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## High airborne level of *Aspergillus fumigatus* and presence of azole-resistant TR34/L98H isolates in the home of a cystic fibrosis patient harbouring chronic colonisation with azole-resistant H285Y *A. fumigatus*

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M. Paluch, S. Lejeune, E. Hecquet, A. Prévotat, A. Deschildre, et al.. High airborne level of *Aspergillus fumigatus* and presence of azole-resistant TR34/L98H isolates in the home of a cystic fibrosis patient harbouring chronic colonisation with azole-resistant H285Y *A. fumigatus*. *Journal of Cystic Fibrosis*, 2019, 18, pp.364 - 367. 10.1016/j.jcf.2019.01.001 . hal-03486401

**HAL Id: hal-03486401**

**<https://hal.science/hal-03486401>**

Submitted on 20 Dec 2021

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## High airborne level of *Aspergillus fumigatus* and presence of azole-resistant

### TR<sub>34</sub>/L98H isolates in the home of a cystic fibrosis patient harbouring

### chronic colonisation with azole-resistant H285Y *A. fumigatus*

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Funding source: No funding was secured for this study.

Financial disclosure: The authors have no financial relationships relevant to this article to disclose.

Conflict of interest: The authors have no conflicts of interest to disclose.

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## Abstract (149 words)

Azole-resistant *Aspergillus fumigatus* (ARAF) has been reported in the domestic environment of patients at risk for aspergillosis. Here, we assessed the mother's and father's homes of an 18-year-old cystic fibrosis patient harbouring chronic colonisation with H285Y CYP51A azole-resistant isolate, in order to explore the link between environmental exposure and ARAF infection. In one dwelling, a very high overall contamination level was found (710-7,240 CFU/m<sup>3</sup>), with a predominance of *A. fumigatus* (640-6,490 CFU/m<sup>3</sup>), and ARAF showing the TR<sub>34</sub>/L98H mutation was isolated. Mycological follow-up of the patient showed the persistence of H285Y isolates, but no acquisition of TR<sub>34</sub>/L98H isolates was observed. This could be due to the low proportion of TR<sub>34</sub>/L98H isolates (<3%), or the establishment of preventative measures and dwelling remediation taken after the environmental investigation. Our data underlines the value of an environmental assessment to establish preventative measures and limit the risk of *A. fumigatus* exposure and ARAF acquisition.

**Keywords:** *Aspergillus fumigatus*, azole-resistance, cystic fibrosis, indoor mould exposure

## 1. Introduction

Azole-resistant *Aspergillus fumigatus* (ARAF) has become an emerging problem worldwide, which has been associated with treatment failure<sup>1</sup>. Azole molecules target lanosterol 14 $\alpha$ -demethylase encoded by the gene *cyp51A*. Various CYP51A mutations have been described throughout the world, including M220 or G54, but the two main resistance mechanisms are TR<sub>34</sub>/L98H and TR<sub>46</sub>/Y121F/T289A, which likely have an environmental origin<sup>1,2,3,4</sup>.

Several studies have described ARAF isolates in France<sup>5,6,7,8</sup>. However little data is available on ARAF in indoor environments. One case of lethal invasive aspergillosis with TR<sub>46</sub>/Y121F/T289A ARAF has been reported recently. The study showed the presence of ARAF isolates in the patient's home or close outdoor environment, which were identified as the potential source of contamination<sup>6</sup>. ARAF has been reported in cystic fibrosis in France with 4,6%-8% prevalence rates in patients colonised with *A. fumigatus*<sup>5,9</sup>. However, the link between environmental exposure and ARAF infection in cystic fibrosis has not been explored so far.

Here we present the case of an 18-year old patient suffering from cystic fibrosis, diagnosed at birth with homozygote  $\Delta$ F508 mutation. The patient was first known to be colonised with *A. fumigatus* in 2010. In May 2012, the patient was diagnosed with allergic bronchopulmonary aspergillosis (ABPA), and antifungal therapy was first initiated with itraconazole (ITZ). In June 2012, determination of antifungal minimum inhibitory concentrations (MICs) using E-Test® yielded an elevated ITZ MIC (2 mg/L), whereas the MICs for voriconazole (VOR) and posaconazole (POS) were low ( $\leq$ 0.125 mg/L). Thus, the antifungal therapy was changed to VOR. The follow-up showed detection of *A. fumigatus* in all 28 sputa sampled between June 2012 and December 2016. In this period, azole MICs were determined for 11 isolates, all of which showed high MICs for ITZ (3-32 mg/L), associated with increased MICs for POS (0.38-2 mg/L), isavuconazole (ISA, 0.75-2 mg/L), and VOR (1.5-3 mg/L; Fig. 1). All of the isolates were amphotericin B and caspofungin-susceptible. *Beta-tubulin* and *Cyp51A* sequencing were performed as described previously<sup>8</sup> in 1 isolate in 2013 and 5 isolates in 2016 (Fig. 1). Sequences were deposited in GenBank under accession numbers MH510837-MH510842, and MH510851-MH510856, respectively. The *beta-tubulin* sequences confirmed the identification of *A. fumigatus sensu stricto* and all the isolates harboured an H285Y CYP51A mutation, which is a rare

mutation reported in our region to date only, in an environmental isolate from the home of a COPD patient<sup>8</sup>. VOR was stopped in March 2016 and treatment with a cystic fibrosis transmembrane conductance regulator (CFTR) modulator, a CFTR corrector in combination with a CFTR potentiator (lumacaftor/ivacaftor Orkambi®) was initiated in October 2016 (Fig. 1).

## 2. Mycological assessment of the patient's home

In December 2016, the patient's close environment was investigated in order to evaluate its potential impact on persistent ARAF colonisation in this patient. Since the parents had separated in September 2016, both the mother's and father's dwellings were evaluated (on 15 and 20 December respectively). For both homes, air samples were collected in a consistent manner, in the living room, kitchen and patient's bedroom using Sampl'air™ Lite impactor (BioMérieux, Marcy l'Etoile, France). For each point (one per room), 100L were sampled on Sabouraud and DG18 agar medium and incubated for 7 days at 30°C and 24°C, respectively. In the patient's bedroom, two further 1000L air samples were collected on Sabouraud agar medium supplemented with either ITZ or VOR (4 mg/L) and stored at 37°C for seven days. In one house, the environmental assessment revealed a moderate airborne contamination level (200-740 CFU/m<sup>3</sup>), low exposure of the patient to *A. fumigatus* (20-70 CFU/m<sup>3</sup>), and no ARAF was detected. In the other house, where the patient was spending most of her time, the contamination level was very high (710-7,240 CFU/m<sup>3</sup>), with a predominance of *A. fumigatus* (640-6,490 CFU/m<sup>3</sup>). These concentrations were well above the 1,000 CFU/m<sup>3</sup> level that has been proposed by ANSES as the threshold to define abnormally high concentrations in dwellings<sup>10</sup>. Although no visible moulds were detected in the dwelling, a 4-meter long wall was damp, following rain passing through the roof into the living room, where the highest contamination levels were detected. Furthermore, old wood windows had been recently replaced with PVC windows to improve isolation, but no ventilation system had been installed to compensate for the loss of natural ventilation resulting from air leaks. When assessing azole-resistance on ITZ medium, a few ARAF isolates were detected (<20 CFU/m<sup>3</sup>) in the patient's bedroom. The identification of *A. fumigatus sensu stricto* was confirmed using *beta-tubulin* sequencing, and *Cyp51A* sequencing of 5 isolates showed only TR<sub>34</sub>/L98H isolates, which had very high MICs for ITZ and high MICs for POS, ISA VOR (12->32

mg/L, 0.75-1 mg/L, 1.5-3 mg/L, and 1.5-2 mg/L, respectively; Fig. 1). The sequences were deposited in GenBank under accession numbers MH510846-MH510850 and MH510860-MH510864 respectively.

### 3. Bio-clinical follow-up

Mycological follow-up of the patient between December 2016 and December 2017 showed the persistence of H285Y isolates in 3/3 sputum samples (in January, May and November 2017, Fig. 1; GenBank accession numbers MH510837-MH510842 and MH510851-MH510857 respectively), but no acquisition of TR<sub>34</sub>/L98H isolates was observed. Serological follow-up showed a 2-fold increase in anti-*A. fumigatus* precipitins in October 2016 (7 precipitins vs. 3 in July 2016) and a slight increase in anti-*Aspergillus* IgG levels in November 2016 (18 UA/mL vs. 11 UA/mL in July 2016), whereas an FEV1 increase, probably related to Orkambi® therapy, was observed in November 2016 (Fig. 2). A further increase in anti-*Aspergillus* IgG levels was observed in March 2017 (reaching 29 UA/mL), but anti-*A. fumigatus* precipitins decreased, and both anti-*A. fumigatus* precipitins and anti-*Aspergillus* IgG levels were lower in December 2017 (reaching 11 UA/mL). FEV1 was quite stable between November 2016 and December 2017.

### 4. Discussion

Since the patient was living in a rural area with potato and maize crop fields treated with agricultural azoles nearby at the time of the first detection of ARAF in 2012, ARAF is likely to have been acquired from the environment during this period. This hypothesis is consistent with the previously reported detection of H285Y ARAF in the environment in our region<sup>8</sup>. However, antifungal pressure exerted by azole therapy (initiated in April 2012) cannot be excluded. Interestingly, when the patient moved to an urban area in 2013 and 2016, ARAF colonisation with H285Y persisted, and despite home exposure to TR<sub>34</sub>/L98H isolates from September 2016 (when she moved to her new home), no colonisation with these isolates was observed. This could be due to the low proportion of TR<sub>34</sub>/L98H isolates (<3%), or, to the establishment of preventative measures (including patient eviction) and dwelling remediation (removal of mould that was discovered under the wallpaper and replacement of wallpaper, installation

of mechanical ventilation) taken after the environmental investigation, which could have decreased the overall exposure to *A. fumigatus* (as supported by the decrease in anti-*Aspergillus* precipitins and IgG levels between April 2017 and December 2017) and TR<sub>34</sub>/L98H isolates.

Overall, our data confirms the potential clinical impact of the newly described H285Y CYP51A mutation. At the environmental level, our data is the first description of TR<sub>34</sub>/L98H isolates in the home of a cystic fibrosis patient, thus confirming that indoor environments can be a source of ARAF for patients at risk. Furthermore, they underline the interest in assessing a patient's dwelling to evaluate *A. fumigatus* exposure and detect ARAF in order to establish preventative measures and limit the risk of *A. fumigatus* exposure and ARAF acquisition.

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## Figure legends

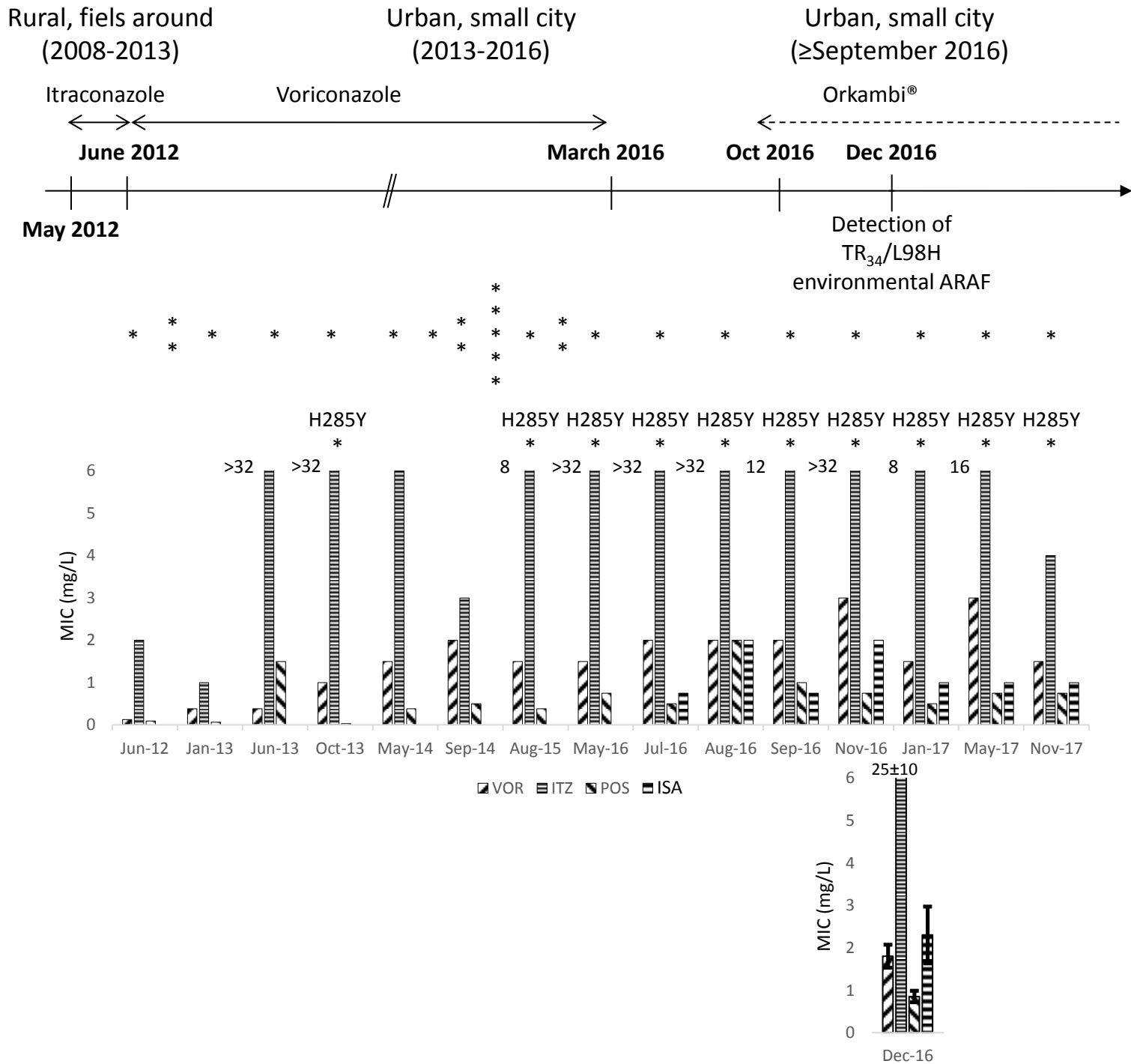
Fig 1. Location of the patient's homes between 2008 and 2017, azole treatment and characterisation of *A. fumigatus* clinical and environmental isolates (*cyp51A* sequencing and azole-antifungal MICs).

MICs of all the clinical and environmental isolates obtained after October 2013 were above the Etest ITZ, VOR, and POS epidemiological cutoff values (ECVs, predictive of non-WT isolates) of 2 mg/L, 0.5 mg/L, and 0.25 mg/L<sup>11,12</sup>, respectively. For informational purposes only, EUCAST criteria which define azole antifungal susceptibility and resistance are the following: an ITZ or VOR MIC of  $\leq 1$  mg/L is considered susceptible, a MIC of 2 mg/L intermediate and of  $> 2$  mg/L resistant; a POS MIC of  $\leq 0.125$  mg/L is considered susceptible, a MIC of 0.25 mg/L is considered intermediate and of  $> 0.25$  mg/L resistant; an ISA MIC of  $\leq 1$  mg/L is considered susceptible, a MIC of  $> 1$  mg/L is considered resistant. Reference is antifungal breakpoints v9.0 February 2018 available at [http://www.eucast.org/clinical\\_breakpoints/](http://www.eucast.org/clinical_breakpoints/)

Fig 2. Anti-*Aspergillus* antibodies and FEV1 follow-up.

Anti-*Aspergillus* IgG level (A) was measured with Platelia<sup>TM</sup> *Aspergillus* kit (Bio-Rad®, Marne-la-Coquette, France). Anti-*A. fumigatus* precipitin titers (B) were determined using Ouchterlony double immunodiffusion. FEV1 (C) is reported in percentage.

**Fig. 1**



**Fig. 2**

