Case report

Paecilomyces variotii central nervous system infection in a patient with cancer

Fallbericht. ZNS-Infektion durch *Paecilomyces variotii* bei einem Krebspatienten

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Summary

Paecilomyces variotii was isolated from two subsequent cerebrospinal fluid (CSF) specimens of a cancer patient. Identification was confirmed through β-tubulin and rDNA ITS sequencing. MICs were determined for seven antifungal agents; the isolate was found to be susceptible to amphotericin B (AMB), itraconazole (ITZ), ketaconazole (KTZ) and 5-fluorocytosine (5FC) but resistant to fluconazole (FLZ) and miconazole (MCZ). Despite antimycotic therapy, the infection proved to be fatal.

Zusammenfassung

Paecilomyces variotii wurde zweimal aus Liquorproben einer Krebspatientin isoliert. Die Bestimmung wurde bestätigt anhand von β-Tubulin und rDNA-ITS-Sequenzierung. MHKs wurden festgestellt gegen sieben Antimykotika; das Isolat war empfindlich für Amphotericin B (AMB), Itraconazol (ITZ), Ketaconazol (KTZ) und 5-Fluorocytosin (5FC), aber resistent gegen Fluconazol (FLZ) und Miconazol (MCZ). Trotz antimykotischer Therapie verstarb die Patientin an dieser Infektion.

Key words: *Paecilomyces variotii*, cerebrospinal fluid, systemic infection, Opportunist.

Schlüsselwörter: *Paecilomyces variotii*, Liquor, systemische Infektion, Opportunist.

Introduction

Species of the hyphomycete genus *Paecilomyces* are common inhabitants of a wide variety of environmental niches such as soil and decaying organic material.[1,2] Some are occasionally encountered as agents of human and animal disease, particularly *Paecilomyces lilacinus* (Thom) Samson and *P. variotii* Bain.[3–9] Most cases are cutaneous or concern transient catheter-related infections, but also deep infections are known.[9] Fogerburg *et al.*[10] reported a cerebrospinal shunt obstruction in a female patient. The present article describes *P. variotii* from cerebrospinal fluid (CSF) of a human patient suffering from cancer.

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Case report

Clinical course

A 60-year-old female patient was admitted to the emergency department of Cerrahpasa Medical Scool Hospital in June 2001, with confusion which had started the previous day and was getting worse. She was diabetic for 18 years and lately her blood sugar was not well-regulated. She was diagnosed to have left invasive ductal breast cancer 19 years ago. Bone metastasis had appeared 5 years ago and a malignant pleural effusion

and lymphangitis carcinomatosa of the right lung appeared 2 years ago. Various chemotherapeutic regimens were applied during the last 2 years. One month ago she was hospitalized with febrile neutropenia following a course of chemotherapy. Ceftriaxon and ciprofloxacin therapy was started empirically. Blood and urine cultures were negative. On the fifth day of antibiotherapy, given the persistance of fever and the presence of an intravenous port, teicoplanin was applied. No growth was observed after repeated culturing of blood, urine and from the intravenous port. Fever dropped on the third day of teicoplanin therapy. Fluconazole was added for oral candidosis and continued for 5 days. Chemotherapy was no longer administered.

On admission patient was arousable with verbal stimulation, but was not cooperable. She was afebrile. Bronchial sounds were absent on left lower lung fields and there was a percussion dullness. Neurological examination revealed a lateral deviation of the left eye. Deep tendon reflexes were bilaterally negative. Her hemoglobin level was 12.8 g/dL, WBC count was 6400/mm^3, and platelet count was 86 000/mm^3. Serum urea, creatinin, electrolytes, and liver function tests were within normal limits. Blood glucose level was 267 mg/dL. A cranial magnetic resonance imaging (MRI) scan was performed (Fig. 1). Multiple lesions with hyperintense pathological signal intensity in T2 and which showed contrast enhancement were detected. Lumbar puncture revealed a clear cerebrospinal fluid (CSF) with a lymphocyte count of 300/mL. The CSF protein concentration was 130 mg/dL and the glucose concentration was 133 mg/dL with a serum glucose concentration of 238 mg/dL. Malignant cells were not detected in CSF, but numerous fungal cells and septate hyphae were observed in mycological examination (Fig. 2) and cocci were reported by bacteriological examination. Cranial MRI was not helpful in distinguishing between cranial metastasis and fungal infection. Cranial radiotherapy was started. The next day lumbar puncture was repeated and numerous fungal cells were observed again. Liposomal amphotericin B (AMB) 100 mg/day intravenous and meropenem 1 gram tid was started empirically. There was no growth on aerobic or anaerobic cultures of CSF specimens. As the patient regained consciousness after the first course of radiotherapy, it was decided to continue radiotherapy until culture results for fungal infection were obtained. The AMB dosage was increased to 150 mg/day and afterwards to 200 mg/day during the following days. After the fourth course of daily radiotherapy the patients' consciousness started to deteriorate. Respiratory failure followed and the patient was intubated and transferred to the intensive care unit (ICU). Patient never became febrile. AMB was continued with a dose of 200 mg/day. There was a slight improvement in the general well-being and consciousness of the patient. She was extubated on the fourth day of admission to the ICU. Subsequently patients' consciousness started to

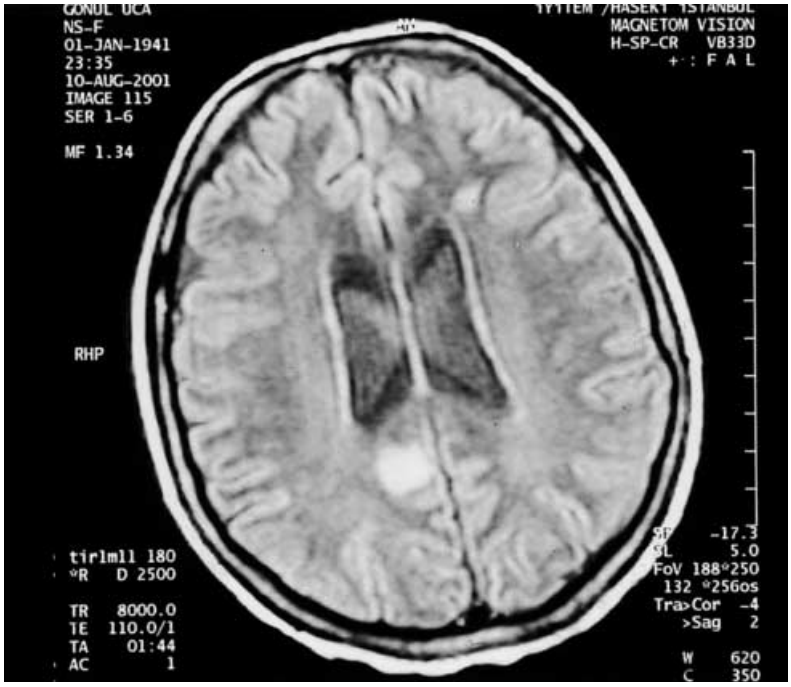

Figure 1 Cranial magnetic resonance image.

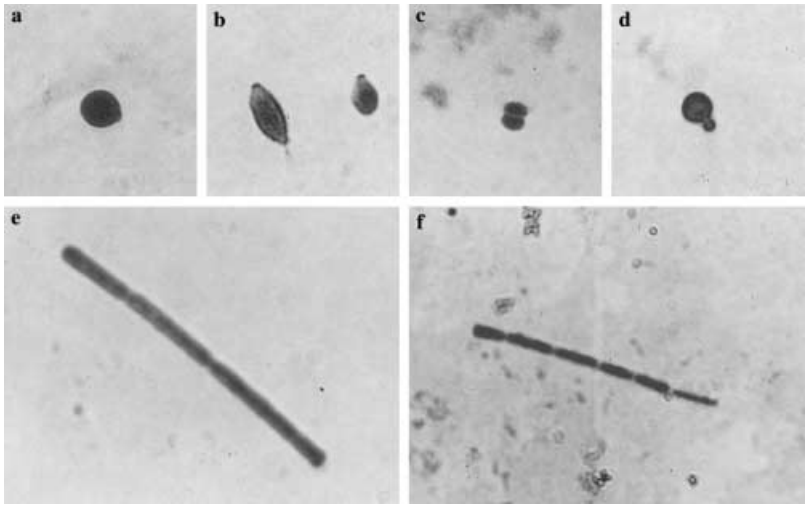

Figure 2 Examples of *in vivo* morphology observed in cerebrospinal fluid specimens [(a–d) stained with Ehrlich Ziehl Neelsen; (e) stained with Giemsa; and (f) stained with methylene blue].

deteriorate again. On the seventh day of ICU admission her left pupil became fixed and dilated. The patient was transferred to a normal room according to the wishes of the patient's family. Next day blood pressure started to drop and body temperature decreased to 35 °C. Blood cultures were taken. The patient deceased the same day, which was the twelfth day of her admission. Blood cultures showed growth of Gram-negative bacteria on day later.

Materials and methods

Isolation

Two successive CSF specimen were investigated. Specimen were centrifuged and the sediment was used for microscopical observation and culture while the upper part was used for cryptococcal latex agglutination (LA) test. The sediment was stained with Gram, Ehrlich Ziehl Neelsen, Giemsa and India ink, and was plated onto Sabouraud glucose agar (SDA), brainheart infusion agar (BHIA), cooked sheep's blood agar (BA) and niger seed agar (NSA) and incubated at 25, 30 and 37 °C. The isolated fungus was transferred to malt extract agar (MEA), Czapek glucose agar (CDA) and potato glucose agar (PDA). Cryptococcal antigen was investigated in the first CSF specimen with the use of a LA kit (Pastorex *Cryptococcus*, Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France). Identification was done by morphological examination of slides in lactophenol cottonblue.

Molecular genetics

Sequences of the rDNA internal transcribed spacer domain (ITS1 and ITS2) and the β-tubuline gene were analysed to verify the species level identification of the isolate. Sequences were identified using a large database of comparable sequences maintained at the Centraalbureau voor Schimmelcultures.

Antifungal susceptibility testing

The case isolate was tested according to the NCCLS reference standard broth macrodilution method (M38-P) for filamentous fungi to determine its susceptibility against seven antifungal agents.[11] Isolate was grown on PDA slants at 25 °C for 8 days. The inoculum was prepared by flooding agar slants with fungal colonies with 5 mL of distilled water, and carefully scraping the surfaces with the tip of a sterile Pasteur pipette to obtain a conidial suspension. The inoculum was adjusted to 95% transmission at 530 nm, vortexed for 15 s, and diluted 1 : 100 in test medium to provide a final inoculum concentration of $4–5 \times 10^4$ CFU/mL.

Antifungal agents and ranges tested included amphotericin B (AMB; Bristol Meyers Squibb, Wallingford, CT, USA), 0.03 to 16 μg/mL; fluconazole (FLZ; Pfizer, Istanbul, Turkey), 0.125 to 64 μg/mL; itraconazole (ITZ; Janssen Pharmaceuticals, Beerse, Belgium), 0.03 to 16 μg/mL; ketaconazole (KTZ; Milen, Istanbul, Turkey), 0.03 to 16 μg/mL; miconazole (MCZ; Selectchemie, Zürich, Switzerland), 0.03 to 16 μg/mL; flucytosine (5FC; Sigma, St Louis, MO, USA) 0.125 to 64μ g/mL; and terbinafine (TRB; Novartis, Basel, Swizerland), 0.03 to 128 μg/mL. Antibiotic Medium 3 (Oxoid, Hampshire, England) supplemented with 2% glucose and buffered with 0.165 mol morpholinepropanesulfonic acid (MOPS; Sigma) was used for testing AMB, and RPMI-1640 (Sigma Chemical Co., St Louis, MO, USA)

with L-glutamine but without sodium bicarbonate and buffered with 0.165 mol MOPS was used for testing the remaining antifungal agents. Tubes were incubated at 35 °C and read at 24-h interval when growth was observed in the drug-free control tube. MICs were determined by visual inspection in terms of the first tube that gave a score of 0 (optically clear) for AMB and a score of 2 (reduction in turbidity of ≥50% in contrast to that of the drug-free control tube) for the remaining agents. The *P. variotii* quality control strain ATCC 22319 with known MIC values, was run in conjunction with the test isolate in the susceptibility test.[12–14]

Results

Mycology

Microscopical examination of CSF preparations revealed yeast cells, chlamydospore-like cells with slightly verrucose walls, globose cells and septate hyphae. Encapsulated yeast cells were not observed in India ink preparations. Cryptococcal LA test was negative. After 8 days of incubation, minute, yellow–brown, powdery, slow growing colonies appeared on SDA. No growth was observed on BHIA, BA and NSA. No other microorganism was isolated from CSF on any of the applied media.

Colonies subcultured on MEA were powdery, in greenish brown. The reverse of colonies was buff with centrally brownish areas. Microscopical preparation revealed elongate phialides and abundant brownish conidia in long chains. The isolate was identified as *Paecilomyces variotii* according to morphological observations and verified by ITS1-2 and β-tubulin sequences of rDNA comparing with the data present in CBS. With ITS, nearest neighbour was CBS 248.48 (*P. variotii*) at 98.5% identity, while with β-tubulin, CBS 109072 (*Talaromyces spectabilis* Udagawa & S. Suzuki, teleomorph of *P. variotii*) was found at 98% identity. The isolate was deposited in the culture collection of the Centraalbureau voor Schimmelcultures with accession number CBS 110036.

Antifungal susceptibility test results

MICs were determined as follows: AMB: 2 μg/mL; FLZ: >64 μg/mL; ITZ: 2 μg/mL; KTZ: 2 μg/mL; MCZ: >16 μg/mL; 5FC: 0.5 μg/mL; and TRB: 1 μg/mL. The isolate was found *in vitro* resistant against FLZ and MCZ.

Table 1 Revirew of human *Paecilomyces variotii* infections.

Infection	References	Age / sex	Underlying conditions	Treatment	Outcome
Pneumonia	15		Hairy cell leukemia		
Pneumonia	16		DM		
Peritonitis	17				
Peritonitis	20	12 / M	CAPD	AMB (10 days)	Cured
Peritonitis	20	60 / F	CAPD	AMB (4 weeks)	Cured
Peritonitis	20	56 / F	CAPD	KTZ (10 days)	Cured
Peritonitis	20	39 /M	CAPD	KTZ	Cured
Peritonitis	21	Infant	CAPD	FLZ	Cured
Peritonitis	18		CAPD		
Peritonitis	19	65 / F	CAPD	AMB	Cured
CNS (disseminated ?)	10	57 / F	VSP		Died
CNS	This study	60 / F	Cancer, CT, DM	AMB	Died
Pyelonephritis	22		Nephrolithiasis, VSP		
Fungemia	23	/ F	BMT	AMB + ITZ	Cured
Soft tissue	24	8 / M	CGD	AMB (7 weeks), ITZ (1 year)	Cured
Sinusitis	25				
Sinusitis	26			Surgical	Cured
Cutaneous	29				
Cutaneous	30	25 / M			
Surgical site infection	31	31 / F	Cesarian incision	Needle aspiration	Cured
Endophtalmitis	27				
Chronic suppurative otitis media	28			KTZ	Cured
Airway colonization	37		CF, LT		

M, male; F, female; DM, diabetes mellitus; CAPD, continuous ambulatory peritoneal dialysis; VSP, ventriculoperitoneal shunt placement; CT, chemotherapy; BMT, allogenic bone marrow transplant; CGD, chronic granulomatous disease; LT, lung transplant; CF, cystic fibrosis.

Discussion

Paecilomyces variotii is a common fungus in the air and can grow in environments with high ambient temperatures. The species has been reported as the causative agent of human mycoses in the literature, mostly occurring in conjunction with prosthetic implants or immunosuppression. Cases include pneumonia,[15,16] peritonitis,[17–21] a fatal infection of a ventriculoperitoneal shunt,[10] a case of pyelonephritis,[22] fungemia,[23] soft tissue infection of the heel,[24] sinusitis,[25,26] endophthalmitis,[27] chronic suppurative otitis media,[28] cutaneous infection,[29,30] and a mycosis at the incision site of a patient who underwent a cesarean section.[31] Essential data are summarized in Table 1.

The first reported case of *P. variotii* associated with CSF was a female patient underwent ventriculoperitoneal shunt placement for noncommunicating hydrocephalus; the shunt was found obstructed by growth of *P. variotii*. In our case possible risk factors were administration of cancer chemotherapy and of corticosteroids, a condition similar to treatment leading central nervous system (CNS) aspergillosis.[32] Additionally, our patient was diabetic and her blood sugar was not well regulated. A case of *Paecilomyces variotii* pneumonia in a patient with diabetes mellitus was also described by Byrd.[16]

The differential diagnosis of infection and brain metastasis created a serious problem because clinical and radiological findings were not helpful in distinguishing between the two entities. The connection between lesions seen in the brain and the fungus in CSF was not established with certainty because of the lack of brain biopsy or autopsy material cultured. Fagerburg *et al.*[10] reported a fatal *P. variotii* infection inoculated with a ventriculoperitoneal shunt. The portal entry of the fungus into the CSF remained unknown in our case. Inoculation with medical devices does not seem likely as the patient had no previous intervention to CNS or any implanted devices, except for an intravenous port. Inhalation might have been another route of entrance. The patient had a right sided pleural effusion on admission, but she was known to have a malignant effusion for 2 years and the radiograms taken on admission to emergency department were not different from the previous ones. CNS infections caused by moulds can be very difficult to treat and often have a fatal outcome. In the present case, the patient died 10 days after recovery of fungus in culture.

The *in vivo* morphology of *Paecilomyces* has been described as pleomorphic, ranging from *Aspergillus*-like hyphae and thin-walled chains of swollen cells to yeast-like cells.[5] In the present case, microscopical examination of the first and second CSF preparations revealed yeast cells, chlamydospore-like, slightly rough-walled, globose cells and septate hyphae. On agar media, microscopic morphology is unmistakable.[33]

The antifungal susceptibility of *Paecilomyces* varies widely with the species.[3] *P. variotii* is almost universally sensitive to AMB and 5FC, while *P. lilacinus* and *P. marquandii* are resistant to these antifungals and sensitive to imidazoles.[3] Marzec *et al.*,[20] reported the succesful antifungal management of four *P. variotii* peritonitis by early removal of the cathether plus AMB administered intravenously in two patients and ketoconazole administered orally in two patients. Shing *et al.*[23] reported a *P. variotii* fungemia in a bone marrow transplant patient who was receiving antifungal prophylaxis with FLZ and successful treatment was achieved by removal of the central venous catheter and application of intravenous AMB and oral ITZ. In a recent study,[34] a new polyene, SPA-S-843 showed similar *in vitro* inhibitory activity against two *P. variotii* strains. Del Poeta *et al.*[35] tested two clinical *P. variotii* strains against pneumocandin L-743,872 and reported MIC ranges ≤0.09 μg/mL. The case isolate had low MIC levels in response to 5FC, TRB, ITZ, KTZ and AMB and demonstrated *in vitro* resistance to FLZ and MCZ.

Microbial infections have long been recognized as one of the most significant causes of morbidity and mortality in cancer patients, often replacing the underlying disease as the leading cause of death. Many organisms that were previously considered to be contaminants when isolated from human specimens have emerged as major causes of disease, especially in the immunocompromised host,[36] among which is *Paecilomyces variotii*.

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