CASE REPORTS

Fungemia Caused by Zygoascus hellenicus in an Allogeneic Stem Cell Transplant Recipient

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Zygoascus hellenicus (Candida helenica) was isolated from a blood culture from a patient who had received an allogeneic stem cell transplant. The isolate displayed an antifungal susceptibility pattern of decreased susceptibility to fluconazole and itraconazole, high susceptibility to voriconazole, and low susceptibility to caspofungin. The organism was misidentified by a commercial yeast identification system. This is the first reported case of human infection with this rare ascomycetous yeast.

CASE REPORT

A 49-year-old man, born in Siberia, presented for medical care in Russia in August 2001 with a large mediastinal mass. He was diagnosed as having non-Hodgkin’s lymphoma and was begun on cytoxan, etoposide, adriamycin, and prednisone chemotherapy. His disease continued to progress, and he was treated from December 2001 to February 2002 with methotrexate, adriamycin, cyclophosphamide, vincristine, prednisone, and bleomycin and intrathecal administration of methotrexate and cytosine arabinoside. Radiation therapy was given in April 2002.

The patient was a Moscow University mathematician who for 10 years had come to the University of Michigan every summer to work as a visiting professor. In September 2002, while he was in Michigan, his lymphoma relapsed with new lung nodules and masses noted in his thorax, abdomen, and pelvis. He was given three courses of ifosfamide, carboplatin, and etoposide, with rituximab for two of the three courses. Allogeneic stem cell transplant from a sister, who lived in Siberia, was performed on 4 December 2002. Engraftment occurred by 14 December. He had received meropenem, vancomycin, and levofloxacin, which were stopped on 24 December. He was additionally treated with ganciclovir for a positive cytomegalovirus shell vial antigen test from bronchoalveolar lavage fluid.

On 25 December, a blood culture that had been drawn on 22 December was noted to be positive for a yeast identified as Candida ciferri by the use of the API 20-C test and cornmeal agar. Other than fever, no specific signs or symptoms were attributable to the fungemia. Bronchoalveolar lavage fluid from 1 January 2003 also yielded the same yeast. Caspofungin had been started empirically on 23 December, and it was decided to continue therapy with that drug until 8 January 2003. The central venous catheter, which was present when fungemia occurred, was retained.

The posttransplant course was complicated by recurrent episodes of diffuse alveolar hemorrhage requiring repeated intubation and ventilator dependence, acute renal failure requiring continuous venous-venous hemofiltration, graft-versus-host disease in the colon, atrial fibrillation, sepsis with Klebsiella oxytoca and Enterococcus faecalis, cholestatic jaundice, recurrent rectal bleeding, and ischemic bowel. He died from multiple complications on 26 February 2003.

Mycological studies. The isolate was received at the Fungus Reference Unit, Centers for Disease Control and Prevention (CDC), as part of a surveillance for invasive fungal infections in transplant recipients (P. G. Pappas, J. Morgan, R. A. Hajjeh, and the Transplant Associated Infection Surveillance Network [TRANSNET], Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1010, 2003). The isolate grew on Sabouraud dextrose agar at 25, 30, and 35°C as cream-colored colonies with dry sectors. On ChromAgar Candida (DRG International) at 30°C, cream-colored colonies appeared by 48 h; by 5 days these colonies were pale pink with a pale blue center. After 72 h, an API 20-C instrument gave a result of 6673276 (positive for assimilation of glucose, glycerol, arabinose, xylose, adonitol, xylitol, galactose, inositol, sorbitol, cellubiose, maltose, sucrose, trehalose, and raffinose), which coded as very good identification for C. ciferri. A cornmeal Dalmau plate showed pseudohyphae with blastospores after 72 h. DNA sequencing was performed as previously described (2) using NL-1 and NL-4 primers which amplified the D1-D2 region of

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the large ribosomal subunit (7). When 540 nucleotides of D1-D2 sequence were used to query the GenBank database, a 99% match was obtained to *Zygoascus hellenicus* AJ508584 and AJ508566 (bit scores 1013: three gaps and one mismatch). This degree of divergence is consistent with the observation made by Kurtzman and Robnett that members of the same species show 0 to 2 nucleotides of divergence in this region of DNA (7). The query sequence also matched that of *Stephanoascus ciferri* (C. ciferri), but at the much lower bit score of 432.

The isolate was sent to the Centraalbureau voor Schimmelcultures for confirmation of identity. The routine identification procedures of the Centraalbureau voor Schimmelcultures included the examination of growth on carbon sources with the ID32C version 2.0 kit (BioMérieux, Paris, France). The numerical profile of 7471774257 was obtained after 3 days, an incubation time applied at the Centraalbureau voor Schimmelcultures that was longer than that recommended by the manufacturer. This profile coded for *Candida helenica*. Finally, the isolate was mated with both mating types of *Z. hellenicus*. Asci and ascospores were produced after mating with the α-mating-type tester strain CBS 6726, thus confirming the identity as a representative of *Z. hellenicus* mating type A. The isolate has been deposited as CBS 9640.

MICs of fluconazole, itraconazole, voriconazole, and caspofungin were determined at CDC by the NCCLS broth microdilution reference procedure (9). The MIC of amphotericin B was determined by the E-test method (12). MIC endpoints were read visually at 48 and 72 h due to slow growth of the organism and were assessed relative to the growth control as a prominent decrease in growth (–50%) for the azoles and complete inhibition of growth for caspofungin and amphotericin B. The fluconazole MIC was 8 μg/ml at 48 h and 16 μg/ml at 72 h. This result was interpreted as susceptible-dose dependent based on NCCLS interpretive criteria (9, 12). The MIC of itraconazole was 0.25 μg/ml at 48 and 72 h and was also interpreted as susceptible-dose dependent (9, 12). The MIC of voriconazole was 0.06 μg/ml at 48 h and 0.12 μg/ml at 72 h. The MIC of caspofungin was 16 μg/ml at both 48 and 72 h. The mean fungicidal concentration was determined for caspofungin by plating 100 μl from the MIC well, one well before and two wells after, onto Sabouraud dextrose agar and incubating it at 35°C for 48 h. The mean fungidal concentration of caspofungin was defined as the drug concentration resulting in less than five colonies and was >32 μg/ml for this isolate. The amphotericin MIC was 0.032 μg/ml at 48 h and 0.064 μg/ml at 72 h.

Although no interpretive breakpoints exist for voriconazole, caspofungin, and amphotericin B, the MIC of amphotericin B for this isolate is significantly below the threshold for resistance, which has been suggested as a MIC of ≥0.5 μg/ml by E-test (12). Likewise, the MICs of voriconazole of 0.06 and 0.012 μg/ml were significantly within the range where most rare *Candida* isolates were inhibited when 314 such strains were tested (MIC at which 90% of the isolates tested are inhibited [MIC90], ~1 μg/ml) (11). When caspofungin was similarly tested against a large number of clinical isolates of *Candida* spp., the majority of isolates were inhibited by 1 μg of this drug/ml (MIC90 of 1 μg/ml), and the least susceptible isolates were those of *Candida guilliermondii*, at a MIC90 of >8 μg/ml (10). At a MIC of 16 μg/ml, this *Zygoascus* isolate displays a similar property of low susceptibility to caspofungin.

**Conclusions.** Fungal agents have long been recognized as significant pathogens in hematopoietic stem cell transplant recipients. Although the majority of systemic yeast infections in these patients are caused by common *Candida* species such as *C. albicans* and *C. glabrata*, infections with less common yeast species have been noted (1, 15). Stem cell transplant recipients commonly receive fluconazole prophylaxis, which is thought to contribute to decreases in systemic disease with species known to be susceptible to this agent (8). Increases in infection with fluconazole-resistant isolates have been observed at some institutions using this prophylaxis regimen (1, 8). In addition to receipt of fluconazole prophylaxis, this patient had also developed bacteremia, cytomegalovirus infection, and graft-versus-host disease, all additional risk factors for development of candidemia with an azole-resistant organism (8).

*Z. hellenicus* (anamorph *C. hellenicus*) has been described from fermenting grape must, a case of bovine mastitis, and various environmental sources. Other synonymous species include *Trichosporon hellenicum*, *Candida steatolytica*, *Candida inositophila*, and *Pichia hangzhouana* (14). To our knowledge, this is the first description of this species as a human pathogen. This report demonstrates that unusual or previously unrecognized organisms may be recovered in this population of patients highly susceptible to fungal infection. These organisms may be misidentified by commonly used commercial identification systems available to clinical laboratories in the United States. This organism was not included in the API 20-C database but was present in the database of the API 32-C, which is not available to clinical laboratories in the United States. When low-prevalence or uncommon yeast isolates are found using commercial systems, the identification should be confirmed by DNA-based methods. In this case, DNA sequence data failed to confirm the identification developed by conventional biochemical testing and pointed instead toward a different taxon.

For this organism, the MIC of caspofungin was much higher than that for most *Candida* species infrequently isolated from blood (10). However, it appeared that the fungemia cleared with treatment with caspofungin alone, thus calling into question the correlation of in vitro susceptibility test results with in vivo outcomes for candidemias. This lack of correlation has been noted previously (12, 13). On the other hand, if in vitro antifungal susceptibility results are viewed in the context of the patient’s overall clinical picture, such results might prove helpful in select cases, especially those of infections due to very unusual pathogens. It should also be noted that *Z. hellenicus* has α-D-linked galactomannans in its cell wall (3), which could explain its resistance to caspofungin, a 1-3-β-D-glucan synthesis inhibitor. Such structural biochemical information may be useful in predicting the susceptibilities of unusual organisms to various classes of antifungals. This also emphasizes the value of correct species identification in predicting antifungal susceptibility when MIC testing is unavailable or unrevealing.

Clinical microbiology staff should be vigilant for the appearance in this patient population of organisms previously not recognized as human pathogens. Such agents have been previously noted (5) and include many soil, water, and airborne fungal plant pathogens or commensals that can cause severe life-threatening infections in the appropriate compromised human host. These organisms may present with atypical morphol-
logy or may not sporulate when plated on common fungal culture media, due to prior antifungal drug exposure or inability to complete their life cycles in the laboratory. Clinical microbiology staff should be aware of such possibilities in examining fungal organisms isolated from this susceptible patient population. In previous surveillance studies, we noted that the most prevalent Candida species isolated from blood cultures in four regions of the United States included C. albicans, C. glabrata, Candida parapsilosis, Candida tropicalis, and Candida krusei (4, 6). Most commercial systems perform well in identifying these species and distinguishing them from one another. However, if a patient isolate generates a code not found in the identification system, codes as a species not commonly isolated in that institution, or suggests a species whose characteristic morphological and biochemical features are not familiar to laboratory staff, laboratories should consider sending the isolate to a reference laboratory for confirmation by DNA-based methods. This is particularly important if the organism is isolated from a patient in a particular high-risk category, if the isolate is believed to be the cause of disease in that patient, and if accurate identification to species level is important for infection control or other epidemiologic purposes.

Nucleotide sequence accession number. The D1-D2 DNA sequence corresponding to isolate CBS 9640 has been deposited in GenBank as AY528676.

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REFERENCES