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Clinical Case of Endocarditis due to *Trichosporon inkin* and Antifungal Susceptibility Profile of the Organism

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A fatal case of *Trichosporon inkin* prosthetic endocarditis is reported. The isolation sites and susceptibility profiles of 10 other isolates are also reviewed. Four strains were recovered from cutaneous or subcutaneous samples, four were recovered from urine, one was recovered from peritoneal liquid, and one was recovered from bone. Voriconazole and amphotericin B had the most potent activities in vitro against the isolates, with MIC geometric means of 0.11 and 0.30 μg/ml, respectively.

CASE REPORT

A 52-year-old male underwent biological valve replacement for an insufficient aortic valve in October 1987. In May 2001, he developed valvular dysfunction with heart failure, and a second aortic valve replacement was performed. Fifteen months later, the patient presented to the Emergency Department with skin lesions on both hands, swelling of the right wrist, and articular pain. Physical examination revealed a temperature of 37.8°C and a leukocyte count of 12.8×10^6 per liter. Blood culture samples were taken, and the patient was discharged with the diagnosis of cellulitis and placed on amoxicillin-clavulanate therapy. Two days later, he came to the Infectious Diseases outpatient clinic with new skin lesions. An examination showed Osler's nodes in the fingers of both hands and in the feet and splinter hemorrhages on the nail folds. Transthoracic echocardiography showed a huge vegetation along the rim of the prosthetic aortic valve. After two new sets of blood cultures were performed, antibiotic therapy with vancomycin (1 g/12 h), gentamicin (80 mg/8 h), and rifampin (600 mg/day) was instituted. Forty-two hours later, the patient developed left hemiparesis and was transferred to another hospital for cardiovascular surgery. Unfortunately, after another 30 hours, he suffered a cardiopulmonary arrest and died before the surgery could be performed. An autopsy was not done.

No growth was detected in the broth blood cultures after 7 days of incubation, but subcultures on Sabouraud glucose agar with gentamicin and chloramphenicol yielded colonies of a cream-colored organism after 48 h of incubation. Microscopic examination revealed budding cells and arthroconidia. Urease activity was positive. The API ID32C clinical yeast identification system (Biomerieux SA, Marcy-L'Etoile, France) was used to identify the isolate. With this commercial kit, we obtained the code 525764371, which is listed in the API ID32C database as a very good identification of *Trichosporon inkin*. However, the strain did not show growth on *N*-acetylglucosamine (the carbon source), a test by which the majority of *T. inkin* strains included in the API database are positive. Therefore, the isolate was sent to the Mycology Laboratory of the National Center for Microbiology of Spain for definitive identification and susceptibility testing.

Identification of the isolate. At the National Center for Microbiology, the strain was labeled as CNM-CL-4811 (Spanish Centro Nacional de Microbiología yeast culture collection). The isolate was identified by routine physiological tests: fermentation of and growth on carbon sources, growth on nitrogen sources, growth at various temperatures, and the ability to hydrolyze urea (27).

Antifungal susceptibility testing. The susceptibility testing followed the National Committee for Clinical Laboratory Standards recommendations for microdilution procedures but included modifications described previously (18, 22). These modifications have recently been included in the recommendations of the European Committee on Antibiotic Susceptibility Testing of the European Society of Clinical Microbiology and Infectious Diseases (21) for antifungal susceptibility testing of fermentative yeasts. Briefly, the susceptibility testing included RPMI 1640 supplemented with 2% glucose as the assay medium, an inoculum size of 10^5 CFU/ml, flat-bottomed trays, and spectrophotometric readings. All microplates were wrapped with film sealer to prevent the medium from evaporating, attached to an electrically driven wheel inside the incubator, agitated at 350 rpm, and incubated at 30°C for 48 h. *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as quality control strains.

The antifungal agents used in the study were as follows: amphotericin B (AMB) (Sigma Aldrich Quimica S.A., Madrid, Spain), 5-flucytosine (5FC) (Sigma Aldrich Quimica), fluconazole (FCZ) (Pfizer S.A., Madrid, Spain), itraconazole (ITZ) (Janssen S.A., Madrid, Spain), and voriconazole (VOR) (Pfizer S.A.). AMB, FCZ, VCZ, and ITZ were dissolved in 100% dimethyl sulfoxide (Sigma Aldrich Quimica). 5FC was

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TABLE 1. Characteristics of *T. inkin*[a]

Carbon source	Reaction after growth for 14 days[b]
Glucose	+
Galactose	+ or −
L-Sorbose	+ D
D-Glucosamine	−
Xylose	+
L-Arabinose	+ or −
D-Arabinose	+ or −
L-Rhamnose	−
Sucrose	+
Maltose	−
Trehalose	+ or −
Cellobiose	+
Salicin	+ D
Melibiose	−
Lactose	+ D
Raffinose	−
Melezitose	+ D
Starch	+ or −
Glycerol	+ or −
Erythritol	+ D
Ribitol	−
Xylitol	+ or −
L-Arabinitol	+ or −
D-Glucitol	+ or −
D-Mannitol	+ D
Galactitol	−
Inositol	+
2-Keto-D-gluconate	+
D-Gluconate	+
D-Glucuronate	+
DL-Lactate	+
Citrate	+ D
Methanol	−
Ethanol	+
Nitrate	−
Nitrite	−
Ethylamine	+
Cadaverine	+
Creatine	−
Cycloheximide (0.01%)	+

[a] All strains grew at 25, 30, 35, 37, and 40°C and hydrolyzed urea. Colonies were white to cream and finely cerebriform. Budding cells and hyphae disarticulated into rectangular or cylindrical arthroconidia. There was no sexual reproduction.

[b] D, delayed reaction; + or −, positive or negative reaction depending on strain analyzed.

TABLE 2. Isolation sites and antifungal susceptibility test results for *T. inkin* isolates analyzed

Strain	Isolation site	MIC (μg/ml) of:				
		AMB	5FC	FCZ	ITZ	VOR
CNM-CL-225	Bone	0.25	32.0	2.0	0.50	0.06
CNM-CL-306	Urine	0.12	0.25	8.0	1.0	0.50
CNM-CL-696	Skin	0.50	16.0	2.0	0.25	0.06
CNM-CL-1996	Urine	1.0	>64.0	2.0	0.25	0.12
CNM-CL-3538	Subcutaneous abscess	1.0	>64.0	32.0	2.0	0.50
CNM-CL-3915	Peritoneal liquid	0.50	32.0	1.0	0.06	0.03
CNM-CL-3916	Urine	0.25	>64.0	2.0	0.25	0.12
CNM-CL-4074	Urine	1.0	4.0	4.0	0.12	0.12
CNM-CL-4157	Skin	0.25	>64.0	1.0	0.06	0.12
CNM-CL-4518	Skin	0.06	8.0	2.0	0.25	0.03
CNM-CL-4811	Blood	0.06	1.0	2.0	1.0	0.25

dissolved in sterile distilled water. All drug stock solutions were frozen at −70°C as 100× stocks until they were used.

The MICs were determined spectrophotometrically at 24 and 48 h. MICs were obtained by measuring the absorbance at 530 nm with an MRXII reader (Dynatech; Cultek, Madrid, Spain). For AMB, the MIC endpoints were defined as the lowest drug concentrations exhibiting a reduction in growth of 90% or more compared with the growth of the control. For 5FZ and the azole drugs, the MIC endpoint was defined as the concentration producing 50% inhibition (7).

Collection of *T. inkin* strains. A collection of 10 clinical isolates was analyzed with the purpose of comparing the biochemical and susceptibility profiles of these isolates with those of the strain causing this case of endocarditis. All strains were recovered during a period of 5 years (1 January 1998 to 31 December 2002) from nine Spanish hospitals. Each clinical isolate represented a unique isolate from a patient. The isolates were sent to the Mycology Laboratory of the National Center for Microbiology of Spain for antifungal susceptibility testing, with a referral note including clinical and microbiological data. Identification and susceptibility testing of isolates were performed as stated above. Four strains were recovered from cutaneous or subcutaneous samples, four were obtained from urine, one was obtained from peritoneal liquid, and one was obtained from a biopsy of bone.

Clinical strains were subcultured on Sabouraud glucose agar. After 10 days at 30°C, colonies were butyrous to membranous and finely cerebriform. Microscopic examination revealed budding cells and hyphae which disarticulated into rectangular and cylindrical arthroconidia. Appressoria were present. Morphological, physiological, and biochemical descriptions of *T. inkin* are listed in Table 1. Results of some physiological tests varied depending on the strain analyzed. Galactose, L-arabinose, D-arabinose, trehalose, starch, glycerol, xylitol, L-arabinitol, D-glucitol, and D-glucuronate were used as carbon sources for some isolates only, but distinct groups were not evident since physiological differences were noted for each isolate.

Table 2 displays MICs of antifungal agents tested. The table includes both MICs for the strain causing endocarditis (CNM-CL-4811) and susceptibility results for the collection of isolates. AMB and VOR exhibited the best activities in vitro against the isolate responsible for the endocarditis case, with MICs of 0.06 and 0.25 μg/ml, respectively. In addition, that strain (CNM-CL-4811) was susceptible in vitro to 5FC, FCZ, and ITZ.

With regard to the collection of *T. inkin* isolates, a wide range of MICs was observed for each antifungal agent. The geometric mean (GM) of MICs of AMB was 0.30 μg/ml (range, 0.06 to 1 μg/ml). The AMB MIC was 1 μg/ml for three strains. No differences in susceptibility were seen for different isolation sites. Seven strains were resistant in vitro to 5FC, and the GM of 5FC MICs for these strains was 17.04 μg/ml. The GMs of MICs of FLZ and ITZ were 2.7 and 0.3 μg/ml, respectively. VOR exhibited a potent in vitro activity (GM of 0.11 μg/ml), with MICs that ranged between 0.03 and 0.50 μg/ml.

The MICs of the five antimicrobial agents for the quality control organisms were consistently within 2 or 3 twofold di-

lutions of each other. No differences were observed between MICs obtained by the NCCLS reference procedure and those achieved by the modified method (18).

The genus *Trichosporon* has recently undergone extensive taxonomic reevaluation. Several morphological and biochemical patterns were recognized among clinical and environmental isolates of *Trichosporon beigelii* (13). Ultrastructural and DNA studies confirmed those findings, and *T. beigelii* has been divided into a number of distinct species (10). The genus *Trichosporon* includes six species pathogenic for humans: *Trichosporon asahii*, *Trichosporon asteroides*, *Trichosporon cutaneum*, *Trichosporon inkin*, *Trichosporon mucoides*, and *Trichosporon ovoides*.

Trichosporon species are causative agents of cutaneous infections and are involved in systemic, localized, or disseminated mycoses, particularly in patients with underlying hematological malignancy (9). *T. inkin*, formerly *Sarcinosporon inkin*, was first reported from scrotal dermatitis in a young male, and it has been suggested that white piedra is caused by this species (12, 15, 23, 24). In addition, *T. inkin* has rarely been reported as a cause of deep fungal infections, but in recent years, a number of severe illnesses have been documented for patients presenting with factors which predispose them to infection. The spectrum of severe human mycoses includes progressive pneumonia (11), lung abscess (20), peritonitis associated with continuous ambulatory peritoneal dialysis (5, 14), vascular access infection (17), and endocarditis (4).

We report a new case of *T. inkin* endocarditis and review sources of isolation and the antifungal susceptibility profile of a collection of *T. inkin* strains which were received at the Mycology Laboratory of the National Center for Microbiology of Spain over the last 5 years. The details of these analyses and their implications with regard to the isolation and the identification of this microorganism are the subject of this work.

There are few reports about endocarditis caused by *Trichosporon* spp., and most of the previously reported cases referred to *T. beigelii* without differentiating among the distinct species of *Trichosporon*. Mooty et al. (16) reviewed clinical characteristics of 11 cases of endocarditis caused by *T. beigelii*. Nine of the cases affected prosthetic valves. Clinical symptoms became apparent between 2 months and 7 years after surgical valve implantation, and embolic complications were observed in 27% of the patients. The medical management of these patients included surgical valve replacement and antifungal therapy, with AMB being prescribed in most of the cases. The prognosis was very poor, with a mortality rate of 82%. This number is significantly higher than the mortality rates reported for prosthetic valve endocarditis caused by other organisms (25 to 60%) (1). Our patient and another previously reported patient with endocarditis due to *T. inkin* (4) showed symptoms and an outcome similar to those described by Mooty et al., indicating that distinct *Trichosporon* spp. may cause cases of endocarditis with like clinical features.

Our patient was probably infected during the operation, as has been described for other prosthetic infections (1). *Trichosporon* spp. do not appear to be associated with the hospital environment. They are found in soil and water and are occasionally found in the human flora, normally associated with skin and nails. Consequently, it is possible that the organism colonized the prosthesis or another intravascular device from the human flora during valve replacement.

Symptoms began 15 months after the surgical valve replacement, and infection was defined as a late prosthetic endocarditis. A remarkable clinical feature of our patient was his presentation with skin lesions that, unfortunately, were overlooked. A skin biopsy might have shown findings consistent with fungal infection, leading to empirical antifungal therapy.

The treatment of clinical forms of systemic trichosporonosis has been generally unrewarding. Therapeutic failures have been described for immunosuppressed patients treated with AMB. For example, a 1990 study defined *T. beigelii* as an emerging pathogen resistant to this antifungal compound (25). Therapy with other systemic antifungal agents was usually inefficient, as well (2, 6). After the taxonomic reevaluation of the genus *Trichosporon*, *T. asahii* is thought to be much more common in cases of systemic infection than other *Trichosporon* spp. (26); notably, *T. asahii* seems to be less susceptible in vitro to AMB than other species (2, 19). The clinical case series on *T. beigelii* infection reported in 1990 (25) probably included *T. asahii* cases, and because of that study, *T. beigelii* has been considered an AMB-resistant organism. However, *Trichosporon* spp. other than *T. asahii* are susceptible in vitro to the polyenes. In this study, MICs of AMB for the *T. inkin* isolates tested ranged from 0.06 to 1 μg/ml, as reported before, and were lower than those observed for *T. asahii* (0.25 to 8 μg/ml) (2, 19).

Regarding azole agents, *Trichosporon* spp. exhibit varied susceptibility profiles, although azole compounds appear to be more potent in vitro than AMB (2, 19). Successful outcomes for treatments with VOR have been documented for some cases of deep trichosporonosis (6; R. Falk, D. G. Wolf, M. Shapiro, and I. Polacheck, Letter, J. Clin. Microbiol. **41:**911, 2003). In addition, a combination of AMB and FCZ was useful in treating systemic infections due to these species (3, 8). In this study, 10 strains of *T. inkin*, including that causing the endocarditis case, were susceptible in vitro to FCZ and ITZ. VOR revealed the most potent activity in vitro against all the isolates, with MICs of ≤0.5 μg/ml. Unfortunately, the patient described in this report died before the trichosporonosis was diagnosed, and antifungal therapy had not yet been prescribed.

The response to therapy of invasive mycosis depends mainly on the correction of the underlying disease, yet susceptibility testing may be able to identify which organisms are unlikely to respond to certain antimicrobial agents. The majority of strains of *T. inkin* are susceptible in vitro to all systemic antifungal compounds, with VOR and AMB being the most active in vitro. It is important to recognize that susceptibility testing does not reliably predict the in vivo response to therapy for human infections. Host factors may have more impact on the infection than on susceptibility results. However, in the case presented herein, the patient was not even treated, underlining the need for methods for the early detection of fungal infections.

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