

## *Aspergillus lentulus*: An Under-recognized Cause of Antifungal Drug-Resistant Aspergillosis

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*Aspergillus lentulus* is a drug-resistant species that is phenotypically similar to *A. fumigatus*. It was discovered as a cause of azole-breakthrough disease, consistent with in vitro resistance. Clinical labs can misidentify the species as *fumigatus* based on phenotypic typing. We describe 4 recent cases and provide a brief review.

**Keywords.** *Aspergillus lentulus*; cryptic species; DNA sequencing; MALDI-TOF MS; multidrug resistance; pulmonary aspergillosis.

*Aspergillus fumigatus*, once considered 1 species, actually encompasses many members in a complex of phenotypically similar but molecularly distinct “cryptic” species [1]. Molecular typing has led to recognition of *A. lentulus* and *A. thermomutatus*, among others [2]. Cryptic species cause 3%–15% of invasive aspergillosis (IA) cases but are under-recognized by labs that use phenotypic identification alone [3–6]. Correct identification is important, as some of these species can be resistant to triazoles and amphotericin B.

*Aspergillus lentulus* was discovered as a cause of IA in hematopoietic stem cell transplant (HSCT) recipients in Seattle, Washington [4, 7]. Although phenotypically identified as *A. fumigatus*, it was found to be genetically distinct and highly drug resistant. It has been suggested that *A. lentulus* infection causes higher mortality compared with *A. fumigatus* (>60%) [3]. We highlight 4 recent cases at 2 US centers, identified after identification methods were enhanced to include matrix-assisted laser desorption ionization–time-of-flight mass spectrometry

(MALDI-TOF MS) and antifungal drug susceptibility testing (minimum inhibitory concentration was determined by the broth microdilution method recommended by CLSI M38-A3).

### PATIENT CASES

The first patient was a 47-year-old man with alcoholic cirrhosis and a liver transplant who had dyspnea and a mass-like consolidation in the right upper lobe on computed tomography (CT). Serum beta-D-glucan (BDG; Fungitell, Associates of Cape Cod, Inc.) and galactomannan (GM; Platelia, BIO-RAD) were positive (>500 pg/mL and 0.82 index, respectively). Tracheal aspirate cultures grew *Aspergillus lentulus*, with susceptibilities noted in Table 1. The patient was treated with voriconazole and micafungin for 2 weeks, then voriconazole monotherapy. Repeat CT chest and serum GM showed improvement after 3 months of therapy. Due to dizziness and vivid dreams, voriconazole was switched to isavuconazole, and he continued to show clinical improvement.

The second case was a 76-year-old man with untreated pulmonary sarcoidosis who presented with months of weight loss and cough. CT showed bilateral upper lobe interstitial fibrosis, bronchiectasis, and reticulonodular opacities. His bronchoalveolar lavage (BAL) cultures grew both *Aspergillus fumigatus* and *Aspergillus lentulus*, with susceptibilities in Table 1. He was treated with voriconazole, with no change in CT findings 4 months later. Voriconazole was discontinued with elevations in liver chemistry tests and biopsy suggestive of drug-induced liver injury. He remained clinically stable despite persistent CT findings.

The third patient was a 54-year-old man with pulmonary sarcoidosis requiring prednisone therapy and metastatic pancreatic cancer treated with fluorouracil who presented with fevers and hypoxia. BAL grew *A. nidulans*, *A. lentulus*, *A. calidoustus*, and *Mycobacterium avium intracellulare*. Susceptibilities for *A. lentulus* are noted in Table 1. Antifungal therapy was initially reserved with negative serum biomarkers (BDG <31 and GM 0.15). After 2 months of observation, he was admitted with fevers and chest CT showing multiple noncalcified pulmonary nodules. With the increase in size of the pancreatic mass and new liver lesions, concern for metastatic cancer was raised. Despite treatment with isavuconazole, micafungin, and antibacterials, his clinical condition deteriorated, and he was transitioned to comfort measures.

The fourth case was a 67-year-old man with transthyretin amyloidosis with cardiac involvement who underwent heart transplantation 1 month before presenting with dry cough. CT showed a new right middle lobe mass, multifocal nodules, and ground glass opacities. BAL grew *A. fumigatus*. Serum BDG

Received 26 May 2021; editorial decision 16 July 2021; accepted 23 July 2021.

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#### Open Forum Infectious Diseases® 2021

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was >500 pg/mL, and serum GM index was 0.42. He was initially treated with voriconazole and micafungin. Voriconazole was changed to liposomal amphotericin after rapid progression in the mass size with cavitation. Sequencing of the isolate was performed by UT Health San Antonio, correcting identification to *A. lentulus*, with susceptibilities in Table 1. Voriconazole was changed to posaconazole, with complete resolution of symptoms but persistent loculated cavitations with surrounding ground glass and stable nodules on imaging after 14 months of therapy.

### Patient Consent

Patients were described after written consent was obtained at Johns Hopkins University (JHU; Baltimore, MD, USA, #1–3) and Virginia Commonwealth University (Richmond, VA, USA, #4). This work did not require local ethical committee review.

### DESCRIPTION AND IDENTIFICATION

*Aspergillus lentulus* was first described in 2004 from BAL samples of 4 patients that represented 5% of HSCT patients in a retrospective study screening for itraconazole resistance [7]. These isolates were identified as a new, genetically distinct species, named *Aspergillus lentulus* [8]. *Lentulus* is derived from the Latin word “lentus,” which means tough and slow, chosen to reflect its sporulation characteristics. Macroscopically, colonies sporulate slowly and appear white, before generating pale

green-blue conidia similar to *A. fumigatus* (Figure 1). Compared with *Aspergillus fumigatus sensu stricto*, *Aspergillus lentulus* has smaller, globose vesicles with shorter conidial heads, and it exhibits increased susceptibility to temperatures >37°C [3, 8]. The species is genetically distinct, enabling molecular identification that relies on DNA sequence (or polymorphism) in internal transcribed spacer (ITS) rDNA and beta-tubulin (benA) [3]. Additionally, MALDI-TOF MS can distinguish between *A. lentulus* and *A. fumigatus* [9].

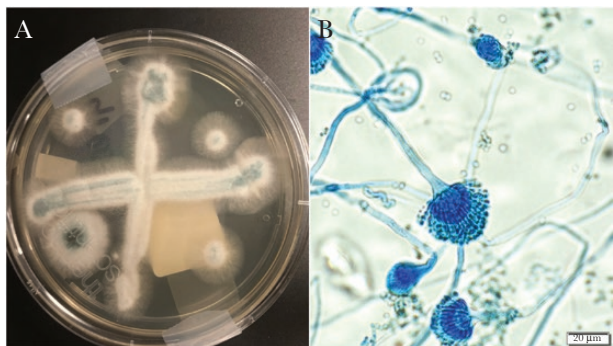
In Baltimore, we identified reported cases in 2020, after the lab initiated routine MALDI-TOF MS (Bruker MALDI Biotyper) with antifungal susceptibility testing on *Aspergillus* isolates recovered from BAL. All 4 isolates in this case series were confirmed by DNA sequencing. Three additional cases of *A. lentulus* infections were identified during preparation and review of the manuscript. These isolates had not been appreciated as a cause of disease at JHU previously, suggesting misidentification using phenotypic methods. Environmental changes promoting epidemiologic shifts and increased case recognition cannot be ruled out. Infections in people from 2 centers minimize the likelihood of common exposures.

In these cases, the organism was associated with IA and/or recovered as a colonizer in the airway in the less immunosuppressed patient with sarcoidosis. This follows the pattern described for all *Aspergillus* species, which cause disease along the spectrum of airway colonization to tissue invasion, influenced

**Table 1. Patient Characteristics of *Aspergillus lentulus* Cases**

4	3	2	1	Patient #
67M	54M	76M	47M	Age, Sex
TTR amyloidosis & heart transplant	Pulmonary sarcoidosis & metastatic pancreatic cancer	Pulmonary sarcoidosis	Liver transplant	Underlying Disease
Tacrolimus, mycophenolate, prednisone	Prednisone & fluorouracil	None	Tacrolimus, prednisone, mycophenolate	Immunosuppression
Lung	Lung	Lung	Lung	Source
Vori: 4 Isavu: 2 Posa: 0.25 Itra: - Mica: <0.015 AmB: 2 Terb: 0.25	Vori: 4 Isavu: 4 Posa: 1 Itra: 2 Mica: <0.008 AmB: 4	Vori: 1 Isavu: 0.25 Posa: 1 Itra: - Mica: 0.008 AmB: 1	Vori: 2 Isavu: 2 Posa: 1 Itra: 1 Mica: <0.008 AmB: 8	Susceptibility (MIC), ug/mL
None	None	None	None	Prior Antifungal
Mass-like consolidation in RML	Noncalcified pulmonary nodules	Interstitial fibrosis and bronchiectasis, scarring, reticulonodular opacities	Mass-like consolidation in RUL	Imaging
>500	<31	-	>500	Serum BDG (Normal <80 pg/mL)
0.42	0.15	-	0.82	Serum GM Index (Normal <0.5)
10.51	0.63	0.17	-	BAL GM Index (Normal <0.5)
Improved but with residual CT changes	Deceased	No follow-up	Improved	Outcome

Abbreviations: AmB, amphotericin B; BAL, bronchoalveolar lavage; BDG, beta-D-glucan (Fungitell, Associates of Cape Cod, Inc.); GM, galactomannan (Platelia, BIO-RAD); Isavu, isavuconazole; Itra, itraconazole; MIC, minimum inhibitory concentration (determined by broth microdilution method recommended by CLSI M38-A3); Mica, micafungin; Posa, posaconazole; RML, right middle lobe; RUL, right upper lobe; Terb, terbinafine; TTR, transthyretin; Vori, voriconazole.



**Figure 1.** Phenotypic images of *Aspergillus lentulus*. A, Morphology image on Sabouraud dextrose agar showing colonies that are whitish, pale green-blue with poor sporulation. B, Microscopic image showing small, globose vesicles with short conidial heads (magnification 60 $\times$ ).

by patient risks. *Aspergillus lentulus* has been reported to cause disease in people with diverse risks, including transplant and oncology patients and people with chronic pulmonary disease, including that caused by cystic fibrosis [10,11].

To our knowledge, this is the first literature description of *A. lentulus* infections in the United States outside of Seattle, where it was first described. Cases have been reported in Spain, Italy, Belgium, Switzerland, Denmark, the Netherlands, Brazil, Argentina, Japan, China, Turkey, Australia, and Canada [5, 10, 11–18, 19–22]. Whether the species has a unique environmental niche is not clear. It has been recovered from soil and beach sand and has been identified as cause of disease in aquatic mammals and cats [23–26]. As it is tolerant to extreme growth conditions, including high pH and salt concentrations, it is likely to be widely dispersed and is being evaluated for biodegradation of toxic compounds [27]. It was among a small number of organisms identified as persistently recovered over 3 flights to the international space station [28]. Environmental azole use may select growth; it was recovered, along with other species, from azole-treated vineyards in Portugal [29].

The majority of cases have been reported in transplant patients and people with other immunocompromising states, including receipt of systemic corticosteroids [5, 10, 12, 13, 17, 19, 20, 30, 31]. A review of 6 cases from China reported immunocompromise, critical illness, and prior antifungals to be risks for invasive *A. lentulus* infections [15]. Reports suggest worse outcomes in patients with mixed *Aspergillus* infections [10, 19, 30]. Similar to other species, *A. lentulus* causes diverse radiographic findings, including consolidations [5, 12, 13, 19, 20], cavitary lesions [5, 12, 31], and nodules [19].

It appears important to identify cryptic species of the *Aspergillus fumigatus* complex because they have different antifungal susceptibility patterns compared with *Aspergillus fumigatus sensu stricto*. A European study reviewed the largest collection of cryptic species, with 51 clinical isolates, 9 *A. lentulus*. Using EUKAST minimum inhibitory concentration

(MIC) breakpoints, *A. lentulus* isolates showed high MICs to all triazoles and amphotericin B. It is one of the few cryptic species (with *A. udagawae*) that shows high MICs to both triazoles and amphotericin B. Although many studies report high MICs to triazoles [4, 7, 15, 32–35], 1 reported favorable susceptibility to isavuconazole [36]. Intrinsic azole resistance appears to be *cyp51A* dependent [3, 37, 38]. Although there are no voriconazole clinical breakpoints for *Aspergillus lentulus*, applying breakpoints for *A. fumigatus* (susceptible  $\leq 0.5$   $\mu\text{g/mL}$ ; intermediate 1  $\mu\text{g/mL}$ ; resistant  $\geq 2$   $\mu\text{g/mL}$ ) suggests voriconazole resistance in cases 1, 3, and 4, and intermediate resistance in case 2.

## LESSONS LEARNED

Infections caused by cryptic species of the *Aspergillus fumigatus* complex are in the minority compared with *A. fumigatus sensu stricto*, but correct identification is important given poor outcomes associated with antifungal resistance. Laboratories may misidentify these species as *A. fumigatus* when only phenotypic methods are used. Providers should consider cryptic species if the laboratory describes an atypical *A. fumigatus* (poor sporulation) or if the lab reports *A. fumigatus* that is resistant to 1 or more triazoles or amphotericin B. In these situations, the practitioner should request additional investigation such as DNA sequencing to confirm the species identification. Alternatively, MALDI-TOF MS can be used as a rapid tool for presumptive identification of *A. lentulus*, but confirmation requires DNA sequencing. With increased *A. fumigatus* resistance and appreciation of cryptic species that have variable susceptibilities, increased reliance on susceptibility testing should be considered. Improvements in both species identification and antifungal susceptibility testing could assist in the management of these complex invasive aspergillosis cases.

## Acknowledgments

**Financial support.** None.

**Potential conflicts of interest.** S. Nematollahi: no financial disclosures. N. Permpalung: consulting/advisory board income from Shionogi Inc.; research grant from Cidara. S. X. Zhang: research grants from IMMY Diagnostics, Vela Diagnostics. M. Morales: no financial disclosures. K.A. Marr: consulting/advisory board income from Cidara, Merck, and Sfunga; equity and licensing revenue from MycoMed Technologies; research grant from Merck. All other authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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