colleagues evaluated the efficacy of posaconazole for the treatment of 176 HIV patients with oropharyngeal and/or esophageal candidiasis who had failed to respond to fluconazole or itraconazole therapy. Posaconazole was administered in one of two dosing regimens: 400 mg twice daily for 28 days, or 400 mg twice daily for 3 days followed by 400 mg once daily for 25 days. Response rates of 75.3% and 74.7% were seen in the low-dose and high-dose groups, respectively.

This study suggests that posaconazole is useful for treating azole-resistant oropharyngeal and esophageal candidiasis. In a previous trial, posaconazole was found to be as effective as fluconazole for azole-susceptible oropharyngeal candidiasis [3]. Patients who received posaconazole were less likely to have recurrence of infection than those who received fluconazole. Future investigations comparing posaconazole with echinocandins or amphotericin B for azole-refractory oropharyngeal and esophageal candidiasis are needed to confirm the important findings of this study.

The authors of this study related the outcomes of 77 patients treated with fluconazole for candidemia to the susceptibility of the Candida species involved to the drug. A mean fluconazole dose/minimum inhibitory concentration (MIC) ratio of 13.3 and an area under curve (AUC)/MIC ratio of 775 were associated with survival, whereas a mean dose/MIC ratio of 7.0 and an AUC/MIC ratio of 589 were associated with a fatal outcome.

Until recently, fluconazole was considered the drug of choice for treating candidemia among non-neutropenic patients. The dose can also be increased to treat infections caused by less susceptible strains (e.g. Candida glabrata). The availability of drugs such as voriconazole [1] and the echinocandins [2] has provided alternative treatment options, although fluconazole therapy is still a reasonable option for susceptible Candida species.

In the present study, the authors investigated the relationship between the pharmacokinetics of fluconazole and the minimum inhibitory concentration (MIC) for the infecting strain. They observed patients who had been treated with fluconazole for candidemia between 2002 and 2005. Data were collected on risk factors, demographics, dose, and duration of fluconazole treatment, and outcome at discharge. Strains were available for 77 episodes of candidemia, and these were analyzed in depth using classification and regression tree analysis. MICs were determined using the Clinical Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) M27 A2 reference method.

Fluconazole treatment at doses of 150–800 mg/day was started at the onset of symptoms for 61 patients (79%), and the remainder were treated within 48 h of onset. C albicans accounted for 49 of the isolates (64%), C glabrata for 11 (14%), and C parapsilosis, C tropicalis, and C lusitaniae for most of the rest. A mean dose/MIC ratio of 13.3 (standard deviation [SD] 10.5) and an area under curve (AUC)/MIC ratio of 775 (SD 739) were associated with survival, whereas a mean dose/MIC ratio of 7.0 (SD 8.0) and an AUC/MIC ratio of 589 (SD 715) were associated with a fatal outcome.

These data confirm the relationship between dose and ratio of AUC to MIC, although the dose/MIC ratio presented in this article is only half that suggested by Pfaller et al. [3], and may reflect the low number of resistant isolates that were found in this study population. The results of this study also support the notion that increasing the dose may lead to a better outcome when dealing with resistant strains. However, it may be prudent to opt for an alternative drug when the offending isolate is resistant to >8 mg/L fluconazole.

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Candidemia in patients with ventricular assist devices
Shoham S, Shaffer R, Sweet L et al.

This case–control study describes the characteristics and outcomes of seven patients with ventricular assist devices who developed device-related candidemia. The median time from device implantation to infection was 25 days. In two patients, the infection was cured with antifungal therapy with device retention. The in-hospital mortality rate was 71% (five of seven patients).

In the REMATCH (Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure) trial, long-term mechanical left ventricular assistance was associated with a relative risk of death of 0.52 compared with medical therapy alone [1]. Hence, ventricular assist devices (VADs) will be increasingly used in patients with end-stage congestive heart failure who are not candidates for cardiac transplantation, and this will probably be accompanied by an increase in cardiac device infections. In the REMATCH trial, the infection rate over the 3 months after device implantation was 28%. Fatal sepsis in infected patients was common.

In this case–control study, the authors describe the characteristics and outcome of the seven out of 117 VAD recipients in their database (covering 1998–2004) who developed candidemia. The median time from VAD placement to the onset of candidemia was 25 days. Five cases were due to Candida parapsilosis, and two were due to C albicans. Five patients died in hospital, four from fungal sepsis. The infection was resolved with antifungal therapy in three patients, two of whom retained the device.

Up to 35% of cultures from infected VADs grow fungal pathogens, the most common of which are Candida spp [2]. The routine administration of prophylactic antibacterial agents in patients with VADs has probably contributed to the emergence of fungal infections. In the setting of cardiac device-related bloodstream infections, fungi are more likely to result in a fatal outcome than Gram-negative or Gram-positive organisms [3].

The ability of Candida to form biofilms is an important characteristic that allows the organism to colonize and infect VADs [4]. Biofilm formation also accounts for the difficulty in eradicating device infections caused by Candida spp. Extended antifungal prophylaxis for all VAD recipients is not cost-effective [5]. Future studies are needed to determine whether restricting prophylaxis to patients at high risk of Candida infection is beneficial.


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Caspofungin in the treatment of symptomatic candiduria
Sobel JD, Bradshaw SK, Lipka CJ et al.

In this case series, the authors describe the clinical characteristics and outcome of six patients with upper urinary tract Candida spp infection who were successfully treated with caspofungin. Although only low levels of caspofungin are attained in the urine, kidney tissue concentrations appear to be favorable.

Candiduria is a common occurrence in the hospital setting among patients with indwelling urinary catheters. The incidence of clinically significant candiduria has recently increased, which probably reflects a greater use of these catheters and a more widespread use of broad-spectrum antibacterial agents [1]. In this retrospective study, Sobel and colleagues describe six cases of symptomatic candiduria that had failed to respond to amphotericin B but were successfully treated with caspofungin. In three of the six cases candiduria was secondary to renal candidiasis, and in the remaining three it reflected invasive, complicated, ascending Candida glabrata infection.

The principal route for the elimination of caspofungin is via the hepatic pathway. Less than 3% of the drug is excreted unchanged in the urine [2]. Hence, caspofungin is not indicated for the primary treatment of genitourinary infections. The current findings are therefore interesting in that they show a potential role for caspofungin in the treatment of candiduria despite unfavorable pharmacokinetics. The authors postulate that, in upper urinary tract infections with Candida spp, serum and tissue concentrations of antifungal agents are more important than urinary concentrations. This theory is supported by a pharmacokinetic study that was performed in healthy individuals [3]. Following a single intravenous dose of caspofungin (70 mg), only 1.4% of the active drug was excreted in the urine. However, when assessing tissue distribution, the concentrations achieved in the kidney were second only to liver concentrations. Kidney tissue concentrations reached a peak at 24 h after drug administration, and the drug was still detectable in the kidney at 288 h after infusion. Coupled with the clinical data provided in this study, these results suggest that caspofungin


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might constitute a viable therapeutic option in the setting of refractory upper urinary tract infections caused by Candida spp. Further studies are needed to confirm these important findings.


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OTHER MYCOSES

Discontinuation of secondary prophylaxis against penicilliosis marneffei in AIDS patients after HAART


This retrospective cohort study investigated the relapse rate of disseminated Penicillium marneffei infection in 33 HIV-infected patients. All patients had previously received long-term prophylaxis with itraconazole and responded well to highly active antiretroviral therapy. No relapse occurred over the median follow-up period of 18 months. These findings suggest that discontinuation of secondary antifungal prophylaxis may be safe in this patient group.

Disseminated infection by Penicillium marneffei is a frequent AIDS-defining opportunistic infection in Southeast Asian HIV-infected patients. The high rate of relapse within 6 months after initial treatment necessitates long-term secondary prophylaxis. However, the duration of secondary prophylaxis needed has yet to be established. The risk of contracting penicilliosis or another opportunistic infection depends on the CD4+ cell count of the patient. As studies on several other opportunistic diseases have shown that prophylaxis may safely be discontinued after initiation of highly active antiretroviral therapy (HAART) and the replenishment of CD4+ cells to counts greater than those associated with a high risk of the development of infection, the authors investigated if the same was true for P marneffei secondary prophylaxis.

This retrospective cohort study included patients with documented P marneffei infection who discontinued secondary prophylaxis after responding to HAART. Review of patient records showed that none of 33 patients relapsed during the median follow-up period of 18 months after discontinuation of secondary prophylaxis. An earlier investigation on the efficacy of itraconazole prophylaxis from the same institution had demonstrated a markedly higher relapse rate in the pre-HAART era [1].

While the present study demonstrates the high safety level associated with discontinuing secondary prophylaxis in the reported cohort, several shortcomings should be noted. Most patients received secondary prophylaxis and HAART for a relatively long time (median 25 months and 13 months, respectively) before discontinuation of itraconazole, which may have confounded the results. As the study was single-centered, changes in local epidemiology and standard of care may have contributed to the results. In addition, the authors discuss the lack of a contemporary control group in their study. Hence, well-designed prospective trials are needed before a recommendation on the length of secondary antifungal prophylaxis after P marneffei infection can be issued.


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Outbreak of keratomycosis attributable to Fusarium solani in the French West Indies


This letter reports on a series of 10 contact lens wearers using Bausch and Lomb (Rochester, NY, USA) ReNu with MoistureLoc contact lens solution who developed corneal abscesses. Corneal scrapings revealed infection by Fusarium solani in five cases and Pseudomonas aeruginosa in four cases. Other outbreaks of keratomycosis in contact lens wearers during the same time period have previously been reported from the US, Singapore, and Hong Kong.

This article reports on a series of 14 patients with corneal abscesses who attended the University Hospital Center of Fort de France (Fort de France, Martinique, French West Indies) between November 2005 and May 2006. Contact lenses were used by 12 of the patients, and 10 of these used Bausch and Lomb (Rochester, NY, USA) ReNu with MoistureLoc contact lens solution (ReNu ML). Corneal scrapings revealed Fusarium solani as the causative pathogen in four patients, Pseudomonas aeruginosa in three patients, and one co-infection by F solani and P aeruginosa. No pathogen was identified in the other patients. All five patients with keratomycosis used ReNu ML and were treated with either topical amphotericin B (three patients, two of whom also received systemic amphotericin B) or voriconazole (two patients; topical and systemic treatment).
One patient with corneal injury by vegetative matter needed penetrating keratoplasty; the others responded to treatment and made a clinical recovery.

In summary, this article reports a case series of fungal keratitis by *Fusarium* spp very probably related to the use of ReNu ML. Other articles have reported similar outbreaks in the US, Singapore, and Hong Kong [1–3]. Most patients showed poor compliance with lens care regimens (overnight wear, reuse of solution, and poor hygiene); ReNu ML shows reduced activity against *Fusarium* spp under these conditions [4–6].

2. Chang DC, Grant GB, O’Donnell K et al. Multistate outbreak of Fusarium keratitis associated with use of a contact lens solution. JAMA 2006;296:593–63.

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Molecular characterization, biofilm analysis and experimental biofouling study of *Fusarium* isolates from recent cases of fungal keratitis in New York State

Dyavaiah M, Ramani R, Chu DS et al.


In the aftermath of reports of fungal keratitis probably connected to use of Bausch and Lomb (Rochester NY, USA) ReNu with MoistureLoc contact lens solution (ReNu ML), Dyavaiah at al. performed a set of analyses on six fungal isolates. Their studies show that ReNu ML effectively kills the infecting fungi and prevents biofilm development when used according to the manufacturer’s directions. However, as the fungi were not killed by short-term exposure to ReNu ML (1 h), the authors conclude that improper lens cleaning regimens may have contributed to the actual infection.

Although the outbreak of fungal keratitis observed between 2005 and 2006 seems clearly connected to the use of Bausch and Lomb (Rochester, NY, USA) ReNu with MoistureLoc contact lens solution (ReNu ML), the actual cause remains elusive. Dyavaiah et al. performed a detailed molecular characterization of six *Fusarium* isolates (three from corneal ulcers, one from an eye, one from a contact lens case, and one from an opened bottle of ReNu ML) obtained from five patients, and showed identical *Fusarium* strains in the contact lens solution and case supplied by a single patient. Unfortunately, no *Fusarium* isolate was recovered from the cornea of that patient. The other strains investigated were distinct from each another, thus undermining the hypothesis that a batch of ReNu ML was contaminated by clonal strains, and confirming the results of earlier studies. Although the authors were able to simulate biofilm formation on contact lenses, this was successfully prevented by proper use of ReNu ML. Antifungal activity was not reduced in ReNu ML solution from containers opened 3 months earlier and stored at room temperature. However, incomplete fungal killing was demonstrated 1 h after inoculation of ReNu ML with *Fusarium* cells. Complete sterilization was only achieved by 4 h of treatment, the duration recommended by the manufacturer.

The authors believe that this finding might have played a role in development of fungal keratitis. However, this delayed effect has already been demonstrated for other contact lens solutions [1], thus incompletely explaining the increased risk of contracting fungal keratitis connected with using ReNu ML.

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Contemporary treatment and outcomes of zygomycosis in a non-oncologic tertiary care center

Sims CR, Ostrosky-Zeichner L.


The authors describe a series of 16 patients without hematological malignancies who were diagnosed with zygomycosis. The most common underlying disease was diabetes mellitus, which was present in seven patients. The mortality rates were higher in patients who did not undergo surgical debridement as part of the treatment approach. The overall mortality rate was 25%, which is lower than in previous reports.

In the present article, Sims and Ostrosky-Zeichner retrospectively assessed the treatment and outcomes of 16 episodes of zygomycosis in a group of 15 non-cancer patients (one patient has recurring fungal peritonitis). In seven of the patients, infection was associated with diabetes mellitus. The overall mortality rate was 25% (four out of 16) and was lower among the group who underwent surgery (17%; two out of 12). These low mortality rates are noteworthy. In their review of 929 cases of zygomycosis, Roden and colleagues quoted a mortality rate of 44% in diabetic patients [1].
Furthermore, the mortality rate among solid organ transplant recipients has been estimated at 49% [2].

The lower mortality rate reported in the current article can be potentially attributed to several factors. The number of patients evaluated in this analysis was low, which makes it difficult to draw reliable inferences about mortality rates. In addition, the authors do not mention the duration of follow-up. Only one patient in this series experienced disease recurrence. A longer follow-up time would probably have yielded a higher disease recurrence rate, especially in patients who remain on heavy immunosuppressive therapy, or in those with diabetes who continue to have poor glycemic control. Of the 15 described cases, eight had an underlying diagnosis of trauma or vascular disease. These patients appear to be in a relatively lower immunosuppressive state than organ transplant recipients or patients with diabetic ketoacidosis. Also, the authors do not provide a clear estimation of the severity of underlying illness (i.e. glycemic control in the patients with diabetes, duration and dose of corticosteroid therapy, and immunosuppressive therapy in the renal transplant recipients). Therefore, further studies involving a larger number of patients are necessary to better estimate the outcomes of subjects with zygomycosis but not cancer.

It would also be interesting to see the effect of the introduction of new antifungal agents on the rate of zygomycosis-associated mortality.

Histoplasma capsulatum α-(1,3)-glucan blocks innate immune recognition by the β-glucan receptor

Rapleye CA, Eissenberg LG, Goldman WE. Proc Natl Acad Sci USA 2007;104:1366–70.

The authors of this article describe a potential mechanism by which the pathogenic fungus Histoplasma capsulatum may evade recognition by the host immune system. They demonstrated that, although β-glucans (immunostimulatory cell wall components) remain conserved in this species, a less common polysaccharide, α-(1,3)-glucan is present in the outermost cell wall layer. Therefore, the authors suggest that α-(1,3)-glucan may contribute to pathogenesis in H capsulatum and other fungi by concealing β-glucan from detection by host phagocytes.

Histoplasma capsulatum is inhaled as hyphae and conidia, which transform into budding yeast cells on exposure to human body temperature. These cells then infiltrate macrophages and monocytes, where they proliferate. In most cases, the infection is self-limiting, remains confined to the lung, and causes a flu-like illness. However, dissemination occasionally occurs, especially in immunosuppressed patients [1]. How Histoplasma evades detection is poorly understood, although α-(1,3)-glucan may play a role in virulence. This polysaccharide forms part of the cell wall of H capsulatum and other medically important fungi. Wild-type isolates of H capsulatum display “rough” colonies on agar and possess α-(1,3)-glucan, whereas “smooth” variants are deficient in this component. The smooth variants are also less virulent in mice than rough types.

Through a series of experiments, the authors of the present paper showed that α-(1,3)-glucan was exclusive to the yeast form of H capsulatum, and was produced during germination. In addition, they demonstrated that the cell wall was probably comprised of layers, with α-(1,3)-glucan being more external than β-glucan. The latter is a pathogen-associated molecule that is recognized by the dectin-1 pattern-recognition receptor found on human macrophages. While yeast cells deficient in α-(1,3)-glucan bound to fibroblasts expressing dectin-1, those containing the polysaccharide did not. Furthermore, there was no adhesion between yeast cells and fibroblasts deficient in dectin-1, confirming that dectin-1 was the pattern-recognition receptor for α-(1,3)-glucan.

Next, the investigators exposed alveolar macrophages to wild-type Histoplasma and found they released only low levels of tumor necrosis factor-α (TNF-α), whereas the variant deficient in α-(1,3)-glucan induced high levels of expression. The authors also showed that cells deficient in dectin-1 produced lower levels of TNF-α after exposure to yeasts deficient in α-(1,3)-glucan. Therefore, TNF-α production was either suppressed by α-(1,3)-glucan directly or through the lack of dectin-1.

In conclusion, the authors propose that α-(1,3)-glucan contributes to pathogenesis by concealing the presence of β-glucan, which prevents it from being recognized by the host. If this is true, it explains the pathogenicity of H capsulatum, and also that of the other agents of endemic mycosis. Moreover, this may explain why Candida albicans is an opportunistic pathogen, rather than a professional pathogen, as this species lacks α-(1,3)-glucan, is recognized by dectin-1 [2], and induces substantial production of TNF-α by macrophages [3].

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Successful treatment of resistant ocular fusariosis with posaconazole (SCH-56592)
Tu EY, McCartney DL, Beatty RF.

This report describes three patients with Fusarium keratitis who had failed to respond to antifungal therapy including voriconazole. The infection was associated with contact lens use in two patients. All three patients exhibited a good response to oral posaconazole without evidence of recurrence after end of treatment.

Fusarium spp are an important cause of fungal keratitis. Geographical variations in the incidence of Fusarium keratitis have been reported, with more infections occurring in tropical and subtropical climates [1]. Since Fusarium spp are commonly isolated from soil, water, and plants, agricultural workers are at particularly high risk of infection following traumatic eye injury. In addition, several outbreaks of Fusarium keratitis have recently been reported in contact lens wearers [2]. In many patients, the infection has been linked to the use of the lens solution ReNu with MoistureLoc (Bausch and Lomb, Rochester, NY, USA).

In this case series, the authors describe the characteristics and outcomes of three patients who were diagnosed with ocular fusariosis and who received oral posaconazole after their infections had failed to respond to conventional antifungal and surgical therapy. All three patients had keratitis, two of them wore contact lenses, and the infection progressed to endophthalmitis in two patients. Treatment with posaconazole resolved the infection in all three patients.

Ocular fusariosis often develops into an intractable infection. However, in up to 16% of cases of Fusarium keratitis, infection can be resolved with topical antibacterial agents alone [3]. More invasive infections require topical and/or systemic antifungal therapy. Therapeutic options include natamycin, itraconazole, amphotericin B, and voriconazole, but treatment failure is not uncommon. In a recent study, topical ceftazidime appeared to be safe and effective in the treatment of keratitis caused by Fusarium solani [4]. Further trials are needed to confirm these findings.

In the present series, one of the patients who responded to posaconazole therapy had an isolate that was resistant to the drug. This illustrates the fact that in vitro resistance does not always correlate with clinical efficacy. Therefore, in treatment-refractory ocular fusariosis, posaconazole can still be a viable therapeutic option even if in vitro susceptibility testing yields high posaconazole minimal inhibitory concentrations.

2. Chang DC, Grant GB, O’Donnell K et al. Multistate outbreak of Fusarium keratitis associated with use of a contact lens solution. JAMA 2006;296:593–63.

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THERAPEUTICS

Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease
Ullmann AJ, Lipton JH, Vesole DH et al.

Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia
Comely OA, Maertens J, Winston DJ et al.

Prophylaxis and aspergillosis – has the principle been proven?
De Pauw BE, Donnelly JP.

In January 2007, two landmark trials on antifungal prophylaxis with posaconazole were published. Ullmann et al. reported on the prophylactic use of posaconazole or fluconazole in patients receiving immunosuppression for graft-versus-host disease after undergoing allogeneic stem cell transplantation. A significant reduction in the incidence of invasive aspergillosis and death attributable to fungal infection in the posaconazole group was shown. A trend towards fewer overall fungal infections was also seen. The other trial, by Comely et al., investigated the prophylactic effect of posaconazole versus fluconazole or itraconazole in patients with long-term neutropenia following remission-induction chemotherapy for acute myelogenous leukemia. Here, posaconazole was significantly superior in preventing invasive fungal infections, reduced the rates of overall mortality and mortality attributable to fungal infections. In an Editorial by De Pauw and Donnelly, the question is raised whether prophylaxis should be the treatment of choice.

Invasive fungal infections (IFIs) are a major cause of morbidity and mortality in patients with hematological diseases. Infection rates are especially high in patients with long-term neutropenia [1], and in those receiving immunosuppression after undergoing allogeneic stem cell transplantation [2]. Numerous studies have tried to prove the efficacy of antifungal prophylaxis for patients with neutropenia, but they have failed to show a marked advantage compared with placebo [3–6]. Fluconazole has been shown to significantly reduce morbidity and mortality rates in patients after hematopoietic stem cell transplantation [7,8]. Posaconazole is a new azole antifungal with a broad spectrum of activity, including most clinically
important yeasts and molds [9]. It has a favorable toxicity profile, and the oral solution is well absorbed.

The study by Ullmann et al. compared the efficacy of antifungal prophylaxis using posaconazole (n=304) or fluconazole (n=298) in patients receiving immunosuppressive therapy for graft-versus-host disease (GVHD). Patients were treated for a fixed 112-day period with either posaconazole or fluconazole. Stratification was performed according to GVHD status. Both groups were well matched according to demographics, underlying disease status, and risk factors.

There were 16 (5.3%) and 27 (9.0%) patients with proven or probable IFI in the posaconazole and fluconazole groups, respectively (p=0.07). Invasive aspergillosis occurred in seven (2.3%) and 21 (7.0%) patients in the respective groups (p=0.006), and mortality rates were 25.2% and 28.1%. Fatal fungal infections occurred in two (0.6%) patients in the posaconazole group and 11 (3.7%) patients in the fluconazole group (p=0.01). Despite the good efficacy of posaconazole in preventing fungal infections, Ullmann et al. were able to demonstrate a marked advantage of using an antifungal drug with activity against molds for prophylaxis.

The other study, conducted by Cornely et al., compared the efficacy of antifungal prophylaxis with posaconazole (n=304) versus fluconazole or itraconazole (n=298) in patients receiving remission-induction chemotherapy for acute myelogenous leukemia. Because of differing local practices, participating centers were allowed to choose between fluconazole and itraconazole as the comparator drug. Patients were treated until complete remission of neutropenia, until the occurrence of an IFI, or up to 12 weeks from randomization to open-label antifungal treatment, whichever came earliest. Again, both groups were well matched. IFIs occurred in seven (2.3%) and 25 (8.4%) patients in the posaconazole and comparator groups, respectively (p<0.001). Of these, two (0.7%) and 20 (6.7%) cases, respectively, were due to Aspergillus spp (p<0.001). The mortality rate was 16.1% in the posaconazole group and 22.5% in the comparator group (p=0.048). Five (1.6%) deaths in the posaconazole group and 16 (5.4%) deaths in the comparator group were related to IFI (p=0.01). This study by Cornely et al. is the first to demonstrate not only an effective prevention of fungal infections, but also a survival benefit of antifungal prophylaxis in patients with neutropenia.

In an Editorial, De Pauw and Donnelly discuss the use of galactomannan for the diagnosis of invasive aspergillosis, as certain drugs are known to affect antigen concentrations [10]. They question why the study of Cornely et al. was performed with open labels. They speculate that less ill patients may have been preselected for the studies, as posaconazole can only be given orally. However, it may be surmised that less ill patients have a lower risk of dying from fungal infection; thus, such preselection would more probably cause a reduced effect of antifungal prophylaxis. The assertion of De Pauw and Donnelly that serum concentrations were not mentioned in the articles at hand is actually wrong, as both provide information on mean plasma concentrations of the study drug. In conclusion, the Editorial advises the reader to rely on pre-emptive treatment strategies in centers with a low incidence of invasive aspergillosis. However, it has yet to be shown how pre-emptive treatment strategies compare with antifungal prophylaxis. While the treatment of IFIs has vastly improved over the last two decades, patients with previous IFIs remain at high risk of relapse during following episodes of cancer treatment [11,12].

The two posaconazole studies show the high potency of antifungal prophylaxis with activity against molds. The study of Cornely et al. is the first to show a significant reduction in overall mortality rate. Although the trial of Ullmann et al. was not sufficiently powered to show a reduction in overall mortality rate, it also provided evidence of a reduction in mortality rate attributable to IFIs using antifungal prophylaxis with posaconazole. Therefore, it is not surprising that the National Comprehensive Cancer Network, the European Conference on Infections in Leukemia, and the German Infectious Diseases Working Party of the German Society of Hematology and Oncology have already changed, or are about to change, their prophylaxis guidelines on the basis of these results. The future will show how well the findings of these studies translate to clinical practice.


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Mycology was well represented during the 2007 joint European Congress for Clinical Microbiology and Infectious Diseases (ECCMID) and the International Congress of Chemotherapy (ICC), with several dedicated oral and poster sessions, as well as official and industry-sponsored symposia. Original studies in the field of epidemiology and antifungal susceptibility testing, pharmacokinetics, diagnosis, and treatment of fungal infections were presented. This report summarizes a part of these presentations.

Epidemiology of candidiasis and antifungal susceptibility

Data from several epidemiological surveys of candidiasis from different European countries highlighted that differences exist between countries. A retrospective study from 1989–2005 in a Swiss intensive care unit (ICU) analyzed 443 episodes of candidemia. A high crude mortality rate of 58% was reported and Candida albicans accounted for 73% of the isolates. Moreover, 94% of C. albicans strains remained highly susceptible to fluconazole. There was a trend toward a decrease in candidemia from 1989–1994 compared with 1995–2004, but no increase in the frequency of non-albicans Candida species was observed [1]. In England and Wales, a retrospective analysis from 1980–2005 showed an increase of candidemia from 0.6 to 3.1 per 100 000 population. Between 1986 and 1998 the distribution of Candida species changed significantly with a decrease of frequency of C. albicans from 75% to 54% [4].

Several other studies reported on the antifungal susceptibility to different antifungals against Candida spp [5,6]. The SENTRY Antimicrobial Surveillance Program tested 965 Candida spp isolates from European patients. Minimal inhibitory concentrations (MICs) of flucytosine, fluconazole, and itraconazole did not change significantly between 1999–2001 and 2003 [5]. However, an increase of voriconazole MICs was seen for C. parapsilosis, C. glabrata, and C. tropicalis in the same period. Similarly, over a 10-year period in Canada, an increased resistance to the azoles was observed for C. glabrata [6]. An evaluation of the in vitro antifungal activity of caspofungin and micafungin, by the European Committee on Antibiotic Susceptibility Testing (EUCAST) reference method, against 1038 isolates from France showed an overall good activity [7]. Micafungin showed lower MIC values, while there was a positive correlation between MICs of both echinocandins.

Diagnosis of invasive fungal infections

The value of galactomannan (GM) detection in serum in non-hematological patients has been evaluated in 75 patients who had an isolate of Aspergillus. It was shown that GM detection is useful for the diagnosis of invasive aspergillosis, with positive and negative predictive values of >83% and 92%, respectively [8].
Another study aimed to detect a zygomycete-specific biological marker in bronchoalveolar lavage (BAL) fluid for diagnosis of invasive zygomycosis. Detection of a water-soluble antigen was performed by immunoblotting with a commercially available anti-Rhizomucor monoclonal antibody in 18 neutropenic patients. All five patients with proven zygomycosis, as well as seven patients without zygomycosis (four with invasive aspergillosis), were positive for antigen. Zygomycete DNA was detected in 11 of 12 BAL samples positive for antigen, indicating the presence of colonization or subclinical infection by a zygomycete [9].

Several studies evaluated molecular diagnostic tools for Candida and Aspergillus [10–13]. A real-time polymerase chain reaction (RT-PCR) assay tested on reference and clinical strains specific for C. albicans was shown to be rapid, sensitive, and 100% specific [10]. Another RT-PCR assay has been developed for detection from blood of six common species of Candida. The test has been validated in ICU patients over a 16-month period, with a specificity of 100%, but a sensitivity of 79% [11]. An RT-PCR assay targeting 18S rDNA for detection of Candida and Aspergillus spp was evaluated by testing 948 blood samples from 127 patients. Four of five patients with aspergillosis and all six patients with candidiasis tested positive [12].

In another study, a commercially available RT-PCR assay, the affigene (Sangtec Molecular Diagnostics AB, Bromma, Sweden) Aspergillus tracer kit, was compared with an in-house nested PCR assay targeting the 18S rDNA, for detection of Aspergillus species [13]. Serum and respiratory samples were obtained from 12 patients at risk for invasive aspergillosis. Overall, both methods were in good agreement for the detection of Aspergillus spp in respiratory samples but did not allow the detection of Aspergillus DNA from serum.

Two interesting studies reported on the use of fluorescence in situ hybridization (FISH) for the identification of different Candida spp directly from blood culture bottles [14,15]. This rapid and easy to perform technique can be used in a routine laboratory of clinical microbiology. In the first study, the probes were evaluated on 14 reference strains and 162 clinical isolates [14]. Subsequent tests on 40 blood culture bottles showed yeasts on the Gram-stain. All probes, except for one of C. dubliniensis showed a sensitivity of 100%. The specificity varied from 94–100%.

The second study utilized the tri-color yeast traffic-light peptide nucleic acid FISH test, where a green fluorescence is observed for C. albicans and C. parapsilosis, a gold fluorescence for C. tropicalis, and a red fluorescence for C. glabrata and C. krusei [15]. The test can be performed within 2.5 h. Evaluation of the test on 50 blood culture samples (40 positive for yeasts and 10 positive for bacteria) showed a sensitivity of 94–100% and a specificity of 100%.

**Clinical efficacy and safety of antifungal drugs**

Several studies evaluated the clinical efficacy and safety of echinocandins [16–22]. Efficacy and safety of caspofungin in 22 solid organ transplant (SOT) patients enrolled in three clinical trials were analyzed [17]. Six patients with invasive candidiasis were treated with caspofungin as primary therapy, with a success rate of 83%. Additionally, 16 patients with invasive aspergillosis were treated with caspofungin as salvage therapy with a success rate of 50%. Although the number of patients was limited, caspofungin appeared to be an effective and well-tolerated treatment in SOT patients.

One clinical trial compared micafungin and caspofungin for treatment of invasive candidiasis and candidemia [21]. Adult patients (n=593) were randomized to receive micafungin at a dose of 100 mg/day or 150 mg/day, or caspofungin at 50 mg/day. Overall, the two micafungin doses were equally effective and were not inferior to caspofungin. Treatment success (defined as a positive clinical and mycological response) was reported in 73.9% and 70.3% of patients in the micafungin 100 mg/day and 150 mg/day arms, respectively, and in 71.4% of those given caspofungin.

There was no difference between the three treatment groups in the incidence of emergent fungal infections, the incidence of post-treatment relapse, and adverse events.

A randomized clinical trial compared micafungin (2 mg/kg/day) with liposomal amphotericin B (3 mg/kg/day) in pediatric patients with invasive candidiasis or candidemia [22]. Treatment efficacy was similar in both arms, with success rates of 69.2% and 74.1% for micafungin and liposomal amphotericin B, respectively. It was showed that micafungin was as effective as liposomal amphotericin B in both neutropenic and non-neutropenic patients.

More specific analyses evaluated the efficacy of micafungin in non-C. albicans infections [16]. Data from two large clinical trials showed that [18]:

- Micafungin at a dose of 100 mg/day was as effective as 3 mg/kg/day of liposomal amphotericin B for all Candida species.
- Micafungin at either the 100 mg/day or 150 mg/day dose was at least as effective as 50 mg/day of caspofungin for all species.
- Micafungin was at least as effective as liposomal amphotericin B or caspofungin for the treatment of invasive candidiasis other than candidemia.

The trial comparing micafungin at 100 mg/day with liposomal amphotericin B also focused on the safety of micafungin, showing an overall safety advantage for micafungin over liposomal amphotericin B [19]. In patients treated with micafungin the estimated glomerular filtration
rate was less decreased and fewer acute infusion-related reactions were noted. Furthermore, the safety of high-dose (100 mg/day) caspofungin has been evaluated in 80 cancer patients and hematopoietic stem cell transplant recipients with invasive fungal infections [20]. In all, 91% of patients received high-dose caspofungin in combination with another antifungal agent, including voriconazole and liposomal amphotericin B. Caspofungin was tolerated without serious hepatic or renal impairment. Reversible hyperbilirubinemia was reported in two patients.

References


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