Use of DNA Sequencing Analysis To Confirm Fungemia Due to *Trichosporon dermatis* in a Pediatric Patient


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This is the first reported case of human disease caused by *Trichosporon dermatis*, an organism recently transferred to the genus *Trichosporon* from *Cryptococcus* and now confirmed to be a human pathogen.

**CASE REPORT**

A 13-month-old male with a history of autoimmune enteropathy developed a fever of unknown origin (FUO) and malaise. The patient was the product of a full-term uncomplicated pregnancy and delivery. At 9 days of life he had a rotavirus infection and subsequently developed a malabsorption-dysmotility syndrome. The etiology of his enteropathy was unknown, but it was suspected to be autoimmune. He had been on immunosuppressant therapy, including prednisolone and tacrolimus; and his caloric needs had been maintained with total parenteral nutrition through a central venous catheter (CVC) since his first month of life. Numerous bacterial line infections had required catheter replacement, and at the time of this presentation he had a Broviac catheter in place in his right internal jugular vein. His physical examination was notable for rhinitis with a temperature of 101.0°F, and the differential diagnosis for his FUO included viral illness versus bacterial infection. The patient had a normal total white blood count of 5.01 × 10⁶ per liter, with relative lymphopenia (54%) and neutrophilia (73%). Following the collection of blood samples for microbiologic analysis, the patient was started on intravenous vancomycin therapy.

The blood specimens were taken from both the central line and a peripheral vein and were inoculated into BACTEC Plus Aerobic/F culture vials and processed in a blood culture system (BACTEC 9240 fluorescent series instruments; BD Diagnostic Systems, Sparks, MD). Growth was detected in the blood cultures after 48 h, with gram-positive organisms and yeast observed by microscopic examination. Subcultures were performed onto Trypticase soy sheep blood agar, which grew a coagulase-negative *Staphylococcus*, and CHROMagar, which produced mucoid mauve-colored colonies at 30°C after 24 h of incubation. Microscopic examination of subcultures grown on cornmeal agar revealed hyphae with the formation of arthroconidia and lateral and terminal blastoconidia (Fig. 1). The organism was found to be urease positive on Christensen's urea and displayed robust growth on Mycosel agar containing 0.01% cycloheximide. Urease positivity and cycloheximide resistance combined with the macroscopic and microscopic mor-

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alignment was performed by using the online BLAST 2 program (National Center for Biotechnology Information, Bethesda, MD), and the sequence obtained from the amplification of the IGS1 region of the isolate was compared to the nucleotide sequence in GenBank for the IGS1 region of *Trichosporon mucoides* (GenBank accession number AB066433) (Table 2). The length (357 bp) obtained for the amplified region was consistent with that for *Trichosporon mucoides*. However, the amplicon showed a significant divergence from the published sequence, including five gap regions; and in the area where there was sufficient homology for comparison, only 196 of 224 (87%) bases matched. At this point it was observed that the IGS1 region for *Trichosporon dermatis*, a species recently added to the genus *Trichosporon* (10), is also 357 bp in length. BLAST comparison of the amplicon with the two identical published IGS1 sequences for *Trichosporon dermatis* (GenBank accession numbers AB066412 and AB072613) showed 99% homology with a difference of only 2 bp (C to T at position 243 and T to C at position 323). The 13-bp species-specific sequence contained within the ITS1 region for *Trichosporon dermatis* was also compared to that of the amplicon and was found to be identical (Table 1). Further analysis showed that the carbohydrate assimilation profiles generated by the VITEK system and the ID32C yeast identification system (bioMerieux) for the isolate were consistent with the profile published for *Trichosporon dermatis* (10). The isolate has been deposited in the American Type Culture Collection (Manassas, VA) under catalog number MYA-3671.

Over the past decade, increasing numbers of systemic human infections by *Trichosporon* spp. have been reported, particularly in susceptible immunocompromised patients (7). In this setting virtually any one of the *Trichosporon* spp. could serve as the causative agent.

### TABLE 1. Comparison of the ITS1 species-specific sequences from *T. mucoides* and *T. dermatis* with the sequence of the blood isolate

<table>
<thead>
<tr>
<th><em>Trichosporon</em> sp.</th>
<th>ITS1 sequence</th>
<th>Accession no.</th>
<th>% Homology to isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. mucoides</em></td>
<td>TCGGTCGAAAATTACT</td>
<td>AB018030</td>
<td>92</td>
</tr>
<tr>
<td><em>T. mucoides</em></td>
<td>TCGGTCAAATTACT</td>
<td>AB018031b</td>
<td>100</td>
</tr>
<tr>
<td><em>T. dermatis</em></td>
<td>TCGGTCAAATTACT</td>
<td>AB05581</td>
<td>100</td>
</tr>
</tbody>
</table>

*a* Clinical isolate.  
*b* Environmental isolate.

### TABLE 2. Comparison of IGS1 regions from *T. mucoides* and the *T. dermatis* isolate

<table>
<thead>
<tr>
<th><em>Trichosporon</em> sp.</th>
<th>Accession no.</th>
<th>% Homology to isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. mucoides</em></td>
<td>AB066433</td>
<td>87b</td>
</tr>
<tr>
<td><em>T. dermatis</em></td>
<td>AB066412c</td>
<td>99</td>
</tr>
<tr>
<td><em>T. dermatis</em></td>
<td>AB072613c</td>
<td>99</td>
</tr>
</tbody>
</table>

*b* The lengths of all sequences were 357 bp.  
c Five gap regions were revealed by nucleotide sequence comparison of the IGS1 region from the isolate to the GenBank sequence for *T. mucoides* (BLAST2 program, National Center for Biotechnology Information). Only 224 bp had sufficient homology for comparison, and 196 of 224 (87%) bases matched.  
c There is 100% base pair homology between the two accession numbers for *T. dermatis* IGS1 region sequences.
Trichosporon has been considered to be medically relevant (3). Most reported cases of Trichosporon fungemia have previously been considered to be nonpathogenic species (4). Disseminated trichosporonosis has also been caused by the presumably nonpathogenic species Trichosporon loubieri (5, 6).

The present case describes fungemia in an immunosuppressed pediatric patient, initially reported as Trichosporon mucoides by morphological and biochemical analysis and retrospectively identified by genetic analysis as Trichosporon dermatis. Although there are a number of case reports in the literature of disseminated infections caused by Trichosporon mucoides, there are to date no case reports of fungemia due to Trichosporon dermatis. These two pathogens share morphological and biochemical characteristics and have virtually identical carbohydrate assimilation profiles (10). Thus, positive identification of the species level can practically be achieved only by analyzing the interspecies divergence revealed at the DNA level. The current classification system makes species-specific identification of Trichosporon feasible, and it has been recommended that species identification be attempted for all relevant clinical isolates (5), as Trichosporon spp. with various antifungal susceptibilities are isolated from increasing numbers of patients (2).

The patient described in this report was treated with voriconazole, to which he had an excellent clinical response. This may be attributed to the efficacy of this drug, the timely removal of the CVC source of infection, and the patient’s normal granulocytic response to fungemia. His tacrolimus level was therapeutic at the time of presentation, and although the patient had a relative lymphopenia, the relative neutrophilia revealed by the leukocyte differential was appropriate for the infection. Tacrolimus inhibits T-lymphocyte activation through interleukin-2 (4) and probably has little direct effect on the phagocytic oxidative burst or bone marrow production of granulocytes.

Despite a steady increase in the number of cases, Trichosporon sepsis is still relatively rare and is often not suspected as the causative agent in a blood culture positive for yeast. Even when trichosporonosis or another emerging mycosis is suspected, positive identification of the causative agent presents a diagnostic challenge because these organisms share morphological and biochemical features with many of the more commonly isolated pathogens. Techniques derived from molecular biology, such as DNA sequencing, can thus complement more traditional laboratory methods for the identification of pathogens (8). In this case report, biochemical and physiologic analysis as well as molecular analysis of the ITS1 sequence incorrectly identified the isolate as T. mucoides. However, sequencing of the IGS1 region correctly identified T. dermatis, supporting the findings of the recent study by Rodriguez-Tudela et al., which demonstrates that sequencing of the IGS1 region is the superior method for the molecular identification of Trichosporon isolates at the species level (7). Although IGS1 sequencing is not always available to the clinical microbiology laboratory, microscopy, susceptibility testing, and traditional biochemical identification methods are adequate for timely diagnosis and management in the majority of cases. However, when it is feasible, identification of the organism at the species level can positively influence patient care, particularly in the future choice of antifungal therapy for specific Trichosporon spp. with demonstrable resistance to certain antifungal drugs.

Nucleotide sequence accession numbers. The rRNA gene sequences of the IGS and ITS1 regions of the clinical isolate described here (isolate UH4277) have been deposited in GenBank under accession numbers DQ228143 and DQ228144, respectively.

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REFERENCES