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Fusarium subglutinans: A new eumycetoma agent ☆

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ABSTRACT

Eumycetoma is a chronic subcutaneous mycosis mainly caused by Madurella spp. Fusarium opportunistic infections in humans are often caused by *Fusarium solani* and *Fusarium oxysporum*. We report a case of eumycetoma by *F. subglutinans*, diagnosed by clinical aspect and culture, and confirmed by PCR sequencing. The patient was successfully treated with oral itraconazole. To our knowledge, this is the second report of human infection and the first case of mycetoma by *Fusarium subglutinans*.

1. Introduction

Mycetoma is an infectious, inflammatory and chronic disease that affects the skin and subcutaneous tissue. Regardless of the aetiologic agent (bacteria or fungi), the clinical disease is essentially the same. The disease is characterized by tumefaction, discharging sinuses, and grains. The disease may disseminate mainly to the fascia, tendons and bones. Actinomycetoma predominates in Latin America, particularly in Mexico, and eumycetoma predominates in the equatorial Trans-African Belt extending from Senegal to the Somali coast being Sudan the highest endemicity area [1]. At least 30 species of hyaline or dematiaceous fungi that cause this infection have been described [2]. The prognosis for each case depends on the causative agent and its resistance to antifungal drugs, the clinical location and the degree of tissue damage.

The genus *Fusarium* includes a wide diversity of species that are mainly considered to be plant pathogens [3]. *Fusarium subglutinans* (Wollenweber & Reinking, Nelson, Toussoun & Marasas; teleomorph: *Giberella subglutinans*) belongs to mating population E of the *G. fujikuroi* species complex and has been reported as a pathogen of maize and other vegetables and cereals [4]. In humans, *Fusarium* species are commonly found in superficial mycoses [5,6], eye infections [7,8], and infections of immunosuppressed patients [9]. To our knowledge, there had been only 1 case of *Fusarium subglutinans* infection documented in the literature, a hyalohyphomycosis case in a 72-year-old seemingly immunocompetent patient [10]. The azoles have been the most commonly used agents for eumycetoma treatment. Itraconazole is preferred as it is better tolerated and is thought to have greater efficacy than ketoconazole [11].

In this document, we report the second case of human infection and the first case of mycetoma caused by with *F. subglutinans*.

2. Case

The patient was a 29-year-old male mason from the city of León, Gto, México. He came to the doctor's office (day 0) with a lesion that had progressed for 8 years (−8 years), located in the distal third of the left leg and in the ankle. He does not remember having a previous traumatic injury. The lesion began with a small painless nodule in the internal malleolar zone, and it evolved to a fistula with an exudate of pus and oedema. The lesion slowly disseminated towards his ankle and leg. He sought medical care services after 2 years (−6 years), and the lesion was twice subjected to biopsy. According the patient's version, the histopathological study showed a chronic inflammatory infiltrate and granulomas. The patient had previously received treatment with trimethoprim/sulfamethoxazole, diamino-dyphenil sulphone and amikacin on several occasions and in non-specified doses over periods from 5 to 6 months without favourable results. In the physical examination (day 0), an increase in volume with a deformity in the left ankle and in the inferior third of the leg is found.

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Tumour-like and ulcerative injuries are observed, and the sinus tracts drain a seropurulent material (Fig. 1a). Other disorders are not found.

Due to the clinical aspect of the injuries, a mycetoma diagnosis was suggested, and the corresponding mycological studies were requested (day 0), including direct examination and culture of the seropurulent material on Sabouraud dextrose agar (SDA, with and without antibiotics) and on Lowenstein–Jensen agar, all in duplicate. The patient did not accept a new biopsy. An X-ray study of the left leg and foot, a complete blood count, glucose, urea, creatinine and urine analyses and an HIV test were also requested (day 0).

The KOH direct examination did not show structures suggesting grains. After 10 days (+10d), in 2 tubes of SDA without antibiotics and 1 tube of Lowenstein–Jensen agar, well-developed whitish filamentous colonies were observed. The results of the microscopic examination of cultures were compatible with *Fusarium* sp. At day +15 the radiographies and laboratory tests were received which showed areas of osteolysis in third lower of the tibia and fibula and in the tarsus bones (Fig. 1b). The other laboratory tests were normal. After these observations (+20 days), the treatment with itraconazole was started as 200 mg twice a day for the following 4 months.

To identify the fungal isolate, it was incubated at 28 °C for 7 days in 4 culture media: SDA, potato dextrose agar (PDA), malt extract agar and yeast extract peptone dextrose (YEPD). The colony morphology varied according to the culture media; it appeared as a yellow colony with a downy aspect and a purplish reverse on PDA and as a white cottony colony with a yellow reverse on YEPD (Fig. 2a–c). The microscopic examination of the isolate after 7 days on PDA revealed abundant cylindrical phialides and polyphialides with narrow necks. Ovoid microconidia with an average size of 2.3 by 16 μm, some of them curved, forming false heads were produced. (Fig. 3a–d). Rare macroconidia with 2 septa were observed. Chlamydospores were not present.

To confirm the identification, the isolate was analysed genetically. From a single-spore culture, the isolate was grown in Sabouraud dextrose broth at 28 °C for 4 days. The mycelial mass was harvested and the DNA was extracted [12]. A fragment of the D1/D2 domain of the 28S ribosomal subunit RNA gene was amplified by PCR [13]. The reaction mixture contained 50 ng of DNA, 1X PCR buffer, 1.2 mM $MgCl_2$, 0.2 $mmol^{-1}$ of each dNTP, 0.4 μM of each primer (sense 5′ CAT ATC AAT AAG CGG AGG AAA AG 3′; antisense 5′ GCT CCG TGT TTC AAG ACG 3′) and 1.25 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). The temperature conditions were as follows: one initial cycle at 94 °C for 5 min; 35 cycles of 1 min at 94 °C, 1 min at 63.3 °C, and 1 min at 72 °C; and one final cycle of 5 min at 72 °C (Thermocycler Perkin Elmer, GeneAmp PCR System 2400). The amplified fragment (650 bp) was visualised on a 2% agarose gel and purified with the PureLink PCR Purification kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The product was sequenced using the Big Dye Terminator Cycling Sequencing Ready Reaction v3.1 kit [14]. The 519 bp sequence was analysed with the program Chromas, version 2.33 and compared to the GenBank database. The species *F. subglutinans* (GenBank accession number HQ876767) was identified by the blast analysis with 100% similarity.

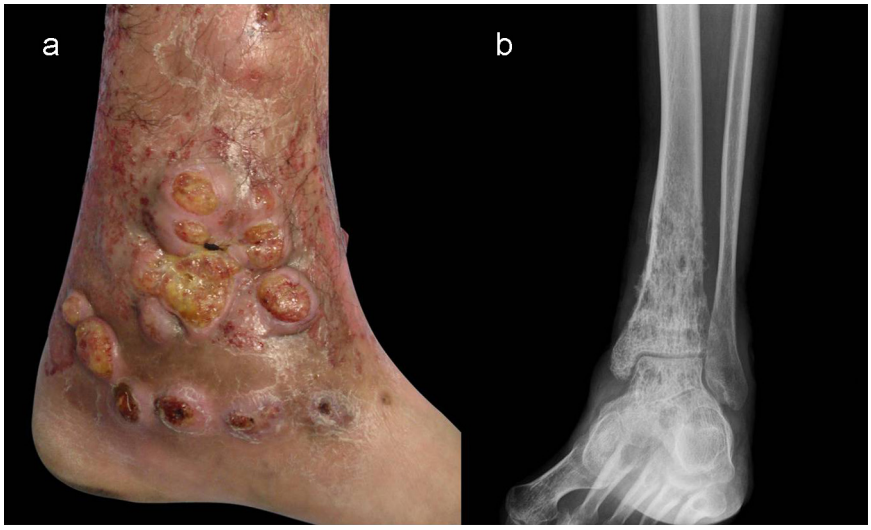

Fig. 1. Mycetoma due to *Fusarium subglutinans*. (a) Tumour-like lesions of the left ankle zone that have softened and ruptured to form sinus tracts. (b) Several lytic areas in the tibia, fibula, astragalus and calcaneous.

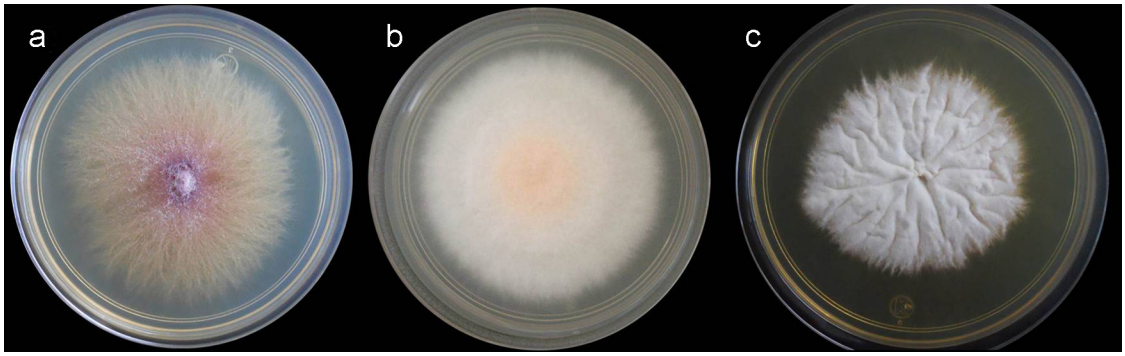

Fig. 2. Macroscopic aspect of *Fusarium subglutinans* grown on different culture media. (a) PDA, (b) SDA, and (c) YPD.

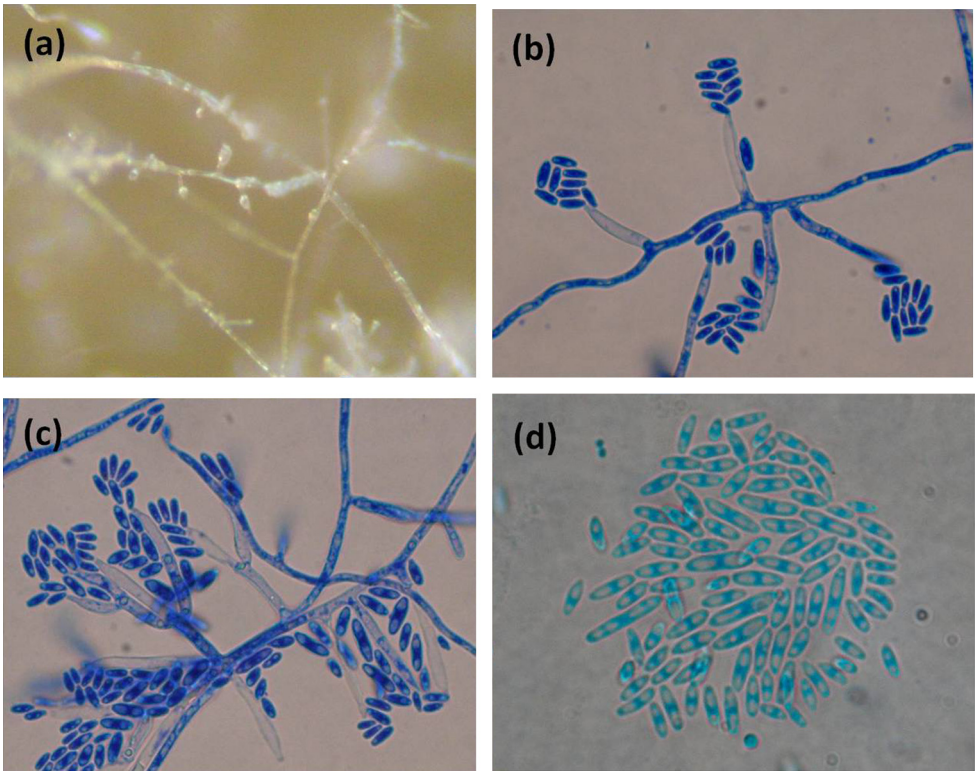

Fig. 3. Microscopic aspect of *Fusarium subglutinans* after 7 days of growth on PDA. (a) Phialides with conidia arranged in false heads observed in the aerial mycelium (× 5). (b) Cylindrical phialides with conidia arranged in false heads (× 100). (c) Polyphialides (× 100). (d) Abundant oval (nearly cylindrical) conidia, some with one septum. (× 100 +zoom 3 ×).

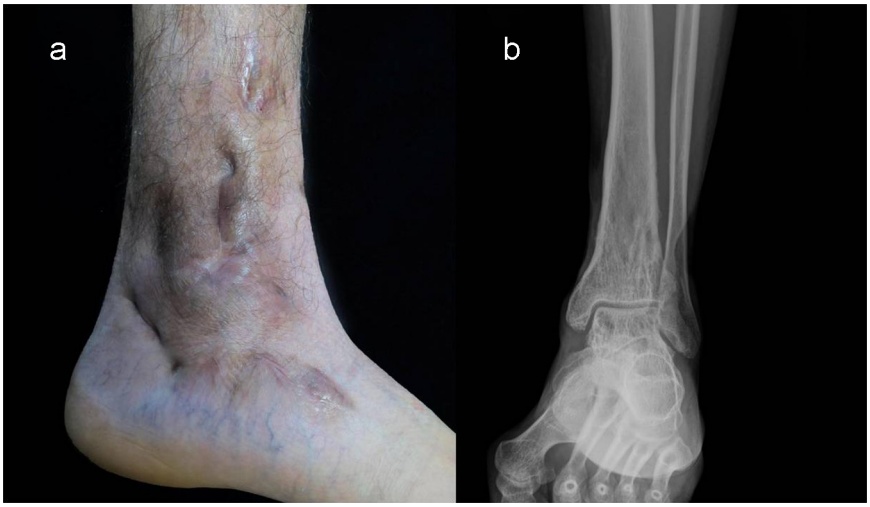

Fig. 4. Mycetoma due to *Fusarium subglutinans* after eighteen months of treatment with itraconazole (400 mg/day). (a) Retractile scars with hyperpigmented zones. (b) The osteolytic areas have been reduced.

In vitro susceptibility testing using the microbroth dilution method according the M38-A2 guidelines resulted in MIC levels for ketoconazole≥16 μg/mL, itraconazole≥16 μg/mL, posaconazole≥ 16 μg/mL, fluconazole≥64 μg/mL, voriconazole=2 μg/mL, amphotericin B 2 μg/mL, terbinafine=8 μg/mL, anidulafungin≥32 μg/mL, and caspofungin≥16 μg/mL. By this method, CLSI established as itraconazole resistance level≥8 μg/mL for non-dermatophyte molds.

After of treatment with itraconazole at month+4, the reduction in the volume of the affected zone was remarkable, and most of the sinus tracts were closed. We decided to continue the same treatment scheme with follow-up examinations. A year and a half after starting the treatment with itraconazole, we observed fibrous hypo- and hyper-pigmented scars. The control X-ray studies showed a reduction in the lytic bone areas. (Fig. 4a-b). Liver function tests are conducted every 4 months, and the results are normal. The serum itraconazole levels in our patient were not tested.

3. Discussion

Mycetoma is a very frequent pathology in Mexico, where 97.8% of the cases are caused by actinomycetes, particularly by *Nocardia brasiliensis* (86.6%) and 2.2% are caused by fungi, mainly *Madurella grisea* and *M. mycetomatis* [15]. The bacterial aetiology predominant

in Mexico was likely a factor that led to the prescription of antibacterial treatment in the present case and did not control the illness but instead allowed its dissemination to the bone tissue. In spite of mycetoma is very frequent in Mexico, often the clinician or laboratory staff is not trained to detect the typical fungal or bacterial structures of this pathology consisting in grains. This case confirms the importance of obtaining an aetiological diagnosis of mycetoma before beginning treatment. The case reported here is very interesting, not only due to the low frequency of eumycetoma in Mexico but also because it was possible to identify *Fusarium subglutinans* as the causative agent.

The diversity of fungal species has made their identification by traditional methods a real challenge, and induced to use faster, more sensitive and reliable procedures, such as PCR and sequencing, to establish the taxonomic status of a particular isolate [16,17]. Sequencing and phylogenetic analysis of the D1–D2 region of the 28S rRNA gene have revealed to be sufficiently discriminative to distinguish fungal species [18]. In the present case, the sequence analysis presented 100% similarity with *F. subglutinans* and this species was corroborated with its morphology: it forms polyphialides and conidia in false heads, and it produces a purplish pigment on PDA; chlamydospores are absent and macroconidia are rare [19].

In the case reported here, diagnosis was firstly established by clinical features and by culture, but later by the therapeutic response to the itraconazole which was remarkable, because in vitro studies *Fusarium* performed by other authors have showed a trend towards resistance to azole antifungals [20]. In spite of no availability of validated susceptibility testing method and interpretative MIC breakpoints for the most of filamentous fungi, the MICs for each antifungal drug in *F. subglutinans* were too high suggesting a full resistance against the most of antifungal agents (except voriconazole and amphotericin B). In our clinical case the therapeutic decision was taken before to know the susceptibility testing result; however, the clinical response was satisfactory and the treatment with itraconzole was continued until the total cure. Therefore, this case provides evidence for a lack of correlation between the in vitro susceptibility test and the clinical outcome.

It is important to determine the causative agent in every patient suspected to present mycetoma to establish an adequate and timely therapeutic plan to avoid the persistence and dissemination of the disease. To our knowledge, this is the second report of human infection by *F. subglutinans,* and it is the first case of mycetoma caused by this agent.

Conflict of interest

There are none.

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