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Case Report

Subcutaneous phaeohyphomycosis caused by plant pathogenic *Corynespora cassiicola*: A case reportJia-Jun Zou^a, Jin Li^a, Shan-Shan Ma^a, Peng-Fei Li^b, Dao-Hong Zhou^{a,*}^a Department of Laboratory Medicine, Daping Hospital, Army Medical University (Third Military Medical University), Chongqing, 400042, China^b Department of Critical Care Medicine, Daping Hospital, Army Medical University (Third Military Medical University), Chongqing, 400042, China

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ABSTRACT

Corynespora cassiicola is a common plant pathogen responsible for leaf-spotting diseases in the tropical and subtropical areas. *C. cassiicola* seldom causes human infections. Here we describe a case of subcutaneous phaeohyphomycosis caused by *C. cassiicola* in a 76-year-old Chinese man, who presented to our hospital with a purulent discharge and painful sensation on his right leg. Skin biopsy revealed an abscess, and culture confirmed *C. cassiicola* to be the causative agent. The result was further identified by sequence analysis of the internal transcribed spacer region. The patient was successfully treated with systemic voriconazole and wound debridement: the lesion disappeared after 20 days.

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Introduction

Subcutaneous phaeohyphomycosis is a rare infection caused by dematiaceous fungi which derive their pigmentation from melanin in cell walls.^{1,2} More than 100 species of fungi, particularly the *Wangiella dermatitidis*, *Alternaria* sp., and *Exophiala jeanselmei*, have been implicated as etiologic agents of phaeohyphomycosis.^{3,4} *Corynespora cassiicola* is a common plant pathogen responsible for leaf-spotting diseases in the tropical and subtropical areas.⁵ This fungus is well-known for its pathogenicity to leaves, stems, flowers, fruits or roots of more than 300 plant species.⁶ *C. cassiicola* rarely causes human infections. Here we present a case of subcutaneous phaeohyphomycosis caused by *C. cassiicola* with prominent tissue necrosis in a male patient.

Case report

A 76-year-old Chinese man was hospitalized for two months for erosions and ulcers on his right leg with purulent discharge and pain. The erythematous skin changes began in the anterior tibia and extended to the right leg, gradually enlarged expanding to

erosion and ulceration. Retrospective analysis of the patient's medical records revealed no symptoms of fever, chills, night sweats or weight loss, nor any history of soil or plant contact.

Physical examination revealed involvement of almost the lower right leg with extensive exudative inflammation, punctuated black changes, and punched-out ulcers (Fig. 1A). Therefore, the patient was initially diagnosed with ulcer of the right lower limb caused by bacterial infection. To further confirm the diagnosis, a series of imaging and laboratory tests were performed. MRI showed abnormal signs in the subcutaneous fat layer and soft tissue, suggesting inflammatory reaction (Fig. 2). Laboratory tests showed a leukocyte count of 18.7×10^9 cells/L and a marked increase of 92.0% in neutrophils. C-reactive protein levels were greater than 14.5 mg/L (normal range: 0–10 mg/L). The levels of aspartate aminotransferase (4941.1 U/L, normal range 15.0–40.0 U/L) and alanine aminotransferase (3194.3U/L, normal range 9.0–50.0 U/L) in liver function were significantly increased. The inflammatory marker procalcitonin was slightly elevated (0.74 ng/mL, normal range 0–0.25 ng/mL).

In order to evaluate the skin ulceration of the patient's right lower extremity and relieve the symptoms, direct microscopic examination, bacterial culture and fungal culture of the purulent discharge were conducted immediately after consultation with trauma surgeons. The purulent discharge was initially observed via microscopy, which showed septate and unbranched hyphae (Fig. 3A). The colonies on sheep blood agar and sabouraud's agar were white and somewhat floccose, becoming grayish at the

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Fig. 1. Subcutaneous phaeohiphomycosis of the right leg in a 76-year-old man caused by *Corynespora cassiicola*. (A) Ulcerating lesions at admission; (B) The lesion disappeared after 20 days.

central zone (Fig. 3B). The isolated strains from a single colony exhibited the following characteristics: conidiophores were straight or slightly flexuous, septate, unbranched and relatively thick-walled; 100–700 μm long and 4–11 μm wide. Conidia were thetic, budded solitarily, but occasionally arranged in chains. The hyphae were septate, hyaline, and thin-walled (Fig. 3C). These morphological characteristics corresponded to those of *C. cassiicola*.

To confirm the identification, the internal transcribed spacer (ITS) region of ribosomal DNA gene was amplified by polymerase chain reaction (PCR) using universal primers ITS1 and ITS4. DNA was extracted from the strain using the Ezup Column Fungi Genomic DNA Purification Kit (Shangon Biotech, Shanghai, China) according to the manufacturer's protocol. Primers were synthesized by Shangon Biotech (Shanghai, China). The PCR amplification was performed using the Applied Biosystems VeritiPro PCR (Thermo Fisher, MA, USA). The ITS1 primer was 5'-TCCGTAGGT-GAACCTGCGG-3', and the ITS4 primer was 5'-TCCTCCGCTTATTGATATGC-3'. The length of the amplified product was 576 bp. The PCR reaction mixture (25 μL) contained genomic DNA extract (1.0 μL), 2 \times Taq Master Mix (12.5 μL), ITS1 primer (1.0 μL), ITS4 primer (1.0 μL), and ddH₂O (9.5 μL). The PCR mixtures were subjected to 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s, with a final extension step of 72°C for 10 min. The PCR product was analyzed by gel electrophoresis of 1% (w/v) for 25 min at 120 V, subsequently observed using a gel documentation system (Bio-Rad, USA). The PCR product was sequenced by Shangon Biotech (Shanghai, China). The isolate showed a 99% similar to *C. cassiicola* (GenBank accession No. KU990882.1). Based on these data, we can conclude that the subcutaneous phaeohiphomycosis was caused by *C. cassiicola*.

The patient was diagnosed as having phaeohiphomycosis caused by *C. cassiicola*. Empirical treatments with fluconazole and voriconazole were used to prompt resolution of his symptoms.

After 20 days of treatment, the ulcers on right leg were shallower and crusted (Fig. 1B). Then the patient was discharged as his relatives required for home care. Long-term treatments with voriconazole and wound debridement were suggested at discharge.

Discussion

Phaeohiphomycosis was first described in 1974 by Ajello et al.⁷ to designate all the cutaneous, subcutaneous and systemic fungal infections. Subcutaneous phaeohiphomycosis usually occurs on limbs in the wrists, fingers, ankles, or knees, following traumatic implantation of the fungal elements through contaminated soil, wood splinters or thorns.⁸ *C. cassiicola* is an anamorphic fungus belonging to the Ascomycota phylum, Dothideomycetes class, Pleosporales order.⁹ The *C. cassiicola* infection is extremely rare in human.

In this study, we report a rare occurrence of subcutaneous phaeohiphomycosis caused by *C. cassiicola*. A review of the literature in PubMed with MeSH searches for phaeohiphomycosis resulted in only five similar cases. The first one was identified as a mycetoma in an Ethiopian farmer.¹⁰ Another two cases were subcutaneous infections by *C. cassiicola*: one following trauma¹¹ and the other due to diabetes mellitus.¹² The rest two cases were corneal and face infections.^{13,14} All of the five cases were treated successfully with antifungal drugs, and good therapeutic effects were achieved. Therefore, antifungal drugs are the main treatment methods to be considered. We should strengthen the identification ability of fungal species.

The mechanisms underlying the pathogenicity of *C. cassiicola* infection in our case were unknown. Although the patient had no history of soil or plant contact, the patient lived in the countryside. Therefore, when we encounter similar signs of infection without obvious trauma in clinical practice, we should be well aware of the

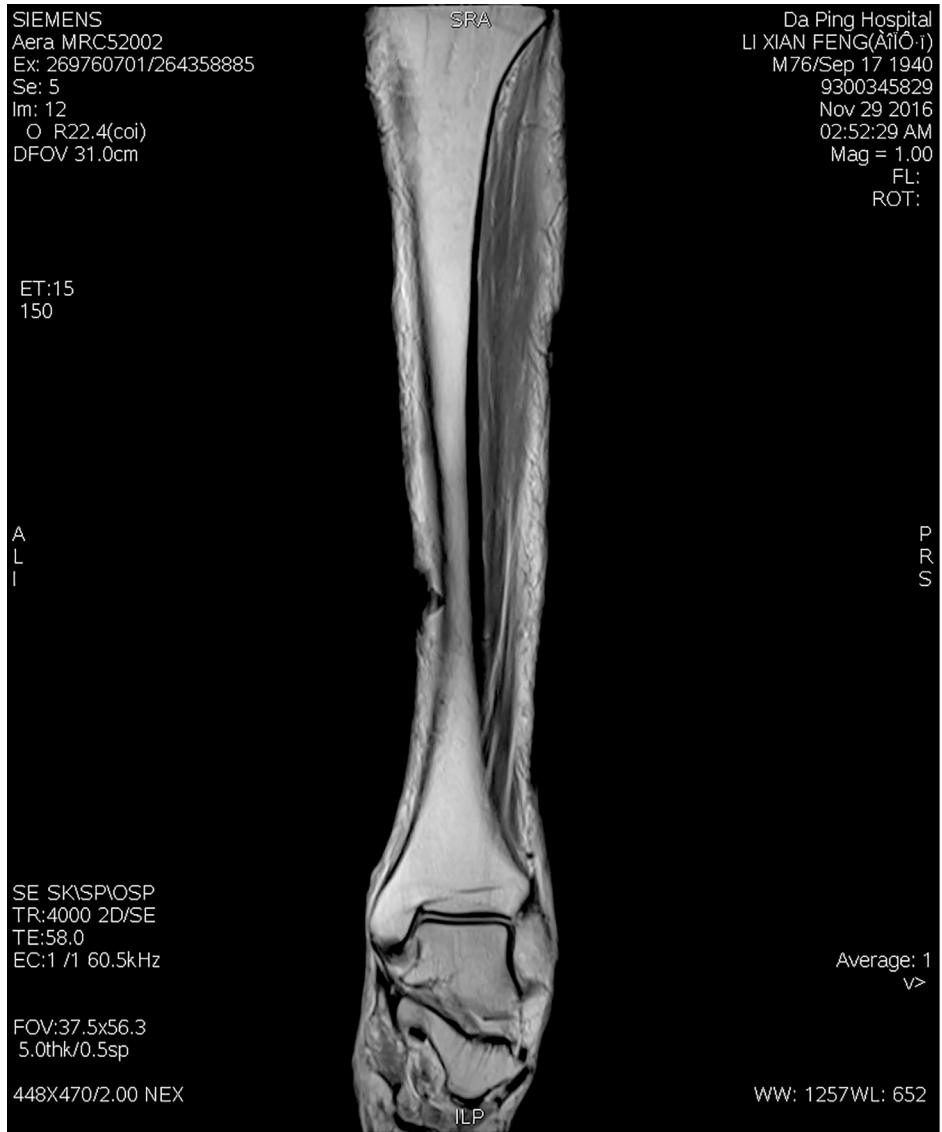


Fig. 2. MRI results of the right leg before admission. Image showed a large number of abnormal signals in subcutaneous fat layer and soft tissue, suggesting an inflammatory reaction (The white arrow refer to inflammatory lesions).

living environment of the patient. If the patient lives in rural areas, or in remote mountainous areas, the possibility of infection with this particular fungus can be considered in addition to conventional infections. In addition, the destructiveness of *C. cassiicola* has been attributed to the host-selective protein toxin of cassiicolin. Cassiicolin, a 27-residue O-glycosylated protein, is able to induce cellular damages.¹⁵

In our case, the diagnosis of phaeohyphomycosis was made by mycological culture, microscopic morphology, and confirmed by molecular techniques. Based on the characteristic microscopical and molecular features, the fungus was identified as *C. cassiicola*. During the diagnosis of this case, although the traditional identification methods of fungi, including fungal culture and microscopic examination, could not identify specific species, they could provide a general direction and provide a basis for ITS region sequencing. The technique of mass spectrometer for the identification of filamentous fungi is not mature enough, and the species of filamentous fungi in the protein library are few. Therefore, results can be

obtained quickly by ITS region sequencing. For molecular diagnosis, the sequencing of ITS regions has been used as the new standard for identification and classification of fungal because of highly conserved sequence in a species.¹⁶ Using this method, the isolated strain was identified as *C. cassiicola* after compared to the GenBank database using the basic local alignment search tool (BLAST) searches. Therefore, ITS region sequencing tests are essential to identify the species of fungal.

In summary, we present a case of subcutaneous phaeohyphomycosis caused by *C. cassiicola* with prominent tissue necrosis in a Chinese man. Systemic voriconazole and wound debridement were effective in managing such infection. The fungus was identified as *C. cassiicola* according to the characteristic phenotypes and molecular methods. This case also indicates that the use of ITS regions sequencing is a great progress compared with traditional phenotypic techniques. It proves a faster and more efficient way to identify the fungus in clinical infections.



Fig. 3. Colony morphology and microscopic morphology of *Corynespora cassiicola*. (A) Direct microscopic examination revealed septate and unbranched hyphae ($\times 100$). (B) Colonies of *C. cassiicola* isolate grown on sabouraud agar and sheep blood agar cultured at 28 °C (left) and 37 °C (right) for 5 days. (C) Micrograph of the isolate. Conidia were tetrads, but occasionally arranged in chains ($\times 100$).

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Ethics statement

The written informed consent has been obtained from the patients or their relatives/guardians. This work was already approved by the Ethics Committee of Daping Hospital (Approval date: 16/03/2021, number: 2021-36).

Declaration of competing interest

All authors have no competing interests.

Author contributions

Jia-Jun Zou, Jin Li and Shan-Shan Ma performed the laboratory measurements. All the authors participated in the experimental design and data analysis. Dao-Hong Zhou contributed largely to the conception and design of the manuscript; Jia-Jun Zou and Jin Li drafted the manuscript. All authors read and approved the final manuscript.

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