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Donor Derived *Candida stellimalicola* in a Clinical Specimen: Preservation Fluid Contamination During Pancreas Procurement

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Abstract We report here a case of possible donor-derived *Candida stellimalicola* infection after pancreas transplantation. *Candida stellimalicola*, an environmental non-filamentous yeast, was isolated from both the peritoneal fluid of the graft donor and the preservation fluid of the transplanted pancreas. Interestingly, this strain exhibited high minimum inhibitory concentrations to azoles. These results justified the use of echinocandins as therapy instead of fluconazole. This switch permitted a favorable outcome. To our knowledge, this is the first report of *C. stellimalicola* from clinical samples and therefore the first reported case of a possible human infection. This case report highlights the need for standardized microbiological procedures in solid organ transplant settings. Moreover, it underlines the importance of using molecular identification technique when routine techniques do not allow successful identification of the pathogen. It is of utmost importance to determine sensitivity profile, even in the absence of species-level identification, because resistance to fluconazole is not uncommon, especially in emergent species.

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Introduction

Invasive candidiasis represents the leading fungal infection, particularly in intensive care unit (ICU) and in immunocompromised patients. Its incidence has markedly increased and diagnosis is usually based on host factors, clinical signs and symptoms associated with mycological evidence [1].

In solid organ transplant (SOT) recipients, *Candida* is the most common cause of fungal infections, particularly in intestinal and multivisceral transplant recipients, with most cases occurring during the first month after surgery [2]. *Candida albicans* is the most frequently encountered species (50%) followed by *Candida glabrata* (30%) mostly in patients having received antifungal therapy [3].

We report here the first isolation to our knowledge in human samples of *Candida stellimalicola*. It was a possible donor-derived candidiasis with contamination of transplant recipient organ preservation fluid [4].

Patient No. 1: Graft Donor, Reims, France

A young woman was hospitalized in 2013 in ICU at the Reims University Hospital (France) for severe traumatic brain injury. Upon admission, she received amoxicillin clavulanate because of otorrhea and an open wound of the right malleolus. After 10 days, the patient evolved to brain death and multiple organ procurement was performed without further complications (heart, lungs, liver, pancreas and kidneys). Culture of post-explant peritoneal fluid allowed isolation of several yeast colonies after incubation in aerobic blood culture bottle for 32 h. Culture after direct inoculation on Sabouraud plates remained negative. Identification could not be performed by neither matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS, software MALDI Biotyper™ 3.0, Bruker, France) nor assimilation tests (Auxacolor, BioRad, France). Other microbiological cultures (pre-explant peritoneal fluid samples, blood cultures, urine, endotracheal suctioning) remained negative as well as preservation fluids of the other organs transplanted to other recipients.

Patient No. 2: Transplant Recipient, Lyon, France

Patient was a 36-year-old female with diabetic nephropathy leading to hemodialysis followed by kidney and pancreas transplantation in 2011. Due to early thrombosis of the pancreatic transplant, a second graft with pancreas from patient 1 was successfully performed in the Transplantation Unit of Edouard Herriot Hospital, Lyon, France, in 2013. Immunosuppressive therapy consisted of prednisolone 15 mg/day, mycophenolate 720 mg twice daily and tacrolimus 8 mg twice daily. Until day 3 post-transplant, C-reactive protein levels remained as low as 10 mg/L consistent with post-operative condition. It reached more than 100 mg/L on day 5 which was associated with fungal growth of the preservation fluid after 24 h of incubation in blood culture bottle. Culture after direct inoculation on Sabouraud plates remained negative. Fungus was identified as a *Candida sp.* without species identification using MALDI-TOF MS (Vitek MS, bioMérieux, Marcy l'Etoile, France). Initial treatment with fluconazole (400 mg/day) was switched to caspofungin (50 mg/day) as high minimum inhibitory concentrations (MIC: E-test®, BioMérieux, France) were found for azoles (fluconazole > 256 µg/mL, voriconazole 6 µg/mL), associated with low MIC to amphotericin B (0.19 µg/mL) and to caspofungin (0.25 µg/mL). These results were congruent with susceptibility testing performed on the yeast isolated in the donor. No bacteria were detected in the preservation fluid. Other drain fluids remained sterile. Afterward, clinical condition of patient 2 improved and antifungal treatment was discontinued after 3 weeks.

Isolates from both patients were identified by molecular analysis using two pairs of universal fungal primers (ITS1/ITS4 targeting internal transcribed spacer 1 and 2 and NL1/NL4 targeting the D1/D2 domain of the 28S RNA). Further analysis of the sequences (GenBank accession numbers: KU295458

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and KU295459) revealed >99% similarity and 100% overlap with *C. stellimalicola* using GenBank (FM199968.1 for ITS and FM180552.1 for D1/D2 blast) and CBS databases (http://www.cbs.knaw.nl/Collections/BioloMICSSequences.aspx?file=all) (strain FM199968 for ITS and strain CBS7853 for D1/D2 blast).

Contamination by the graft was suspected, and we concluded to a possible *C. stellimalicola* donor-derived fungal infection.

Discussion

Incidence of invasive fungal infections (IFI) in SOT ranges from 1.3% up to 11.6% depending on organ type with *Candida* and *Aspergillus* accounting for more than 80% of all episodes of IFI [5, 6]. They often have pejorative prognosis with overall 12-week mortality of 29.6% [3]. *Candida* remains the leading cause of fungal infections in SOT recipients particularly in small bowel and pancreas transplant patients (85 and 76% respectively of IFI) [6]. IFI in SOT are associated with increased mortality [3]. They also independently predict for severe pancreatic graft dysfunction [7].

Two major routes of infection are described in transplanted patients. The first is colonization leading to infection by means of host defenses impairment (disruption of normal immune barriers, immunosuppression) allowing candidiasis of endogenous origin to occur. It is increased by the use of broad-spectrum antibiotics, diabetes, corticosteroids, prolonged stay in ICU, central venous catheters, dialysis or abdominal surgery [8]. The second type of infection is surgical site infection and is related to the transplantation surgery. Most cases occurring during the first month after surgery, infection seems acquired during the graft or organ procurement procedure [2, 9]. *Candida* can be transmitted particularly through contamination of organ [9] or preservation solution [10].

Regarding pancreas transplantation (PT), it is most often a part of a multivisceral transplant, especially kidney–pancreas transplantation (KPT) in diabetic insulin-dependent patients, and only scarce information about epidemiology and microbiological characteristics of IFI following PT is available [7, 11]. Opening of the donor duodenum is associated with *Candida* infection following KPT. Species other than *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis* and *C. lusitaniae* represent less than 1% of cases [6]. This highlights the need to sample and monitor organ preservation solution in search for microbiological contamination [12]. According to the guidelines of the French Biomedicine Agency and the French Microbiology Society [13], preservation solutions and any fluid detected at the incision should be sampled for detection of bacteria and fungi and inoculated in both aerobic and anaerobic blood culture bottles and on blood agar plate for a 5-day incubation time, owing to the frequently low fungal load [14]. It is recommended to identify every isolated organism at the species level, to determine antimicrobial susceptibility by a validated system and to store all the strains isolated from organ preservation solution for at least 12 months at −80 °C. These recommendations refer to liver, kidney, lung and heart transplants, but to our knowledge there are no specific guidelines regarding pancreatic grafts. However, in practice, approach is identical to those followed for other organ transplants. It allowed us to compare the strains isolated in each of our two centers (donor and recipient).

Conservative liquids contamination could transmit potential life-threatening infections [9, 10]. As it was the case here, it may also lead to uneventful benign infection and should not be managed with mandatory graft removal but with well-standardized antifungal treatment regimens [15]. Indeed, IDSA recommends that antifungal therapy should be started for kidney transplant recipient if donor preservation fluid is positive for yeast, whereas limited data are available in PT patient [4]. Most of the therapeutic experience in SOT is only based on small studies and expert opinions with a low level of evidence.

Candida stellimalicola has first been described in 1994 by Suzuki et al. [16]. It was isolated from star apples (*Averrhoa carambola*), collected in the markets around Bangkok and the nearby provinces in Thailand in December, 1978. This yeast has since been isolated worldwide from various decaying fruits [17–19].

Candida stellimalicola is phylogenetically situated within the *Starmera* genus [18, 19], unrelated to any of the usual *Candida* species known to cause infection. It has been proposed by Freitas et al. that the species *C. stellimalicola* be renamed *Starmera stellimalicola* with the mention *forma asexualis* (*f.a.*), as teleomorph has not yet been described. As there seems to be uncertainty about the validity of the genus *Starmera*, we have used *C. stellimalicola* in this article [19].

Candida stellimalicola is a non-filamentous yeast, with round or ovoid cells (1.8 × 4.0 – 4.2 µm). It usually grows within a few days at 17–25 °C and produces smooth cream-colored colonies [16, 17, 20–22]. It presents the following biochemical criteria: assimilation of D-arabinose and DL-lactic acid, inability to assimilate ribitol and L-lysine, absence of alcoholic fermentation and use of ubiquinone Q7.

As most environmental yeasts, *C. stellimalicola* grows well at temperature lower than those of human body. This thermal preference may explain the difficulties encountered with isolation of this yeast. Interestingly, in our samples, *C. stellimalicola* did not grow on standard fungal media in both centers (Can2, BioMérieux and CHROMagar *Candida* Becton–Dickinson) plated with centrifuged fluids. Furthermore, peritoneal and preservation fluids only grew from inoculated blood cultures bottles incubated at 37 °C. A small fungal load leading only to mild symptoms could also explain the difficulty of isolation, requiring this enrichment phase.

After isolation from two clinical samples in two different laboratories, we could not identify *C. stellimalicola* using routine identification methods: morphological and biochemical criteria as well as MALDI-TOF MS. In medical microbiology laboratories, MALDI-TOF MS is a very reliable tool, now widely used routinely for identification of yeasts [23], but its application is limited regarding rare species such as *C. stellimalicola* which are represented neither in the database of the Bruker Fungi Library V1.0 (Bruker Daltonics) nor in that of Vitek MS IVD V2.0 (BioMérieux).

The difficulties encountered in identifying this yeast make use of molecular biology techniques essential. Sequencing of the ITS region provides "universal DNA barcodes" for fungi, facilitating reliable identification of practically all fungi pathogenic to humans. In our case, sequencing of ITS area and D1/D2 domain allowed successful identification of our clinical strains at the species level.

Our strain of *C. stellimalicola* exhibited high MIC values to azole compounds. In the absence of *in vivo* data, it is difficult to extrapolate high MIC to clinical azole resistance but *in vitro* profile and our patient clinical outcome advocates use of echinocandins in *C. stellimalicola* infections. Susceptibility testing needs to be performed on a greater number of *C. stellimalicola* strains in order to determine its susceptibility pattern.

Candida stellimalicola is rare and likely has low virulence, which could explain that we reported the first isolation in a clinical specimen. Other human cases of *C. stellimalicola* candidiasis may have occurred, but the yeast was either not isolated or might have been misidentified or it might have not been reported. In conclusion, we report the first case of possible infection by *C. stellimalicola,* which occurred in a pancreas-transplanted patient and had a favorable outcome after antifungal treatment. *C. stellimalicola* was probably responsible for preservation fluid contamination during organ procurement or processing. This highlights the need of a good communication channel between centers to initiate an early and appropriate antifungal treatment.

Although *C. albicans* is the most frequently involved yeast in organ-transplanted patients, other *Candida* species must not be ignored. It is therefore important to use molecular identification technique when routine techniques do not allow successful identification. Moreover, sensitivity profile must be determined, even in the absence of species-level identification, because resistance to fluconazole is not uncommon, especially in emergent species.

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Compliance with Ethical Standards

Conflict of interest The authors have no relevant conflicts of interest.

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