**Candida quercitrusa Candidemia in a 6-Year-Old Child**

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We present the first case of candidemia due to *Candida quercitrusa* in a pediatric patient. The identification of the isolate was protracted and ultimately dependent upon sequence analysis of the internal transcribed spacer region. To further define the antifungal susceptibility characteristics of this species, we performed antifungal susceptibility testing of clinical and type strains. In light of the antifungal susceptibility testing results, we caution against the use of fluconazole for treating *C. quercitrusa* infections.

**CASE REPORT**

The patient was a 6-year-old male with a history of malignant sacrococcygeal teratoma requiring sacrectomy and radiation therapy during infancy. He had subsequently developed neurogenic bladder, requiring a Mitrofanoff appendicovesicostomy, and neurogenic bowel, requiring a diverting ileostomy. He had gastroparesis with chronic emesis and was therefore dependent on gastrojejunal feeds and total parenteral nutrition (TPN). His medical history was notable for multiple infectious complications involving his central venous line (CVL), and he had received multiple courses of micafungin, voriconazole, and fluconazole separately in time from 2009 onward. Five months prior to admission, he had been diagnosed with a *Mycobacterium abscessus* tunnel infection of his CVL with an associated chest wall abscess. The abscess had been incised and drained, and the patient had been placed on chronic azithromycin therapy. During the same hospitalization, he was found to have *Candida glabrata* candidemia (the isolate was identified in house), which was treated with liposomal amphotericin B at a dosage of 3 mg/kg of body weight every 24 h initially, followed by 4 mg/kg micafungin every 24 h for a total of 14 days. Two months prior to admission, he was hospitalized with *Candida famata* candidemia (the isolate was identified at an outside institution), and he received 2 mg/kg micafungin every 24 h for 6 days, followed by 9 mg/kg voriconazole every 12 h for 10 days based on antifungal susceptibility testing data. His contaminated CVL was removed and a new peripherally inserted central catheter (PICC) was inserted 6 weeks prior to admission. He was then placed on oral nystatin (300,000 units twice a day) for antifungal prophylaxis.

The patient presented to our facility with low-grade fevers that had persisted for approximately 1 month. His laboratory values were notable for thrombocytopenia (platelets, 92,000/µL) and a mildly elevated level of C-reactive protein (3.9 mg/dL). Three independent aerobic blood cultures (Bact/Alert Pediatric FAN; bioMérieux, Marcy l’Etoile, France) obtained from his PICC line on hospital days 1, 2, and 5 grew yeasts on hospital days 4, 5, and 10, respectively. The yeast isolates were prepared for identification by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) on the Bruker Biotyper platform (Bruker Daltonics, Bremen, Germany) using an extraction method (1). When queried against the database (Biotyper version 3.1.66 [5,627 entries]), the resultant mass spectra returned no identification (i.e., a log[score] value of <1.7). Therefore, the internal transcribed spacer (ITS) region (including ITS-1, the 5.8S rRNA gene, and ITS-2) of the initial isolate obtained on day 4 of hospitalization was amplified using PCR and sequenced (2). Sequencing data were queried against GenBank (3), and the best match returned was *Candida quercitrusa* type strain CBS 4412, with 100% identity (556 bp/556 bp), while the next best match was *Candida natalensis* strain UCMA3722, with 95.6% identity (498 bp/521 bp). Therefore, these data are highly supportive of a species-level identification of *C. quercitrusa*.

Antifungal susceptibility testing was attempted using the Sentitre YeastOne YO9 panel (Thermo Scientific [TREK], Lenexa, KS, USA); however, no growth was detected after 48 h incubation in ambient air at 35°C. The team managing the patient initiated empirical therapy with 2 mg/kg micafungin intravenously every 24 h. Given the patient’s dependence on catheters, a decision was made to retain his PICC line for the duration of therapy, which was 15 days in total. An echocardiogram and dilated funduscopic exam showed no evidence of disseminated candidiasis. Upon completion of antifungal therapy, the patient’s PICC line was removed and a new CVL was placed. His fevers resolved and he returned to his clinical baseline.

To the best of our knowledge, this is only the second report describing an infection due to *C. quercitrusa* in humans (PubMed [http://www.ncbi.nlm.nih.gov/pubmed]; terms “Candida” and “quercitrusa”). In the initial report by Xiao and colleagues, three independent cases of candidemia due to *C. quercitrusa* were documented in a single institution within a 2-month period (4). Initially, the isolates were identified using the Vitek 2 YST system (bioMérieux) as *Candida pulcherrima* (89% probability) and by
the API 20C AUX system (bioMéreux) as Candida lusitaniae (86.2% identification), while MALDI-TOF MS (Bruker Biotype platform) was unable to identify the isolates. Ultimately, the isolates were identified as C. quercitrusa using nucleic acid-based methods. In agreement with the study by Xiao and coworkers, the identification of our isolates was protracted and ultimately dependent upon DNA sequencing of the ITS region. The routine identification system in our laboratory, MALDI-TOF MS (Bruker Biotype platform), failed to identify the isolates due to the absence of C. quercitrusa in the database; however, importantly, the isolates were not misidentified.

To further describe the C. quercitrusa-specific characteristics of strains isolated from clinical specimens, the isolate obtained on hospital day 4 was analyzed using a combination of microscopic and phenotypic methods. The microscopic morphology of the isolate on cornmeal-Tween 80 agar (Thermo Scientific [Remel], Lenexa, KS, USA) after incubation at 30°C in ambient air for 4 days using the Dalmau plate method revealed pseudohyphae constricted by septae. Growth in Sabouraud brain heart infusion agar (Thermo Scientific [Remel]), and Sabouraud brain heart infusion agar with sheep blood supplemented with gentamicin and chloramphenicol (Thermo Scientific [Remel]), inhibited mold agar (Thermo Scientific [Remel]), and Sabouraud brain heart infusion agar (Thermo Scientific [Remel]) but no growth on mycobiotic agar (Thermo Scientific [Remel]). Finally, the Vitek 2 YST card yielded an identification of C. pulcherrima (89% probability; bioinnumber, 440014441100170) with a good confidence level, while the API 20C AUX v4.0 system generated a doubtful identification (profile, 614235) of either Candida parapsilosis (61.7% identification), C. lusitaniae (19.6% identification), or Candida dubliniensis (16.9% identification).

As described previously, antifungal susceptibility testing was initially attempted using the Sensititre YeastOne YO9 panel; however, due to insufficient growth at 35°C, the testing was terminated. As part of our characterization effort, we attempted to define the antifungal susceptibility profile of the initial C. quercitrusa isolate obtained prior to the administration of antifungal therapy during this hospitalization (i.e., the isolate obtained on day four of hospitalization) using the Vitek 2 AST-YS06 card (bioMéreux). Due to insufficient growth, antifungal testing using the Vitek 2 AST-YS06 card was terminated for all agents except caspofungin after 36 h incubation. The resultant MIC for caspofungin was ≥4 μg/ml.

Subsequently, we performed antifungal susceptibility testing (AST) on RPMI 1640 agar with MOPS (morpholinepropanesulfonic acid) and 2% glucose (Thermo Scientific [Remel]) at 30°C and 35°C using the Etest (bioMéreux) method as recommended by the manufacturer. MICs were recorded after 48 h incubation. In addition to the initial clinical isolate obtained on day 4 of hospitalization, we also tested the C. quercitrusa type strain (C. quercitrusa ATCC 564665) and quality control organisms (Candida dubliniensis ATCC 90028, C. krusei ATCC 6258, and C. parapsilosis ATCC 22019). Data are tabulated in Table 1. Quality control testing at both temperatures was acceptable according to the MIC ranges specified by the manufacturer. For all quality control organisms, there were no major differences between MICs recorded at 30°C and at 35°C, and all MICs were equivalent or within one 2-fold dilution difference. However, with the exception of amphotericin B, and micafungin for the clinical isolate, the antifungal agent MICs for the C. quercitrusa isolates were notably different at 30°C and 35°C. The MICs recorded after growth at 30°C were elevated compared to those after growth at 35°C. Based on the results of quality control testing and the in vitro growth characteristics of C. quercitrusa, we believe that this difference can be explained by the poor growth of this organism at 35°C rather than the enhanced activity of the antifungal agents at 35°C.

Using the manufacturer’s interpretive guidelines, the susceptibility profiles of both C. quercitrusa isolates were determined. Both C. quercitrusa isolates tested susceptible to fluconazole and micafungin at 30°C and 35°C. The clinical isolate tested susceptible to voriconazole at both temperatures, while the type strain was

### Table 1: Antifungal susceptibility testing of the C. quercitrusa clinical isolate, C. quercitrusa type strain, and quality control organisms after 48 h of incubation at 30°C and 35°C using the Etest method

<table>
<thead>
<tr>
<th>Organism</th>
<th>Amphotericin B</th>
<th>Fluconazole</th>
<th>Flucytosine</th>
<th>Micafungin</th>
<th>Voriconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans ATCC 90028</td>
<td>0.25 ± 0.25</td>
<td>0.25 ± 0.25</td>
<td>2 ± 1.5</td>
<td>0.012 ± 0.016</td>
<td>0.012 ± 0.008</td>
</tr>
<tr>
<td>C. krusei ATCC 6258</td>
<td>0.5 ± 0.5</td>
<td>0.25 ± 0.25</td>
<td>&gt;32 ± 32</td>
<td>0.125 ± 0.19</td>
<td>0.25 ± 0.38</td>
</tr>
<tr>
<td>C. parapsilosis ATCC 22019</td>
<td>0.38 ± 0.38</td>
<td>0.125 ± 0.125</td>
<td>0.125 ± 0.19</td>
<td>0.125 ± 0.19</td>
<td>0.016 ± 0.032</td>
</tr>
<tr>
<td>C. quercitrusa ATCC 564665</td>
<td>0.125 ± 0.125</td>
<td>&gt;256 ± 4</td>
<td>0.19 ± 0.004</td>
<td>1.5 ± 0.25</td>
<td>0.125 ± 0.125</td>
</tr>
<tr>
<td>C. quercitrusa clinical isolate</td>
<td>0.25 ± 0.25</td>
<td>0.25 ± 0.25</td>
<td>2 ± 1.5</td>
<td>0.094 ± 0.004</td>
<td>0.5 ± 0.5</td>
</tr>
</tbody>
</table>

**a** Interpretive criteria were as follows: for amphotericin B, not available; for fluconazole, susceptible, ≤8 μg/ml; susceptible-dose dependent, 16 to 32 μg/ml; resistant, ≥64 μg/ml; for flucytosine, susceptible, ≤4 μg/ml; intermediate, 8 to 16 μg/ml; resistant, ≥32 μg/ml; for micafungin, susceptible, ≤2 μg/ml; for voriconazole, susceptible, ≤1 μg/ml; susceptible-dose dependent, 2 μg/ml resistant, ≥4 μg/ml.

**b** Quality control MIC ranges (in micrograms per milliliter) for C. albicans ATCC 90028: amphotericin B, 0.125 to 0.5; fluconazole, 0.125 to 0.5; flucytosine, 0.5 to 2; micafungin, 0.004 to 0.032; voriconazole, 0.004 to 0.016.

**c** Quality control MIC ranges (in micrograms per milliliter) for C. krusei ATCC 6258: amphotericin B, 0.125 to 0.5; fluconazole, 128 to 256; flucytosine, 32 to 64; micafungin, 0.032 to 0.25; voriconazole, 0.25 to 1.

**d** Quality control MIC ranges (in micrograms per milliliter) for C. parapsilosis ATCC 22019: amphotericin B, 0.25 to 1; fluconazole, 1 to 8; flucytosine, 0.064 to 0.25; micafungin, 0.25 to 2; voriconazole, 0.016 to 0.064.

**e** The isolate exhibited very poor growth, making MIC interpretation questionable.
resistant to voriconazole at 30°C (MIC, 4 μg/ml) and susceptible at 35°C (MIC, 0.125 μg/ml). The most dramatic difference in antifungal AST was observed for fluconazole. The C. quercitrusa type strain exhibited high-level fluconazole resistance at 30°C (MIC, >256 μg/ml) yet tested susceptible at 35°C (MIC, 4 μg/ml). The clinical isolate tested susceptible-dose dependent at 30°C (MIC, 24 μg/ml) and susceptible at 35°C (MIC, 4 μg/ml). We believe that elevated MICs of fluconazole at 30°C are a species-specific trait, as this characteristic was observed in both isolates, including the type strain, which was originally isolated from an insect (4). Importantly, while there are no interpretive guidelines for amphotericin B, both C. quercitrusa isolates exhibited low MICs for amphotericin B at 30°C and 35°C, thus implying susceptibility in vivo. It is unclear if the temperature-dependent AST results observed in vitro are clinically significant, as the poor in vitro growth kinetics of C. quercitrusa at 35°C make disseminated infection highly unlikely. However, C. quercitrusa could readily infect sites of low temperature, e.g., skin, or colonize a catheter, and considering our AST data, fluconazole would be a poor candidate for therapy.

Candida species are a significant cause of bloodstream infections, and in pediatric patients, Candida albicans and C. parapsilosis are the most common agents of candidemia (6). However, as the number of patients with profound immunosuppression continues to rise, invasive infections due to uncommon non-C. albicans species are also increasing (7). In this report, we document the first pediatric case of candidemia due to C. quercitrusa. The patient had several underlying comorbidities and had a long history of antifungal therapy. In addition, he was receiving TPN and had an intravascular catheter. Interestingly, two of the three patients documented by Xiao and colleagues also had intravascular catheters and were receiving TPN (4). Therefore, the presence of an intravascular catheter and TPN administration could be risk factors for infection with this species.

Micafungin was empirically used to treat our patient and appeared to sterilize the patient’s PICC line blood cultures. Subsequent AST data revealed that the clinical isolate tested susceptible to micafungin. However, as peripheral blood cultures remained negative for C. quercitrusa throughout the patient’s hospitalization, we believe that the PICC line was the source of the infection and only upon removal of the PICC line was the C. quercitrusa infection eliminated. In the setting of candidemia, it is generally advised that intravascular catheters be removed (8), but depending on the patient’s underlying condition, this is not always immediately possible.

In summary, we present only the second report describing C. quercitrusa infection in humans. At present, it is apparent that molecular methods are required for the identification of this species, but mass spectrometric methods could be useful upon inclusion of this species in mass spectrometric databases. Both the type strain and the assayed clinical isolate demonstrated elevated fluconazole MICs at 30°C. Furthermore, the clinical isolates characterized by Xiao and colleagues displayed fluconazole MICs between 16 and 32 μg/ml (4). Therefore, we caution against the use of fluconazole for treating C. quercitrusa infections.

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REFERENCES


