Triazole-resistant *Aspergillus luchuensis*, an industrially important black *Aspergillus* spp. used in fermentation in East Asia, isolated from the patient with invasive pulmonary aspergillosis in China

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**ABSTRACT**

*Aspergillus luchuensis*, an industrially important member of *Aspergillus* species belonging to section *Nigri* used in fermentation in East Asia, was isolated from an immunocompromised patient with probable invasive pulmonary aspergillosis who failed voriconazole therapy in China. This isolate showed non-wild-type susceptibility to itraconazole, voriconazole, isavuconazole, and posaconazole. A G1378A mutation in *cyp51A*, resulting in the G441S amino acid substitution, which is the homolog to G448S conferring triazole-resistance in *A. fumigatus*, was detected in the *A. luchuensis* isolate.

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**Introduction**

*Aspergillus* species (spp.) are the causative pathogens of invasive aspergillosis (IA) with considerable morbidity and mortality. Although *Aspergillus fumigatus* continues to be the most prevalent spp., other *Aspergillus* spp. such as *Aspergillus* section *Nigri* have been increasingly recognized to cause invasive disease [1]. *Aspergillus* section *Nigri* is widespread in the environment and used in industrial manufacture to produce pharmaceuticals, food ingredients, and enzymes [2]. *A. luchuensis*, a member of *Aspergillus* section *Nigri*, is widely used in food fermentation in East Asia, such as meju and nuruk in Korea, awamori in Japan, and Puerh tea in China [2]. *A. luchuensis* is often associated with otomycosis [3] and is not reported to cause IA. Here, we report the first case of probable invasive pulmonary aspergillosis (IPA) caused by *A. luchuensis* exhibiting triazole-resistance with a G441S mutation in *cyp51A* gene.

**Methods and results**

A 60-year-old male patient complained of recurrent cough with bloody sputum for seven months. He also suffered from myalgia, tinnitus, and hearing loss. Chest computed tomographic (CT) scan demonstrated bilateral pulmonary masses. Anti-neutrophil cytoplasmic/proteinase-3 antibodies (c-ANCA/PR3) test was positive. Granulomatosis with polyangiitis (GPA) was diagnosed. He was initially treated with oral prednisone (1 mg/kg/d). Five months later, he developed a deteriorating cough with brown sputum. CT scan revealed bilateral pulmonary cavitary lesions. The sputum sample was culture positive for *Aspergillus* spp. Therefore, the diagnosis of IPA was suspected [4] and oral voriconazole (VRC, 200 mg twice daily) was initiated. Prednisone was continued for the GPA treatment. However, the cough with sputum persisted, and breathlessness and fever developed. A bronchoscopy was performed and bronchoalveolar lavage fluid (BALF) was culture positive for *Aspergillus* spp. which was identified by macroscopic and microscopic
characteristics on potato dextrose agar (PDA), Czapek agar (CZA) and malt extract agar (MEA) at 25°C for 7 days (Figure 1(A)), and by sequencing of β-tubulin and calmodulin genes (GenBank accession number: MZ028459, MZ028460). The isolate was identified as *A. luchuensis* (ID number BMU10878). Because of the poor response to VRC, antifungal susceptibility of BMU10878 to itraconazole (ITC), VRC, amphotericin B (AMB), caspofungin (CAS), posaconazole (POS), and isavuconazole (ISA) was determined using E-test and disk diffusion (Figure 1(B)).

**Figure 1.** (A) Morphology of *A. luchuensis* BMU09478 and BMU10878 following 7-day-culture at 25°C. PDA: black granular colony; CZA: cottony, brown-yellow colony; MEA: velvet-like, yellow-green colony; corolla-like conidial heads with conidiogenous cells; scattered spores. (B) Antifungal susceptibilities of BMU09478 and BMU10878 to ITC, VRC, POS, AMB, CAS determined by E-test; ITC (80 μg), VRC (10 μg), POS (10 μg), ISA (80 μg) determined by disk diffusion. ITC, itraconazole; VRC, voriconazole; POS, posaconazole; ISA, isavuconazole; AMB, amphotericin B; CAS, caspofungin. (C) (a) Crops are exposed to triazole fungicides. (b) Crops applied with fungicides are further fermented with *A. luchuensis*. (c) Triazole-resistant isolates of *A. luchuensis* are selected by residues of the fungicides. (d) Triazole-resistant spores inhaled by immunocompromised patient causing IPA and failing in triazole-therapy.
posaconazole (POS), isavuconazole (ISA), amphotericin B (AMB), caspofungin (CAS) was determined by the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) M38-A3 document [5]. Another isolate of *A. luchuensis*, BMU09478 was included for comparison. According to the epidemiological cutoff values (ECVs) for *A. niger* [6], no clinical breakpoints (CBPs) and ECVs were established for *A. luchuensis*. BMU10878 showed non-wide-type susceptibility to ITC, VRC, ISA (all MICs > 16 μg/mL), and POS (MIC = 1 μg/mL), while the MICs of ITC, VRC, POS, ISA against *A. luchuensis* BMU09478 control isolate were 0.25 μg/mL. The MICs of AMB against both isolates were 2 μg/mL. Additionally, E-test and disk diffusion were performed and the results (Figure 1(B)) were consistent with those observed by the broth microdilution method. BALF galactomannan (GM) was 15.23 and with those observed by the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) M38-A3 document [5]. Another isolate of *A. luchuensis*, BMU09478 was included for comparison. According to the epidemiological cutoff values (ECVs) for *A. niger* [6], no clinical breakpoints (CBPs) and ECVs were established for *A. luchuensis*. BMU10878 showed non-wide-type susceptibility to ITC, VRC, ISA (all MICs > 16 μg/mL), and POS (MIC = 1 μg/mL), while the MICs of ITC, VRC, POS, ISA against *A. luchuensis* BMU09478 control isolate were 0.25 μg/mL. The MICs of AMB against both isolates were 2 μg/mL. Additionally, E-test and disk diffusion were performed and the results (Figure 1(B)) were consistent with those observed by the broth microdilution method. BALF galactomannan (GM) was 15.23 and with those observed by the broth microdilution method. Hence, we cannot rule out the possibility that the mutation was acquired in the environment, since *A. luchuensis* is widely used in fermentation and can be exposed to agricultural azoles during growth and storage of the fermentation products. The resulting spores with triazole-resistance could be inhaled by immunocompromised patients to cause IPA without triazole-therapy (Figure 1(C)). For IPA caused by those isolates of *Aspergillus* spp. with triazole-resistance, liposomal-AMB has strongly been recommended [1]. And the case in this report has also confirmed that liposomal-AMB is an effective alternative for the treatment of triazole-resistant IPA.

In conclusion, triazole fungicides being applied to fermentable crops may be a potential driver of triazole-resistance in industrial *Aspergillus* spp. used for fermentation. Testing of these and other environmental isolates could help to confirm an environmental route of resistance selection. If confirmed, our observation would provide evidence for fungicide resistance selection beyond *A. fumigatus*, with implications for antifungal stewardship both in environmental and clinical use of triazole compounds.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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