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# Case report. Isolation of *Cladosporium cladosporioides* from cerebrospinal fluid

## Fallbericht: Isolierung von *Cladosporium cladosporioides* aus Liquor

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**Key words.** *Cladosporium cladosporioides*, cerebrospinal fluid, systemic infection.
**Schlüsselwörter.** *Cladosporium cladosporioides*, Liquor, Systemische Infektion.

**Summary.** *Cladosporium cladosporioides* was isolated from three subsequent cerebrospinal fluid specimens and from a brain biopsy specimen of a human patient. Susceptibility testing of the isolate was performed against seven antifungal agents.

**Zusammenfassung.** *Cladosporium cladosporioides* wurde aus verschiedene Liquorproben und ebenfalls aus einer Hirnbiopsie-Probe eines menschlichen Patienten isoliert. Empfindlichkeitstestung gegen sieben Antimykotika wurde durchgeführt.

## Introduction

*Cladosporium* species are found ubiquitously as saprobes in soil and on rotten plant material. Despite their prevalence, only a limited number of species have been documented as agents of human mycotic infections. *Cladosporium cladosporioides* (Fres.) de Vries, *Cl. elatum* (Harz) Nannf., *Cl. herbarum* (Pers.) Link:Fr., *Cl. oxysporum* Berk. and Curt and *Cl. sphaerospermum* Penz. have been listed as occasional agents of phaeohyphomycosis [1, 2]. Infections by *Cl. cladosporioides* are extremely rare and predominantly concern superficial cases [2], although also systemic infections have been reported [3, 4]. This paper reports the first *Cl. cladosporioides* strain isolated from the central nervous system (CNS) of a human patient.

## Materials and methods

### *Isolation*

The materials investigated were three subsequent cerebrospinal fluid (CSF) specimens and a brain biopsy specimen of the same patient. Centrifugated sediment of the first CSF specimen was stained with Gram, Ehrlich–Ziehl–Neelsen, Giemsa and India ink and was also examined directly to determine whether unstained fungal elements were melanized. The sediment was plated onto Sabouraud glucose agar (SDA), brain–heart infusion agar (BHIA), cooked sheep's blood agar (BA) and niger seed agar (NSA), and incubated at 25, 30 and 37 °C. The plates were sealed with parafilm to maintain adequate humidity. Further CSF specimens sent 3 days and 1 month afterwards were treated similarly. In addition a brain biopsy specimen was obtained; as it was extremely small, specimens were placed on SDA and incubated at 30 °C without staining.

### *Identification*

The isolate was identified phenotypically and by Internal Transcribed Spacer (ITS) ribosomal DNA (rDNA) sequencing and comparison with sequences deposited in the public domain with a large *Cladosporium/Mycosphaerella* databank present at CBS (G. S. de Hoog, P. Zalar & E. Abeln, unpublished data).

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*Antifungal susceptibility testing*

*In vitro* susceptibility of the patient's isolate against seven antifungal agents were performed according to National Committee for Clinical Laboratory Standards (NCCLS) M38-P reference broth macrodilution method [5]. Antibiotic medium 3 (Oxoid, Hampshire, UK) supplemented with 2% glucose and buffered with 0.165 M morpholinepropanesulphonic acid (MOPS; Sigma, St Louis, MO, USA) was used for testing amphotericin B (AMB; Bristol-Meyers Squibb, Wallingford, CT, USA) (0.03–16 μg $ml^{-1}$), and RPMI-1640 (Sigma) with L-glutamine but without sodium bicarbonate and buffered with 0.165 M MOPS was used to prepare for testing fluconazole (FCZ; Pfizer, Istanbul, Turkey) (0.125–64 μg $ml^{-1}$), itraconazole (ITZ; Janssen Pharmaceuticals, Beerse, Belgium) (0.03–16 μg $ml^{-1}$), ketoconazole (KTZ; Milen, Istanbul, Turkey) (0.03–16 μg $ml^{-1}$), miconazole (MCZ; Selectchemie AG, Zürich, Switzerland) (0.03–16 μg $ml^{-1}$), 5-fluorocytosine (5-FC; Sigma) (0.125–64 μg $ml^{-1}$) and terbinafine (TBF; Novartis, Basel, Switzerland) (0.03–128 μg $ml^{-1}$). AMB, ITZ, KTZ and MCZ were dissolved in dimethyl sulphoxide (DMSO), 5-FC and FCZ were dissolved in sterile distilled water and 10 twofold serial dilutions of the antifungal agents were prepared. TBF was dissolved in DMSO containing 5% Tween-80 [6]. The isolate and *Paecilomyces variotii* ATCC 36257 control strain were grown on potato glucose agar slants at 25 °C for 8 days. The inoculum was prepared by flooding the agar slants with the fungi with 5 ml of distilled water, and carefully scraping the surfaces with the tip of a sterile Pasteur pipette. Then, the inoculum suspensions were 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 $0.4–5 \times 10^4$. Aliquots of 0.01 ml were spread onto SDA plates, incubated at 35 °C, and were observed daily for the presence of growth. To control the final inocula concentration, the colonies were counted as soon as possible after the observation of visible growth. A drug-free growth control tube was included with the tubes containing the patient's isolate and control strain. In addition, 1 ml of uninoculated, drug-free medium was included as a sterility control. The control strain was tested in the same manner. The tubes were incubated at 35 °C, and minimal inhibitory concentrations (MICs) were determined by visual inspection at the first 24-h interval when growth was observed in the drug-free control tube. MICs were defined 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.

## Case report

The patient was a 30-year old apparently healthy, human immunodeficiency virus seronegative Caucasian male with a history of diplopia and showing stains at the brainstem on computed tomographic scan and magnetic resonance imaging.

*Mycology*

Microscopical examination of first CSF preparations revealed a moderate number of irregularly swollen, septate, subhyaline to pale brown hyphal elements and chlamydospore-like, spherical or oblong, thick-walled conidial chains (Fig. 1), individual conidia with visible scars at one or both ends and a few multi-celled germinating conidium. From this specimen no fungal growth was obtained on BHIA and BA, but on SDA cultures yielded a few initially yeast-like colonies which later became velvety and turned dark greyish-green with greenish-black reverse. The second CSF specimen sent 3 days later and the third specimen submitted after 1 month showed the same elements plus individual yeast cells and short arthroconidium-like elements. All stained microscopical slide preparations were carefully examined and typical fungal structures were photographed [7]. The same fungus grew from second and third CSF specimens on SDA plates. Growth was observed just around the biopsy specimens. The fungus was identified morphologically as *Cl. cladosporioides*, which was confirmed by ITS 1-2 rDNA sequencing. It has been deposited into the culture collection of the Centraalbureau voor Schimmelcultures as CBS 109501.

*Antifungal susceptibility*

The MICs were determined as follows: AMB, 8 μg $ml^{-1}$, FCZ, 0.25 μg $ml^{-1}$, ITZ, 0.06 μg $ml^{-1}$, KTZ, < 0.03 μg $ml^{-1}$, MCZ, <0.03 μg $ml^{-1}$, 5-FC, < 0.125 μg $ml^{-1}$ and TBF, 0.5 μg $ml^{-1}$. The isolate was found to be susceptible to all azole antifungals tested and to flucytosine.

## Discussion

Fungal elements were observed in direct microscopical CNS specimens and the fungus was isolated repeatedly on culture media. The elements in culture were morphologically similar to those seen *in vivo*, suggesting that this fungus was the aetiological agent of the infection. *Cladosporium cladosporioides* has previously been isolated from a pulmonary fungus ball [3], from keratitis [8], from phaeohyphomycosis [9], and from cutaneous and

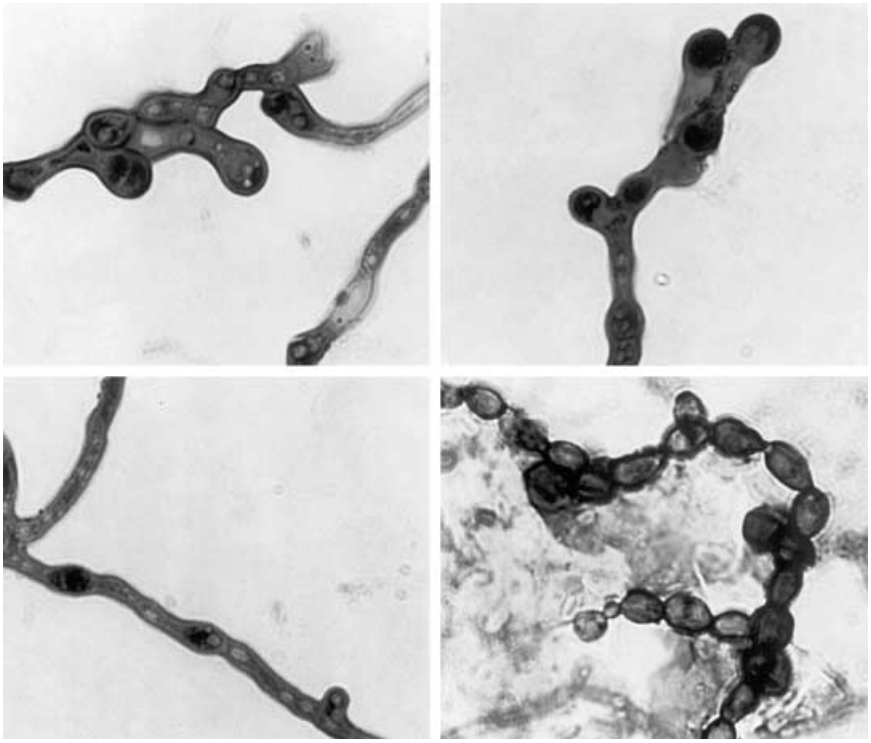

**Figure 1.** (a–d) Examples of *in vivo* fungal elements observed in three subsequent CSF specimens of the patient. (a–c) Irregularly swollen hyphal elements (stained with Ehrlich–Ziehl–Neelsen); (d) chains of swollen cells and septate hyphal element (stained with Giemsa).

subcutaneous infections [10–13]. The clinical spectrum of the species is very wide and unordered, suggesting that *Cl. cladosporioides* is a coincidental opportunist. *Cladosporium* species are extremely rare as agents of deep mycosis, neurotropism generally being limited to morphologically similar but phylogenetically unrelated *Cladophialophora* species, *Cladophialophora bantiana* in particular [14].

The patient's isolate was found to be susceptible to all azole antifungals tested and to flucytosine. Few data are available about antifungal susceptibility profile of *Cladosporium* species. Guarro *et al.* [15] determined MICs of three *Cl. cladosporioides* isolates: AMB, 0.125–1 and 0.5–8 μg $ml^{-1}$; for FCZ, 16 to >64 and 32 to >64 μg $ml^{-1}$; for ITZ, 0.06–8 and 1 to >64 μg $ml^{-1}$; for KTZ, <0.03 to 4 and 0.5 to >64 μg $ml^{-1}$; for MCZ, 0.25 to >64 μg $ml^{-1}$ and 0.06–8 μg $ml^{-1}$; and for 5-FC 0.125–128 and 0.125 to >128 μg $ml^{-1}$ for hyphal and conidial inocula, respectively. McGinnis and Pasarell [16] reported MIC ranges of three *Cl. cladosporioides* isolates as 0.03–2 μg $ml^{-3}$ for AMB and 0.03–0.25 μg $ml^{-3}$ for ITZ. Llop *et al.* [17] tested one *Cl. cladosporioides* among five *Cladosporium* spp. and determined MIC ranges for AMB, 0.125–2 μg $ml^{-1}$; for ITZ, 0.25–32 μg $ml^{-1}$; for KTZ, 1–2 μg $ml^{-1}$ and for 5-FC, 0.5–256 μg $ml^{-1}$ by visual reading. In another study [18], one *Cl. cladosporioides* among five *Cladosporium* isolates was tested and MICs were found as 0.25 μg $ml^{-1}$ against AMB, 0.5 μg $ml^{-1}$ against FLZ, 0.5 μg $ml^{-1}$ against ITZ, 2 μg $ml^{-1}$ against KTZ, 1 μg $ml^{-1}$ against MCZ, 0.25 μg $ml^{-1}$ against 5-FC and 8 μg $ml^{-1}$ against TBF. Yücel and Kantarcioğlu [19], reported MICs for one *Cl. cladosporioides* isolate against AMB, FLZ, ITZ, KTZ, MCZ and 5-FC as 2 μg $ml^{-1}$, 0.125 μg $ml^{-1}$, 0.25 μg $ml^{-1}$, 1 μg $ml^{-1}$, 0.06 μg $ml^{-1}$, and >64 μg $ml^{-1}$, respectively.

*In vitro* susceptibility of the patient's isolate was generally found in agreement with the above mentioned reports except for FLZ values reported by Guarro *et al.* [15]. This may in part be the result of differences in inoculum preparation procedures. Guarro *et al.* [15] prepared two types of inocula using conidia and hyphae separately, whereas in our study inoculum suspensions were prepared as described by NCCLS M38-P methodology, using homogeneous suspensions of mixtures of conidia and hyphal fragments. In general, FLZ susceptibility shows some variance, as some investigators reported high MIC values [15] while others [18, 19] revealed FLZ-susceptible isolates. Our data are in line with the last mentioned reports [18, 19, 20]. Recently a clinical case of subcutaneous phaeohyphomycosis caused by *Cl. cladosporioides* was reported to respond very well to treatment with FCZ [19]. Further studies are needed for a more definitive evaluation of antifungal susceptibility profile of this species.

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