

# First Report of *Westerdykella dispersa* as a Cause of an Angioinvasive Fungal Infection in a Neutropenic Host

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**Angioinvasive fungal infections (AFIs) are an important cause of morbidity and mortality among immunocompromised patients. However, clinicomicrobiological characteristics and treatment of many AFI agents remain poorly defined. We report the first human case of infection with *Westerdykella dispersa*, an emergent cause of AFI, which was successfully treated in a neutropenic pediatric patient.**

## CASE REPORT

A 12-year-old male with a history of pre-B-cell acute lymphoblastic leukemia presented with fever and relapsed disease 1 year following an HLA-matched bone marrow transplant. He was admitted for intravenous antibiotics and reinduction chemotherapy, and a peripherally inserted central catheter (PICC) was placed in his left upper extremity on hospital day 2. He received piperacillin-tazobactam and high-dose Ara-C (1,000 mg/m<sup>2</sup>), etoposide (150 mg/m<sup>2</sup>), and plerixafor and subsequently developed neutropenia (absolute neutrophil count [ANC] = 0 per mm<sup>3</sup>) and an abrasion distal to the site of his PICC 1 week following chemotherapy. He was otherwise well but on hospital day 16 developed new fevers and increasing pain at the abrasion site, with associated drainage, central necrosis, and spreading erythema of his lesion, measuring 1.5 cm in diameter (Fig. 1A). Blood cultures and serum galactomannan were sent for testing. A skin biopsy specimen was sent for a histopathology and microbiology workup. He was continued on antibiotics, and liposomal amphotericin B (5 mg/kg of body weight daily) was empirically added for presumed invasive fungal cellulitis.

Histopathology revealed angioinvasive fungal hyphae (Fig. 2B and C). Intravenous voriconazole (4 mg/kg every 12 h [q12h]) was started, and the dose of liposomal amphotericin B was increased (7.5 mg/kg daily). Results of analyses of blood cultures and serum galactomannan were negative, but tissue cultures grew a filamentous fungus from day 2 to day 5 that was initially identified as a *Phoma*-like species. Further workup demonstrated the presence of multiple pulmonary nodules that were 2 to 3 mm in diameter in an image from a noncontrast chest computed tomography (CT) scan (Fig. 1C) that was highly suspicious for fungal infection despite a lack of respiratory symptoms. Due to ongoing fever, neutropenia, and hypotension, daily granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte infusions were initiated on hospital day 22 and were continued until neutrophil count recovery (day 35). Additional debridement of the upper-extremity lesion was performed on hospital day 21 and showed fungal elements upon histologic examination, though cultures remained negative.

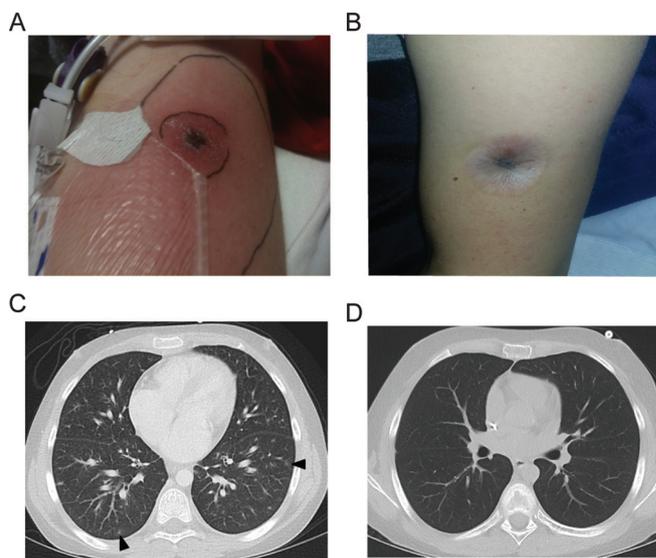


FIG 1 (A) Erythematous ulceration around the PICC line injection site prior to the onset of the disease. (B) Resolution of ulceration post-antifungal treatment and local debridement. (C) CT chest scan, with arrowheads showing opacities and highlighting nodules at the onset of the disease. (D) CT chest scan showing marked improvement after antifungal treatment.

Following debridement and institution of antifungal therapy, the patient's skin lesion significantly improved over the next 4 weeks (Fig. 1B), and a repeat CT scan at the time of discharge (Fig.

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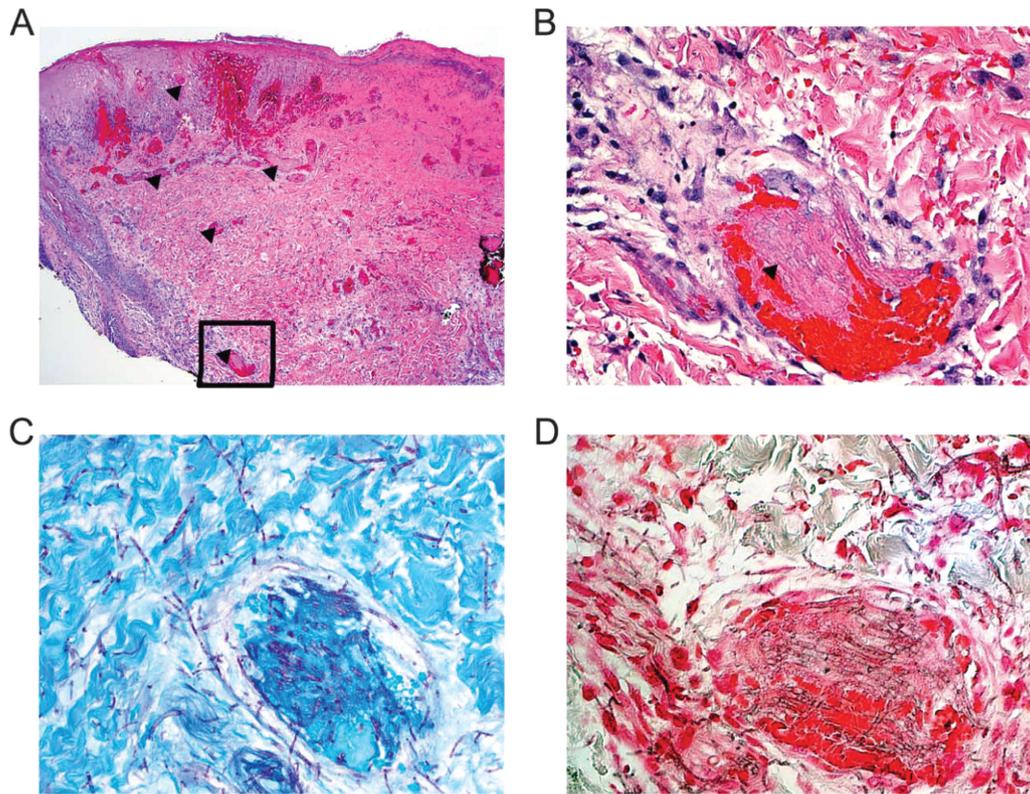
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**FIG 2** (A) Low-power ( $\times 40$ ) magnification of an H&E slide of a skin biopsy specimen demonstrates superficial ulceration, inflammation, and several foci of possible intravascular thrombi (arrowheads); the area marked with a square was chosen to be examined under high-power magnification. (B) High-power ( $\times 160$ ) magnification of an H&E slide demonstrates a thrombotic nidus of angioinvasive, filamentous fungal hyphae. (C) PAS staining highlights septate filamentous fungal hyphae with angioinvasion. (D) Fontana-Masson staining highlights fungal melanin within the hyphae.

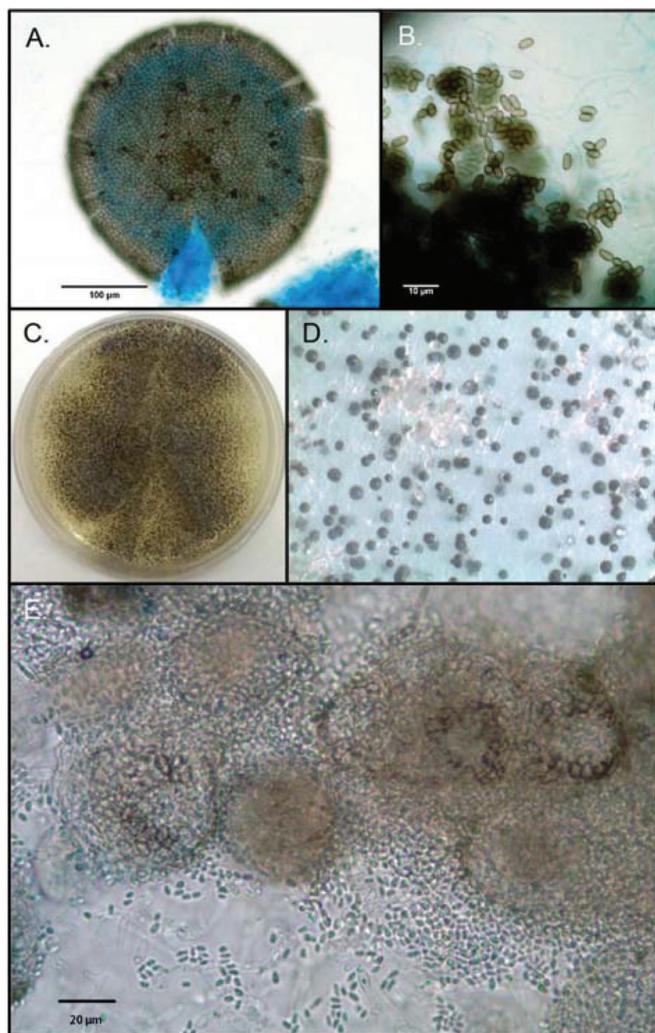
1D) showed a decrease in the number and size of bilateral opacities consistent with a resolving infectious process. The patient demonstrated neutrophil count recovery by week 5 and was discharged home at that time to complete a 6-week course of liposomal amphotericin B treatment followed by ongoing voriconazole prophylaxis.

**Histopathology.** The first biopsy specimen from the periphery of the 1.5-cm-diameter left-arm ulceration was sent for hematoxylin and eosin (H&E), Giemsa, and periodic acid-Schiff (PAS) staining. Microscopic examination showed superficial ulceration with thrombosis and friability of dermal vasculature (Fig. 2A), and angioinvasive septate hyphal forms were also present in H&E and PAS staining (Fig. 2B and C). Subsequent Fontana-Mason staining highlighted light melanin pigmentation (Fig. 2D). The second debridement specimen was also sent for histopathology examination and showed the presence of fungal hyphal elements.

**Mycology.** Calcofluor white staining on the first biopsy tissue specimen demonstrated numerous hyphal elements, and the cultures subsequently grew a filamentous fungus. The fungus was recovered on Sabouraud and brain heart infusion agar media and was able to grow at 37°C. As revealed by microscopic examination, the organism produced pycnidial conidiomata suggestive of a *Phoma* species at day 7. The organism was further referred to the Fungus Testing Laboratory (FTL) at the University of Texas Health Science Center at San Antonio (UTHSCSA) for identification by morphology and phenotypic characteristics (UTHSCSA DI14-299). It was subcultured onto potato flake agar (PFA) and

carnation leaf agar (this plant medium is routinely used to promote the production of conidiomata and/or ascomata), prepared in-house, and incubated at 25°C. After 14 days of incubation, both the pycnidial *Phoma* anamorph stage and the cleistothecial teleomorph were present. Cleistothecia were black and globose (Fig. 3A), asci contained 32 spores, and ascospores were smooth, reddish-brown, and cylindrical to slightly reniform, measuring 5 to 6  $\mu\text{m}$  in length by 2.5 to 3.5  $\mu\text{m}$  in width (Fig. 3B). On the basis of the features described above, the isolate was identified as a *Westerdykella* species. Additional subcultures of the isolate onto PFA for 9 days at 30°C provided better documentation of the anamorphic stage, demonstrating pycnidia developing on PFA (Fig. 3C and D), ostioles (openings) present on the pycnidia, and the small subglobose to pyriform conidia (Fig. 3E). Both the calcofluor white stain and culture results were negative in the second debridement sample.

**Molecular identification.** DNA sequencing identification of this organism was also performed using previously described methods (1). Briefly, after DNA extraction, the internal transcribed spacer (ITS) region and the D1 and D2 (D1/D2) region of 28S ribosomal DNA were amplified (ITS region, 487 to 501 bp; D1/D2 region, 571 to 581 bp) and sequenced. Resultant sequence results were analyzed by the use of SmartGene (SmartGene, Inc., Raleigh, NC) software and library data and also were subjected to BLAST analysis using the NCBI database. The sequencing results from the D1/D2 and ITS regions matched 100% and 99%, respectively, with those from the corresponding regions of *West-*



**FIG 3** (A) Dark, globose cleistothecium of *Westerdykella dispersa*. (B) Reddish-brown, smooth, cylindrical to slightly reniform ascospores of *W. dispersa*. Panels A and B show the results of growth on PFA after 14 days of incubation. (C and D) Pycnidial anamorph of *Westerdykella dispersa* on PFA plates after 9 days of incubation at 30°C. (E) Pycnidial ostioles (openings) and subglobose to pyriform, hyaline conidia.

*erdykella dispersa* type strain CBS 297.56 (GenBank accession no. GQ203753.1 and GQ203797.1).

In addition, an attempt to directly identify the fungus present in the formalin-fixed paraffin-embedded (FFPE) tissue block of the first biopsy specimen tissue was performed at the Microbiology Service, Department of Laboratory Medicine, and the Molecular Diagnostics Unit, Laboratory of Pathology, National Institutes of Health. Briefly, a piece of a 5- $\mu$ m-diameter FFPE tissue block was obtained. It was deparaffinized using Xylene Substitute (Sigma-Aldrich), and DNA was extracted using a QIAamp DNA FFPE kit (Qiagen GmbH, Hilden, Germany). The extracted DNA was amplified using ITS primers, and the amplicon was sequenced. The sequence results were searched against the NCBI database and exhibited 99% identity to *Westerdykella dispersa* type strain CBS 297.56 (GenBank accession no. GQ203797.1).

**Antifungal susceptibility testing.** Antifungal susceptibility testing was performed by broth microdilution according to CLSI methods for filamentous fungi (M38-A2) (2). MICs for amphotericin B, itraconazole, posaconazole, and voriconazole were read as the lowest concentration of each agent that resulted in 100% inhibition of growth compared to the growth control after 48 h of incubation. For micafungin, the minimum effective concentration (MEC) was read as the lowest concentration of this echinocandin that resulted in abnormally branched, stubby hyphae. Of the antifungals tested, amphotericin B (MIC, 0.06  $\mu$ g/ml) and micafungin (MEC, 0.25  $\mu$ g/ml) had the most potent *in vitro* activity, followed by voriconazole (MIC, 0.5  $\mu$ g/ml), posaconazole (MIC, 1  $\mu$ g/ml), and itraconazole (MIC, 4  $\mu$ g/ml).

In preparation for this report, three additional *Westerdykella* isolates that had been previously sent to the FTL for identification were also evaluated. Their species identities were confirmed by sequence analysis of the ITS and D1/D2 regions and the beta-tubulin gene at the UTHSCSA Advanced Nucleic Acids Core Facility (3–5). All isolates were recovered from lower-extremity wounds or tissue of patients in different locations in the United States. As shown in Table 1, two of the isolates (UTHSCSA DI-301 and DI-302) were identified as *Westerdykella reniformis* by sequence analysis, and one (UTHSCSA DI-300) was identified as *W. dispersa*. Interestingly, in both of the *W. dispersa* isolates (the isolate whose data are presented in this case report and the one previously sent to the FTL), a *Phoma* pycnidial anamorph was present along with ascospores that were mostly cylindrical to slightly reniform. In contrast, in the two *W. reniformis* isolates, the

**TABLE 1** Sequence results and morphological characteristics for the current *Westerdykella* spp. and for those previously sent to the FTL for identification

Isolate	Species identity	Sequencing target and % identity (GenBank accession no.)			Morphological characteristics
		ITS	D1/D2	Beta-tubulin	
UTHSCSA DI14-299 <sup>a</sup>	<i>W. dispersa</i>	99 (GQ203797)	100 (GQ203753)	ND	<i>Phoma</i> anamorph; cylindrical to slightly reniform ascospores
UTHSCSA DI14-300	<i>W. dispersa</i>	99 (DQ468031)	99 (GQ203753)	93 (GQ203716)	<i>Phoma</i> anamorph; cylindrical to slightly reniform ascospores
UTHSCSA DI14-301	<i>W. reniformis</i>	97 (JX235700)	98 (GQ203720)	99 (JX235706)	No <i>Phoma</i> anamorph; reniform (kidney-shaped) ascospores with a distinct central groove
UTHSCSA DI14-302	<i>W. reniformis</i>	99 (JX235700)	99 (JX235704)	99 (JX235706)	No <i>Phoma</i> anamorph; reniform (kidney-shaped) ascospores with a distinct central groove

<sup>a</sup> Case isolate.

ascospores contained a distinct central groove giving them a pronounced reniform shape; no *Phoma* anamorph was present in these isolates.

The case isolate has been deposited in the University of Alberta Microfungus Collection & Herbarium (UAMH) and assigned strain number UAMH 11810.

**Discussion.** *Westerdykella* species are saprobic fungi and exist worldwide on a variety of substrates, including soil, mud, dung, and plant material (6). *Westerdykella* was first identified in 1955 by Stolk (7) and is an ascomycete fungus that belongs to the family Sporormiaceae within the order Pleosporales. It is distinguished from other genera in the family by the production of cleistothecoid ascospores containing small asci (<50 µm in length), enclosing one-celled ascospores without germ slits. To date, 10 species, including *W. ornata*, *W. angulata*, *W. aurantiaca*, *W. cylindrica*, *W. dispersa*, *W. globosa*, *W. multisporea*, *W. nigra*, *W. purpurea*, and *W. reniformis*, have been described within *Westerdykella* (6). Species delineation within the genus has been based primarily on shapes of asci and ascospores and, more recently, on ITS ribosomal DNA and β-tubulin gene sequences (8). Several *Westerdykella* spp., including *W. nigra* and *W. globosa*, can present as an asexual *Phoma* state (*Phoma* anamorph), which was also observed in the *W. dispersa* strain isolated in our case patient.

While a disseminated infection caused by a *Westerdykella* sp. has recently been reported in a dog (9), no fungus from this genus has ever been reported to cause a human infection. For related *Phoma* spp., over 20 cases of human infection have been reported in the literature (10), with invasive disease occurring almost exclusively in immunosuppressed patients (10, 11). *Westerdykella* spp. have otherwise been isolated in only three prior human sources, as described above, from isolates sent to the FTL in San Antonio. In our case, *W. dispersa* demonstrated clear evidence of angioinvasion (Fig. 2) on histopathology, and while our blood culture results remained negative, the marked radiological improvement seen using a lung CT scan following treatment of our patient is suggestive of disseminated infection. The pulmonary nodules were likely associated with this organism because they were not present prior to this episode and they were significantly improved after antifungal therapy. Given the development of the lesion at the site of a previous abrasion, it is likely that the skin served as the portal of entry in our patient. His PICC site itself showed no evidence of erythema or induration throughout this episode and following multiple negative blood culture analyses, and therefore the line was ultimately retained for the duration of his course of treatment.

No current treatment recommendations for *Westerdykella* infection exist, and little is known about antifungal susceptibility of *Westerdykella* spp. In the aforementioned veterinary case of disseminated mycosis in a German shepherd dog caused by a *Westerdykella* sp., the reported MIC values for amphotericin B and itraconazole were similar to those for the isolate described in our patient (9). However, the posaconazole MIC was lower in that veterinary case (0.125 µg/ml). Although the data are scarce, the *in vitro* results from these two cases suggest that itraconazole has reduced *in vitro* potency compared to the other second-generation triazoles posaconazole and voriconazole. As a result, an ineffective clinical response was observed in the veterinary case treated

with itraconazole (9). Due to a lack of clinical MIC breakpoints for this organism, we opted to treat our patient with combination antifungal therapy using amphotericin B and voriconazole, both of which demonstrated relatively low MICs by *in vitro* susceptibility testing. While angioinvasive fungal infections can be life-threatening, particularly in severely immunocompromised hosts (12), our case illustrates that early local debridement in conjunction with appropriate antifungal agents and neutropenia-targeting therapies may be an effective strategy to minimize morbidity and mortality in immunocompromised patients.

In conclusion, this is to our knowledge the first reported case of a human infection caused by a *Westerdykella* species (*W. dispersa*). Our identification of the etiologic agent is supported by the histopathology results and was confirmed by phenotypic and molecular characterization, and we observed a successful outcome in our patient following a management strategy of resection, targeted antifungal therapy, and granulocyte infusion. Our case highlights *W. dispersa* as a potential pathogen and source of angioinvasive fungal infection among immunocompromised patients and demonstrates the need for accurate identification and susceptibility testing of novel fungal pathogens in severely immunocompromised patients.

**Nucleotide sequence accession numbers.** The D1/D2 and ITS sequence data were deposited into GenBank under accession numbers [KM008550](https://www.ncbi.nlm.nih.gov/nuccore/KM008550) and [KM008551](https://www.ncbi.nlm.nih.gov/nuccore/KM008551), respectively.

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