



# Invasive *Curvularia* Infection in Pediatric Patients With Hematologic Malignancy Identified by Fungal Sequencing

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*Curvularia* is a saprophytic dematiaceous mold and a rare human pathogen. Here, we report three severely immunocompromised pediatric patients who developed invasive *Curvularia* infection. Diagnosis was achieved or confirmed in all cases by fungal ribosome sequencing, which hastened species identification and targeted treatment for the patients reported. There are no treatment guidelines for invasive *Curvularia* infection, though we report three patients who were cured of their infection through a combination of surgical resection and various anti-fungal therapies, indicating a relatively low virulence and good prognosis in comparison to other angioinvasive molds.

**Keywords:** *Curvularia*; leukemia; fungal sequencing.

Angioinvasive molds are opportunistic pathogens in children undergoing cancer therapy. The diagnosis of invasive mold infection depends on histopathologic and microbiologic evaluations of tissue. Although mold can be visualized in tissue specimens, genus-level identification is often hindered by the poor sensitivity of cultures.

The vast majority of mold infections identified in immunocompromised pediatric patients with cancer are caused by *Aspergillus* and *Mucorales* species. Dematiaceous molds, distinguished by their production of melanin, are uncommon in this patient population. Localized infection occurs as a result of traumatic inoculation of cutaneous tissues. Invasive or disseminated disease is rare. Differentiating between common invasive molds and dematiaceous fungi is important for prognosis and treatment of infection.

*Curvularia* species are dematiaceous molds that are rarely reported as human pathogens. Here, we report the first case series of pediatric patients with cancer and invasive *Curvularia* infection. The diagnosis was made or confirmed in each case by fungal ribosome sequencing, which was faster than phenotypic identification and highlights the importance of using molecular diagnostics for detecting opportunistic fungal infections.

## CASE REPORTS

Patient A was a 6-year-old boy with T-cell acute lymphoblastic leukemia (ALL) who presented with febrile neutropenia, which

prompted inpatient admission. During his hospitalization, he sustained a minor injury to his posterior left ankle. Two days later, the resulting ankle lesion became tender and nodular with central erosion (Figure 1A). Cutaneous nodules developed on his left thigh and scalp simultaneously. He was treated empirically with liposomal amphotericin B (L-AMB) and voriconazole. Histopathologic examination of a biopsy specimen revealed deep subcutaneous fungal hyphae with angioinvasion. A tissue culture grew mold within 3 days, and this mold was identified as a *Curvularia* spp by sequencing performed on culture day 4 and subsequently confirmed by phenotypic testing on culture day 7. Susceptibility testing revealed a low minimum inhibitory concentration (MIC) for each of the antifungal agents tested (Table 1). A computed tomography (CT) scan of his chest, abdomen, and pelvis revealed scattered bilateral pulmonary nodules and a hepatic lesion, consistent with disseminated disease. He experienced neutrophil recovery 2 weeks after the diagnosis of fungal infection and continued treatment with L-AMB and voriconazole.

Six weeks after his diagnosis of fungal infection, patient A developed new subcutaneous nodules on his legs and chest. An abdominal ultrasound revealed an increase in the size of a liver lesion from 0.4 to 2.5 cm. Evaluation of biopsy specimens from subcutaneous nodules revealed necrotizing granulomas and rare fungal organisms with degenerate morphology. Tissue cultures of biopsy specimens were negative. These findings coincided with lymphocyte recovery and, thus, were suspected to be a result of immune reconstitution. Six weeks later, a CT scan revealed improvement in his pulmonary and hepatic nodules. The L-AMB was discontinued, and patient A was treated with voriconazole monotherapy. He completed intensive chemotherapy and experienced no further episodes of neutropenia.

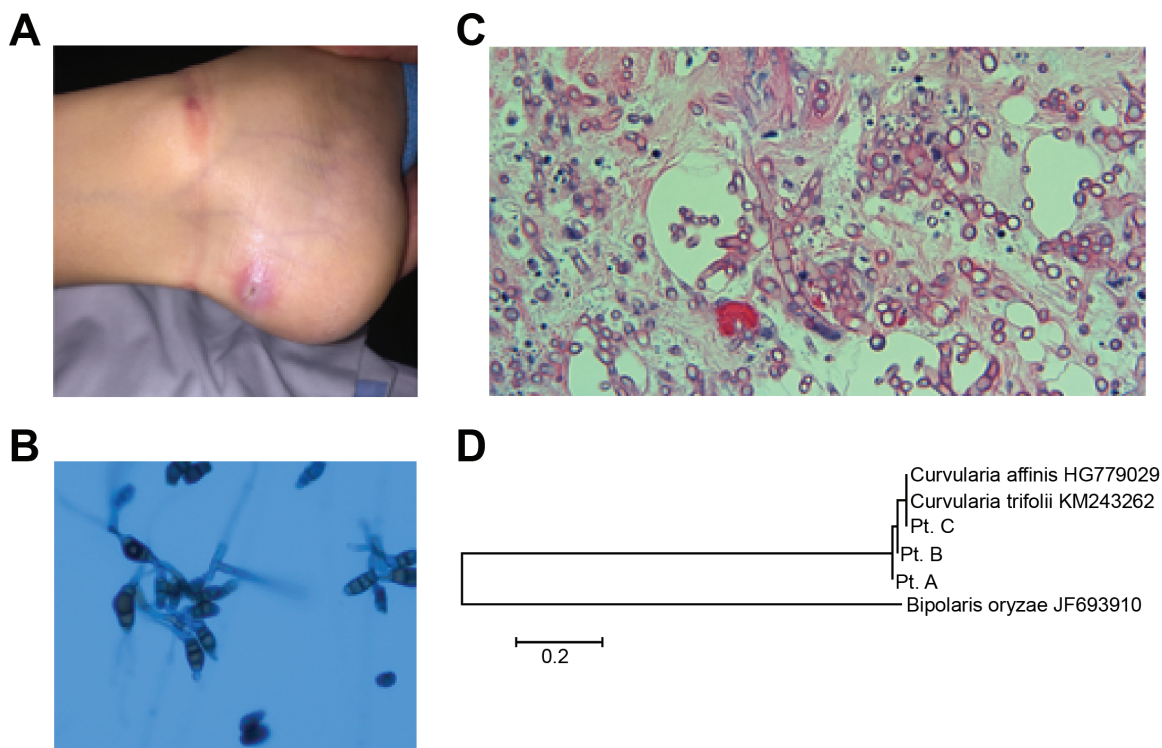
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**Figure 1.** Gross and microscopic appearance of tissue-invasive *Curvularia* spp and sequence analysis of molds from our 3 patients. (A) Cutaneous lesion on the left ankle of patient A. Shown is a tender, macerated nodule on the posterior ankle with central erosion. Another nodular lesion is located in a more anterior position. (B) Microscopic appearance of a *Curvularia* spp isolated from patient B. Shown is a tape preparation of a culture isolate from patient B stained with lactophenol aniline blue. Characteristic central swellings of *Curvularia* spp conidia are visible, as is a brown pigment (melanin), suggestive of a dematiaceous mold (400× magnification). (C) Histological findings for patient C. Numerous fungal organisms are present, with variably formed hyphae and acute angle branching. Invasion of organisms into dermal blood vessels is visible. (D) Each patient's *Curvularia* spp had a unique sequence. Shown is a neighbor-joining consensus tree (1000 bootstrap replicates) of fungal 28S ribosomal subunit sequences (320-bp amplicon) from the 3 patients (culture isolates from patients A and B, direct specimen from patient C) along with closest-match *Curvularia* spp reference sequences; a *Bipolaris* spp reference sequence was used as the outgroup (GenBank accession numbers are shown). Evolutionary analyses were conducted with Molecular Evolutionary Genetics Analysis version 7 (MEGA7).

Five months after the diagnosis of fungal infection and while he was still receiving voriconazole, patient A's subcutaneous nodules reappeared. A CT scan revealed an increase in the size of his pulmonary and hepatic nodules. Evaluation of a biopsy specimen from a subcutaneous nodule revealed granulomatous

inflammation without fungal elements, and culture results were negative. The patient's voriconazole levels had been mostly therapeutic; thus, because of concern for voriconazole failure, patient A was transitioned to caspofungin treatment, which he received for 12 weeks. Nine months after his diagnosis of fungal

**Table 1. Characteristics of Patients Diagnosed With Invasive *Curvularia* Infection**

Patient	Age (years)/Sex	Oncologic Diagnosis	Clinical Manifestation	Organism Identification	Susceptibility Testing (MIC [ $\mu$ g/mL]) <sup>a</sup>	Treatment (Duration)
A	6/M	IR T-ALL	Subcutaneous nodules on left heel, thigh, and scalp; nodules in lungs, liver, and kidney	Culture with rapid sequence identification and later phenotypic confirmation	AMB, 0.25; Caspo, 0.5; Posa, 0.06; Vori, 0.25; Terb, 0.25	L-AMB + Vori (12 wk); Vori (10 wk); L-AMB (4 wk); Caspo (2 wk) (total duration, 9 mo)
B	6/F	T-LL	Subcutaneous nodule on right forearm	Culture with rapid sequence identification and later phenotypic confirmation	AMB, 0.25; Vori, 0.25	L-AMB + Vori (7 wk); L-AMB (4 wk) (total duration, 11 wk)
C	7/M	Relapsed B-ALL	Diffuse nodular skin rash	Sequence-based identification directly from tissue	Not done	L-AMB (8 wk); Vori (>6 mo) (total duration, >8 mo)

Abbreviations: ALL, acute lymphoblastic leukemia; AMB, amphotericin B; Caspo, caspofungin; IR, intermediate risk; MIC, minimum inhibitory concentration; L-AMB, liposomal amphotericin B; LL, lymphoblastic lymphoma; Terb, terbinafine; Vori, voriconazole.

<sup>a</sup>Antifungal susceptibility testing was done at the University of Texas San Antonio Fungal Testing Laboratory. There are no interpretive criteria for the antifungal MICs reported.

infection, imaging revealed resolution of the pulmonary nodules but a persistent stable liver nodule. A biopsy specimen of the liver lesion revealed acellular fibrosis consistent with scarring; thus, his antifungal treatment was discontinued. Patient A was free of infection 1.5 years after his initial diagnosis of fungal infection.

Patient B was a 6-year-old girl with a history of T-lymphoblastic lymphoma who presented with febrile neutropenia 2 weeks after beginning consolidation chemotherapy. She had persistent fevers and developed an erythematous papule with a central eschar on her right forearm. Evaluation of a biopsy specimen revealed fungal organisms with angioinvasion. Culture of the biopsy specimen grew a dematiaceous mold within 3 days, which was identified as *Curvularia* species by sequencing on culture day 4 and subsequent phenotypic confirmation on day 14 (Figure 1B). She was treated empirically with amphotericin and voriconazole, and the lesion was excised with clear margins. Imaging evaluation with a CT scan revealed no evidence of dissemination to her eyes, lungs, or abdominal organs.

Susceptibility testing revealed a low MIC for AMB and voriconazole (Table 1). Two months after starting voriconazole, the patient had an episode of hepatic encephalopathy concerning for voriconazole toxicity. She was treated with L-AMB alone for an additional 4 weeks. She was not neutropenic throughout this treatment course. Subsequent CT imaging and physical examination revealed no signs concerning for recurrent infection. Patient B remained free of infection >1 year after her diagnosis.

Patient C was a 7-year-old boy with relapsed pre-B-cell ALL. After completing reinduction chemotherapy, he developed fevers and a necrotic skin lesion on his right knee after a traumatic injury. A 3-mm punch biopsy was performed at an outside hospital, and histopathology of the specimen revealed fungal elements extending to the surgical margins, which the pathologist reported as suggestive of *Aspergillus* species (Figure 1C). He was transferred to our institution and treated empirically with L-AMB, and he underwent wide excision of the nodule. Fungal culture of both the punch and excisional biopsy specimens yielded no growth. To aid in diagnosis and treatment, fungal ribosome sequencing of paraffin-embedded tissue from the punch biopsy was performed, and *Curvularia* species was identified.

Two days after resection, the patient developed a papular rash over his head, face, abdomen, and back. A biopsy specimen of the rash revealed a lymphocytic infiltrate with microabscesses in the papillary dermis. Bacterial, fungal, and viral testing yielded no causal organism; however, the rash was consistent with disseminated *Curvularia* infection. A CT scan of the chest, abdomen, and sinuses did not reveal additional sites of fungal infection. He experienced neutrophil recovery approximately 1 week after resection, at which point the rash improved.

After 2 months, patient C transitioned to voriconazole monotherapy. He underwent allogeneic bone marrow transplantation 4 months after his diagnosis of fungal infection and remained on antifungal prophylaxis throughout the peri-transplant period (Table 1). One year after the diagnosis of fungal infection, patient C remained infection free.

## METHODS

Total nucleic acid was extracted from isolated mold colonies derived from patients A and B with the MagNA Pure system using an LC kit (Roche, Indianapolis, Indiana). From patient C, DNA was extracted from five 5- $\mu$ m scrolls of formalin-fixed paraffin-embedded tissue by using a QIAamp DNA FFPE tissue kit (Qiagen, Germantown, Maryland). All extracted DNA was normalized to 10 ng of input and amplified with primers specific for ~320 bp of the 28S ribosomal subunit in AmpliTaq Gold master mix (Thermo Fisher, Waltham, Massachusetts) following manufacturer specifications. The amplified product was cleaned using ExoSAP-IT (Thermo Fisher) before Sanger sequencing strand synthesis using BigDye Terminator v1.1 (Thermo Fisher) following manufacturer instructions. Sequencing products were purified using Performa DTR gel-filtration cartridges (EdgeBio, San Jose, California) and run on a 3500 genetic analyzer from Thermo Fisher. Sequence analysis was performed in Molecular Evolutionary Genetics Analysis version 7 (MEGA7) [1].

## DISCUSSION

We report cutaneously acquired *Curvularia* infection in 3 pediatric oncology patients, with evidence of hematogenous dissemination in 2 cases. Invasive *Curvularia* infection in pediatric solid organ transplant recipients and in neonates has been described [1, 2]. However, to our knowledge, this is the first reported case series of invasive *Curvularia* infection in children with lymphoid malignancy, and these cases emphasize the important role of direct-tissue or isolate sequencing for accurate diagnosis and faster identification of fungal pathogens.

*Curvularia* spp are rarely reported as human pathogens. In immunocompetent hosts, the most common manifestations of *Curvularia* infection are superficial, including allergic bronchopulmonary disease, allergic sinusitis, and noninvasive cutaneous lesions. Invasive infection is infrequent outside of immunocompromised hosts. Among immunocompromised patients, including those with myeloid malignancy and those undergoing a solid organ or stem cell transplant, *Curvularia* spp can cause invasive infection that originates most commonly from traumatic inoculation of the skin or contamination of a defective anatomic barrier [2–4]. Vulnerability to invasive *Curvularia* infection is likely mediated by profound immunosuppression. Our patients included 2 with T-lymphoblastic

malignancy and 1 with recurrent B-cell ALL who developed invasive *Curvularia* infection. These patients all had high-risk malignancies and therefore received intensive chemotherapy leading to significant immunosuppression, placing them at high risk for invasive fungal infection.

No typical presentation of invasive *Curvularia* infection has been reported, although 2 of our patients developed an initial lesion at the site of a traumatic injury, consistent with previously reported mechanisms of inoculation. The third patient did not have an identified injury that preceded infection, although she had a small papule that developed into an ecthyma gangrenosum-like lesion. A similar presentation of cutaneous *Curvularia* infection in other severely immunocompromised patients has been reported [5]. The mechanism of infection likely includes inoculation of an unrecognized skin lesion. Two of our patients developed disseminated disease to distant skin sites and/or internal organs. This was presumed to be secondary to angioinvasion and hematogenous dissemination which has previously been reported in immunocompromised patients with *Curvularia* as well as other invasive mold infections [4].

Like other invasive molds, the diagnosis of *Curvularia* has traditionally relied on histopathologic features and culture. However, the histopathologic appearance of *Curvularia* spp is of septate hyphae that can be branched or unbranched, which does not distinguish the organism from other molds [2], as illustrated by the case of patient C for whom the morphology was reported as “suggestive of *Aspergillus*.” *Curvularia* spp can be identified by the morphology of the cultured organism, which produces sympodial, pale-brown, slightly curved conidia. However, culturing *Curvularia* spp is often difficult due to a low burden of organisms, slow growth, and exposure to antifungal agents in vivo. In addition, the mold appears light in color during the early days of its growth, and it can be slow to produce conidia (Figure 1B); thus, it cannot be differentiated quickly as a dematiaceous mold, even when growth is visible. Finally, recent studies found that the morphological identification of *Curvularia* spp does not correlate well with molecular identification [6]. Previous reports of molecular diagnostics for dematiaceous fungi using in situ hybridization were not able to distinguish among the genera [7]. One case in the literature reported use of Sanger sequencing to diagnose culture-negative *Curvularia* infection, but use of this method has not been widespread [4]. For all of our cases, *Curvularia* was identified by sequencing of the fungal ribosome from culture or tissue, which ensured faster identification from culture and an unexpected diagnosis in the culture-negative patient originally reported to have *Aspergillus* spp infection. Molecular identification also enabled prognostication of invasive mold infection in severely immunocompromised patients, given the relatively low virulence of *Curvularia* spp in contrast to that of other opportunistic molds.

While sequence-based diagnostics are expensive and labor intensive, cheaper and faster isolate-identification options, such as matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, are emerging and might enable a reduction in the time needed to identify *Curvularia* spp in culture.

One limitation of direct-specimen molecular diagnostics is that it does not provide antifungal susceptibility data, although identifying the genetic determinants of antifungal resistance is an area of intense research. No specific guidelines for the treatment of *Curvularia* infection exist. A large in vitro study of 99 clinical *Curvularia* spp isolates found that echinocandins, amphotericin B, and posaconazole showed the most reliable activity [6]. Very few reports of invasive *Curvularia* infection in immunocompromised patients exist in the literature; however, among immunocompromised adults, amphotericin B is the treatment reported most commonly [3, 8]. One child with acute myeloid leukemia was also reported to have improvement of hepatosplenic *Curvularia* infection after treatment with amphotericin B [9]. Itraconazole was used in 2 cases of locally invasive *Curvularia* infection that ultimately resolved, but only after surgical intervention [10, 11]. The treatment regimens in these reported cases were temporally biased, because many of them occurred before the availability of extended-spectrum azoles. Among the more recent reports, amphotericin B has remained the most commonly used treatment for invasive infection, and although voriconazole has a slightly higher MIC, it has been used successfully for treating invasive *Curvularia* infection in an immunocompromised adult host [4]. Fluconazole should be avoided in the treatment of *Curvularia* infection, because it has a high in vitro MIC, and treatment failures have been reported [4].

Our report highlights *Curvularia* spp as opportunistic pathogens in children with hematologic malignancies, and the potential for widely disseminated disease exists. We have demonstrated the importance of molecular diagnostic testing through fungal ribosome sequencing, which established a faster microbiologic diagnosis that enabled targeted treatment. Finally, despite ongoing significant immunosuppressive therapy, all 3 patients were cured of their infection through a combination of surgical source control and antifungal therapy, which indicates that *Curvularia* infection is curable even in severely immunocompromised hosts.

## Notes

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