

Case Report

Subcutaneous phaeohyphomycosis due to *Cladophialophora bantiana*: a first case report in an immunocompetent patient in Latin America and a brief literature review

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Abstract

We report a rare case of subcutaneous phaeohyphomycosis caused by *Cladophialophora bantiana* in an immunocompetent patient in Amazonas, Brazil. This dematiaceous fungus has been mainly associated with life-threatening infections affecting the central nervous systems of immunosuppressed patients. We present the clinical, laboratory, and therapeutic aspects, and in vitro susceptibility test results for different antifungal drugs. A brief review of the cases reported in the literature over the past 20 years has also been discussed. According to the literature review, the present case is the first report of subcutaneous phaeohyphomycosis due to *C. bantiana* in an immunocompetent patient in Latin America.

Keywords: *Cladophialophora bantiana*. Subcutaneous phaeohyphomycosis. Antifungal susceptibility.

INTRODUCTION

Phaeohyphomycosis is a group of mycotic diseases caused by dematiaceous fungi; these diseases range from superficial to deep infections and affect both immunocompetent and immunocompromised patients. In clinical practice, this term is most commonly used for some sporadic mycoses with wide clinical and etiological diversity, thus excluding classic clinical entities such as chromomycosis, black-grain mycetoma, and tinea nigra. Underlying disease, treatment with immunosuppressive drugs, solid organ transplantation, and traumatic inoculation are among the main reported predisposing factors. Several fungal species have been isolated, including *Exophiala jeanselmei*, *E. spinifera*, *E. dermatitidis*, *Cladosporium* sp., *Cladophialophora* sp., *Curvularia* sp., and *Alternaria* sp.¹⁻³

In a recent review of the literature, we found few reports of subcutaneous phaeohyphomycosis caused by *C. bantiana*. The majority of cases associated with this fungal species were cerebral fungal infections usually causing single or multiple brain abscesses with rapid evolution and high mortality rates. *C. bantiana* has been highlighted as the main causative agent of phaeohyphomycosis of the central nervous system and is, therefore, considered a true neurotropic fungus. Extracerebral infectious conditions caused by this fungus, such as pulmonary infections, sinusitis, septic arthritis, and subcutaneous phaeohyphomycosis, are uncommon^{3,4}.

Subcutaneous phaeohyphomycosis has a diverse etiology with different clinical types and manifests as polymorphic lesions, including subcutaneous cysts, erythematous-squamous lesions, pustules, granulomatous crusts, cutaneous abscesses, and serosanguineous exudate, and other manifestations. The infection mostly occurs following traumatic inoculation of a saprobic fungus into the subcutaneous tissue. The phaeohyphomycotic cyst is the most recurrent reported form and the most common presentation is a solitary, asymptomatic, well-encapsulated subcutaneous mass. *Exophiala jeanselmei*

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and *E. dermatitidis* are among the most common isolated agents from this clinical form^{1,3,5-7}.

CASE REPORT

A 60-year-old male rural worker from Humaitá (Amazonas state, Brazil) presented with a cutaneous asymptomatic lesion in the distal third of his left forearm that had persisted for two months before the office visit. The patient was a diabetic and well-controlled hypertensive, with normal blood glucose and blood pressure levels due to the use of antidiabetic and antihypertensive drugs. The clinical examination revealed an encrusted, friable, erythematous, violaceous plaque with multiple orifices and purulent drainage (**Figure 1A**). Laboratory findings such as blood count, renal and hepatic function, and glycosylated hemoglobin levels were normal. HIV serology was negative. Nocardiosis and cutaneous tuberculosis were the initial diagnostic hypotheses. For laboratory confirmation, biopsy fragments from two different sites were collected using a punch biopsy instrument (4 mm). The samples obtained were submitted for histopathological and culture examination for bacteria and fungi.

Direct mycological examination with KOH (20%) revealed some dematiaceous, septate, and tortuous hyphae and isolated or paired dematiaceous yeast-like cells (**Figure 2A**). Histopathological examination showed the epidermis with hyperkeratosis, parakeratosis, fibrin-leukocyte crust, and irregular acanthosis. A pronounced inflammatory infiltrate consisting of lymphocytes, histiocytes, plasmocytes, polymorphonuclear neutrophils, and multinucleated giant cells was observed in the dermis. Septate and brownish hyphae were seen and small rounded, brownish structures were present in the interstice and inside of multinucleated giant cells. (**Figure 2B**). The fungal colony grew on Mycosel agar (DIFCO®) and Sabouraud dextrose agar (DIFCO®) with chloramphenicol (0,05 g/L). Based on its phenotypic features, the fungus was identified as *C. bantiana*. Macromorphological analysis on potato dextrose agar (PDA) revealed a colony with moderately fast growth, olive green color, velvet-like texture, and discreetly grooved topography (**Figure 2C**). Micromorphological analysis using the Riddell technique demonstrated dematiaceous septate hyphae, blastic-acropetal conidiogenesis with long chains of ellipsoidal conidia commonly developing directly from the primary hyphae or from short and poorly differentiated conidiophores, and frequent chains of conidia without branches or with only sparse ramifications (**Figure 2D**).

The species was confirmed by the amplification and sequencing of the rDNA intergenic spacer region (ITS/rDNA) (ribosomal DNA internal transcribed spacer region). For DNA extraction, a DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) was used according to the manufacturer's protocol after mechanical lysis of the cell wall by maceration of 100 mg of the frozen mycelium at -20°C. The target region was amplified using the ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) primer pair in a final volume of 25 µL containing buffer solution, 200 µM of dNTPs, 1.5 mM of MgCl₂, 0.3 µM of each primer, and 2.5 U

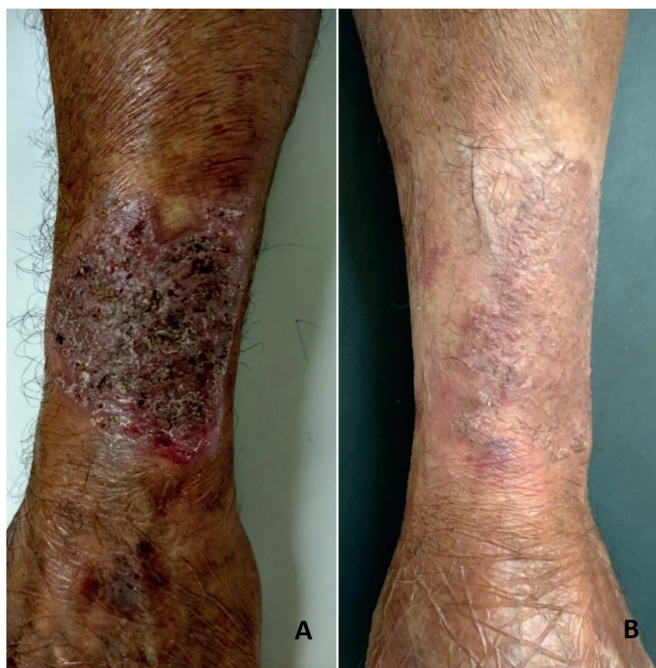


FIGURE 1: (A) Subcutaneous phaeohiphomycosis caused by *Cladophialophora bantiana*. Crusted plaque on the left forearm. (B) Lesion aspect after 10 months of treatment with itraconazole.

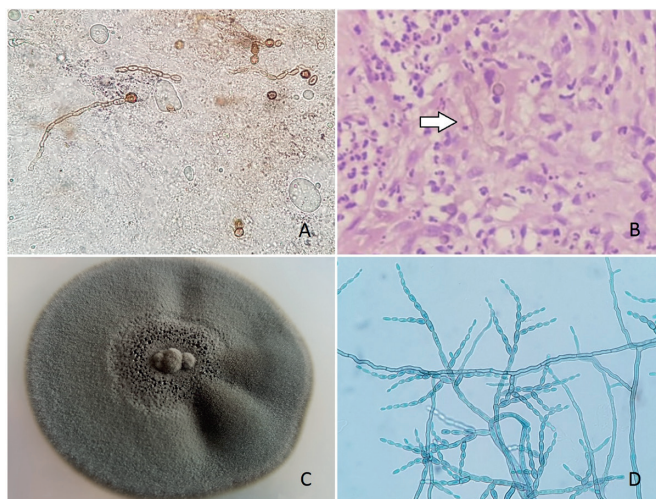


FIGURE 2: (A) KOH (20%) mount showing dematiaceous hyphae and yeast-like cells. (B) Histopathological examination revealing septate dematiaceous hyphae (400×). (C) Macromorphological features of *Cladophialophora bantiana* on PDA medium showing a velvety and olive green colony after 20 days growth. (D) Microscopic morphology of *C. bantiana* from a Riddell slide culture, showing the long chains of ellipsoidal conidia and poorly differentiated conidiophores (400×).

of Taq DNA polymerase. Polymerase chain reaction (PCR) was performed with an initial denaturation at 96°C for 5 min, followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were sequenced on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using a BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, USA) according to the manufacturer's guidelines.

TABLE 1: Summary of Cases of Subcutaneous Phaeohyphomycosis Caused by *C. bantiana*.

Reference	Country	Sex/Age	Clinical aspects	Immunological status	Treatment
Jacyk et al., 1997	-	M/63	Large tumorous mass on the right thigh over 3 months.	Immunosuppressed. Prolonged use of prednisone and cyclosporin.	ITZ for 3 months with complete healing of the lesion.
Lacaz, et al., 2002	Brazil	F/30	Ulcerogenic subcutaneous lesion.	Immunosuppressed due to renal transplant.	Surgical excision, remaining evident cicatricial process after one year.
Jain, Agrawal & Jain, 2003	India	F/45	Small, painless, stellate lesion on the face. Pyogranulomatous foci and low inflammation. Encapsulated cyst.	Immunocompetent.	Corticosteroid and amphotericin B. After 3 months the patient presented scraping negative for fungi.
Arnoldo, et al., 2005; Hussey et al., 2005; Werlinger & Yen Moore, 2005	USA	F/32	Erythematous hardened plaques and floating nodules with purulent drainage. Crust formation on the back.	Immunosuppressed due to treatment of SLE and RA during pregnancy.	FCZ and ITZ with no response. Surgical treatment was adopted with grafting and ITZ for 8 months. Healing was achieved.
Petrini, et al., 2006	Thailand	M/F couple	Purulent infections on the legs following tsunami-associated injuries and subsequently skin grafts after multiple surgical revision.	Local immunosuppression. Concomitant infections by <i>C. bantiana</i> and <i>Mycobacterium abscessus</i> .	Voriconazole for one month, clarithromycin and amikacin. Skin biopsies after this period were negative for fungi.
Neoh et al., 2007	China	F/56	Erythematous plaque on the face with 4 years of evolution after trauma with a branch.	Immunocompetent.	3 months of persistence and recurrence after electrocautery, use of ITZ and surgical excision. Cure after terbinafine treatment for 5 months.
Pincus et al., 2009	USA	M/26	Erythema, edema, and tenderness around the scar on the right arm following a motor vehicle accident in Puerto Rico.	Local immunosuppression after four months of serial intralesional corticosteroid injections.	Incision, drainage and systemic antifungal therapy with ITZ (ended due to intolerance), then oral voriconazole for 4 months.
Schoeffler et al., 2011	France	M/48	Single, painless and non-suppurative nodular lesion on the right, after a trip to Vietnam.	Local immunosuppression due to corticosteroid injections in the cicatricial lesions.	Surgical excision and no recurrence.
Khader, et al., 2015	India	F/38	Granuloma-like nodules (lower limbs), dermatophytosis-like plaques (foot), and subcutaneous cysts (hand).	Immunosuppressed due to prolonged use of corticosteroids and cyclosporine.	ITZ for 6 months. Complete resolution of all lesions.
Patel, Kapadiya, & Shah, 2016	India	F/50	Non-healing wound with blackening of skin at local site after abdominal hernia repair surgery.	Immunosuppressed due to uncontrolled diabetes mellitus.	Voriconazole for 6 weeks, terbinafine for 14 days and amphotericin-B. Complete wound healing.

FCZ: Fluconazole; **ITZ:** Itraconazole; **SLE:** Systemic Lupus Erythematosus; **RA:** Rheumatoid Arthritis.

The DNA sequence chromatogram files were analyzed using Phred and BioEdit software (quality ≥ 20) and the nucleotide sequence was added to the National Center for Biotechnology Information (NCBI) GenBank (accession number MH445303). The nucleotide sequence was compared to those deposited in the database using the BLASTn tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>); the sequence had a 99% identity with more than 20 *C. bantiana* isolates (strains LT837815.1, KY432483.1, KP131825.1, and AF131079.1, among others). In addition, we performed phylogenetic analysis based on the ITS sequences and constructed a phylogenetic tree using MEGAX software with 100 bootstrap replicates to show the relationship between our strain and other strains of *C. bantiana* (data not shown).

In vitro antifungal susceptibility tests were performed according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 guidelines. The minimum inhibitory

concentrations (MICs) of posaconazole, itraconazole, amphotericin B, terbinafine, and fluconazole were 0.25, 2, 4, 8, and >16 $\mu\text{g/mL}$, respectively. Initially, the patient was administered fluconazole without clinical response after 30 days of treatment. Currently, the patient is under treatment with itraconazole 200 mg twice daily with lesion regression but without complete healing after 10 months (**Figure 1B**). The patient remains on antifungal treatment and cryotherapy was recently performed.

DISCUSSION

To our knowledge, this report describes the first case of *C. bantiana* diagnosed in an immunocompetent patient in Latin America. The patient was a well-controlled diabetic with normal laboratory findings and no evidence of an immunocompromised status.

There are few reports of subcutaneous phaeohyphomycosis caused by *C. bantiana* and many questions about its eco-epidemiology and pathogenesis lack elucidation. Despite this, we can observe some common features in most cases reported so far. We found 12 reports published between 1987 and 2018; however, three described the same patient in Texas, USA⁸⁻¹⁰.

Therefore, 10 cases reported involvement in adults of all age groups, including seven women and four men; one of the case reports involved a Swedish man and woman that sustained injuries during a tsunami catastrophe in Thailand¹⁵. Most of the cases were immunosuppressed due to underlying diseases and prolonged use of corticosteroids. Only two cases occurred in immunocompetent patients; both were female and were affected on the face. In the first case, the patient possibly contracted the infection through the continuous use of cosmetics in a beauty parlor (attributed to her sales profession) that caused microscopic lesions on her face, favoring the inoculation of the fungus⁵. In the second case, the transmission was via the conventional route, in which the patient suffered traumatic inoculation on the face due to an accident involving a twig⁷. **Table 1** summarizes these cases.

The natural habitat of *C. bantiana* is unknown, although most reports refer to traumatic inoculation of the fungus due to accidents with material contaminated with soil or plants, as in the present case. According to the cases listed in **Table 1**, the limbs and trunk are the most affected anatomical sites.

Three similar cases were reported with probable implantation of fungi after traumatic accidents. In the first case, the active infection occurred 16 years after the injury due to the establishment of systemic immunosuppression caused by systemic lupus erythematosus and prednisone therapy⁸⁻¹⁰. The other cases occurred after corticosteroid injections that caused local immunosuppression¹¹⁻¹³. The authors suggest possible fungal latency for years and the reactivation of infection after the development of an immunosuppressive state.

We observed different clinical aspects, such as erythematous plaques and nodular and ulcerogenic lesions, from those reported in most cases by other agents of subcutaneous phaeohyphomycosis, which are usually a solitary cystic lesion or an encapsulated tumor mass. Khader, et al. (2015) reported a case of an immunosuppressed patient who presented three types of lesions with distinct morphological patterns: pyogenic granuloma-like nodules in the lower limbs, dermatophytosis-like plaques in the foot, and subcutaneous cyst in the hand¹⁴.

Concomitant late tissue infections by *C. bantiana* and *Mycobacterium abscessus* were reported by Petrini, et al. (2006), following tsunami-associated injuries and subsequent partial-thickness skin grafts after multiple surgical revisions¹⁵.

This report describes a case of subcutaneous phaeohyphomycosis caused by the unusual *C. bantiana* fungus. The evidence pointed to traumatic implantation of the fungus into the patient's skin due to his occupation as a rural worker. Unlike most reported cases, no significant immunosuppressive factor was evidenced in this case. However, he remains without cure after 10 months of treatment with itraconazole. While the optimal therapy has not yet been established, itraconazole is the drug of choice in the dose range of 200–600 mg per day.

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Conflict of interest

The authors declare that there is no conflict of interest.

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