

# First detection of *Aspergillus fumigatus* azole-resistant strain due to Cyp51A TR46/Y121F/T289A in an azole-naïve patient in Spain

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## Abstract

We report the first isolation of a voriconazole-resistant *Aspergillus fumigatus* strain harbouring the azole resistance mechanism TR46/Y121F/T289A, recovered from an azole-naïve patient in Spain with chronic obstructive pulmonary disease. This new finding in Spain suggests the spread of this resistance mechanism and reinforces the need for antifungal susceptibility surveillance.

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**Keywords:** *Aspergillus fumigatus*, azole resistance spread, cyp51A, resistance mechanism

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A 76-year-old man with increased basal dyspnoea, pleuritic-type chest pain and fever was admitted to the Hospital Gregorio Marañón, Madrid, in May 2014. The patient had been diagnosed with chronic obstructive pulmonary disease in 1997, and since then his baseline situation had worsened; he now manifested chronic respiratory failure treated with high-dose corticotherapy. In the present admission, *Aspergillus fumigatus* was cultured from sputum; however, galactomannan detection, calcofluor stain and PCR assay in bronchoalveolar lavage fluid were negative. The patient had no obvious clinical signs of invasive aspergillosis, and this isolation was considered a colonization following the revised EORTC/MSG criteria [1].

Strain identification (TP90812) was confirmed as *A. fumigatus* by PCR amplification and sequencing. The full coding sequences of the gene encoding the azole target (*cyp51A* gene including its

promoter) was PCR amplified and sequenced [2], showing the newly described azole resistance mechanism, consisting of a 46 bp tandem repeat in the promoter region of *cyp51A* along with two punctual mutations in the coding sequence of the *cyp51A* gene (a433t and a936g), leading to substitutions of tyrosine to phenylalanine at codon 121 and a threonine to alanine at codon 289 (TR46/Y121F/T289A) [3].

Antifungal susceptibility test was performed using the broth microdilution method described by the European Committee on Antimicrobial Susceptibility Testing [4]. The *A. fumigatus* strain azole minimum inhibitory concentrations were itraconazole 2.0, voriconazole (VRC) >8.0 and posaconazole 0.5 and would be considered resistant to VRC and with intermediate minimum inhibitory concentration values to itraconazole and posaconazole [5].

The number of clinical isolates of azole-resistant *A. fumigatus* is gradually increasing. Triazole resistance can evolve during therapy, but resistant isolates are also being detected in azole-naïve patients [6]. We report the first isolation of a VRC-resistant *A. fumigatus* strain TR46/Y121F/T289A in an azole-naïve patient in Spain.

To date, *A. fumigatus* azole resistance is mostly associated to modifications of the target site (encoded by *cyp51A*) and its overexpression. The main azole resistance mechanism is mediated by a tandem repeat of 34 bases in the promoter of

**TABLE 1.** Reports of azole-resistant strains carrying TR46/Y121F/T289A mutation in *cyp51A* gene, by country

Continent	First reported	No. of strains	Year	Origin	Study
Europe	Germany	1	2012	Clinical	[8]
	The Netherlands	21	2009–11	Clinical	[3]
		14	2013	Environment	
	Belgium	1	2012	Clinical	[7]
	Denmark	1	2014	Clinical	[9]
Africa	Spain	1	2014	Clinical	This work
Africa	Tanzania	4	2014	Environment	[10]
Asia	India	6	2012–13	Environment	[10]

*cyp51A* gene and a substitution in position 98 (TR34/L98H) [3]. This mechanism is spreading across Europe, and it has been related to azole fungicide use in agriculture [6]. A new resistance mechanism responsible for VRC resistance, TR46/Y121F/T289A in the *cyp51A* gene, was first detected in 2009 in a Dutch patient [4] and has recently been reported in other countries [7–10] (Table 1).

To our knowledge, this is the first case report of this specific mutation in an *A. fumigatus* clinical isolate in Spain. Although there has yet been not recovery of a TR46/Y121F/T289A azole-resistant *A. fumigatus* strains from the environment in Spain, its isolation from an azole-naïve patient is an interesting finding, suggesting that an effective analysis of clinical and environmental sources must be done to detect azole resistance in *A. fumigatus*. The emergence and spread of this new azole resistance mechanism in *A. fumigatus* is of major concern because it conferred high resistance to VRC and is associated with treatment failure in patients with invasive aspergillosis [6,7].

### Conflict of interest

None declared.

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