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LETTER TO THE EDITOR

Case Report. Fungaemia due to *Penicillium piceum*, a member of the *Penicillium marneffei* complex

Fallbericht. Fungämie durch *Penicillium piceum*, einem Mitglied des *Penicillium marneffei* Komplexes

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Schlüsselwörter. *Penicillium piceum*, *Penicillium marneffei*, *Biverticillata*, Fungämie.

Summary. Due to the inability of most *Penicillium* species to grow at 37 °C, systemic non-*marneffei* infections are very rare in the human host. We describe a case of fungemia due to *Penicillium piceum* in a female patient, who died a few days after repeated isolation of this fungus from blood cultures. The species is a member of the section *Biverticillata* of *Penicillium*, as was confirmed by rDNA Internal Transcribed Spacer (ITS) sequence data, and hence may share virulence factors with *P. marneffei.*

Zusammenfassung. Bedingt durch die Tatsache, daß die meisten *Penicillium*-Spezies nicht fähig sind, sich bei 37 °C zu vermehren, sind systemische nicht-*marneffei* Infektionen beim Menschen sehr selten. Wir beschreiben einen Fall einer Fungämie durch *Penicillium piceum* bei einer Patientin, die wenige Tage nach wiederholter Isolation dieses Pilzes aus Blutkulturen verstarb. Diese Spezies ist ein Angehöriger der Sektion *Biverticillata* von *Penicillium*, was durch rDNS ITS-Sequenzen bestätigt wurde; möglicherweise besitzt es ähnliche Virulenzfaktoren.

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Introduction

In clinical practice, *Penicillium* species isolated from non-AIDS patients are mostly discarded as contaminants. The main reason is their ubiquitous occurrence as airborne contaminants, which are generally unable to grow at 37 °C. In the present article we report on a fungus that was repeatedly isolated from blood of a patient with cholangiocarcinoma, which initially grew with sterile mycelium and only in a later stage produced conidiophores typical of the genus *Penicillium*. The species was identified as *P. piceum*, a close relative of *P. marneffei* and hitherto unknown in clinical pathology.

Case report

A 57-year-old German woman with chronic sinusitis and a history of alcohol abuse suffered from pain in her back for a few weeks and had observed significant weight loss during the last two months before admission to a regional hospital. A CT-scan showed multiple lesions in the patient's liver. Histopathology of tissue material from those lesions revealed tumour metastases. Two weeks later the patient was transferred to the University Hospital of Bonn, Germany,

for the establishment of a definite diagnosis. No primary cancer could be found by CT-scan or sonographically. Clinical chemistry gave the following results, with normal parameters shown in brackets: γ-glutamyltransferase: 229 Ul^{-1} (4–18 Ul^{-1}), glutamic oxalacetic transaminase; 46 Ul^{-1} (15 Ul^{-1}), alkaline phosphatase; 1050 Ul^{-1} (55–170 Ul^{-1}), lactate dehydrogenase; 384 Ul^{-1} (100–200 Ul^{-1}), urea; 11.0 mg dL^{-1} (2.3–6 mg dL^{-1}), C-reactive protein; 17.6 mg dL^{-1} (max. 0.8 mg dL^{-1}), leucocytes; 36.2 g l^{-1} (4.0–9.0 g l^{-1}), cholinesterase; 984 Ul^{-1} (2800–8500 Ul^{-1}) and haemoglobin: 8.8 g dL^{-1} (11.5–16.5 g dL^{-1}). Remaining tests showed no abnormalities. A gallstone constipating the biliary duct could be observed. Two weeks later the patient developed fever of approximately 38.5 °C accompanied by cough. Pulmonary infiltrates were diagnosed by CT-scan. Blood cultures were sent for microbiological examination. After five days of incubation at 37 °C, the blood culture system (Bactec 9240, Becton Dickinson, Heidelberg, Germany) showed growth in the aerobic culture bottle. Gram-stained smears of the culture fluid showed masses of septate hyphae. Amphotericin B therapy was started, but two weeks later the patient died of cardiovascular disorder. Two days before death, another blood culture was taken, from which the same fungus was isolated. In autopsy specimens of the liver, kidneys and pleura, a cholangiocarcinoma was diagnosed, but no fungal elements were found with Gomori's methenamine-silver stain. Because no serum samples were available and tests for HIV during the patient's life had not been performed, both blood culture fluids were tested for HIV (in-house Reverse Transcriptase-PCR), hepatitis B (in-house PCR) and hepatitis C (in-house RT-PCR). All of these yielded negative results.

Mycology

Subculturing on Sabouraud glucose agar yielded growth after 3 days at 37 °C and after 4 days at 30 °C. Colonies were yellow, soon becoming green. On Columbia agar supplemented with 5% sheep blood, colonies were yellow to orange. Microscopic examination of yellow parts showed compacted sterile hyphae, while green patches revealed *Penicillium*-like morphology. A voucher strain was deposited in the CBS Culture Collection as CBS 102383 (Centraalbureau voor Schimmelcultures Utrecht, The Netherlands). Identification with *P. piceum* was performed on the basis of cultural characteristics and microscopic morphology (Fig. 1), and was confirmed by sequencing of the Internal Transcribed Spacer (ITS) domain of the rDNA gene. The sequence was compared to four reference sequences of *P. piceum*, amongst which was the type strain CBS 361.48, as well as *P. marneffei* and related species of the subgenus *Biverticillata.* The strains with final identification as *P. piceum* all proved to have closely similar ITS sequences (data not shown).

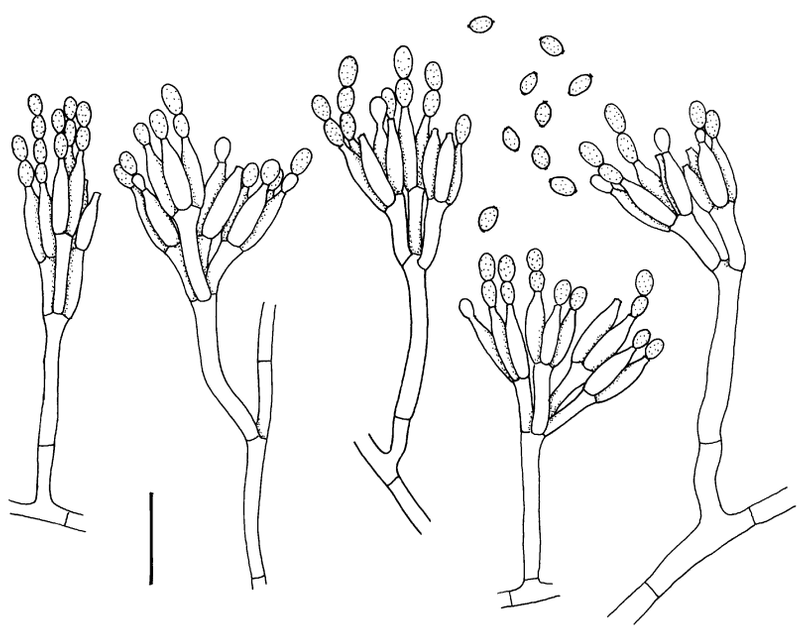

Figure 1. Microscopic morphology of *Penicillium piceum.*

Discussion

Penicillium piceum [1] is an uncommon representative of the genus *Penicillium*, which does not belong to the normal flora of hospital air. The same fungus was isolated from two different blood cultures with an interval of two weeks. No other microorganisms were encountered. This strongly suggests that this fungus was present as an agent of disseminated disease and that it may have contributed to the patient's death. Species of the related genus *Aspergillus* in general cannot be isolated using blood cultures; however this is possible with *P. marneffei* [2, 3] and the *Penicillium* species, which is currently recognized as pathogen, *P. piceum*. Both species belong to the subgenus *Biverticillata* of *Penicillium*. This group contains species showing close phylogenetic coherence, are morphologically similar and frequently produce red pigments into the agar. A remarkably large number of strains have been isolated from systemic infections in humans and warm-blooded animals (G. S. de Hoog & N. Poonwan, unpublished data). *P. piceum* shows faster growth at 37 °C than at 25 °C. This suggests an opportunistic potential of *P. piceum*. The species may share some virulence factors with *P. marneffei,* which is found at a short phylogenetic distance. As in our case, *P. marneffei* infection is frequently characterized by fever, weight loss, chronic cough and abnormal liver function [2, 4]. Unlike our *P. piceum* case, *P. marneffei* is almost exclusively found in patients with AIDS. Until recently *P. piceum* was known as a soil fungus [2, 3], but the culture collections of the CBS and the International Mycological Institute (IMI) hold several clinical isolates, from human sputum (CBS 250.56 and CBS 435.62) and pig lung tissue (IMI 140718). The species were previously reported as a cause of two cases of mycotic abortion in cattle [5]. With a systemic infection, disease processes may have been aggravated due to the fungus' production of the mycotoxin verruculogen, a tremorgenic toxin previously known as toxin-X [6]. This toxin was shown to cause solitary or multiple lesions in the liver of mice and chickens after feeding with contaminated foods [7]. Our patient was initially observed to have abnormalities in the liver; the possibility that the fungus had an impact on liver function cannot be excluded.

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