

# Susceptibility Patterns of Contemporary *Aspergillus fumigatus* Isolates from the U.S. to Azole Antifungals

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## BACKGROUND & OBJECTIVE

- Invasive aspergillosis remains a significant cause of morbidity and mortality in highly immunocompromised patients. *Aspergillus fumigatus* is the most prevalent species of this genus to cause invasive disease.
- The mainstays of antifungal prophylaxis and treatment include the azole antifungals voriconazole, posaconazole, and isavuconazole.
- However, there is growing concern regarding azole resistance in *A. fumigatus*, which has been documented following both clinical and environmental exposure to azoles.
- Our objective was to evaluate the in vitro susceptibility of clinical isolates of *A. fumigatus* from the United States to voriconazole, posaconazole, and isavuconazole.

## MATERIALS & METHODS

- Clinical isolates of *Aspergillus* cultured from humans sent to the Fungus Testing Laboratory at the UT Health Science Center San Antonio for testing between September 2015 and March 2019 were included.
- Species identification was performed by combined phenotypic characteristics and DNA sequence analysis of the  $\beta$ -tubulin (*BenA*) and calmodulin (*CaM*) genes.
- Minimum inhibitory concentrations (MICs) for voriconazole, posaconazole, and isavuconazole were determined by broth microdilution susceptibility testing according to the methods in the CLSI M38 reference standard (M38Ed3).
- MIC ranges, MIC<sub>50</sub> and MIC<sub>90</sub> values, and geometric mean (GM) MIC values were determined.

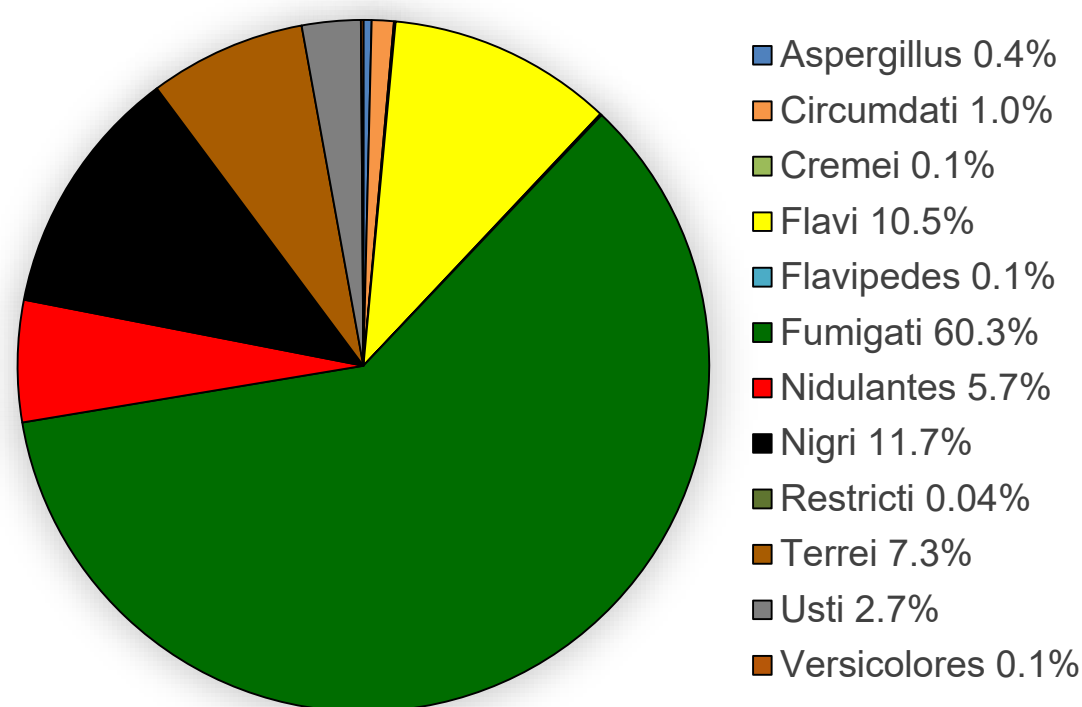
## RESULTS

- Species identifications were performed on 2620 *Aspergillus* isolates. The most common sections were *Fumigati*, *Flavi*, *Nigri*, *Terrei*, and *Nidulantes*.
- The most common species was *A. fumigatus* (n = 1534 [58.1%]).
- 1391 voriconazole, 773 posaconazole, and 1534 isavuconazole MIC values against *A. fumigatus* sensu stricto isolates were available for analysis.
- MIC ranges were  $\leq 0.03$  -  $>16$  mg/L for each triazole, and the GM MIC was lowest for posaconazole (0.12 mg/L) followed by voriconazole (0.37 mg/L) and isavuconazole (0.66 mg/L). Similar trends were observed for MIC<sub>50</sub> and MIC<sub>90</sub> values (Table 1).
- Per published epidemiological cut-off values established using the CLSI M38 broth microdilution method (published in CLSI M59 document), 3.49% - 5.15% of *A. fumigatus* isolates would be considered non-wild type to at least one of these triazoles (3.49% for posaconazole, 3.52% for voriconazole, and 5.15% for isavuconazole).
- Per the proposed CLSI voriconazole *A. fumigatus* clinical breakpoints, 92.38% of isolates were susceptible to voriconazole (MIC  $\leq 0.5$  mg/L), 4.10% were intermediate (MIC 1 mg/L), and 3.52% were resistant (MIC  $\leq 2$  mg/L).

## CONCLUSIONS

*Aspergillus fumigatus* remains the predominant *Aspergillus* species cultured from patients in the U.S. Overall, the majority of *A. fumigatus* isolates received by our reference laboratory during this 3.5-year period are wild type based on epidemiological cut-off values established per CLSI methods. However, non-wild type isolates and those considered resistant to voriconazole based on proposed CLSI clinical breakpoints were identified throughout this surveillance period. Clinicians and microbiology laboratories in the U.S. need to be aware of azole resistance in *A. fumigatus* and continued vigilance is needed.

**Figure 1.** Distribution of *Aspergillus* sections among 2620 clinical isolates from the U.S. between 2015-2018.



**Table 1.** MIC ranges, MIC<sub>50</sub>/MIC<sub>90</sub> values, GM MICs of voriconazole, posaconazole, and isavuconazole against *A. fumigatus* sensu stricto. Values are expressed in mg/L. Percent wild-type per CLSI ECV values are presented for each triazole, and percent susceptible, intermediate, and resistant are presented for voriconazole based on CLSI clinical breakpoints (CBP).

MIC Parameter	Voriconazole	Posaconazole	Isavuconazole
MIC Range	$\leq 0.03$ - $>16$	$\leq 0.03$ - $>16$	$\leq 0.03$ - $>16$
MIC <sub>50</sub>	0.25	0.125	0.5
MIC <sub>90</sub>	0.5	0.25	1
GM MIC	0.37	0.12	0.66
ECV % Wild Type	96.48%	96.51%	94.85%
ECV % Non-Wild Type	3.52%	3.49%	5.15%
<b>Voriconazole CLSI CBP</b>	<b>Susceptible</b>	<b>Intermediate</b>	<b>Resistant</b>
Percent	92.38%	4.10%	3.52%

**Table 2.** Distributions of five most common species within most prevalent *Aspergillus* sections.

Section <i>Fumigati</i> (n = 1580)		Section <i>Flavi</i> (n = 277)		Section <i>Nigri</i> (n = 307)		Section <i>Terrei</i> (n = 192)		Section <i>Nidulantes</i> (n = 149)	
Species	Percent	Species	Percent	Species	Percent	Species	Percent	Species	Percent
<i>A. fumigatus</i>	97.09%	<i>A. flavus</i>	85.92%	<i>A. tubingensis</i>	39.74%	<i>A. terreus</i>	68.75%	<i>A. sydowii</i>	30.20%
<i>A. lentulus</i>	1.27%	<i>A. tamarri</i>	4.33%	<i>A. welwitschiae</i>	26.71%	<i>A. hortai</i>	14.06%	<i>A. nidulans</i>	22.82%
<i>A. hiratsukae</i>	0.57%	<i>A. nomius</i>	3.97%	<i>A. niger</i>	13.03%	<i>A. citrinoterreus</i>	7.81%	Undetermined	11.41%
<i>A. thermomutatus</i>	0.44%	<i>A. parasiticus</i>	1.44%	Undetermined	10.75%	<i>A. alabamensis</i>	6.25%	<i>A. spinulosporus</i>	8.72%
<i>A. udagawae</i>	0.32%	<i>A. pseudonomius</i>	1.44%	<i>A. luchuensis</i>	3.91%	Undetermined	2.60%	<i>A. jensenii</i>	5.37%