Evaluation of the Activity of Triazoles Against Non-*fumigatus* *Aspergillus* and Cryptic *Aspergillus* Species Causing Invasive Infections Tested in the SENTRY Program

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The activity of isavuconazole and other triazoles against non-*fumigatus* (non-AFM) *Aspergillus* causing invasive aspergillosis was evaluated. A total of 390 non-AFM isolates were collected (1/patient) in 2017–2021 from 41 hospitals. Isolates were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry and/or internal spacer region/β-tubulin sequencing and tested by Clinical and Laboratory Standards Institute (CLSI) broth microdilution. CLSI epidemiological cutoff values were applied, where available. Isavuconazole showed activity against *Aspergillus* sections *Flavi* (n = 122; minimum inhibitory concentration [MIC]$_{50/90}$, 0.5/1 mg/L), *Terrei* (n = 57; MIC$_{50/90}$, 0.5/0.5 mg/L), *Nidulantes* (n = 34; MIC$_{50/90}$, 0.12/0.25 mg/L), *Versicolores* (n = 7; MIC$_{50}$, 1 mg/L), and *Circumdati* (n = 2; MIC range, 0.12–2 mg/L). Similar activity was displayed by other triazoles against those *Aspergillus* sections. Most of the isolates from *Aspergillus* sections *Fumigati* (n = 9), *Nigri* (n = 146), and *Usti* (n = 12) exhibited elevated MIC values to isavuconazole (MIC$_{50/90}$, 2/–, 2/4, and 2/8 mg/L), voriconazole (MIC$_{50/90}$, 2/–, 1/2, and 4/8 mg/L), itraconazole (MIC$_{50/90}$, 2/–, 2/4, and 8/>8 mg/L), and posaconazole (MIC$_{50/90}$, 0.5/–, 0.5/1, and >8/>8 mg/L), respectively. Isavuconazole was active (MIC values, ≤1 mg/L) against *Aspergillus parasiticus*, *Aspergillus tamarii*, *Aspergillus nomius*, *Aspergillus nidulans*, *Aspergillus unguis*, *Aspergillus terreus*, *Aspergillus alabamensis*, and *Aspergillus hortai*, while isavuconazole MIC values between 2 and 8 mg/L were observed against cryptic isolates from *Aspergillus* section *Fumigati*. Isavuconazole inhibited 96.1% of *Aspergillus niger* and 80.0% of *Aspergillus tubingensis* at ≤4 mg/L, the CLSI wild-type cutoff value for *A niger*. Voriconazole, itraconazole, and posaconazole showed similar activity to isavuconazole against most cryptic species. Isavuconazole exhibited potent in vitro activity against non-AFM; however, the activity of triazoles varies among and within cryptic species.

Keywords. antifungal; cryptic species; invasive aspergillosis; triazole resistance; *Aspergillus* non-fumigatus.

Invasive fungal infections due to opportunistic molds are most frequently due to *Aspergillus fumigatus* but recent years have seen an increase in infections due to non-*fumigatus* species of *Aspergillus* [1, 2]. Furthermore, the application of molecular methods has identified numerous cryptic species, several of which exhibit decreased susceptibility (elevated or non-wild type [NWT] minimum inhibitory concentration [MIC] values) to 1 or more systemically active antifungal agents [1, 3–5].

The reported frequency of non-*fumigatus* species of *Aspergillus* from clinical specimens ranges from 11% to 54%, with considerable variation according to geographic location [2]. Notably, non-*fumigatus* species appear to be the leading cause of invasive aspergillosis (IA) in tropical and subtropical regions and in individuals with primary immunodeficiencies [2, 6, 7]. After the broader use of molecular and/or proteomic methods for species identification, it is estimated that the frequency of cryptic species of *Aspergillus* in the clinical setting is between 10% and 15% [1, 3] or even greater (27%) in patients with chronic pulmonary aspergillosis [8]. The cryptic and other non-*fumigatus* species of *Aspergillus* have not been well-recognized due to their rare occurrence in clinical settings compounded by taxonomic changes and, most importantly, the lack of accurate species identification in diagnosis [2, 9].

Voriconazole and isavuconazole are considered first-line therapy for IA [10], but the performance of these mold-active triazoles is uncertain against the cryptic *Aspergillus* species [3, 11]. The emergence of resistance to the azole class of antifungal agents among isolates of *Aspergillus fumigatus* is well-known

[9, 12]. In contrast, detailed epidemiology and antifungal resistance profiles of non-*fumigatus* species of *Aspergillus*, including cryptic species, is lacking; the reason is infrequent isolation in culture heightened by the lack of proper species identification and antifungal susceptibility testing [2]. The recognition of cryptic species, such as *Aspergillus lentulus* in the *Aspergillus* section *Fumigati*, emphasize how newly recognized cryptic species exhibit significantly different susceptibility profiles than *A fumigatus* and are often less susceptible [2, 9]. Infections with these non-*fumigatus* species are generally much more difficult to treat due to acquired or intrinsic resistance to available antifungal agents [2, 9]. As such, correct and prompt identification at the species level and antifungal susceptibility testing is critical to determine which therapy is appropriate to improve patient outcomes [2, 9].

In the present study, we examined the frequency of non-*fumigatus* and cryptic species in a collection of 1236 clinical isolates of *Aspergillus* species obtained during the SENTRY Antimicrobial Surveillance Program survey for the years 2017–2021. Isolates were obtained from the microbiology laboratories of 45 medical centers worldwide. All isolates were identified centrally to the species level by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) or DNA sequencing methods and tested for susceptibility to isavuconazole and 3 azole comparators (itraconazole, posaconazole, and voriconazole) using standard broth microdilution methods according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

MATERIALS AND METHODS

Organisms

A total of 1236 nonduplicate isolates of *Aspergillus* species were collected from 45 medical centers participating in SENTRY during 2017–2021: North America (n = 419; 18 centers), Europe (n = 610; 18 centers), Asia-Pacific (n = 187; 8 centers), and Latin America (n = 20; 1 center). For the purposes of this study, all isolates of *A fumigatus* sensu stricto (n = 846 [68.4%]) were omitted from consideration, leaving a total of 390 (31.6%) non-*fumigatus* isolates: North America, n = 137

(32.7%); Europe, n = 161 (26.4%); Asia-Pacific, n = 85 (45.5%); and Latin America, n = 7 (35.0%) (Table 1). The non-*fumigatus* isolates were recovered from patients with respiratory tract infections (241 isolates), bloodstream infections (8 isolates), skin and skin structure infections (35 isolates), and miscellaneous other sites (106 isolates) as determined by local criteria.

Identification Methods

Species identification was confirmed using DNA sequencing and proteomic methods [13, 14]. Mold isolates were subcultured on potato dextrose agar (Remel, Inc, Lenexa, Kansas) after arrival at the coordinating laboratory and grown for up to 7 days to assess purity and viability. Isolates confirmed as pure were inoculated into Sabouraud Liquid Broth Modified (Becton, Dickinson and Company, Sparks, Maryland) and the hyphae harvested and prepared for formic acid extraction. Isolates then were submitted to MALDI-TOF MS using the MALDI Biotyper (Bruker Daltronics, Billerica, Massachusetts). Isolates not scoring ≥2.0 by spectrometry were identified using sequencing of the 28S ribosomal subunit, followed by analysis of β-tubulin or internal spacer regions (ITSs) [13–16]. Nucleotide sequences were analyzed using Lasergene software (DNASTAR, Madison, Wisconsin) and compared to sequences using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Susceptibility Testing

All isolates of non-*fumigatus* species were tested by broth microdilution using CLSI methodologies [17] against isavuconazole (testing range, 0.008–8 mg/L), itraconazole (0.008–8 mg/L), posaconazole (0.008–8 mg/L), and voriconazole (0.008–8 mg/L). Frozen-form microdilution panels using RPMI 1640 broth supplemented with morpholinepropane sulfonic acid buffer were inoculated with $0.4–5.0 \times 10^4$ colony-forming units (CFU)/mL conidial suspensions for a final concentration of $0.2–2.5 \times 10^4$ CFU/mL. MICs were visualized after 48 hours. MIC endpoints were read at the lowest concentration producing visually clear wells. Quality control was performed in accordance with CLSI M38 guidelines using *Aspergillus flavus* ATCC 204304 and *A fumigatus* ATCC MYA-3626. MIC values were within the quality control ranges.

Clinical breakpoints (CBPs) have been published by CLSI for *Aspergillus* section *Fumigati* and voriconazole (susceptible, ≤0.5 mg/L; intermediate, 1 mg/L; resistant, ≥2 mg/L) and, most recently for isavuconazole (susceptible, ≤1 mg/L; intermediate, 2 mg/L; resistant, ≥4 mg/L) [18]. Epidemiological cutoff values (ECVs) have been developed for *Aspergillus* section *Fumigati* and isavuconazole (ECV, 1 mg/L), itraconazole (ECV, 1 mg/L), posaconazole (ECV, 0.5 mg/L), and voriconazole (ECV, 1 mg/L) [19–21]. CBPs have not been established for any antifungal agent and the non-*fumigatus* species.

Table 1. Frequency of *Aspergillus fumigatus*, Non-*fumigatus*, and Cryptic Species by Geographic Region—SENTRY Antimicrobial Surveillance Program, 2017–2021

Location	No. of Isolates	*Aspergillus fumigatus*, No. (%)	Non-*fumigatus* Species, No. (%)	Cryptic Species, No. (%)
All regions	1236	846 (68.4)	390 (31.6)	63 (5.1)
Asia-Pacific	187	102 (54.5)	85 (45.5)	12 (6.4)
Europe	610	449 (73.6)	161 (26.4)	21 (3.4)
Latin America	20	13 (65.0)	7 (35.0)	0 (0.0)
North America	419	282 (67.3)	137 (32.7)	30 (7.2)

However, in addition to *Aspergillus* section *Fumigati*, ECVs have been developed by CLSI for *Aspergillus* section *Flavi*, section *Nigri*, section *Nidulantes*, section *Nigri*, and section *Terrei* for isavuconazole, itraconazole, posaconazole, and voriconazole [20–23]. Isavuconazole, itraconazole, and voriconazole MIC values of >1 mg/L were considered NWT for section *Flavi* and section *Terrei*; isavuconazole, itraconazole, and posaconazole MIC values of >1 mg/L and voriconazole MIC values of >2 mg/L were considered NWT for section *Nidulantes*. Posaconazole MIC values of >0.25 mg/L were considered NWT for section *Flavi* and MIC results of >0.5 mg/L were NWT for section *Nigri* and section *Terrei*; isavuconazole and itraconazole MIC values of >4 mg/L and posaconazole and voriconazole MIC values of >2 mg/L were NWT for section *Nigri*. Isolates for which triazole MIC results exceeded the ECV were considered NWT [18, 19]. For the purposes of this study, we chose to apply the respective ECVs for each triazole and *Aspergillus* section to those cryptic species within the appropriate section.

RESULTS

Organisms

Of the 1236 clinical isolates of *Aspergillus* species collected during the 2017–2021 survey, 846 (68.4%) were *A fumigatus*, 390 (31.6%) were non-*fumigatus* species, and 63 (5.1%) were cryptic species (Table 1). The frequency of non-*fumigatus* species ranged from 26.4% in Europe to 45.5% in the Asia-Pacific region. The frequency of cryptic species was 0.0% among isolates from Latin America, 3.4% among European isolates, 6.4% among isolates from the Asia-Pacific region, and 7.2% among North American isolates (Table 1). There were 8 different sections or species complexes identified that comprised non-*fumigatus* species including section *Fumigati* (9 isolates; 3 species), section *Circumdati* (2 isolates; 1 species), section *Flavi* (122 isolates; 4 species), section *Nidulantes* (34 isolates; 3 species), section *Nigri* (146 isolates; 3 species), section *Terrei* (57 isolates; 4 species), section *Usti* (12 isolates; 2 species), and section *Versicolores* (7 isolates; 2 species) (Table 2).

In Vitro Activity of Triazoles Against Non-*fumigatus* Species of *Aspergillus* Split by *Aspergillus* Sections

Among the 8 different *Aspergillus* sections listed in Table 2, 4 had ECVs were available for all 4 of the triazoles tested: section *Flavi*, section *Fumigati*, section *Nigri*, and section *Terrei*. Most isolates within sections *Flavi* (98.4%–100.0% wild type [WT]), *Nigri* (92.4%–100.0%), and *Terrei* (98.2%–100.0%) were classified as WT using the ECVs published by CLSI for the respective sections and antifungals tested. *Aspergillus* section *Fumigati* contained 3 species that are well-known to express resistance to triazoles as well as other classes of antifungal agents: *A lentulus*, *Aspergillus thermomutatus*, and *Aspergillus udagawae*

Table 2. Activity of Triazoles Against Non-*fumigatus* *Aspergillus* Split by Sections

| Aspergillus Section | Aspergillus Species Included | No. of Isolates | MIC$_{50/90}$ (MIC Range; % WT[b]), mg/L | | | | |
			Isavuconazole	Voriconazole	Itraconazole	Posaconazole
Circumdati	*A sclerotiorum*	2	0.12/– (0.12–2; NA)	0.12/– (0.12–0.5; NA)	1/– (1; NA)	0.25/– (0.25–0.5; NA)
Flavi	*A parasiticus, A tamarii, A nomius, A flavus* SC	122	0.5/1 (0.12–2; 98.4%)	0.5/1 (0.25–2; 100%)	0.5/1 (0.12–1; 100%)	0.5/0.5 (0.12–1; 99.2%)
Fumigati	*A lentulus, A thermomutatus, A udagawae*	9	2/– (2–8; 0%)	2/– (2–8; 0%)	2/– (1–4; 33.3%)	0.5/– (0.5–1; 66.7%)
Nidulantes	*A nidulans, A unguis, A nidulans* SC	34	0.12/0.25 (0.015–0.5; NA)	0.12/0.25 (0.03–0.5; NA)	0.5/1 (0.12–4; NA)	0.25/0.5 (0.06–1; NA)
Nigri	*A niger, A tubingensis, A niger* SC	146	2/4 (0.06–>8; 95.2%)	1/2 (0.06–4; 97.9%)	2/4 (0.12–>8; 92.4%)	0.5/1 (0.12–2; 100%)
Terrei	*A alabamensis, A hortai, A terreus, A terreus* SC	57	0.5/0.5 (0.06–2; 98.2%)	0.25/0.5 (0.06–1; 100%)	0.5/1 (0.12–1; 100%)	0.25/0.5 (0.12–0.5; 100%)
Usti	*A calidoustus, A ustus* SC	12	2/8 (0.12–8; NA)	4/8 (0.25–>8; NA)	8/>8 (0.25–>8; NA)	>8/>8 (0.5–>8; NA)
Versicolores	*A versicolor, A sydowii*	7	1/– (0.5–2; NA)	1/– (0.25–1; NA)	1/– (0.5–2; NA)	1/– (0.25–1; NA)

Abbreviations: MIC, minimum inhibitory concentration; NA, not available; SC, species complex; WT, wild type.

^{a}MIC$_{90}$ was not calculated for *Aspergillus* sections with <10 isolates.

^{b}Clinical and Laboratory Standards Institute M57 (2022) epidemiological cutoff value criteria for *A fumigatus, A flavus, A niger,* and *A terreus* were applied to their corresponding *Aspergillus* section isolates.

Table 3. Activity of Triazoles Against Cryptic *Aspergillus* Species

Aspergillus Section	*Aspergillus* Species	No. of Isolates	MIC Range (% WT[a]), mg/L			
			Isavuconazole	Voriconazole	Itraconazole	Posaconazole
Circumdati	*A sclerotiorum*	2	0.12–2 (NA)	0.12–0.5 (NA)	1 (NA)	0.25–0.5 (NA)
Flavi	*A parasiticus*	2	0.5 (100%)	0.5–1 (100%)	0.5–1 (100%)	0.25–0.5 (100%)
	A tamarii	2	0.12–0.25 (100%)	0.25–0.5 (100%)	0.25–0.5 (100%)	0.12–0.25 (100%)
	A nomius	1	0.5 (100%)	0.5 (100%)	0.5 (100%)	0.5 (100%)
Fumigati	*A lentulus*	6	2–4 (0.0%)	2 (0.0%)	1–2 (50.0%)	0.5 (100.0%)
	A thermomutatus	2	2–8 (0.0%)	4–8 (0.0%)	4 (0.0%)	1 (0.0%)
	A udagawae	1	2 (0.0%)	2 (0.0%)	2 (0.0%)	1 (0.0%)
Nidulantes	*A nidulans*	24	0.015–0.25 (NA)	0.03–0.5 (NA)	0.12–1 (NA)	0.06–0.5 (NA)
	A unguis	3	0.25–0.5 (NA)	0.06–0.25 (NA)	0.5–4 (NA)	0.25–1 (NA)
Nigri	*A tubingensis*	5	2–8 (80.0%)	1–4 (80.0%)	2–>8 (80.0%)	0.5–2 (100.0%)
Terrei	*A alabamensis*	2	0.25 (100%)	0.25–0.5 (100%)	0.5 (100%)	0.25 (100%)
	A hortai	1	0.25 (100%)	0.12 (100%)	0.5 (100%)	0.12 (100%)
Usti	*A calidoustus*	5	2–8 (NA)	4–>8 (NA)	4–8 (NA)	4–>8 (NA)
Versicolores	*A versicolor*	6	0.5–2 (NA)	0.25–1 (NA)	0.5–2 (NA)	0.25–1 (NA)
	A sydowii	1	2 (NA)	1 (NA)	2 (NA)	1 (NA)

Abbreviations: MIC, minimum inhibitory concentration; NA, not available; WT, wild type.

[a]Clinical and Laboratory Standards Institute M57 (2022) epidemiological cutoff value criteria for *A fumigatus*, *A flavus*, *A niger*, and *A terreus* were applied to cryptic species from the same *Aspergillus* section.

(Table 2). These isolates had elevated MIC values of 2–8 mg/L for both isavuconazole and voriconazole and were classified as NWT to those agents. Both itraconazole and posaconazole were slightly more active against these species, with 33.3% WT to itraconazole and 66.7% WT to posaconazole at their respective ECVs. ECVs are not available for the remaining *Aspergillus* sections (Table 2). *Aspergillus* section *Usti* isolates showed elevated MIC₉₀ values to all 4 triazoles ranging from 8 mg/L for isavuconazole and voriconazole to >8 mg/L for itraconazole and posaconazole (Table 2).

Triazole Activity Against Cryptic *Aspergillus* Species

For purposes of comparison, we applied the ECVs available for the 4 triazoles and each *Aspergillus* section to the individual cryptic species in that section. The activity of the triazoles against the 63 isolates of cryptic species of *Aspergillus* are shown in Table 3. Isavuconazole was active (MIC values, ≤1 mg/L) against *Aspergillus parasiticus* (100.0% WT), *Aspergillus tamarii* (100.0% WT), *Aspergillus nomius* (100.0% WT), *Aspergillus nidulans* (MIC range, 0.015–0.25 mg/L [no ECV]), *Aspergillus unguis* (MIC range, 0.25–0.5 mg/L [no ECV]), *Aspergillus alabamensis* (100.0% WT), and *Aspergillus hortai* (100.0% WT), while isavuconazole MIC values between 2 and 8 mg/L were observed against cryptic species from *Aspergillus* section *Fumigati* as well as *Aspergillus tubingensis*, *Aspergillus calidoustus*, and *Aspergillus sydowii*. Isavuconazole inhibited 80.0% of *A tubingensis* isolates at ≤4 mg/L, the ECV for *Aspergillus* section *Nigri*. Voriconazole, itraconazole, and posaconazole showed similar activity to isavuconazole against most cryptic *Aspergillus* species (Table 3). Among the 63 isolates comprising the cryptic species, there were 20 isolates representing 6 different species (*A lentulus*, *A thermomutatus*, *A udagawae*, *A tubingensis*, *A calidoustus*, and *A sydowii*) for which the MIC values for both isavuconazole and voriconazole were elevated (≥2 mg/L) or NWT (Table 3). Most of these species were from North America (n = 10 [50.0%]) and Europe (n = 6 [30.0%]), but 4 isolates (20.0%) were from the Asia-Pacific region. No isolates were from Latin America.

Triazole Activity Against the 3 Most Frequent Non-*fumigatus* Species: *A flavus* Species Complex, *A terreus*, and *A niger*

The most common clinical isolates of non-*fumigatus* *Aspergillus* species are *A. flavus* species complex (30.0% of non-*fumigatus* species), *Aspergillus niger* (19.7%), and *Aspergillus terreus* (6.9%) (Table 4). These species were classified as WT to isavuconazole: *A flavus* species complex (98.3% WT), *A terreus* (100.0% WT), and *A niger* (96.1% WT). Voriconazole, itraconazole, and posaconazole all showed similar activity to isavuconazole against these 3 species, with 96.1%–100.0% WT using CLSI ECVs (Table 4).

DISCUSSION

The most frequent cause of IA is *A fumigatus*. Accordingly, the mold-active triazoles, isavuconazole and voriconazole, have been developed with this species in mind [10]. *Aspergillus fumigatus* and the 3 most frequently isolated non-*fumigatus* species, *A flavus*, *A niger*, and *A terreus*, are generally identified using morphological criteria in the routine clinical laboratory [2]. Despite the recognition of cryptic species of *Aspergillus*, only a limited number of isolates have been described clinically, and the data regarding antifungal treatment are even more scarce [2, 3]. This lack of data is understandable when most

Table 4. Activity of Triazoles Against Worldwide *Aspergillus flavus* Species Complex, *Aspergillus terreus*, and *Aspergillus niger* From 2017 to 2021

Aspergillus Section	*Aspergillus* Species	No. of Isolates	MIC$_{50/90}$ (MIC Range; % WT[a]), mg/L			
			Isavuconazole	Voriconazole	Itraconazole	Posaconazole
Flavi	*A flavus* species complex	117	0.5/1 (0.12–2; 98.3%)	0.5/1 (0.25–2; 100%)	0.5/1 (0.12–1; 100%)	0.5/0.5 (0.12–1; 99.1%)
Terrei	*A terreus*	27	0.5/0.5 (0.06–1; 100%)	0.25/0.5 (0.12–0.5; 100%)	0.5/1 (0.25–1; 100%)	0.25/0.5 (0.12–0.5; 100%)
Nigri	*A niger*	77	2/4 (0.5–>8; 96.1%)	1/1 (0.25–4; 98.7%)	2/4 (0.25–>8; 97.4%)	0.5/1 (0.25–1; 100%)

Abbreviations: MIC, minimum inhibitory concentration; WT, wild type.

[a]Using Clinical and Laboratory Standards Institute M57 (2022) epidemiological cutoff value criteria.

patients in antifungal therapy trials for IA are enrolled based on galactomannan antigen testing or diagnostic imaging, so culture is usually not obtained and the frequency of cryptic species or other rare non-*fumigatus* species cannot be determined [5]. Despite these difficulties, reports of cryptic species appear to be increasing either because of better awareness of cryptic species, the use of improved molecular identification methods and databases, or a real trend of increasing frequency [1]. At present, our understanding of antifungal susceptibility patterns and response to antifungal therapy is limited for the non-*fumigatus* and cryptic species of *Aspergillus* [1–3, 5]. Infection with these species is generally more difficult to treat than *A fumigatus* due to their lower susceptibility to available antifungal agents [3, 9]. As such, correct and prompt identification at the species level and antifungal susceptibility testing is critical for appropriate therapy to be selected, which would improve patient outcomes [1–5, 9, 24].

Isavuconazole MIC values for the non-*fumigatus* species of *Aspergillus* from this most recent 5-year surveillance period (2017–2021) demonstrated little or no change compared to reports from previous years [15, 16, 25, 26], with activity comparable to that of itraconazole, posaconazole, and voriconazole. Isavuconazole and the other mold-active triazoles continue to be highly active against the more common non-*fumigatus* species, section *Flavi*, section *Nigri*, and section *Terrei*, but are less potent against several of the cryptic species such as *A lentulus*, *A thermomutatus*, *A udagawae*, *A tubingensis*, *A calidoustus*, and *A sydowii* (Table 3).

The frequency of non-*fumigatus* and cryptic species varied across geographic regions in the present survey with the highest frequency of non-*fumigatus* species observed in isolates from the Asia-Pacific region (45.5%; Table 1). Only 26.4% of European isolates were non-*fumigatus* species compared to 32.7% of isolates from North America. The highest frequency of cryptic species were from North America (7.2%; Table 1). Despite the robustness of the in vitro data in this study, the collection of isolates could create a bias in the species prevalence due to the targeting of certain regions and the absence of samples of other geographic areas; thus, these data might not be extrapolated globally.

In general, the more common non-*fumigatus* sections of *Aspergillus* were susceptible/WT to all the mold-active triazoles tested: section *Flavi* (98.4%–100.0% WT), section *Nigri* (92.4%–100.0% WT), and section *Terrei* (98.2%–100.0% WT) (Table 2). We demonstrated that resistance/NWT to 2 or more of the triazoles tested was frequent among 6 different cryptic species (*A lentulus*, *A thermomutatus*, *A udagawae*, *A tubingensis*, *A calidoustus*, and *A sydowii*) representing 5.1% of all non-*fumigatus* species and 1.6% of all *Aspergillus* species tested (Table 3). Half (50.0%) of these isolates originated from North America. These triazole-NWT non-*fumigatus* species, along with emerging multidrug-resistant strains of *A fumigatus*, must be actively sought in clinical material and undergo accurate species identification as well as antifungal susceptibility testing to ensure optimal patient management [1–5, 9, 24]. It is important to note that an analysis of real-world usage, along with an analysis of clinical trial samples, showed that drug concentrations of >1 mg/L may be achieved with standard doses of isavuconazole [27]. Recently published data indicated that high-dose isavuconazole treatment (eg, 400 mg once daily) might be an option in selected patients infected with an *Aspergillus* isolate for which the isavuconazole MIC value was 2 mg/L [22].

In summary, the application of molecular and proteomic methods of identification reveals numerous cryptic non-*fumigatus* species within each *Aspergillus* section. Isavuconazole exhibited excellent activity against the more common non-*fumigatus* species as well as several of the cryptic species in each section save section *Fumigati* (*A lentulus*, *A thermomutatus*, and *A udagawae*) and section *Usti* (*A calidoustus*) (Table 3). The cryptic species *A tubingensis* (section *Nigri*) and *A sydowii* (section *Versicolores*) also show elevated MIC values to isavuconazole and at least 1 additional triazole. The finding of relatively resistant cryptic species within a section of otherwise susceptible species points to the importance of accurate identification as well as the need for antifungal susceptibility testing. Misidentification may lead to inappropriate treatment decisions, worsening the infection outcome [2]. In light of accumulating evidence that in vitro triazole resistance has an adverse impact on the survival of patients infected with common species such as *A fumigatus*

[28] and *A flavus* [29], one may reasonably assume that any resistant/NWT *Aspergillus* species infection would be associated with a poor prognosis [2]. Although most species of *Aspergillus* remain susceptible to isavuconazole and the other triazoles, emergence of acquired or intrinsic resistance during therapy, especially in patients with previous antifungal exposure, must be acknowledged [2, 9]. Given the extensive use of mold-active triazoles in the prevention and treatment of IA, emergence of cryptic species of *Aspergillus* with intrinsic or acquired resistance as breakthrough infections is a clear threat.

Notes

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