Endocarditis due to a co-infection of *Candida albicans* and *Candida tropicalis* in a drug abuser

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In recent decades the incidence of *Candida* endocarditis has increased dramatically. Despite the application of surgery and antifungal therapy, *Candida* endocarditis remains a life-threatening infection with significant morbidity and mortality. We report a 37-year-old male drug abuser presenting with high fever, chest pain, loss of appetite and cardiac failure. His echocardiography revealed mobile large tricuspid valve vegetations. Fungal endocarditis was confirmed by culturing of the resected vegetation showing mixed growth of *Candida albicans* and *Candida tropicalis*, although three consecutive blood cultures were negative for *Candida* species. Phenotypic identification was reconfirmed by sequencing of the internal transcribed spacer (ITS rDNA) region. The patient was initially treated with intravenous fluconazole (6 mg kg$^{-1}$ per day), followed by 2 weeks of intravenous amphotericin B deoxycholate (1 mg kg$^{-1}$ per day). Although MICs were low for both drugs, the patient’s antifungal therapy combined with valve replacement failed, and he died due to respiratory failure.

**Introduction**

In recent decades, the incidence of fungal agents causing endocarditis has increased dramatically, especially in patients infected with human immunodeficiency virus (HIV), immunocompromised hosts, intravenous drug abusers, and those who present underlying valvular heart diseases, implantation of prosthetic valves or prolonged use of intravenous catheters (Pierrotti & Baddour, 2002; Prendergast, 2006). Despite valve replacement and antifungal therapy, fungal endocarditis (FE) is a life-threatening infection with significant morbidity, mortality and healthcare costs (Moreillon & Que, 2004; Nadir & Rubinstein, 2004), particularly in developing countries (Baddley et al., 2008; Jain et al., 2011). *Candida albicans* is the major and most frequently reported agent, followed by *Candida parapsilosis*, *Candida tropicalis* and *Candida guilliermondii* (Millar et al., 2005; Kumar et al., 2010). It has been noted that intravenous drug abusers with symptomatic HIV infection, patients with severe immunodeficiency and patients with valvular involvement are at high risk of death due to *Candida* infective endocarditis (Nahass et al., 1990; Barrau et al., 2004). Here we describe the unsuccessful treatment and fatal outcome of mixed endocarditis due to *C. albicans* and *C. tropicalis*, despite both medical and surgical intervention.

**Case report**

A 37-year-old male drug abuser was admitted to the Tehran Heart Center presenting with a history of high-grade fever, dry cough, chest pain and loss of appetite. He also had cardiac failure and was a candidate for tricuspid valve replacement.

**Abbreviations:** FE, fungal endocarditis; HIV, human immunodeficiency virus; IFRC, Invasive Fungi Research Center.

The GenBank/EMBL/DDBJ accession numbers for the ITS rDNA region sequences of the *C. tropicalis* and *C. albicans* isolates are KC422426 and KC422428, respectively.
valve replacement. He complained of localized chest pain on the left side, and his past medical history was significant for endocarditis with tricuspid valve replacement. Initial cardiovascular examination revealed a fever of 38.7°C, hypertension, paleness, haemodynamic instability, respiratory distress, breathing with gasping, a sinusoidal heart rhythm and apnoea. He was admitted to the intensive care unit, because of respiratory failure and septic shock. A computed tomography scan and pulmonary angiogram demonstrated a septic embolus in the left branch of the pulmonary artery. Transthoracic echocardiography showed normal left ventricle size, an abnormal septal motion and mobile masses attached to the atrial side of the tricuspid valve (vegetation 13 × 6 mm). In addition, there was moderate tricuspid regurgitation, pulmonary artery pressure of about 40–45 mmHg, and a normal right ventricular size and function (Fig. 1). A full laboratory assay demonstrated a white blood cell count of 40 cells mm⁻³ with 69.4% neutrophils and 20.9% lymphocytes, a red blood count of 3.35 × 10⁶ cells mm⁻³, 110 μg ferritin 1⁻¹ and 0.09 μg procalcitonin 1⁻¹. The C-reactive protein level was 31.4 mg l⁻¹ with a normal erythrocyte sedimentation rate and urine analysis. Both serum creatinine and blood urea nitrogen were normal. Ophthalmological and nephrological consultations were not ordered. Serum electrolytes, liver function and glucose concentration were within normal ranges. Bacterial blood cultures were sterile. Serology testing for HIV and hepatitis C antibodies was negative. Ceftriaxone (1 g per day) and gentamicin (1.5–2 mg kg⁻¹ per day) treatments were initiated for suspected bacterial endocarditis; however, FE was strongly considered because of the patient’s large vegetation, his intravenous drug abuse and his prolonged broad-spectrum antibacterial therapy. Although three consecutive blood cultures were negative for Candida species, intravenous fluconazole (6 mg kg⁻¹ per day) was initiated, followed by 2 weeks of intravenous amphotericin B deoxycholate (1 mg kg⁻¹ per day). Subsequently, surgical excision of the entire vegetation with tricuspid valve replacement was performed, and a sample was sent for histopathological and microbiological examination. The obtained specimen consisted of loose leaflet tissue with chord connection and papillary muscle of 25 × 15 × 0.4 cm in size with a large vegetation in the valve (13 × 6 mm). Histology analysis showed fungal hyphae with infiltration by giant cells, neutrophils, lymphocytes and eosinophils. These granulomas and giant cells contained pseudohyphae and blood vessels (Fig. 2b). After the surgical excision, the patient continued treatment with intravenous amphotericin B deoxycholate (1 mg kg⁻¹ per day) and fluconazole (6 mg kg⁻¹ per day) for 1 week; however, his condition deteriorated rapidly and he developed respiratory distress, followed by haemoptysis with severe and uncontrollable gastrointestinal bleeding. Ultimately the patient died due to respiratory failure. The presentation of this case was approved by the ethics committee of Tehran Heart Center and Tehran University of Medical Science, and written informed consent was obtained from the patient’s next of kin for publication of this report.

Results

Mycological and molecular investigation

Diagnosis of FE was made by culturing and histopathology of the resected vegetation. Growth of yeast-like fungi on Sabouraud dextrose agar (Difco) was observed after 1 day, and these fungi were initially identified as C. albicans and C. tropicalis with API 20C AUX (bioMérieux), chromogenic Candida agar (bioMérieux) and standard morphological methods (Fig. 2a). For reconfirmation of the identity of the isolates that were identified as C. albicans and C. tropicalis based on conventional methods, voucher strains were deposited into the reference culture collection of the Invasive Fungi Research Center (IFRC), Sari, Iran, under accession numbers IFRC41 and IFRC39, respectively. For sequencing, DNA was extracted from 3-day-old Sabouraud dextrose agar cultures with an Ultraclean microbial DNA isolation kit (Mo Bio Laboratories) according to manufacturer’s protocol and stored at −20 °C. The internal transcribed spacer (ITS rDNA) region was amplified and sequenced using primers ITS1 (5’-TCCGTAGGTGAACCTGCGG-3’) and ITS4 (5’-TCCGATATTGATATGC-3’), which have been described elsewhere (White et al., 1990). Briefly, the amplification of ITS rDNA was performed with a cycle of 5 min at 94 °C for primary denaturation, followed by 40 cycles at 94 °C (30 s), 52 °C (30 s) and 72 °C (80 s), with a final 7 min extension step at 72 °C. PCR products were first run in 1.5% agarose gels and visualized with UV after etidium bromide staining, and then subsequently were purified using GFX PCR DNA (GE Healthcare). Amplicons were then subjected to direct sequencing using ABI prism BigDye™ terminator cycle sequencing kit (Applied Biosystems) and analysed on an ABI Prism 3730XL Sequencer. Sequence data obtained were adjusted using Lasergene SeqMan software version 9.0.4 (DNASTAR) and compared with GenBank and through localBLAST with a molecular database maintained for research purposes at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands. The DNA sequence of the ITS rDNA region matched that of C. albicans (IFRC41=GenBank accession no. KC422428) and C. tropicalis (IFRC39=GenBank accession no. KC422426) by showing 99.8 and 99% similarity with the ex-type strain, respectively. The molecular results confirmed the mycological and histopathological diagnosis of mixed FE due to C. albicans and C. tropicalis.

In vitro antifungal susceptibility testing

The in vitro antifungal susceptibility testing of obtained isolates was carried out by E-test according to the manufacturer’s instructions (AB Biodisk). The MIC results (Table 1) show that the tested drugs had potent activity against both isolates (C. albicans and C. tropicalis). MICS of amphotericin B were lower than 0.032 mg l⁻¹ for C. albicans and 0.5 mg l⁻¹ for C. tropicalis, and both isolates were susceptible to fluconazole. Although the results
showed the low MICs in vitro, the patient’s fluconazole and amphotericin B deoxycholate therapy failed.

Discussion

FE is one of the most severe fungal infections occurring in patients with underlying cardiac diseases, previous cardiac surgery, implantation of prosthetic valves, central venous catheters and intravenous drug abusers (Pierrotti & Baddour, 2002; Prendergast, 2006). The most common risk factor for FE is prolonged antibiotic use with implanted central venous catheters in place, or intravenous drug abusers as presented in the current case report (Sousa et al., 2012; Athan et al., 2012). The left side of the heart (aortic and mitral) is mostly involved in FE (Pierrotti & Baddour, 2002; Moreillon & Que, 2004). It has been reported that tricuspid valve endocarditis occurs in 5–10 % of FE, and nearly 4 % of tricuspid valve endocarditis cases are caused by Candida species (Saito et al., 2001). The most common agent of FE is C. albicans (24–46 %), followed by non-albicans Candida species (Prendergast, 2006; Baddley et al., 2008). C. tropicalis infections are rare, and only a few cases of FE have been reported that were successfully treated with surgical excision and antifungal therapy (Kumar et al., 2010). The current initial recommendation for antifungal treatment of Candida endocarditis is liposomal amphotericin B (3–5 mg kg\(^{-1}\) per day), with
Table 1. *In vitro* antifungal susceptibility E-test profiles of *C. albicans* and *C. tropicalis* expressed in μg ml⁻¹

<table>
<thead>
<tr>
<th>Collection &amp; accession no.</th>
<th>Species</th>
<th>AMB</th>
<th>FLC</th>
<th>ITC</th>
<th>VOR</th>
<th>POS</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFRC41, KC422428</td>
<td><em>C. albicans</em></td>
<td>0.032</td>
<td>0.25</td>
<td>0.5</td>
<td>0.19</td>
<td>0.032</td>
<td>0.064</td>
</tr>
<tr>
<td>IFRC39, KC422426</td>
<td><em>C. tropicalis</em></td>
<td>0.5</td>
<td>1</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
<td>0.5</td>
</tr>
</tbody>
</table>

or without oral 5-flucytosine, or fluconazole alone for at least 6 weeks after valve replacement (Kumar et al., 2010; Melamed et al., 2009). Moreover, the *in vitro* and *in vivo* susceptibility of caspofungin revealed potent activity against *C. albicans* biofilms in comparison to both amphotericin B and fluconazole, so it might provide an effective medical treatment option for *Candida* endocarditis in selected patients (Rajendram et al., 2005; Talarmin et al., 2009). However, a review of several cases of *Candida* endocarditis treated with caspofungin showed that the outcome of treatment with caspofungin was also poor (Bacak et al., 2006). Here, we describe the unsuccessful outcome of a case of a drug-abusing patient with mixed infection of *Candida* endocarditis (*C. albicans* and *C. tropicalis*) treated by amphotericin B deoxycholate and fluconazole combined with the surgical excision of a large infected vegetation on the tricuspid valve. Polymicrobial endocarditis is a variant of infective endocarditis that is uncommon and often fatal, as was the case in our patient (Wang’ondu & Murray, 2011; Daas et al., 2009). Molecular techniques have allowed an increased ability to detect and identify causal organisms associated with FE. Relatively few reports of the employment of fungal PCR to detect mycological causal agents of endocarditis have been described (Millar & Moore, 2004; Millar et al., 2001). In our case, fungal identity was confirmed by DNA sequencing of the ITS rDNA regions. MIC testing of the initial fungal isolates demonstrated susceptibility to all antifungals. Studies have shown that *Candida krusei* isolated from immunocompromised patients was mainly resistant to fluconazole; *Candida glabrata*, *C. tropicalis* and *Candida utilis* isolated from immunocompetent hosts also showed fluconazole resistance (Jiang et al., 2013; Oz et al., 2013). Therefore, it is crucially important to routinely determine the *in vitro* antifungal susceptibility. To the best of our knowledge, this is the first reported case of tricuspid valve endocarditis due to mixed *Candida* species in a patient with a history of drug abuse. Clinicians with management of patients with suspected FE should consult with a microbiologist and, where appropriate, discuss employment of newer molecular technologies and antifungal susceptibility testing so that improved outcomes may be achieved.

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**References**


