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CASE REPORT

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Detection of invasive *Trichosporon asahii* in patient blood by a fungal PCR array

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Abstract

Rare invasive fungal infections are increasingly emerging in hosts with predisposing factors such as immunodeficiency. Their timely diagnosis remains difficult, as their clinical picture may initially mimic infections with more common fungal species and species identification may be difficult with routine methods or may require time-consuming subcultures. This often results in ineffective drug administration and fatal outcomes. We report on a patient in their early twenties with mixed cellularity classical Hodgkin lymphoma with a disseminated *Trichosporon asahii* (*T. asahii*) infection. Even though pathogen detection and identification was possible via the standard procedure consisting of culture followed by matrix-assisted laser desorption ionisation–time of flight (MALDI-TOF) mass spectrometry, the patient passed away in the course of multi organ failure. Herein, we report on a retrospectively applied experimental diagnostic fungal PCR-analysis used on an EDTA blood sample and consisting of two pan-fungal reactions and seven branch-specific reactions. Regarding invasive *T. asahii* infection, this PCR array could considerably shorten time to diagnosis and switch to a targeted therapy with triazoles.

INTRODUCTION

Immunosuppression due to medical therapy has extended life expectancy of patients with previously fatal diseases and is therefore playing a crucial role in the increasing incidence of invasive fungal infections [1]. The selective pressure caused by broad-spectrum antifungal prophylaxis is important for the emergence of non-*aspergillus* mould or non-*candida* yeast infections [1, 2]. Globally, invasive *trichosporonosis* is reported to be the second most common non-*candida* yeast infection in patients suffering from haematological malignancies [3], oftentimes leading to fatal outcomes despite appropriate therapy [4–6]. The genus *Trichosporon* consists of 50 known species of which at least 15 are considered to be of medical relevance. The main infections caused by these species include white piedra, hypersensitivity pneumonitis and invasive infections [3], the latter predominantly caused by *T. asahii* [4, 7]. To assure successful patient outcome, all serious fungal infections need appropriate therapy. Different fungal species show a high heterogeneity in their antifungal susceptibility as resistance development is already an evolving challenge. Since only a few classes of antifungal drugs are available [8], one of the essential keys in successful case management including disseminated *trichosporonosis* is an early and accurate identification of these pathogens assuring an efficient antifungal drug administration [4, 9]. Due to its effectiveness against *Candida* spp., caspofungin is the preferred empirical therapy for blood stream infections with yeasts [10], but it is ineffective against *T. asahii* [5]. Herein, we report a lethal case of invasive *T. asahii* infection in a patient in their early twenties suffering from a mixed cellularity classical Hodgkin lymphoma. We emphasise the use of an experimental diagnostic PCR that would have shortened time to diagnosis by at least 24 h. Rapid diagnosis of *Trichosporon* spp. in blood using qPCR and enabling an early switch to targeted therapy may improve patient outcome.

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Abbreviations: CT, computed tomography; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; FUO, fever of unknown origin; ITS, internal transcribed spacer; LPCB, lactophenol cotton blue wet mount; MALDI-TOF, matrix-assisted laser desorption ionisation – time of flight; MIC, minimal inhibitory concentration; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; qPCR, real-time polymerase chain reaction; RNA, ribonucleic acid.

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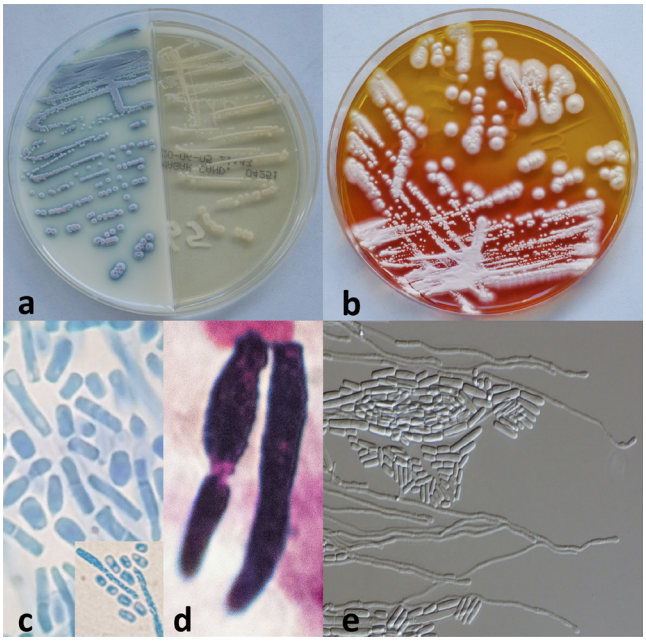

Fig. 1. *T. asahii* morphology on culture and microscopy, subcultures from the positive blood culture bottle: (a) On candida medium biplate after 4 days of incubation. Left side with dry blue/petrol-coloured colonies on chromogenic sabouraud agar, right side with white to cream-coloured colonies with raised surfaces on sabouraud agar. (b) Dermasel agar plate after 4 days of incubation showing brilliant-white fluffy growth. (c) Microscopic morphology of *T. asahii*: septate hyphae and arthroconidia (lactophenol cotton blue wet mount). (d) Gram-staining. (e) differential interference contrast microscopy.

CASE REPORT

A young male was diagnosed in September 2019 with an Ann Arbour stage IV mixed cellularity classical Hodgkin lymphoma with multiple organ involvement. He received two cycles of escalated combination therapy with bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone (escBEACOPP) between September 2019 and January 2020. At the end of March 2020, he was hospitalised due to a reduced general condition with relapsing fever of unknown origin (FUO). A restaging scan including chest CT and abdominal MRI was performed and showed a progression of the liver involvement with an increase in organ size. Due to the progressive liver disease, an immediate relapse therapy was initiated according to the IGEV-protocol (ifosfamide 2000 mg m^2 on days 1 to 4, gemcitabine 800 mg m^2 on days 1 and 4, vinorelbine 20 mg m^2 on day1, and prednisolone 100 mg on days 1 to 4) [11]. Additionally, extensive diagnostic testing was conducted to rule out possible infectious causes of the fever. One blood culture grew *Enterococcus faecalis*. Transoesophageal echocardiography showed no vegetation. An antibiotic lock therapy of the port catheter system was performed with vancomycin. The patient received empirical antibiotic treatment with piperacillin-tazobactam and vancomycin. Under this therapy, further clinical decline with increasing inflammation markers and persistent fever was observed. In addition, chest radiography showed new bi-pulmonary infiltrates. The anti-infective therapy was empirically escalated to meropenem, linezolid, amikacin and caspofungin. Additionally, prophylaxis against *Pneumocystis* pneumonia with sulfamethoxazole-trimethoprim was started. After an initial improvement, global respiratory failure necessitating endotracheal intubation developed before completion of the first chemotherapy cycle. The port catheter was explanted and new blood culture samples were collected. During severe neutropenia, the clinical picture of sepsis progressively evolved resulting in multiple organ dysfunction. Due to acute kidney failure, dialysis was begun.

Table 1. Minimal inhibitory concentration (MIC) values against antifungal agents of the *T. asahii* isolate obtained at the National Reference Centre for Invasive Fungal Infections by microdilution (according to the EUCAST requirements)

Antifungal agent	MIC (µg ml^{-1})
Amphotericin B	16.0
Fluconazole	8.0
Voriconazole	0.25
Posaconazole	0.5
Anidulafungin	>8.0
Caspofungin	32.0
Itraconazole	1.0
Isavuconazole	0.25

After 9 days of ventilation without detection of a causative pathogen, a single blood culture bottle indicated growth (time to positivity: 28 h). Gram-staining (Fig. 1d) revealed yeast-like structures and sub-culture on Sabouraud agar was performed (Fig. 1a, b). A lactophenol cotton blue wet mount (LPCB) was performed with the material from the agar plates showing septate hyphae and arthroconidia (Fig. 1c). After further sub-cultivation on Sabouraud agar for 24 h, the fungal pathogen was identified as *T. asahii* by matrix-assisted laser desorption ionisation–time of flight (MALDI-TOF) mass spectrometry with an accuracy of 99.9% using VITEK MS (bioMérieux, Marcy l'Etoile, France). In addition, cultures from a lower respiratory sample (semi-quantitative: sparse, i.e. growth only in a densely seeded area of the plate) and a urine sample (pathogen count: 1000 ml^{-1}) from the same day showed growth of *T. asahii* (equally identified by MALDI-TOF) on Sabouraud and non-selective agar plates. Strains are deposited in the Jena Microbial Resource Collection (JMRC:NRZ:2547 and JMRC:NRZ:2548).

Antifungal therapy was switched to 400 mg voriconazole twice daily and susceptibility testing was done by the German National Reference Centre for invasive fungal infections in Jena by microdilution according to EUCAST standards. The minimal inhibitory concentrations (MIC) of possible therapeutic substances were determined with the results listed in Table 1. The clinical condition of the patient

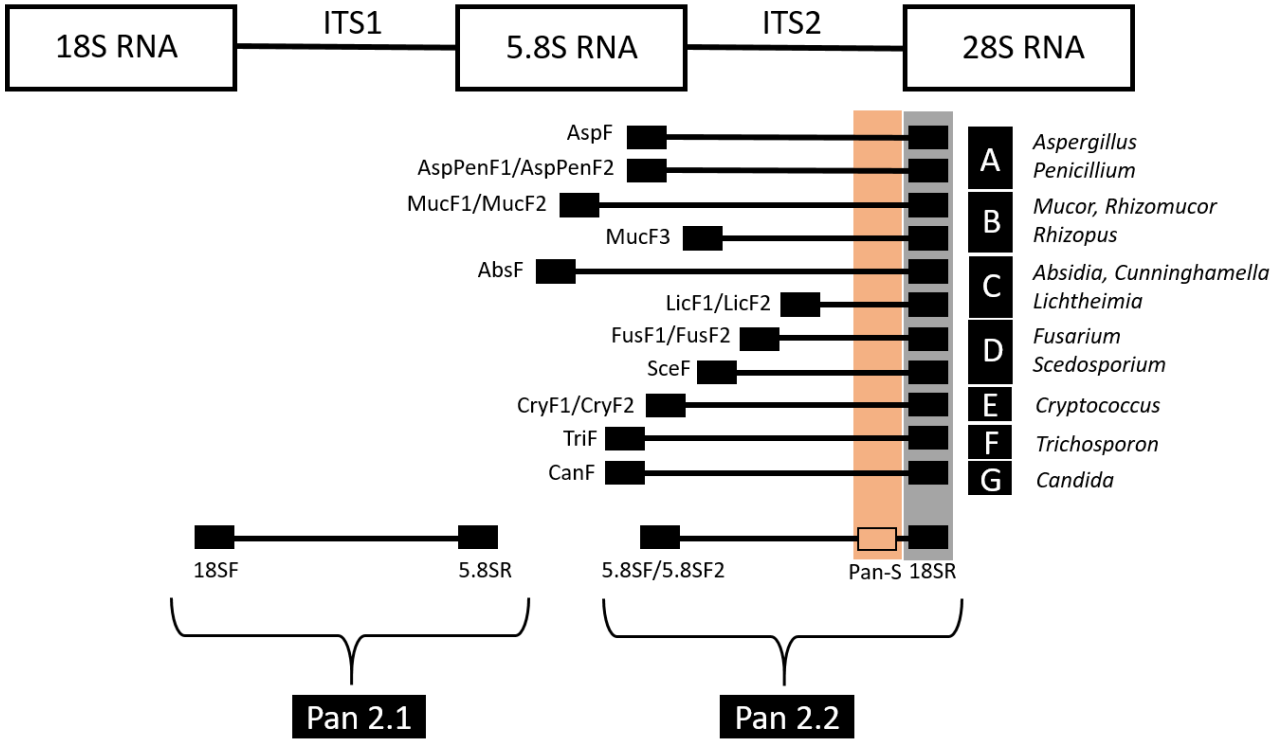

Fig. 2. Binding regions of primers and the probe of the fungal PCR array. ITS: nuclear ribosomal internal transcribed spacer region. The binding region of the probe is marked in orange.

improved and extubation was possible on the fourth day of voriconazole treatment. Chest CT showed a reduction of pulmonary lesions. However, 8 days later septic symptoms recurred and the patient developed acute respiratory failure and massive fluid accumulation in the lungs. His condition required re-intubation. The patient subsequently died due to multi organ failure. Despite four pairs of blood cultures and twice daily respiratory specimens being used for culture, no pathogen could be detected in the patient's final episode of illness.

In a retrospective analysis, we tested the original positive blood culture bottle, multiple subcultures in blood culture bottles and in addition tested EDTA blood received for other reasons using a fungal PCR array (fuPCR). The array consists of (Fig. 2):

(1) A set of two pan-fungal reactions using
   a. universal primers targeting the 18S RNA- and 5.8S RNA-encoding region (Pan 2.1) and
   b. universal primers targeting the 5.8S RNA- and 28S RNA-encoding region (Pan 2.2).

(2) Seven branch-specific reactions (A-G) for the detection of common moulds (*Aspergillus*, *Penicillium* (A), *Rhizopus, Mucor, Rhizomucor* (B), *Absidia, Lichtheimia, Cunninghamella* (C), *Fusarium* and *Scedosporium* (D) and yeasts (*Cryptococcus* (E), *Trichosporon* (F) and *Candida* (G)) using a branch-specific primer targeting the nuclear ribosomal internal transcribed spacer 2 (ITS) region and a universal primer targeting the 28S RNA-encoding region.

The PCR array and primer sequences have been published elsewhere [12]. For increased specificity of the branch-specific reactions, a PCR probe (Pan-S: 6FAM-TAAGTTCAGCGG GTAGTCCTACCTGATT-BHQ1) targeting the ITS2 region was added. With reaction F (Fig. 2), *Trichosporon* spp. DNA was detected in subcultures from all blood culture bottles and, importantly, also in the patient's EDTA blood sample from the first day of voriconazole treatment. Two further branch-specific reactions (B and C, Fig. 2) showed cross-positivity. Sequencing of the PCR product with subsequent homology search via BLAST [13] and CBS-database [14] allowed species identification of *T. asahii* in all samples (Fig. 3).

DISCUSSION

This report reiterates the importance of considering *T. asahii* as an invasive fungal pathogen in patients at high risk for

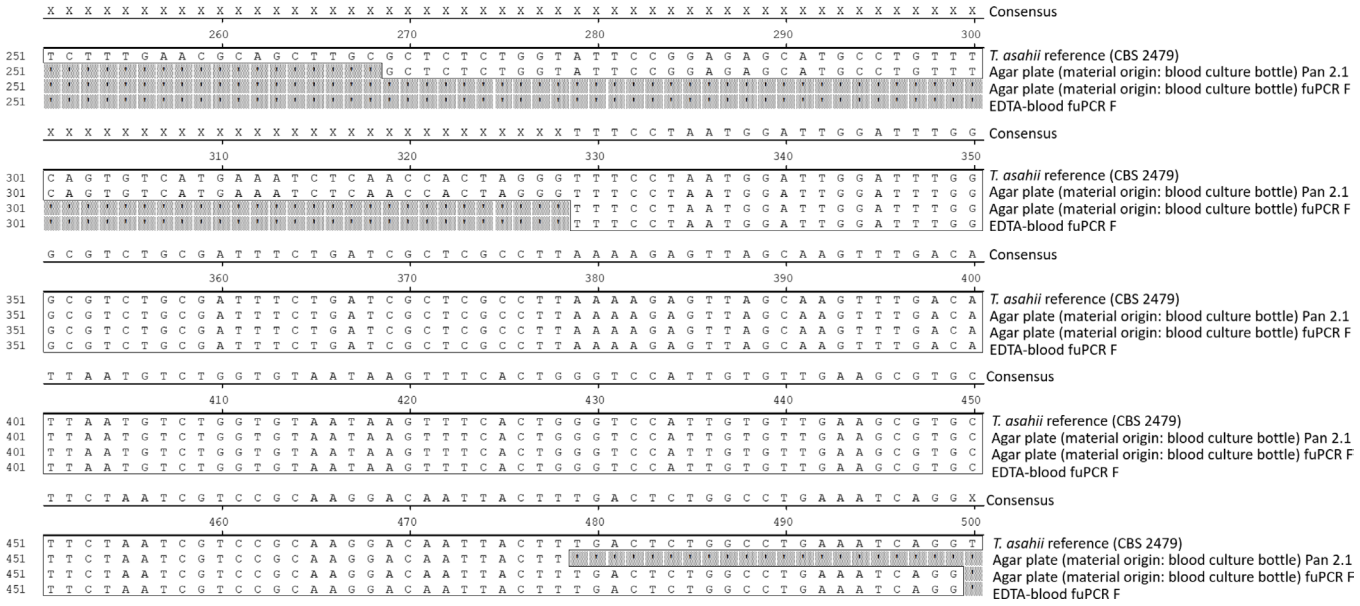

Fig. 3. ITS-Sequence alignments.

fungal infections and demonstrates that *T. asahii* can be detected and identified from patient blood with the PCR array method described here and without prior culturing.

Trichosporon spp. can cause a broad spectrum of disease ranging from self-limiting cutaneous infections to fatal invasive infections [3]. Diagnosis of disseminated *Trichosporon spp*. infections may be complicated by a lack of standardisation for species identification [15]. In addition, invasive *trichosporonosis* may be indistinguishable from invasive *candidiasis* in both clinical manifestation as well as histological appearance [9, 15]. *T. asahii* is increasingly reported to cause systemic opportunistic infections, especially in patients suffering from haematological malignancies [16–18]. Even though invasive *trichosporonosis* is a more common problem in neutropenic patients [19], rarer cases without neutropenia have been described [20].

Trichosporon spp. can be isolated from multiple sites including blood, urine, wounds, ascites, pleural effusion, bile, cerebrospinal fluid, sputum and stool [5]. The minimum requirement for diagnosis of a disseminated infection is at least one positive blood culture or positive cultures from two different sites [21]. We were able to isolate the pathogen at three different sites: blood, urine and tracheal aspirate. The microscopic investigation of our samples showed a typical morphology of *T. asahii* presenting septated hyphae that disarticulate into arthroconidia (Fig. 1). In MALDI-TOF mass spectrometry, all of our samples were identified as *T. asahii*.

Two sequential culturing steps (with 28 and 24 h duration, i.e. 52 h in total) were necessary for species detection and identification allowing a switch to voriconazole treatment. In contrast, the application of the PCR array was able to identify a high probability of non-*candida* infection within 4 h and species identification after subsequent sequencing, i.e. within 28 h and thereby 24 h faster than the conventional methods. Faster sequencing methods, which are already available, could shorten this time even further. In contrast to previously described methods for faster identification of invasive fungal infections [22], the approach we describe here aims for species identification without need for a prior culturing step.

The material we investigated was aligned using the Basic Local Alignment Search Tool [23]. The alignment shows a 100% match to the ITS sequence of ex-type strain of *T. asahii* (CBS 2479). This allows a definite identification of the species (Fig. 3).

Currently, there is no standard treatment for invasive *trichosporonosis*, since there is a high variety in susceptibility against antifungal compounds [4, 9]. Also, numerous break-through infections under antifungal prophylaxis have been reported [17, 18, 24]. *Trichosporon* spp. have been reported to show high MIC-values for echinocandines and 5-flucytosine [4, 5, 25]. Triazoles have emerged as a promising alternative [5, 26], with voriconazole developing as the preferred compound due to good *in vitro* activity and *in vivo* outcome [5, 21, 27].

Based on our case, we would like to emphasize the importance of considering *T. asahii* as a causal agent of invasive fungal infection. Implementing a pan-fungal PCR array, like the one described here, in routine diagnosis has the potential to significantly shorten the time between sampling and genus identification. While culture is necessary to obtain susceptibility testing results, we conclude that in patients with an increased risk of life-threatening infections with rare fungal pathogens, a rapid detection method allowing pathogen identification like the one presented here can be an important addition. By using a method developed for efficient DNA

extraction from (filamentous) fungi, an increased specificity of the branch-specific reactions of the fuPCR could shorten time to definitive diagnosis even further and help achieving the best possible outcome for the patients [28].

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Approval by an ethics committee is not required for case reports according to local regulations.

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