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BRIEF COMMUNICATION

Chaetomium atrobrunneum and *Aspergillus fumigatus* in multiple tracheal aspirates: Copathogens or symbiosis



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Abstract *Chaetomium atrobrunneum* has never been reported to be associated with pneumonia. We report the isolation of *C. atrobrunneum* and *Aspergillus fumigatus* from a Chinese elderly patient with fatal pneumonia. Branched, long, and septate hyphae were observed in potassium hydroxide preparations and Gram-stained smears, and confluent *C. atrobrunneum* growth and a few *A. fumigatus* colonies were found in tracheal aspirates (nine separate occasions). These isolates were identified by conventional morphological methods and by sequencing the internal transcribed spacer and the D1/D2 domain of the 26S rRNA gene. The patient responded poorly to the combination therapy of amphotericin B and caspofungin. This report adds *C. atrobrunneum* to the list of fungal pneumonia in immunocompromised hosts. This case report also illustrated the presence of a growth symbiosis between *Chaetomium* species and *A. fumigatus*.

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Introduction

Invasive mold infections are an important cause of mortality in immunocompromised patients.^{1–3} Although aspergillosis represents the most common invasive mold

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infection, less commonly encountered molds, such as the mucormycetes, and *Fusarium*, *Scedosporium*, dematiaceous molds, and *Acremonium* species are increasingly being reported.^{1–3} *Chaetomium* fungi are dematiaceous molds found worldwide in soil and plant debris as a saprophyte; it is also found in warm, dry areas, including on dung, straw paper, textiles, and bird feathers.⁴ *Chaetomium* species affect healthy as well as immunocompromised individuals.^{5–19} *Chaetomium globosum* is the most common species; other pathogenic species described are *Chaetomium atrobrunneum*, *Chaetomium strumarium*, *Chaetomium perlucidum*, *Chaetomium funiculum*, and *Chaetomium murorum*.^{5,8,13,14} Clinical features of infection have included those in association with onychomycosis, keratitis, sinusitis, lung empyema, pneumonia, and fatal disseminated cerebral mycosis.^{5–19} However, *C. atrobrunneum* has not been previously reported to be associated with lung infection.^{4,14–18}

Case Report

An 89-year-old man presented at the Emergency Department of the Peking Union Medical College Hospital with a 7-day history of nausea, vomiting, constipation, cough with scanty sputum, and a noticeably decreased urine output. His medical history included biliary tract infection, colonic adenoma, and lung embolism, and he had been bed ridden for 2 years. Laboratory studies revealed leukocytosis (a white blood cell count of $15.6 \times 10^9/L$), thrombocytopenia ($116 \times 10^9/L$), an elevated serum creatinine level of $607 \mu\text{mol/L}$, and an elevated procalcitonin level of 7.75 ng/mL . An initial chest X-ray and computed tomography demonstrated multiple foci of consolidation in both the lungs and bilateral pleural effusions. Meropenem (200 mg every 6 hours), moxifloxacin (400 mg daily), and intravenous fluconazole (200 mg daily) were initiated for the empirical treatment of pneumonia. Over the next 3 days, he developed septic shock, multiorgan failure, and worsening thrombocytopenia, and required mechanical

ventilation for respiratory support. Teicoplanin (200 mg every 3 days) was administered intravenously.

Direct microscopic examination by Gram staining and potassium hydroxide (KOH) preparations of a tracheal aspirate sample collected on the 9th hospital day revealed numerous branched, long, and septate hyphae suggestive of *Aspergillus* species. The Ziehl–Neelsen stain analyses were negative for acid-fast bacilli. Cultures obtained from the tracheal aspirate sample yielded a few colonies of *Aspergillus fumigatus*; in addition, heavy and confluent growth of *Chaetomium* species, 3 days later, was identified by routine morphological methods (Figures 1A and 1B).¹ Tests for HIV-1/HIV-2 antibodies (Abbott, Wiesbaden, Germany) were negative. Fluconazole was ceased, and it was replaced by caspofungin 50 mg daily and amphotericin B deoxycholate (50 mg daily). Meropenem and teicoplanin were discontinued, and cefoperazone–sulbactam (3 g every 12 hours) and minocycline were added. The serum 1-3- β -D glucan test (Gold Mountain River, Tianjin, China) was conducted on four occasions throughout hospitalization, and each returned a negative result ($<10 \text{ ng/mL}$). Over the next 18 days, repeated tracheal aspirate samples were collected for fungal cultures; confluent growth of *Chaetomium* species along with scattered growth of *A. fumigatus* was present on the trypticase soy agar on 5% sheep blood agar (Figure 1A) and on Sabouraud dextrose agar (Thermo Fisher Scientific, Waltham, MA, USA) plates on each occasion from nine separated tracheal aspirate samples. Due to uncontrolled lung infection and progressive multiorgan failure, the patient died on the 58th hospital day.

Microbiological investigation

The surface of the colonies of *the Chaetomium* isolates on Sabouraud dextrose agar and sheep blood agar (Figure 1A) was white in color and woolly in texture on Day 3 of growth, but became gray to olivaceous after Day 7 of culture. Lactophenol cotton blue mounts from slide cultures showed septate hyphae and flask-shaped black perithecia

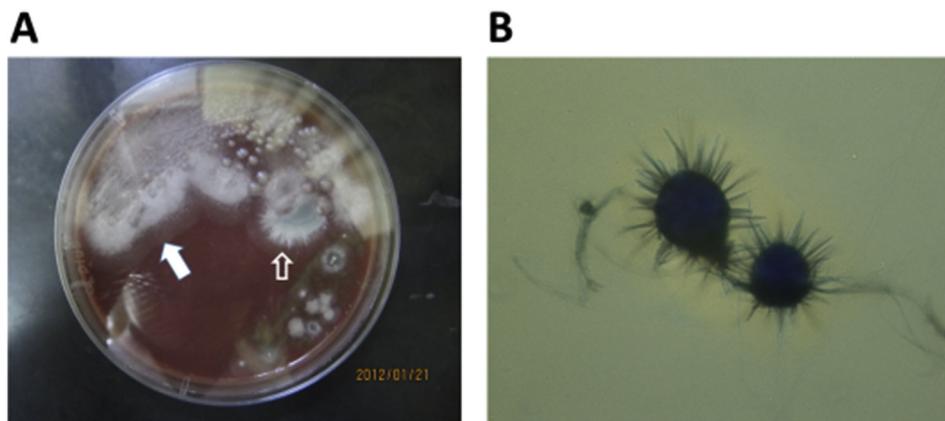


Figure 1. Microbiological findings of *C. atrobrunneum*. (A) Confluent growth of *C. atrobrunneum* (solid arrow) and a few colonies of *A. fumigatus* (hollow arrow) found 3 days after the culture of the bronchial aspirate on the trypticase soy agar supplemented with 5% sheep blood. The surface of colonies of *C. atrobrunneum* was white and woolly on the 3rd day after culture and became gray to olivaceous 7 days after culture. (B) Lactophenol cotton blue stain (400 \times magnification) of slide culture of *C. atrobrunneum* showing brown to black perithecia with long, brown, and erect setae.

(Figure 1B). The perithecia contained asci and single-celled ascospores. Ascospores were oval or lemon-shaped spores that were olive brown in color and released from the ostiole of a matured perithecium.

Amplification of the fungal internal transcribed spacer (ITS) region and the D1/D2 domain of the 26S rRNA gene of the *Chaetomium* species was performed as previously described using the primer pairs ITS1/ITS4 and F63/R635, respectively.^{20,21} Species identification was performed by comparing the obtained ITS and D1/D2 domain sequences with those contained in the Centraalbureau voor Schimmcultures (CBS) Fungal Biodiversity Center database as well as the GenBank database using the BioloMICSNet and BLASTn software, respectively.^{20,21} The results of ITS region and D1/D2 domain sequencing of the 26S rRNA gene of the *Chaetomium* species showed 100% (894/894bp) and 100% (1016/1016bp) sequence similarity to the ITS and D1/D2 domain sequences of *C. atrobrunneum*-type strain CBS379.66, respectively (GenBank accession numbers JX280771.1 and JX280666.1, respectively).^{20,21} Identification of *A. fumigatus* was based on conventional morphological identification methods.²²

Minimum inhibitory concentrations (MICs) of voriconazole and amphotericin B, as determined by the Etest method (bioMérieux, Marcy l’Etoile, France), were 0.047 µg/mL and 0.047 µg/mL, respectively, for *A. fumigatus*, and 0.75 µg/mL and 0.064 µg/mL, respectively, for *C. atrobrunneum*. Only two Etest strips of antifungal agents (voriconazole and amphotericin B) were available in our laboratory, and MICs were determined after physicians’ request.

Discussion

In the current study, we report a patient who developed mixed lung infection caused by *C. atrobrunneum* and *A. fumigatus* and who died from uncontrolled lung infection, despite antifungal therapy, and lung infection-triggered progressive multiorgan failure. A total of five cases of human infection due to *C. atrobrunneum* have previously been reported (Table 1).^{4,14–18} In 1998, *C. atrobrunneum* was first detected as the pathogen of a cerebral abscess in a bone marrow transplant recipient, followed by another case, also of cerebral infection.^{4,14} In 2010, a case of mixed cutaneous infection caused by *C. atrobrunneum* and *Clavisspora lusitaniae* was described in a boy with acute and severe infection around his left eyelid.¹⁵ A further case of *C. atrobrunneum* keratitis was described in an adult male.¹⁷ Of the six patients infected with *C. atrobrunneum* (with the addition of the present patient here), three have died: two from cerebral abscesses and one from severe pneumonia (the present patient) (Table 1). Our patient extends the spectrum of infection caused by *C. atrobrunneum* to include pneumonia. Pneumonia caused by *C. globosum* has previously been reported in a patient with refractory acute myeloid leukemia.¹² Interestingly, histological examination of a lung biopsy specimen revealed only branched septate hyphae, suggestive of *Aspergillus* species. The finding was similar to that observed in multiple tracheal aspirates in our patient. In another report, Hoppin et al¹⁹ described a fatal case of lung empyema in a patient

Table 1 Summary of six patients with infections caused by *C. atrobrunneum*.

Patient no. (country)	Year of report	Age/sex	Associated underlying conditions	Type of infection	Source of isolation	Molecular methods for species identification	Antifungal treatment regimen	Outcome	Authors (reference)
1 (USA)	1998	31/M	Bone marrow transplant recipient	Cerebral abscess	Brain biopsy (abscess)	No (conventional morphological methods and scanning electron microscopic studies)	Amphotericin B and itraconazole	Died	Guppy et al ⁴
2 (USA)	1999	Not available	Bone marrow transplant recipient	Cerebral abscess	Brain biopsy (abscess)	No (conventional morphological methods)	Not available	Died	Thomas et al ¹⁴
3 (China)	2010	1.5/M	Nil	Cellulitis (left eye) and conjunctivitis	Eye discharge	Yes (ITS & D1/D2 of 26S RNA gene sequencing analysis)	Itraconazole and fluconazole	Resolved	Zhang et al ¹⁵
4 (USA)	2010	11/M	Hodgkin lymphoma	Retinitis and subhyaloid abscess	Vitreous aspirate	No (conventional morphological methods)	Voriconazole	Improved	Tabbara et al ¹⁶
5 (India)	2012	44/M	Nil	Keratitis	Corneal scrapings	Yes (ITS sequencing analysis)	Topical natamycin and oral ketoconazole	Resolved	Balne et al ¹⁷
6 (China)	89/M	89/M	Bed-ridden for long term	Pneumonia	Tracheal aspirates	Yes (ITS & D1/D2 of 26S RNA gene sequencing analysis)	Amphotericin B and caspofungin	Died	Present study

ITS = internal transcribed spacer; M = male.

with acute lymphocytic leukemia where *Chaetomium* species was cultured from the extrapleural fluids on two separate occasions. However, the species of the fungus was not specified.

In the present case, the presence of confluent growth of *C. atrobrunneum* accompanied by only a few colonies of *A. fumigatus* isolated from multiple respiratory secretions over an 18-day period supported the diagnosis of *C. atrobrunneum* pneumonia and *A. fumigatus* might have played a helper's or a stimulator's role, as suggested earlier.^{13,23} The genus of *Chaetomium* requires a cellulose-rich medium for sporulation, and growth of *C. globosum* has been noted to be often stimulated in the presence of *A. fumigatus*, which excretes compounds such as sugar phosphates and phosphoglyceric acid.^{13,23} Aru et al²³ reported a 73-year-old woman with chronic maxillary sinusitis and an infundibulectomy was performed.²³ Histological examination showed necrotic material with hyphae of *A. fumigatus* and perithecia of *Chaetomium* species. Lamoth et al²⁴ demonstrated that with two consecutive β -D-glucan tests, the specificity was 98.9% and the estimated negative predictive value was 94.6%. The repeated (4 separated occasions) negative results for β -D-glucan test were obtained during hospitalization, indicating that *A. fumigatus* is less likely to be the main pathogen in our patient.²⁴ However, there were no reports to indicate the usefulness for β -D-glucan test for diagnosing invasive infection due to *Chaetomium* species.^{4,5-19,24,25} Whether 1,3- β -D-glucan is also a main cell wall constituent of *Chaetomium* species is not known; however, a β -1,3-glucanase has been detected in the culture broth of *Chaetomium* species.²⁶ Consequently, the heavy growth of *C. atrobrunneum* from multiple deep respiratory secretions and the repeatedly negative β -D-glucan tests in our patient suggest a more important role of *C. atrobrunneum* than *A. fumigatus* as a pathogen associated with lung infection. However, pneumonia caused by dual pathogens (*C. atrobrunneum* and *A. fumigatus*) cannot be excluded because the heavy growth of *C. atrobrunneum* might be stimulated by the presence of *A. fumigatus* in the respiratory secretions.²³

The appropriate regimen for the treatment of lung infections due to *Chaetomium* species is not well established.^{4,5-19} In our patient, both amphotericin B and caspofungin were used for the treatment of lung infection, but a poor response was noted, although the MICs of amphotericin B against both *C. atrobrunneum* and *A. fumigatus* isolates were low. Two previous studies on *in vitro* susceptibility testing of *Chaetomium* species demonstrated that these organisms were resistant to flucytosine and fluconazole, and none of the other commonly used antifungal agents at that time (ketoconazole, itraconazole, miconazole, or amphotericin B) exhibited good *in vitro* fungicidal activity against this organism.^{27,28} Serena et al²⁸ demonstrated that among the 19 isolates of *Chaetomium* species tested, micafungin was not active at all, while the geometric mean MICs and minimum effective concentrations of the three triazoles (voriconazole, ravuconazole, and albaconazole) were <0.5 g/mL and <0.4 g/mL, respectively. As for the seven isolates of *C. atrobrunneum* tested, the ranges of MIC and MIC₉₀ were 0.5–4 μ g/mL and 2 μ g/mL, respectively, to amphotericin B; 64 μ g/mL and 64 μ g/mL, respectively, to micafungin;

0.12–0.5 μ g/mL and 0.5 μ g/mL, respectively, to voriconazole; 0.06–1 μ g/mL and 0.25 μ g/mL, respectively, to ravuconazole; and 0.12–1 μ g/mL and 0.25 μ g/mL, respectively, to albaconazole.²⁸ Caspofungin was effective as a salvage therapy in patients with invasive aspergillosis who were refractory to or intolerant of the standard therapy.²⁹ However, the *in vitro* activity of caspofungin against *Chaetomium* species was limited.¹¹ Serena et al²⁸ also demonstrated that neither amphotericin deoxycholate, alone or in combination with itraconazole, nor its liposomal formulation has been effective in treating patients with *Chaetomium* infections.

In summary, we first report a fatal case of pneumonia most likely caused by *C. atrobrunneum*, and *A. fumigatus* probably played the role of a stimulator in an elderly patient, although the possibility of dual infection with *A. fumigatus* could not be excluded. This report adds *C. atrobrunneum* to the list of fungal pneumonia in immunocompromised hosts. This case report also illustrated the presence of growth symbiosis between *Chaetomium* species and *A. fumigatus*.

Conflicts of interest

None declared.

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