Case Report

Fungal arthritis secondary to *Colletotrichum gloeosporioides*

Jonathan C. Cho,¹ Rajendra S. Sharma,² Deanna A. Sutton,³
Nathan P. Wiederhold,⁴ Carmita Sanders,³ Brian L. Wickes⁵
and Sandy J. Estrada¹

Correspondence

Sandy Estrada
sandy.estrada@leememorial.org

¹Pharmacy Department, Lee Memorial Health System, Fort Myers, FL, USA

²Lee Physician Group – Infectious Diseases, Lee Memorial Health System, Fort Myers, FL, USA

³Department of Pathology, University of Texas Health Science Center, San Antonio, TX, USA

⁴Departments of Pathology & Medicine/Infectious Diseases, University of Texas Health Science Center, San Antonio, TX, USA

⁵Department of Microbiology and Immunology, University of Texas Health Science Center, San Antonio, TX, USA

Introduction: *Colletotrichum* spp. are common plant-pathogenic fungi that usually occur as asymptomatic endophytes on aerial organs of host plants. Of the many *Colletotrichum* spp. described, only four have been associated with infections in humans, one of these being *Colletotrichum gloeosporioides*.

Case presentation: Infections cited in humans due to *Colletotrichum* spp. typically involve the eye (keratitis/keratomycosis/endophthalmitis) or are subcutaneous in nature and secondary to trauma. We report a clinical case of septic arthritis caused by *Colletotrichum gloeosporioides* in an immunocompetent 55-year-old male. DNA sequencing was performed for identification of the organism. Antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute M38-A2 standard for broth microdilution testing against filamentous fungi.

Conclusion: To the best of our knowledge, this is the first case of a *Colletotrichum* sp., specifically *Colletotrichum gloeosporioides*, causing septic arthritis in humans. This report highlights the successful treatment of a case of septic arthritis due to *Colletotrichum gloeosporioides* with voriconazole.

Keywords: arthritis; *Colletotrichum gloeosporioides*; fungal; voriconazole.

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Introduction

Coelomycete genera (forming their conidia within an enclosed or semi-enclosed asexual structure referred to as a conidioma), were originally described by the same system that applied to the anamorphic system of classification for the Hyphomycetes (those fungi bearing their conidia free to the air, such as *Aspergillus* spp.). Several genera of coelomycetes produce their conidia within a more enclosed structure known as a pycnidium (order *Sphaeropsidales*);

however as *Colletotrichum* spp. produce their conidia within a semi-enclosed cup-like structure known as an acervulus, they were placed in the order *Melanconiales*, form-family *Melanconiaceae*. With the advent of molecular characterization, this classification system is now obsolete; however, there is still good correlation with these classical features and molecular sequence data. Most species names, including *Colletotrichum gloeosporioides*, now reside within a particular phylogenetic 'clade' and represent a species complex (Weir *et al.*, 2012).

Colletotrichum spp. are common plant-pathogenic fungi, usually occurring as asymptomatic endophytes on aerial organs of the host plants (Hyde *et al.*, 2009; Cannon *et al.*, 2012). They affect many different crops causing post-harvest rots, and anthracnose spots and blights of plants. *Colletotrichum gloeosporioides* is most frequently associated

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the ITS, GAPDH and β -tubulin sequences determined in this study are KP101083–KP101085.

with anthracnose of papaya; however, it has also been associated with coffee berry disease and red rot of sugar cane (Guarro *et al.*, 1998; Weir *et al.*, 2012).

While relatively few human fungal infections have been attributed to *Colletotrichum* spp., the ubiquity of the genus has suggested that clinical disease may be under-reported, or isolates have been misidentified as belonging to other genera/species. Infections cited in humans typically involve the eye (keratitis/keratomycosis/endophthalmitis) or are subcutaneous in nature and secondary to trauma. Species associated with infection have included *Colletotrichum dematium*, *Colletotrichum gloeosporioides*, *Colletotrichum graminicola*, *Colletotrichum crassipes*, *Colletotrichum truncatum*, *Colletotrichum graminicola* and *Colletotrichum coccodes* (Guarro *et al.*, 1998; Sutton, 1999; Castro *et al.*, 2001; Figtree *et al.*, 2013; Stchigel & Sutton, 2013). We describe a patient case of fungal septic arthritis secondary to *Colletotrichum gloeosporioides*. To the best of our knowledge, this is the first case of a *Colletotrichum* sp. causing septic arthritis in humans.

Case report

A 55-year-old male presented to his primary care provider with major complaints of pain in his joints involving the lower right leg. His past medical history included hypertension, dyslipidemia, anxiety disorder, neuropathy and hepatitis B. He denied any physical activity that could have contributed to his joint pain but did maintain a garden. He was tested for human immunodeficiency virus multiple times in the past with all tests being negative, the last test being 2 months prior to this visit. Anti-inflammatories and activity modifications did not resolve the pain.

A month prior to the patient's visit at the infectious diseases clinic, he saw a surgeon and received two to three aspirations of the knee joint without much relief, and the joint pain, swelling and redness of the knee kept recurring. The patient's knee pain was consistent with patella-femoral pain and prepatellar bursitis, and a bursectomy was performed. Routine cultures consisting of aerobic bacteria, anaerobic bacteria, acid-fast bacilli, *Mycobacterium* and fungi were also obtained at this time. Upon follow-up, the patient did not have full relief of knee pain and swelling, and the cultures were positive for an unidentified fungus thought to be a yeast. The patient was referred to the infectious diseases clinic for further management.

During the patient's first visit with an infectious diseases physician, he was found to have complaints of leg and joint swelling, tenderness, effusion and problems with his gait. He also complained of 5/10 pain in the knee joint area and had a low-grade fever of 100.5 °F for several days with chills, sweats and no rigors. Repeat aspiration of the right knee was ordered to confirm fungal infection. Magnetic resonance imaging of the knee was ordered to rule out osteomyelitis/septic arthritis, and the patient was started empirically on fluconazole. Cultures from the fluid taken

from the knee grew a fungus originally thought to be a *Cylindrocarpon* sp. The patient was switched from fluconazole to voriconazole, and the culture was sent to the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio for species identification and susceptibility testing. Repeat cultures were negative.

The patient was seen in the office for his 1-month follow-up and his knee dramatically improved. There was no tenderness, warmth or erythema noted with a noticeable decrease in swelling. Some joint swelling was still noted on examination, but the pain level had markedly decreased (from 5/10 to 1/10). The patient was doing well on voriconazole, which was due to continue for 3 months. On follow-up appointment 1 month later, the patient was doing significantly better. His right knee had linear healthy scars, minimal swelling present, no erythema, tenderness or indurations, and had full movements of his knee joints. Towards the end of the treatment, the patient had red maculopapular rashes on all four extremities. He saw a dermatologist and was given fluocinonide cream, which helped. The rash could have been a reaction to voriconazole therapy, but the patient only had 2 days of treatment remaining. At this time, he had completed nearly 3 months of therapy. On further follow-up appointments at 2 and 3 months after his initial visit to the infectious diseases clinic, the patient denied any pain, redness or swelling of the knee joint and responded well to 3 months of voriconazole therapy.

Diagnosis

Mycology

The isolate submitted to the Fungus Testing Laboratory, given the accession number UTSHCSA DI14-254, produced grey, woolly colonies after 8 days of incubation at 25 °C on potato flakes agar (PFA, prepared in house) (Fig. 1a). Conidia were produced within acervuli and were approximately 10–14 × 3–4 µm with a rounded apex and truncate base (Fig. 1b). Brown, lobed appressoria were also noted on PFA after 6 days at 25 °C (Fig. 1c).

Molecular identification

DNA sequencing identification of this organism was also performed using methods described previously (Romanelli *et al.*, 2010). After DNA extraction, the internal transcribed spacer (ITS) rRNA gene region (primers V9G and ITS4) and the β-tubulin (primers BT2a and BT2b) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; primers GPD1 and GPD2) genes were amplified and sequenced (White *et al.*, 1990; Glass & Donaldson, 1995; Berbee *et al.*, 1999; Gerrits van den Ende *et al.*, 1999). The resultant sequence results were queried in GenBank using the BLASTn algorithm at the NCBI site (<http://www.ncbi.nlm.nih.gov>), and were also compared with those available in the CBS-KNAW Fungal Biodiversity Centre database (<http://www.cbs.knaw.nl>). The

Fig. 1. Phenotypic features of UTSHCSA DI14-254. (a) Grey, woolly colony produced on a 60 mm PFA plate after 8 days of incubation at 25 °C. (b) Conidia with rounded apices and truncate bases. (c) Brown, lobed appressoria. Panels (b) and (c) are lactophenol cotton blue mounts.

ITS sequence results had 100 % similarity with *C. gloeosporioides* OCAC24 (GenBank accession no. KJ813602, 334/334 bp match), the β -tubulin sequence had 99.3 % similarity with *C. gloeosporioides* (GenBank accession no. HM575217, 436/439 bp match) and the GAPDH sequence results had 99.5 % similarity with *C. cf. gloeosporioides* (GenBank accession no. HQ022565, 411/413 bp match). The isolate was deposited in the University of Alberta Microfungus Collection and Herbarium (UAMH 11856).

Antifungal susceptibility testing

Antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 standard for broth microdilution testing against filamentous fungi (CLSI, 2008). MICs for fluconazole, posaconazole, voriconazole and miconazole were read as the lowest concentration of each antifungal that resulted in 100 % inhibition of growth compared with growth control after 48 h of incubation. For micafungin, the minimum effective concentration was read as the lowest concentration that resulted in abnormal morphology (i.e. abnormally branched, stubby hyphae). Of the azoles tested, miconazole had the greatest potency *in vitro* with a MIC of $\leq 0.03 \mu\text{g ml}^{-1}$. The MICs for both posaconazole and voriconazole were $0.5 \mu\text{g ml}^{-1}$, the minimum effective

concentration for micafungin was $0.03 \mu\text{g ml}^{-1}$, and fluconazole had no *in vitro* activity (MIC $> 64 \mu\text{g ml}^{-1}$).

Discussion

Fungal infections associated with *Colletotrichum* spp. have rarely been implicated in human diseases. There have not been any reported cases of septic arthritis in humans due to a *Colletotrichum* sp., specifically *Colletotrichum gloeosporioides*. The cases that have been reported usually involved the eye, causing a form of keratitis or endophthalmitis, or are subcutaneous in nature and secondary to trauma (Guarro *et al.*, 1998; Sutton, 1999; Stchigel & Sutton, 2013). After a thorough examination, this patient was found to be relatively healthy and non-immunocompromised but did have a history of hepatitis B. He was tested for human immunodeficiency virus multiple times in the past with all prior tests being negative. To the best of our knowledge, this is the first case of a *Colletotrichum* sp. causing septic arthritis in humans.

Septic arthritis is most commonly caused by bacterial pathogens such as *Staphylococcus* spp., *Streptococcus* spp. and Gram-negative bacilli (Liu *et al.*, 2011). Fungal arthritis has an insidious onset and indolent course; the diagnosis is usually confirmed with synovial fluid cultures or biopsy. *Colletotrichum gloeosporioides* has not been reported previously as a cause of septic arthritis. We speculate that the organism was residing on his skin, due to his hobby as a gardener, and subsequent multiple aspirations served as a point of entry for the organism to cause this patient's fungal arthritis. The patient was not on antibiotics at the time, and previous therapy consisting of anti-inflammatories, steroid injections and activity modifications in an attempt to relieve his joint pain was unsuccessful.

Treatment of fungal arthritis usually consists of an oral azole or parenteral amphotericin B, but treatment is species dependent. This patient was initiated on fluconazole when yeast was identified in the synovial fluid culture. The yeast was preliminarily identified as a type of *Cylindrocarpon* sp. The patient's fluconazole regimen was changed to voriconazole based on previous reports showing successful treatment of *Cylindrocarpon* spp. with voriconazole (Mittra *et al.*, 2009). The final identification of the fungus was *Colletotrichum gloeosporioides*. Previous case reports have shown successful treatment of this organism using voriconazole (Figtree *et al.*, 2013). Antifungal susceptibility testing was performed using the CLSI M38-A2 standard for broth microdilution testing against filamentous fungi and showed resistance to fluconazole (MIC $> 64 \mu\text{g ml}^{-1}$) but potent activity for miconazole ($\leq 0.03 \mu\text{g ml}^{-1}$), posaconazole ($0.5 \mu\text{g ml}^{-1}$) and voriconazole ($0.5 \mu\text{g ml}^{-1}$).

This case report highlights successful treatment of a 55-year-old, immunocompetent man following 3 months of voriconazole therapy for a septic arthritis due to *Colletotrichum gloeosporioides*.

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