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Trichosporon beigelii, an Emerging Pathogen Resistant to Amphotericin B

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***Trichosporon beigelii* caused fatal disseminated infections that were resistant to amphotericin B in two granulocytopenic patients. In vitro susceptibility studies demonstrated that both index strains of *T. beigelii* were inhibited but not killed by amphotericin B at achievable concentrations in serum. The minimum lethal concentration for both isolates was ≥18 μg/ml. Five of seven other isolates were found to have a similar pattern of amphotericin B resistance. The fact that the minimum lethal concentration of *T. beigelii* was many times greater than its MIC was consistent with a resistance pattern of tolerance. We concluded that *T. beigelii* may be resistant in vitro to amphotericin B and that this in vitro resistance was correlated with refractory, disseminated trichosporonosis in granulocytopenic patients. *T. beigelii* should be included in the expanding list of amphotericin B-resistant fungi.**

Trichosporon beigelii (Kuchenmeister et Rabenhorst) Vuillemin is the etiologic agent of white piedra in immunologically normal patients and of disseminated trichosporonosis in immunocompromised hosts (18). Disseminated trichosporonosis is an uncommon, but increasingly reported and frequently fatal, fungal infection in immunocompromised patients. Disseminated trichosporonosis in granulocytopenic patients commonly develops as a rapid onset of fever, fungemia, funguria, azotemia, pulmonary infiltrates, and cutaneous lesions with invasion of the kidneys, lungs, skin, and other tissues (6, 7, 17–19). Serum from such patients may react with the cryptococcal latex agglutination assay (10). *Trichosporon beigelii* isolated from blood, skin biopsy, or urine specimens of an immunocompromised patient should be regarded by the clinical microbiology laboratory as a true pathogen unless proven otherwise.

Disseminated infection caused by *T. beigelii* is frequently fatal in granulocytopenic patients, despite the administration of amphotericin B. The cause for this refractoriness of *T. beigelii* to amphotericin B therapy is not known. There are an increasing number of reports of amphotericin B resistance by *Candida* spp., including *Candida tropicalis* (4, 15), *Candida lusitaniae* (1, 5, 12), *Candida guilliermondii* (3), and *Candida parapsilosis* (16). The fact that amphotericin B-resistant *T. beigelii* may also be a cause of disseminated trichosporonosis that is refractory to amphotericin B therapy is possible but has not been well documented.

There is a paucity of data available to clinical microbiology laboratories which correlate clinical response and in vitro activity of antifungal compounds against invasive opportunistic fungi, particularly for *T. beigelii*. As the frequency of opportunistic fungal infections increases and as newer antifungal agents become available, there is clearly a need, however, to document correlations between in vitro antifungal susceptibility and clinical outcome, especially for the more refractory mycoses. Such correlations between in vitro amphotericin B resistance and therapeutic resistance to amphotericin B have been reported previously for *C. tropicalis* (4), *C. lusitaniae* (1, 5, 12), *C. guilliermondii* (3), and *C. parapsilosis* (16).

We report here two patients with *T. beigelii* infections that caused fatal disseminated trichosporonosis with persistent fungemia that was refractory to amphotericin B. In order to investigate this problem of refractory trichosporonosis further, we studied the in vitro antifungal susceptibilities of the two index isolates and seven additional isolates of *T. beigelii* to amphotericin B, flucytosine, and antifungal azoles. We also studied the activities of the two index isolates in timed kill curve assays of amphotericin B and flucytosine.

MATERIALS AND METHODS

Case reports (i) Case 1. The first patient was an 18-year-old male with aplastic anemia and granulocytopenia (<100 granulocytes per μl) who was treated with antithymocyte globulin. He received ceftazidime, vancomycin, and metronidazole for a possible intra-abdominal infection. Amphotericin B, 0.5 mg/kg per day, was initiated empirically 1 week later for persistent fever, despite the administration of antibacterial antibiotics. His fever initially resolved but recurred at 39.7°C 2 weeks later in association with progressive oral mucositis, right-side flank pain, and hematuria. *T. beigelii* grew from blood cultured by an isolator lysis centrifugation method (Dupont Diagnostics, Wilmington, Del.) on 5% horse blood–Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with pyridoxal (Remel, Lenexa, Kans.) within 48 h of incubation. *T. beigelii* also grew at this time from urine plated on 5% sheep blood–Trypticase soy agar and from a culture of oropharyngeal secretions swabbed onto 5% sheep blood–Trypticase soy agar incubated at 35°C. Multiple colonies of *T. beigelii* were found on culture plates of blood, skin, and urine specimens and oropharyngeal secretions. *T. beigelii* was the only organism cultured from blood, skin, and urine specimens. A serum cryptococcal latex agglutination reaction was 1:256. Flucytosine adminis-

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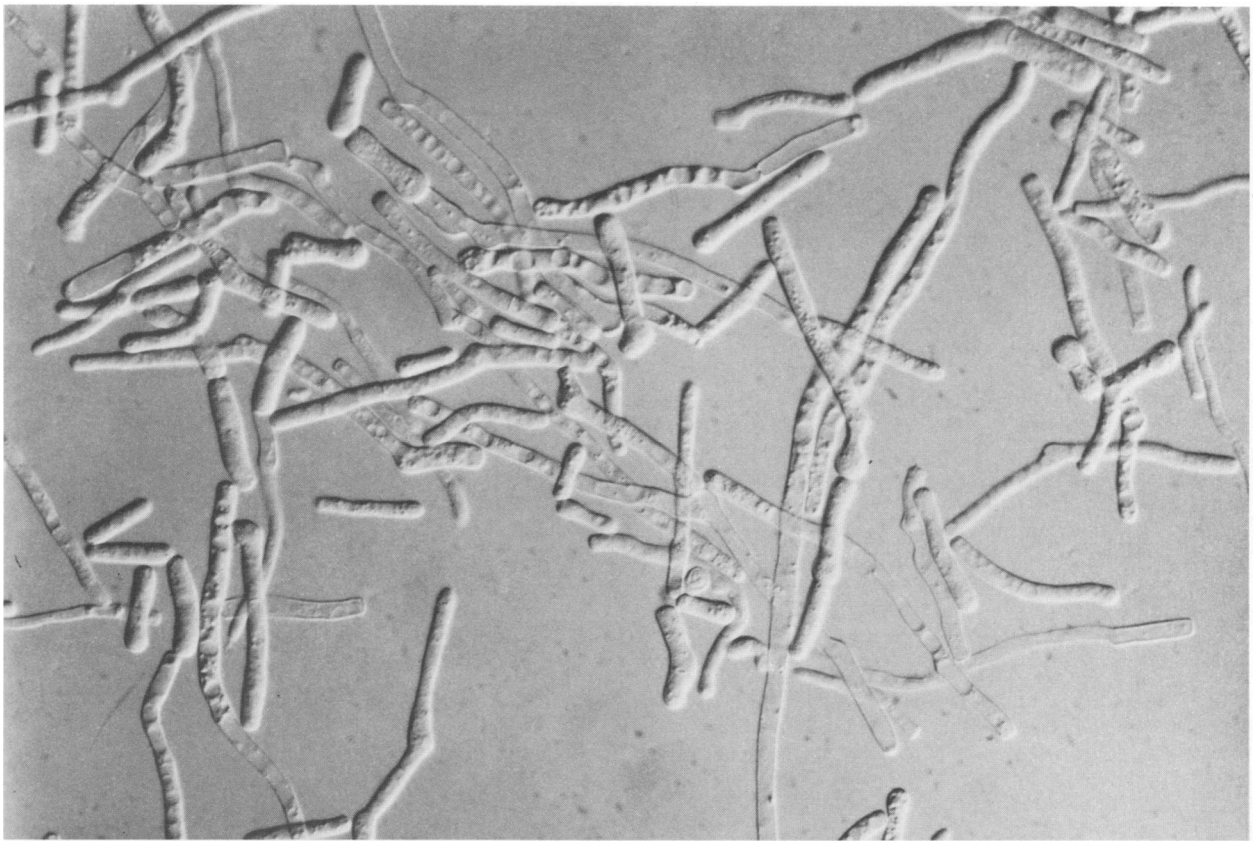

FIG. 1. Wet mount of *T. beigelii* isolated from the blood of case 2 (strain TSA). When grown on Sabouraud glucose agar and grown at 37°C for 48 h, this isolate on wet mount demonstrated many hyphae, some arthroconidia, and few blastoconidia. Differential interference contrast microscopy was used. Magnification, ×400.

tered at 37.5 mg/kg every 6 h orally (later, it was administered intravenously) was added at this time, and the dose was adjusted to maintain concentrations in serum of between 50 and 60 μg/ml. During the week following the initial isolation of *T. beigelii*, a chest radiograph revealed new nodular pulmonary infiltrates. Hemorrhagic maculopapular cutaneous lesions also developed at this time on the patient's trunk and extremities. Biopsies of these cutaneous lesions grew *T. beigelii* and histologically demonstrated hyphae, arthroconidia, and blastoconidia invading the papillary dermis, as illustrated elsewhere (18). Since blood cultures continued to grow *T. beigelii*, despite the combination of amphotericin B and flucytosine therapy, the central and peripheral venous catheters were removed. Culture of the central venous catheter on 5% horse blood–Trypticase soy agar plates grew *T. beigelii*. Trichosporonemia persisted, and the cutaneous lesions increased in size and number. There were no cardiac murmurs, and an echocardiogram revealed no vegetations. Creatinine in serum increased from 0.8 to 1.9 mg/dl, despite appropriate hydration. A trial of itraconazole at 200 mg twice per day by mouth was attempted but was unsuccessful because of the patient's inability to swallow. Trichosporonemia persisted and the patient died, with all 16 sets of blood cultures drawn over 24 days growing *T. beigelii*.

(ii) Case 2. A 65-year-old male presented with newly diagnosed acute lymphocytic leukemia and fever for which he received induction chemotherapy and empirical antibiotics. His fever resolved initially with cefazolin, carbenicillin, and gentamicin; however, a new fever developed on day 20 of granulocytopenia. Cefazolin was discontinued and vancomycin was added. A blood culture drawn at this time grew a yeastlike fungus 3 days later from the 6A bottle of the BACTEC 460 radiometric blood culture system (Becton-Dickinson Diagnostic Instrument Systems, Towson, Md.) at 35°C, at which time amphotericin B given intravenously at 0.5 mg/kg per day was instituted. The fungus was identified as *T. beigelii* (Fig. 1). Despite administration of amphotericin B, blood cultures remained persistently positive for *T. beigelii*, and erythematous maculonodular lesions developed on the patient's trunk and lower extremities. Biopsy of these lesions demonstrated infiltrating arthroconidia, blastoconidia, and pseudohyphae, which were morphologically consistent with *T. beigelii*. A chest radiograph revealed new bilateral interstitial infiltrates in association with the new onset of hypoxemia and the requirement of mechanical ventilation. The dosage of amphotericin B was increased to 1.0 mg/kg per day without any resolution of fungemia or clinical improvement. A serum latex agglutination assay for cryptococcal antigen was positive at a titer of 1:8. The patient became hemodynamically unstable and sustained a cardiac arrest from which he could not be resuscitated. Postmortem cultures of lung and skin specimens grew only *T. beigelii*. Histopathological examination demonstrated *Trichosporon* bilateral necrotizing pneumonitis; myocardial septic infarction; and infiltration of the liver, spleen, kidneys, and skin (Fig. 2).

Microbiological studies. (i) Study isolates. Nine isolates of

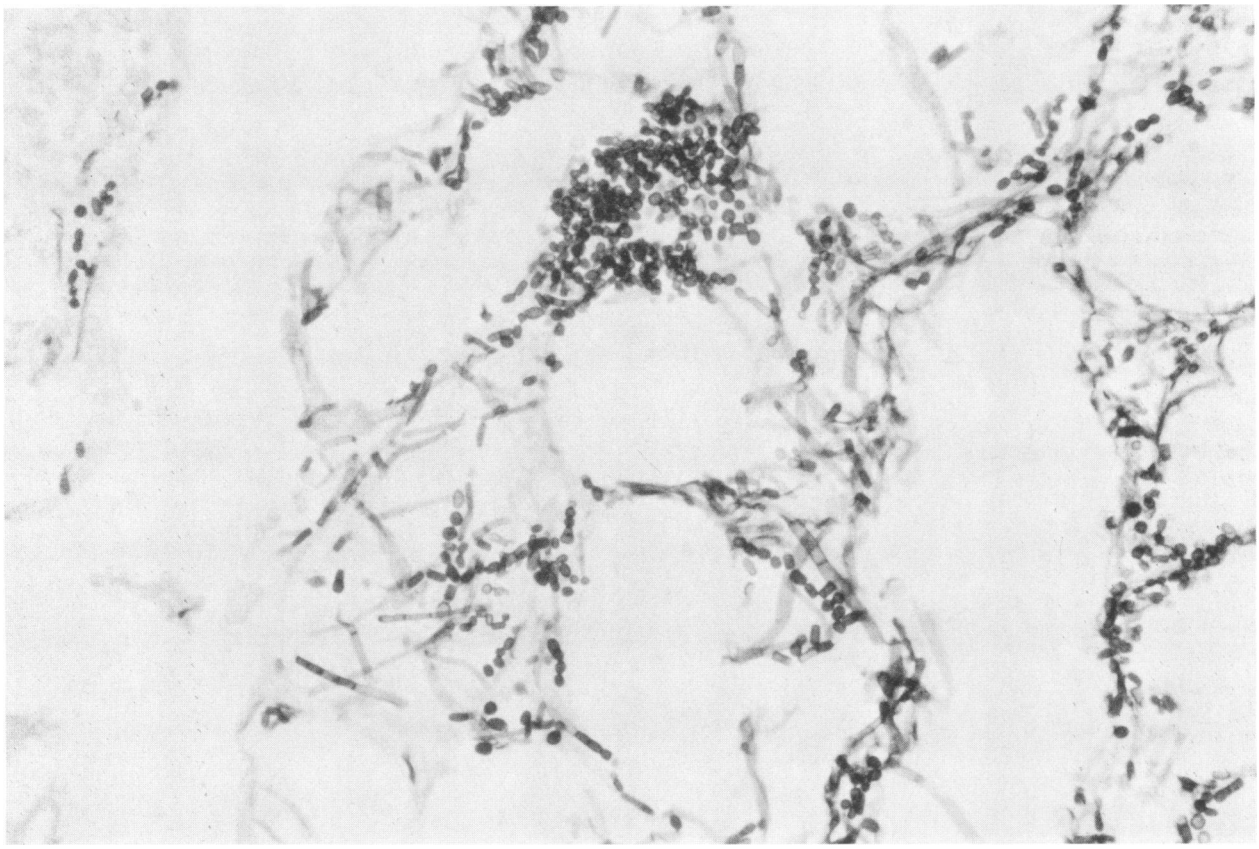

FIG. 2. Histopathological specimen of lung from case 2 demonstrated many hyphae, arthroconidia, and blastoconidia consistent with *T. beigelii* infiltrating pulmonary alveolar septa and some alveolar spaces. Gomori methenamine silver stain was used. Magnification, ×400.

T. beigelii were obtained from the following laboratories: Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, Md.; Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio; and the New York State Department of Health, Albany. The isolate from the first index case is referred here as TCM, and that of the second index case is referred to as TSA. Among the nine isolates studied, seven were from human sources and two were from veterinary sources. Among the seven human isolates, five were from blood, one was from stool, and one was from the vagina. These isolates were maintained in specimen collections for between 1 and 6 years. The clinical source, reference laboratory, and code number for each isolate of *T. beigelii* studied are listed in Table 1.

Isolates of *T. beigelii* from the Warren Grant Magnuson Clinical Center of the National Institutes of Health were maintained on the surface of potato glucose agar slants at −70°C. Isolates of *T. beigelii* from the University of Texas Health Science Center at San Antonio were maintained on the surface of potato flake agar at −70°C. Isolates from the New York State Department of Health were lyophilized and maintained at room temperature. All isolates of *T. beigelii*, when studied for susceptibility, were subcultured from maintenance cultures onto the surface of Sabouraud glucose agar slants.

(ii) Isolation and identification. The skin biopsy specimen from case 1 was cut into small pieces with a sterile scalpel, and the specimen was placed into a sterile mortar and pestle, to which several drops of filter-sterilized water (Millipore Corp., Bedford, Mass.) was added to facilitate grinding. The homogenized specimen was transferred with a sterile pipette onto 5% sheep blood–Trypticase soy agar (BBL Microbiology Systems) incubated at 35°C.

All study isolates of *T. beigelii* were further identified on Sabouraud glucose agar by the presence of hyaline arthroconidia, blastoconidia, hyphae, and pseudohyphae. All isolates grew at 37°C and were urease positive. All isolates were studied on the API 20C system (Analytab Products, Plainview, N.Y.) and yielded assimilation profiles ranging from good likelihood to excellent identification as *T. beigelii*. All isolates were maintained on Sabouraud glucose agar during the identification and antifungal susceptibility studies.

(iii) Susceptibility by macrodilution methods. In vitro susceptibility studies were performed by previously described macrodilution methods (8, 9). Briefly, the isolates of *T. beigelii* and the standard controls (*Saccharomyces cerevisiae* ATCC 3633750 and *C. tropicalis* ATCC 13803) were grown for 48 h at 35°C on Sabouraud glucose agar and suspended in sterile distilled water. The suspension was adjusted with a spectrophotometer to 90% transmission at 530 nm and diluted 10-fold. Concentration ranges for amphotericin B (E. R. Squibb & Sons, Princeton, N.J.) were 0.14 to 18.5 μg/ml, for flucytosine (Roche Diagnostics, Div. Hoffmann-La Roche, Inc., Nutley, N.J.) they were 10.1 to 323 μg/ml, for miconazole (Janssen Pharmaceutica, Piscataway, N.J.) they were 0.6 to 10.0 μg/ml, for itraconazole (Janssen Pharmaceutica) they were 0.018 to 10 μg/ml, for fluconazole (Pfizer Central Research, Groton, Conn.) they were 1.25 to 80 μg/ml, and for ketoconazole (Janssen Pharmaceutica)

TABLE 1. Susceptibilities of isolates of *T. beigelii* to antifungal compounds

Code (site)[a]	Time (h)[b]	Amphotericin B		Flucytosine		Miconazole		Ketoconazole		Fluconazole		Itraconazole	
		MIC (μg/ml)	MLC (μg/ml)	MIC (μg/ml)	MLC (μg/ml)	MIC (μg/ml)	MLC (μg/ml)	MIC (μg/ml)	MLC (μg/ml)	MIC (μg/ml)	MLC (μg/ml)	MIC (μg/ml)	MLC (μg/ml)
TCM (blood)[c]	24	≤0.14	4.62	<10.1	161	≤0.6	2.5	0.8	>12.8	10	>80	≤0.018	10
	48	0.58	18.5	<10.1	>323	≤0.6	2.5	0.8	>12.8	10	>80	≤0.018	>10
TSA (blood)[d]	24	0.58	>18.5	≤10.1	323	≤0.6	2.5	3.2	>12.8	20	40	≤0.018	5
	48	1.16	>18.5	≤10.1	323	≤0.6	2.5	3.2	>12.8	20	>80	0.15	>10
82-100 (vaginal)	24	≤0.14	0.58	≤10.1	40.34	≤0.6	2.5	0.4	12.8	5	5	≤0.018	>10
	48	0.29	0.58	20.17	>323	≤0.6	2.5	0.4	12.8	5	>80	0.07	>10
82-102 (blood)	24	<0.14	0.58	≤10.1	10.1	≤0.6	2.5	1.6	>12.8	5	20	0.3	10
	48	0.58	4.62	≤10.1	323	≤0.6	2.5	1.6	>12.8	5	>80	0.3	>10
82-112 (canine)	24	≤0.14	1.16	≤10.1	≤10.1	≤0.6	2.5	0.1	3.2	≤1.25	>80	≤0.018	1.25
	48	≤0.14	2.31	≤10.1	>323	≤0.6	2.5	0.1	12.8	≤1.25	>80	≤0.018	1.25
M297-87 (stool)	24	≤0.14	0.58	≤10.1	323	≤0.6	5.0	0.8	>12.8	20	80	≤0.018	>10
	48	9.24	>18.5	≤10.1	>323	≤0.6	5.0	0.8	>12.8	20	80	0.30	>10
M135-88 (blood)	24	0.58	4.62	≤10.1	161	≤0.6	2.5	0.8	>12.8	20	>80	0.07	>10
	48	1.16	>18.5	≤10.1	>323	≤0.6	5.0	0.8	>12.8	20	>80	0.30	>10
M1181-82 (blood)	24	0.58	18.5	≤10.1	>323	≤0.6	1.25	0.8	>12.8	5	80	≤0.018	10
	48	1.16	>18.5	≤10.1	>323	≤0.6	2.5	0.8	>12.8	10	>80	0.035	>10
R-888 (blood)	24	≤0.14	0.58	≤10.1	>323	≤0.6	1.25	0.4	>12.8	10	>80	0.035	10
	48	0.58	4.62	≤10.1	>323	≤0.6	2.50	1.6	>12.8	10	>80	0.035	>10

[a] Institutional sources of isolates: TCM National Institutes of Health, Bethesda, Md.; TSA, University of Texas Health Science Center at San Antonio; 82-100, 82-102, and 82-112, Montana State University, Bozeman; M297-87, M135-88, M1181-82, and R-88, New York State Department of Health, Albany.
[b] Time elapsed between inoculation and incubation in broth.
[c] Index case 1.
[d] Index case 2.

they were 0.8 to 12.8 μg/ml. Amphotericin B was tested in antibiotic medium 3, flucytosine was tested in buffered yeast nitrogen base, and azoles were tested in synthetic amino acid medium-fungal (SAAMF; American Biorganics Inc., North Tonawanda, N.Y.). The tubes were inoculated with 50 μl of the inoculum suspension and incubated at 35°C for 48 h. MICs and minimum lethal concentrations (MLCs) were measured at 24 and 48 h. The MLC was determined by dispensing and streaking 10 μl of broth from those tubes exhibiting no growth onto Sabouraud glucose agar for each concentration. The MIC was defined as the smallest concentration of antifungal compound in which growth was not observed. The MLC was defined as the smallest concentration of antifungal compound from which three or fewer colonies were present on the Sabouraud glucose agar plate. All tests were run in duplicate.

(iv) Timed kill curve studies. To characterize the pattern of amphotericin B resistance in *T. beigelii* further, timed kill studies were performed. Isolates of *T. beigelii* from both index cases were further examined by timed kill studies against amphotericin B and flucytosine, alone and in combination. These studies were performed in SAAMF in order to provide a medium suitable for the study of amphotericin B and flucytosine alone and in combination. Three concentrations of amphotericin B (1.0, 4.0, and 20.0 μg/ml) and one concentration of flucytosine (50 μg/ml) were studied.

The inoculum for the timed kill study was prepared by growing the isolate for 48 h at 37°C on Sabouraud glucose agar, inoculating colonies into a starter broth of 50 ml of Sabouraud glucose broth, and incubating the colonies for 2 h in a shaking water bath at 37°C. A total of 1 ml of this suspension was transferred into 50 ml of fresh SAAMF broth in each of four 250-ml Erlenmeyer flasks and incubated at 37°C for 16 h. The suspension was centrifuged, washed three times, adjusted for concentration with a hemacytometer, inoculated into 250-ml Erlenmeyer flasks containing 50 ml of SAAMF broth alone or SAAMF plus antifungal compound to yield a final concentration 3.0×10^5 CFU/ml, and confirmed by quantitative culture. These flasks were incubated simultaneously in a shaking water bath at 37°C. The growth suspensions were sampled at 0, 2, 4, 6, and 24 h and plated in dilutions of 10^0, 10^{-2}, 10^{-3}, and 10^{-4} on Sabouraud glucose agar. Colonies were counted after 48 h of incubation at 37°C, and the calculated CFU per milliliter was plotted for each time point.

(v) Assays of antifungal agents. Amphotericin B was measured in the serum obtained from case 1 by a bioassay as described previously (8, 9). Briefly, this procedure measured the antifungal activity of amphotericin B in serum by an agar-well diffusion assay with *Paecilomyces varioti* ATCC 36257. A semilogarithmic standard curve was plotted by using four known amphotericin B concentration standards (0.125, 0.25, 0.5, and 1.0 μg/ml). The zone of inhibition was measured for each sample of serum and plotted along the standard curve. Concentrations of amphotericin B greater than 1.0 μg/ml were determined by analysis of serially diluted samples. The coefficient of variance for replicate samples was 3.6%.

Flucytosine also was measured in the serum obtained from case 1 by a bioassay as described previously (8, 9). Concentrations of flucytosine were measured in the same manner as those of amphotericin B. The antifungal activity of flucytosine in serum was determined by using an agar-well diffusion assay with *Candida kefyr* ATCC 46764. A semilogarithmic standard curve was plotted by using known flucytosine concentration standards (2.5, 5.0, 10, 20, 40, and 80 μg/ml).

RESULTS

MICs and MLCs. The MICs and MLCs of amphotericin B and other antifungal compounds against TCM, TSA, and the other isolates of *T. beigelii* are summarized in Table 1. At 24 and 48 h, all strains of *T. beigelii* showed inhibition by safely achievable (≤2.0 μg/ml) concentrations of amphotericin B in serum. However, seven of the nine strains, including the two

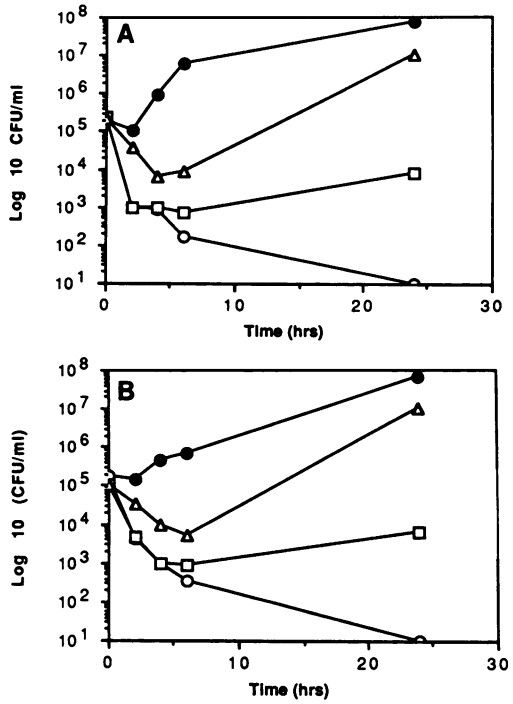

FIG. 3. Timed kill curves of *T. beigelii* TCM (A) and TSA (B) at different concentrations of amphotericin B. Amphotericin B against strain TCM showed initial inhibition and then growth at 1.0 μg/ml, sustained inhibition but no killing at 4 μg/ml, and killing of >99.9% CFU/ml at 20 μg/ml (A). Amphotericin B against strain TSA showed initial inhibition and then growth at 1.0 μg/ml, sustained inhibition but no killing at 4 μg/ml, and killing of >99.9% CFU/ml at 20 μg/ml (B). Symbols: ●, SAAMF; ○, amphotericin B at 20 μg/ml; □, amphotericin B at 4 μg/ml; △, amphotericin B at 1 μg/ml.

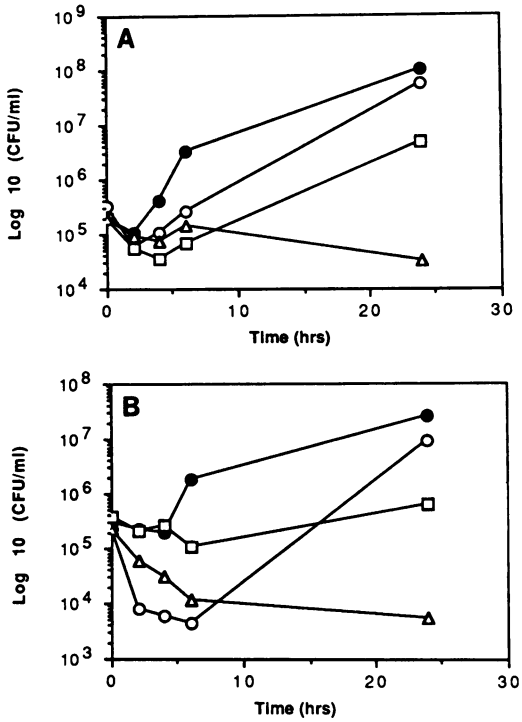

FIG. 4. Timed kill curves of *T. beigelii* TCM (A) and TSA (B) with the combination of amphotericin B and flucytosine. The combination of amphotericin B at 1.0 μg/ml plus flucytosine at 50 μg/ml produced a >90 but <99% decline of CFU per milliliter and was more active than either amphotericin B or flucytosine alone at the same concentrations against TCM (A). The combination of amphotericin B at 1.0 μg/ml plus flucytosine at 50 μg/ml produced a >90 but <99% decline of CFU per milliliter and was more active than either amphotericin B or flucytosine alone at the same concentrations against TSA (B). Symbols: ●, SAAMF; ○, amphotericin B at 1 μg/ml; □, flucytosine at 50 μg/ml; △, amphotericin B and flucytosine.

index case isolates, were not killed by safely achievable concentrations of amphotericin B at 24 and 48 h. The MLCs for the two index case isolates were 4.62 and 18.5 μg/ml at 24 h and ≥18.5 μg/ml at 48 h. All isolates were inhibited by flucytosine, but not of them were killed by flucytosine at therapeutically achievable concentrations in serum. Most isolates, including those from the two index cases, were inhibited at achievable concentrations of miconazole, ketoconazole, itraconazole, and fluconazole in serum. Miconazole was the most actively fungicidal compound at achievable concentrations in serum.

Timed kill studies. Timed kill curves of both index case isolates were similar (Fig. 3). At 1.0 μg of amphotericin B per ml, initial inhibition and then growth occurred. At 4.0 μg/ml, sustained inhibition but no killing was observed. Killing of >99.9% CFU per milliliter occurred only at 20 μg/ml. The combination of amphotericin B at 1.0 μg/ml plus flucytosine at 50 μg/ml produced a >90% but <99% decline of CFU per milliliter and was more active than either amphotericin B or flucytosine alone at the same concentrations. The combination of amphotericin B (1.0 μg/ml) and flucytosine (50 μg/ml), however, did not achieve complete killing of *T. beigelii*. The only completely fungicidal agent was amphotericin B alone at the highest concentration (20 μg/ml) (Fig. 4).

Concentrations in serum. Simultaneous concentrations of amphotericin B and flucytosine in serum were measured in the first patient (case 1) 0.5 h after infusion of amphotericin B on days 2 and 4 of combination antifungal chemotherapy. The concentrations of amphotericin B in serum were 1.13 and 1.40 μg/ml, and those of flucytosine were 30 and 28.3 μg/ml, respectively. The concentrations of amphotericin B in serum were at least eight times the MIC at 24 h and twice the MIC of strain TCM at 48 h. By comparison, the concentrations of amphotericin B in serum were ≤24% of the MLC at 24 h and ≤6% of the MLC at 48 h.

DISCUSSION

Isolates of *T. beigelii* from the two index cases of lethal disseminated trichosporonosis refractory to amphotericin B were found to be resistant to amphotericin B by macrodilution and timed kill studies. The isolates of *T. beigelii* from these index cases were inhibited but not killed by safely achievable concentrations of amphotericin B in serum. The fact that *T. beigelii* is resistant to amphotericin B may be more common than has been previously appreciated, especially given the fact that five of seven other medical and veterinary isolates demonstrated this same pattern of amphotericin B resistance. There did not appear to be any attenuation of virulence associated with amphotericin B resistance. Both patients used as cases in this study demonstrated progressive renal, pulmonary, and hemodynamic deterioration, despite the administration of amphotericin B.

Resistance to amphotericin B associated with the fatal

outcome of disseminated trichosporonosis in both granulocytopenic patients in this study would have been predicted by the MLCs but not by the MICs. Strain M1181-82 (Table 1) was also from a granulocytopenic patient with acute myelogenous leukemia who died with disseminated trichosporonosis while receiving amphotericin B. The MIC at 24 h was 0.58 μg/ml and MLC at 24 h was >18.5 μg/ml. Thus, the MLC may be a more important determinant in predicting the activity of antifungal compounds against *T. beigelii* in granulocytopenic patients, in whom fungicidal activity would be especially important for a successful outcome.

Inhibition but not killing of resistant *T. beigelii* isolates by amphotericin B may be inadequate in granulocytopenic patients, in whom there are no granulocytes to facilitate host clearance and in whom fungicidal activity is needed. Timed kill curves of amphotericin B against the two index case isolates demonstrated killing only at the highest concentration of amphotericin B (20 μg/ml). This concentration of amphotericin B desoxycholate is not achievable in serum, even at the highest tolerated dosages. Although *T. beigelii* was treated more effectively in vitro by the combination of amphotericin B plus flucytosine than it was by either compound alone, the fungicidal effect was not as complete as that of amphotericin B at the highest levels. Thus, antifungal synergy may still permit the persistence of *T. beigelii* if the combination is not completely fungicidal.

The time at which MICs and MLCs were determined (24 versus 48 h) influenced the percentage of isolates found to be resistant to amphotericin B. Four isolates were resistant (MLC, ≥2.0 μg/ml) at 24 h and eight isolates were resistant at 48 h. This difference between values at 24 and 48 h may reflect a selection over time of a more amphotericin B-resistant subpopulation. The source of the isolate may also be a factor related to amphotericin B resistance. Four of the five blood isolates of *T. beigelii* had patterns of resistance to amphotericin B for which the MLC was ≥2.0 μg/ml at both 24 and 48 h. By comparison, among the four veterinary (canine) or mucosal (vaginal and stool) isolates, none had MLCs of ≥2.0 μg/ml at 24 h. Moreover, among resistant isolates, the MLC was higher for blood isolates than for the nonblood isolates at 24 and 48 h. Four of five blood isolates had MLCs to amphotericin B of ≥18.5 μg/ml at 24 or 48 h, in comparison with one of four nonblood isolates. These findings suggest a pattern for more resistant strains of *T. beigelii* isolated from blood, in comparison with strains of *T. beigelii* isolated from mucosal or veterinary sources. Perhaps the MLC read at 24 h may have been sufficient to detect most blood isolates of *T. beigelii* that are resistant to amphotericin B.

Resistance to amphotericin B has been associated with a fatal outcome in other mycoses, including disseminated candidiasis caused by amphotericin B-resistant strains of *C. lusitaniae* (5) and *C. guilliermondii* (3). Another study in bone marrow transplant recipients demonstrated that isolates of *C. albicans* and *C. tropicalis* with intermediate in vitro resistances to amphotericin B were associated with a significantly higher proportion of fatal disseminated candidiasis that was refractory to amphotericin B (14).

We suggest that recovery of *T. beigelii* from blood, tissue biopsy, or urine specimens in the clinical microbiology laboratory should be regarded as clinically significant until proven otherwise. A dialog between clinical microbiologists and clinicians caring for such patients should promptly plan further evaluation, including repeat blood cultures and evaluation of serum for cross-reacting antigen by the cryptococcal latex agglutination assay. Cell wall antigens of *T. beigelii* cross-react with those of *Cryptococcus neoformans*, resulting in antigenemia that is detectable by cryptococcal antigen latex agglutination assays (10, 11). An elevated cryptococcal latex agglutination reaction in a granulocytopenic patient with trichosporonemia is consistent with disseminated trichosporonosis. Cryptococcal latex agglutination reaction titers were positive to 1:256 in case 1 and 1:8 in case 2 in our study. If repeated blood cultures continue to yield *T. beigelii* while the patient is receiving amphotericin B, then modifications of antifungal therapy are warranted.

Antifungal susceptibility studies of isolates of *T. beigelii* from immunocompromised patients, especially those with refractory fungemia, may provide useful data in guiding antifungal therapy. If such studies are to be performed, both MICs and MLCs would be valuable. Should the findings from these studies indicate that the isolate of *T. beigelii* is inhibited but not killed by the usually achievable concentrations of amphotericin B in serum, then other therapeutic options may be considered, e.g., increasing the dose of amphotericin B, adding flucytosine to amphotericin B, administering an antifungal azole, or using liposomal amphotericin B. It is not known whether antifungal susceptibility data will improve the outcomes of patients with trichosporonosis. Nevertheless, use of high doses of amphotericin B with flucytosine has proved useful in other types of refractory mycoses, including invasive pulmonary aspergillosis (2) and hyalohyphomycosis caused by *Fusarium* spp. (13). Whether these approaches are applicable to *T. beigelii* with elevated MLCs remains to be determined. New strategies for disseminated trichosporonosis, however, are clearly needed. The in vitro activity of miconazole suggests that it may have promising activity against *T. beigelii*; however, the new antifungal triazoles, such as itraconazole or fluconazole, may also be important alternatives to amphotericin B. Antifungal triazoles may still have in vivo activity against *T. beigelii*, despite reduced or absent in vitro activity. Susceptibility data from isolates of *T. beigelii* that cause refractory disseminated trichosporonosis in immunocompromised patients may provide useful information in guiding antifungal therapy. While antifungal susceptibility testing is currently not standardized, progress continues to be achieved in this area and reference mycology laboratories may provide reproducible results.

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