First Probable Case of Subcutaneous Infection Due to *Truncatella angustata*: a New Fungal Pathogen of Humans?

Tomasz Jagielski, a Iwona Żak, b Jerzy Tyrak, c Agata Bryk d

Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland; a Department of Clinical Microbiology, Children’s University Hospital of Cracow, Cracow, Poland; b Anaesthesiology and Intensive Care Unit no. 1, The University Hospital in Cracow, Cracow, Poland; c The University Hospital in Cracow, Cracow, Poland

*Truncatella angustata* is a coelomycetous fungus, typically associated with vascular plants as either an endophyte or a pathogen. This organism has not previously been implicated in human disease. This report describes a case of *T. angustata* subcutaneous infection in an immunocompetent patient. A conclusive diagnosis was achieved through partial sequencing of ribosomal DNA (rDNA) cluster. The patient was successfully treated with voriconazole followed by itraconazole.

CASE REPORT

In March 2011, a 50-year-old woman, a practicing pediatrician, was examined in the Department of Allergy and Clinical Immunology, University Hospital in Cracow. At that time, she presented with disseminated skin lesions on her right leg. The most pronounced lesion was on the medial portion of the calf and consisted of a poorly defined area of redness and swelling, with blistering and seropurulent drainage (Fig. 1A). Adjacent to the main lesion, small (ca. 2- to 3-mm diameter) and occasionally confluent erythematous papules (satellite lesions) could be observed. Similar erythematopapular eruptions were scattered over the entire thigh up to the hip line. The primary calf lesion had developed 6 months earlier (September 2010) after a cut with a rotten deck board on the porch of the lodge in the forest, while on her summer holidays in Connecticut, USA. The wound was washed with saline, disinfected with octenidine hydrochloride solution (Ocetenisep), and covered with a sterile dressing and protective bandage. As the wound did not heal for several days, the woman, who had already returned to Poland, sought dermatological and surgical consultation. She was advised topical treatment with ethacridine lactate (Rivanol) compresses, bacitracin ointment, Granuflex Extra Thin hydrocolloid dressings, and activated charcoal impregnated with metallic silver dressings, all but the latter producing no improvement in the condition of the patient. It was only when the activated carbon and silver dressing had been applied to a wound that it began to dry and finally closed (December 2010). Soon after that, small, itchy erythematous papules emerged, at first in the vicinity of the primary lesion only but later spreading over the entire limb. During the next several weeks, the woman received different treatments, as prescribed by a dermatologist, based largely on topical glucocorticosteroids, including betamethasone dipropionate with gentamicin (Diprogenta) or with gentamicin and clotrimazole (Triderm). Not only did they not alleviate the lesions, but they resulted in further deterioration of the condition. The papules appeared in greater numbers on the calf and extended to the abdomen and limbs. Moreover, the itching increased noticeably, and methylprednisolone was administered orally (8 mg once a day [q.d.], along with antihistamines ( cetirizine at 10 mg q.d.), and yet with only slight improvement (January and February 2011). Shortly after this episode, the patient was admitted and examined in our clinic. (Written informed consent was obtained from the patient for publication of this case report and accompanying images.) Here, investigation for any systemic disease was performed. However, no systemic involvement was observed. The patient was in general good health and apyretic, reporting no symptoms of weight loss, fatigue, or night sweats. The lungs were clear, and the routine biochemical test results, including those for blood and urine chemistry and liver and renal function profiles, were within normal limits. After the examination and laboratory evaluations, the condition was thought to represent either mycobacterial or fungal infection. Consequently, a surgical biopsy of subcutaneous tissue, down to the fascia, was carried out, and the biopsy specimens were sent for histopathology and microbiology assessment. Tissue sections stained with hematoxylin and eosin revealed nonvasculitic panniculitis with acute inflammation and fat necrosis. Neither bacterial nor fungal pathogens were found. Interestingly, direct microscopy of 10% KOH wet-mount smears of the biopsy material showed hyaline, septate, and branched hyphae with no associated spores. Both Ziehl-Neelsen staining and culture, in an automated radiometric system (Bactec 460 TB), were negative for acid-fast bacilli, thus excluding tubercular etiology of the lesion. Mycological culture was performed on Sabouraud dextrose agar (SDA) either with chloramphenicol or with chloramphenicol and cycloheximide, and the culture was incubated at 25°C and 37°C. After 5 days, greyish-white colonies of a mold could be observed on SDA at 25°C (Fig. 1C); there was no growth at 37°C or in the presence of cycloheximide. A wet preparation stained with lactophenol cotton blue (LCB) again revealed septate fungal elements without conidia. For diag-
nostic purposes, a subculture was made on potato dextrose agar (PDA) to induce sporulation. Subcultures were incubated at 25°C for a total of 4 weeks and checked periodically (once a week) for the formation of conidia or other generative structures. Conidia started to be visible after 2 weeks but were best demonstrated after 4 weeks of incubation. They were spindle shaped and 4 celled, with median cells medium to dark brown and apical and basal cells subhyaline (Fig. 1D). However, the fungus could not be identified by its colonial and microscopic features. Therefore, a subculture of the isolate was sent to the Department of Applied Microbiology, University of Warsaw, for specific identification. This was achieved by molecular typing, which involved extraction of chromosomal DNA from the fungal mycelium (1), PCR amplification of internal transcribed spacer (ITS) regions ITS1 and -2 (ITS1/2) along with the D1/D2 variable domains of the 28S rRNA gene, and subsequent sequencing of those loci in both forward and reverse directions, essentially as described elsewhere (2). The obtained sequences were aligned against the GenBank database using the BLAST algorithm (www.ncbi.nlm.nih.gov/blast/Blast.cgi). The cutoff for species delineation was set at 99% sequence similarity. The 616-bp ITS1/2 sequence showed a perfect match with a corresponding sequence of *Truncatella angustata* strain G26 (GU566260.1), whereas the 607-bp D1/D2 sequence showed 99% identity to *T. angustata* strain ICMP7062 (AF382383.1). The results of the molecular species identification were further compared with the morphology description hitherto collected and were reassessed by means of electron microscopy. The scanning electron micrographs of a 2-week-old slide culture of the isolate, grown on PDA medium at 25°C, demonstrated, concordantly with previous observations, fusiform, smooth-walled conidia, ca. 14 by 5 μm in size, with three transverse septa, and apical cells significantly smaller than the middle ones. Occasionally, conidia possessed hyaline, single, double, or multiple long appendages, at the apex only (Fig. 1E). The morphology of the isolate fully corresponded to the description of *T. angustata* species (3).

By the time the definitive diagnosis was established, the susceptibility of the fungus to a panel of conventional antifungal drugs had been determined *in vitro* by means of the Etest method, as described by the manufacturer (bioMérieux, France). An important modification was that the drug susceptibility profiling was performed at 25°C instead of 37°C, as in the original protocol. This was because the isolate grew only at room temperature. The MIC values for the isolate were as follows: amphotericin B, 0.012 μg/ml; 5-fluorocytosine, 32 μg/ml; fluconazole, 256 μg/ml; itraconazole, 32 μg/ml; posaconazole, 32 μg/ml; ketoconazole, 8 μg/ml; voriconazole, 0.012 μg/ml; anidulafungin, 32 μg/ml; caspofungin, 3 μg/ml; and micafungin, 32 μg/ml. Given the very low MICs of amphotericin B and voriconazole, the isolate was assumed to be susceptible to these drugs. The patient was thereupon started on voriconazole at 200 mg administered intravenously once daily for 2 weeks and, after that, orally (200 mg twice daily [b.i.d.]) for 5 months. The patient was then continued with oral itraconazole treatment (100 mg b.i.d.) for a period of 3 months, followed by another 3 months of itraconazole given as 1-week pulse therapy per month (100 mg b.i.d.) (Fig. 1B). Starting from the third week of voriconazole therapy (June 2011), the skin lesions commenced to resolve gradually. Complete resolution was noted 3 months later. In the course of treatment, no adverse effects were observed except for the slight phototoxic dermatitis of the facial skin in August 2011. Throughout the treatment, the liver and kidney functions were monitored on a regular basis, showing no abnormalities. The follow-up biopsy specimen, taken 8 months after the initiation of treatment (March 2012), demonstrated complete resolution of the skin lesions. A conclusive result was obtained from the culture of the biopsy specimen with any fungal growth.

![FIG 1](A and B) Clinical aspect of involved leg before treatment (A) and after 11-month therapy with voriconazole and itraconazole (B). (C) Culture of *Truncatella angustata* on Sabouraud’s dextrose agar (25°C, 4 weeks). (D) Micromorphology of mycelium and conidia; lactophenol cotton blue stain (magnification, ×400). (E) Scanning electron microscopy (magnification, ×4,000; bar, 2 μm).
Pestalotiopsis have been implicated in human disease (13, 14). It is tioid fungi and other as-
olive (14–18). In severely or moderately immunocompromised patients
fusiform, and appendage-bearing conidia. Members of the pestalotioid genera have a wide geographic distribution and are commonly associated with plants, either as endophytes or as pathogens. This is particularly evident in the Truncatella genus, largely restricted to an endophytic or parasitic lifestyle, with the ability to colonize plant tissues without causing any harm to the host or to incite a variety of diseases in a wide array of plant spe-
cies, respectively. The most prominent representative of 21 spe-
cies-containing genera (http://www.indexfungorum.org/names
amorphous fungi in the ascomycetous family Amphisphaeriaceae (Xylariales), characterized by having
fungi that would impair the immunological function of the patient. Although this somewhat contrasts with the majority of the coelomycete-induced infections, favored by local or systemic immuno-
suppression, infections by these fungi have also been de-
scribed in healthy individuals (15, 19). Noteworthy, in this case, is
affected by epidemiology, a source of plant-associated fungi). Interest-
ing the origin of plant or soil. The inoculation of T. angustata most probably occurred through a cut from a rotten wooden board on the porch. (The whole porch deck was in a poor condition, with many broken or deteriorated boards covered with decaying plant material, thus being an excellent source of plant-associated fungi).

The identification of Coelomyces may be somewhat challen-
ging as they are beyond the repertoire of fungi routinely encoun-
tered in clinical samples. Most of the coelomycetous fungi are
quite easily recovered from tissues and usually grow readily on
standard mycological media. However, due to their environ-
ment-dependent plasticity and pleomorphism, proper identification by
using morphological criteria, especially in culture, may pose a
serious difficulty. This difficulty is even further compounded by
the inability of some of the strains to amply produce reproductive
structures (conidiomata and conidia), which are of utmost diag-
nostic value. To induce sporulation, enriched media, such as PDA,
as in this study, need to be employed. But even if the diagnostic
structures are present, their morphological variability or subtle intetaxon differences often preclude an accurate identification.

Therefore, molecular typing is a suitable alternative. Diagnosti-
cally, the most powerful approach seems to be sequencing of ei-
er ITS or D1/D2 regions within the rDNA cluster. In this study,
the diagnosis was optimized by combining clinical history, culture
findings, histopathology, and molecular analysis based on seq-
encing of both ITS and D1/D2 loci. Whereas anamnesis was
merely suggestive of infection by a plant- or wood-associated fun-
gus, histopathology revealed a nonspecific inflammatory process,
with fungal cells probably missed or misinterpreted as artifacts,
culture-based microscopy allowed determination of the mor-
phology of the fungus but failed to allocate it to any species or
genus; only sequencing analysis yielded a definitive diagnosis.

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Case Report

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There is no consensus therapeutic strategy against coelomyceteous fungi. This is partly because of the paucity of data on the efficacy of conventional antifungal drugs in both in vitro and in vivo studies. The methodology of the in vitro susceptibility testing has not been standardized, and no interpretative breakpoints have been established. Furthermore, discrepancies between in vitro activity and clinical response have commonly been observed (14). Consequently, treatment is empirically developed and the in vitro data provide guidance rather than rigid instruction when formulating drug regimens. In this case, the clinical outcome did correspond to the drug susceptibility pattern of the fungus isolated. Of the two drugs (amphotericin B and voriconazole) which showed the lowest MICs (0.012 μg/ml), voriconazole was used with apparent efficacy. Amphotericin B was refused by the patient because of the potential risk of nephrotoxicity. Although the lesions resolved completely after 3 months of voriconazole therapy, the drug was continued for further 2 months and was then replaced by itraconazole, given for 6 months in total, to prevent relapse.

In conclusion, this is the first report on Truncatella species associated with human disease. This case illustrates the expanding spectrum of coelomyceteous fungi as potential human pathogens, even when no underlying condition is present. The difficulty in the identification of the fungus, based on morphological criteria, highlights the importance of molecular methods for an unambiguous diagnosis.

**Nucleotide sequence accession numbers.** The 616-bp ITSI/2 sequence was deposited in GenBank under accession no. KC241879.1. The 607-bp D1/D2 sequence was deposited in GenBank under accession no. KC241880.1.

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**REFERENCES**


