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In Vitro Sensitivity of *Torulopsis glabrata* to Amphotericin B, 5-Fluorocytosine, and Clotrimazole (Bay 5097)

MELVIN I. MARKS,[1] PAUL STEER, AND THEODORE C. EICKHOFF
Departments of Medicine and Pediatrics, University of Colorado Medical Center, Denver, Colorado 80220

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Thirty-five strains of *Torulopsis glabrata* were tested by a tube dilution method for their susceptibility to amphotericin B, 5-fluorocytosine, and clotrimazole (Bay 5097). Amphotericin B was the most active in vitro, inhibiting all strains at a concentration of 1 μg/ml and killing all strains at 2 μg/ml. 5-Fluorocytosine inhibited over 80% of strains at 0.24 μg/ml, but three strains required ≥7.8 μg/ml for killing. A concentration of 2 μg of clotrimazole per ml inhibited less than 50% of strains, and 8 μg/ml killed only 10% of strains. Most strains of *T. glabrata* were killed by therapeutically achievable concentrations of amphotericin B and 5-fluorocytosine, but not clotrimazole.

Recognition of the yeast *Torulopsis glabrata* as an opportunistic pathogen in man has emphasized the need for studies of the susceptibility of this fungus to antifungal agents (3). Amphotericin B has been successfully employed in the treatment of *T. glabrata* infections (1); however, this drug is often toxic and must be administered intravenously. 5-Fluorocytosine is an orally administered antifungal agent that appears to be less toxic than amphotericin B and is active against many *Candida* species, *Cryptococcus neoformans*, and *Aspergillus fumigatus* (6). Preliminary studies with a new oral antifungal agent, clotrimazole (Bay 5097), indicate this drug is active against some strains of *Candida* species as well (5). In the present study, 35 recent clinical isolates of *T. glabrata* were tested for susceptibility to amphotericin B, 5-fluorocytosine, and clotrimazole.

MATERIALS AND METHODS

Thirty-five clinical isolates of *T. glabrata* from 33 patients at the University of Colorado Medical Center were selected for study. Seventeen of the yeasts were isolated from urine, seven from blood, four from wounds, three each from stool and sputum, and one from bile. Two patients had been treated with amphotericin B before these fungi were isolated, and none had been exposed to either of the other drugs used in this study. The yeasts were cultured and identified as previously described (4) and maintained at room temperature on Sabouraud dextrose agar until tested.

Commercial amphotericin B (Fungizone-Squibb) was prepared at the time of each test. 5-Fluorocytosine (R02-9915) was supplied as a pure powder by Hoffmann-La Roche Inc. (lot no. AP3) and was maintained as a stock solution of 10,000 μg/ml at −70 C. Clotrimazole (Bay 5097) was supplied by Delbay Pharmaceuticals and a fresh stock solution of 2,000 μg/ml was prepared in 100% ethyl alcohol at the time of testing. The ethyl alcohol solvent diluted in water without drug had no inhibitory effect with concentrations equivalent to 32 μg/ml or less of Bay 5097.

The tube dilution method for the study of yeast susceptibilities has been previously described (2). A 10^{-3} dilution of an overnight broth culture was employed as the inoculum for these tests. Sabouraud dextrose broth was used for the amphotericin B and Bay 5097 studies and yeast-nitrogen base broth supplemented with L-asparagine and dextrose was used for the 5-fluorocytosine testing (6). The tests were incubated at 37 C and the minimum inhibitory concentrations, defined as the lowest concentration of drug in tubes without visible growth, were estimated at 48 hr. Samples of 0.05 ml from tubes without visible growth were subcultured to Sabouraud dextrose agar and reincubated at 37 C for 48 hr. Minimum fungicidal concentrations, defined as the lowest concentration of drug in tubes from which there was no growth on subculture, were recorded at 48 hr.

RESULTS

The results of the susceptibility testing of 35 strains of *T. glabrata* are illustrated in Fig. 1-3. The fungistatic and fungicidal concentrations of amphotericin B were within the achievable therapeutic range for all strains tested (Fig. 1). Over 90% of the isolates were sensitive to ≤1 μg of amphotericin B per ml and only one isolate

[1] Present address: Montreal Children's Hospital, Montreal 108, Quebec, Canada.

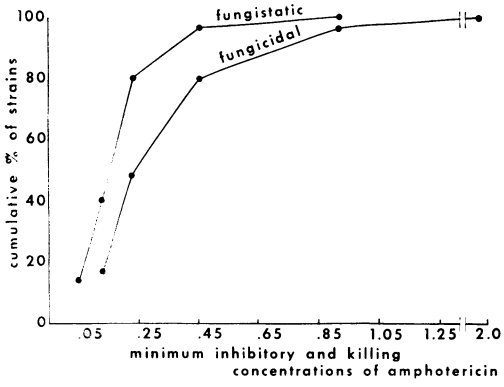

FIG. 1. *Susceptibility of 35 strains of Torulopsis glabrata to amphotericin B.*

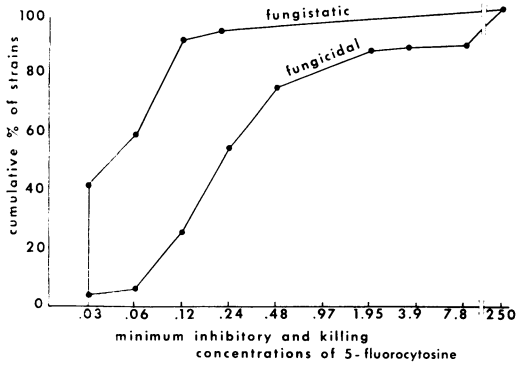

FIG. 2. *Susceptibility of 35 strains of Torulopsis glabrata to 5-fluorocytosine.*

required 2 μg/ml for a fungicidal effect. The difference between the fungistatic and fungicidal concentration was greater with 5-fluorocytosine than with amphotericin B. However, 90% of strains were inhibited by ≤0.2 μg of 5-fluorocytosine per ml (Fig. 2). Three strains required ≥7.8 μg/ml for killing.

Susceptibility of the strains of *T. glabrata* to clotrimazole is recorded in Fig. 3. There is a marked difference between the fungistatic and fungicidal concentrations; less than 50% of the isolates were inhibited by ≤2 μg/ml, but only 10% of the isolates were killed by ≤8 μg/ml.

DISCUSSION

The decision to treat a patient from whom *T. glabrata* has been isolated is often difficult and is based on many considerations. These include the sensitivity of the yeast to antifungal agents, the toxicity of these agents, and the differentiation of colonization from true infection. Amphotericin

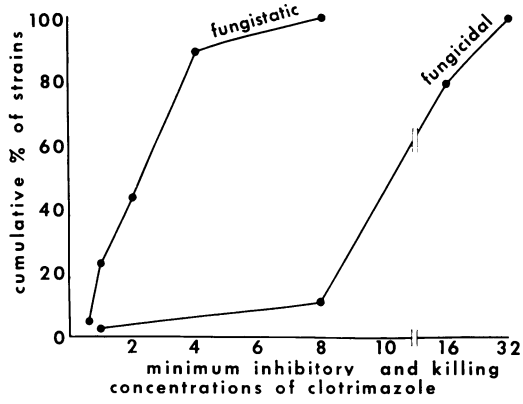

FIG. 3. *Susceptibility of 35 strains of Torulopsis glabrata to clotrimazole (Bay 5097).*

B appears to be the most active of the three agents against *T. glabrata* in vitro; however, it is also the most toxic of these drugs. 5-Fluorocytosine is less toxic, is available as an oral preparation, and has significant antifungal activity against *T. glabrata*. Thirty-two out of 35 isolates tested were killed by concentrations of ≤7.8 μg/ml. Serum concentrations of this drug in humans are 10 to 150 μg/ml and urine concentrations 295 to 1,900 μg/ml after oral doses of up to 150 mg per kg per day (6; P. Steer et al., *in preparation*). Thus, 5-fluorocytosine may be an effective drug for most strains of *T. glabrata*. A disc test has been described which may provide a simple and rapid method for in vitro sensitivity testing (2). Clotrimazole inhibited only a small proportion of the strains of *T. glabrata* tested at achievable therapeutic concentrations which, although variable, average 1 to 3 μg/ml after oral doses of 40 mg/kg (F. Falco, *personal communication*). Further studies correlating in vitro and in vivo activity of these antifungal agents are necessary to confirm these results.

The present data suggest that 5-fluorocytosine or amphotericin B may be effective in the treatment of *T. glabrata* infections in man. 5-Fluorocytosine deserves further clinical study, as it possesses the advantages of oral administration and less toxicity than amphotericin B.

ACKNOWLEDGMENTS

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