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CASE REPORTS

Endophthalmitis Caused by *Fusarium proliferatum*

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Fusarium proliferatum **caused endophthalmitis after cataract surgery. Diagnosis was established by classical microbiology and molecular biology methods (PCR and DNA typing). The treatment with local amphotericin B, oral ketoconazole, and topical natamycin was successful.**

CASE REPORT

A 66-year-old man underwent cataract extraction and intraocular lens implantation in his right eye. Four months after surgery, he complained of eye discomfort and was prescribed a topical steroid (dexamethasone) and tobramicin. Fifteen days later, his symptoms persisted, and the eye began to show severe palpebral edema. Slit lamp examination showed conjunctival hyperemia, hypopyon, capsular fibrosis, and corneal edema, and the intraocular lens had moved to the nasal site. Intraocular pressure was 28 mm Hg, and fundus examination showed vitreous haze. An aqueous humor sample was taken using a 30-gauge needle following examination of the eye. Diagnostic techniques included standard microbiological tests (culture and stains) and PCR. The aqueous humor sample was cultured on several media including Columbia agar plates supplemented with 5% sheep blood chocolate agar, MacConkey agar, and thioglycolate broth and brain heart infusion broth (all media were from Biomérieux, biomérieux Sa, Marcy L'Etoile, France) at 37°C in ambient air. The sample was also inoculated into Sabouraud dextrose agar with chloramphenicol and incubated at 30°C. Two different PCRs were carried out: the first focused on bacterial 16S rRNA gene (16) amplification, and the second focused on specific detection of the fungal internal transcribed spacer (ITS)/5.8S DNA region (5). All the tests on the aqueous humor sample (stains, cultures, and PCRs) were negative. Next, a vitreous sample was taken, and microbiological and molecular tests were repeated. Direct smear of the vitreous sample showed septate hyphae of a filamentous fungus with neutrophils (Fig. 1). PCR with specific fungal primers was positive, and PCR with bacterial primers was negative. This preliminary result was obtained 6 h after the sample was taken. Antifungal treatment was given as soon as the fungal hyphae were seen in the vitreous sample by direct smear. Intravitreal amphotericin B (5 μg/0.1 ml) and oral fluconazole (200 mg/day) were administered the same day the sample was taken.

Amplified DNA from fungal PCR was submitted for sequence analysis (PE Applied Biosystems, Foster City, Calif.) and compared to DNA sequences in the BLAST alignment program of the GenBank database (National Institutes of Health) and the EMBL fungal DNA database using Fasta3 sequence similarity searches, which allowed the species identification 48 h after the sample was obtained. DNA database comparison of the sequence showed 100% identity with *Fusarium proliferatum* (NRRL 31071; USDA Agricultural Research Service Collection, Peoria, Ill.), 99.8% identity with *Fusarium fujikuroi*, and 99.8 to 99.6% identity with *Fusarium annulatum*, all of which belong to the *Gibberella fujikuroi* complex (10, 26).

When the molecular identification showed *F. proliferatum* as the causal agent, a second intravitreal injection of 50 μg/ml amphotericin B in 0.1 ml and topical treatment with natamycin (one 50-mg/ml drop four times at day) were administered. The oral fluconazole treatment was changed to ketoconazole (200 mg/12 h). Oral ketoconazole and topical natamycin were maintained for 3 months and 1 month, respectively. After 3 months, the anterior chamber and vitreous sample showed no inflammatory activity, and visual acuity had improved to 0.6. Twenty months after finishing the treatment, the patient showed no symptoms of infection. Two weeks after the sample was taken, the susceptibility of the fungus to six antifungal drugs (amphotericin B, ketoconazole, fluconazole, flucytosine, itraconazole, and voriconazole) was determined by the Sensititre YeastOne microdilution antifungal susceptibility test (TREK Diagnostics, Cleveland, Ohio) (18, 20). MICs were >64 μg/ml of flucytosine, >256 μg/ml of fluconazole, >32 μg/ml of ketoconazole, >32 μg/ml of itraconazole, 4 μg/ml voriconazole, and 0.5 μg/ml of amphotericin B. Given these results, the treatment was not changed.

F. proliferatum was recovered from the vitreous sample on Sabouraud dextrose agar (Pronadisa Lab. Conda, Madrid, Spain) after 7 days of incubation at 30°C. Colonies were 40 mm in diameter, white, floccose, and attached to the surface of the

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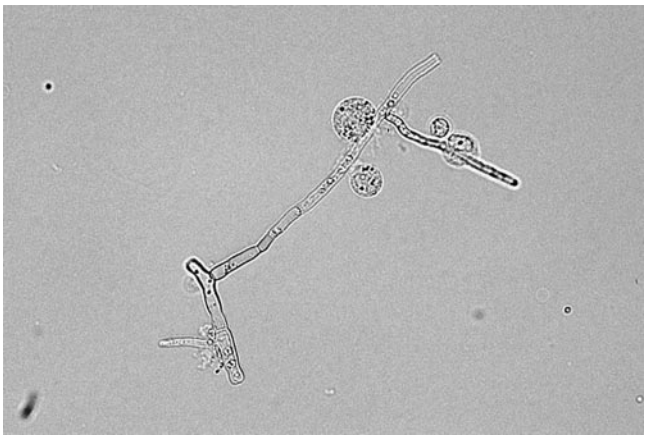

FIG. 1. Direct smear microscopic observation of vitreous sample (×1,000 magnification). Fungal hyphae with attached neutrophils are shown.

medium. For identification, the recommended media are potato dextrose agar (PDA), cornmeal agar (CMA), malt extract agar (MEA), synthetic nutrient-poor agar, and carnation leaf agar (3, 10, 24, 35). In our center, the subcultures were made with PDA (Oxoid Ltd., Basingstoke, Hampshire, England), CMA (Oxoid Ltd., Basingstoke, Hampshire, England), and MEA (Pronadisa Lab. Conda, Madrid, Spain), and we also used SDA (Pronadisa Lab. Conda, Madrid, Spain) because most microbiologists observe this species on this medium before subculturing it onto identification media. Incubation was carried out at 30°C and at room temperature. Colonies were about 30 to 40 mm after 5 days in all media, although macroscopic differences were detectable. CMA cultures showed a thinly tufted, floccose, white growth. SBA, PDA, and MEA growth at room temperature was at first (7 days) floccose and white to pale pink, soon becoming cotton grass-like and salmon-pink colored, with a vinaceous ring at the center of the PDA colony. The reverse was creamy buff, becoming pink-orange, with a small zone of vinaceous purple at the center in the PDA-developed colony (Fig. 2). Soluble pigment and odor were lacking in all media assayed.

Microscopic descriptions were prepared based on material from all medium plates and slide cultures on SBA blocks. Common features in all microscopic observations were the presence of abundant microconidia and septate hyphae frequently in clusters (Fig. 3A), with mono- and polyphialidic conidiogenous cells arising laterally from aerial hyphae. Microconidia were clavate with truncate base, pyriform to ovoidal, and borne in chains. The length of the microconidia was 5 to 9 μm, and thickness was 2 to 2.5 μm. Macroconidia were very scarce and slightly fusiform or nearly straight, with a distinct foot cell, usually three septate, 22 to 25 μm long and 2.5 to 5.0 μm thick (Fig. 3B). Chlamydospores were absent in PDA, CMA, and MEA, although hyphal swellings were observed on SBA.

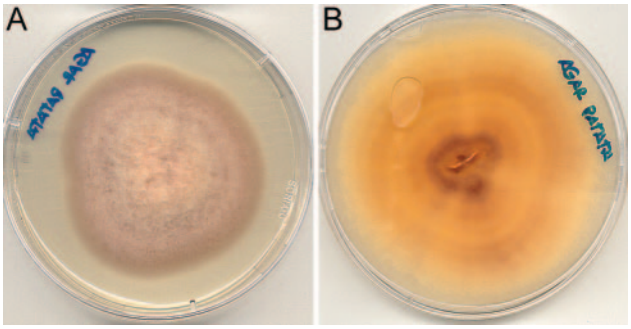

FIG. 2. Colony grown for 14 days on PDA. (A) Obverse. (B) Reverse.

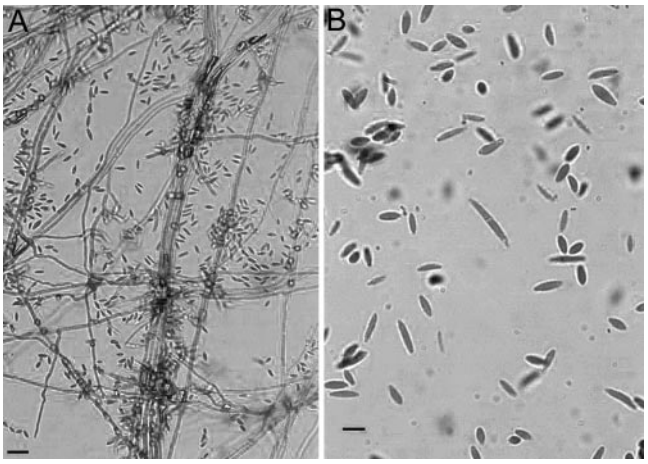

FIG. 3. Microscopic observation of slide culture stained with lactophenol blue. (A) Hyphae in clusters and microconidia bearing in chains (magnification, ×400). Scale micron bar, 30 μm. (B) Microconidia and macroconidia (magnification, ×1,000). Scale micron bar, 10 μm.

Special attention was paid to morphologically differentiate *F. proliferatum* from *F. annulatum* and *F. fujikuroi*. These species showed a high degree of homology with the DNA sequence obtained from the vitreous sample. The typical curved shape of *F. annulatum* microconidia (10), which differs substantially from that described for *F. proliferatum*, allows microscopic differentiation of both species. In addition, the strong conidial chain formation (Fig. 3A) tends to exclude the weakly chain-forming *F. fujikuroi* (10, 35).

Living culture was deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS 116324), and the Spanish Type Culture Collection, Valencia, Spain (CECT 20546).

Fusarium is a filamentous fungus widely found on plants and soil. The genus currently contains over 100 species (10). The most common human pathogens are *Fusarium solani* and *Fusarium oxysporum*, with *F. solani* being the most virulent (21, 22). Numerous cases of keratitis (1, 4, 6, 7, 13) and endophthalmitis (17, 19, 27, 30, 33, 37, 39) caused by *F. solani* and *F. oxysporum* have been reported. Other species, such as *Fusarium dimerum* (8, 38) and *Fusarium verticillioides* (7), are rarely involved in ocular infections. Fungal endophthalmitis is a destructive intraocular infection that has an extremely poor visual prognosis, the worst being when *Fusarium* is the genus involved (4). We can include the lack of adequate treatment for the

species (4), the delay in the application of the treatment (9), and the fact that *Fusarium* produces extracellular proteases resulting in tissue matrix degradation (11) among the causes of this bad prognosis.

Fusarium is one of the most drug-resistant fungi. Among the *Fusarium* species, *F. solani* in general tends to be the most resistant of all. *Fusarium* strains yield quite high MICs of flucytosine, ketoconazole, miconazole, fluconazole, itraconazole, and posaconazole. The antifungal drugs that yield relatively low MICs for *Fusarium* species are amphotericin B, econazole, and natamycin (28, 29, 32, 36). There is a tendency to treat the fungal ocular infections with oral fluconazole and one local dose of amphotericin B. When this infection is caused by *Fusarium*, the only effective treatment is amphotericin B due to the fact that *Fusarium* is resistant to fluconazole (20, 28). Furthermore, it is not uncommon to find *Fusarium* strains that are resistant to amphotericin B (12, 20, 37), leaving the patient untreated. In other cases, a combined therapy with oral imidazoles (fluconazole or ketoconazole) and topical natamycin was administered but was ineffective (15, 31, 37, 39). Sometimes, the treatment was adequate, but the delay in application meant penetrating keratoplasty was needed in the case of keratitis (9), or in other cases, vision was lost (30). In ocular infections, it is crucial not just to identify the species but also to do so as quickly as possible, as the patient's outcome depends on this. Confirmation of the diagnosis of fungal endophthalmitis and the identification of the fungal agent are essential to give an adequate therapy because the treatment strategies are completely different. The diagnosis of fungal endophthalmitis is established by demonstration of the presence of fungus in intraocular fluid. This can be carried out by traditional microbiological means or by PCR and DNA typing. In our case, the fungal detection was performed by direct visualization of fungal hyphae in the vitreous sample, leading to administration of the usual treatment in these cases, intravitreal amphotericin and oral fluconazole. However, the fungal identification by sequencing allowed us to identify the species and change fluconazole to ketoconazole; furthermore, a second dose of intravitreal amphotericin B was administered, and topical natamycin (5%) was given for over 1 month. Two weeks later, when the test of antifungal drugs was performed, sensibility to amphotericin B and resistance to fluconazole "in vitro" was demonstrated. The lowest MIC of azoles was that of ketoconazole, although it was slightly over the established minimum. There is a questionable correlation between in vitro susceptibility and in vivo efficacy of the antifungal agents, a common feature of *Fusarium* infections. In any case, both the knowledge of the species and the "in vitro" antifungal test can help us to give the most adequate therapy. Due to the diversity of fungi that have been reported as opportunistic pathogens, it is imperative that their specific identification is made correctly by an experienced microbiologist. The isolation of *Fusarium* is simple, but species identification of *F. proliferatum* is difficult. The macroscopic and microscopic morphological features of the genus *Fusarium* often change in subculture. The species identification of these fungi is somewhat difficult because of their special growth conditions and subtle morphological differences and the need for correct interpretation of their morphological features. In the majority of ocular fungal infections, the species remains unidentified (9, 25). In this case, the fungal identification was given by ITS/5.8 S rRNA gene analysis. We could limit the probability to three species, *F. proliferatum*, *F. fujikuroi*, and *F. annulatum*, in order of sequence homology. Later, the classical microbiological study led us to identify *F. proliferatum*, eliminating *F. annulatum* and *F. fujikuroi* due to their microscopic features. These three species belong to the *Gibberella fujikuroi* complex, and they are phylogenetically very close, making their differentiation very difficult based on the ITS sequence (26). In this case, we have been able to reach species level based on the ITS sequence with the aid of classical microbiology to ensure the result, but other authors have previously suggested sequencing other loci such as elongation factor alpha to reach species level (40).

Although the genus *Fusarium* has been described as a causal agent in numerous cases of keratitis and endophthalmitis, to our knowledge, *F. proliferatum* has not been known to cause either endophthalmitis or keratitis. *F. proliferatum* has been found as causing human infections in four cases: three were disseminated infections (2, 14, 34) and one was a superficial suppurative thrombophlebitis (23), and three of them were immunocompromised patients. In our case, the patient was immunocompetent, and the only immunosuppressor treatment he received was local treatment (dexamethasone), and he received the treatment after the infection had appeared. This drug was removed immediately after the fungal hyphae were observed. The patient finished the treatment 20 months ago, and to date, the infection has not recurred.

Application of PCR and molecular methods in this case provided a rapid diagnosis and resulted in administration of specific and effective therapy. To our knowledge, the present report describes the fifth case of human infection caused by *F. proliferatum*.

Nucleotide sequence accession number. The EMBL accession number for the sequence obtained from our isolate is AJ810449.

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