NOTES

Exophiala oligosperma Causing Olecranon Bursitis

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Received 17 March 2003/Returned for modification 9 May 2003/Accepted 2 June 2003

A 62-year-old male with a history of Wegener's granulomatosis and immunosuppressive therapy presented with chronic olecranon bursitis. A black velvety mould with brown septiculate hyphae and tapered annelidases was isolated from a left elbow bursa aspirate and was identified as an Exophiala species. Internal transcribed sequence rRNA sequencing showed the isolate to be identical to Exophiala oligosperma. The patient was successfully treated with aspiration and intrabursal amphotericin B.

Exophiala species are dematiaceous, or dark-pigmented, dimorphic hyphomycetes that are found at low density in relatively extreme microenvironments, such as oligotrophic waters or preservative-treated wood. The species most commonly involved in human infection are Exophiala jeanesmei complex members, Exophiala spinifera, and Exophiala dermatitidis, with other species at low frequency (4, 6, 28). They are generally associated with phaeohyphomycosis, which encompasses a heterogeneous group of infections that are histologically defined by the presence of yeast-like or hyphal forms of the fungus in superficial subcutaneous locations or disseminated systemic disease. The increased detection of Exophiala species as agents of human disease is also believed to be related to the increased number of immunocompromised hosts (2, 10, 24, 26, 27). We report an unusual case of olecranon bursitis attributed to the newly recognized species Exophiala oligosperma (7).

(Presented at the 102nd General Meeting of the American Society for Microbiology, 21 May 2002 [abstract F-80].)

Case report. A 62-year-old male with Wegener’s granulomatosis presented to the University of Iowa rheumatology clinic in July 2001 with chronic painless left elbow swelling. The patient was known to rest his elbows on a sink while irrigating his sinuses twice daily for chronic sinusitis. The elbow was not warm or erythematous. Raised scabbed areas were observed on the skin over the olecranon process that had been present for at least 8 months. The patient exhibited no other signs of infection, and his white blood cell count was within normal limits. The patient’s vasculitis had been treated with prednisone, 20 mg daily, which was tapered to 5 mg every other day, and cyclophosphamide, 100 mg daily, for several months. The olecranon bursa was aspirated, and 15 ml of fluid was removed.

No organisms were seen on Gram or calcofluor white stains. Fungal cultures on potato dextrose agar initially grew yeast-

like colonies that developed into an olivaceous black velvety mould with aerial hyphae and a black reverse (Fig. 1). Microscopically, the hyphae were septeate and pale brown. Conidia were formed in small clusters both from intercalary loci along undifferentiated hyphae and from tapered annelidic conidiogenous cells (Fig. 2), features characteristic of Exophiala species. Two subsequent aspirations from the left olecranon bursa ~1 month apart (3 and 7 weeks after the initial aspiration) also grew Exophiala species.

The isolate was submitted for species identification to the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio, and accessioned into their stock collection as UTTHSC 01-1637. Subcultures onto potato flake agar incubated at 25°C produced small dark yeast-like colonies within 6 days. With extended incubation, the colonies became more filamentous, and at 16 days they were brown pigmented and measured ~18 mm in diameter. Temperature studies on potato flake agar indicated growth at 25 and 30°C but no growth at 35°C. The isolate was positive for nitrate assimilation and grew on media containing cycloheximide. Microscopically, single-celled, small, apiculate annelloconidia (1 to 1.5 by 1.5 to 2.5 μm) were produced from relatively short, somewhat inflated annelidases, as well as from intercalary loci. The yeast cells were larger (4 by 5.5 to 8 μm), somewhat inflated like those of Exophiala piscipila, and had prominent annellidic projections. As this isolate did not appear to represent any of the known Exophiala species, it was referred to A. Grooters for sequencing.

A segment of the nuclear rRNA gene that included the entire internal transcribed sequence 1 (ITS1) region and portions of the 18S and 5.8S subunits was amplified using primers EXO1 (5' - CTCAGAGCCGGA-AACTTGGTC-3') and EXO2 (5' - CCAGGCTCATTTGTCTTTGG-3'), which had been designed by one of the authors (A. M. G.) for amplification of Exophiala species. The resultant products were sequenced from both the 5' and 3' ends with a dye-labeled terminator kit and automated sequencer. The sequence of this isolate, GenBank accession number AY231163, was compared with those
of other isolates recently described and clustered by G. S. de Hoog et al. (7). The isolate was found to be strictly identical to the newly defined type strain E. oligosperma Calendron ex de Hoog et Tintelnot (7, 29).

**Susceptibility testing.** Antifungal susceptibility was determined in the Clinical Microbiology Laboratory at the University of Iowa using a broth microdilution assay according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (22). The MIC endpoints were read visually after 48 h of incubation at 35°C. The MICs of flucytosine, itraconazole, and voriconazole were defined at ~50% inhibition. The amphotericin B endpoint was read at complete inhibition of growth. Quality control testing was performed with *Candida parapsilosis* ATCC 22019. The isolate showed in vitro susceptibility to flucytosine, itraconazole, voriconazole, and amphotericin B, with corresponding MICs of 4.0, 0.25, 0.12, and 1.0 μg/mL, respectively. Even though NCCLS antimicrobial susceptibility breakpoints have not been set for these fungal species, the low MICs suggest that the isolate would be susceptible to all of these antifungal agents.

Therapy was initiated 2 months after the initial positive culture and consisted of weekly aspiration of bursal fluid and intrabursal injection of 1 (initial dose) or 2 mg of amphotericin B once a week for 4 weeks (total dose, 7 mg). The patient initially received a test dose of 0.1 mg of amphotericin B with significant pain. Thereafter, the patient received 1% lidocaine 10 to 30 min before the amphotericin injection. Calcifluor stains and cultures of fluid aspirated prior to the third injection and 11 weeks after the final injection were negative for fungal organisms. Cool swelling of the left olecranon bursa continued to be noted 5 months after completion of therapy, but complete resolution of the bursitis was documented 10 months after the final amphotericin injection.

**Discussion.** This report describes the case of a rare infection of the olecranon bursa by the newly recognized species *E. oligosperma*. Twenty-three isolates of *E. oligosperma* have been identified, and the majority are from environmental sources (honey, swimming pool, and soil) (7). Of the seven other human *E. oligosperma* isolates, three were originally considered *E. jeansenbei*, one was named *Rhinochloridiella* sp., and the remaining three were not given a species designation (7). *Exophiala* species are dematiaceous hyphomycetes exhibiting a black yeast synanamorph and conidial production predominantly via annelaidic conidiogenous cells. They are related to the phaeoid moulds, including *Fonsecaea*, *Cladosphialaphora*, *Phialocephala*, *Rhinochloridiellia*, and *Ramichloridium*. *E. jeansenbei* was the first species identified, from a mycetoma of the foot in 1928 by Jeansenle on Martinique Island (20, 26), and it was given the name *Tordula jeansenbei* by Langeron. The *Exophiala* genus was erected by Carriehel in 1966, describing an aquatic hyphomycete of trout fry, *Exophiala salmonis* (1). In 1977, after many changes in nomenclature, *T. jeansenbei* was transferred to the *Exophiala* genus (19) based on its conidiogenesis, which has remained a defining criterion for the genus. The type strain of *E. oligosperma*, *Melanchroenus oligospermus*, was isolated by Calendron in 1953 and is defined by molecular methods in this issue (7).

Species identification has historically involved separation based on morphological and biochemical assimulative properties (3, 5). The colony morphology of *E. oligosperma* most closely resembles those of *E. jeansenbei* and *E. spinifera*, with an oliveaceous black color, a black reverse, and a velvety appearance due to the aerial mycelium (28). Other species, such as *E. dematitidis*, have a colony morphology that is flat with a scar-like annelated zone of brown pigment diffused into the agar, while *E. psciphila* has a dry and hairy appearance. Microscopically, annelloconidiation is a defining morphological feature of *Exophiala* species. Septate hyphae form tapered tubes, or annelides, that eventually bear ovoid conidiogenous daughter cells (annelcoconidia) from their ends (Fig. 2). The annelloconidia are usually one celled and can be found at the tapered ends of the annelides or along the sides of the hyphae in small clusters of cells (28). Some of the biochemical characteristics that separate *E. oligosperma* from *E. jeansenbei*, though tested with a limited number of isolates, are the ability to utilize xylose and creatinine and the absence of growth with lactose, inositol, or citrate (29). *E. oligosperma* differs from *E. dematitidis* in that it assimilates nitrate, nitrite, creatinine, and creatine but not inositol. Physiologically, *E. oligosperma* most closely resembles *E. psciphila*, differing only in its inability to utilize lactose and inositol. More recently, molecular techniques have been employed for species identification and genus comparison, including restriction fragment length polymorphism (32), random amplified polymorphic DNA (31), and DNA sequencing. Sequencing of rRNA genes, ITS sequences,
and small-subunit genes has proven particularly robust because of the ability to show relatedness of the dematiaceous fungi and still separate the individual species at a fundamental level (4, 12, 33). The rRNA ITS results were used to confirm the identity of this isolate as *E. oligosperma* (7).

*Exophiala* species are most commonly isolated clinically from cutaneous lesions or subcutaneous nodules following traumatic inoculation or contamination of a wound or surgical site (8, 26). Phaeohyphomycosis, or the presence of pigmented yeast-like or hyphal forms in tissue, is the most common presentation. However, some species occasionally appear as thick-walled muriform cells with intersecting cross walls (sclerotic bodies) in superficial or subcutaneous nodules, referred to as chromoblastomycosis. Mycetoma is another form of infection by dematiaceous fungi defined by the presence of black mycotic granules, usually with subcutaneous tumefaction.

*Exophiala* species have been isolated from several human tissues besides superficial and subcutaneous sites, including ophthalmologic, cardiac, bone, and central nervous system tissues (14, 26, 31). Pulmonary infection is associated with inhalation of the organism (18), and the organism has been isolated from the sputa of patients with cystic fibrosis (11, 23). Reports of central nervous system infection have been published particularly for *E. dermatitidis* (13, 30) and for the *Exophiala* species now recognized as *E. oligosperma* (7, 29). Review of the literature reveals only one report of isolation of an *Exophiala* species, *E. jeaneselmei*, from a joint (knee) (A. J. Roncoroni and J. Smayevsky, Letter, Ann. Intern. Med. 108:773, 1988). Roncoroni and Smayevsky describe a complex case of *E. jeaneselmei* infection of the right knee synovium and of a ventricular septal defect patch in a patient who presented with multiple bouts of septic fungal emboli to the lungs and heart. His treatment with up to 3,000 mg of amorfinic B and 200 mg of ketoconazole/day for over a year was not successful in eradicating the fungus. Postmortem examination revealed an abscess of the interventricular septum and multiple pulmonary emboli with mycotic granulomas in the parenchyma. The tissue origin of the infection could not be determined, as initial knee aspirations failed to grow any fungus and no direct trauma to the knee was ever reported.

The rise in recovery of and recognition of infections by *Exophiala* and other dematiaceous fungi appears to parallel the increase in the number of immunocompromised patients. Multiple reports describe the infection of organ transplant recipients on high-dose steroid and other immunosuppressive agents by *Exophiala* species (9, 25, 26, 27). In addition, partial defects in cell-mediated immunity have been associated with both human infection and infection in animal models (16, 17).

Most reports of curative therapy describe the use of surgical debridement and long-term antifungal medication and illustrate the difficulty in treating these types of fungal infections. Long-term therapy that is interrupted or prematurely terminated raises the concern for developing antifungal resistance. Meletiadis et al. reported that 11 clinical isolates of *E. spinifera*, *E. dermatitidis*, and *Exophiala castellani* were susceptible to miconazol, terbinafine, itraconazol, voriconazol, and amorfinic B, with MICs at which 90% of isolates were inhibited ranging from 0.125 to 1.0 µg/mL (21). They found that terbinafine was the most active drug against all of the isolates. Others have reported susceptibility to commonly used antifungal agents as well (9, 18, D. Whittle and I. S. Kominos, Letter, Clin. Infect. Dis. 21:1068, 1995). However, one report by Rath et al. (23) found that the 30% inhibitory concentration (the concentration required to reduce growth to 30% of the growth control) for 11 strains of *E. dermatitidis* indicated susceptibility to amorfinic B, ketoconazol, and itraconazol but not to fluconazol or fluconysine. Another report by Kotylo et al. (15) described fluconysine and amorfinic B resistance in *E. spinifera*. At present, it appears that the difficulty of infection eradication is not likely to be due to antifungal resistance as measured in vitro. Notably, the isolate in the present case was quite susceptible to all agents tested, and the infection was managed by aspiration and local instillation of amorfinic B into the bursa.

**Nucleotide sequence accession number.** The GenBank accession number for the *E. oligosperma* nuclear rRNA gene segment reported in this paper is AY231633.

**REFERENCES**


