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Cutaneous Phaeohyphomycosis caused by *Paraconiothyrium cyclothyrioides*

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Abstract

Paraconiothyrium cyclothyrioides is a recently-described coelomycetous fungal species. We present a case in a renal transplant patient with chronic skin lesions of the lower extremities caused by *P. cyclothyrioides*. Treatment with posaconazole led to complete resolution of the lesions. *P. cyclothyrioides* should be considered as an opportunistic human pathogen in immunocompromised patients.

Case Report

A 49-year-old Latin American man with end stage renal disease and cadaveric renal transplant (two years prior to the current admission) was hospitalized with cutaneous lesions in his lower extremities. The patient had history of type II diabetes, hypertension and atrial fibrillation. One and half years previous to admission, the patient received thymoglobulin for acute T-cell rejection with improvement of the episode. Approximately 6 months before current admission, the patient first noticed a painless, non-pruritic, mildly tender scaly papule on the left tibia just inferior to the knee joint and applied a local adhesive band to prevent friction injury. Over the next few months, other lesions appeared on both tibial surfaces, which then began to coalesce into violaceous, necrotic, plaque-like lesions with some areas of ulceration and crusting and mild self-limiting sanguineous discharge without accompanying fevers. The patient lives in Brownsville, TX , works in an office as a manager and denied any travel outside Texas, trauma, animal or insect bites. Patient's hobby included performing mechanical work on car engines. Medications included leflunomide, prednisone, tacrolimus, doxazosin, clonidine, carvedilol, minoxidil, amlodipine, trimethoprim-sulfamethoxazole, insulin, famotidine, gemfibrozil and pravastatin. Complete blood count was notable for platelet count of 73 and creatinine of 2.4

mg/dL. On physical exam he was afebrile and vital signs were stable. Cardiac exam evidenced an irregular rate and a II/VI murmur loudest in the apex. The lungs were clear and the rest of the examination was unremarkable except for the lower extremities. He had a crusted, ulcerated plaque on the anteromedial aspect of the left knee (measuring 6 x 3 cm), and several similar-appearing lesions of smaller size on the same area (**Figure 1A**). The patient was started on vancomycin (1.25 gm every 24 h), radiographs of the knee did not reveal any bone abnormality. A skin biopsy yielded a presumptive diagnosis of pyoderma gangrenosum, antibiotics were stopped and the patient was discharged with wound care instructions and silver sulfadiazine.

Two months prior to the current admission a fungal culture from the skin tissue, was positive for a single mold which grew on Sab Dex agar but was not identified at the time. Oral voriconazole (200 mg orally every 12 h) was initiated. A month later after his discharge, he presented to the dermatology clinic with evidence of progression of his skin lesions and was again admitted to the hospital. He denied new recent trauma, travel history and reported compliance with voriconazole. New punch biopsies revealed pseudo-epitheliomatous hyperplasia, suppurative granulomas and fungal elements were seen invading the dermis (**Figure 1B**). Cultures yielded a mold (only one colony type) with the same morphology of the previous culture in Sab Dex agar, that was subsequently identified as *Paraconiothyrium cyclothyrioides* (see below). The patient was started on amphotericin B lipid complex, 400 mg IV every 24 h and micafungin 100 mg IV every 24 h. Due to the elevation in the creatinine level, amphotericin B was stopped and posaconazole (400 mg orally every 12 h with meals) was initiated. Micafungin was discontinued. The skin lesions markedly improved 10 days after initiation of posaconazole therapy. Follow up at three weeks after hospital discharge showed complete resolution of the skin lesions with only

some remaining scaling of the skin in the area. Posaconazole was continued for an additional four weeks.

Extensive morphological and molecular characterization of the isolate was required to obtain a definitive identification. Accessioned into the Fungus Testing Laboratory culture collection as UTHSC 11-2294, the isolate was subcultured onto potato flakes agar (PFA), V-8 agar, and carnation leaf agar (CLA), all prepared in-house, at 25°C. A photomicrograph of the macroscopic morphology on PFA after 6 days incubation at 30°C shows a woolly, olivaceous-gray colony with a white periphery (**Figure 2A**). A brown diffusing pigment developed after three weeks incubation at 25 ˚C . Temperature studies on PFA indicated optimal growth at 27°C and minimal growth (1+) at 35°C. After 4 weeks incubation on CLA at 25°C thick-walled, submerged, reddish-brown conidiomata were present on CLA (**Figure 2B**). The hyaline, discrete or integrated, phialidic conidiogenous cells (**Figure 2C**) producing brown, cylindrical, 1-celled conidia measuring approximately 1.5 x 3-4 µm (**Figure 2D**) were suggestive of taxa known in the pycnidial coelomycete genera *Microsphaeropsis/Paraconiothyrium/Coniothyrium*. In order to accurately identify the isolate, molecular characterization by DNA sequencing was performed and the sequence was deposited under accession number R-4779 at University of Texas Health Science Center Fungus Testing laboratory culture collection. Briefly, DNA was recovered from a 24 hour culture grown on potato dextrose agar (Difco, Detroit, MI) and amplified by PCR using the ITS1 and NL4 primers as previously described (2). The amplicon was sequenced in the UTHSCSA Advanced Nucleic Acids Core facility to yield ITS and D1/D2 sequences (2). A BLASTn search of GenBank was performed using the ITS sequence and cutoff values of ≥97% identity at a query length ≥90% were used for consideration of conspecificity. The results of this

search identified *Microsphaeropsis arundinis* (accession # JN851034.1; 99% identity over 551 nucleotides), *Bipolaris sorokiniana* (accession# DQ337383.1 99% identity over 541 nucleotides) and *Paraconiothyrium cyclothyrioides* (accession# AY642529.1, 99% identity over 505 nucleotides). The D1/D2 sequence was not informative because there is no Genbank deposit for *Paraconiothyrium cyclothyrioides.* As ITS and D1/D2 sequencing failed to discriminate between *P. cyclothyrioides* and *M. arundinis,* both of which showed 99% nucleotide identity to the case isolate, our sequence data was forwarded to the Centraalbureau voor Schimmelcultures (CBS) for further analysis by G.J.M. Verkley and J.B. Stielow and subsequent comparison with their nucleotide sequence database of known isolates (3). With additional sequencing of γ-actin and β-tubulin loci (primers ACT512f/ACT783r and Btub4rd/Btub4fd , respectively) (3), the sequence identity of R-4779 with the type strain of *P. cyclothyrioides* (CBS 972.95) was 100%, 97%, and 99.1% for ITS, actin, and β-tubulin, respectively; suggesting that the case isolate is *P. cyclothyrioides*. The ITS and D1/D2 sequences were deposited in GenBank under accession numbers JQ681303 and JQ681304, respectively. The case isolate has also been deposited in the University of Alberta Microfungus Collection and Herbarium under the accession number UAMH 11641.

Coelomycetous fungi are common, anamorphic (asexual), plant and soil organisms that produce conidia within some type of -closed (pycnidial) or semi--closed (acervular) structure.
The coelomycete genus *Paraconiothyrium* was proposed to accommodate species resembling those in *Microsphaeropsis* and *Coniothyrium* that do not match species in these genera morphologically and phylogenetically (2). Due to the variability and plasticity in morphological characters, especially in culture, it is difficult to distinguish members of these genera

morphologically, but generally *Coniothyrium* and *Microsphaeropsis* mostly include species with true pycnidia (conidiomata with an opening) while most species of *Paraconiothyrium* form more complex conidiomata lacking a distinct ostiole. *Coniothyrium* spp. exhibit annellidic conidiogenesis, while those of the other two genera mostly show phialidic conidiogenesis, sometimes with percurrent proliferations. The species thus far included in *Paraconiothyrium* were formally described based on their anamorph or asexual reproductive stage in vitro and currently include *P. cyclothyrioides,* first isolated from a soil sample collected in Papua New Guinea in 1995*, P. estuarinum, P. brasiliense* and *P. fungicola* (3). Molecular phylogenetic data places all three genera within the order Pleosporales and resolves them as three distinct clades (3).

To the best of our knowledge, this is the second reported case of infection with *Paraconiothyrium cyclothyrioides*. We present unequivocal evidence that the organism was the pathogen in this case since it invaded the skin of the patient and lesions resolved with appropriate antifungal therapy. Balajee et al. (1) described a case in which a three-year-old child presented with crusted nodules on the lower extremity one year after receiving a liver transplant. ITS sequencing and comparison with a large coelomycete database at the United States Department of Agriculture (accessible at http://nt.ars-grin.gov/sbmlweb/fungi/index.cfm) identified the isolate as *P. cyclothyrioides*. Localization of the lesions (lower extremities) was very similar to our case but no information was provided in terms of the clinical evolution or treatment of the infection (1). Interestingly, a fungus isolated from a skin lesion of a 56 year old immunocompromised patient was recently identified as *Paraconiothyrium maculicutis*, (4) a

close relative of *P. cyclothyrioides*, supporting the fact that *Paraconiothyrium* spp. are potential human pathogens.

Our case highlights the importance of obtaining tissue samples for culture when immunocompromised patients present with chronic indolent skin lesions since the differential diagnosis is broad and includes fungi, mycobacteria and *Nocardia* spp., among others. In fact, several biopsies were taken during the course of this infection which increased the likelihood of isolating the etiologic agent. In our case, although susceptibilities were not performed , the clinical course of our patient suggest that *P. cyclothyrioides* has important differences in susceptibility to azole antifungals; indeed, our patient did not respond to voriconazole but showed a remarkable improvement when switched to posaconazole with resolution of the lesions. We were unable to evaluate the response to amphotericin B since this compound had to be stopped due to renal toxicity.

In summary, the rare soil fungus *P. cyclothyrioides* has pathogenic potential in chronic, crusted and ulcerated skin lesions in transplant patients and should be considered in the differential diagnosis of invasive fungal infections in immunocompromised patients. The most accurate identification among *Coniothyrium* like fungi and allied genera can be achieved by β-tubulin sequencing using the suggested primer pair. Posaconozale appears to be effective in treating these infections.

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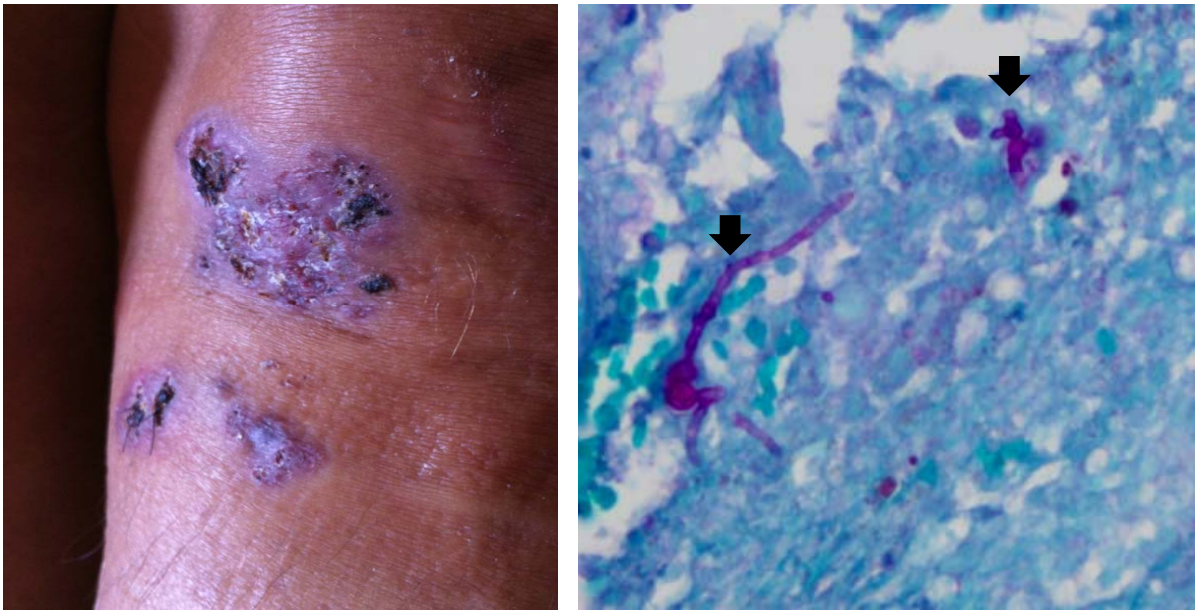
Legends to Figures

Figure 1. **Panel A**, Crusted, ulcerated skin lesions of the left knee; **Panel B**, Periodic acid-schiff (PAS) stain showing fungal elements (hyphae, black arrows) invading the dermis (60 X magnification).

Figure 2. **Macroscopic and microscopic features (lactophenol cotton blue mounting medium) of case isolate of *Paraconiothyrium cyclothyrioides*. Panel A,** Colony after 6 days incubation at 30°C on potato flakes agar; **Panel B,** thick-walled conidioma showing conidia that are reddish-brown, in mass, within the cavity; **Panel C,** Phialidic conidiogenous cells protruding into the cavity of the conidioma; **Panel D,** Small, 1-celled, brownish cylindrical conidia.



A **B**

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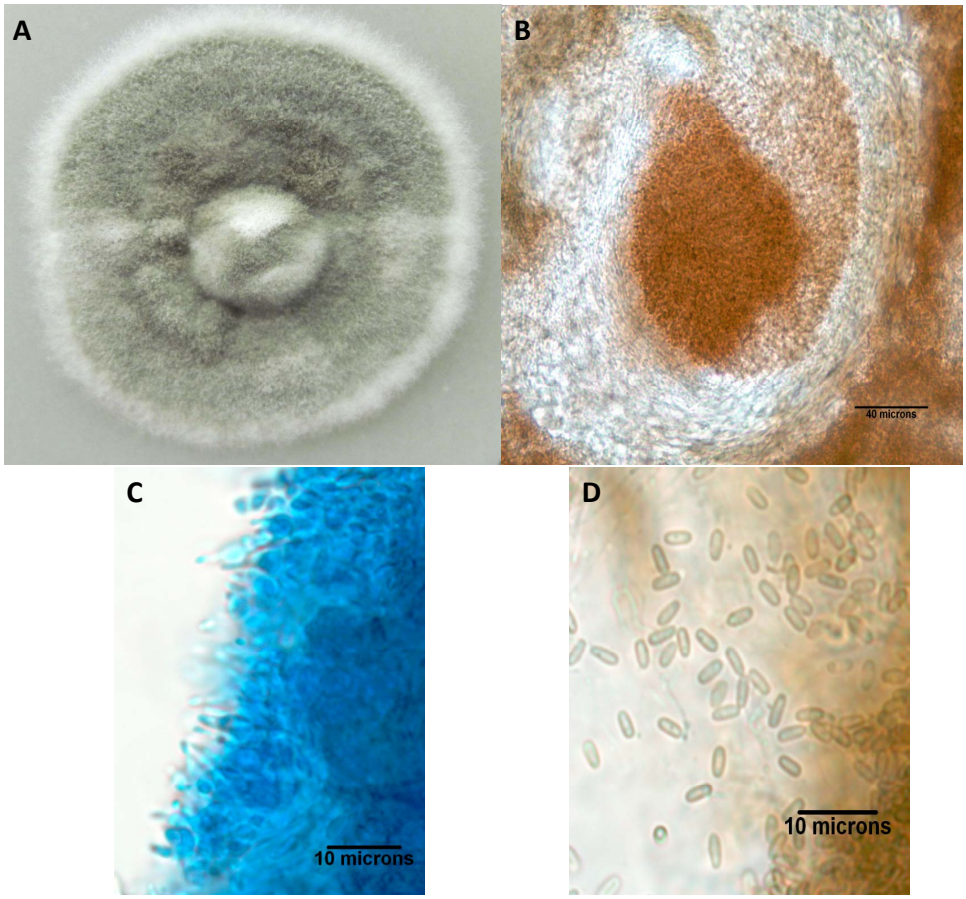

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