Case of Keratitis Caused by an Uncommon *Fusarium* Species

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***Fusarium polyphialidicum* caused a corneal ulcer in a Spanish man. Diagnosis was established by a histopathological study and repeated cultures. The isolate was clearly resistant in vitro to the antifungal agents tested. This is the first case of human or animal mycosis by this rare fungus.**

CASE REPORT

A 65-year-old Spanish male was referred to the Ophthalmology Service of the Lozano Blesa University Hospital in Zaragoza on 5 June 2000 because of acute keratitis in his left eye. Approximately 1 month earlier, the patient suffered a nonpenetrating foreign-body injury when he was working on his farm. Since then, he had complained of increasing disturbance in the eye. On examination, he showed a central corneal ulcer of ca. 4 mm in diameter with irregular borders, slight infiltration, and a thinned weakness but with no risk of perforation. A hypopyon 1.2 mm high was also observed. Visual acuity was 1 to 200. The right eye showed no abnormality. Results of other local and systemic examinations were negative.

Deep corneal scrapings were collected with a sterile scalpel blade for fungal and bacteriological cultures. The corneal scrapings were inoculated directly onto Sabouraud glucose agar (Oxoid, Basingstoke, England) and incubated at 25 and 37°C. After 2 days, numerous small whitish colonies appeared on all cultures. All the colonies were apparently identical, and the fungus was identified as *Fusarium* sp. The results of routine bacteriological cultures were negative. Since the fungus was detected, treatment was initiated with 0.5% amphotericin B applied topically every hour. However, after a week, the condition of the eye worsened; the corneal infiltration and hypopyon increased. The ulcer became torpid and enlarged into a deepening corneal abscess and endophthalmitis. More corneal scrapings were collected for new cultures, which were again negative for bacteria and positive for the same fungus recovered previously. A corneal transplant was performed on 30 June. Cultures from a biopsy of the excised cornea again yielded the same *Fusarium* sp. Portions of the cornea were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Sections of the stained tissue revealed short, contorted, hyaline, septate, hyphal fragments (Fig. 1). The transplanted eye improved considerably, and therefore the patient was discharged. In September 2000,

the patient presented a visual acuity of 20 to 40 in the affected eye.

The clinical strain was sent to the Faculty of Medicine of the Rovira i Virgili University in Reus, Spain, to be identified and to determine its antifungal susceptibility.

Mycological study and diagnosis. For identification purposes, the fungus was subcultured on potato dextrose agar (PDA; Difco Laboratories, Detroit, Mich.) and oatmeal agar (30 g of oat flakes, 1 g of $MgSO_4 \cdot 7H_2O$, 1.5 g of KH_2PO_4, 15 g of agar, 1,000 ml of tap water) and incubated at 25, 37, and 40°C in darkness. After 12 days at 25°C, the colonies on PDA attained a diameter of 68 to 70 mm and those on oatmeal agar covered the whole agar surface. Colony morphologies were very similar on both media: white, cottony, and floccose towards the edge, with a colorless or pale yellow reverse. Microscopically, mono- and polyphialidic conidiogenous cells, which were hyaline and measured 10 to 48 μm long by 2.5 to 3.5 μm wide, were observed (Fig. 2A and B). Polyphialidic conidiogenous cells were predominant and usually presented 2 to 5 conidiogenic loci. Only microconidia were produced (Fig. 2C). They were abundant, grouped in slimy masses, hyaline, usually nonseptate, fusiform, or subclavate and measured 5 to 14 by 2 to 3 μm. Intercalary, smooth- and thick-walled chlamydospores, up to 15 μm in diameter, were observed, although only on PDA (Fig. 3). Colonies on PDA at 37°C attained a diameter of 12 to 14 mm after 5 days. They were white and floccose, with a yellowish reverse and without sporulation. The fungus did not grow at 40°C.

The combination of the morphological features indicated above is not typical of any fusarial species described as pathogenic to humans (5). On the basis of the macroscopic features, the abundant production of polyphialidic conidiogenous cells, and the microconidial morphology, we tentatively identified the case strain as *Fusarium polyphialidicum*. However, the fungus could not be definitively identified because macroconidia and rough-walled chlamydospores (typical of that species) were never developed by the strain. To confirm the identification, the internal transcribed spacer (ITS) region was sequenced. DNA extraction, amplification, and sequencing were performed as described by Solé et al. (18). A search of the GenBank database (1) revealed a high level of similarity (99%)

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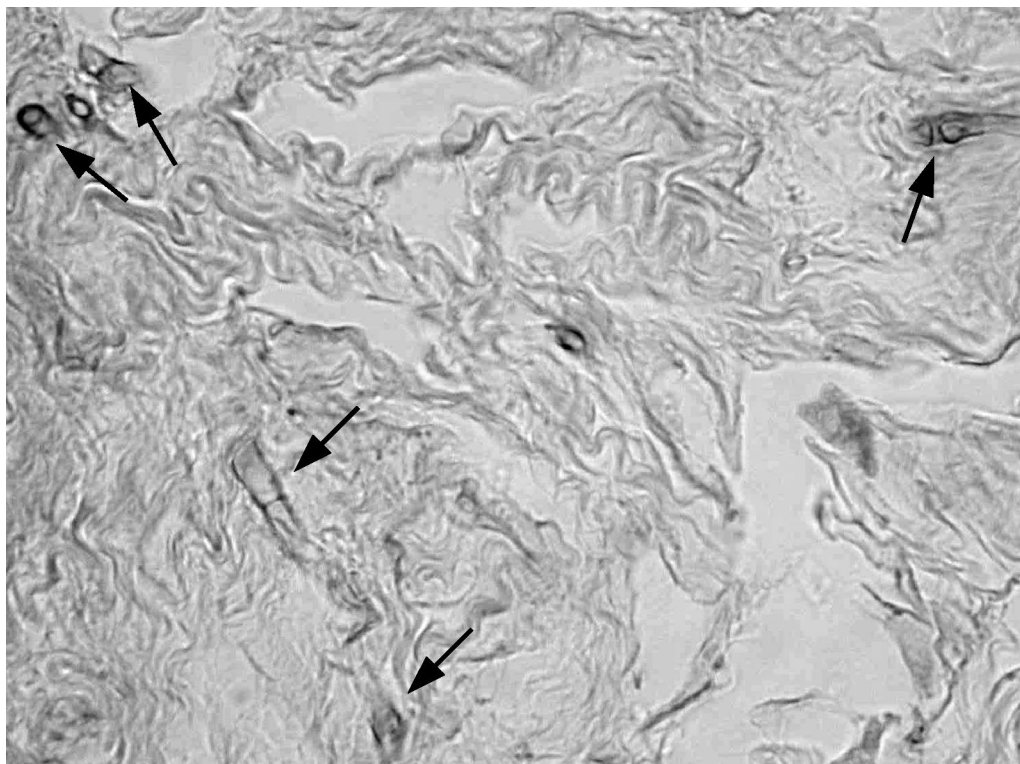
FIG. 1. Hematoxylin and eosin stain from the corneal tissue showing short hyphal fragments (arrows). Magnification, ×1,280.

with the sequence of another *F. polyphialidicum* strain (GenBank accession no. X94172). Living cultures were deposited in the Faculty of Medicine, Reus, Spain (FMR 7804), in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS 111770), and in the Institute of Hygiene and Epidemiology, Brussels, Belgium (IHEM 19748).

Antifungal susceptibility testing. The susceptibility of the fungus to eight antifungal drugs (amphotericin B, ketoconazole, fluconazole, flucytosine, itraconazole, terbinafine, ravuconazole, and voriconazole) was determined by a previously described microdilution method (16), mainly following the guidelines of the National Committee for Clinical Laboratory Standards for molds (13). The tests were performed by using RPMI 1640 medium buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid, an inoculum of 1.7×10^4 CFU/ml, an incubation temperature of 32°C, an incubation time of 48 h, and an additive drug dilution procedure. MICs were >128 μg of flucytosine/ml, 64 μg of fluconazole/ml, 16 μg of ketoconazole/ml, 8 μg of itraconazole and voriconazole/ml, 4 to 8 μg of terbinafine/ml, 2 to 4 μg of amphotericin B/ml, and 2 μg of ravuconazole/ml.

Discussion. Keratomycosis is a relatively common disease that can rapidly lead to progressive corneal destruction and loss of vision in most cases (3). Numerous predisposing factors, such as use of corticosteroids or antibiotics, foreign bodies, postoperative infections, pre-existing eye diseases, contact lenses, systemic illness, age, sex, climate, and season, have been reported (3). The human cornea can be infected by multiple fungal species, of which *Candida*, *Aspergillus*, and *Fusarium* spp. are the most common. In most cases, *Fusarium* species

have clearly been predominant. For example, in a review of 156 cases of mycotic keratitis performed in India, 32.3% were caused by *Fusarium*, 29% by *Aspergillus*, 12.9% by *Alternaria*, and 6.4% by *Candida* (20). However, species of other genera are regularly being added to the long list of agents able to cause keratomycosis. Recently, we reported three Brazilian cases caused by *Curvalaria senegalensis* (9), *Phaeoisaria clematidis* (8), and *Sarcopodium oculorum* (6), three species not previously reported as causing human keratomycoses.

The keratitis caused by *Fusarium* is clinically similar to that caused by other fungi, but its prognosis is worse (22). The presence of malignant glaucoma is a severe complication of fusarial keratitis (10). The most common *Fusarium* species that cause ocular infections is *F. solani* (5). This species has also been reported to cause keratitis related to contact lenses (19). Other species, such as *F. dimerum*, *F. oxysporum*, *F. sacchari*, and *F. verticillioides*, are more rarely involved in ocular infections (7, 22). Here, we describe a case of keratitis in an otherwise healthy patient caused by *F. polyphialidicum*. The present report is the first one to show that it can infect animals and humans.

F. polyphialidicum is a rare species that was described in 1984 from plant debris collected in South Africa (11). It is mainly characterized by its well-developed polyphialides, from which fusiform microconidia are produced in false heads; its long and falcate, usually 5-septate macroconidia with a distinct curved apical cell; its smooth or verrucose chlamydospores; and its cottony or floccose, fast-growing white colonies. Although an *F. polyphialidicum* isolate recovered from a man was deposited in the CBS culture collection (4), no case of human
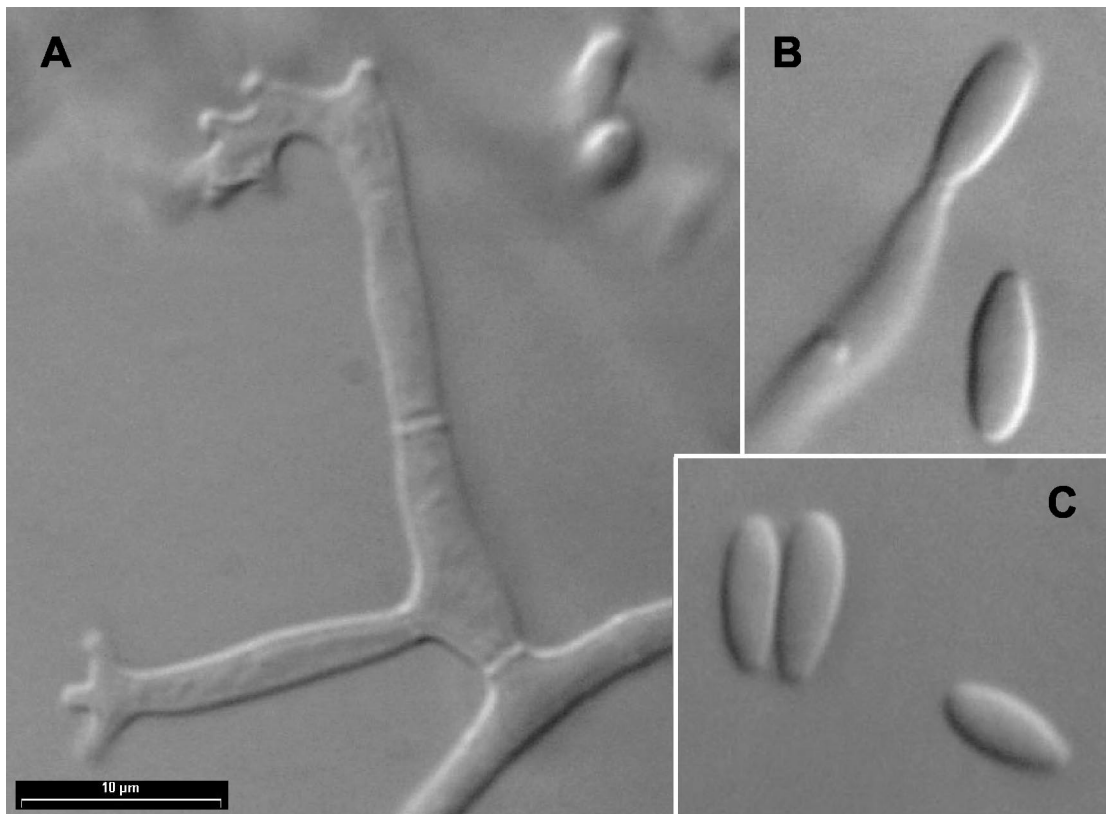
FIG. 2. *F. polyphialidicum* FMR 7804. (A) Polyphialidic conidiogenous cells. (B and C) Monophialidic conidiogenous cells and microconidia.

or animal infection by this fungus has been reported so far. *F. polyphialidicum* presents morphological features similar to those of other *Fusarium* species occasionally found in human infections, such as *F. chlamydosporum* and *F. semitectum*. How-

FIG. 3. *F. polyphialidicum* FMR 7804. Chlamydospores are shown.

ever, these two species can be easily distinguished because of the colony color on PDA (white to pink to brown with a carmine red reverse in *F. chlamydosporum* and tan to brown with a reverse of the same color in *F. semitectum*) and because of the size of their macroconidia, which are shorter and narrower than those produced by *F. polyphialidicum*.

A molecular analysis of ribosomal ITS region sequences of several *Fusarium* species of medical interest demonstrated that the ITS1 sequences of *F. napiforme*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. sacchari*, *F. subglutinans*, and *F. verticillioides* were very similar to one another, while the sequence of *F. polyphialidicum* was considerably different (21). This finding suggests that sequencing this region could be useful for distinguishing this fungus from other occasionally pathogenic species.

It has been suggested that the reason for the marked virulence of *Fusarium* for the cornea is that it produces substances that help it to penetrate or toxins that help to produce the lesions (10). It has also been suggested that the ocular virulence of *Fusarium* may be due to its capacity for angioinvasion, which occludes the intraocular vascular channels with secondary infarction, hemorrhage, and necrosis (15). The potential virulence for ocular structures of *F. solani*, the most frequent pathogenic *Fusarium* species, has been confirmed in different animal models. The inoculation of this fungus into the anterior chambers of rats (14) or intravenously in mice (12) caused severe endophthalmitis in both cases. The very quick clinical
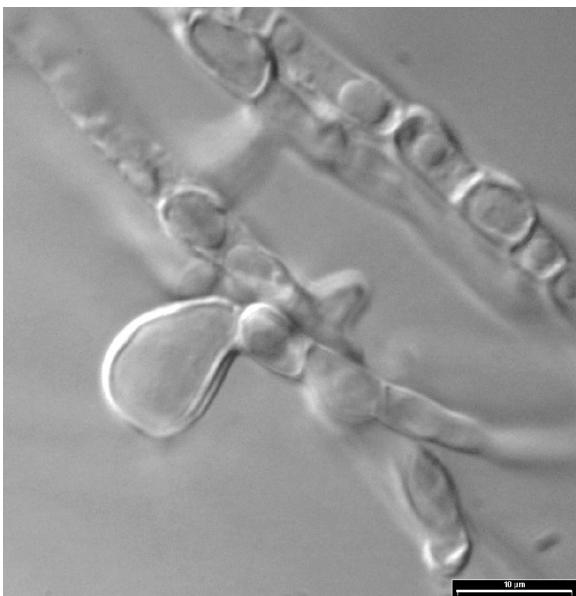
evolution of the infection in the present case seems to indicate that *F. polyphialidicum* is particularly virulent for ocular structures. However, further experimental studies are required if this is to be confirmed.

Although the in vitro antifungal susceptibility of *F. polyphialidicum* had not been previously tested, the low susceptibility shown by the case strain was partly expected because of the demonstrated resistance of *Fusarium* species to the available antifungal drugs (2, 17). The high MIC of amphotericin B for this isolate correlated with the clinical outcome of the patient.

Nucleotide sequence accession number. The GenBank accession number for the sequence (522-bp long) obtained from the case strain is AJ538042.

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