Home Environment as a Source of Life-Threatening Azole-Resistant Aspergillus fumigatus in Immunocompromised Patients

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A case of fatal aspergillosis due to a TR46/Y121F/T289A azole-resistant Aspergillus fumigatus is reported. Environmental investigations at the patient’s residence led to the recovery of TR46/Y121F/T289A isolates, genotypically indistinguishable from the clinical isolate, supporting for the first time the direct role of household as potential source of azole-resistant invasive aspergillosis.

Keywords. Aspergillus fumigatus; azole resistance; TR46/Y121F/T289A; environment; invasive aspergillosis.

Since the early description of itraconazole-resistant Aspergillus fumigatus in 1997 in California, azole resistance in A. fumigatus is increasingly observed worldwide and in various clinical contexts. The most recognized resistance mechanism involves mutations in the CYP51A gene that encodes for lanosterol 14α-demethylase [1]. To date, 2 routes of azole resistance development involving mutations in the CYP51A gene have been described: (1) acquired mutations through long-termazole drug exposure and (2) acquisition of azole-resistant A. fumigatus from environmental origin due to the use of azole fungicides. Environmental resistance is characterized by 2 main genetic alterations affecting the CYP51A gene and tandem repeat (TR) duplications in its promotor region: TR34/L98H was first discovered [2], followed by TR46/Y121F/T289A [3]. Isolates harboring the TR46/Y121F/T289A alteration, conferring high-level resistance to voriconazole, have been found in both clinical and environmental samples [3]. To the best of our knowledge, no TR46/Y121F/T289A isolates have been recovered from the immediate environment of patients infected or colonized with such isolates. We report here a case of fatal invasive aspergillosis due to a TR46/Y121F/T289A A. fumigatus isolate. Importantly, the clinical isolate and environmental TR46/Y121F/T289A isolates from the patient’s home were genetically indistinguishable, supporting the hypothesis of living environment as a source of azole-resistant A. fumigatus.

CASE REPORT

A 66-year-old retired farmer was admitted to the intensive care unit in May 2014 because of bilateral pneumonia with alveolar consolidations and bilateral and diffuse ground-glass opacities on computed tomography. He was treated with infliximab (330 mg every 8 weeks) since 2012 for rheumatoid arthritis and had no history of antifungal exposure, azoles included. On admission, the patient was treated empirically with piperacillin/tazobactam (4 g/0.5 g every 8 hours) and ciprofloxacin (0.5 g twice a day) while awaiting microbiological investigations. Direct examination of bronchoalveolar fluid revealed septate, branching hyphae, and Aspergillus section Fumigati grew on Sabouraud agar media. Both serum galactomannan (Bio-Rad, Marnes-la-Coquette, France) and serum (1→3)-β-D-glucan (Associates of Cape Cod Inc, East Falmouth, Massachusetts) were positive (index 0.69 pg/mL and 175 pg/mL, respectively) together with a positive bronchoalveolar galactomannan (index 5.69). Antifungal susceptibility testing using the Etest method (bioMérieux, Marcy-l’Étoile, France) showed a high level of in vitro resistance to voriconazole (minimum inhibitory concentration [MIC] > 32 µg/mL) and itraconazole (MIC = 8 µg/mL). Because the isolate displayed low MICs of echinocandins and the patient had a high aspartate aminotransferase level, he was treated with caspofungin (0.50 g twice a day) while awaiting microbiological investigations.

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located in close proximity to the patient’s home were collected. In October, soil samples in the patient’s vegetable garden (n = 11) and surface samples inside the patient’s house (n = 10) were collected (Table 1). Each sample was cultivated onto Sabouraud agar plates at 43°C. Aspergillus-positive samples were then cultivated onto Sabouraud agar plates supplemented with 4 mg/L itraconazole or 4 mg/L voriconazole (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Aspergillus from 6 of 34 environmental samples grew on media containing azole: 1 from the fields (barley), 2 from the garden, and 3 from the patient’s house. In the house, many colonies were found originating from the sample taken from the top of the nightstand and from the workbench located in the basement. Forty-six isolates (up to 5 isolates per sample) that grew on antifungal-supplemented media were subjected to both β-tubulin and CYP51A gene sequencing. All isolates were identified as *A. fumigatus* and all harbored the environmental alterations: the 5 isolates from the barley fields showed a TR34/L98H alteration whereas 41 isolates from the garden and the patient’s house carried the TR46/Y121F/T289A alteration. The clinical isolate and 26 environmental isolates (TR34/L98H [n = 4]; TR46/Y121F/T289A [n = 22]) able to grow on media containing voriconazole were subjected to genotyping using 9 short tandem repeat loci [5]. Interestingly, all TR46/Y121F/T289A isolates, including the patient isolate, were genotypically indistinguishable from each other (Table 1). All TR34/L98H isolates from the fields were genotypically indistinguishable but different from TR46/Y121F/T289A isolates. In addition, the present TR46/Y121F/T289A isolates were also genetically distinct from previously described TR46/Y121F/T289A isolates from France, the Netherlands, and Germany (data not shown).

**DISCUSSION**

In this study, we report a case of fatal invasive aspergillosis due to an azole-resistant strain harboring the TR46/Y121F/T289A alteration in a patient receiving anti–tumor necrosis factor (TNF) therapy with infliximab. Importantly, we found azole-resistant isolates at the patient’s home carrying the same environmental alteration and being genotypically indistinguishable from each other.

*Aspergillus fumigatus* is an opportunistic mold that is responsible for a large spectrum of pulmonary diseases, including invasive infection in immunocompromised patients. Although progress has been made in the management of invasive aspergillosis, partially due to the introduction of voriconazole, the mortality remains high. However, as discussed elsewhere, the current situation with the emergence of azole resistance is becoming a worrisome problem [6].

To date, clinical TR46/Y121F/T289A isolates have been reported from patients with various underlying diseases such as hematological diseases, cystic fibrosis, solid organ transplantation, or inflammatory diseases. Our patient was immunocompromised related to the anti-TNF therapy prescribed for rheumatoid arthritis. Treatment with TNF-α blockers is a recognized host factor that predisposes patients to invasive fungal diseases [7] and has been highlighted in a 3-year prospective French registry [8]. Interestingly, although there is no real doubt regarding the diagnosis of invasive aspergillosis in our patient, the revised European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria for probable invasive aspergillosis [7] were not met due to the radiological presentation that was not strictly specific for aspergillosis. This underlines again that these criteria, although being of paramount importance for clinical trials and epidemiological studies, cannot be applied to every patient population [9].

The environmental origin of the TR46/Y121F/T289A alteration was first suggested by van der Linden et al in a study conducted in the Netherlands where both clinical and

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**Table 1. Description of the Localization of Sampling and Resistance Investigation Results**

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of Samples</th>
<th>No. of Positive Samples</th>
<th>No. of Positive isolates</th>
<th>No. of Colonies</th>
<th>CYP51A Mutations*</th>
<th>No. of Isolatesb</th>
<th>STRA1 Genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley field</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>TR46/Y121F/T289A</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Corn field</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat field</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garden</td>
<td>11</td>
<td>6</td>
<td>2</td>
<td>&gt;10</td>
<td>TR46/Y121F/T289A</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Living room</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedroom</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>&gt;10</td>
<td>TR46/Y121F/T289A</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Bathroom</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>TR46/Y121F/T289A</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Basement</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>&gt;10</td>
<td>TR46/Y121F/T289A</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Clinical isolate</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>&gt;10</td>
<td>TR46/Y121F/T289A</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

*Up to 5 colonies per sample were subjected to CYP51A gene and its promoter sequencing.

b Genotyping was restricted to isolates growing on Sabouraud agar plates supplemented with voriconazole.
environmental TR46/Y121F/T289A azole-resistant isolates were genetically related [3]. Unfortunately, in this study it was not specified whether or not environmental isolates were isolated from the domiciles of infected patients. More recently, a case of invasive aspergillosis due to a TR34/L98H isolate in a French farmer was investigated by sampling inside and outside the patient’s home. This study led to the recovery of a single TR46/L98H resistant isolate from a barley crop located in front of the patient’s home. However, genotyping failed to show evidence of a genetic relationship between the clinical and environmental isolates [10]. Previously, we described the first TR46/Y121F/T289A isolate in France in a patient with cystic fibrosis. An environmental investigation in the same region demonstrated that TR46/Y121F/T289A isolates were present in soil samples (wheat field) but were located far from the patient’s home [4].

The present study is the first description of voriconazole-resistant TR46/Y121F/T289A isolates from an azole-naive patient with invasive aspergillosis, along with genetically related isolates from the patient’s home. Although no related TR46/Y121F/T289A isolate was found in soil samples from the fields surrounding the home, TR46/Y121F/T289A isolates were detected in garden soil recovered from a rototiller blade, suggesting that the TR46/Y121F/T289A isolates found in the house came from outdoors. Taken together, there is strong evidence to believe that our patient was infected with primary azole-resistant A. fumigatus from his living environment. Although Vazquez et al. recently suggested that the TR46/Y121F/T289A alteration could occur during prolonged voriconazole therapy [11], our report showed molecular evidence supporting the environmental origin of TR46/Y121F/T289A A. fumigatus isolates.

The fact that we were able to find TR46/Y121F/T289A isolates in the patient’s home >4 months after his death indicates that such resistant isolates can probably persist over months in the environment. This patient lived in an agricultural area in northern France with potentially high-level fungicide pressure for crop protection, as illustrated by the simultaneous recovery of TR34/L98H isolates.

Because azole resistance could be associated with treatment failure, experts recently recommended the use of liposomal amphotericin B or a combination of voriconazole plus an echinocandin for the treatment of invasive aspergillosis in regions with a high resistance rate (>10%) or azole-resistant invasive aspergillosis [6, 12]. Importantly, in clinical practice, antifungal susceptibility testing should be performed, when available, in regions with a high resistance rate when azole therapy is needed. Environmental and clinical surveillance of azole resistance should be considered to evaluate rates of azole resistance in each region/country. Finally, this case highlights the need for further studies aiming to evaluate the cost-effectiveness of environmental sampling in the home surroundings of immunocompromised outpatients, as it could represent a potential life-threatening source of infection with azole-resistant isolates.

Notes

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References