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Fatal Pulmonary Infection Caused by the Basidiomycete *Hormographiella aspergillata*

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A fatal case of a pulmonary infection caused by *Hormographiella aspergillata*, the anamorph of the mushroom *Coprinus cinereus*, is reported for a patient receiving treatment for a second relapse of acute lymphoblastic leukemia. The filamentous basidiomycete was identified with restriction fragment length polymorphism patterns of PCR-amplified internally transcribed spacers and small subunit ribosomal DNA with four restriction enzymes. The patient failed to respond to treatment with amphotericin B and itraconazole. The fungus was cultured from the lungs at autopsy: the MIC of amphotericin B for the fungus was low (0.5 mg/liter), and that of itraconazole was high (8 mg/liter).

Aspergillus fumigatus is the most common cause of fungal pneumonia in immunocompromised patients (2), although other filamentous fungi such as non-*fumigatus Aspergillus* species, *Fusarium* species, zygomycetes, and *Scedosporium apiospermum* are noted (2, 8). Until now, filamentous basidiomycetes are rarely reported as a cause of invasive infections (10, 11), despite the fact that airborne spores are abundantly present in the environment and that several species grow well at 37°C. *Hormographiella* is a new hyphomycete genus, described by Guarro et al. (9) in 1992, which has three species: *H. aspergillata*, *H. verticillata*, and *H. candelabrata. H. aspergillata* was found to be the anamorph of the mushroom *Coprinus cinereus* (7). Here, we report a case of fatal pneumonia due to *H. aspergillata* in a patient receiving intensive cytotoxic treatment.

A 24-year-old man was diagnosed with acute lymphoblastic leukemia in 1988. He responded to intensive induction, consolidation, and maintenance therapy. A first relapse was diagnosed in 1993, and treatment with peripheral stem-cell transplantation resulted in complete remission. However, a relapse was diagnosed for the second time in September 1995. Cytotoxic therapy was initiated, and after 13 days while he was still granulocytopenic, he developed fever again despite empiric ceftazidime therapy. Cultures of blood and oral lesions remained sterile, and radiographs of the chest were unrevealing. On day 18, he complained of right-sided pleuritic chest pain, and a new chest radiograph showed focal pulmonary infiltrates in the right lower lobe. Because of severe respiratory insufficiency, a diagnostic intervention (biopsy or lung lavage) was not possible. Treatment with amphotericin B desoxycholate (1 mg/kg/of body weight per day) was started on suspicion of invasive aspergillosis. A chest radiograph made on day 23 showed progression of the right-sided infiltrate, and new infiltrates in the left upper and lower lobes of the lung. The patient remained profoundly neutropenic with fever and headache. A right-sided hemiparesis developed, but a computed tomography scan of the brain was normal. Therapy with amphotericin B was changed to itraconazole (400 mg/day) on day 26 because of amphotericin B-associated nephrotoxicity. By then the patient had received a cumulative dose of 310 mg of amphotericin B. The clinical condition deteriorated rapidly, and the patient died from respiratory failure on day 35.

An autopsy was performed 20 h after death. Tissue samples were obtained from all major organs and from any macroscopic abnormalities, fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with periodic acid-Schiff, Gomori methenamine silver with eosin counterstain, or hematoxylin and eosin. Tissue samples were obtained aseptically for microbiological cultures from the lungs, liver, spleen, and kidneys; homogenized (Stomacher 80 Lab-Blender; Seward Medical, London, United Kingdom); and streaked onto sheep blood agar plates and Levine medium and then were incubated at 37°C for 48 h. Homogenized tissue samples were also streaked onto Sabouraud glucose (2%) agar containing chloramphenicol and incubated at 28 and 42°C for 4 days. Identification of the cultured fungus was performed by PCR amplification of DNA with primers NS1 and NS24 (amplicon small subunit [SSU]) and internally transcribed spacers (ITS) 1 and 4 primers (amplicon ITS1 and 2, including 5.8S ribosomal DNA [rDNA]) (17). Amplicons were digested with the restriction enzymes *Hin*fI, *Hae*III, *Rsa*I, and *Dde*I and electrophoresed on 1.4% agarose gels (Fig. 1). Representative strains of *Schizophyllum commune*, *Coprinus cinereus*, *Phanerochaete chrysosporium*, *Sporotrichum dimorphosum*, and *Bjerkandera adusta* and type strains of *H. aspergillata*, *H. candelabrata*, and *H. verticillata* were run on the same gels. Patterns were compared by using the Image Master (Pharmacia, Roosendaal, The Netherlands) software package for evaluation of molecular weights. Antifungal susceptibility testing was performed by the agar incorporation method with RPMI-1640 agar buffered with 0.165 M morpholinepropanesulfonic acid (MOPS) with 0.03% (wt/vol) L-glutamine added (5). Doubling dilutions of amphotericin B (Bristol-Myers Squibb, Woerden, The Netherlands), fluconazole (Pfizer, Capelle a/d IJssel, The Netherlands), itraconazole (Janssen-Cilag Biotech, Tilburg, The Netherlands), and voriconazole (Pfizer, Sandwich, United Kingdom) from 64 to 0.03 mg/liter were prepared in 2-ml volumes of sterile water. Volumes (18 ml) of molten agar were added, and the components were mixed, poured into petri dishes, and allowed to set. Spore suspensions were adjusted to a density of 10^7 conidia/ml with sterile water. Plates were

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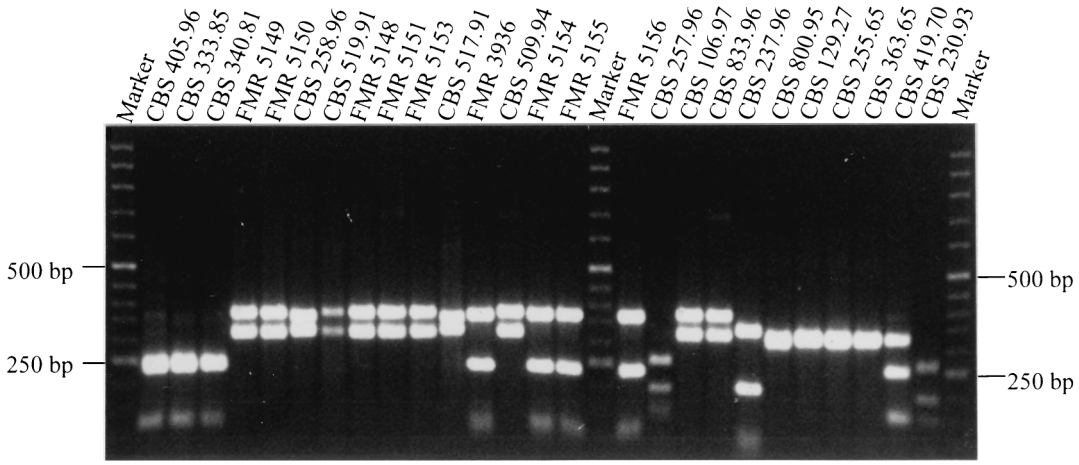

FIG. 1. Restriction profiles of ITS1 and -2 for *Hin*fI of filamentous basidiomycetes. Lanes 1, 17, and 29 contain a 100-bp ladder. CBS 405.96 = CBS 333.85 = CBS 340.81 = *S. commune*; FMR 5149 = FMR 5150 = *C. cinereus*; CBS 258.96 = *Coprinus* sp.; CBS 519.91 (type strain of *H. aspergillata*) = FMR 5148 = FMR 5151 = FMR 5153 = *C. cinereus*; CBS 517.91 = *H. candelabrata*; FMR 3936 = *H. verticillata*; CBS 509.94 = *C. cinereus*; FMR 5154 = FMR 5155 = FMR 5156 = *H. verticillata*; CBS 257.96 = *Hormographiella* sp.; CBS 106.97 = *C. cinereus*; CBS 833.96 = UAMH 8819 =present isolate; CBS 237.96 = basidiomycete sp. 1; CBS 800.95 = basidiomycete sp. 2; CBS 129.27 = CBS 255.65 = CBS 363.65 = *P. chrysosporium*; CBS 419.70 = *S. dimorphosporum*; CBS 230.93 = *B. adusta*. CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; FMR, Facultad de Medicina, Reus, Spain; UAMH, University of Alberta Microfungus Collection and Herbarium.

inoculated with the spore suspensions with a multipoint inoculator and incubated at 35°C. MICs were read at 72 h. The MIC was defined as the lowest concentration of drug at which there was no visible growth. The isolate was sent to Michael G. Rinaldi, Fungus Testing Laboratory, The University of Texas Health Science Center, San Antonio, for confirmation by the proposed National Committee for Clinical Laboratory Standards for yeasts (M27-P) (6a) for a macro-broth dilution method with RPMI-1640 for fluconazole and itraconazole and antibiotic medium 3 for amphotericin B.

Histopathology. At autopsy, a necrotizing bronchopneumonia was found which had affected both lungs. On macroscopic examination, multiple abscesses were found in both lungs. Periodic acid-Schiff and Gomori methenamine silver stains showed that the center of the lesions was occupied by a large mass of hyphae that were present in the cavity of the abscess and that also had invaded the surrounding tissue. The hyphae were septate, measuring between 1.9 to 4.6 μm in diameter, sometimes showing irregularly shaped swellings, and branching at an acute angle or dichotomously (Fig. 2). There was no

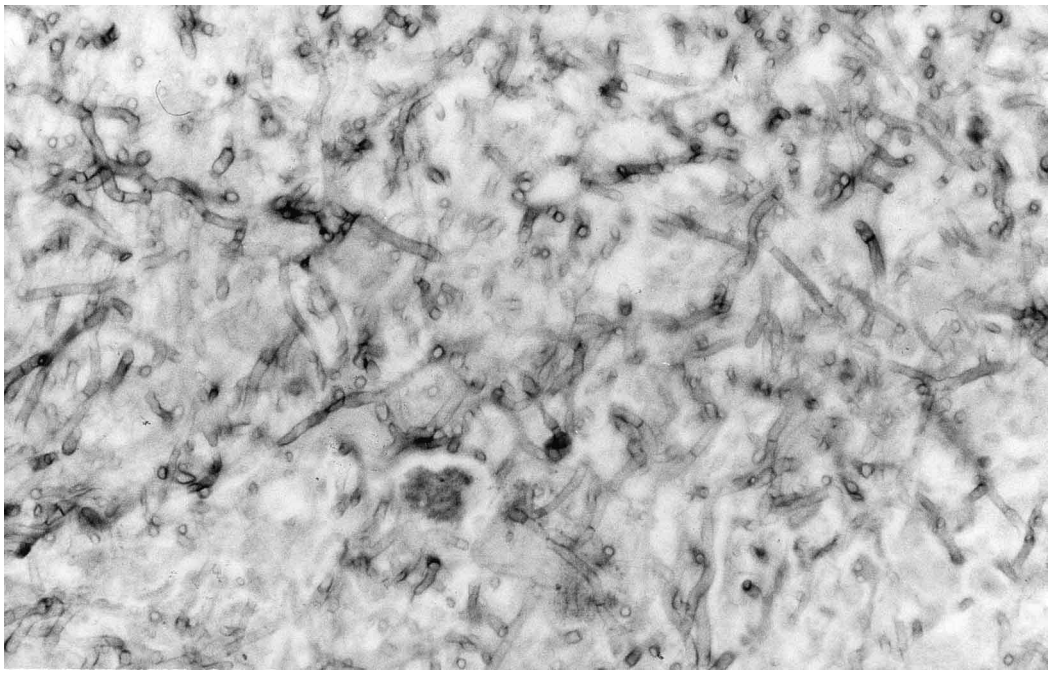

FIG. 2. Hyphae with irregular staining and formation of clews. The sample was stained with Gomori methenamine silver with eosin counterstain. Magnification, ×400.

FIG. 3. *H. aspergillata* cultured from lung tissue after 4 days of incubation on Sabouraud agar.

evidence of dissemination of the infection to other organs, although autopsy of the brain was not permitted.

Mycology. After 2 days, cultures of the left and right lungs yielded multiple white to cream-colored, cottony and dense colonies of a rapidly growing fungus (Fig. 3), while cultures from all other organs remained sterile. All inoculated media (blood agar and Sabouraud plates at 28 and 42°C) yielded pure cultures of the fungus. Microscopically the fungus showed hyaline, septate hyphae and the formation of macronematous conidiophores, but no clamp connections were seen (Fig. 4). Straight or curved, thin-walled conidia were adherent, often accumulating around the conidiophore. Definitive identification of the isolated fungus was rather difficult, because it produced undiagnostic arthroconidia. Therefore the fungus was identified as *H. aspergillata* (*C. cinereus*) (CBS 833.96, UAMH 8819) on the basis of SSU and ITS restriction fragment length polymorphism patterns, which were strictly identical in all strains of *H. aspergillata* and *C. cinereus* analyzed. The other

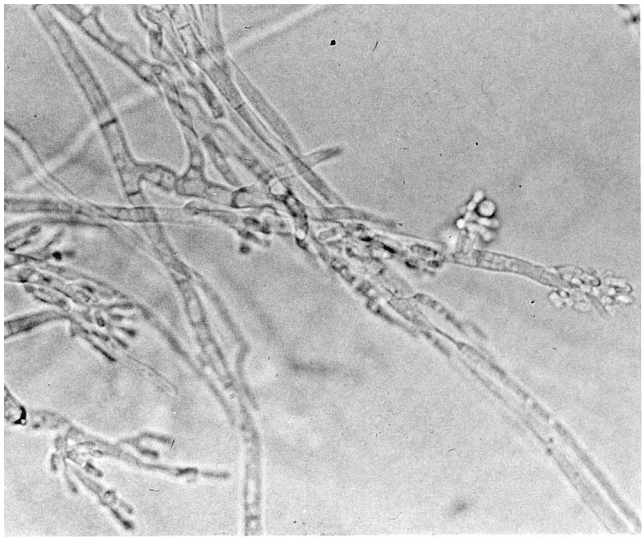

FIG. 4. Slide culture preparation of *H. aspergillata* showing hyaline septate hyphae without clamp connections. Magnification, ×

TABLE 1. Results of in vitro susceptibility testing by agar incorporation and broth macrodilution of the *H. aspergillata* isolate cultured from the lungs of an infected patient

Method	Reading time (h)	MIC (mg/liter)[a]			
		Amb	Itra	Flu	Vori
Agar incorporation	72	0.03	32	>64	0.5
Broth macrodilution	48	0.5	8	>64	ND[b]

[a] Amb, amphotericin B; Itra, itraconazole; Flu, fluconazole; Vori, voriconazole.
[b] ND, not done.

Hormographiella species differed in three ITS restriction patterns, while the remaining basidiomycetes generated uncomparable patterns. The in vitro susceptibility of the isolate is shown in Table 1. Both the agar incorporation method and the macrobroth dilution method indicated that the MICs of fluconazole and itraconazole for the isolate were high.

Filamentous basidiomycetes are commonly isolated from clinical specimens (1, 10), but in many cases, the clinical significance remains unclear. *S. commune* (3) has been recovered from the sputum of patients with chronic lung disease. This is the basidiomycete most commonly reported to cause local invasive (12, 14, 15) or disseminated disease (13) in both immunocompetent and immunocompromised patients. *H. aspergillata* (= anamorph of *C. cinereus*) and *H. verticillata* have been isolated from skin lesions and a catheter (7). However, documented infections are very rare, despite the fact that humans are regularly exposed to their spores, since the fungi can be isolated from soil, leaves, compost, and air (15). These fungi have been proven to cause endocarditis in two patients (6, 16). Both patients presented with endocarditis several months after the implantation of a prosthetic valve, and the valve was thought to have been contaminated by airborne spores during the operative procedure (16). The present case expands the spectrum of disease caused by members of the Basidiomycotina.

The epidemiology of invasive infections in humans caused by *Hormographiella* species may be changing. Although invasive infections by members of this genus until now have been extremely rare, the Centraalbureau voor Schimmelcultures (Baarn, The Netherlands) reported that seven *Hormographiella* isolates from human specimens, including the fungus cultured from the patient reported in this study, were received for identification in 1996 (4). The isolates originated from four European countries, including The Netherlands, Belgium, Germany, and Austria. The fungus was cultured from lung tissue, bronchoalveolar lavage fluid, brain tissue, cerebrospinal fluid, and from an eye, which suggests invasive potential. Despite the possible increase in the number of infections caused by *Hormographiella* species, filamentous basidiomycetes remain poorly recognized in the clinical laboratory and therefore may have been overlooked as potential opportunistic pathogens. This is mainly due to the difficulty of identifying filamentous basidiomycetes correctly.

Our isolate initially could not be identified because it failed to sporulate. Furthermore, key diagnostic features such as clamp connections may be absent, and the arthroconidial shape is undiagnostic. Definitive determination was only possible at a reference laboratory (Centraalbureau voor Schimmelcultures, Baarn, The Netherlands) with additional molecular techniques using restriction fragment length polymorphism patterns of PCR-amplified ITSs and SSU rDNA. Recently guidelines for the identification of these fungi have been reviewed which may

help to recognize basidiomycetes when cultured from clinical specimens (15).

Overall, the incidence of opportunistic fungal infections is increasing, and the epidemiology is changing. Until 10 years ago, *Candida* species were found to be the most frequent cause of fungal infection in hematologic patients at autopsy, followed by *Aspergillus* species (2). Now *A. fumigatus* has become the most important pathogen, and the number of unidentified or novel fungal pathogens is increasing (8). Although the number of emerging fungal pathogens causing infection in hematologic patients at present is low, the rising incidence may call for a more profound diagnostic approach. For each granulocytopenic patient who is suspected of a fungal infection, the risk of obtaining a specimen by bronchoscopy or by an invasive procedure for histology and culture is weighed against the risk of treating the patient empirically without a correct diagnosis. The latter approach was frequently followed because the physician relied on the expectation that the number of possible fungal pathogens was limited and that all would be susceptible to first-line antifungal agents such as amphotericin B or itraconazole. The present case illustrates that this approach may fail, because clinical signs and symptoms produced by emerging fungi and the radiological presentation are indistinguishable from invasive aspergillosis, but the susceptibility to first-line or, in our case, second-line antifungal agents may vary.

At present, limited data about the susceptibility of *Hormographiella* species to antifungal agents are available. *C. cinereus* and *H. verticillata* were previously found to be susceptible to antifungal azoles (with the exception of fluconazole), variably susceptible to amphotericin B, and resistant to flucytosine by use of a broth microdilution method (7). However, in the present study, two different susceptibility tests suggested that *H. aspergillata* may be resistant to itraconazole. Since the number of infections by unusual fungi is increasing, reproducible and meaningful in vitro tests need to be developed for susceptibility testing of these fungi, and the correlation of the MICs with clinical outcome needs to be ascertained. Clearly, this field of research will need to be addressed in the near future.

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