JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1987, p. 2395–2397
0095-1137/87/122395-03$02.00/0

Vol. 25, No. 12

# NOTES

## First Report of Subcutaneous Phaeohyphomycosis of the Foot Caused by *Phoma minutella*†

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Received 13 April 1987/Accepted 28 August 1987

***Phoma minutella*, a dematiaceous hyphomycete, was isolated to the exclusion of all other fungi from a subcutaneous inflammatory process on the foot of a farmer undergoing corticosteroid therapy for myasthenia gravis. Isolation was achieved on several nutrient media. Examination of stained smears and sections revealed dematiaceous fungal elements consistent with the mold. This is the first reported association of *P. minutella* with a human infection and only the second reported case involving a *Phoma* sp. as the etiologic agent of a subcutaneous infection.**

The incidence of mycotic diseases, especially opportunistic infections, has increased in recent years as a paradoxical consequence of various clinical and therapeutic advances (14). The use of cytotoxic and immunosuppressive drugs, corticosteroids, and broad-spectrum antibiotics in the management of neoplastic disorders, organ transplants, and other serious underlying diseases has created a large population of severely compromised patients. Fungi that hitherto have been encountered rarely in the clinical laboratory, as well as new species of clinically common genera, have been reported associated with human infections (1, 6, 9).

*Phoma* (*Peyronellaea*) spp. are dematiaceous hyphomycetes routinely isolated from soil and commonly found as phytopathogens (3, 4, 8, 17). They have gradually assumed increased importance as pathogens in humans and in lower animals (2, 5, 11, 16, 18). Except in the case reported by Young and associates (18) in which a *Phoma* sp. was recovered from a subcutaneous abscess, the fungus has been associated with only superficial or cutaneous infections.

We present the first authenticated human case of *Phoma minutella* infection and only the second case of a *Phoma* sp. infection involving subcutaneous tissue. The fungus was observed by direct microscopic examination of tissue homogenates and aspirates from the affected site as well as in stained biopsy specimens. In addition, the mold was recovered on several isolation media inoculated with portions of the same clinical specimens.

(Preliminary results were presented previously [J. G. Baker, I. F. Salkin, P. Forgacs, J. H. Haines, and M. E. Kemna, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, F77, p. 401].)

**Clinical history.** A 75-year-old farmer, originally from the Dominican Republic, who had a history of diabetes managed with oral hypoglycemic agents, had also been treated for 3 years with corticosteroids for myasthenia gravis. He presented at Lahey Clinic Medical Center with a 1-year history of chronic drainage, pain, and swelling of the left foot. On physical examination, both lower extremities were swollen. The dorsalis pedis and posterior tibial pulses of the left leg were absent, and popliteal pulses were markedly decreased. Fluctuance and multiple sinus tracts associated with induration, tenderness, and increased heat extended from the left second toe to the dorsum of the foot. Roentgenograms revealed a degenerative arthritis of the first metatarsal phalanx and calcification of the blood vessels of the lower extremity consistent with a diagnosis of arteriosclerosis, but no bone involvement. The infection could not be associated directly with any previous trauma.

Debridement of the left foot and excision of the sinus tracts revealed a burrowing subcutaneous infection with minimal purulence. Poor postsurgical healing at the debridement site led to a 4-day empiric drug regimen with tobramycin, ampicillin, and clindamycin. A second specimen of the debridement site taken 1 month after surgery grew *Pseudomonas aeruginosa*, which was treated with a further course of tobramycin and metronidazole. However, the patient's condition did not improve, and the development of gangrene necessitated the amputation of the left leg below the knee approximately 5 weeks after the initial surgery. The amputation site healed satisfactorily. The patient was discharged but died 1 year later of pancreatic cancer.

**Laboratory studies.** Debrided tissue and aspirate from the subcutaneous abscess were obtained for microbiologic, cytologic, and histologic studies.

Part of the tissue was fixed in 10% Formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, periodic acid-Schiff, and Grocott-Gomori methenamine-silver stains.

Another portion of the abscess tissue was homogenized in sterile saline with a sterile mortar and pestle. Portions of the aspirate and tissue homogenate were Gram stained and auramine-rhodamine acid-fast stained, as well as mounted in sterile saline for microscopic examination.

Other portions of the aspirate and tissue homogenate were also cultured for aerobic and anaerobic bacteria, mycobacteria, and fungi. To prepare aerobic and anaerobic bacterial

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† Published in part as contribution 518 of the New York Science Service.

cultures, the aspirate and homogenate were streaked onto the following media obtained from BBL Microbiology Systems (Cockeysville, Md.): brucella horse blood agar, chocolate agar, Columbia-colistin-nalidixic acid with 5% sheep blood agar, Levine eosin-methylene blue agar, and anaerobic Columbia with 5% sheep blood agar. In addition, tryptic soy and thioglycolate broths (GIBCO Laboratories, Lawrence, Mass.) were seeded with the same clinical specimens. To isolate aerobic bacteria, the first four agar media indicated above were incubated in 7.5% $CO_2$ at 35°C, while tryptic soy broth cultures were incubated in ambient air at the same temperature. For anaerobic bacteria, the anaerobic Columbia with 5% sheep blood agar was incubated in an atmosphere consisting of 85% $N_2$, 10% $H_2$, and 5% $CO_2$ at 35°C, and the thioglycolate broths were incubated in ambient air at the same temperature.

To isolate mycobacteria, portions of the aspirate and tissue homogenate were streaked onto Lowenstein-Jensen and Mycobactosel agars and added to Dubos broth (all media were obtained from BBL). All cultures were incubated in 10% $CO_2$ at 35°C.

For fungal isolation studies, the aspirate and homogenate were used to seed the following GIBCO media: Emmons modified Sabouraud dextrose agar (SAB); Mycobiotic agar; and brain heart infusion agar supplemented with chloramphenicol. All cultures were incubated in air at 30°C.

A modification of the macrotube broth dilution method described by Shadomy et al. (15) was used to study the in vitro susceptibility of *P. minutella* to amphotericin B and flucytosine. *Candida albicans* (Squibb 1539) was the control. All susceptibility tests were conducted concurrently at 30°C and observed daily for growth for 3 days.

The MIC of each antimycotic for the *C. albicans* control, i.e., the lowest concentration at which no growth was observed, was 0.625 μg/ml ± 1 dilution for amphotericin B and 0.06 μg/ml ± 1 dilution for flucytosine.

**Microscopic studies.** Microscopic examination of the stained sections revealed a necrotizing, granulomatous inflammation infiltrated with polymorphonuclear leukocytes and numerous septate hyphae and pseudohyphal fragments (Fig. 1). While no fungal structures were observed in stained preparations of the tissue homogenates and aspirates, numerous lightly pigmented hyphal elements, similar to those observed in tissue sections, were found in the sterile saline mounts. No mycotic granules were seen.

**Bacteriology studies.** *Staphylococcus epidermidis* was the only bacterium isolated from the tissue homogenate. However, colonies of a dematiaceous mold were recovered on all aerobic bacterial and mycobacterial agar cultures.

**Mycology studies.** A dematiaceous mold identical to those isolated on bacterial cultures was recovered on SAB and brain heart infusion agar with chloramphenicol seeded with aspirate and tissue homogenate. Colonies on SAB were slow growing, attaining a diameter of 4 cm in 4 weeks at room temperature, with an olive-gray obverse and black reverse and radial furrows with a velvety texture. The mold grew at 27 and 37°C but failed to grow at 42°C on SAB. It also did not grow on Mycobiotic agar and did not hydrolyze gelatin.

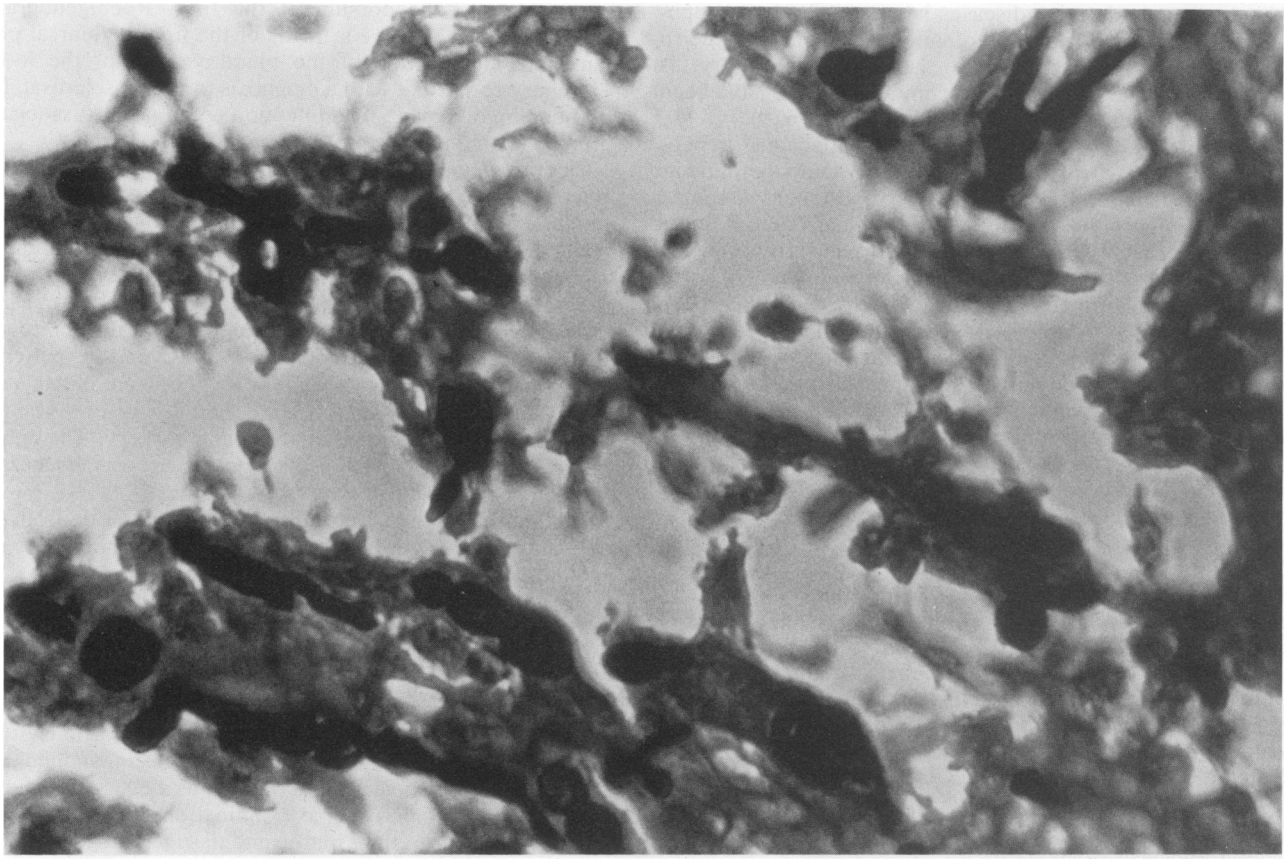

FIG. 1. Grocott-Gomori methenamine-silver-stained pseudohyphallike fungal elements of *Phoma minutella* in foot abscess tissue. Magnification, ×1,900.

Portions of growth from the periphery of colonies on SAB were transferred aseptically to cornmeal and potato dextrose agars (both obtained from BBL) and to 2% cereal agar prepared by one of us (I.F.S.) as described by McGinnis (10). After incubation for 3 to 4 weeks at 30°C, pyriform, ostiolate, partially submerged pycnidia without setae were found on all three media. Small (2 to 3 μm by 0.5 to 1 μm), hyaline, slightly curved conidia produced from phialidic cells were found within the pycnidia.

Since these structures are characteristic of *Phoma* spp., a subculture of the isolate was submitted to E. Punithalingam of the Commonwealth Mycological Institute (Kew, England), who identified the isolate as *P. minutella*.

In vitro susceptibility studies with the mold revealed an MIC of 0.125 μg/ml with amphotericin B and 0.23 μg/ml with flucytosine.

The observation of septate, pigmented, hyphal elements in the tissue homogenate and aspirate as well as in stained tissue sections—and the isolation of *P. minutella* to the exclusion of all other fungi and bacteria (except *S. epidermidis*) on all bacterial, all mycobacterial, and two of three fungal isolation media—strongly suggest that this mold was the etiologic agent of the subcutaneous granulomatous inflammatory process. Our inability to find hyphal elements in the Gram-stained smears of the homogenates confirms the unreliability of bacteriologic staining for detection of fungal structures in clinical specimens (7).

*Phoma* spp. are distinguished from other dematiaceous molds by the formation of dark brown, ostiolate pycnidia without setae; the pycnidia contain gelatinous masses of conidia formed on sessile phialides (12). While species identification is best undertaken by experts in *Phoma* taxonomy, *P. minutella* is differentiated by the presence of globose to pyriform, partially submerged, ostiolate pycnidia containing punctiform, hyaline, very sparingly allantoid conidia measuring 2 to 3 μm by 0.5 to 1 μm (12).

The inability of *P. minutella* to form fruiting structures and conidia on SAB emphasizes the need to use sporulation media for identification of suspected pathogenic molds. Several nutrient-deficient agar media, such as cornmeal, potato dextrose, malt, and 2% cereal, are useful in inducing conidial formation and should be part of the routine identification procedures in clinical mycology (10, 13).

We have presented this case report to increase laboratorians' awareness of *Phoma* spp. as potential human pathogens and to provide the salient morphologic characteristics of the genus to permit their identification in a clinical setting. *Phoma* spp. are apparently weak pathogens, as evidenced by their association predominantly with superficial or cutaneous infections. However, in immunocompromised patients, as indicated in the present case and that presented by Young and co-workers (18), *Phoma* spp. may also cause severe subcutaneous infections.

We thank Ed Lapa for his analysis of the in vitro susceptibility of our isolate.

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