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Catheter-related fungemia due to fluconazole-resistant *Candida nivariensis*

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Running title: fluconazole-resistant *Candida nivariensis* candidemia

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We report a case of fungemia due to fluconazole-resistant *Candida nivariensis* (MIC, ≥128 μg/ml). Internal transcribed spacer PCR followed by microchip gel electrophoresis with positive-testing blood culture revealed a unique pattern different from those of other pathogenic yeast.

CASE REPORT

A 70-year-old woman with rheumatoid arthritis was hospitalized for treatment of nutritional disorder in September 2006. The patient had received total parenteral nutrition for two months before admission. On day 20, the patient had a fever of 37.8°C, which continued for 5 days. Urine and stool cultures yielded *C. albicans*, while catheter-tip and blood cultures were negative. Although no source of infection was clinically apparent, she was treated with intravenous fluconazole (400 mg daily) for 27 days. On day 77, however, the patient developed a high fever of 40°C. Blood was drawn for culture and the central venous catheter was removed. Hematological investigations revealed a hemoglobin level of 11.6 g/dl, platelet count of 139×10^9/liter, and a white cell count of 6.6×10^9/liter. C-reactive protein was 6 mg/liter, and (1→3)-β-D-glucan level was 153.7 μg/liter. Serum *Candida* antigen was positive with the Unimedi Candida monotest (Unitica Ltd.,

Tokyo, Japan). The Bact/Alert blood culture system (Organon Teknika Corp., Durham, NC) signaled microbial growth on the day after blood collection. As Gram staining of the blood culture showed yeast, intravenous fluconazole (400 mg daily) was commenced as empirical therapy. Catheter-tip culture also grew yeast in pure culture. Despite fluconazole administration, the patient remained febrile, and blood culture performed during fluconazole treatment was again positive for yeast. Therefore, the treatment was changed to intravenous voriconazole (400 mg daily) and micafungin (150 mg daily) for 7 days based on the results of broth microdilution susceptibility testing performed using a commercial kit (6). The MICs of the isolate were as follows: 0.5 μg/ml for amphotericin-B, 2.0 μg/ml for 5-fluorocytosine, 2 μg/ml for miconazole, ≥16 μg/ml for itraconazole, ≥128 μg/ml for fluconazole, 4 μg/ml for voriconazole, and 0.06 μg/ml for micafungin. The fever resolved 4 days later, and the patient was given MCFG for a further 10 days. There was no evidence of relapse during the next month, when she was transferred to another hospital.

Nuclear DNA extraction from the yeast-positive blood culture bottles and PCR amplification of internal transcribed spacers (ITS) followed by microchip gel electrophoresis are routinely performed in our laboratory as described previously (3,4) with the following changes: (i) usage of a MagExtract DNA extraction kit was omitted, and (ii) PCR cycling set at 25 cycles. It took approximately 50 min to extract DNA from positive blood culture bottles. The PCR procedure required 35 min, and electrophoresis took 5 min. Therefore, the overall turnaround time of the PCR ITS assay was approximately 1.5 h. ITS-PCR of the three isolates (two from blood cultures and one from catheter-tip culture) with universal primers ITS1 and ITS4 yielded fragments 765 to 768 bp in length, while universal primers ITS3 and ITS4 yielded fragments 411 to 414 bp in length. The ITS-PCR pattern of the isolate has not yet been found in our in-house ITS-PCR database (3). The three isolates were identified as *Candida glabrata* with the ID32C yeast identification system (bioMerieux, Marcy l'Etoile, France). The ID32C code obtained was 0001-0002-01, which showed an excellent match with the profile for *C. glabrata* in the APILAB database (ID96.9%, T0.94). However, the ITS-PCR pattern differed from those of *C. glabrata* (Fig. 1). Furthermore,

the colonies of the isolates on CHROMagar Candida medium were white in contrast to those of *C. glabrata*, which are pink. The ITS2 region was sequenced to confirm identification of the isolate using an ABI Prism 377 automated DNA sequencer (Applied Biosytems, Foster City, CA) with a BigDye Terminator cycle sequencing kit (Applied Biosystems). The primers used for sequencing ITS regions were ITS1, ITS2, ITS3, and ITS4 (7). The ITS2 sequence was compared using nucleotide-nucleotide BLAST (blastn) with default settings, and the isolate showed a high level of similarity (99% nucleotide identity, 218 of 219) with the sequence of a *C. nivariensis* (GenBank accession no. AY620957); the isolate differed only one bp for the ITS2 region (a conversion of T to A in position 80) from the *C.nivariensis* type strain (CBS 9983T). The lengths of ITS1 and ITS2 regions of the blood isolate were 283 bp and 232 bp, respectively. In contrast, the length of ITS1 of *C. glabrata* ranged from 399 bp to 402 bp, and that of ITS2 ranged from 233 to 238 bp (GenBank accession no. AB032177, AF167993, AM492797, and AM429798).

C. nivariensis is a recently described pathogenic yeast closely related to *C. glabrata*, and only four cases of *C. nivariensis* infection have been reported;

three from Spain (1) and one from Indonesia (GenBank accession no. EF056322). *C. nivariensis* isolates have been misidentified as *C. glabrata* using commercial identification kits (1). The same misidentification was made with the strains isolated from the patient in the present study. CHROMagar Candida medium seems to be useful to differentiate *C. glabrata* and *C. nivariensis.* However, a PCR-based technique is needed for rapid and specific identification of *C. nivariensis* (1,2). It is well known that *C. glabrata* and *C. krusei* are resistant or intermediately susceptible to fluconazole (5), but no susceptibility testing for *C. nivariensis* has been reported. Although the blood isolate was resistant to fluconazole, further studies are needed to determine whether strains belonging to *C. nivariensis* species are resistant to azole antifungal agents.

In conclusion, if the patient had been treated previously with an azole, the possibility of *C. nivariensis* infection must be considered as shown in the present study. ITS PCR pattern analysis can not only reliably identify *C. nivariensis,* but is also straightforward and can be completed within 1.5 h from positive-testing blood culture bottles.

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FIG. 1. ITS-PCR patterns of *C.nivariensis* K16661 (A), *C.glabrata* K432 (B), *C.glabrata* K12107 (C), and *Cglabrata* K10212 (D). Arrows indicate DNA size markers of 100 bp (left) and 1,000 bp (right), and asterisks indicate PCR products (A, 412 and 768 bp; B, 419 and 884 bp; C, 422 and 878 bp; and D, 418 and 884 bp). Int., intensity.

Fig 1