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Multiple Fungicide-Driven Alterations in Azole-Resistant *Aspergillus fumigatus*, Colombia, 2015

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To the Editor: We read with interest the report by van der Linden et al. about the prevalence of azole-resistant *Aspergillus fumigatus* isolates from 19 countries, including 2 from the Americas (Brazil and the United States) (1). Recent reports have suggested a link between use of fungicides in agricultural practices and the presence of triazole-resistant *A. fumigatus* among azole-naïve persons (2). These resistant strains harbored the TR34/L98H and TR46/Y121F/T289A mutations in the *CYP51A* gene and its promoter region. These novel mechanisms of resistance have been reported both in environmental and clinical samples in Europe, Asia, and Africa, suggesting a broad geographic spread. However, clinical isolates from 22 states in the United States (3) and a few isolates from Latin America (1,4) failed to show any fungicide-driven resistance in *A. fumigatus* in these continents, even though use of pesticides is a widespread practice in the Americas. Colombia was ranked fourth in the world in 2010 for the use of pesticides, reportedly using 14.5 tons/1,000 ha, 30% of which were fungicides (5). Among the fungicides approved by Colombia's regulatory agency, the Colombian Agricultural Institute (6), tebuconazole and difenoconazole are largely used in the flower industry, more specifically in Cundinamarca, where 60% of Colombia's flowers are produced.

In 2015, we conducted a study for which 60 soil samples from flower fields and greenhouses were collected in the outskirts of Bogota, Cundinamarca. Samples were

inoculated on Sabouraud agar at 43°C, and positive samples were screened for azole-resistance on agar supplemented with either itraconazole (4 mg/L) or voriconazole (4 mg/L). Of the 38 resistant *Aspergillus* strains, 20 were selected (up to 5 colonies for each positive culture), identified as *A. fumigatus* by β -tubulin gene sequencing, and analyzed for *CYP51A* gene alterations (7). Results showed great diversity in molecular resistance with the presence of TR46/Y121F/T289A (n = 17), TR34/L98H (n = 1), and TR53 (n = 1) mutations; 1 isolate had a wild-type *CYP51* sequence (8).

Our study highlights the presence of *A. fumigatus* harboring fungicide-driven alterations in Colombia, South America. The results indicate the importance of initiating active agricultural surveillance along with close monitoring of drug resistance in clinical isolates from naïve and azole-exposed patients in these countries. Clinical management of *Aspergillus* disease can be challenging because of unfavorable clinical outcomes after patients have acquired multi-azole-resistant strains from the environment (9). Additional studies are needed to evaluate the extent to which pesticide use in floriculture and agriculture (e.g., coffee and banana) contributes to azole resistance in Colombia.

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Azole Resistance of *Aspergillus fumigatus* in Immunocompromised Patients with Invasive Aspergillosis

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To the Editor: First-line antifungal therapy for invasive aspergillosis (IA) is voriconazole, which is challenged by the emergence of azole resistance (1). A recent article reported a 3.2% prevalence of *Aspergillus fumigatus* isolates that are resistant to azole from 3,788 isolates screened in Europe (2). Of the 1,911 patients from whom the isolates were collected, IA developed in 10 (3 proven, 1 probable, 6 possible). Prevalence of azole-resistant *A. fumigatus* disease among patient populations at risk of IA was unavailable.

As described (3), we screened every *A. fumigatus* isolate recovered from respiratory specimens from patients with probable or proven IA in our hospital in Paris, France, during January 2012–December 2014. Every isolate recovered from 2% malt extract agar plates or Sabouraud dextrose agar slants (Bio-Rad, Marnes-la-Coquette, France) was incubated at 30°C and tested as individual isolates or multiple ones from a single sample by using itraconazole,

voriconazole, and posaconazole Etest strips (bioMérieux, Marcy l'Étoile, France). Resistance was assessed for MICs >2.0 µg/µL for voriconazole and itraconazole and >0.25 µg/µL for posaconazole by using European Committee on Antimicrobial Susceptibility Testing clinical breakpoints for fungi (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Antifungal_breakpoints_v_7.0.pdf).

Every 4 months, a local multidisciplinary medical team classified each IA case by using the 2008 criteria established by the 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 (4). For 148 patients (127 with hematologic malignancies and 21 with other conditions), the team recorded 152 episodes: 9 proven and 143 probable IA episodes. Possible IA was not analyzed because of a lack of microbiologic criteria. For 51 probable IA episodes, galactomannan positivity in blood or bronchoalveolar lavage fluid samples was the only microbiologic criterion used for classification. Cultures of respiratory samples (i.e., bronchoalveolar lavage fluid, tracheal aspirate, and sputum) or biopsies were positive for 99 episodes: 68 with *A. fumigatus* isolates and 31 with other *Aspergillus* spp. isolates. Among the 68 *A. fumigatus* isolates, 1 (1.5%) associated with probable IA was resistant to azoles (5). The isolate harbored the TR₃₄/L98H mutation (5), leading to a rate of IA caused by azole-resistant *A. fumigatus* of 0.7% (1/152) for total episodes recorded and 1% (1/99) for culture-positive episodes only. Nineteen (36%) of 53 culture-negative patients and 35 (37%) of 95 culture-positive patients died.

Azole resistance of *A. fumigatus* warrants specific surveillance in hospitals treating immunocompromised patients. Prevalence of resistant isolates can differ by hospital location and underlying disease (e.g., immunodeficiency vs. chronic lung diseases). When focusing on patients with probable or proven IA, we did not observe an emergence of azole-resistant *A. fumigatus* isolates during 2006–2009 (3) and 2012–2014 in France. Consequently, our center does not question the use of voriconazole as first-line treatment or of posaconazole as prophylaxis.

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