First Reported Case of Catheter-related fungemia due to *Candida mengyuniae*

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Abstract

We report a case of intravenous catheter-associated funguemia caused by the recently described species *Candida mengyuniae*, a yeast not previously associated with human disease. The infection occurred in an 84-year-old woman with pancreas adenocarcinoma. Yeast isolates recovered from catheter and blood were identified as *C. mengyuniae* by sequencing 18S, 5.8S-ITS and D1/D2-domains of rDNA.
The increased incidence of systemic fungal infections in the past two 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 towards less frequent Candida spp. Candida glabrata, C. parasilopsis, 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 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). By this reason DNA-based methods are being largely used for yeast systematic and phylogeny.

In the present work, we have recovered from a single patient three yeast isolates from different clinical specimens. The three isolates had identical biochemical pattern and were identified as Candida spp., most probably C. utilis, by commercial miniaturized methods. Further DNA analyses demonstrated that such 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 Williopsis saturnus (characterized by hat-shaped ascospores or Saturnispores) and other species of the genus 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) re-analyzed the phylogenetic relationships among species assigned to the genera Pichia, Issatchenka and Williopsis by multigene sequence analysis.
In the herein explained study we describe the first reported case of catheter-related fungemia due to *C. mengyuniae*. We present a phylogenetic analysis based on rDNA sequences comparison to undoubtedly certificate that the three isolates belong to the new species *C. mengyuniae*. 
Case report. An 89 year-old woman patient was admitted on 8th May 2007 at urgencies in the Hospital Universitario Ntra. Sra. de Candelaria (HUNSC) showing acute abdomen 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 presented 37.2°C, 70/40 blood pressures (BP) and 111 beats per minute. The abdomen was relaxed but painful when examined. Abdomen Rx showed pneumoperitoneal lesions. Four days before she attended to urgencies, she was examined by colonoscopy because she showed hemorrhagic lower digestive tract and malaises in left side. During that colonoscopy, APC (Argon Plasma Coagulator) was applied to 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%; haemoglobin, 13.4%; red blood cell count, 4.53E12/L; white blood cell count, 2.2E09/L (neutrophils, 1.52E9/L; lymphocytes, 0.37E9/L); platelet count, 285E9/L; protrombin time, 18.4 s; protrombin activity, 54%; INR, 1.57; TTPA, 31.0 s; urea, 61 mg/dL; creatinin, 1.70 mg/dL; sodium, 133.0 mmol/L; potassium, 4.20 mmol/L; and normal levels of transaminases, amylase and colestasis enzymes.

Surgical intervention evidenced a generalized faecal peritonitis due to a 0.5 cm of diameter perforation. Prophylactic antimicrobial therapy with piperacillin/tazobactam (4 g each 8 h) and metronidazol (500 mg each 8 h), was implemented. During 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 peritoneal liquid: Escherichia coli and Bacteroides fragilis.
Clinical progression was then positive, without fever and a central venous catheter had been positioned for parenteral hyper alimentation.

Five days after surgery, the patient felt generally worst and showed hemodynamic instability, including septic shock criteria with secondary coagulopathy. After 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 of diameter malignant-like lesion was detected in the pancreas tail. Sampling biopsies was not possible then because of hemodynamic instability that obliged to suspend intervention. Under a high suspect of adenocarcinoma in pancreas tail, CA19-9 marker level was asked for and it resulted in 158358 UI/mL. Computed tomography confirmed the diagnostic.

In day 14th after the second surgical intervention, the patient was febrile (38.5°C) with trembling. At the moment, two sets of blood cultures bottles were collected and the central venous catheter was removed, and they were sent to the Microbiology Service. Within 48 h, all two aerobic blood culture bottles and central venous catheter became positive for yeast (see below). At that moment, treatment with 200 mg fluconazol i.v. was implemented for 20 days. After that period, the patient showed clinical improvement and hospital discharge was decided 40 days after her admission to the hospital. Then she was moved to the palliative cares unit. The patient died one month later as a result of cancer progression and deterioration.

**Yeast isolates and phenotypic characterization.** Three yeast isolates were recovered at the Microbiology Service of the HUNSC from blood cultures and intravenous catheter. Culture media, fermentation procedures and other standard growth tests for yeast taxonomy are given by Yarrow (11). Assimilation profiles of each isolate were examined in triplicate using
different batches of ID 32C panel and ID-YST card for Vitek 2 (bioMérieux). Fermentation
tests were performed with a standardized Yeast Nitrogen Base (Difco, Detroit, USA) (4).

Blood cultures were performed with continuously monitored non-invasive system,
BacT/Alert (bioMerieux Inc., Durham, NC). The yeasts were subcultured onto Sabouraud
chloramphenicol agar (BioMérieux). The three isolates shared a unique identical cluster of
phenotypic properties. Colonies on Potato Dextrose Agar (PDA) 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,
glossy with pale edges at 48 h, by five days these colonies where pink with a dark centre.

The biochemical identification for the three isolates at the species level after all these
trials was C. utilis. Identification by Vitek 2 (ID-YST Card) was C. utilis (96.80%). Besides,
identification by API ID 32C after 48 h. was C. utilis (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, ITS region (including the 5.8S rRNA gene) and 26S RNA gene D1/D2 domain were
sequenced from the PCR products using previously described primers (NS1 and NS5; ITS1
and ITS4; NL1 and NL4 respectively) (9). Amplicons were sequenced on an ABI PRISM 310
genetic analyzer using a Big Dye terminator cycle sequencing ready reaction kit (Applied
Biosystems Japan Co. Ltd., Tokyo, Japan) as recommended by manufacturers. The sequences
were compared with those in the GenBank database. The three isolates had an identical
nucleotide sequence in the three ribosomal DNA regions. Based on the BLAST searches
(National Center for Biotechnology Information, USA) the 18S, 5.8S-ITS and 26S rDNA
gene 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 *W. saturnus* var *saturnus* NRRL Y-17396\textsuperscript{T} was 97.0% and with *C. utilis* NRRL Y-1542\textsuperscript{T} was 92.0%.

The 5.8S-ITS and 26S DNA sequences of HUNSC isolates were deposited in the GenBank database with the following accession numbers: EU819146 and FJ456891.

**Physiological characteristics.** The three recovered isolates differed from *W. saturnus* and *C. utilis* in their physiological characteristics. The main differences were: Arbutina, Cellobiose, Salicin, Succinate (positive in *W. saturnus* and *C. utilis*, and negative in *C. mengyuniae*), L-Rhamnose, Xylitol (positive in *W. saturnus* and negative in *C. mengyuniae* and *C. utilis*), Maltose, Melezitose (positive in *C. utilis*, and negative in *C. mengyuniae* and *W. saturnus*) D-Galactose (positive in *C. mengyuniae* and negative in *W. saturnus* and *C. utilis*) Methyl α-D-glucoside, Trehalose (positive in *C. mengyuniae* and *C. utilis*, and negative in *W. saturnus*). On the other hand, *C. mengyuniae* was able to grow at 45°C while other species are inhibited at this temperature (7, 11).

**Antifungal susceptibility testing.** For the Sensititre YeastOne 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* ATCC22019 and *C. krusei* ATCC6258 were tested as quality control isolates. Colorimetric MICs were interpreted as the lowest antifungal concentration that corresponded to the first
purple or blue well after 24 h of incubation at 35ºC (3). The MIC values and the 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 h and 48 h. Results of susceptibilities analysis showed the same MIC values for each isolate/drug combination. On the other hand, colony growth was inhibited on 0.01% cycloheximide medium.

Conclusions. Systemic fungal infections represent a major cause of morbidity and mortality in immunocompromised patients. Moreover, the increasing role of other species different from C. albicans enhances this critical situation. Besides, the intrinsic or potential development of resistance of some emerging species to certain antifungal agents makes rapid species-level identification especially important for informed therapeutic decisions in 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 the
intravenous catheter that had previously been identified as *C. utilis* using API ID 32C (profile 4271250101, 99.8% agreement) and VITEK 2 systems (96.8%).

Ribosomal DNA sequences comparative analyses permitted 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*, even further this is the first time that human body infection by this yeast is documented. These data could be enough to consider *C. mengyuniae* an opportunistic human pathogen.

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References


TABLE 1. Susceptibilities of the three yeast isolates to the eight antifungal agents.

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