First Reported Case of Catheter-Related Fungemia Due to Candida menguniae

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We report a case of intravenous catheter-associated fungemia caused by the recently described species Candida menguniae, a yeast not previously associated with human disease. The infection occurred in an 89-year-old woman with pancreatic adenocarcinoma. Yeast isolates recovered from a catheter and blood were identified as C. menguniae by sequencing of the 18S, 5.8S internal transcribed spacer, and D1/D2 26S ribosomal DNA domains.

CASE REPORT

An 89-year-old woman was admitted on 8 May 2007 to the emergency department of the Hospital Universitario Nuestra Señora de Candelaria (HUNSC) showing acute abdominal stress with clinical signs of intestinal perforation. Her anamnesis included arterial hypertension, diabetes mellitus (type II) under treatment with oral antidiabetics, chronic arterial fibrillation, and hyperuricemia. She had a body temperature of 37.2°C, a blood pressure of 70/40 mm Hg, and a heart rate of 111 beats per minute. The abdomen was relaxed but painful when examined. An abdominal X-ray showed pneumoperitoneal lesions. Four days before she was admitted to the emergency department, she was examined by colonoscopy because she showed a hemorrhagic lower digestive tract and pains on left side. During that colonoscopy, argon plasma coagulation was administered for angiodysplasia of the colon. Moreover, the patient had chronic hepatitis B surface antigenicity.

The patient’s blood tests gave the following results: hematocrit, 40.3%; hemoglobin, 13.4%; red blood cell count, 4.53 × 10¹²/liter; white blood cell count, 2.2 × 10⁹/liter (neutrophils, 1.52 × 10⁹/liter; lymphocytes, 0.37 × 10⁹/liter); platelet count, 285 × 10⁹/liter; prothrombin time, 18.4 s; prothrombin activity, 54%; international normalized ratio, 1.57; activated partial thromboplastin time, 31.0 s; urea, 61 mg/dl; creatinine, 1.70 mg/dl; sodium, 133.0 mmol/liter; potassium, 4.20 mmol/liter; and normal levels of transaminases, amylase, and cholestasis enzymes.

Surgical intervention evidenced a generalized fecal peritonitis due to a 0.5-cm-diameter perforation. Prophylactic anti-microbial therapy with piperacillin-tazobactam (4 g every 8 h) and metronidazole (500 mg every 8 h) was implemented. During the intervention, abdominal lavage was performed and peritoneal liquid samples were taken and submitted to the microbiology service (HUNSC). Growth was observed after 24 h of incubation at 36°C in an aerobic atmosphere, and two bacteria were recovered from the peritoneal liquid, Escherichia coli and Bacteroides fragilis.

Clinical progression was then positive, without fever, and a central venous catheter was installed for parenteral hyper-alimentation.

Five days after surgery, the patient felt generally worse and showed hemodynamic instability, including septic shock signs with secondary coagulopathy. After an exploratory laparotomy, dirty serum liquid was found and clinical samples were taken from it. The cultures of these samples were positive for E. coli and Pseudomonas aeruginosa.

During abdominal cavity exploration, a 3-cm-diameter malignancy-like lesion was detected in the pancreatic tail. Sampling of biopsy specimens was not possible then because of hemodynamic instability that necessitated cessation of the intervention. CA19-9 marker level testing under high suspicion of adenocarcinoma of the pancreatic tail showed a level of 158,358 UI/ml. Computed tomography confirmed the diagnosis.

On day 14 after the second surgical intervention, the patient was febrile (38.5°C) with trembling. At that time, two sets of blood culture bottles were collected, the central venous catheter was removed, and they were all sent to the microbiology service. Within 48 h, both aerobic blood culture bottles and the central venous catheter were positive for yeast (see below). Intravenous treatment with 200 mg fluconazole/day was implemented for 20 days. After that period, the patient showed clinical improvement and was discharged from the hospital 40 days after admission. Then she was moved to the palliative care unit. The patient died 1 month later as a result of cancer progression and deterioration.

Yeast isolates and phenotypic characterization. Three yeast isolates were recovered from blood cultures and an intravenous catheter at the HUNSC microbiology service. Culture
media, fermentation procedures, and other standard growth tests for yeast taxonomy were previously described by Yarrow (11). Assimilation profiles of each isolate were determined in triplicate using different batches of the ID 32C panel and the ID-YST card for Vitek 2 (bioMérieux; API ID 32C). Fermentation tests were performed with a standardized yeast nitrogen base (Difco, Detroit, MI) (4).

Blood cultures were performed with a continuously monitored noninvasive system, BacT/ALERT (bioMérieux Inc., Durham, NC). The yeasts were subcultured onto Sabouraud chloramphenicol agar (bioMérieux). The three isolates shared a unique cluster of identical phenotypic properties. Colonies that formed on potato dextrose agar over 48 h of incubation at 25°C were white to cream colored, soft, and smooth. When using CHROMagar Candida chromogenic growth medium, we found that the colonies of the three isolates were pink and glossy with pale edges at 48 h; by 5 days, these colonies where pink with a dark center.

After all of these trials, the three isolates were biochemically identified to the species level as C. utilis. Vitek 2 (ID-YST card) identified them as C. utilis (96.80%). In addition, API ID 32C identified them as C. utilis after 48 h (profile 4271250101, 99.8% agreement).

Sequencing and molecular phylogenetic analysis. To confirm the biochemical identification, genomic DNA was isolated from fungal colonies as previously described (5). The 18S ribosomal DNA (rDNA), internal transcribed spacer (ITS; including the 5.8S rDNA), and 26S rDNA D1/D2 domains were sequenced from the PCR products using previously described primers (NS1 and NS5, ITS1 and ITS4, and NL1 and NL4, respectively) (9). Amplicons were sequenced on an ABI PRISM 310 genetic analyzer using a BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems Japan Co. Ltd., Tokyo, Japan) as recommended by the manufacturer. The sequences were compared with those in the GenBank database. The three isolates had identical nucleotide sequences in the three rDNA regions. Based on BLAST searches (National Center for Biotechnology Information), the 18S, 5.8S ITS, and 26S rDNA D1/D2 domain sequences had 99.9% identity with the sequences of C. mengyuniae (EU043157, EU043159, and EU043158) (2). The identity of the 26S rDNA sequences with Williopsis saturnus var. saturnus NRRL Y-17396T was 97.0%, and that with C. utilis NRRL Y-1542T was 92.0%.

Physiological characteristics. The three isolates recovered differed from W. saturnus and C. utilis in their physiological characteristics. The main differences were as follows: arbutin, cellobiose, salicin, and succinate, W. saturnus and C. utilis positive and C. mengyuniae negative; 1-rhamnose and xylose, W. saturnus positive and C. mengyuniae and C. utilis negative; maltose and melezitose, C. utilis positive and C. mengyuniae and W. saturnus negative; α-D-galactose, C. mengyuniae positive and W. saturnus and C. utilis negative; methyl α-D-glucoside and trehalose, C. mengyuniae and C. utilis positive and W. saturnus negative. On the other hand, C. mengyuniae was able to grow at 45°C while the other species were inhibited by that temperature (7, 11).

Antifungal susceptibility testing. For the Sensititre Yeast-One system, a suspension of yeast in demineralized water was adjusted to match the turbidity of a 0.5 McFarland standard by using a Sensititre nephelometer. The Sensititre antifungal susceptibility test method was performed according to the manufacturer’s instructions. Two reference strains, C. parapsilosis ATCC 22019 and C. krusei ATCC 6258, were tested as quality control isolates. The colorimetric MIC was interpreted as the lowest antifungal concentration that corresponded to the first purple or blue well after 24 h of incubation at 35°C (3). MIC values and susceptibility categories were evaluated according to CLSI standards (7).

Table 1 summarizes the in vitro susceptibilities of the three isolates to the eight antifungal agents tested at 24 and 48 h. Results of susceptibility analysis showed the same MIC values for each isolate-drug combination. On the other hand, colony growth was inhibited by 0.01% cycloheximide medium.

The increased incidence of systemic fungal infections in the past 2 decades has been overwhelming. Fungemia is an infection that is usually nosocomial and that has grown as the global population of immunocompromised patients has increased. Among nosocomial mycotic infections, there has been a gradual and significant shift away from Candida albicans toward less frequent species. Candida glabrata, C. parapsilosis, C. tropicalis, and C. krusei, among others, are emerging as opportunistic pathogens (8). Some of these species may display reduced susceptibility to azole antifungal drugs compared to that of C. albicans (10).

This global change in systemic fungal infections has emphasized the need to recognize this increasingly large group of potential fungal pathogens. Less frequent Candida spp. may be misidentified by commonly used commercial identification systems available to clinical laboratories (1, 3, 4). For this reason, DNA-based methods are being largely used for yeast systematics and phylogeny.

In the present work, we recovered three yeast isolates from different clinical specimens from a single patient. The three isolates had identical biochemical patterns and were identified as belonging to a Candida species, most probably C. utilis, by commercial miniaturized methods. Further DNA analyses demonstrated that this identification was erroneous and that the three isolates belong to the recently described species C. mengyuniae, which was originally isolated from metsulfuron-methyl-contaminated soil collected in Jiangsu Province, China (2). This novel species is phylogenetically related to the teleomorphic species W. saturnus (characterized by hat-shaped

### Table 1. Susceptibilities of the three yeast isolates to the eight antifungal agents tested in this study

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/ml)</th>
<th>24 h</th>
<th>48 h</th>
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<tbody>
<tr>
<td>Amphotericin</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
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<tr>
<td>Caspofungin</td>
<td>0.008</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Flucytosine</td>
<td>2</td>
<td>16</td>
<td></td>
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<tr>
<td>Fluconazole</td>
<td>0.5</td>
<td>2</td>
<td></td>
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<tr>
<td>Itraconazole</td>
<td>0.125</td>
<td>0.25</td>
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<tr>
<td>Ketocazole</td>
<td>0.03</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.03</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.25</td>
<td>0.25</td>
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</tbody>
</table>

#### Footnotes:

2. EU043157, EU043159, EU043158
3. National Center for Biotechnology Information
4. BLAST searches
5. GenBank database
6. 18S, 5.8S ITS, 26S rDNA D1/D2 domain sequences
7. CLSI standards
8. Opportunistic pathogens
9. Sensititre Yeast-One system
10. Candida glabrata, C. parapsilosis, C. tropicalis, C. krusei
11. W. saturnus
12. C. utilis
13. C. mengyuniae
14. W. saturnus
15. C. utilis
16. C. mengyuniae
17. Susceptibilities of the three yeast isolates to the eight antifungal agents tested in this study
18. MIC (µg/ml)
19. 24 h
20. 48 h
21. Drug
22. Amphotericin
23. Caspofungin
24. Flucytosine
25. Fluconazole
26. Itraconazole
27. Ketocazole
28. Voriconazole
29. Posaconazole
ascospores or saturnospores) and other species of the genera Candida and Pichia, including C. utilis (teleomorph: Pichia jadinii). However, the sexual state was not observed in C. mengyuniae in any of the sporulation culture media described by Yarrow (11). Kurtzman et al. (6) reanalyzed the phylogenetic relationships among species assigned to the genera Pichia, Issatchenka, and Williopsis by multigene sequence analysis.

Here we describe the first reported case of catheter-related fungemia due to C. mengyuniae. We present a phylogenetic analysis based on rDNA sequence comparisons to undoubtedly certify that the three isolates belong to the new species C. mengyuniae.

**Conclusions.** Systemic fungal infections represent a major cause of morbidity and mortality in immunocompromised patients. Moreover, the increasing role of species other than C. albicans enhances this critical situation. In addition, the intrinsic or potential development of resistance of some emerging species to certain antifungal agents makes rapid identification to the species level especially important for making informed therapeutic decisions about acutely ill patients. Currently available biochemical systems for yeast identification perform admirably with the most commonly encountered species but are inadequate to identify the more unusual potential pathogens.

This report demonstrates that unusual or previously unrecognized organisms may be recovered in a population of patients highly susceptible to fungal infections. These organisms may be misidentified by commonly used commercial identification systems. 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. In this study, we used 5.8S ITS, 18S, and 26S rDNA sequencing to undoubtedly identify yeasts isolates from the patient’s blood and an intravenous catheter that had previously been identified as C. utilis using the API ID 32C (profile 4271250101, 99.8% agreement) and Vitek 2 systems (96.8% agreement).

Comparative rDNA sequence analyses permitted us to conclude that the three isolates belong to the recently described species C. mengyuniae. Thus, this is the first reported case of catheter-related fungemia due to C. mengyuniae; further, this is the first documented human infection with this yeast. These data could be enough to consider C. mengyuniae an opportunistic human pathogen.

**Nucleotide sequence accession numbers.** The 5.8S ITS and 26S rDNA sequences of the HUNSC isolates described here were deposited in the GenBank database under accession numbers EU819146 and FJ456891.

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**REFERENCES**