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CAPA Case Report

## Multi-triazole-resistant *Aspergillus fumigatus* and SARS-CoV-2 co-infection: A lethal combination

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### ABSTRACT

We report a case of severe COVID-19 pneumonia complicated by fatal co-infection with a multi-triazole resistant *Aspergillus fumigatus* and highlight the importance of recognising the significance of *Aspergillus* sp. isolation from respiratory samples. Early diagnosis and detection of triazole resistance are essential for appropriate antifungal therapy to improve outcome in patients with coronavirus associated invasive aspergillosis.

### 1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing COVID-19 infection, is a newly recognised pathogen that has been traced to Wuhan (Hubei province) in China [1]. The clinical spectrum of COVID-19 varies from asymptomatic infection to severe pneumonia requiring mechanical ventilation. The overall case fatality rate is estimated to be 2.7%, which increases to 4.5% in those  $\geq 60$  years old [2]. Secondary infections with bacterial and fungal pathogens have been reported in patients with COVID-19 pneumonia which may be due to virus induced mucosal damage and/or the dysregulated immune response seen in patients with acute respiratory distress syndrome [3,4].

We report severe COVID-19 pneumonia with multi-triazole-resistant *Aspergillus fumigatus* co-infection in a patient not known to be immunocompromised to highlight the importance of early diagnosis and detection of triazole resistance.

### 2. Case

A 66-year-old male presented to the emergency department with a history of progressive shortness of breath (SOB), myalgia, headaches, non-productive cough and fever.

Two days previously, he had contacted his general practitioner (GP) describing a seven-day history of fever and cough. He had returned from the United Kingdom eight days earlier after visiting a relative who was subsequently diagnosed with COVID-19. He was advised to self-isolate and monitor his symptoms. Due to progressive SOB, he re-presented to his GP who referred him to the hospital for further management.

The patient had Type 2 diabetes mellitus, hypertension, hyperlipidaemia and obesity with a body mass index of 35.5 (weight 90kg) with no hospital admissions in the past eight years. His medications included metformin, aspirin, simvastatin and ramipril. He had no history of prior triazole antifungal therapy. He was an ex-smoker but had not been previously diagnosed to have chronic lung disease. He works as a ground maintenance personnel where he is exposed to fungicides daily.

On presentation, a portable chest radiograph was performed which showed unilateral peripheral left basal airspace shadowing (Fig. 1A). In view of the clinical presentation, radiographic findings and the recent exposure history, the patient was admitted under contact and droplet precautions.

On admission (Day 7 of COVID-19 illness), the patient was noted to be pyrexial (temperature of 38.6 °C), tachycardic (pulse rate of 174 beats per minute) with new onset atrial fibrillation, normotensive (BP

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117/69 mmHg), tachypnic (24 breaths per minute) with oxygen saturation of 84% on room air. He was commenced on a trial of continuous positive airway pressure with oxygen therapy. Preliminary laboratory results revealed white cell count of  $4.7 \times 10^9/L$  (reference range  $4.0\text{--}11.0 \times 10^9/L$ ), neutrophil count  $3.0 \times 10^9/L$  (reference range  $2.0\text{--}7.0 \times 10^9/L$ ), lymphocyte count  $0.8 \times 10^9/L$  (reference range  $1.0\text{--}3.0 \times 10^9/L$ ) and C-reactive protein of 118 mg/L (reference range  $< 5.0$  mg/L). Nasopharyngeal and oropharyngeal swabs detected SARS-CoV-2 using real-time reverse transcriptase polymerase chain reaction on day 3. He was commenced on azithromycin (500 mg on day 1 and 250 once daily on days 2 and 3) and hydroxychloroquine (200 mg twice daily) orally as per our local protocol at that time.

On day 4 (Day 11 of COVID-19 illness) he developed worsening hypoxic respiratory failure with a Sequential Organ Failure Assessment (SOFA) score of 6 necessitating intensive care unit (ICU) admission for ventilatory support. The patient was prone initially for 15 hours which did not improve his oxygenation. This, combined with significant hemodynamic instability, were contraindications for further proning. His condition continued to deteriorate progressing to multi-organ failure on day 7 (Day 14 of COVID-19 illness) which included acute respiratory failure, acute kidney injury requiring continuous renal replacement therapy and vasopressor support due to septic shock. His SOFA score increased to 12. He had continuing pyrexia and copious amounts of purulent respiratory secretions prompting a repeat septic screen which included culture of blood and endotracheal aspirate (ETA).

Empiric antimicrobial therapy for hospital-acquired pneumonia with intravenous (IV) piperacillin-tazobactam (4.5g three times a day) was started. Subsequently, ETA culture grew *Klebsiella varicola* (susceptible to piperacillin-tazobactam), *Aspergillus* sp. and *Candida albicans*. Antifungal therapy with IV liposomal amphotericin B (3 mg per kg once daily) was commenced due to the patients rapidly deteriorating status and uncertainty of the full identification of the mould isolate which may be resistant to triazoles. The *Aspergillus* sp. was referred for further identification and antifungal susceptibility testing, as were serum samples for detection of 1–3,  $\beta$ -d-glucan (BDG) antigen and galactomannan (GM) antigen and ETA for GM antigen.

His SOFA score on day 11 (Day 18 of COVID-19 illness) increased to 16. Antibiotic therapy was escalated to IV meropenem (1g twice daily) and vancomycin, with dosing based on renal function, due to ongoing pyrexia and deteriorating status. He was not prescribed systemic steroids at any time during his critical illness.

A repeat portable chest radiograph on day 12 (Day 20 of COVID-19 illness) showed progression to additional right upper and lower zone peripheral airspace shadowing with no evidence of cavitation (Fig. 1B).

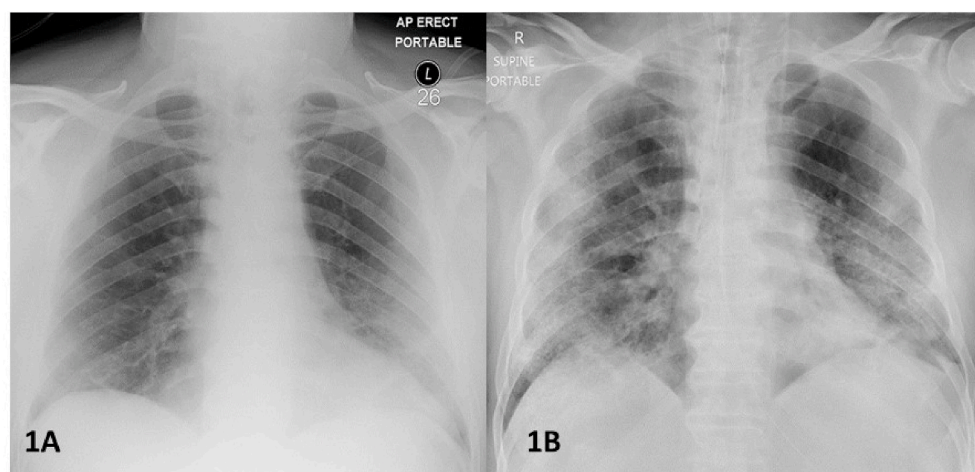
He remained haemodynamically unstable which precluded further radiological investigations. In view of his non-responsive multi-organ failure, and after discussion with his family, the patient's life-support was withdrawn. He died on day 14 of hospitalization (Day 22 of COVID-19 illness).

The *Aspergillus* sp. isolated from the ETA was confirmed to be *A. fumigatus*. Phenotypic testing utilising a 4-well triazole resistance screen (VIP check™, Mediaproducts BV, The Netherlands) (Fig. 2A and B) as well as determination of minimum inhibitory concentrations (MIC) utilising gradient strips (Liofilchem, Waltham MA, USA) were suggestive of triazole resistance (Table 1) [5]. The MIC to amphotericin was 0.125 mg/L (susceptible  $\leq 1$  mg/L) [5]. Genotypic testing utilising a commercial assay (Aspergenius™, Pathonostics B.V. The Netherlands) on the ETA detected *A. fumigati* complex with the TR<sub>34</sub>L98H resistance mutation in the *cyp51A* gene, the most common mutation in triazole-resistant *A. fumigatus* originating from an environmental source. The serum and ETA GM optical density index (ODI) were 1.1 and 5.5 respectively (Platelia™ *Aspergillus* Ag EIA, WA, USA). Serum BDG was 202 pg per milliliter (Fungitell™ Assay, Associates of Cape Cod, USA). These results (Table 1) were consistent with severe COVID-19 pneumonia and triazole-resistant invasive pulmonary aspergillus co-infection.

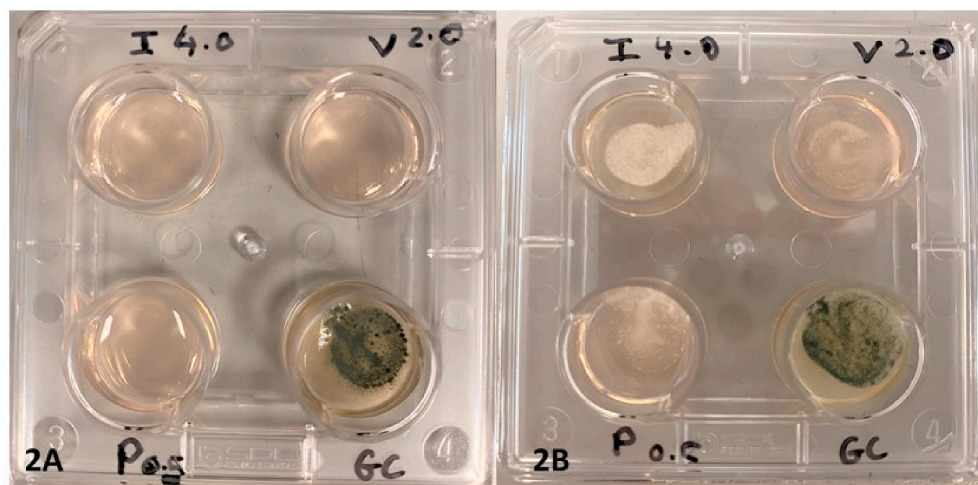
### 3. Discussion

Previously known to cause infections in severely immunocompromised patients, *A. fumigatus* is now recognised as an emerging pathogen in critical care patients suffering chronic respiratory disorders and as a complication of severe influenza infection [6]. *Aspergillus* colonisation can rapidly lead to invasive aspergillosis following severe influenza infection due to multiple pathways including structural lung damage coupled with disruption of mucociliary clearance, leukopenia, Th1/Th2 imbalance and diffuse damage to the respiratory mucosa [7]. In addition, severe respiratory viral infections such as influenza have been identified as an independent risk factor for IPA with high mortality [6]. Although our patient did not receive steroids, their use in critical care patients is linked to increased risk of invasive fungal infections [6]. Like other causes of viral pneumonias, SARS-CoV-2 may impair local mucosal and systemic immune defenses [3].

Thirty-four cases of COVID-19 associated invasive aspergillosis (CAPA) have been reported to date [8–17]. These were all patients with severe pneumonia with adult respiratory distress syndrome, most of whom had no known history of immunocompromise. These has led to challenges in early diagnosis since most of them do not have the classical



**Fig. 1.** A. Portable chest radiograph taken on day of admission showing unilateral peripheral left basal airspace shadowing. B. Portable chest radiograph taken on Day 12 of hospitalization (Day 20 of COVID-19 infection). The endotracheal tube and bilateral central lines are in satisfactory position. There has been interval progression of the left peripheral airspace shadowing with additional right upper and lower zone peripheral airspace shadowing with no evidence of cavitation.



**Fig. 2.** A and B VIPcheck™ plate, a four well triazole resistance screen plate which contains itraconazole (I) 4 mg per liter, voriconazole (V) 2 mg per liter, posaconazole (P) 0.5 mg per liter, and growth control (GC). This is a simple phenotypic test used to screen for triazole resistance due to the common mutations in the *cyp51A* gene in *A. fumigatus* from an environmental source. The photos show a susceptible control strain AF293 (2A) where growth is only seen on GC well and the patient's isolate (2B) with growth in all wells consistent with findings seen in *A. fumigatus* with the *cyp51A* TR<sub>34</sub>/L98H mutation.

**Table 1**  
Laboratory results.

	Patient's Isolate <i>A. fumigatus</i>	*Clinical breakpoint > Resistant
Antifungal Susceptibility Test		
Minimum inhibitory concentration (MIC) mg/L		
Voriconazole	2.0	1
Itraconazole	>32	1
Posaconazole	1.0	0.25
Amphotericin B	0.125	1
Fungal antigens <sup>b</sup>		
Galactomannan Optical Density Index (ODI)	Patient's results	Cut-off
Serum	1.1	≥ 0.5
Tracheal aspirate	5.5	N/A
1,3 β-d-glucan pg/mL		
Serum	202	≥ 80

<sup>a</sup> Based on the European Committee on Antimicrobial Susceptibility testing guidelines.

<sup>b</sup> Fungal antigens: Galactomannan cut-off based on Platelia™ *Aspergillus* Ag EIA and 1–3 β-d-glucan cut-off based on Fungitell™ assay.

risk factors for invasive aspergillosis.

Histopathology and culture from sterile site samples and biopsy remain the gold standard for proven IPA however the definition of probable or “putative” IPA has expanded but remains to be a composite of the host factors, clinical features and mycological evidence [6,18]. Although severe viral pneumonia is not considered a risk factor for IPA or other invasive mould disease according to the European Organisation for Research and Treatment of Cancer/Mycoses Study Group definitions, the structural damage to the lung parenchyma, in addition to the dys-regulated immune response, can lead to IPA [6]. Recently a panel of experts proposed a case definition for influenza associated invasive aspergillosis (IAPA) which may be useful to classify COVID-19 patients with invasive aspergillosis. Following this case definition a patient with COVID-19 detected in respiratory sample by PCR, pulmonary infiltrates and a positive serum or bronchoalveolar lavage galactomannan has criteria consistent with probable CAPA [19]. Our patient had radiographic findings consistent with COVID-19 pneumonia. Unfortunately, his clinical status did not allow for computed tomography (CT) to be performed. Other mycological evidence of IPA include culture of *Aspergillus* sp. from non-sterile sites or detection of fungal antigens such as serum BDG. Our patient had COVID-19 pneumonia, *A. fumigatus* from a tracheal aspirate with elevated serum BDG and GM and elevated ETA GM, findings consistent with COVID-19 and probable IPA co-infection.

Multi-triazole resistance in *A. fumigatus* has recently emerged and is

linked to the use of triazole-containing compounds as agricultural fungicides or less commonly prolonged triazole use [20]. The former mechanism of resistance typically affects azole naïve patients and is characterised by elevated MICs to itraconazole, voriconazole and posaconazole as was found in our patient. This is of serious concern since triazoles are recommended as the first-line and most effective treatment for IPA [21]. Of the 34 cases reported so far, only 7 cases had reported susceptibility results of which one was triazole resistant *A. fumigatus* [16] similar to our case. Our patient's exposure to fungicides daily in his work has most likely led to exposure and colonisation with triazole-resistant *A. fumigatus* which was further supported by detection of *cyp51A* TR<sub>34</sub>L98H mutation from our patient's ETA, the most prevalent triazole resistance mutation from environmental source [20].

This case highlights the importance of early evaluation of patients with COVID-19 pneumonia because of the risk of secondary or co-infection with fungal pathogens. Case definitions have been proposed for IAPA [6,19] which can be modified for early recognition of CAPA. The occurrence of multi-triazole resistance in this case emphasises the urgent need for antifungal drug susceptibility testing of *Aspergillus* isolates using a rapid and simple phenotypic method and/or by detection of *Cyp51* gene associated triazole resistance mutations directly on respiratory samples.

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## Declaration of competing interestCOI

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