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LETTER TO THE EDITOR

Triazole Antifungal Susceptibility Patterns among *Aspergillus* Species in Québec, Canada

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Invasive aspergillosis (IA) is an important cause of morbidity and mortality among immunocompromised patients (1–3), with over 500 patients developing IA in Canada each year (4). The overall mortality rate of IA is approximately 25% (5, 6) but increases to over 60% in the setting of infection with azole-resistant *Aspergillus fumigatus* (ARAF) (7–9). Given the spread of ARAF to countries in Europe (10), Asia (11, 12), Africa, the Middle East, and the Americas (13), we sought to determine the prevalence of azole resistance among *Aspergillus* isolates in the province of Québec, Canada.

We performed antimicrobial susceptibility testing on all *Aspergillus* species isolated from sterile sites and respiratory specimens that were processed at the McGill University Health Centre (MUHC) between March 2018 and December 2018. The MUHC is a university-affiliated tertiary-care hospital in Montreal that serves as the central mycology laboratory for 10 hospitals and a population of 1.8 million in the province of Québec.

Clinical isolates of *Aspergillus* were identified to the species level by conventional microscopy techniques. For a given isolate, sporulating colonies were picked and subcultured on VIPcheck plates (Mediaproducts BV, the Netherlands) and incubated per the manufacturer's instructions. The VIPcheck plate has a sensitivity of 98% and a specificity of 93% for detecting azole resistance in *Aspergillus* species (14). Briefly, a moist, sterile swab collected conidia from the *Aspergillus* colony. A suspension with a 0.5 to 2 McFarland standard was then created in sterile water. All 4 wells of the VIPcheck plate were inoculated with a single drop of the suspension. Finally, the plate was incubated and read after 24 and 48 h. Growth in any well except the control well indicated the possibility of an azole-resistant isolate. Isolates with positive screening results were sent to the Laboratoire de Santé Publique du Québec (LSPQ; the provincial reference center) for molecular identification (by sequencing of internal transcribed spacer [ITS] and BenA regions) and antifungal susceptibility confirmatory testing by broth microdilution per the Clinical and Laboratory Standards Institute method (15, 16). Epidemiological cutoff values (ECV) were used to infer antifungal susceptibility (17).

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TABLE 1 Antifungal susceptibility testing results with VIPcheck plates

Antifungal agent	*A. fumigatus* ($n = 86$)	*A. flavus* ($n = 12$)	*A. niger* ($n = 5$)	*A. versicolor* ($n = 4$)	*A. glaucus* ($n = 2$)	*Aspergillus calidoustus* ($n = 2$)	Other species ($n = 2$)[a]
Itraconazole (no. [%])[b]	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
Voriconazole (no. [%])	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
Posaconazole (no. [%])	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
Any azole (no. [%])	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)

[a]The other species included *Aspergillus terreus* and *Aspergillus nidulans*.
[b]Values refer to the number and percentage of isolates that had a positive screening test with VIPcheck plates.

In total, 113 isolates were received from 5 different hospital centers across the province of Québec. The complete list of species and the results of antifungal susceptibility testing are presented in Table 1. The most common species identified were *Aspergillus fumigatus* ($n = 86$; 76.1%) followed by *Aspergillus flavus* ($n = 12$; 10.6%), *Aspergillus niger* ($n = 5$; 4.4%), and *Aspergillus versicolor* ($n = 4$; 3.5%). Only a single isolate (0.8% of total tested) of *Aspergillus glaucus* screened positive for azole nonsusceptibility in the VIPcheck plate. This isolate had MICs to itraconazole, voriconazole, and posaconazole of 0.25 mg/liter, 0.5 mg/liter, and 0.12 mg/liter, respectively. While there are no defined ECV for *A. glaucus*, the values are below the ECV for other *Aspergillus* species (17) and the isolate was thus presumed susceptible. The prevalence of azole resistance in our population is estimated at 0% (95% confidence interval [CI], 0 to 3.2%).

While patients with IA treated with an azole may require changes in therapy due to a lack of clinical efficacy, these cases can often be successfully treated with a different azole antifungal (18). Our data suggest that microbiologically confirmed azole resistance is rare in the province of Québec. Therefore, antifungal resistance to triazole antifungals is unlikely a significant contributor to clinical failure in patients with IA in our geographic area, and other factors, including host immune status, antifungal pharmacokinetics, and incorrect diagnosis, should be considered first.

Our results must be interpreted in the context of the study characteristics. Our epidemiologic study did not examine patient outcomes and, therefore, only indirectly addresses the potential causes of treatment failure with azole antifungals. Although the VIPcheck is not 100% sensitive, assuming even a 5% pretest probability of azole resistance, the negative predictive value of the VIPcheck remains excellent at 99.9%, which gives us confidence in our estimate. Our results are thus informative regarding the risk of antifungal resistance across a large geographical area and suggest that the rate of azole antifungal resistance among *Aspergillus* species is low in the province of Québec.

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