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# Fatal Disseminated Infection with *Fusarium petroliphilum*

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**Abstract** Members of the *Fusarium solani* species complex (FSSC) are causing the majority of the fusariosis in humans. Disseminated fusariosis has a high mortality and is predominantly observed in patients with leukemia. Here, we present the case of a fatal infection by a *Fusarium* strain with a degenerated phenotype, in a patient with acute lymphatic leukemia. Multiple nasal and skin biopsies as well as blood cultures yielded fungal growth, while in direct and histopathological examination of biopsy material septate hyphae were visible. Initial colonies were white with slimy masses with microconidia reminiscent of *Fusarium/Acremonium*, but with conidiospore production directly on the hyphae. Multi-locus sequence typing discerned a pionnotal—morphologically degenerated—colony of the recently recognized *F. petroliphilum* as etiological agent. The culture returned to a typical *F. solani* species complex morphology only after several weeks of growth in culture. Antifungal susceptibility tests indicate amphotericin B as best drug for this FSSC member rather than any of the azoles or echinocandins.

**Keywords** Acute leukemia · Antifungal susceptibility tests · *Fusarium petroliphilum* · *Fusarium solani* species complex · Pionnotal cultures

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## Introduction

The types of infection caused by *Fusarium* spp. in humans range from infections of nail, skin, and eye in immunocompetent hosts to invasive and disseminated infections in mainly immunocompromised patients [1–3]. In particular, the different types of leukemic patients are vulnerable to disseminated infections. For disseminated fusariosis, prolonged neutropenia and T cell immunodeficiency are two of the main risk factors [1]. Disseminated fusariosis is often fatal and presents with characteristic skin lesions with necrotic center and positive blood cultures.

At least six species complexes and several single species with the large genus *Fusarium* have been

responsible for human infections. Such species complexes are often species rich, while many sibling species are morphologically near indistinguishable. The *Fusarium solani* species complex (FSSC) members are responsible for approximately two-thirds of all cases of fusariosis [4]. Besides causing human infections, members of FSSC are commonly encountered as economically important plant pathogens as well as saprophytes [5].

To recognize the different sibling species within the FSSC, we currently rely on multi-locus sequence typing (MLST) [4–7]. Many of the recognized MLST haplotypes have no binomial names as yet, but some were recently identified, like *F. petroliphilum* and *F. keratoplasticum* [8]. Some morphologically recognizable etiological agents like *F. falciforme* and *F. lichenicola* lacking the fusiform macroconidia typically associated with *Fusarium* have now also been placed based on MLST data within the FSSC [9]. A specially curated database is available for the identification of *Fusarium* strains based on sequence data [6]. However, isolated etiological agents may not be recognized at first to belong to the genus *Fusarium* as pionnotal—degenerated—appearances that are frequently encountered in human isolates, especially in the FSSC [2, 8].

Guidelines for the treatment of deep and disseminated infections by *Fusarium* and other hyalohyphomycoses were recently published by a consortium of experts [10]. *Fusarium* species are known for their refractory behavior toward antifungals [11]. Treatment options seem limited to amphotericin B, voriconazole, and posaconazole, while echinocandins are better avoided [10]. Within species complexes variation exists in susceptibility for specific antifungal compounds [e.g., 12, 13]. Occasionally, human isolates and species that are multiple resistant to all available drugs are encountered [14]. With (MLST) identification of species level rather than (morphological) recognition of species complexes and with antifungal susceptibility test (AFST) data on species level, we may tailor patient treatment better to the encountered etiological agent.

## Case Presentation

The 48-year-old female patient who had symptoms of shortness of breath and palpitations was hospitalized with the diagnosis of acute lymphoblastic leukemia to the hematology department of the Uludag University Hospital. The patient developed fever on the second day of remission induction therapy. The initial clinical suspicion was a community-acquired infection, and treatment with cefepime ($3\times$ 2 g $day^{-1}$ i.v.) and clarithromycin ($2\times$ 500 mg $day^{-1}$ for 10 days i.v.) was started. In spite of an initial decrease in fever, the patient's body temperature increased again on the 17th day of chemotherapy and cefepime was switched to imipenem ($4\times$ 500 mg $day^{-1}$ i.v.). Due to the presence of a central venous line, vancomycine ($2\times$ 1 g $day^{-1}$ i.v.) was also added. Two days later, a black-crusted lesion was observed in the patient's nasal mucosa. Although the paranasal sinus and chest computerized tomography (CT) did not indicate a fungal infection, fresh microscopic examination (KOH %10) of the nasal biopsy sample revealed septate hyphae, and liposomal amphotericin B at 3 mg/kg/day was added to patient's regimen on the 19th day of the chemotherapy. On the second day of amphotericin B therapy, the patient had another episode of dyspnea and the chest X-ray indicated bilateral pneumonia. Thus, levofloxacin ($1\times$ 500 mg $day^{-1}$ i.v.) and trimethoprim-sulfamethoxazole ($3\times$ 960 mg $day^{-1}$ i.v.) were added to patient's regimen on the 20th day of chemotherapy.

Septate hyaline hyphae were seen in histopathological examination of nasal biopsy, and culture of biopsy material yielded a growth of a mold resembling *Fusarium*/*Acremonium* spp. The patient developed 0.5–1-cm-wide maculopapular lesions with necrotic centers and hyperemic margins on the 22nd day of chemotherapy (Fig. 1). Two skin biopsy samples and a blood culture obtained during the febrile period yielded the same growth of mold resembling *Fusarium*/*Acremonium* spp. The patient's condition gradually deteriorated, and on the 28th day of chemotherapy and on the 8th day of liposomal amphotericin B therapy, the patient died. The cultures were sent to the CBS-KNAW Fungal Biodiversity Centre, Netherlands for further identification.

## Identification of the Fungal Etiological Agent

From nasal biopsy material (two times), skin biopsy materials (two times), and blood culture, mycelial growth was obtained. Direct and histopathological

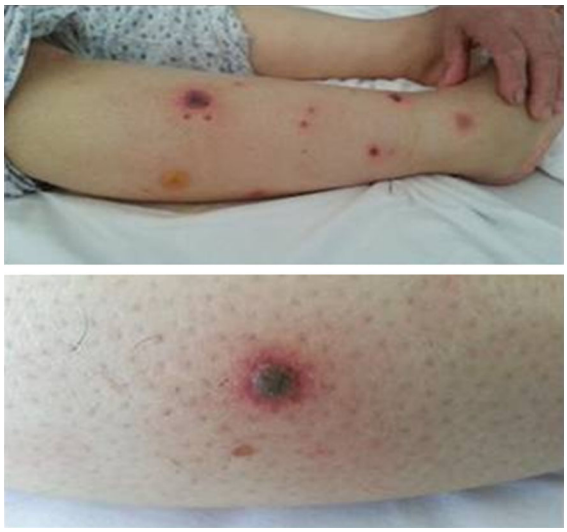

**Fig. 1** Necrotic maculopapular skin lesions of the patient

examination of the biopsy material showed hyaline septate hyphae. The colonies grown on Sabouraud-dextrose medium (SABG) were white, with slimy heads full with slightly crooked microconidia reminiscent of *Acremonium* or *Fusarium*. However, the colonies did not show typical conidiophores, but spores (Fig. 2a) were produced directly on the hyphae, where also small round scars were visible (Fig. 2b, c). The culture was deposited in the CBS culture collection, Utrecht, The Netherlands, under number CBS 135955.

DNA was isolated with a cetyltrimethyl ammonium bromide (CTAB)-based method [15]. The barcoding internally transcribed spacer (ITS) region and the 28S large ribosomal subunit (LSU) were used for identification of genus level; for identification of species level, partial transcription elongation factor 1 alpha (*tef*1α) and a subunit of the RNA polymerase (*rpb*2) sequences were used under conditions as described before [16]. The PCR fragments were sequenced with the ABI Prism_Big DyeTM Terminator v. 3.0 ready reaction cycle sequencing kit (Applied Biosystems, Foster City, CA) and analyzed on an ABI PRISM 3700 Genetic Analyzer (Applied Biosystems). Sequences were compared via BLAST to sequences in GenBank (http://www.ncbi.nlm.nih.gov/genbank/), and to the *Fusarium* MLST database (http://www.cbs.knaw.nl/Fusarium/) [6]. The obtained sequences (Genbank accession numbers KJ867425 (ITS), KJ867423 (*LSU*), KJ867424 (*tef*1α), and KJ867426 (*rpb*2)) identified the etiological agent as a FSSC type 2 strain and hence an isolate of *F. petroliphilum*.

Re-examination of older and subcultured colonies showed that the culture slowly reverted to morphology as expected from species belonging to the FSSC with long conidiophores and macroconidia (Fig. 2d). In this particular strain, the pionnotal phenotype proved reversible. Pionnotal *Fusarium* strains—even ones totally lacking spore production—have regularly been implicated in clinical infections [2, 8].

## Antifungal Susceptibility Testing

In vitro AFST was performed for anidulafungin (ANI; Pfizer Central Research, UK), amphotericin B (AMB;

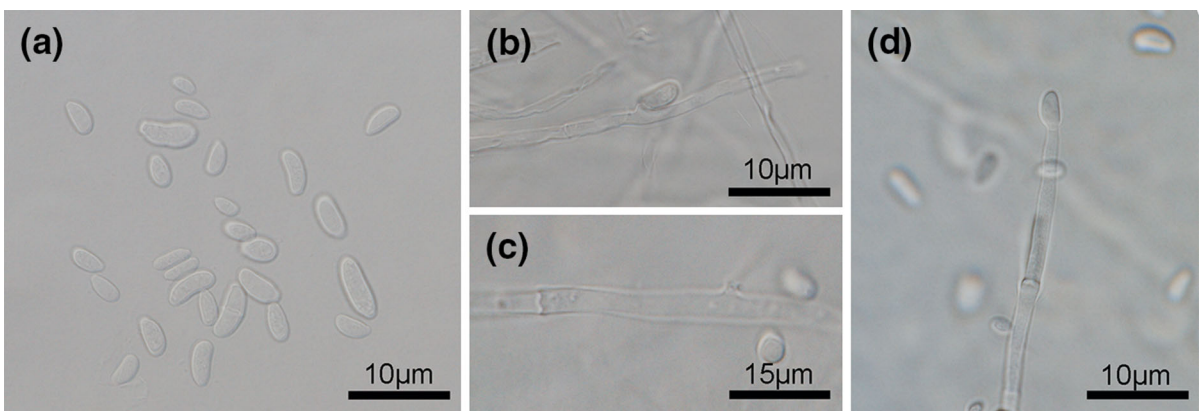


**Fig. 2** **a** Microconidia that is predominantly 0-septate, oval, ellipsoid, and reniform. **b** Microconidium formed directly on the side of hyphae in the pionnotal mycelium. **c** Scar tissue on hyphae after microconidia production. **d** Reverted colonies with long conidiophores typical for members of the FSSC

**Table 1** MIC values of 8 antifungal compounds (mg/l) of individual *F. petroliphilum* strains tested according to CLSIM38A2

| CBS number | ANI* | AMB* | FLC* | ISA* | ITC* | MICA* | POS* | VOR* |
|---|---|---|---|---|---|---|---|---|
| CBS 224.34 | >8 | 1 | >64 | >16 | >16 | >8 | >16 | 16 |
| CBS135779 | >8 | 1 | >64 | >16 | >16 | >8 | >16 | 16 |
| CBS135789 | >8 | 1 | >64 | >16 | >16 | >8 | >16 | 16 |
| CBS135799 | >8 | 0.5 | >64 | >16 | >16 | >8 | >16 | 8 |
| **CBS135955** | **>8** | **1** | **>64** | **>16** | **>16** | **>8** | **>16** | **8** |

The strain involved in the clinical case described here is indicated in bold

* *ANI* anidulafungin, *AMB* amphotericin B, *FLC* fluconazole, *ISA* isavuconazole, *ITC* itraconazole, *MICA* micafungin, *POS* posaconazole, *VOR* voriconazole

**Table 2** Comparison of the ranges of the MIC values of 8 antifungal compounds (mg/l) of *Fusarium solani* sensu *latu*, *F. petroliphilum*, *F. falciforme,* and *F. lichenicola*

| Species (number of isolates) | ANI* | AMB* | FLC* | ISA* | ITC* | MICA* | POS* | VOR* | References |
|---|---|---|---|---|---|---|---|---|---|
| *F. solani* sensu *latu(66)* | >16 | 0.5–8 | >64 | 8–16 | >16 | 8–256 | >16 | 1–32 | [21, 22] |
| *F. petroliphilum(6)* | >8 | 0.5–1 | >64 | >16 | >16 | >8 | >16 | 8–16 | This study, [4] |
| *F. falciforme(4)* | >16 | 0.5–4 | >64 | | >8–>32 | >16 | >8 | 4 | [4, 23], unpublished data |
| *F. lichenicola(3)* | >16 | 0.25–4 | >64 | | >8–100 | >16 | >8 | 1.56–8 | [4, 24, 25] |

* *ANI* anidulafungin, *AMB* amphotericin B, *FLC* fluconazole, *ISA* isavuconazole, *ITC* itraconazole, *MICA* micafungin, *POS* posaconazole, *VOR* voriconazole

Sigma, The Netherlands), fluconazole (FLC; Pfizer Central Research, Sandwich, UK), isavuconazole (ISA; Basilea Pharmaceuticals, Basel, Switzerland), itraconazole (ITC; Janssen-Cilag,Tilburg, The Netherlands), micafungin (MICA; Astellas, Japan), posaconazole (POS; Merck, Whitehouse Station, USA), and voriconazole (VOR; Pfizer Central Research). All antifungal compounds were obtained from their manufacturers as pure powders. The CLSI microdilution method M38-A2 for filamentous fungi [17] was followed using treated 96-wells tissue culture plates from Costar®, RPMI1640 with glutamine without $NaHCO_3$ from Gibco, and MOPS from Sigma. Minimal inhibitory/effective concentrations (MIC/MEC) for CBS 135955 were ANI (>8 mg/l), AMB (1 mg/l), FLC (64 mg/l), ISA (>16 mg/l), ITC (>16 mg/l), MICA (>8 mg/l), POS (>16 mg/l), and VOR (8 mg/l); the results for the other tested *F. petroliphilum* strains from the CBS collection were similar (Table 1).

Comparing these results to other published AFST data for the FSSC or particular members of the FSSC, we can observe that the FSSC MICs of VOR are in the range from 1 to 32 mg/l, hence from susceptible to resistant, whereas all tested *F. petroliphilum* had MIC values of ≥8 mg/ml (Table 2). For ANI, FLC, ISA, ITC, MICA, and POS both *F. petroliphilum* and the other FSSC members are resistant. Therefore, amphotericin B seems to be the drug with the lowest MIC against *F. petroliphilum*.

## Discussion

In immunocompromised patients, *Fusarium* spp. either penetrate the skin barrier or cause a pulmonary infection by airborne transmission. In many patients, skin lesions may be the first sign of a disseminated infection and are commonly seen in the early stages of the disease [1, 18]. In 40 % of the cases, *Fusarium* species can be isolated from blood cultures. In the presence of skin lesions, the identification rate may increase up to 56 % [19]. In the great majority of patients, fungaemia developed within 5 days after skin lesions become evident [20]. In our patient, the causative agent was first isolated from a nasal biopsy, and skin lesions developed later. Similar as reported in the literature, the agent was isolated from blood cultures 4 days after the onset of skin lesions.

Survival rate of patients with fusariosis who are persistent neutropenic remains around 4 % in spite of

aggressive therapy [19]. The recently published guidelines for treatment of fusariosis recommend amphotericin B and voriconazole for infections caused by *Fusarium* [10], while posaconazole—often used as prophylaxis—has also activity against some members of the genus *Fusarium* [1]. However, our antifungal susceptibility tests of the different *F. petroliphilum* isolates indicate that for this particular species in the FSSC, the azoles and echinocandins are not effective, leaving only amphotericin B as potentially effective treatment.

Despite the fact that our patient received amphotericin B therapy, she died. Hence, more important than the antifungal drug in effective treatment of disseminated fusariosis in a leukemic patient is reversal of neutropenia and T cell dysfunction. Disseminated infections in other types of immunocompromised patients like SOT patients or HIV patients are extremely rare [1, 2]. In non-leukemic patients, invasive fusariosis remains localized, suggesting that especially neutrophils and T cells have an important role in limiting spread of *Fusarium* via blood. The prognosis of disseminated fusariosis is poor and is determined largely by the degree of immunosuppression [1].

*Fusarium petroliphilum* is not a new species causing opportunistic infections in humans, only its status as separate species within the *Fusarium solani* species complex is recent [8]. We tested five clinical isolates, the oldest being CBS 224.34, first isolated from onychomycosis in 1934. The AFST tests of the 8 antifungal compounds proved very uniform, showing how refractory the species always have been to any antifungal compound available.

In clinical infections, pionnotal *Fusarium* strains—even ones totally lacking spore production—have regularly been described [2, 8]. In the etiological agent studied here, this degenerate pionnotal phenotype proved reversible. Re-examination of colonies that were subcultured again or kept for several weeks showed that our culture reverted to morphology as expected from species belonging to the FSSC. It seems that for the opportunistic pathogen *Fusarium* the complex conditions within the human body select for different growth characteristics. Direct production of microconidia on hyphal fragments in tissue could be a reason why *Fusarium* is one of the few fungi regularly isolated from blood cultures.

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