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Emerging *Aspergillus lentulus* infections in Taiwan: clinical and environmental surveillance

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Objectives: This study aimed to investigate the prevalence and characteristics of *Aspergillus lentulus* clinical and environmental isolates in Taiwan.

Methods: *Aspergillus* isolates obtained from patients at three hospitals and from 530 soil samples across Taiwan were screened. *A. lentulus*, confirmed by calmodulin sequencing, was subjected to antifungal susceptibility testing and *cyp51A* analyses. Soil samples yielding *A. lentulus* were analysed for residues of 25 azole fungicides.

Results: Nine *A. lentulus* isolates were identified, which included seven (1.2%, 7/601) isolates from three antifungal-naïve patients out of 601 *Aspergillus* section *Fumigati* clinical isolates and two (0.3%, 2/659) isolates out of 659 *Aspergillus* soil isolates. All isolates developed white colonies and failed to grow at 48°C. They were susceptible to anidulafungin but showed reduced susceptibility to amphotericin B (AmB), voriconazole and azole fungicides. One heart transplant recipient with proven invasive pulmonary aspergillosis (IPA) initially showed suboptimal response to voriconazole monotherapy but was cured with a combination of voriconazole–caspofungin, liposomal AmB (LAmB)–caspofungin, along with surgery, followed by voriconazole maintenance therapy. Among two critically ill patients with probable IPA, one survived with micafungin, while the other died of aspergillosis despite sequential isavuconazole and LAmB monotherapy. Clinical and environmental isolates sharing identical Cyp51A sequence are identified, matching the Cyp51A sequence of *A. lentulus* NIID0096. Flusilazole (0.0009 mg/kg) was detected in one soil sample.

Conclusions: This study raises concerns about health threat posed by human pathogenic *A. lentulus* originating from natural environments and underscores the need for increased clinical and laboratory vigilance regarding *A. lentulus* infections.

Introduction

Aspergillus lentulus, a cryptic species within *Aspergillus* section *Fumigati*, was first described in a 2004 report, where it was recovered from patients undergoing stem cell transplantation and characterized by slow sporulation and decreased susceptibilities to amphotericin B (AmB), itraconazole and voriconazole.[1] *A. lentulus* is of medical importance due to its potential to cause human diseases and inherent drug resistance.[2] To enhance epidemiological knowledge, this study describes the prevalence and characteristics of clinical and environmental isolates of *A. lentulus* in Taiwan.

Materials and methods

Clinical isolates of *A. lentulus* were screened among consecutive *Aspergillus* isolates obtained from patients at Chi Mei Medical Center, Liouying (CMMC), National Cheng Kung University Hospital (NCKUH) and National Taiwan University Hospital (NTUH) between 2011 and 2022. *Aspergillus* isolates from CMMC and NCKUH were identified using calmodulin gene sequencing.[3] *Aspergillus* isolates from NTUH were identified using internal transcribed spacer (ITS) sequencing,[3] and those belonging to *Aspergillus* section *Fumigati* and developing white colonies were further identified using calmodulin sequencing. *Aspergillus* disease was defined according to the European Organization for the Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) definition.[4]

Environmental isolates of *A. lentulus* were screened among 659 *Aspergillus* isolates obtained from cultivation of 530 soil samples collected from 265 farms (two samples per farm) across Taiwan during 2018–19. *A. lentulus* isolates were identified based on their inherent reduced susceptibility to azoles using Sabouraud dextrose agar (SDA) plates supplemented with itraconazole (2 μg/mL), voriconazole (1 μg/mL) and posaconazole (0.25 μg/mL), respectively, as previously described.[5] *Aspergillus* isolates capable of growing on any of the azole agar plates were subsequently identified by calmodulin sequencing. In our prior observations, isolates with voriconazole MICs of ≥1 μg/mL were able to grow on the voriconazole-containing SDA plate.[5]

A. lentulus isolates, confirmed by calmodulin sequencing, underwent ITS- and calmodulin-based phylogenetic analysis using MEGA X. Additionally, growth capability at 48°C, species identification via matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) (VITEK®, bioMérieux), susceptibility testing of antifungal drugs used in clinical and agricultural settings following CLSI M38-A3 guidelines, and *cyp51A* analyses were conducted using previously described methods.[6,7] Soil samples yielding *A. lentulus* were analysed for residues of 25 azole fungicides using gas chromatography/triple quadrupole mass spectrometry.

The details of materials and methods are provided in the Supplementary Data (available as Supplementary data at *JAC-AMR* Online).

Results

Nine *A. lentulus* isolates were identified, which included seven (1.2%, 7/601) isolates from three patients (A, B and C) at three hospitals out of 601 *Aspergillus* section *Fumigati* clinical isolates and two (0.3%, 2/659) soil isolates from two farms out of 659 *Aspergillus* isolates (Table 1). In contrast to *A. fumigatus*, all *A. lentulus* isolates grew as fluffy white colonies, consisting predominantly of vegetative hyphae after 5 days of incubation (Figure 1), failed to grow at 48°C and received a confidence value of 99.9% as *A. lentulus* scored by the VITEK® MS v3.2 IVD library. Calmodulin-based phylogenetic analysis differentiates *A. lentulus* from other cryptic species within section *Fumigati* more accurately than ITS-based phylogeny (Figure S1).

All nine isolates were susceptible to anidulafungin with a low minimum effective concentration (≤0.004 μg/mL). However, they displayed reduced susceptibility to AmB with minimum inhibitory concentrations (MICs) of 1–4 μg/mL and intermediate resistance or resistance to voriconazole with MICs of 1–4 μg/mL based on CLSI breakpoints for *A. fumigatus* (susceptible ≤ 0.5 μg/mL, intermediate 1 μg/mL, resistant ≥ 2 μg/mL).[8] MICs of other azoles were as follows: 1–4 μg/mL for isavuconazole, 0.12–0.5 μg/mL for posaconazole and 0.5 to >16 μg/mL for itraconazole. Elevated MICs were observed for azole fungicides: difenoconazole 8–16 μg/mL and tebuconazole > 32 μg/mL (Table 1).

All *A. lentulus* isolates shared an identical Cyp51A amino acid sequence with that of *A. lentulus* NIID0096 (accession no. LC649065),[9] except for two isolates (NCK0965 and NCK0966 from Patient A) (A9P) and one soil isolate (19S031S-1) (V23A), each of which had a SNP, resulting in an amino acid substitution (Table S1).

Patient A, a 60-year-old man diagnosed with proven invasive pulmonary aspergillosis (IPA), presented with mass lesions in both lungs 3 weeks after undergoing heart transplantation [Day 1 (D1)]. Initially, he received treatment with voriconazole (400 mg/day) from D24 to D32. However, due to the progressive enlargement of the lung lesions with cavitation, voriconazole was combined with caspofungin (50 mg/day) from D33 to D40. Segmentectomy of the right upper lung and wedge resection of the left lower lung were performed on D35 for source control. Fungal culture of the bilaterally resected lung tissue samples both yielded *A. lentulus*, and histopathology revealed multiple foci of aggregates of septate fungal hyphae with acute angle branching (Figure 1). Postoperatively, voriconazole–caspofungin combination was followed by caspofungin–liposomal AmB (LAmB) (4 mg/kg/day) combination therapy (D41–D46), LAmB (4 mg/kg/day) monotherapy (D47–D52) and voriconazole (400 mg/day) maintenance therapy (D53–D188). The patient recovered well without relapse of IPA during a 4-year follow-up. Both isolates displayed reduced susceptibility to AmB (MIC 1 μg/mL) and voriconazole resistance (MIC 2 μg/mL). Patient B was a 48-year-old man with diabetes mellitus, decompensated liver cirrhosis and chronic steroid use. He developed probable IPA due to *A. lentulus* while receiving intensive care for infectious enteritis and ventilator-associated pneumonia and survived with micafungin (100 mg/day). Patient C, an 80-year-old man initially diagnosed with community-acquired pneumonia, developed acute respiratory distress syndrome and subsequently received treatment with methylprednisolone (120 mg/day). Endotracheal aspirates obtained at 14 and 17 days after admission yielded *A. lentulus*, accompanied by newly observed pulmonary consolidation on chest imaging. Isavuconazole (200 mg/day) was administered for probable IPA, but despite 7 days of isavuconazole therapy, endotracheal aspirate cultures continued to yield *A. lentulus*, along with a markedly elevated serum galactomannan index (6.96). The patient eventually died of IPA despite treatment with isavuconazole followed by LAmB (5 mg/kg). All three isolates exhibited reduced susceptibility to AmB and isavuconazole (both MICs 4 μg/mL). Three patients were antifungal-naïve, defined as having no systemic antifungal exposure within the past 30 days.

Of the two soil samples yielding *A. lentulus*, flusilazole (0.0009 mg/kg) was detected in one soil sample; none of the 25 azole fungicides tested was detected in the other.

Table 1. Isolate collection and data of nine *A. lentulus* clinical and environmental isolates in this study

Hospital/ Patient	Age (yr)/ Sex	Underlying conditions	Strain no.	Sample	Year of collection[a]	EORTC IPA category	Serum/ BAL GM	Antifungal therapy (days)	Outcome	MIC (μg/mL)[c]							MEC[c]
										AmB	ITR	VRC	PSC	ISA	DFC	TBC	ANF
Clinical																	
NCKUH: Two *A. lentulus* isolates from Patient A (1.0%, 2/208) out of 208 *Aspergillus* section *Fumigati* isolates collected during August 2011–December 2020																	
A	60/ M	DM, heart transplant,	NCK0965	Lung	2019 (d0)	Proven	>5.86/ >5.86	VRC (9), VRC/ CPF (8), CPF/	alive	1	2	2	0.25	2	16	>32	≤0.004
		CAD, CKD, steroids[b]	NCK0966	Lung	2019 (d0)			LAmB (6), LAmB (6), VRC (136)		1	2	2	0.25	2	16	>32	≤0.004
CMMC: Two *A. lentulus* isolates from patient B (1.2%, 2/165) out of 165 *Aspergillus* section *Fumigati* isolates collected during January 2015–May 2021																	
B	48/ M	DM, LC, enteritis,	LCM062	ETA	2015 (d0)	Probable	nd/nd	MCF (7d)	alive	4	0.5	1	0.12	1	16	>32	≤0.004
		chronic pancreatitis, steroids[b]	LCM065	ETA	2015 (d0)					4	0.5	1	0.12	1	8	>32	≤0.004
NTUH: Three *A. lentulus* isolates from patient C (1.3%, 3/228) out of 228 *Aspergillus* section *Fumigati* isolates collected during January 2015–22																	
C	80/ M	DM, steroids[b]	P042	ETA	2022 (d0)	Probable	7.39/nd	ISA (10), LAmB (7)	died	4	>16	4	0.5	4	16	>32	≤0.004
			P023	ETA	2022 (d3)					4	>16	4	0.5	4	16	>32	≤0.004
			P043	ETA	2022 (d11)					4	>16	4	0.5	4	16	>32	≤0.004
Environment: Two *A. lentulus* isolates (0.3%, 2/659) identified among 659 *Aspergillus* isolates																	
Sugar apple farm			19S031S-1	Soils	2019					2	1	4	0.25	2	16	>32	≤0.004
Mango farm			19S033S-1	Soils	2019					2	1	4	0.25	2	16	>32	≤0.004
A. fumigatus ATCC-MYA3626										0.5	0.5	0.5	0.12	1	2	4	≤0.004

AmB, amphotericin B; ANF, anidulafungin; BAL, bronchoalveolar lavage; CAD, coronary artery disease; CKD, chronic kidney disease; CPF, caspofungin; DFC, difenoconazole; DM, diabetes mellitus; ETA, endotracheal aspirate; GM, galactomannan index; IPA, invasive pulmonary aspergillosis; ISA, isavuconazole; ITR, itraconazole; LAmB, liposomal amphotericin B; LC, liver cirrhosis; MCF, micafungin; MEC, minimum effective concentration (μg/mL); nd, not done; PSC, posaconazole; TBC, tebuconazole; VRC, voriconazole.

[a](d0) and (dn) refer to the day 0 and *n* days after the first isolation of *A. lentulus,* respectively.

[b]Glucocorticoid treatment with prednisolone equivalent of 20 mg or more per day.

[c]MIC/MEC (μg/mL) were determined using CLSI M38-A3. *A. fumigatus* ATCC MYA-3626 and *Candida parapsilosis* ATCC 22019 were used as quality controls.

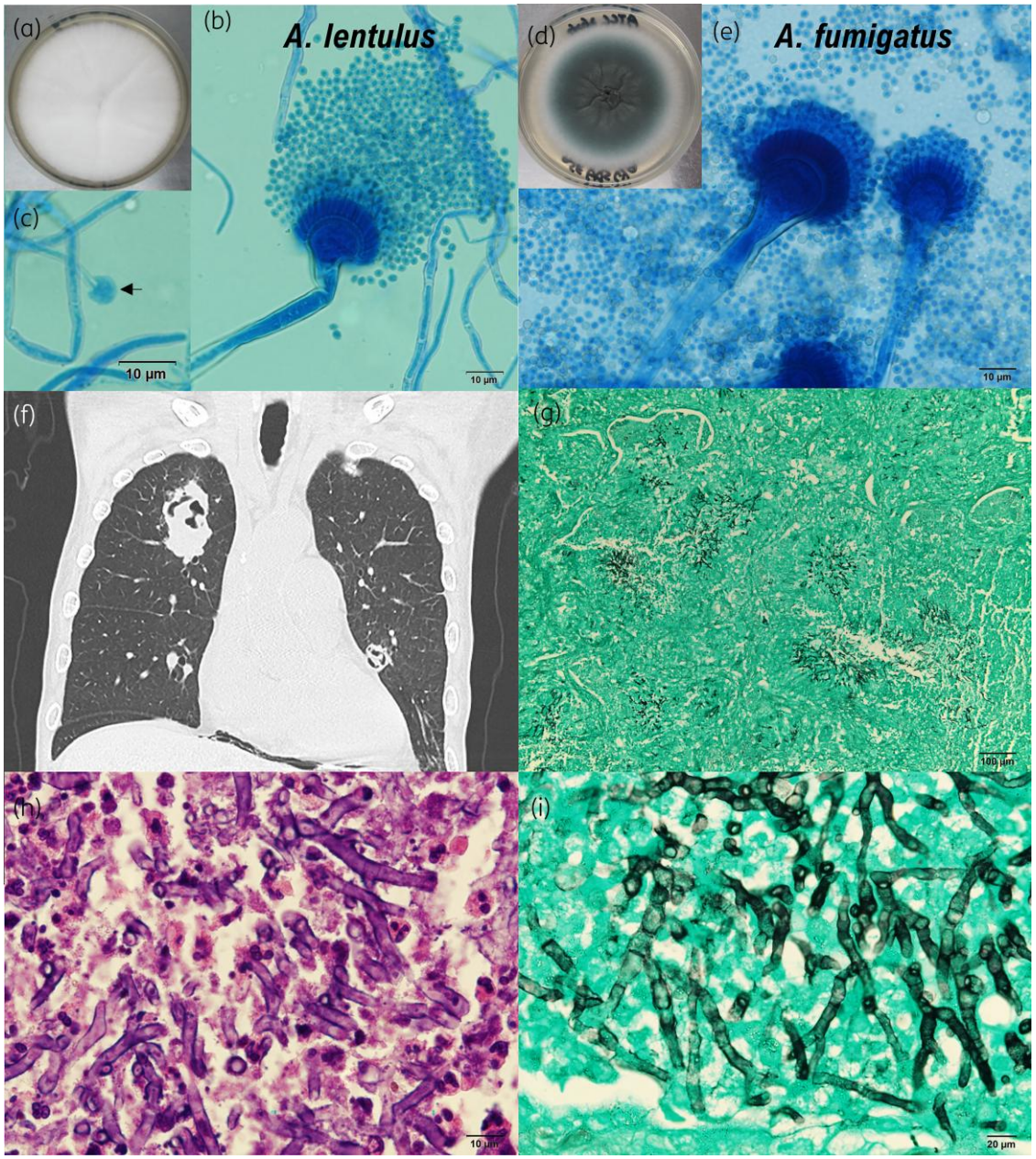

Figure 1. Mycological features of *A. lentulus* NCK0965 from Patient A. Colony and microscopic appearance with lactophenol cotton blue staining of NCK0965 (a–c) after incubation for 5 days and *A. fumigatus* ATCC MYA-3626 (d, e) after incubation for 3 days on SDA at 28°C. Both *A. lentulus* and *A. fumigatus* display uniseriate conidiophores with columnar conidial heads, but the *A. lentulus* colony appears white-coloured due to slow sporulation (a). Mature conidiophores bearing conidia (b) were rarely found for *A. lentulus*, but numerous developing conidiophores (arrow) (c) were identified. Computed tomography imaging at 4 weeks after heart transplantation revealed bilateral cavitary lung lesions (f); histopathology with Grocott's methenamine silver staining (100× and 1000× magnification) (g, i) and haematoxylin and eosin staining (1000× magnification) (h) revealed multiple foci of the infected lung tissue invaded by septate, acute angle branching fungal hyphae.

Discussion

This study reports a low prevalence of *A. lentulus* among clinical and environmental samples. Nevertheless, it is worth noting that these isolates exhibited reduced susceptibility to AmB and both medical and agricultural azoles. Additionally, the clinical isolates were associated with life-threatening pulmonary aspergillosis, with two patients showing an inadequate response to azole monotherapy.

Although less explored, environmental isolates of *A. lentulus* have been recovered from various sources, including air, beach sands, forest soils, cocoa beans, *Coffea* spp., soils from maize or pepper fields and soils treated with herbicides.[10,11] Additionally, *A. lentulus* with reduced susceptibility to penconazole has been

recovered from vineyard soils that were either not exposed to azoles or treated with penconazole.[12] Our study recovered two *A. lentulus* isolates from fruit farmland soils where no or minimal azole fungicide residues were detected.[12] These observations suggested that *A. lentulus* could be found in both azole-treated and azole-untreated environments.

A search on the NCBI database for Cyp51A of *A. lentulus* revealed two major amino acid sequence patterns, represented by strains NIID0096 and CM1290.[9,13] Using NIID0096 as a reference, SNPs M11T, Y29F and H352Q were found in CM1290 and in strains from the USA and South Korea (Table S1). Previous genetic experiments showed that the Cyp51A of *A. lentulus* NIID0096 and CM1290 are responsible for their voriconazole resistance, with MICs of 2 and 4 μg/mL, respectively.[9,13] In this study, the Cyp51A sequences of six *A. lentulus* isolates are identical to that of NIID0096, explaining their observed voriconazole resistance. Additional amino acid substitutions (A9P or V23A) in the remaining three isolates are unlikely to contribute to resistance, as they are located away from the voriconazole binding site of Cyp51A.[14] However, whether *A. lentulus* Cyp51A is responsible for cross-resistance to difenoconazole and tebuconazole warrants further studies. While the environmental use of azole fungicides has been linked to acquired azole resistance in *A. fumigatus*,[15] this may not be the case in reduced azole susceptibility observed for *A. lentulus*, given the high frequency of azole resistance reported in both clinical and environmental *A. lentulus* isolates to date.[2,10,12] Although the trait associated with resistance development was not explored here, the shared *Cyp51A* sequence between environmental isolates and clinical isolates from azole-naïve patients suggests an environmental origin for drug-resistant *A. lentulus* infections.

In this study and previous reports summarized in Table S2, most *A. lentulus* isolates displayed immediate resistance or resistance to voriconazole (MICs 1–16 μg/mL), reduced susceptibility to AmB (MICs 1–8 μg/mL) and posaconazole MICs ranging from 0.125 to 1 μg/mL. Patient A initially showed a suboptimal response to voriconazole monotherapy but was cured with a combination of voriconazole–caspofungin and LAmB–caspofungin therapy, along with surgery, followed by voriconazole maintenance therapy. Patient C deteriorated despite sequential isavuconazole and LAmB monotherapy. These observations align with the 2017 ESCMID-ECMM-ERS recommendation, which advises against azole monotherapy for *A. lentulus* infection.[16] Notably, a voriconazole–echinocandin combination has been recommended for IPA caused by *A. fumigatus* with a voriconazole MIC ≥ 2 μg/mL.[16] In the literature, three cases with *A. lentulus* infections showed improvement with voriconazole–echinocandin combination therapy (voriconazole MICs 2–4 μg/mL), and one patient responded to micafungin–LAmB combination therapy (AmB MIC 2 μg/mL) (Table S2).[17–19] Collectively, combination therapy appears to be a reasonable approach for managing *A. lentulus* infections. It is also worth noting that high-dose posaconazole with serum trough levels exceeding 3 mg/L has been successful in treating azole-resistant aspergillosis and other mould infections caused by isolates with posaconazole MICs of 0.25–2 μg/mL.[20] Further studies are needed to determine whether posaconazole is a feasible treatment option and to establish optimal therapeutic strategies based on MIC values of AmB and azole drugs in correlation with clinical outcomes. Given the suboptimal response to voriconazole monotherapy, early species identification and antifungal susceptibility testing are crucial. *A. lentulus* should be suspected in fluffy, whitish colonies that fail to grow at 48°C. It can be identified presumptively by MALDI-TOF MS and confirmed through calmodulin or β-tubulin sequencing.[2]

In conclusion, this study raises concerns about health threat posed by human pathogenic *A. lentulus* originating from natural environments and underscores the need for increased clinical and laboratory vigilance regarding *A. lentulus* infections.

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Transparency declarations

All authors have no competing interests to declare.

Ethical approval

The study was approved by the Institutional Review Board of CMMC (IRB numbers 10607-L01, 10707-L05 and 10711-L03), NCKUH (IRB numbers B-ER-101-342, B-ER-104-057 and B-ER-109-364) and NTUH (IRB number 202203102RINC).

Supplementary data

Figure S1 and Tables S1 and S2 are available as Supplementary data at *JAC-AMR* Online.

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