

## *Pichia farinosa* Bloodstream Infection in a Lymphoma Patient<sup>∇</sup>

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**We describe a case of *Pichia farinosa* bloodstream infection in a lymphoma patient. Phenotypic methods failed to identify the isolate, which was identified by sequence-based methods. This case highlights the importance of implementing molecular methods for the identification of rare fungal pathogens.**

### CASE REPORT

A 13-year-old boy with anaplastic large-cell lymphoma presented with fever for several hours and vomiting. The diagnosis of lymphoma was made 3 months previously, and a Broviac catheter was inserted into the right jugular vein. The patient was treated with chemotherapy that included systemic dexamethasone, cyclophosphamide, and methotrexate, along with prophylactic antimicrobial therapy with trimethoprim-sulfamethoxazole. The patient had one episode of *Pseudomonas* sp. sepsis that was treated with ciprofloxacin. The fifth chemotherapy course was administered 2 weeks prior to his admission, and severe neutropenia (absolute neutrophil count, <500 cells/ $\mu$ l) was present from 2 days before his admission. On admission, the patient appeared alert, his temperature was 40°C, his heart rate was 160 beats per minute, and the rest of the physical examination was normal. Treatment with piperacillin and gentamicin was initiated after drawing of two sets of blood cultures (BACTEC; Becton Dickinson) through the Broviac catheter. Three days after his admission, growth of yeasts was detected microscopically in three blood culture sets, and local redness and swelling were observed along the catheter tunnel. Treatment with amphotericin B was started, and the Broviac catheter was removed.

The fungus that grew on Sabouraud dextrose agar medium was identified by using the API 20 C AUX identification system (bioMérieux, France). The system code (6412044) was interpreted as *Candida boidinii* (% identification = 99.8%;  $T = 0.51$ ). Further characterization was attempted by using the ID 32 C identification system (bioMérieux, France). The system code (1001711217) with the closest match was *Pichia farinosa*, but since two tests contradicted with the software's data (assimilation in L-sorbose and lack of assimilation in *N*-acetylglucosamine), it was reported as an "unacceptable profile."

Molecular identification was attempted by amplification and sequencing of a fragment that includes internal transcribed spacer 1 (ITS 1), the 5.8S rRNA gene, and ITS 2 (9) as previously described, with minor modifications of the PCR conditions. The PCR assay was performed with 1  $\mu$ l DNA in a total volume of 50  $\mu$ l. The assay mixture contained 5  $\mu$ l BIOTAQ

10 $\times$  PCR buffer (Bioline, United Kingdom), 3.0 mM magnesium chloride, 1.0  $\mu$ l of a 10 mM deoxynucleoside triphosphate mixture, 50 pmol of each of the respective primers, and 2.5 U BIOTAQ DNA polymerase (Bioline, United Kingdom) per 50  $\mu$ l. After initial denaturation at 94°C for 5 min, 35 cycles were performed, consisting of a denaturation step at 94°C for 40 s, an annealing step at 49°C for 40 s, and an extension step at 72°C for 45 s, with a final extension step at 72°C for 2 min following the last cycle. For amplification of the ribosomal large subunit (LSU) D1/D2 variable regions, primers were chosen as previously described (7). The same PCR setup was used, except that the annealing temperature was increased to 54°C. DNA sequences were then aligned and compared to other sequences in the GenBank database by using BLAST. Both amplicons exhibited perfect identity (100%) with sequences of *P. farinosa* (GenBank accession numbers AY821846.1 and AY821846.1) but <98% identity with sequences of any other species. These results were then confirmed by comparing our sequence data to those for reference strains of *P. farinosa* and *C. boidinii* from the Centraalbureau voor Schimmelcultures (CBS) by using ClustalW alignment. The LSU D1/D2-based phylogenetic tree of *P. farinosa* and *C. boidinii* CBS strains is presented in Fig. 1. Our strain exhibited 100% and 99.6% similarities with the *P. farinosa* type strain CBS 185 LSU D1/D2 and ITS 1/2 regions, respectively. However, only 85.3% and 56.4% similarities were obtained with sequences from the type strain of *C. boidinii*, CBS 2428. Interestingly, we identified a subcluster of strains within the *P. farinosa* cluster that contained CBS 2006, the type strain of a current synonym of *P. farinosa*, namely, *Pichia miso*, which exhibited a lower level of similarity with our isolate (the LSU D1/D2 and ITS 1/2 regions had 99.6% and 99.1% similarities, respectively). The susceptibility of our isolate to the most commonly used antifungal agents was determined by using both the Etest system (AB BIODISK, Sweden) and broth microdilution assay. Antifungal susceptibility testing by the Etest method was performed according to the manufacturer's instructions, using RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 2% glucose and buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid buffer (MOPS; Sigma), an inoculum density of an  $\sim$ 0.5 McFarland standard, and incubation at 35°C for 24 h. *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 served as quality control organisms for all tests. MICs were determined by the broth microdilution method according to the CLSI recommendation for yeasts (10). The

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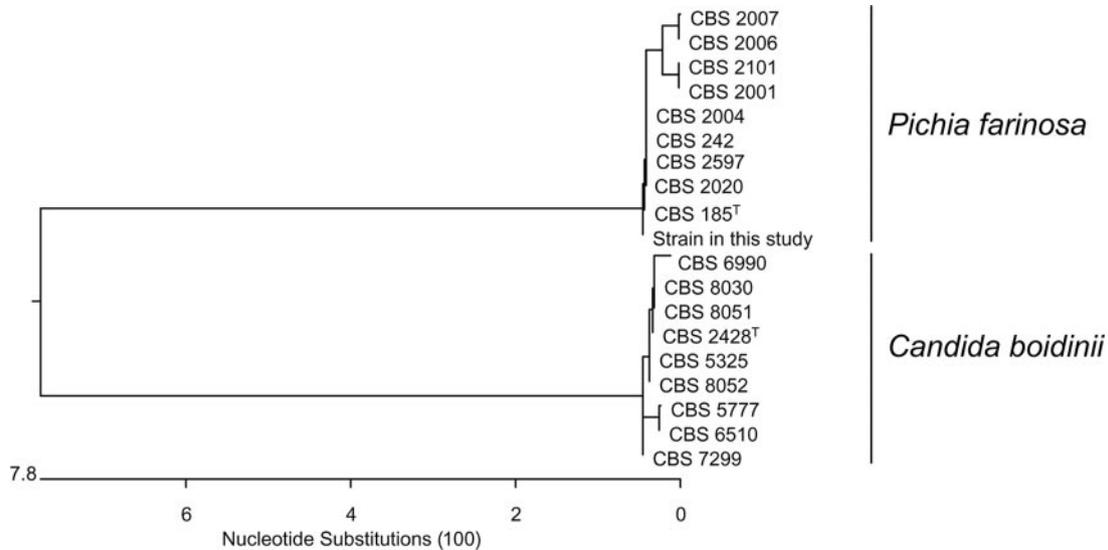


FIG. 1. Phylogenetic tree based on neighbor-joining analysis of LSU D1/D2 sequences showing the identity of the isolate as *Pichia farinosa*. The phenotypic identification of *Candida boidinii* by the API 20 C AUX system is not supported.

MIC was determined visually as the lowest concentration of drug showing no growth for amphotericin B and caspofungin and a prominent reduction of growth (80%) for fluconazole compared to the drug-free control growth. *Candida albicans* ATCC 90028 served as the quality control organism.

The MICs (mg/liter) determined by the Etest method and broth microdilution (in parentheses) were as follows: amphotericin B, 0.125 (0.062); fluconazole, 6.0 (6.25); and caspofungin, 0.25 (0.097). These MICs were below the breakpoint for *C. albicans*; no data are available for *P. farinosa*.

The patient became afebrile after 2 days of treatment, and the neutropenia resolved. He was discharged and completed a course of oral fluconazole for 2 weeks. No evidence of recurrence or complications was noted in follow-up visits.

To the best of our knowledge, our case is the second published case of human infection caused by *P. farinosa* (1). *P. farinosa* is a halotolerant yeast and is the teleomorph of *Candida cacaoi*. It is characterized by the production of a salt-mediated killer toxin that kills yeasts of several genera, including *Saccharomyces cerevisiae* (15). *P. farinosa* has been found in different regions of the world and from various sources, ranging from miso soup to giraffe dung (CBS Yeast Database [http://www.cbs.knaw.nl/yeast/BioMICS.aspx]). The only published case of human disease due to *P. farinosa* was described for a 12-year-old girl with teratoma who had apparent catheter-related fungemia that resolved after removal of the catheter (1). Colonization of oral mucosa with *P. farinosa* was reported for two immunocompromised patients (6, 14). In recent years, the number of reports of human infections caused by other *Pichia* species, including *Pichia anomala* (2, 5, 13), *Pichia ohmeri* (11, 12), and *Pichia fabianii* (3), has increased. In most of these studies (2, 5, 11, 12), *Pichia* species were first correctly diagnosed by phenotypic methods. In one study (3), phenotypic methods, including the use of the API 20 C AUX identification system (bioMérieux, France), failed to identify *P.*

*fabianii*. In our case, the API 20 C AUX identification system (bioMérieux, France) lacks the species *P. farinosa* in its database and misidentified the isolate as *C. boidinii*. Interestingly, the ID 32 C identification system (bioMérieux, France) closest match was indeed *P. farinosa*, but the software regarded this identification as “unacceptable” due to the results of the L-sorbose and *N*-acetylglucosamine assimilation tests. Although this system is regarded as the method of choice for phenotypic identification of medically important yeasts (4), compared with molecular methods its identification efficacy may be lower than 80%, especially when uncommon species are tested (8).

In conclusion, our case emphasizes the importance of molecular methods as tools for the identification of medically important yeasts in an era when the significance of non-*C. albicans* *Candida* species and other uncommon yeasts as human pathogens is increasing. We believe that these methods should be used in any case of significant infection when the results obtained by phenotypic methods are ambiguous or a rare organism is identified.

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