A case of endocarditis caused by the yeast *Pichia fabianii* with biofilm production and developed *in vitro* resistance to azoles in the course of antifungal treatment

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*Pichia fabianii*, a yeast rarely causing human infections, was isolated from the blood of a patient with aortic valve endocarditis. The isolates were initially identified biochemically as *Candida pelliculosa*, but based on direct sequencing of the ITS2 region of rRNA, they were subsequently reidentified as *P. fabianii*. Antifungal therapy with fluconazole and later with voriconazole led to the development of resistant variants which had high MIC values to both antifungals. Strong biofilm formation by this yeast could also have played a role in the development of its resistance and allowed for its persistence on the infected valve during antifungal therapy. To our knowledge, this is the first published case of endocarditis and the fourth human infection caused by this yeast species.

**Keywords** *Pichia fabianii*, systemic mycoses, ITS sequencing, antifungal resistance, biofilm production

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**Introduction**

Infectious endocarditis can be caused by a wide range of microbial species, with an incidence ranging from 1.9–6.2 cases per 100,000 inhabitants [1]. While fungal endocarditis is rather rare, it has a mortality rate exceeding 80% [2]. For a long time, amphotericin B was the drug of choice, despite its toxicity, and its use was usually combined with cardiac surgery. At present, antifungals with comparable therapeutic effect, such as caspofungin or voriconazole, are recommended [3,4] in cases of fungal endocarditis. *Pichia* is an ascomycetous yeast genus representing teleomorph stages of several *Candida* species. Only a few *Pichia* species have been reported to cause human infections. We present a case of infectious endocarditis caused by a yeast which was identified through the use of routine diagnostic techniques as *Candida pelliculosa*, the anamorph stage of *Pichia anomala*. Later, based on direct sequencing of the internal transcribed spacer 2 (ITS2) region, the yeast was reidentified as *Pichia fabianii*. Since *P. fabianii*, unlike *P. anomala*, is not included in databases of commonly used biochemical kits, its misidentification is possible in routine practice. Identification based on nucleic acid sequence information can be more reliable than evaluation of phenotypic characteristics. The ribosomal DNA gene complex is the most frequently used target for molecular genetic identification of unusual pathogens. Up to now, we have found only three reports of *Pichia fabianii* human infections in the literature. However, because of the possible misidentification of *Pichia fabianii*, other cases of endocarditis caused by this species may have occurred in the past (e.g., [5]).
A 40-year-old man had been monitored for an extended period of time for congenital combined aortic incompetence of the mitral valve. He was hospitalized in July 2005 due to an ischemic stroke with paralysis of the left limbs, paresis of the left nerve VII and left hemianopsia. Transesophageal echocardiography (TEE) detected no vegetations on the aortic valve. Because of expansion of the ischemic area with cerebral edema, decompressive craniectomy was performed and as a result, sepsis developed with repeated isolation of yeasts from the bloodstream. However, at that time no exact identification was made except for the exclusion of *Candida albicans*. The patient was treated with fluconazole, 400 mg per day intravenously for 18 days and then 150 mg per day orally for the next 30 days. After this period of time, the antifungal therapy was terminated because of no clinical signs of infection and no mycological findings. However, on the day of therapy termination, a yeast identified as *Candida pelliculosa* was detected in the bloodstream. After the second recovery of this same yeast in blood cultures, the finding of subfebrile temperatures and laboratory signs of inflammation, resulted in the reinitiation of antifungal therapy with 200 mg of voriconazole per day orally for 21 days. Subsequently, the same yeast was isolated twice more from the bloodstream. As the latter isolates were found to be markedly resistant in vitro to voriconazole, the therapy was changed to amphotericin B, 50 mg intravenously for 35 days. Apart from the yeasts, *Acinetobacter* sp. and *Propionibacterium acnes* were isolated from blood cultures and *Proteus mirabilis* along with *Escherichia coli* from urine. Therefore, gentamicin and cefotaxime were added to the antibiotic regimen (3 × 80 mg intravenously for 14 days and 3 × 2 g intravenously for 45 days, respectively). Transthoracic echocardiography revealed vegetations on the aortic valve and an area of heterogeneous tissue (size 20 × 19 × 25 mm) suggesting an abscess in the sinus of Valsalva (Fig. 1a). Cardiac surgery was recommended after 4 weeks of intensive antifungal and antibiotic treatment. During the treatment, the patient was afebrile, CRP decreased to 43 mg/l and blood cultures were negative.

Before surgery, however, ischemic stroke recurred, with severe mixed speech impairment and bilateral sensorineural loss. Using magnetic resonance imaging, a new ischemic area was found in the left temporoparietal region. TEE detected depleted abscess cavity in the left sinus of Valsalva (Fig. 1b). The aortic valve was replaced with a homograft after 11 days. Although blood cultures were negative during and after treatment with amphotericin B, a yeast was isolated from the infected valve after surgery. At present, the patient is stabilized, rehabilitating, without signs of infection, but with serious neurological involvement.

Initially, blood cultures were incubated using the BacT/Alert automated detection system (bioMérieux, Marcy l’Etoile, France). Subsequently, eight isolates were stored for study purposes. Yeast colonies that formed after three-day subculturing on Sabouraud glucose agar with chloramphenicol (0.05 g/l) at 37°C were 2 mm in diameter, tannish-white in color, round, glossy, butyrous, with entire margins. Pinkish-white colonies were seen on CHROMagar Candida (CHROM-agar, Paris, France). The isolates were capable of growing at 42°C but markedly slower in comparison with 37°C. Microscopically, spheroidal to ellipsoidal, (1.0–4.5) × (2.0–6.5) μm yeast cells occurring singly, in pairs and small clusters were seen after three days of incubation in glucose-yeast extract-peptone broth at 25°C (Fig. 2a). The isolates were chlamydospore-negative, and produced moderately branched pseudohyphae with mostly solitary, laterally arranged blastoconidia on rice agar with Tween 80 after seven days at 25°C (Fig. 2b). Carbon assimilation profiles were performed using the ID 32C kit (bioMérieux),
with the same biocode 4275350111 always generated after 72 h at 30°C [6]. Using the APILAB Plus software (bioMérieux), the isolates were identified as Candida pelliculosa (Pichia anomala) (%id = 57.3; T = 0.85) with an inability to assimilate erythritol as the only discrepant test result. The second identification choice was Candida utilis (Pichia jadinii) (%id = 42.6; T = 0.84) but the isolates ability to utilize sorbitol but not gluconate were not consistent with this identification. The isolates fermented glucose, sucrose and maltose, but not lactose and galactose. Ascospores were not evaluated due to a lack of a complementary mating strain.

Sequencing of the ITS2 of one of the isolates (HE-650) was performed later. Amplification was done using the UNF1 (5’gca tcg atg aag aac gca gc 3’) and UNF2 (5’ttg ata tgc tta agt tca gcg g 3’) primers. The PCR product was sequenced directly from both sides using the UNF1 and UNF2 primers, respectively. All sequences were processed by the VBC Genomics Bioscience Research (Vienna, Austria) sequencing service. The acquired sequence (154 bp, GenBank Accession Number EF613117) was compared with those published in the Entrez Nucleotide Database of the National Center for Biotechnology Information using the BLAST algorithm to identify sequences showing the highest homology. This procedure revealed 100% homology with the 154 bp ITS2 sequence of Pichia fabianii type strain (CBS 5640, GenBank Accession Number AF335967), while the sequence homology with Pichia anomala type strain (CBS 605, GenBank Accession Number AF218991) was only 56%. Selected differences in phenotypic characters among Candida pelliculosa (Pichia anomala), Candida utilis (Pichia jadinii) and Candida fabianii (Pichia fabianii) are summarized in Table 1 [7,8].

Pulsed-field gel electrophoresis was performed under conditions described previously for Pichia anomala [9], and results were used to check the identity of the isolates, with the same banding pattern being found in all of them. The isolate HE-650 has been deposited and is available under the strain number CCY 38-20-5 from the Culture Collection of Yeasts, Bratislava, Slovakia.

Susceptibility to six systemic antifungals – amphotericin B, ketoconazole, itraconazole, fluconazole, voriconazole and caspofungin – was determined using E-test strips (AB Biodisk, Solna, Sweden) on the RPMI 1640 medium. The MICs of the three isolates initially recovered (i.e., prior to the antifungal treatment) from the bloodstream were significantly lower when compared with those obtained after 48 days of fluconazole therapy. Moreover, the last bloodstream isolate recovered after 21-day voriconazole treatment showed

### Table 1 Selected phenotype differences among Pichia fabianii, Pichia anomala and Pichia jadinii.

<table>
<thead>
<tr>
<th>Test</th>
<th>Teleomorph</th>
<th>Anamorph</th>
<th>P. fabianii</th>
<th>C. fabianii</th>
<th>P. anomala</th>
<th>C. pelliculosa</th>
<th>P. jadinii</th>
<th>C. utilis</th>
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<tr>
<td>42°C growth*</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+/−</td>
<td>+/−</td>
<td>−</td>
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<tr>
<td>erythritol assimilation</td>
<td>−</td>
<td>−</td>
<td>+</td>
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*Thermotolerance data from Ref. 7, other data from Ref. 8; + = positive; − = negative; +/− = variable; +/− = positive or weak; + (−) = usual positive.
resistance to this agent, as well as a follow-up increase of fluconazole MIC (Table 2). Biofilm production was tested by the microtiter plate method [10], with all isolates showing strong biofilm production.

Discussion

Our case report is of interest because it is the first to identify Pichia fabianii as the causative agent of endocarditis. We performed a PubMed search of cases through December 2007 using both Candida fabianii and Pichia fabianii as search terms and found only three cases in which this yeast species was reported to cause human infection, with none of them involving endocarditis. The first case, published by Dooley et al. in 1990, was prostatitis in a 57-year-old male with chronic lymphocytic leukemia and a 20-year history of urinary tract infections [11]. He had multiple relapses on ketoconazole, but responded to amphotericin B. In 2006, Bhally et al. reported fungemia in a five-week-old premature baby girl successfully treated with amphotericin B [12]. In the same year, Valenza et al. described a fatal case treated by fluconazole followed by caspofungin in a 46-year-old male who initially suffered from severe pneumococcal septicemia and later developed polymicrobial secondary infection [13]. In the latter two reports, the correct identification was only obtainable through the use of sequencing data, as in our case. This suggests that Pichia fabianii could have been commonly misidentified in the past, most probably as Pichia anomalala [5,14]. Pichia fabianii is not yet included in databases of the most widely used diagnostic kits (API 20C AUX, ID 32C and Vitek-2) and, therefore, both species are difficult to distinguish based on physiological characteristics in routine practice. On the other hand, misidentification seems to be unlikely if formation of ascospores is investigated as only P. fabianii has an ascosporogenous state in nature [8,15,16] (Table 1). However, the long incubation time to induce ascospores and the requirement of a special medium may preclude its practical use in most laboratories. Nevertheless, identification based on nucleic acid sequence information (obtained using, e.g., the MicroSeq D2 LSU rDNA fungal sequencing kit) could be more reliable than evaluation of phenotypic features [17]. Secondly, this strain developed in vitro resistance to triazoles in the course of antifungal treatment. According to the CLSI interpretive breakpoints, the isolates were primarily susceptible to fluconazole, itraconazole and voriconazole [18,19]. Markedly higher MICs to azoles were found after 48 days of fluconazole therapy in contrast to relatively stable MICs to amphotericin B and caspofungin. Later, when treatment with voriconazole was started, resistance to this antifungal agent occurred relatively rapidly. This could have been the result of the development of cross-resistance to azoles observed particularly in Candida glabrata strains [20,21]. High levels of biofilm formation can also prevent access of antimycotics to the yeast cells and, in addition, allow persistence of the strain on the infected valve during antifungal therapy [10].

To our knowledge, we report the first case of mycotic endocarditis due to the emerging yeast species Pichia fabianii, confirmed by ITS2 sequencing. This emphasizes the need for commercially available molecular genetic-based assays in medical mycology.

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