In Vitro Antifungal Susceptibilities of Uncommon Basidiomycetous Yeasts

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The in vitro activities of eight antifungal drugs against 50 isolates of basidiomycetous yeasts were determined by a microdilution method. In general fluconazole and micafungin were inactive. Terbinafine was active only against Sporobolomyces salmonicolor. The activities of the other antifungals were variable and depended on the species tested. The new triazoles showed the lowest MICs, but amphotericin B and itraconazole were the only drugs active against Cryptococcus albidus.

Basidiomycetous yeasts are anamorphs (asexual states) of members of jelly fungi (Tremellales) or smuts (Ustilaginales). Some of these yeasts, such as Cryptococcus neoformans and Malassezia spp., are well-known human pathogens. However, many other species are also able to cause human infections, mainly in immunosuppressed patients. Among these, Cryptococcus laurentii, Cryptococcus albidus, Sporobolomyces salmonicolor, Rhodotorula glutinis, and, more commonly, Trichosporon asahii have been reported to cause severe infections (1, 4, 10, 12, 19, 23). In general the most common treatment for yeast infections is based on the use of amphotericin B (AMB) and fluconazole (FLC). However, against infections caused by the five above-mentioned species, these drugs have repeatedly failed (3, 5, 10, 12, 19, 21). Such a limitation, associated with AMB toxicity, determines the interest in evaluating the potential antifungal role of the new azoles and echinocandins. Although some of these species have been tested in vitro, only the responses of a reduced number of isolates are known (6, 24). Trichosporon has received the most attention (2, 3, 21–23), but in most studies the strains tested were identified as Trichosporon beigelli, which is a not valid name, and so it is not known which current species were actually tested.

In this study we have evaluated the in vitro activity of eight antifungal drugs against the five opportunistic species mentioned above. Although a reference method for testing these species does not exist, we have used M27-A2 (17), which has been shown to be very useful for testing the more-common yeasts.

A total of 50 isolates were tested (10 species each of C. albidus, C. laurentii, R. glutinis, S. salmonicolor, and T. asahii). Most of them are clinical isolates provided by the BCCM/IHEM Biomedical Fungi/Yeast collection or Centraalbureau voor Schimmelcultures. The isolates were stored lyophilized and were subcultured on Sabouraud dextrose agar for the study. T. asahii, S. salmonicolor, and R. glutinis were incubated at 35°C for 24 to 72 h, and C. albidus and C. laurentii were incubated at 30°C for 48 to 72 h. Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were included in each batch of tests as a quality control.

Antifungal agents were obtained as pure powders. AMB (USP, Rockville, Md.), albaconazole (ABC; J. Uriach & Cia, Barcelona, Spain), voriconazole (VRC; Pfizer Inc., Madrid, Spain), itraconazole (ITC; Janssen Pharmaceutica, Beerse, Belgium), ravuconazole (RVC; Bristol-Myers Squibb Company, New Brunswick, N.J.), and terbinafine (TBF; Novartis, Basel, Switzerland) were diluted in dimethyl sulfoxide (Panreac Química S.A., Barcelona, Spain). Micafungin (MFG; Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) and FLC (Pfizer Inc., Madrid, Spain) were diluted in sterile distilled water. Microdilution plates were prepared as described in the document M27-A2 (17). Final drug concentrations on the microdilution plates were mainly those recommended by the NCCLS guidelines for yeasts (17) or were based on previous data on basidiomycetous yeasts published by other authors (11, 18, 20). They ranged from 128 to 0.25 μg/ml for FLC, from 64 to 0.12 μg/ml for MFG, and from 16 to 0.03 μg/ml for the other antifungal agents.

Yeast suspensions were adjusted to 10⁶ CFU/ml with a hemocytometer. Suspensions of T. asahii were filtered through sterile gauze in order to eliminate hyphal elements prior to the count. Inoculum concentrations were checked by quantitative colony counts on Sabouraud dextrose agar plates.

Antifungal susceptibility testing was performed by a broth microdilution method that followed the NCCLS guidelines for yeasts (17). The MICs of AMB, TBF, and MFG were defined as the lowest concentrations resulting in 100% inhibition of growth, and those for all the azoles were defined as the lowest concentrations at which there was 50% inhibition of growth compared with a drug-free control. Geometric means, ranges, and MICs at which 90% of the isolates were inhibited (MIC₉₀) were obtained for each species-drug combination tested.

Minimal effective concentration (MEC) was determined for T. asahii and was defined as the lowest drug concentration that produced morphological changes in fungal hyphae (15). Mac-
MICs of this drug (mean MICs = 3.03 μg/ml) were observed against S. salmonicolor.

Recently Wolf et al. (23) also studied the AMB susceptibilities of six isolates of T. asahii using the microdilution and Etest methods and obtained a low correlation between methods. The MICs for some isolates were very high, even higher than 32 μg/ml, by the microdilution method. We tested those six strains, and our results were much more homogenous. The use of a different medium, RPMI 1640 medium supplemented with 2% glucose, by Wolf et al. could explain these important discrepancies.

One of the most remarkable aspects of the study was the low activity of FLC (MIC90 ≥ 64 μg/ml for all isolates tested). This agrees with results reported by other authors (6, 8, 9, 23, 24) and correlates with the failure of this drug to resolve infections by T. asahii (3, 5) and C. laurentii (14). By contrast, the other azoles generally showed good activity against all the fungi tested with the exception of C. albicans. MICs of all the azoles for some isolates of this species were very high, which confirms the data published by other authors (9). As the MICs for other species tested were very low, these important differences in antifungal susceptibility to azoles among the different species of Cryptococcus emphasize the need for a correct identification at species level of clinical isolates of Cryptococcus. ITC in general worked well against all the species tested, with the exception of R. glutinis (mean MICs = 2.65 μg/ml). The new triazoles showed mean MICs lower than 0.20 μg/ml against C. laurentii, S. salmonicolor, and T. asahii. The good activity of VRC and RVC against some of these species had also been reported previously (6, 18; R. Falk, D. G. Wolf, H. Shapiro, and I. Polacheck, Letter, J. Clin. Microbiol. 41:911, 2003; T. Peláez, V. García-Arias, L. Alcalí, A. Bláquez, J. V. Guinea, P. Muñoz, and E. Bouza, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 3829, 2003). By contrast, VRC showed poor activity against Rhodotorula spp., as also demonstrated by Zaas et al. (24).

MFG was inactive against all the species tested, which was to some extent expected because it had been demonstrated that echinocandins are not active against other basidiomycetous yeasts, such as Cryptococcus neoformans, Trichosporon cutaneum (20), and Rhodotorula spp. (24). MECs of this drug were determined only for T. asahii and did not differ from MICs.

TBF seemed generally inactive for all the species tested but surprisingly showed very low MICs against S. salmonicolor (mean MICs = 0.08 μg/ml). However, there are no data on the use of this compound in clinical cases where this species was involved in order to confirm these findings.

In summary, and although further in vivo studies are re-
quired, these results seem to indicate that the new triazoles and even ITC could be alternatives for the treatment of infections by the yeasts tested here.

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