Invasive Pulmonary Aspergillosis Due to *Aspergillus Lentulus* in a Liver Transplant Recipient: a Case Report and Literature Review

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Case report

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Abstract

Background: *Aspergillus lentulus*, a new species discovered in 2005, is a rare fungal pathogen with reduced sensitivity to antifungal agents.

Case presentation: Here we reported a 58-year-old male who underwent a liver transplantation on January 2020 and followed by maintenance immunosuppressive therapy. Then *A.lentulus* was cultured from his sputum twice and identified by using the MALDI-TOF-MS and confirmed by the reference of RUO database. In the susceptibility tests of the isolate, minimal inhibitory concentrations for amphotericin B, voriconazole, posaconazole, and caspofungin were found to be 0.5 mg/L, 1.0 mg/L, 0.5 mg/L and 8 mg/L, respectively. The patient was improved after treatment with a voriconazole 0.2g q12h orally plus caspofungin 50mg qd intravenously.

Conclusion: *A. lentulus i*s easily confused with A. fumigatus and its susceptibility to amphotericin B and azoles antifungal agents is reduced. Therefore, the identification of strain and drug susceptibility test are very important for clinical treatment.

Background

Aspergillosis have become a major problem among immunocompromised patients, and always led to life-threatening infection in patients with prolonged neutropenia, allogeneic hematopoietic stem cell transplant (HSCT), solid organ transplant (SOT), corticosteroid use, and others[1]. In SOT recipients,invasive fungal disease is now a major cause of postoperative death[2].

Aspergillus species are widely found in soil, air samples and decayed materials all over the world[3]. Human infection due to *Aspergillus* species was mainly caused by typical *A. fumigatus* isolates. However recent taxonomic studies showed that the *Aspergillus* genus includes at least eight subgenus, subdivided into 22 complexes or sections. The main sections of medical importance are *Fumigati*, *Flavi*, *Terrei*, *Nigri*, *Usti*, and *Nidulantes*, which can also cause invasive infections[4, 5].

Aspergillus lentulus was first found by Balajee and his colleagues[6] in a medical center in the United States, and subsequently, the species was also isolated from patients in other countries as well as environmental samples. The study showed this pathogen morphologically closely resembling *A. fumigatus*, once has been misidentified as a variant of *A. fumigatus* sensu stricto and was found to exhibit decreased antifungal susceptibilities[7].

Herein we report a probable pulmonary invasive aspergillosis caused by *A. lentulus* in an adult patient after liver transplantation, which was successfully treated with voriconazole plus caspofungin.

Case Presentation

A 58-year-old Chinese male with chronic kidney failure and a 20-year history of hepatitis B was submitted to the hospital because of decompensation liver cirrhosis, then he underwent liver transplantation on January 2020 and followed by maintenance immunosuppressive therapy consist of high-dose methylprednisolone, tacrolimus, and mycophenolate mofetil. No graft rejection occurred later. On post-transplantation day 5, severe anuria and hyperkalemia led to renal replacement therapy. At day 26 after transplantation, multi-drug resistant *Klebsiella pneumoniae* was isolated from sputum culture, which was treated with ZAVICEFTA (Ceftazidime/Avibactam) 0.94g every 12 hours for 14 days considered his renal function. His temperature was 36.8°C, laboratory investigation showed leucocyte count was 6.47*10^9/L, neutrophil percentage was 88.8%, C-reactive protein (CRP) was 71.2 mg/L and procalcitonin (PCT) was 1.96 ng/mL. An X-ray showed: Exudation in both lungs, thickness of blood vessels in both hilar, pleural effusion. At day 33 after transplantation, *Enterococcus faecium* was isolated from blood culture, then we treated with vancomycin started with a 1 g loading dose (LD) and 0.5 g maintain dose (MD) once daily. At day 36 after transplant, a chest x-ray performed because of his persistent signs of inflammation, but without fever, showed a new infiltrate in the right pulmonary. The lung computed tomography (CT) scan also demonstrated bilateral inflammatory infiltrate. Two respiratory samples (sputum) were obtained. At this time, the patient had no fever and his temperature was 36.8°C, his laboratory tests showed anemia (hemoglobin 7.6 g/dL), leukocytes 11.05*10^9/L, neutrophils 92.9% and thrombocytopenia (38*10^9/L), serum creatinine 134 μmol/L (normal range 44-115 μmol/L), and serum urea 23.4 mmol/L (normal range 2.9-8.2 mg/dL), CRP was 60.1 mg/L and PCT was 0.86 ng/mL. The (1- 3)-β-D-glucan (BDG) level was 23.6 pg/ml and GM test was 0.808ng/mL. Then *A. lentulus* was isolated twice from both of these sputum specimens. Invasive pulmonary aspergillosis was diagnosed according to host factors (liver transplantation and immunosuppression), radiological, and microbiological findings.

In order to recover *Aspergillus* species from the sputum specimens, 0each sample was inoculated onto two Sabouraud dextrose agar (Autobio Diagnostics, China). These plates were incubated at 25°C and 35°C for growth. The original colonies were then subcultured onto another SDA agar and incubated at 35°C for observation of their macroscopic and microscopic morphology.(Fig. 1,see Additional file Figure_1) The surface of the colonies was at first white, then turning cottony and greenish with a white border. The reverse was white (Fig. 1A). Fluorescence microscopy was used to determine the microscopic morphology of the isolates. Branched conidiophore structures and reduced sporulation were observed at 400x magnifications (Fig. 1B). MALDI-TOF-MS (bioMerieux, France) was used for the identification of the isolates which revealed the identity of 75.9% to the *A. lentulus* reference structure according to the RUO database.

Antifungal drug susceptibility toward itraconazole, voriconazole, posaconazole and amphotericin B was tested on the isolate according to Clinical Laboratory Standards Institute guideline M61 using Sensititre^{TM} YeastOneTM YO10 AST plate (ThermoFisher Scientific, USA), which revealed minimum inhibitory concentrations (MIC) of 0.5 mg/L, 1.0 mg/L, 0.5 mg/L and 8 mg/L, respectively. Antifungal therapy with a voriconazole LD of 0.4g q12h followed by a MD of 0.2g q12h orally plus caspofungin 70 mg (LD) followed by 50mg MD intravenously was started immediately. Four days after start of voriconazole, the steady-state serum trough level was 2.1mg/L. Thirteen days after initiation, sputum culture turned negative and laboratory tests showed a reduction in leukocytes

7.2*10^9/L, neutrophils 82.7%, CRP 48.3 mg/L and PCT 1.27 ng/mL, but an increase in BDG level (53.5 pg/ml). However repeated CT scan showed a reduction of the lesions, and the patient's condition improved gradually and recovered eventually.

Conclusions

To our knowledge, this is the first case of invasive pulmonary aspergillosis after liver transplantation in China caused by *A. lentulus*. In 2004, Balajee[6] first reported a distinct variant of *A. fumigatus* that causes invasive infection in four HSCT recipients and demonstrated decreased susceptibilities to multiple antifungal drugs. The isolates sporulated slowly and needed prolonged incubations to be similar to typical isolates. Sequencing showed the mitochondrial cytochrome b gene sequences of all the new isolates were unique, which suggested the potential presence of a genetically unique. Phenotypic methods revealed that the variant isolate has smaller conidial heads with diminutive vesicles compared to *A. fumigatus* and is not able to survive at 48°C, which was then designated *A. lentulus*[7]. From then on, this new species has been found in soil, environmental samples[8] and was reported in patients in America, Japan, Spain, Brazil and some other countries, described as a new sibling species of *A. fumigatus* due to their morphological resemblance.

According to the literature[9], approximately 4-5% of "*A. fumigatus*" isolated from patients have later turned out to be the related species, due to their morphological resemblance, which makes the accurate identification of *A. lentulus* difficult for clinical microbiology laboratories. Therefore, the reported prevalence of *A. lentulus* might be underestimated. Hence, molecular analysis is required for the identification of *A. lentulus*. Tamiya and his colleagues[10] used liquid chromatography/time-of-flight mass spectrometry to identify the secondary metabolites secreted as virulence factors by *A. lentulus*. Among all the secondary metabolites, auranthine in conidia and culture filtrate was only found in *A. lentulus*, but not in *A. fumigatus* or the other related species. In culture filtrate, the *A. fumigatus* isolates produced significantly higher amounts of gliotoxin compared with other species including *A. lentulus*.

Since the first documented description, only 11 cases (except the present one) of IA caused by *A. lentulus* have been reported around the world, related to 17 patients. The characteristics and details of the cases in the literature are shown in Table 1. (see Additional file Table_1)

The median age at diagnosis was 50 years (range, 12-82 years). Except two cases with incomplete information, nine patients (77.8%) were male in the remaining 9 cases. Among the 17 patients, 8 (47%) had a history of HSCT, 5 (29.4%) experienced SOT, and 4 patients had other underlying diseases (COPD, cystic fibrosis, refractory nephrotic syndrome, ANCA-associated vasculitis). *A. lentulus* and *A. fumigatus* were isolated simultaneously in one patient with COPD. All SOT patients received immunosuppressive therapy and three patients with no history of transplantation, also received immunosuppressive therapy including glucocorticoids. 6 (35.2%) patients had a history of antifungal drug usage before diagnosis.

Except two cases with incomplete information, voriconazole and amphotericin B (including amphotericin B liposomes) were used in four (4/9, 44.4%) and four (4/9, 44.4%) cases as primary antifungal treatment, respectively. However, 6 patients (6/9, 66.6%) showed a fatal clinical outcome.

As we all know, *A. fumigatus* are intrinsically sensitive to azoles and amphotericin B, but *A. lentulus* isolates usually have higher MIC values for these drugs and caspofungin, and even show primary resistance to azole drugs. Nowadays, the most common acknowledged mechanism concerning azole resistance in *A. lentulus* is mutations in the cyp51A gene. Mellado[11] performed heterologous expression in an *A. fumigatus* cyp51A deficient strain, confirming that cyp51A is responsible for the differences in *A. lentulus*-azole drug resistance. Since there is no breakpoint for *A. lentulus* according to CLSI and EUCAST, and the breakpoint of *A. fumigatus* cannot be referred to, so we did some therapeutic drug monitoring to keep the voriconazole trough concentration above 1.0 mg/L.

According to other researches, Tamiya[10] tested drug susceptibility for *A. fumigatus* and all three related species including *A. lentulus*, revealed that amphotericin B was effective against all four species. In contrast, *A. lentulus* had higher MICs to azoles such as voriconazole and itraconazole. Environmental isolates had decreased susceptibility to voriconazole and itraconazole than clinical isolates. As pointed out by Mortensen[12], susceptibility tests indicated that *A. lentulus* demonstrates higher MICs to amphotericin B, itraconazole and voriconazole but a MIC of 0.5 for posaconazole. Interestingly, the susceptibility characteristics of the isolates in our case are slightly different from the susceptibility reported in the published literature. It showed higher MICs to amphotericin B (MIC, 8 mg/L), but normal MICs to voriconazole (MIC, 1.0 mg/L), itraconazole and posaconazole (MIC, 0.5 mg/L). This highlights the importance of microorganism identification and antimicrobial susceptibility testing. However, it should be understood that high in vitro MICs may not only necessarily consist with decreased susceptibility in vivo, clinicians and laboratories should also take notice of patients' poor condition, drug dose, frequency and so on. In our present case, we administered voriconazole (LD 0.4g q12h, MD 0.2g q12h) plus caspofungin (LD 70mg once,MD 50mg qd), and kept the voriconazole plasma concentration within the recommended range (1.0-5.5mg/L) by therapy drug monitoring. Finally, his sputum culture turned negative and laboratory tests reduced gradually, repeated CT scan showed a reduction of the lesions, and the patient improved eventually.

In conclusion, here we reported the first case of probable invasive aspergillosis caused by *A. lentulus* after liver transplantation in our country, which showed a good clinical response to voriconazole and caspofungin. We exemplify the important role of accurate species identification and antimicrobial susceptibility testing due to the differential MICs of *Aspergillus*-related species. More clinical features and treatment of infection with *A. lentulus* should be discussed in the future.

Abbreviations

HSCT: hematopoietic stem cell transplant; SOT: solid organ transplant; CRP: C-reactive protein; PCT: procalcitonin; LD: loading dose; MD:maintain dose; CT: computed tomography; BDG: β-D-glucan; MIC: minimum inhibitory concentrations.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Zhongshan Hospital, Fudan University (Approval No. B2021-054).

Consent for publication

Written informed consent was obtained from our patient to publish this case report and its accompanying images.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the study conception. YYZ performed the microbiological cultures, microscopic identification, molecular analyses and antifungal susceptibility testing; YS and XPS contributed to data collection and literature search; TW and XPS helped to treat the patient; YS was a major contributor in writing the manuscript and all authors commented on previous versions of the manuscript; YS, XPS and JX contributed to the final manuscript review. All authors have read and approved the final manuscript.

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Table

Table 1 Summary on published clinical cases of IA caused by *A. lentulus*

year	patient	underlying condition	prior antifungal therapy	Immuno-suppressive drugs	infection site	disease classification	susceptibility pattern (MIC)	treatment
2004[6]	American Four patients	HSCT	FLU+AMB FLU FLU AMB+VRC	—	lung+kidney lung+mouth lung lung+mouth	proven or probable	ITRC 0.5-1 VRC 2 AmB1-2 ITRC 1 VRC 2 AmB1-2ITRC1 VRC 2 AmB2 ITRC 1 VRC 2 AmB1-2	Not reported
2008[13]	Spain 68y male	COPD	—	GC	sputum	probable	AmB 4 ITRC 3 VRC 1.5 CPFG>32	VRC
2009[14]	American Four patients	HSCT	—	—	Lung	proven or probable	ITRC 0.5 VRC 4 AmB 2 POSC 0.25ITRC 0.5 VRC 2 AmB 2 POSC 0.25ITRC0.25 VRC 1 AmB 0.5 POSC 0.25 ITRC 0.5 VRC 4 AmB 2 POSC 0.25	Not reported
2009[15]	Argentina 43y female	Kidney transplant	AmB	MMF CsA GC	Lung (BALF)	probable	VRC 2 AMB 2 CPFG16	L-AmB 5g over 30 days
2010[16]	France 20y male	CF	ITRC	—	sputum	colonization	ITRC 2 VRC 8 AmB 4	Not reported
2012[17]	Switzerland 68y male	Heart transplant	—	GC CsA MMF	Lung (BALF, sputum)	proven	ITRC 0.25 VRC 1 POSC0.125 AmB 2 CPFG MEC16	VRC→AmB+CPFG→POSC
2013[18]	Turkey 36y male	Kidney transplant	—	FK506 MMF GC	Lung (sputum, BALF)	proven	AmB 0.5 VRC 0.25 PSC 0.125 CPFG 0.25	VRC 2*200mg/day
2015[19]	Japan 62y male	Liver transplantation	—	GC FK506	Lung (BALF, tracheal specimens)	proven	ITRC 0.5 VRC 2 AmB 4 MCF MEC <0.015	LAmB→+LAmB+CPFG→CPF
2015[10]	Brazil 61y male	Kidney transplant	—	GC sirolimus	Lung	proven	ITRC 2 AmB 2 VRC 4	empirical AmB
2017[20]	Japan 12y boy	refractory nephrotic syndrome	—	Rituximab MMF GC	Lung (sputum, BALF)	probable	AmB 4 VRC 4 FLU>64 MCF MEC 0.015.	VRC100mg bid →MCF
2019[21]	Japan 82y female	AAV	—	GC	Lung (BALF)	proven	—	L-AmB→VRC 150mg bid

HSCT, hematopoietic stem cell transplantation; COPD, chronic obstructive pulmonary disease; CF, cystic fibrosis; AAV, ANCA associated vasculitis; VRC, vorico fluconazole; AmB, amphotericin B; LAmB, liposomal amphotericin B; ITRC, itraconazole; POSC, posaconazole; CPFG, caspofungin; MCF, micafungin; GC, gluc mycophenolate mofetil; CsA, cyclosporine; FK506,tacrolimus; BALF, bronchoalveolar lavage fluid; MIC, minimum inhibitory concentration; MEC, minimum effe

The characteristics and details of the 11 cases caused by *A. lentulus* in the literature reported around the world since the first documented description.

Figures

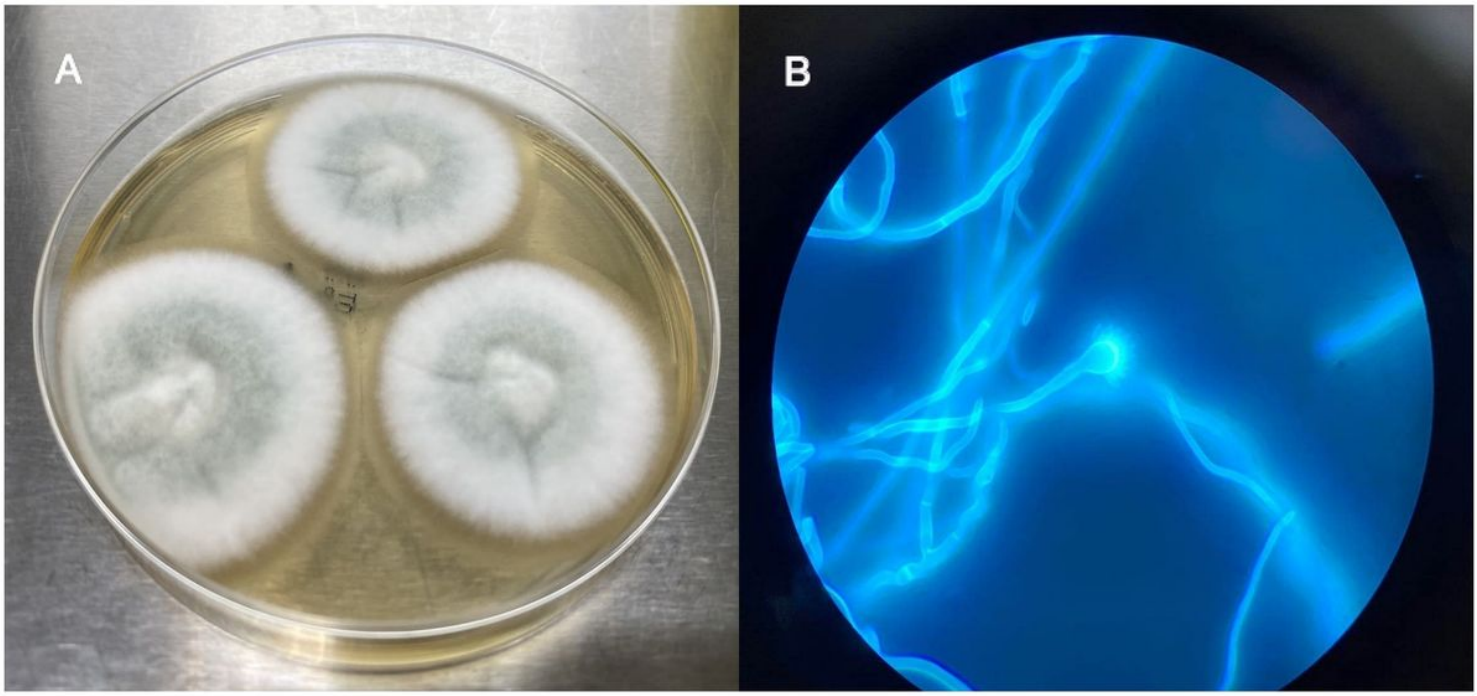

Figure 1

Macroscopic view colonies after incubation A: Aspergillus lentulus: macroscopic morphology after 5-days of incubation at 35°C; B: Aspergillus lentulus: microscopic morphology under fluorescence microscope.