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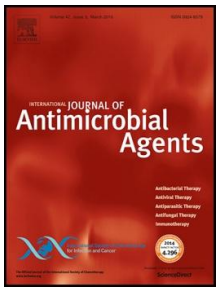

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First isolation of the pan-azole-resistant *Aspergillus fumigatus cyp51A* TR46/Y121F/T289A mutant in a UK patient

Sir,

Antifungal resistance in *Aspergillus fumigatus* owing to a number of mutations has been reported from many regions of the globe. The *cyp51A* gene TR46/Y121F/T289A mutation is an emerging mechanism conferring resistance to azole antifungal drugs. It is unclear whether these mutations are acquired from specific ecological niches or are generated by long-term exposure to azoles during suboptimal therapy. Previous surveys of azole resistance in the UK have not found this mutation [1–3].

A man in his early forties was admitted to the Adult Burns Centre of University Hospital of South Manchester (Manchester, UK) in April 2016 following self-inflicted burns involving 44% of total body surface area. He also had an associated inhalation injury requiring immediate intubation and ventilation. His wounds became colonised with *Candida parapsilosis* and he was administered fluconazole (Days 34–40) and subsequently transitioned to anidulafungin (Table 1). His hospital course was complicated by bowel ischaemia requiring subtotal colectomy on Day 34 of admission as well as bilateral necrosis of his fingers distally.

On Day 47, *A. fumigatus* was isolated from a non-directed bronchoalveolar lavage (BAL) specimen. Lung computed tomography (CT) demonstrated large bilateral pleural effusions with associated atelectasis. A directed bronchoscopy was performed but no features suggestive of airway aspergillosis were seen. Abdominal

complications following bowel ischaemia and surgery were ongoing, requiring further drainage and laparoscopy and washout on Day 83 (Table 1). His ventilator requirements increased in association with this.

On Day 70, *Aspergillus flavus* was isolated bilaterally from the patient's hands. On abdominal CT performed due to intra-abdominal complications, images from the lung bases demonstrated bilateral dense consolidation. He was commenced on micafungin and liposomal amphotericin B (AmB) mainly to cover the *A. flavus* from the necrotic areas of his hands. These were stopped on Days 141 and 200, respectively.

The patient was discharged to rehabilitation on Day 228 with no signs of ongoing infection and having discontinued all antifungal agents.

The patient worked in a marble plant where he was involved in resizing imported marble from Spain and Italy. His last travel abroad (Spain) was ca. 3 months prior to admission. He had no history of prior use of azole antifungals.

Respiratory, wound and blood samples were collected regularly from Day 1 onwards (Table 1). *Aspergillus fumigatus* resistant to itraconazole, voriconazole, posaconazole and isavuconazole [minimum inhibitory concentrations (MICs) of >8, >8, 1 and >8 mg/L, respectively] was first isolated from a non-directed BAL on Day 47 post-admission. Pan-azole-resistant *A. fumigatus* isolates were also reported from a variety of respiratory samples on Days 53, 57, 69 and 74. All of these isolates

were susceptible to AmB and echinocandins. Prior to Day 47, twelve respiratory samples taken as part of routine care had been reported negative for fungi.

Weekly environmental monitoring of indoor and outdoor air was performed throughout the patient's admission because of construction work adjacent to the Burns Centre. All *A. fumigatus* isolates from air samples were susceptible to all azoles, echinocandins and AmB.

Nucleic acids were extracted from the patient isolates taken on Days 47 and 57. Identification of *A. fumigatus* was confirmed by sequencing the internal transcribed spacer (ITS) region as well as β-tubulin and calmodulin genes. In addition, the entire *cyp51A* gene, including 360 bases 5′ upstream of the start codon, was amplified by PCR. The amplified gene product was purified and sequencing, revealing a TR46 repeat insertion (TCTAGAATCACGCGGTCCGGATGTGTGCTGAGCCGAATGAAAGTTG) in the 5′ region upstream of *cyp51A*. In addition, the mutations Y121F and T289A were detected. No other mutations were found.

Here we report the first case of a pan-azole-resistant *A. fumigatus cyp51A* TR46/Y121F/T289A mutant in the UK. The source of this isolate is not clear, but it is unlikely that resistance evolved in the patient considering his minimal exposure to azole antifungals during hospitalisation. Although extensive environmental sampling was performed in the Burns Centre and outside, no other similar isolates were identified. It is possible that the patient carried the azole-resistant *A. fumigatus* in his airways prior to admission. However, it is unlikely that it would have remained

dormant in the airways for 47 days, especially considering how profoundly immunocompromised a patient with 44% burns and an inhalation injury is. It is even more unlikely that the patient would have carried the *A. fumigatus* conidia in his airways for over 4 months since his last trip abroad. Therefore, it can be assumed that he had obtained the pan-azole-resistant *A. fumigatus cyp51A* TR46/Y121F/T289A mutant from the environment within the UK.

This is of clinical importance because first-line therapy for pulmonary aspergillosis is voriconazole [4] as azole resistance is not acknowledged in treatment-naïve patients. In addition, previous reports have associated the *A. fumigatus cyp51A* TR46/Y121F/T289A mutant with invasive disease and therapy failure [5]. The extent of azole resistance due to this or other mutations is in the UK is impossible to estimate as susceptibility testing is not routinely performed for clinical and environmental mould isolates. Nevertheless, it is likely that this case represents a 'tip of the iceberg' and that there is an environmental origin. We advocate the introduction of UK-wide genetic analysis of azole-resistant isolates of *A. fumigatus* to enable monitoring of environmental transmission.

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Competing interests: None declared.

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References

[1] Howard SJ, Cerar D, Anderson MJ, Albarrag A, Fisher MC, Pasqualotto AC, et al. Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. Emerg Infect Dis 2009;15:1068–76.

[2] van der Linden JWM, Arendrup MC, Warris A, Lagrou K, Pelloux H, Hauser PM, et al. Prospective multicenter international surveillance of azole resistance in *Aspergillus fumigatus*. Emerg Infect Dis 2015;21:1041–4.

[3] White PL, Posso RB, Barnes RA. Analytical and clinical evaluation of the PathoNostics AsperGenius assay for detection of invasive aspergillosis and resistance to azole antifungal drugs during testing of serum samples. J Clin Microbiol 2015;53:2115–21.

[4] Patterson TF, Thompson GR 3rd, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 2016;63:e1–60.

[5] Verweij PE, Chowdhary A, Melchers WJG, Meis JF. Azole resistance in *Aspergillus fumigatus*: can we retain the clinical use of mold-active antifungal azoles? Clin Infect Dis 2016;62:362–8.

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Table 1. Summary of the case

Clinical history	Day	Fungal culture and biomarker findings	Antifungal therapy
Admission, intubated, ventilated	1		
	4	Non-directed BAL: no fungal growth	
Burns theatre: debridement	5		
	9	Endotracheal secretions: no fungal growth	
Ventilator-associated pneumonia	11	Sputum: no fungal growth	
	12	BAL ×2: no fungal growth for either	
	15	Wound swabs, L and R hands: yeasts + grown in both, serum BDG positive	
Abdominal distention, CT abdomen: faecal loading and enema	16		
Increasing oxygen requirements, abdominal distention, pressors	17		
	20	Non-directed BAL: no fungal growth	MFG (100 mg once daily) initiated
	21	Wound swabs ×2, R forearm: scanty yeasts in both	
	22	Non-directed BAL: no fungal growth	

CT abdomen: bowel wall haematoma	25		
Percutaneous tracheostomy (bedside)	26		
Burns theatre: removal of Biobrane	27	Non-directed BAL: no fungal growth. Wounds swabs ×10, various body sites: *Candida parapsilosis* species complex	MFG stopped
	28		
Burns theatre: alcohol and saline wash	29	Non-directed BAL: no fungal growth. Wound swab, R hand: yeasts +	
	30	Serum BDG positive	
	32	Non-directed BAL: no fungal growth	
	33		
Burns theatre: subtotal colectomy (ischaemic bowel)	34	Serum BDG positive	FLC initiated [a]
	36	Wounds swabs ×11, various body sites: yeasts +	
	39	Non-directed BAL: no fungal growth	
	40		FLC stopped, AFG (100 mg once daily) initiated
	41	Non-directed BAL: no fungal growth	

	42	Wounds swabs ×3, various body sites: *C. parapsilosis* species complex
Abdominal collection: drain placed under US guidance	43	
	45	Serum BDG positive
	47	Non-directed BAL: *Aspergillus fumigatus* +
Abdominal drain removed	49	
	53	Sputum: *A. fumigatus* ++. Wound swabs from various body sites: *C. parapsilosis* complex. Wound swab, R chest: *Trichosporon* sp.
	54	Non-directed BAL: no fungal growth, serum BDG positive
Right iliac fossa drain placed under US guidance	55	Sputum GM positive
Left iliac fossa drain placed under US guidance	56	
	57	Non-directed BAL: *A. fumigatus* +
	61	Wounds swabs ×6, various body sites: *C. parapsilosis* complex
	62	Non-directed BAL: no fungal growth
	63	Pleural fluid L & R: no fungal growth

	67		AFG stopped, FLC initiated [a]
	68		
	69	BAL (RUL and LUL): *A. fumigatus* ++	
	70	Wounds swabs from both hands and L shoulder: *Aspergillus flavus* on all. Wound swab from R flank: yeasts +. Sacrum swab: *Candida albicans* +	
	71	Non-directed BAL: no fungal growth	
TEE, no evidence of endocarditis	74	BAL: *A. fumigatus*. BAL GM positive. BAL *Aspergillus* PCR positive	
	77	Non-directed BAL: no fungal growth	FLC stopped, MFG (150 mg once daily) and L-AmB (3 mg/kg) initiated
	81	Sputum: no fungal growth	
	82	Non-directed BAL: no fungal growth	
Abdominal wash out. Large collection, T tube inserted	83		
	89	Non-directed BAL: no fungal growth	
	90	Confirmed *cyp51A* resistance mutations TR46 insertion and Y121F, T289A in isolates from Days 47 and 57	

99	Confirmed *A. fumigatus* identification by ITS, β-tubulin and calmodulin sequencing	
141		MFG stopped
200		L-AmB stopped
228		Discharged to rehabilitation

BAL, bronchoalveolar lavage; L, left; R, right; BDG, β-1-3-D-glucan; CT, computed tomography; MFG, micafungin; FLC, fluconazole; AFG, anidulafungin; US, ultrasound; GM, galactomannan; RUL, right upper lobe; LUL, left upper lobe; TEE, transoesophageal echocardiography; L-AmB, liposomal amphotericin B; ITS, internal transcribed spacer.

[a] Details of dosing not available.