Azole-resistant *Aspergillus fumigatus* at a university hospital in Belgium: A laboratory-based surveillance

Montesinos I[1], Argudin MA[1], Dodémont M[1], Dagyaran C[1], Bakkali M[1], Etienne I[2], Hites M[3], Patteet S[4], Lagrou K[4]
[1] Microbiology Department [2] Pneumology Department [3] Infectious Diseases Department. Hopital Erasme, Brussels. [4]Belgian Reference Center for Mycosis, UZ Leuven. Belgium

Introduction and Purposes:

Azole-resistant *Aspergillus fumigatus* is an emerging worldwide problem with major clinical implications. Mould active triazoles are commonly used as first line treatment and prophylaxis of invasive aspergillosis (IA). Mutations in the *cyp51A* gene, represent the most commonly reported mechanism conferring azole resistance and consequently treatment failure in *A. fumigatus.*

A clinical case of *A. fumigatus* containing the TR_{46}/Y121F/T289A mutation in the *cyp51A* gene was detected in 2013 at Hôpital Erasme in Brussels. A laboratory-based surveillance of unselected *A. fumigatus* was set up in order to determine the azole-resistance frequency and resistance mechanisms.

Methods:

From June 2015 to October 2016, 212 *A. fumigatus* isolated from 109 patients hospitalized at Hôpital Erasme were screened by VIPcheck™. All isolates able to grow on at least one of the azole-containing wells were further investigated for their minimal inhibitory concentrations (MICs) by Sensititre YeastOne Epidemiological cutoff's based on CLSI guidelines were used for interpretation of the MIC values (0.5µg/mL for posaconazole, and 1 µg/mL for both voriconazole and itraconazole). Resistance genotyping were performed by *cyp51A*, *cyp51B* and *hapE* sequencing. Demographic and clinical data were collected from patient's charts..

Results:

Two hundred and twelve positive samples for *A. fumigatus* were isolated from 109 hospitalized patients' respiratory specimens and screened by VIPcheck™. The most prevalent underlying diseases amongst these 109 patients were as follows: 23% (n=25) cystic fibrosis patients, 21% (n=23) lung transplant patients and 13% (n=14) chronic obstructive pulmonary disease (COPD). Seventeen percent (n=19) of these patients were diagnosed with IA, 4% (n=5) with allergic bronchopulmonary aspergillosis (ABPA), and the remaining 78% of patients (n=85) were considered to be colonized by *A. fumigatus*. Twenty five specimens from 14 patients had azole-resistant *A. fumigatus* isolates, translating into a prevalence of azole-resistance of 12.8% among all patients and of 10.5% (2/19) among patients with proven or probable IA. Mutations at the *cyp51A* gene by resistance genotyping were observed in 23 *A. fumigatus* isolates from 12 patients, while missense mutations were observed in two cases. The TR_{34}/L98H was the most prevalent mutation (58%), followed by TR_{46}/Y121F/T289A (33%). Seven *A. fumigatus* isolates with mutations at the *cyp51A* gene were recovered from one patient, and they carried either the mutation TR_{34}/L98H (n=5) or G448S (n=2). An isolate with a TR_{34}/L98H mutation from another patient showed also a deletion of eight nucleotides in the *cyp51B* promotor. No isolates showed mutations at *hapE*. MICs, resistance genotyping results and clinical and demographics data from patients harboring azole-resistant *A. fumigatus* are summarized in Table 1. Prevalence of azole-resistance among cystic fibrosis and lung transplant patients was 16% and 17%, respectively.

TABLE 1 *cyp51A* mutations, MICs results and demographic data from patients harboring azole-resistant *A. fumigatus* isolated at Erasme Hospital from June 2015 to October 2016.

Patient n°	Age (years)	Underlying disease[a]	Source[b]	Colonization /IA[c]	Prior azole exposition	MIC[d] (mg/L)			cyp51A mutations
						ITC	VRC	POS	
1	39	Cystis fibrosis	BA/S	colonization	VRZ, ITZ	1 0.5	2 1	0.5 0.25	TR_{34}/L98H G448S
2	81	Solid malignancy	BA/S	colonization		1	2	0.5	-
3	53	Haematological malignancy	BA/S	colonization	POS	1	2	0.5	TR_{34}/L98H
4	47	Cystis fibrosis	BA/S	colonization		1	1	0.5	-
5	18	Cystis fibrosis	BA/S	colonization		1	2	0.5	TR_{34}/L98H
6	26	Intestinal malabsorption	BA/S	colonization		1	2	0.5	TR_{34}/L98H
7	67	Lung transplant	BAL	probable IA		1	1	0.5	TR_{34}/L98H
8	58	Heart transplant	BAL	probable IA	VRZ	0.5	>8	0.5	TR_{46}/Y121F/T289A
9	26	Cystis fibrosis	BA/S	colonization		0.5	>8	0.5	TR_{46}/Y121F/T289A
10	86	Solid malignancy	BA/S	colonization		16	1	1	TR_{34}/L98H[e]
11	66	COPD[1]	BAL	colonization		1	2	0.5	TR_{34}/L98H
12	48	Lung transplant	BA/S	colonization	VRZ	0.5	>8	0.5	N248K
13	55	Lung transplant	BA/S	colonization	VRZ	0.5	>8	0.5	TR_{46}/Y121F/T289A
14	65	Lung transplant	BA/S	colonization		0.5	>8	0.25	TR_{46}/Y121F/T289A

[a] COPD, chronic obstructive pulmonary disease.
[b] BA/S, bronquial aspiration or sputum; BAL, Bronchoalveolar lavage.
[c] IA, invasive Aspergillosis.
[d] MIC, Minimal inhibitory concentration; ITC itraconazole; VRC, voriconazole; POS, posaconazole.
[e] A deletion of eight nucleotides in the *cyp51B* promotor was also observed for this isolate.

Conclusions:

This laboratory-based surveillance of unselected *A. fumigatus* and screening with VIPcheck™ identified a high prevalence of azole-resistance among all patients (approximately 13%), and among patients with probable or proven IA (approximately 11%). High prevalence was observed among cystis fibrosis and lung transplant patients. Further surveillance of azole-resistance in *A. fumigatus* at Erasme hospital is warranted.