**Pichia farinosa** Blood-Stream Infection in a Lymphoma Patient

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Abstract

We describe a case of *Pichia farinosa* blood-stream infection in a lymphoma patient. Phenotypic methods failed to identify the isolate that was identified by sequence-based methods. The case highlights the importance of implementing molecular methods for the identification of rare fungal pathogens.
A 13-year-old boy with Anaplastic Large-Cell Lymphoma presented with fever for several hours and vomiting. The diagnosis of Lymphoma was made three months previously, a Broviac catheter was inserted to the right Jugular vein. The patient was treated with chemotherapy that included systemic dexamethasone, cyclophosphamide, methotrexate, along with prophylactic antimicrobial therapy with trimethoprim-sulphamethoxazole. The patient had one episode of *Pseudomonas* species sepsis that was treated with ciprofloxacin. The fifth chemotherapy course was administered two weeks prior to his admission and severe neutropenia (absolute neutrophil count < 500 cells/µl) was present from two days before his admission. On admission, the patient appeared alert, his temperature was 40 ºC, 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, USA) 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 was removed.

The yeast that grew on Sabouraud dextrose agar medium was identified by using API 20 C AUX identification system (BioMérieux, France). The system code (6412044) was interpreted as *Candida boidinii* (%ID=99.8%; T=0.51). Further characterization was attempted by using ID 32 C identification system (BioMérieux, France). The system code (1001711217) 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*-acetylglucoseamine) it was reported as "unacceptable profile".

Molecular identification was attempted by amplification and sequencing of a fragment that includes the internal transcribed spacer (ITS) 1, the 5.8S ribosomal
DNA gene, and the ITS 2 (9) as previously described, with minor modifications of the PCR conditions: The PCR assay was performed with 1 µl DNA in a total volume of 50 µl. The assay mixture contained 5 µl BIOTAQ™ 10x PCR buffer (Bioline, UK), 3.0 mM magnesium chloride, 1.0 µl of a 10 mM dNTP mixture, 50 pmol of each of the respective primers and 2.5 U BIOTAQ DNA polymerase (Bioline, UK) per 50 µl. 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 the amplification of the ribosomal large subunit (LSU) D1/D2 variable regions, primers were chosen as previously described (7). The same PCR set-up as above was used, except that the annealing temperature was increased to 54°C. DNA sequences were then aligned and compared to other sequences by in the GenBank database by using BLAST. Both amplicons exhibited perfect identity (100%) with sequences of *P. farinosa* (GenBank accession numbers [AY821846.1](http://www.ncbi.nlm.nih.gov/nuccore/AY821846.1) and [AY821846.1](http://www.ncbi.nlm.nih.gov/nuccore/AY821846.1)) but less than 98% with any other species. These results were then confirmed by comparing our sequence data to those of reference strains of *P. farinosa* and *C. boidinii* from the Centraalbureau voor Schimmelcultures (CBS) by using clustalW alignment. The large subunit (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% similarity with that of the type strain of *P. farinosa* CBS 185 LSU D1/D2 and ITS 1+2 regions, respectively. However, only 85.3% and 56.4% similarity was obtained with sequences from the type strain of *C. boidinii* CBS 2428. Interestingly, we identified a sub-cluster of strains within the *P. farinosa* cluster that contained CBS 2006, the type strain of a current synonym of *P. farinosa*, namely *Pichia miso*, that...
exhibited lower level of similarity with our isolate (LSU D1/D2 and ITS 1+2 had 99.6% and 99.1% similarity, respectively).

The susceptibility of our isolate to the most commonly used antifungal agents was determined by using both Etest® system (AB BIODISK, Sweden) and broth microdilution assay. The antifungal susceptibility testing by Etest method was determined according to the manufacturer’s instruction 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); inoculum density ~ 0.5 McFarland standard; incubation at 35°C for 24 h. Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 served as quality control organisms for all tests. MIC were determined by the broth microdilution method according to the CLSI recommendation for yeasts M27-A2 (10). The 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 growth control. Candida albicans ATCC 90028 served as quality control organism.

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

The patient became afebrile after two days of treatment and the neutropenia resolved. He was discharged and completed a course of oral fluconazole for two weeks. No evidence for 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 it is the teleomorph of Candida cacaoi. It is characterized by the production of salt-mediated
killer toxin that kills yeasts of several genera, including *Saccharomyces cerevisiae* (15). *P. farinosa* was found in different regions of the world and from various sources, ranging from miso soup to Giraffe dung (16). The only published case of human diseases due to *P. farinose* was described in 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 in 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 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 in the L-sorbose and N-acetylglucoseamine assimilation tests. Although this system is regarded as the method of choice for phenotypic identification of medically important yeasts (4), compared with molecular method its identification efficacy may be lower than 80%, especially when uncommon species are tested (8).

In conclusion, our case emphasizes the importance of molecular-based methods as tools for identification of medically important yeasts. In an era when the importance of non-*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.
References


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Legend to Figure 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 as *Candida boidinii* by API 20 C AUX is not supported.