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Catheter-Related Fungemia Due to *Candida thermophila*

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We report a case of bloodstream infection caused by *Candida thermophila*, a yeast not previously associated with human disease. The infection occurred in a 13-year-old boy with medulloblastoma who presented with 1 day of fever. Multiple blood cultures were positive for yeast. Removal of the catheter resulted in prompt resolution of the fever and sterilization of the blood cultures. The species was identified by sequencing domains 1 and 2 of the large subunit rRNA gene. Antifungal susceptibility testing was also performed.

CASE REPORT

A 13-year-old boy with medulloblastoma presented to the emergency department because of a 1-day history of fever up to 39.3°C, decreased oral intake, and increased fatigue. The tumor was diagnosed 14 months prior to his presentation. The patient was treated according to the Children's Oncology Group A-9961 protocol with surgical resection followed by reduced-dose craniospinal irradiation and alternate cycles of cisplatin, vincristine, and cyclophosphamide. The last cycle was given 3 weeks prior to his presentation. A central venous catheter, in place for a year, was used for administration of chemotherapy and hyperalimentation. The patient also received *Pneumocystis jiroveci* pneumonia prophylaxis with trimethoprim-sulfamethoxazole (160 mg of the trimethoprim component twice daily for three consecutive days each week).

Physical examination showed a febrile but otherwise well-appearing boy. The central line site showed no signs of infection or inflammation. Total white blood cell count was 4,300/mm^3 with 3,400 neutrophils/mm^3 and 560 band forms/mm^3, hemoglobin was 8.1 g/dl, and platelets were 59,000/mm^3. Findings on a chest radiograph were normal. A blood culture was drawn from the central line; the patient was given a dose of ceftriaxone and was sent home. The blood culture grew yeast after 24 h, and the patient was called and admitted to the hospital. At that time he was still well appearing and afebrile. An additional set of central and peripheral blood cultures was obtained, and administration of intravenous liposomal amphotericin (AmBisome) at 200 mg (5 mg/kg of body weight) once a day was begun. Altogether, eight sets of standard blood cultures (BACTEC Peds plus/F and standard anaerobic/F for each) and four sets of fungal blood cultures (ISOLATOR 1.5; Wampole Laboratories) were drawn over a 5-day period, and nine (five of the standard blood culture bottles and all fungal cultures) grew yeast. Sterilization of the blood was achieved only following removal of the central venous catheter on the fifth day of the antifungal therapy. The patient completed 6 weeks of liposomal amphotericin therapy and recovered without complications.

Laboratory findings. The yeast isolate from the patient grew after 24 to 48 h of incubation at 37°C. The colonies were moist and white in color. The germ tube test was negative. No hyphae or pseudohyphae were observed. The isolate was evaluated by the Microscan Walkaway system with a yeast identification plate (Dade Behring) and the API 20C AUX system (bioMerieux). Both gave an identification of *Hansenula polymorpha*. When biochemical reactions were run independently of the rapid systems, the isolate was negative for urease and positive for nitrate and glucose. The yeast grew at 37 and 42°C but not at 50°C.

Since the yeast could not be identified satisfactorily with the Microscan and API identification systems, DNA sequencing was conducted to provide identification. The isolate was identified as *Candida thermophila* (9) from its unique DNA sequence in domains 1 and 2 (D1/D2) of the large subunit rRNA gene by the National Center for Agricultural Utilization Research in Peoria and by the University of Texas Health Sciences Center in San Antonio. As described earlier (5, 6), genomic DNA was extracted from the yeast cells and combined with primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5′-GGTCCGTGTTTCAAGACGG) in a PCR. The resulting D1/D2 amplicon of ca. 600 nucleotides in length was purified, and both DNA strands were sequenced using primers NL-1 and NL-4 and an ABI (Applied Biosystems) automated DNA sequencer. The GenBank accession number for this sequence is DQ402185. The sequence of the isolate differed from that of *Candida thermophila* (GenBank accession AF283568) by one nucleotide. Other phylogenetically closely related organisms included *Pichia salicis* (GenBank accession AF403148; 99% identity), a presently undescribed species, and *Pichia angusta* (GenBank accession U75524; 98% identity) (4, 6). Our isolate has been deposited with the ARS Culture Collection as NRRL Y-27863 and with the American Type Culture Collection (ATCC MYA-3665).

Antifungal drug susceptibility testing was performed by the broth microdilution method based on the CLSI (formerly NCCLS) guidelines (8). Briefly, RPMI medium was used. The inoculation size was 5×10^4 CFU. MICs were read

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TABLE 1. MIC and minimum fungicidal concentration results

Drug	MIC (μg/ml) at 24 h	MIC (μg/ml) at 48 h	MFC[a] (μg/ml)
Amphotericin	≤0.03	0.125	0.25
Caspofungin	0.12	0.5	1
Flucytosine	0.12	2	≥128
Fluconazole	1	2	16
Itraconazole	0.06	0.25	0.5
Ketoconazole	≤0.03	0.125	1
Voriconazole	≤0.03	≤0.03	≤0.03

[a] MFC, minimum fungicidal concentration.

at 24 and 48 h by comparing the turbidity of test wells to that of the untreated controls. A change in turbidity equal to or greater than 90% compared to drug-free control results was used to establish MIC breakpoints. The minimal fungicidal concentration results were obtained by recording colony counts on plates. Results are summarized in Table 1.

Discussion. Invasive candidiasis is an important cause of morbidity and mortality in chronically or critically ill patients (2, 3). Infections caused by *Candida* species are the fourth most common cause of nosocomial bloodstream infection in the United States (1, 11), with species other than *Candida albicans* emerging as pathogens. The non-*C. albicans* yeasts are often associated with resistance to antifungal azoles and with higher mortality. We describe the first reported case of *Candida thermophila* causing a human infection.

C. thermophila was described as a thermophilic soil yeast capable of growth at 50°C (9). Although the current isolate did not grow at 50°C, it did grow well at 37 and 42°C. Since it is difficult to identify this species with either commercial or conventional biochemical assays, this characteristic of growth in elevated temperature can be an indication for further analysis such as rRNA gene sequencing. Identification of yeasts from the large subunit rRNA gene D1/D2 sequence comparisons has been highly reliable. Strains of the same species ordinarily show only 0 to 3 nucleotide differences (6), but a few exceptions to this pattern have been found. For example, *Candida guilliermondii* and *Candida fermentati* differ by 3 nucleotides in large-subunit D1/D2 but show only 40% relatedness when compared by nuclear DNA reassociation (10). Consequently, these two taxa are closely related but not conspecific. The current isolate was identified as *C. thermophila* based on its close genetic similarity to the type strain of this species. The single nucleotide difference with the type strain has been interpreted as intraspecies strain variation. Gene sequence analysis has been successfully used for the identification of pathogenic fungi in addition to analysis of morphological and biochemical characteristics (7).

Although this is the first reported case of *C. thermophila* causing candidemia in a human, this may not be the first case of invasive disease due to this recently identified yeast, because identification and differentiation of yeasts on the basis of morphological and biochemical characteristics can be difficult. Therefore, the incidence and prevalence of this organism and its pathogenic role might be underestimated.

Many *Candida* species causing invasive infections have been non-*C. albicans* yeasts such as *C. krusei* and *C. glabrata*. These species can be inherently (primarily) or secondarily resistant to fluconazole and may be more difficult to treat. The isolate from our patient was susceptible to all antifungals in vitro, and the patient was treated successfully with liposomal amphotericin, although fluconazole might have been as effective.

In summary, as the population of immunocompromised hosts grows, organisms previously not considered as pathogens might cause invasive disease. *C. thermophila* should be added to the long list of yeasts that can cause bloodstream infections in the immunocompromised or critically ill patient.

Nucelotide sequence accession number. The sequence of the D1/D2 amplicon described in this study has been deposited under GenBank accession no. DQ402185.

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