

In Vitro Activity of Systemic Antifungal Agents against *Malassezia furfur*

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The activity of four antifungal agents against 15 systemic (blood and vascular catheter) and 10 superficial (skin) *Malassezia furfur* isolates was evaluated. MIC ranges were similar for the two groups of organisms: amphotericin B, 0.3 to 2.5 µg/ml; flucytosine, >100 µg/ml; miconazole, 0.4 to 1.5 µg/ml; and ketoconazole, 0.025 to 0.4 µg/ml.

Malassezia furfur is a lipid-dependent yeast commonly found on the skin of adolescents and adults and the causative agent of tinea versicolor, a chronic, superficial skin infection (9). The organism has recently been isolated from the skin of neonates in intensive care nurseries and also from deep-line vascular catheters and catheter blood cultures in patients with signs and symptoms of sepsis (1, 4, 7, 8). Administration of parenteral fat emulsions for caloric supplementation via a deep-line catheter is highly associated with subsequent catheter colonization and probable sepsis (4, 7, 8). However, *M. furfur* has not been recovered directly from fat emulsion fluids, and we hypothesize that catheter colonization and associated sepsis occur subsequent to skin colonization, while the fat emulsions provide the necessary lipid growth requirement for the organism. The organism has also been recovered from peripheral blood and lung tissue at biopsy and autopsy, suggesting the systemic nature of this infection (8). Although most patients have been successfully managed by removal of the infected catheter, vascular access is often critical, and treatment of patients with antifungal therapy while the catheter remains in place is an alternative form of management.

To determine whether antifungal agents might be useful in treatment of *M. furfur*-associated catheter sepsis, we performed in vitro susceptibility tests with 15 catheter tip or blood culture isolates of *M. furfur* against four antimicrobial agents used in the systemic therapy of fungal infection: amphotericin B, flucytosine (5-FC), miconazole, and ketoconazole. For comparison, we also tested 10 skin isolates of *M. furfur* from infants without catheter sepsis.

(A portion of these results were presented previously [M. J. Marcon, D. A. Powell, D. E. Durrell, and W. J. Buesching, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 836, 1986].)

M. furfur-associated catheter sepsis isolates were recovered from Isolator (Du Pont Co., Wilmington, Del.) blood cultures (6) drawn via a deep-line catheter or by peripheral venipuncture from infected infants or from the catheter tip at the time of removal. The following data support the association of these *M. furfur* isolates with catheter sepsis: (i) peripheral blood cultures drawn at the same time as catheter blood cultures were also positive for *M. furfur* in 4 of 10 patients tested; (ii) 10 of 10 catheter tip cultures plated by the

semiquantitative method of Maki et al. (5) grew >15 CFU (most grew >100 CFU) of *M. furfur*; (iii) most patients had multiple positive catheter blood cultures collected over several days; (iv) the patients appeared septic, and laboratory data supported this observation (fever, apnea, bradycardia, thrombocytopenia, leukocytosis, elevated neutrophil band counts); (v) patients responded dramatically to catheter removal; and (vi) no other infectious agent was recovered from blood or catheter tip culture. We previously reported the clinical presentation of five of these patients (7). Skin isolates were obtained from scrapings of the back or chest of infants hospitalized in an intensive care unit. Organisms were isolated on glucose-yeast extract-peptone agar (GYPS) supplemented with glycerol monostearate (0.25%), Tween 80 (0.2%), and olive oil (2%) (6). Isolates were subcultured once or twice on GYP-S, harvested after 2 or 3 days of growth at 35°C, and stored frozen at -70°C in whole sheep blood.

Amphotericin B was obtained from E. R. Squibb & Sons, Princeton, N.J.; 5-FC was obtained from Hoffman-La Roche Inc., Nutley, N.J.; and both miconazole and ketoconazole were obtained from Janssen Pharmaceutica, Piscataway, N.J. All drugs were obtained as powders from single lots and stored desiccated in the dark at 4°C. Stock solutions of amphotericin B were prepared fresh in dimethyl sulfoxide, stored at -70°C, and used within 1 month; solutions of the other agents were prepared in the solvents indicated and stored at -70°C for up to 3 months until used (5-FC, distilled water; miconazole, dimethyl sulfoxide; ketoconazole, 0.2 M HCL). All dilutions of stock solutions were prepared in the susceptibility test medium (broth dilution) or 0.85% saline (agar dilution).

Amphotericin B and 5-FC were tested by broth macrodilution as described by Shadomy et al. (10). In addition, select isolates were tested by a modification of this method for adaptation to microdilution trays. Tests with amphotericin B were performed in antibiotic medium 3 (Penassay broth; Difco Laboratories, Detroit, Mich.), while unbuffered yeast nitrogen base (YNB; Difco) supplemented with 1% glucose and 0.15% asparagine was used for tests with 5-FC. Both media were additionally supplemented with 0.5% (final concentration) Tween 80 to support the growth of *M. furfur*. Each isolate was tested as least two times on separate days. Inocula were prepared in saline from 2- or 3-day-old growth on GYP-S agar and adjusted to the turbidity of a McFarland

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TABLE 1. In vitro activity of four antifungal agents against systemic (blood or catheter tip culture) and superficial (skin culture) isolates of *M. furfur* and quality control organisms

Organism and source	Range of MICs ($\mu\text{g/ml}$) ^a			
	Amphotericin B	5-FC	Miconazole	Ketoconazole
<i>M. furfur</i> , blood or catheter tip (15 isolates)	0.6–2.5 (0.6)	>100	0.4–1.5 (0.8)	0.05–0.4 (0.05)
<i>M. furfur</i> , skin (10 isolates)	0.3–0.6 (0.3)	>100	0.4–0.8 (0.4)	0.025–0.05 (0.05)
<i>S. cerevisiae</i> ATCC 36375	0.08–0.15 (0.15)	0.025–0.1 (0.05)	0.1–0.4 (0.2)	0.4–0.8 (0.4)
<i>C. pseudotropicalis</i> ATCC 28838	0.15–0.3 (0.15)	0.1–0.4 (0.2)	0.2–0.4 (0.2)	0.1–0.4 (0.1)

^a Numbers in parentheses are mode values.

no. 1 standard. This yielded a suspension of approximately 1×10^6 to 2×10^6 CFU/ml. The suspension was diluted 1:10 in antibiotic medium 3 or YNB supplemented with 1% Tween 80, and 0.5-ml volumes were inoculated into test tubes containing 0.5-ml volumes of twofold dilutions of the antimicrobial agent to be tested. Thus, the final inoculum concentration was about 5×10^4 to 1×10^5 CFU/ml. Growth and sterility control tubes were included, and each assay was performed in duplicate. The following quality control organisms were included with each assay: *Saccharomyces cerevisiae* ATCC 36375 and *Candida pseudotropicalis* ATCC 28838. Tubes were incubated at 30°C and examined at 24 and 48 h; endpoints were read as soon as growth was visible in the growth control tubes. Generally, this was 24 h for amphotericin B in antibiotic medium 3 and 48 h for 5-FC in YNB. The MIC was defined as the lowest concentration of drug which inhibited obvious visible growth, ignoring a faint haze. Minimal fungicidal concentrations of amphotericin B were determined by subculturing 10 μl of broth from each negative tube onto GYP-S agar, with subsequent incubation at 30°C for 72 h. The minimal fungicidal concentration was defined as the lowest concentration of drug which yielded fewer than two colonies (99.9% kill).

Susceptibility tests of the imidazoles were initially performed by broth macro- and microdilution as described by Shadomy et al. (10) using either buffered YNB or Casitone (Difco)-yeast extract-glucose media, both supplemented with 0.5% Tween 80. However, we were unable to obtain published target MICs with both *S. cerevisiae* 36375 and *C. pseudotropicalis* 28838 control organisms (10). Specifically, the MICs of both miconazole and ketoconazole for these organisms were greater than eightfold higher than published values and also than results of MIC tests performed without Tween 80 added to the media. It is possible that Tween 80 at the concentration (0.5%) tested antagonized the activity of the imidazoles. Because of these observations, the imidazoles were tested by agar dilution on diagnostic sensitivity test agar (Oxoid USA, Inc., Columbia, Md.) as described by Faergemann and Bernander (2, 3). The agar medium was supplemented with glycerol monostearate (0.25%) and Tween 80 (0.2%) and adjusted to a final pH of 5.6. Solutions of the imidazoles were prepared as described above and incorporated into the agar medium at the time of preparation. All plates were used on the day of preparation, and each isolate was tested at least two times on separate days. Inocula were prepared by suspending 2- or 3-day-old growth from GYP-S agar in saline and diluting the suspension so that 10 μl contained approximately 10^4 CFU of *M. furfur*. After the agar dilution plates were spotted with the inocula, they were incubated at 30°C and read after 1, 2, and 3 days when growth control plates (without drug) showed good colony development. All experiments were performed in duplicate, and quality control organisms for which MIC

endpoints were known were always run in parallel, as described for amphotericin B and 5-FC. The MIC endpoint was defined as the lowest concentration of drug that inhibited development of colonial growth or allowed just a slight haze when viewed with incident light.

The results of the in vitro broth and agar dilution susceptibility tests are shown in Table 1. The MIC results for amphotericin B and 5-FC obtained by macro- and microbroth dilution were the same or within one twofold dilution, and therefore only one result is shown for these drugs. No difference was observed in MICs for systemic versus skin isolates. The most active drug in vitro was ketoconazole; the least active was 5-FC. Both amphotericin B and miconazole were intermediate in in vitro activity. Minimum fungicidal concentrations of amphotericin B ranged from 1.2 to 10 $\mu\text{g/ml}$ (data not shown). These concentrations are higher than reported achievable peak levels in serum (11). Modal MICs for both quality control organisms tested were within one dilution of published target values (10).

There are limited published data on the in vitro activity of commonly used antifungal agents against systemic *M. furfur* infections. Faergemann and Bernander tested 19 skin isolates against miconazole; for 16 of these, MICs ranged from 0.05 to 1.5 $\mu\text{g/ml}$ (3). However, for three isolates, MICs were 50 $\mu\text{g/ml}$. Faergemann also tested six isolates with ketoconazole; for five isolates, MICs were 0.05 $\mu\text{g/ml}$, while the MIC for one isolate was 0.02 $\mu\text{g/ml}$ (2). These data are in agreement with our results (except for Faergemann's three isolates for which miconazole MICs were high). Danker and Spector reported a single *M. furfur* catheter blood culture isolate for which MICs were 2.5 μg of amphotericin B per ml and >100 μg of 5-FC per ml (1). Redline et al. reported an isolate for which MICs of amphotericin B and 5-FC were 0.8 and 0.05 $\mu\text{g/ml}$, respectively (8). Our data agree with that of the single isolate tested by Danker and Spector but not with the 5-FC data on the isolate reported by Redline and associates.

Performance of in vitro antifungal susceptibility tests is subject to many variables, including composition of the medium, inoculum size, and time and temperature of incubation. In this study, we found that the addition of 0.5% Tween 80 to broth medium did not alter target MICs of amphotericin B and 5-FC for control organisms when tested by a standard method (10); both macro- and microbroth dilutions are suitable methods for testing *M. furfur* against these agents. However, the imidazoles could not be tested by standard broth dilution methods with the addition of 0.5% Tween 80, and we chose to use a published agar dilution procedure for this purpose (2, 3). This procedure yielded acceptable results with quality control organisms. Based on our own in vitro data, clinical trials of miconazole or ketoconazole, and perhaps amphotericin B, in cases of *M.*

furfur-associated catheter sepsis are warranted. However, the presence of a foreign body in patients with catheter sepsis presents a significant factor complicating the ability of an antifungal agent to eradicate an infection. Thus, in vitro susceptibility test data for *M. furfur* must be viewed cautiously, and patients treated with antifungal agents (based on in vitro data) without catheter removal must be monitored closely.

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