



Antifungal Susceptibility Testing : Issues and Controversies

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Microbiological resistance to antifungals, particularly azoles, has been increasingly reported. Despite the increase in frequency of resistance, reports of clinical antifungal failures or single stains becoming resistant to antifungal therapies remain distinctly uncommon. Recently NCCLS guidelines for susceptibility testing of yeasts and moulds have been approved. In addition, other methods which are easier, less expensive or more rapid, such as agar based screening techniques, colorimetric assays and the E-test are also available for susceptibility testing. With the increased availability of antifungal susceptibility testing it is important to assess the clinical significance of antifungal resistance and to develop strategies for optimizing use of these procedures. The presence of less susceptible yeasts in oropharyngeal candidiasis, particularly with species other than *Candida albicans*, is common but clinical responses to azoles remain excellent. Prior to the advent of highly active antiretroviral therapy, antifungal resistant oropharyngeal yeasts were common. Using data mostly from patients with oropharyngeal candidiasis, breakpoints for the first-generation azoles, fluconazole and itraconazole were established. Resistant yeasts (defined for fluconazole as an MIC \geq 64) were associated with significantly poorer responses to therapy. A new category of dose-dependent susceptibility (S-DD) was established for both fluconazole (MIC 16-32) or itraconazole (MIC 0.25-0.5) in which higher doses (for fluconazole) or increased serum levels (for itraconazole) were likely required for clinical response. In other clinical settings of patients at high risk for serious fungal infection, including patients undergoing bone marrow and organ transplantation, azole resistance remains uncommon, particularly in *Candida albicans*. Azole prophylaxis in those patients has significantly reduced serious *Candida* although species with decreased susceptibility or resistance have been reported particularly in those few patients who develop a breakthrough infection. In highly immunosuppressed patients and in patients with fungemia and other deep sites of infection, antifungal resistance has important clinical consequences. The empiric use of azoles for the treatment of serious *Candida* infections has been used in the management of patients at high risk for infection because the diagnosis of invasive candidiasis is difficult and is associated with high mortality. In such patients the dose of azole therapy should be chosen to adequately treat serious infection but may need to be changed or increased in patients with less susceptible yeasts such as *Candida glabrata*.

Recently the fungal armamentarium has expanded to include the echinocandins as well as newer triazoles, such as voriconazole which possess an expanded spectrum of activity that includes resistant yeasts such as *Candida krusei* and *Candida glabrata*. While the newer azoles have only limited clinical studies supporting their use in serious *Candida* infections due to these organisms, they possess excellent activity *in vitro* and in animal models of *Candida* infection. Notably, while the *in vitro* activity of

these drugs is significantly better than fluconazole against many of these strains, there is a linear relationship between the MICs of voriconazole and those of fluconazole so that organisms highly resistant to fluconazole will have an increased MIC to voriconazole as well. Recently proposed breakpoints for voriconazole have been proposed, with resistance at 4 and Intermediate MIC 1-2.

The echinocandins offer potential fungicidal activity against *Candida* species, including fluconazole-resistant and -susceptible strains, as well as yeasts other than *Candida albicans*. Only a few species have increased MICs, such as *Candida parapsilosis*, although the clinical significance of those findings are not clear. While resistance through mutation in the target enzyme has been reported, the development of resistance on therapy thus far has been limited and the role of testing yeasts for susceptibility to this class of compound has not yet been established.

For most yeasts, it is more important to devote resources to the species level identification than it is to perform actual susceptibility testing. For example, the isolation of *Candida krusei* will be inherently resistant to fluconazole yet susceptible to amphotericin, the echinocandins and newer azoles, such as voriconazole. For some isolates, particularly *Candida glabrata* or for persistent infection or breakthroughs with azole prophylaxis, susceptibility testing may be of clinical value.

The importance of antifungal resistance in moulds appears to be a growing clinical problem. While resistant *Aspergillus* species have been reported, with more strains undergoing testing it appears that polyene resistance particularly against non-*fumigatus* species is strikingly common. Some *Aspergillus* species, especially *Aspergillus terreus* but also others like *A. flavus* are frequently resistant to amphotericin B so that the newer azoles may be the drug of choice against that organism. Notably, cross-resistance between itraconazole and posaconazole has been reported which could be of clinical importance, as voriconazole susceptibility was maintained. More frequent has been the isolation of less susceptible or resistant fungi, such as the Zygomycetes, which are resistant to most azoles, and *Scedosporium apiospermum* or *Fusarium*, which are both frequently resistant to amphotericin B and susceptible to the newer azoles. In those settings as in the case of non-*albicans* yeasts, identification to the species level is more important than the actual performance of susceptibility testing.

The number of serious invasive fungal infections has continued to increase due to the fact that more immunosuppressed patients are at risk for these infections. Fortunately, the antifungal agents against these organisms have also increased with more effective and less toxic alternatives. Clearly the importance of clinical resistance continues to increase so that the injudicious use of antifungals should be avoided. Adequate dosing and targeted use of azole therapy will optimize the utility of these agents in the therapy of invasive fungal infection and will keep antifungal resistance a manageable clinical problem.

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