Pseudallescheria fusoidea, a New Cause of Osteomyelitis

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CASE REPORT

An otherwise healthy 29-year-old male was admitted to the hospital with progressive pain, intermittent swelling, tenderness, and erythema of the right foot. Three years earlier, the patient injured his foot while playing baseball, resulting in a laceration of the skin which was subsequently left untreated. Physical examination exhibited a diffusely swollen right foot with tenderness and erythema; no sinus tract or drainage was observed. The patient denied any fevers, chills, numbness, or use of medications. He also had no history of travel outside the United States. Magnetic resonance imaging (MRI) of the right foot revealed chronic osteomyelitis extending into the metatarsal shafts of the first through the fifth digit. The patient was initially placed on vancomycin therapy until a subsequent bone biopsy demonstrated fibrovascular tissue with chronic inflammation, including many plasma cells, and fungal hyphal elements (Fig. 1).

Culture of the bone tissue on potato dextrose agar produced growth of a white, woolly colony that turned slightly gray with age. Microscopic morphology revealed a hyaline mold with a few pyriform conidia resembling Scedosporium sp. The isolate was sent to the Centers for Disease Control and Prevention (CDC) fungus reference unit for confirmation. The mold was subcultured ontoSabouraud agar, which induced abundant pyriform conidia produced on short, annellidic conidiophores (Fig. 2). Optimal growth of the isolate was observed at 30°C, with slower growth at 25°C and 37°C, restricted growth at 40°C, and no growth at 45°C. DNA was extracted from the mold, and PCR amplification of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) was performed using 20 μM each of the primers ITS5 (5′-ACAAGG-3′) and ITS4 (5′-TCTTCCGCTTATTGATATG-3′) (22). 0.2 mM of each deoxynucleotide triphosphate, and 1.25 U of Taq polymerase diluted in standard PCR buffer (Roche Diagnostics Corp., Indianapolis, IN). Two microliters of the DNA extract was added to the PCR mixture, and the target DNA was amplified using an Applied Biosystems 9700 thermocycler for 40 cycles of 94°C, 51°C, and 72°C for 1 min each. The sequence of the amplified product was obtained using a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and evaluated using a 3730 DNA analyzer (Applied Biosystems). The resulting sequence was compared to those deposited in GenBank, using the BLASTn program. The 569-bp sequence of the ITS region exhibited a 97 to 98% match to that of P. fusoidea. The 544-bp sequence displayed a 99% similarity to the sequence of the type strain of P. fusoidea. At the Rovira i Virgili University mycological laboratory in Spain, further sequence analysis of the TUB region of the β-tubulin gene, using the primers TUB-F/TUB-R (5, 7), identified the mold as Pseudallescheria fusoidea. The 544-bp sequence displayed a 99% similarity to the sequence of the type strain of P. fusoidea (CBS 106.53). The isolate was placed into the CDC culture collection (CDC no. B7315).

Subsequently, antifungal testing of the patient isolate was performed at the CDC antifungal drugs unit, using the broth microdilution assay, following the Clinical and Laboratory Standards Institute (CLSI) M38-A reference method (4). The culture and incubation conditions recommended by this document for antifungal susceptibility testing of P. boydii were followed, with the following exceptions: (i) the isolate was grown on Sabouraud agar, as this agar induced sporulation to a greater extent than the recommended potato dextrose agar, and (ii) due to the slow rate of sporulation observed with this isolate, conidia suspensions used in the assay were obtained after 4 weeks of growth at 30°C instead of the recommended 7 days at 35°C. As recommended by CLSI document M38-A, the MIC was determined after incubation at 35°C for 72 h and defined as the lowest drug concentration that caused complete (100%) inhibition of growth. Antifungal susceptibility testing with amphotericin B was determined using the Etest (17). Susceptibility to the echinocandins was interpreted by using the minimum effective concentration (MEC), which was defined as the lowest concentration of a drug that produced a morphological change from filamentous growth to microcolonies. The P. fusoidea isolate displayed elevated MICs against amphotericin B (>32 μg/ml), fluconazole (256 μg/ml), and itraconazole (16 μg/...
ml), and ketoconazole (16 μg/ml). MICs of 1 μg/ml were observed with both voriconazole and posaconazole. Of the three echinocandins tested, anidulafungin displayed the lowest MIC of 0.015 μg/ml, and caspofungin and micafungin displayed higher MICs of 8 μg/ml and 4 μg/ml, respectively.

The patient was placed on a 6-week course of voriconazole but did not return for a follow-up examination until a year later. At that time, he reported that his symptoms had improved after finishing the initial antifungal course but had now returned due to new onset of pain in his right foot. An X ray of the patient’s foot revealed radiographic stability with continued osteopenia and loss of bony tissue as a result of the initial infection. A bone biopsy was performed; however, the results of microbiological cultures were negative. Unfortunately, histopathologic examination was not performed. All laboratory values were within normal limits, except for an elevated C-reactive protein (50 mg/liter) value. Because the patient had a history of not being seen for extended periods of time and the possibility that he had an undetected infection, he was placed on broad-spectrum antibiotics for 6 weeks. The patient was seen in the clinic 4 months after antibiotic therapy was discontinued and continues to show clinical stability, with no further progression of disease.

Osteomyelitis, an infection of bone and bone marrow, occurs either secondarily by hematogenous spread or through implantation due to physical trauma or surgery. Whereas osteomyelitis is most often the result of bacterial infection, fungal osteomyelitis occurs less frequently, usually resulting in a chronic, more indolent course of infection. Fungal osteomyelitis following a traumatic injury is typically due to contamination of the wound with soil containing fungi such as \textit{Fusarium} spp. (12, 18), \textit{Scedosporium prolificans} (19, 20), or \textit{Pseudallescheria boydii} (6, 11, 13, 14). The clinical and radiological presentation of fungal osteomyelitis differs little from that of osteomyelitis caused by bacteria. If the infection is treated empirically, the diagnosis of fungal osteomyelitis may be delayed and may not even be considered until standard antibacterial treatment fails.

This report describes for the first time a case of fungal osteomyelitis caused by the mold \textit{Pseudallescheria fusoidea}, demonstrating the chronic and insidious nature of this disease. Molds in the genus \textit{Pseudallescheria} are commonly found in the soil in temperate regions of the world (10). The most common species isolated are \textit{Pseudallescheria boydii} and \textit{Scedosporium apiospermum}, traditionally considered the anamorphic (asexual) state of \textit{P. boydii}, but recent molecular studies have demonstrated that they are two different species (8).
A recent taxonomic investigation has demonstrated that *P. boydii* is a complex of closely related but distinct species including *P. angusta*, *P. ellipsodea*, and *P. fusoidea* (7). Phylo-
typic species identification is based on the morphology of their ascospores (7, 21), observed during the teleomorphic state of growth. However, when they are in the amorphic state, which is the state most often observed in the clinical laboratory, these species are generally indistinguishable. Conse-
sequently, a mold phenotypically identified as *P. boydii*, isolated from patients with osteomyelitis (6, 11, 13, 14) or other clinical forms of disease (2, 3, 16), may have been one of these other species, including *P. fusoidea*. To accurately determine the species identification of these molds, genotypic analysis is re-
quired.

The rDNA gene sequence has been used most commonly for the molecular identification of fungal species. However, as in the current case, the discriminatory power of the rDNA gene sequence was not great enough to distinguish between species of this genus. The use of an additional gene sequence, the β-tubulin gene in this case, was necessary to determine the final identification (7, 9). The TUB region of the β-tubulin gene has been shown to be the most informative in the phylo-
genic analysis of *P. boydii* and its relatives, as demonstrated in a previous multilocus sequence study (7).

The number of fungal species known to cause significant disease is continually increasing (15). With more antifungal drugs now available for the treatment of fungal infections (1), determining the specific identification of these fungi is impor-
tant. Diagnosis by histopathology alone may not be sufficient, since some of these fungi may have similar morphological characteristics but vary greatly in their drug sensitivities. For example, species of the genus *Aspergillus* are typically suscept-
table to antifungal drugs such as itraconazole and amphotericin B (15), while *Pseudallescheria boydii*, which can be difficult to differentiate from *Aspergillus* histopathologically, is resistant to these drugs (9). The *P. fusoidea* isolate described in this report displayed high MICs to many of the commonly used antifungal agents including amphotericin B, fluconazole, and itracon-
azole. Antifungal drugs that demonstrated the lowest MICs against *P. fusoidea* were the more recently developed extended-
spectrum azoles, voriconazole and posaconazole, and the echinocandin anidulafungin. The MIC pattern observed with this isolate was consistent with that of others reported previously (9).

Currently, very little is known about the epidemiology of *P. fusoidea*. Up to now, only three other strains of this species are known, but all have an environmental origin, i.e., the type strain was recovered from goat dung in India, and the other two originated from forest soil of Cuba and Zaire. This is the first isolate of *P. fusoidea* that has been recovered from a clinical source and causes significant morbidity. Genotypic identification of more isolates from both clinical and environ-
mental sources will be necessary to further our understanding of the epidemiology and public health significance of this or-
ganism.

**Nucleotide sequence accession numbers.** The ITS region and the TUB region sequences were submitted to GenBank (accession numbers EU370965 and EU370966, respectively).

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the CDC.

**REFERENCES**

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