Cutaneous Infection Caused by *Cylindrocarpon lichenicola* in a Patient with Acute Myelogenous Leukemia

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*Cylindrocarpon lichenicola* is a saprophytic soil fungus which has rarely been associated with human disease. We report the first case of localized invasive cutaneous infection caused by this fungus in a 53-year-old male from the rural midwestern United States with relapsed acute myelogenous leukemia. On admission for induction chemotherapy, the patient was noted to have an abrasive laceration between the fourth and fifth metacarpophalangeal joints and on the dorsum of the right hand, which progressed to frank ulceration following chemotherapy. A biopsy provided an initial diagnosis of an invasive fungal infection consistent with aspergillosis based on the histopathological appearance of the mold in tissue. Multiple positive fungal cultures which were obtained from the biopsied tissue were subsequently identified by microscopic and macroscopic characteristics to be *C. lichenicola*. The infection resolved following marrow regeneration, aggressive debridement of the affected tissue, and treatment with amphotericin B. This case extends the conditions associated with invasive disease caused by *C. lichenicola*.

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**CASE REPORT**

A 53-year-old male social worker who was also a farm hobbiest with a history of polycythemia vera presented with fever and extreme fatigue. Review of the peripheral blood smear showed 70% circulating blasts, consistent with AML (FAB-M2). On admission for induction chemotherapy, the patient was noted to have an abrasive laceration between the fourth and fifth metacarpophalangeal joints and on the dorsum of the right hand, which he stated happened “while herding pigs.” Cultures of the hand were negative for bacterial pathogens, and Polysporin ointment (Burroughs Wellcome, Research Triangle Park, N.C.) was applied to the cutaneous lesions. He required two cycles of induction chemotherapy with idarubicin and cytarabine to achieve a remission. Fligelastrom was started 24 h after the last dose of chemotherapy. Three days after completion of chemotherapy, the laceration on the right hand had progressed to frank ulceration, and the orthopedic staff were consulted for possible debridement. A plain X ray of the right hand showed no evidence of osteomyelitis. A punch biopsy of the right hand revealed numerous septate branching hyphae that the pathologist considered consistent with *Aspergillus* species (Fig. 1). Based on a diagnosis of an invasive mold infection, intravenous amphotericin B (AmB) was started at a dose of 0.75 mg per kg of body weight per day. Culture from the biopsy material subsequently grew a white mold that was identified as *C. lichenicola*. Removal of infected tissue when disease is localized is considered standard treatment at our institution for patients with a hematological malignancy undergoing high-dose chemotherapy. Since the skin lesion was too large for surgical debridement, an amputation was performed, with removal of the fourth and fifth digits and part of the palm of the right hand two days after AmB therapy was started. Fungal cultures of tissues removed from the lesion also grew *C. lichenicola*. The patient was discharged 29 days after admission for induction chemotherapy, in stable condition with an absolute neutrophil count of 1,360 cells per μL. AmB therapy was continued daily in the outpatient clinic for 6 weeks, and itraconazole (Itr) was additionally prescribed (200 mg per day) for long-term maintenance. He subsequently relapsed with AML and successfully underwent reinduction chemotherapy without reactivation of the invasive fungal infection. During reinduction, a pulmonary nodule was noted on a chest X ray which was resected using a video-assisted thoracoscopy procedure. No evidence of a mold infection following histopathological examination of this lung tissue was noted.

**MATERIALS AND METHODS**

**Mycology.** The tissue isolate was forwarded to the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center (UTHSC) at San Antonio, for characterization and susceptibility testing. It was entered into the UTHSC stock collection under accession number UTHSC 98-2175. To induce conidiation, the isolate was subcultured onto potato flakes agar (PFA) plates (prepared in-house) (15) and PFA slide cultures, both incubated at 25°C. The microscopic morphology was examined from colonies on PFA plates and from the biopsy material subsequently grown on PFA for 7 days at 25°C. Cultures of the hand were negative for bacterial pathogens, and Polysporin ointment (Burroughs Wellcome, Research Triangle Park, N.C.) was applied to the cutaneous lesions. He required two cycles of induction chemotherapy with idarubicin and cytarabine to achieve a remission. Fligelastrom was started 24 h after the last dose of chemotherapy. Three days after completion of chemotherapy, the laceration on the right hand had progressed to frank ulceration, and the orthopedic staff were consulted for possible debridement. A plain X ray of the right hand showed no evidence of osteomyelitis. A punch biopsy of the right hand revealed numerous septate branching hyphae that the pathologist considered consistent with *Aspergillus* species (Fig. 1). Based on a diagnosis of an invasive mold infection, intravenous amphotericin B (AmB) was started at a dose of 0.75 mg per kg of body weight per day. Culture from the biopsy material subsequently grew a white mold that was identified as *C. lichenicola*. Removal of infected tissue when disease is localized is considered standard treatment at our institution for patients with a hematological malignancy undergoing high-dose chemotherapy. Since the skin lesion was too large for surgical debridement, an amputation was performed, with removal of the fourth and fifth digits and part of the palm of the right hand two days after AmB therapy was started. Fungal cultures of tissues removed from the lesion also grew *C. lichenicola*. The patient was discharged 29 days after admission for induction chemotherapy, in stable condition with an absolute neutrophil count of 1,360 cells per μL. AmB therapy was continued daily in the outpatient clinic for 6 weeks, and itraconazole (Itr) was additionally prescribed (200 mg per day) for long-term maintenance. He subsequently relapsed with AML and successfully underwent reinduction chemotherapy without reactivation of the invasive fungal infection. During reinduction, a pulmonary nodule was noted on a chest X ray which was resected using a video-assisted thoracoscopy procedure. No evidence of a mold infection following histopathological examination of this lung tissue was noted.

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**Antifungal susceptibility testing.** Susceptibility testing was performed on the case isolate by utilizing the National Committee for Clinical Laboratory Standards Macrophotob Dilution Method M27-A, modified for mold testing (13). Briefly, the case isolate and the *Paecilomyces* control strain (UTHSC 90-459) were grown on PFA for 7 days at 25°C and the inocula were standardized...
spectrophotometrically. The PFA slants were overlaid with sterile distilled water, and suspensions were made by gently scraping the colonies with the tip of a Pasteur pipette. Heavy hyphal fragments were allowed to settle, and the upper, homogenous suspensions were removed. Suspensions were adjusted to a 95% transmission at 530 nm and then diluted 1:10 in medium to provide a 1.0 \times 10^4 inoculum concentration as determined by plate counts. Final drug concentration ranges were as follows: AmB (E. R. Squibb & Sons, Princeton, N.J.), 0.03 to 16 \mu g/ml; 5-fluorocytosine (5-Fc; Roche Laboratories, Nutley, N.J.), 0.125 to 64 \mu g/ml; and Itr, (Janssen Pharmaceutica, Titusville, N.J.), 0.015 to 8 \mu g/ml. AmB was tested in Antibiotic Medium 3 (Difco, Detroit, Mich.); other antifungal agents were tested in RPMI 1640 with L-glutamine and morpholinepropanesulfonic acid (MOPS) buffer at a concentration of 165 mM and without sodium bicarbonate (American Biorganics, Inc., Niagara Falls, N.Y.). Previously prepared, frozen drug tubes containing 0.1 ml of drug were allowed to thaw and were inoculated with 0.9 ml of the hyphal medium suspension. The tubes were incubated at 35°C, and MICs were read at the first 24-h interval when growth was observed in the drug-free growth control. MICs were defined as the first tube that yielded a score of 0 (optically clear) for AmB and a score of 2 (reduction in turbidity that was equal to or greater than 80% of the turbidity of the drug-free control tube) for 5-Fc and Itr. Minimum lethal concentrations (MLCs) for AmB were determined by plating 100-m\l samples onto Sabouraud dextrose agar (SBA) plates from tubes containing the following: drug-free control, AmB at the MIC, and AmB at concentrations above the MIC, all incubated at 35°C. The MLC was defined as the lowest concentration of antifungal compound resulting in five or fewer colonies on the SBA plate, which represented 99.9% killing (16).

Molecular testing. The complete internal transcribed spacer (ITS) 1 region, 5.8S rDNA region, and the ITS 2 region of \textit{C. lichenicola} was amplified and sequenced using a previously described procedure (9). Comparison of the case isolate sequence to GenBank database sequences was performed using a non-gapped, advanced BLAST search. The similarities to other sequences was determined with the expectation frequency minimized to 0.0001. Sequences were not filtered for low complexity.

Nucleotide sequence accession number. The nucleotide sequence for \textit{C. lichenicola} of this region was deposited into the National Center for Biotechnology Information (NCBI) GenBank database under accession no. AF133843.

RESULTS

The case isolate was identified as \textit{C. lichenicola} (C. Massal, D. Hawksworth) based upon macroscopic and microscopic features (1). Colonies on PFA exhibited rapid growth, attaining a diameter of approximately 35 mm in 6 days at 25°C. The colonies were velvety to floccose, initially white, later yellowing and becoming pale brown at maturity (Fig. 2). The reverse ranged from buff at the periphery to a darker brown centrally, with a brown diffusing pigment. Hyphae were septate and hyaline, and the conidiophores were long and simple or poorly branched (Fig. 3). Subulate (slender and tapering to a point) conidiogenous cells (phialides) were 38 by 50 \mu m in length and 3 by 5 \mu m in diameter and sometimes had a distinct collarette at the apices. Macroconidia were borne singly and in clusters at the apices of phialides (Fig. 4). They were smooth, hyaline, rounded at the tip, distinctly truncate at the base with offset basal pedicels (17), predominately 3-septate but occasionally up to 5-septate, and ranged from 19.6 to 32 \mu m long by 5 \mu m wide (Fig. 5) (4). Septations appeared as rings around the macroconidia. In old cultures, chlamydooconidia formed and in face view appeared as distinct globose cells within the multicellular macroconidia (Fig. 6). Microconidia were absent. Numerous chlamydooconidia also were produced after 2 weeks of incubation from terminal or short lateral branches. In older

FIG. 1. Methenamine silver stain of skin tissue showing hyphal elements and globular structures that resemble conidia. Magnification, \times 580.

FIG. 2. Colony of \textit{C. lichenicola} ATCC 204306 (case isolate) on PFA agar after 3 weeks at 25°C.

FIG. 3. Long, simple conidiophore, macroconidia, and clusters of chlamydoconidia of \textit{C. lichenicola}. Magnification, \times 460.
cultures, chlamydoconidia were globose, hyaline to pale brown, smooth to spinulose, 8 to 15 μm in diameter, and occurred singly (Fig. 3), in clusters (Fig. 3 and 6), and in short chains. Temperature studies revealed poor growth at 35°C and no growth at 42°C.

The drug susceptibility data for the case isolate showed the MICs to be 2 μg per ml for AmB at 24 and 48 h, >64 μg per ml for 5-Fc at 24 h, and >8 μg per ml for Itr at 24 h. MLCs for AmB remained at 2 μg per ml at 48 h (16). As interpreted by these in vitro data, the isolate appeared resistant to all agents tested based upon standard dosing regimens.

The resulting nucleotide base sequence of the ITS 1-5.8S-ITS 2 region of the case isolate did not align with any of the known sequences following a BLAST search within the NCBI GenBank database. No nucleotide sequences of this target region are available from other strains of *C. lichenicola* for comparison.

**DISCUSSION**

The genus *Cylindrocarpon* was described in a monograph by Booth as containing 27 species and six varieties arranged into four groups depending upon the presence or absence of chlamydoconidia and microconidia (1). All species produce slimy macroconidia in basipetal succession (the youngest conidium at the base) which do not adhere in chains. Of these species, only *Cylindrocarpon destructans*, *C. lichenicola*, and *Cylindrocarpon vaginae* have been implicated as causes of human disease, with *C. lichenicola* the only species described in the literature as causing invasive infection (2, 11, 12, 18, 20). *C. lichenicola* (C. Massal, D. Hawksworth), previously known as *Cylindrocarpon tonkinense* Bugnicourt, has a wide geographic distribution. Although it appears somewhat uncommonly in temperate regions, it occurs frequently in tropical climates, having a wide host range for woody and herbaceous plants and being a common agent of postharvest fruit invasion (6). The present study describes the first case of localized cutaneous invasive disease caused by *C. lichenicola* following trauma in a patient with AML recently undergoing chemotherapy.

Our patient’s infection was evident upon admission to the hospital 7 days after trauma to the hand. Bacterial cultures were tested at the time of admission, and no pathogen was identified. Fungal cultures were not tested at this time. At the time of admission, the patient was neutropenic with acute
granulocytopenia and leukemia. Three days following completion of two rounds of chemotherapy, it was noted that the abrasive laceration on the right hand had progressed and a biopsy was taken. The histological appearance of the fungus in the skin tissue was suggestive of an Aspergillus or Fusarium species, with an identification of either genus indicating a grave prognosis in an immunocompromised patient (3, 10). Along with septate branching hyphae, large globular structures mostly associated with the hyphae were also noted in the tissue (Fig. 1). This was inconsistent with the typical findings observed in tissue infections with the aspergilli and fusaria species, even though under some conditions it is not unusual for the Aspergillus species to exhibit atypical morphological features in histopathological sections (5). A retrospective reexamination of the tissue suggested that the globular structures may have been associated conidia, since they were similar in size (8 to 11 μm in diameter) and shape to the conidia formed in culture. Invasive molds rarely produce conidia in tissues, and then only in lesions exposed to ambient air, such as in a pulmonary aspergillosis fungus ball or primary cutaneous aspergillosis in burned patients (5). In our case, the presence of the fungus on the cutaneous surface, and therefore exposure to ambient air, may have allowed the Cylindrocarpon organisms to produce conidial structures.

Five days after culture of the tissue, a mold which had both cultural and microscopic characteristics resembling a Fusarium species was detected. To further characterize the isolate and for susceptibility testing, the isolate was submitted to a reference laboratory, where the identification of C. lichenicola was made. It had been reported that Cylindrocarpon species appear closely related morphologically and taxonomically to Fusarium species, with both sharing teleomorphs in the genus Nectria (11, 17). Because of morphological similarities, separation of these two genera by cultural characteristics becomes problematic at times (8). C. lichenicola most closely resembles Fusarium solani, a common agent of keratomycoses and disseminated disease in neutropenic and/or immunocompromised hosts. The isolate differed macroscopically from F. solani by forming macroconidia which were predominately straight rather than curved, by having apical cells that were rounded rather than tapering, by having basal cells with truncate and offset rather than attenuated pedicels (foot cells), by lacking microconidia, by having pigmented chlamydoconidia, and by the formation of a brown rather than cream-colored colony on PFA (Fig. 3 to 6) (6).

Molecular methods have been suggested as a means to identify fungal pathogens (9). Amplification and sequencing of the ITS 1-5.8S-ITS 2 region was successful; however, no other examples of this sequence from other strains of C. lichenicola were available for comparison. This case strain represents the first sequence from this region deposited into the NCBI GenBank. Sequencing of other strains of this species are needed to verify intraspecies and interspecies variations within the targeted sequence.

In a previous case of disseminated infection caused by C. lichenicola, James et al. reported clinical improvement of disease following marrow regeneration and treatment with 1 mg of Amb per kg (11). In this reported case, the isolate demonstrated susceptibility to Amb (MIC <0.25 μg per ml) and resistance to Itr using an antifungal susceptibility test described by Warnock (19). In our present patient’s case standard protocol in our institution, extensive debridement of the area occurred 2 days after the start of Amb therapy. Susceptibility data for the present case suggested that the isolate was resistant to both Amb and Itr. In both the reported case and the present case of invasive disease caused by C. lichenicola, the patient cleared the infection; however, it appears from both cases that the role of antifungal therapy in the overall clinical response of these patients was unclear, since recovery of the marrow was a critical part of the patient’s condition (7). Additionally, in our present case, prompt surgical debridement of the isolated lesion on the right hand was a major part of the patient’s management.

The identification of a localized invasive cutaneous infection caused by C. lichenicola in a patient from a rural area with leukemia suggested that immunosuppressed patients must be appropriately educated as to the risks of gardening or farming may engender. This case also reemphasizes the need for more rapid diagnostic tests to properly identify fungal pathogens and a requirement for reliable antifungal susceptibility testing so that patients with invasive mold infections may be treated in the most efficacious method possible.

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REFERENCES