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Susceptibility Testing of Common and Uncommon *Aspergillus* Species against Posaconazole and Other Mold-Active Antifungal Azoles Using the Sensititre Method

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ABSTRACT We tested 59 common and 27 uncommon *Aspergillus* species isolates for susceptibility to the mold-active azole antifungal agents itraconazole, voriconazole, and posaconazole using the Sensititre method. The overall essential agreement with the CLSI reference method was 96.5% for itraconazole and posaconazole and was 100% for voriconazole. By the Sensititre method as well as the CLSI reference method, all of 10 *A. fumigatus* isolates with a *cyp51* mutant genotype were classified as being non-wild-type isolates (MIC > epidemiological cutoff value [ECV]) with respect to triazole susceptibility.

KEYWORDS *Aspergillus*, Sensititre, antifungal susceptibility testing

In contrast to other but emerging molds (1), *Aspergillus* species, particularly *Aspergillus fumigatus*, remain the most common causes of invasive fungal diseases in both North America and Europe (2, 3). Because of the availability of (tri)azole antifungal agents, survival of immunocompromised patients with invasive aspergillosis has improved dramatically and could be further improved by optimizing antifungal treatments (4). A key component of this optimization should be the regular *in vitro* antifungal susceptibility testing of the patients' *A. fumigatus* isolates to detect azole resistance (5). Unfortunately, in most clinical microbiology laboratories, antifungal susceptibility testing of aspergilli (and other molds) is not routinely performed (6), thus underestimating the true prevalence of fungal resistance (4).

The azole antifungal agents for clinical use include itraconazole, voriconazole, posaconazole, and, most recently, isavuconazole (7). Despite their role—unlike voriconazole, itraconazole and posaconazole are not approved as first-line agents—in treatment of invasive aspergillosis (4), the Clinical and Laboratory Standards Institute (CLSI) did not set clinical breakpoints (CBPs) for common *Aspergillus* species and mold-active triazoles, e.g., itraconazole and posaconazole (8), in contrast to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (9). However, CLSI-based epidemiological cutoff values (ECVs) were established—instead of CBPs—for *Aspergillus* species (*A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, *A. nidulans*, and *A. versicolor*) and for triazoles to aid in the early identification of clinical isolates with acquired resistance mechanisms (10, 11). Isolates of these six *Aspergillus* species for which triazole MICs exceed the ECV are considered to be non-wild type (non-WT) and may harbor mutations in the *cyp51a* gene—the best-known mechanism of triazole resistance in the *A. fumigatus* species—or other mutations (12). Interestingly, whereas the significance of ECVs in clinical practice needs to be understood, the ECVs defined to date—albeit mainly for *Candida*

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species—are based not only on CLSI or EUCAST methods but also on the Sensititre YeastOne (SYO; Thermo Fisher Scientific, MA; reviewed in reference 13) method (8). Whereas we have shown previously that the SYO microdilution panel—with which amphotericin B, echinocandins, and triazoles can be tested in parallel—is a reliable tool for antifungal resistance surveillance in *Candida* species (14), only limited data have been reported for *Aspergillus* (and other mold) species (15, 16).

In the present study, we used the SYO method for determining the activities of itraconazole, voriconazole, and posaconazole against clinical *Aspergillus* isolates, including WT and non-WT (MIC $>$ ECV) isolates, of common (59 isolates) and uncommon (27 isolates) *Aspergillus* species. All isolates were also tested against triazoles by the CLSI reference microdilution method, and data corresponding to species-specific and overall essential agreement (EA; $\pm$ 2 2-fold dilutions) were determined for each triazole.

Before testing was performed, a set of 86 *Aspergillus* isolates that represented either strains from clinical collections (held at the University Hospitals of Rome [Italy] and Nijmegen [The Netherlands]) or strains freshly isolated from clinical specimens were (re)identified at the species level by both molecular and proteomic analyses. First, comparative sequence analyses of the fungal ribosomal DNA internal transcribed spacer (ITS) region for intersection identification and of the beta-tubulin/calmodulin gene for intrasection identification (i.e., at the species level) were performed according to expert recommendations (17). Second, species-level identification was confirmed or exclusively obtained (e.g., for *Aspergillus oryzae* isolates) with matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry analysis as described previously (18; also see references 19 and 20), using an in-house database. Isolates with itraconazole and/or voriconazole CLSI MIC values of $>$1 μg/ml—the ECV developed for *A. fumigatus*, *A. flavus*, and *A. terreus* (10)—were submitted to *cyp51* gene sequence analysis for detection of azole resistance-associated mutations (4, 12). The 86 *Aspergillus* species isolates were tested for *in vitro* susceptibility to the triazoles (itraconazole, voriconazole, and posaconazole) by using the broth microdilution method of CLSI (21) and the SYO manufacturer-recommended protocol. By the CLSI method, the final range of antifungal concentrations tested was 0.03 to 16 μg/ml for all triazoles; by the SYO method, the antifungal concentrations of the YO10 panel (i.e., the SYO-10 version that includes 10 antifungal agents) ranged from 0.008 to 8 μg/ml for voriconazole and posaconazole and from 0.015 to 16 μg/ml for itraconazole (14). The MIC results for all triazoles were read after 48 h of incubation, and the MIC values were determined visually as the lowest drug concentrations that caused complete (100%) inhibition of growth relative to that of the growth control. As prolonged incubation times (i.e., $>$24 h) of YO10 panels were required, visual readings of MICs obtained with the SYO method were performed regardless of colorimetric changes. To allow comparability between the methods, MIC values of 0.008 to 0.03 μg/ml for voriconazole and posaconazole and MIC values of 0.015 to 0.03 μg/ml for itraconazole, obtained with the SYO method, were reported as $\leq$0.03 μg/ml. Otherwise, MIC values of $\geq$16 μg/ml for voriconazole and posaconazole, obtained with the CLSI method, and similar values for itraconazole, obtained with the SYO method, were reported as $>$8 μg/ml. The SYO MIC results were compared with those of the CLSI method in order to determine the EA between MIC values. High off-scale MIC results were converted to the next highest concentration, and low off-scale MIC results were left unchanged. Discrepancies of at least $\pm$2 2-fold dilutions among MIC results were used to calculate the EA (see Table S1 in the supplemental material). Thus, percent EA was calculated by using the number of test results in EA as the numerator and the total number of organisms tested as the denominator. Finally, according to the triazole ECVs established for *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, and *A. nidulans* (10), percentages of isolates from these species that were classified as WT (MIC $\leq$ ECV) or non-WT (MIC $>$ ECV) with respect to each antifungal agent using either SYO or CLSI were calculated. MIC values of the triazoles for *Candida krusei* ATCC 6258, *A. fumigatus* ATCC MYA-3626, and *A. flavus* ATCC 204304, which were used as quality control isolates, were all within the expected ranges (data not shown).

TABLE 1 *In vitro* susceptibilities of 86 *Aspergillus* species isolates to azole antifungal agents as determined by the SYO method[a]

Species (no. of isolates tested)	Species complex or section	Antifungal agent[c]	No. of isolates (no. of mutants[b]) with MIC (μg/ml) of:									
			≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
Common species												
A. fumigatus (21)	*Fumigati*	PSC		5	6		2 (2)	8 (8)				
		VRC			2	5	4	1 (1)	1 (1)	2 (2)	1 (1)	5 (5)
		ITC			2	7	3 (1)	4 (4)	1 (1)			4 (4)
A. flavus (19)	*Flavi*	PSC		7	12							
		VRC			1	14	3	1				
		ITC	1	9	8	1						
A. terreus (12)	*Terrei*	PSC		9	3							
		VRC		1	8	3						
		ITC		3	8	1						
A. niger (7)	*Nigri*	PSC	1	2	1	3						
		VRC				6	1					
		ITC			2	3	2					
Uncommon species												
A. tubingensis (6)	*Nigri*	PSC			4	2						
		VRC				1	5					
		ITC				3	3					
A. nidulans (5)	*Nidulantes*	PSC		3	2							
		VRC			2	3						
		ITC			4	1						
A. oryzae (5)	*Flavi*	PSC			2	2	1 (1)					
		VRC					2	2				1 (1)
		ITC			3	1	1 (1)					
A. lentulus (3)	*Fumigati*	PSC	2		1							
		VRC							2	1		
		ITC		1	2							
A. (*Neosartorya*) species (3)[d]	*Fumigati*	PSC			2	1						
		VRC					1	1	1			
		ITC				2	1					
A. foetidus (3)	*Nigri*	PSC			2	1						
		VRC					2	1				
		ITC					3					
A. awamori (2)	*Nigri*	PSC		2								
		VRC				2						
		ITC			1	1						

[a]MICs were determined visually after 48 h of incubation and were defined as the antifungal concentrations at which complete (100%) inhibition of growth of the *Aspergillus* species isolates was observed. As a prolonged incubation (i.e., >24 h) of SYO colorimetric plates was required, visual readings of MICs were performed regardless of color changes.

[b]Mutant isolates were defined as isolates carrying a *cyp51a* mutation (e.g., a leucine-for-histidine substitution), together with a tandem repeat of a 34-bp (or 46-bp) sequence in the gene promoter that is known to be associated with azole resistance in *A. fumigatus* (4, 12). One of 5 *A. oryzae* isolates was found to carry the T788G mutation in the *cyp51c* gene that has been reported as an azole resistance mechanism in the closely related species *A. flavus* (22).

[c]PSC, posaconazole; VRC, voriconazole; ITC, itraconazole.

[d]Data include 1 isolate each of *A.* (*Neosartorya*) *hiratsukae*, *A. thermomutatus* (*Neosartorya pseudofischeri*), and *A.* (*Neosartorya*) *udagawae*. In accordance with the recent taxonomists' recommendations for species for which a single-name nomenclature (i.e., keeping the name *Aspergillus* for all species of this genus) must be applied (17), the old teleomorphic name is indicated in brackets.

Table 1 depicts the MIC distributions for posaconazole, voriconazole, and itraconazole for the 13 species (4 common and 9 uncommon) of *Aspergillus* tested by the SYO method. The numbers of *cyp51* mutant strains detected in *A. fumigatus* and *A. oryzae* are listed in parentheses. Overall, 78 of 86 (90.7%) isolates from all *Aspergillus* species were captured at a posaconazole MIC of 0.5 μg/ml. The posaconazole MIC was 1 μg/ml for all 8 isolates of *A. fumigatus* characterized as being non-WT for posaconazole (ECV, 0.5 μg/ml). In contrast, 81 (94.2%) and 72 (83.7%) of 86 isolates from all *Aspergillus* species were captured at MICs of 1 μg/ml for itraconazole and voriconazole, respectively. Among the 14 *Aspergillus* isolates characterized as being non-WT for voriconazole (ECV, 1 μg/ml) or as having high voriconazole MIC values, the voriconazole MIC was ≥2 μg/ml for 9 *A. fumigatus* isolates (range, 2 to >8 μg/ml) and 3 *A. lentulus* isolates (MICs, 2; 2; and 4 μg/ml), 2 μg/ml for 1 *A.* (*Neosartorya*) *udagawae* isolate, and >8 μg/ml for 1 *A. oryzae* isolate. Only 4 of 9 *A. fumigatus* isolates were also classified as non-WT for itraconazole (ECV, 1 μg/ml), and the itraconazole MIC was 2 μg/ml for 1

isolate and >8 μg/ml for 3 isolates. The 1 remaining *A. fumigatus* isolate that was non-WT for itraconazole (MIC, >8 μg/ml) was instead WT for voriconazole. In summary, 2 of the 10 *A. fumigatus* isolates found to contain mutations in the *cyp51a* gene were WT for posaconazole (MIC, 0.5 μg/ml), whereas 5 and 1 of these isolates were WT for itraconazole (MICs, 0.5 to 1 μg/ml) and voriconazole (MIC, 1 μg/ml), respectively. Likewise, 1 *A. oryzae* isolate that contained the T788G mutation in the *cyp51c* gene exhibited a drug MIC value of 0.5 μg/ml for both posaconazole and itraconazole; such genetic alteration had previously been found in 1 *A. flavus* isolate exhibiting elevated CLSI voriconazole and itraconazole MICs (8 and 2 μg/ml, respectively) as described elsewhere (22). Taken together, our data indicate that the *in vitro* activity of posaconazole against both WT and *cyp51* mutant strains of *Aspergillus* species was comparable to that of voriconazole and itraconazole tested by the SYO method. These findings are in agreement with those of Gheith et al., who found that the voriconazole and posaconazole MICs were below the ECVs for all 48 clinical *Aspergillus* isolates (17 *A. niger* isolates, 18 *A. flavus* isolates, 9 *A. tubingensis* isolates, 2 *A. fumigatus* isolates, 1 *A. westerdijkiae* isolate, and 1 *A. ochraceus* isolate) tested, whereas only 2 of these isolates (2 *A. tubingensis* isolates; 22%) exhibited itraconazole MICs that were >ECV (15). Although it is plausible that lower itraconazole susceptibility of *A. tubingensis* isolates is related to the occurrence of a *cyp51a* mutation—similarly to the mutation described in *Aspergillus awamori* (another species of the section *Nigri*; see reference 23), the finding of high voriconazole susceptibility in the *Aspergillus* species studied by Gheith et al. (15) argues for the use of voriconazole as the first-line treatment of invasive aspergillosis in hospital settings, in keeping with the international recommendations (24). However, these recommendations need to be cautiously assessed in confirmed cases of azole-resistant aspergillosis (25).

Table 2 shows the comparative levels of *in vitro* activity of the three azoles against the 13 *Aspergillus* species using the SYO and CLSI methods. Whereas the posaconazole MIC results were comparable for the two methods, the MIC values obtained for voriconazole were generally higher and for itraconazole were lower with the SYO method than with the CLSI method. The overall EA between SYO MICs and CLSI MICs was 100% for voriconazole and 96.5% for both itraconazole and posaconazole. Determined only for isolates of *A. fumigatus* ($n = 21$), *A. flavus* ($n = 19$), *A. terreus* ($n = 12$), and *A. niger* ($n = 7$), the EA value was unchanged for voriconazole (100%), whereas it increased for posaconazole (98.3%) and decreased for itraconazole (94.9%). As detailed (see Table S1 in the supplemental material), the lowest EA value (66.7%) was seen with 3 isolates of *A.* (*Neosartorya*) *hiratsukae*, *A. thermomutatus* (*Neosartorya pseudofischeri*), and *A.* (*Neosartorya*) *udagawae*. However, the *N. udagawae* isolate had SYO and CLSI MICs that disagreed for 3 2-fold dilutions, although 2 other isolates had SYO and CLSI MICs that were in agreement at ±0 2-fold dilutions. The categorical agreement between the methods was 96.9% (62/64 isolates) for posaconazole, 98.4% (63/64 isolates) for voriconazole, and 93.7% (60/64 isolates) for itraconazole in interpreting the MICs according to CLSI ECVs for the 21 *A. fumigatus*, 19 *A. flavus*, 12 *A. terreus*, 7 *A. niger*, and 5 *A. nidulans* isolates studied.

Table 3 summarizes the SYO and CLSI triazole MICs for 10 *A. fumigatus* isolates with *cyp51* alterations. All but 2 isolates exhibited non-WT phenotypes for posaconazole and voriconazole (or itraconazole) according to their decreased susceptibilities (MIC > ECV) obtained with both the SYO and CLSI methods. It is worth noting that for 2 (v075-77 and v128-51) of 5 isolates with a TR_{34}/L98H mutation, a posaconazole non-WT phenotype was determined by the CLSI method (MICs, 1 μg/ml) but not by the SYO method (MICs, 0.5 μg/ml). Interestingly, for the v128-51 isolate, a voriconazole non-WT phenotype was determined by the SYO method (MIC, 2 μg/ml) but not by the CLSI method (MIC, 1 μg/ml). In general, discrepancies between the methods—with respect to their capability of discriminating non-WT from WT isolates—were noticed among the *A. fumigatus* isolates for which ±1-dilution MIC differences fell into ranges of 0.5 to 1 μg/ml or 1 to 2 μg/ml and thus include the posaconazole or voriconazole (and itraconazole) ECVs of 0.5 and 1 μg/ml, respectively. Consistently, all of 5 *A. fumigatus*

TABLE 2 Comparison of *in vitro* activities of posaconazole, voriconazole, and itraconazole tested against *Aspergillus* species by SYO and CLSI methods[a]

Species (no. of isolates tested)[b]	Test method	MIC (μg/ml)								
		PSC			VRC			ITC		
		Range	Mode(s)	% EA	Range	Mode(s)	% EA	Range	Mode(s)	% EA
A. fumigatus (21)	SYO	0.06 to 1	1	100	0.125 to >8	0.25	100	0.125 to >8	0.25	95.2
	CLSI	≤0.03 to 2	1		0.06 to >8	0.125		0.25 to >8	1	
A. flavus (19)	SYO	0.06 to 0.125	0.125	100	0.125 to 1	0.25	100	0.03 to 0.25	0.06	94.7
	CLSI	≤0.03 to 0.25	0.125		0.06 to 0.25	0.125		0.06 to 0.5	0.125	
A. terreus (12)	SYO	0.06 to 0.125	0.06	100	0.06 to 0.25	0.125	100	0.06 to 0.25	0.125	91.7
	CLSI	≤0.03 to 0.25	0.25		0.06 to 0.125	0.06		0.125 to 0.5	0.25	
A. niger (7)	SYO	≤0.03 to 0.25	0.25	85.7	0.25 to 0.5	0.25	100	0.125 to 0.5	0.25	100
	CLSI	0.06 to 0.25	0.25		0.06 to 0.25	0.25		0.5 to 1	0.5	
A. tubingensis (6)	SYO	0.125 to 0.25	0.125	100	0.25 to 0.5	0.5	100	0.25 to 0.5	0.25, 0.5	100
	CLSI	0.125 to 0.5	0.125, 0.25, 0.5		0.125 to 0.5	0.25		0.5 to 2	0.5	
A. nidulans (5)	SYO	0.06 to 0.125	0.06	100	0.125 to 0.25	0.25	100	0.125 to 0.25	0.125	100
	CLSI	≤0.03 to 0.06	0.03		0.06 to 0.25	0.06, 0.125		0.25 to 1	0.5	
A. oryzae (5)	SYO	0.125 to 0.5	0.125, 0.25	80.0	0.5 to >8	0.5, 1	100	0.125 to 0.5	0.125	100
	CLSI	≤0.03 to 1	0.03		0.125 to 2	0.125		0.25 to 1	0.25	
A. lentulus (3)	SYO	0.03 to 0.125	0.03	100	2 to 4	2	100	0.06 to 0.125	0.125	100
	CLSI	≤0.03 to 0.06	0.03		0.5 to 2	ND		0.25 to 0.5	0.25	
A. (*Neosartorya*) species (3)[c]	SYO	0.125 to 0.25	0.125	66.7	0.5 to 2	ND	100	0.25 to 0.5	0.25	100
	CLSI	≤0.03 to 0.125	0.125		0.5 to 1	1		0.5 to 1	0.5	
A. foetidus (3)	SYO	0.125 to 0.25	0.125	100	0.5 to 1	0.5	100	0.5	0.5	100
	CLSI	≤0.03 to 0.25	ND		0.25 to 0.5	0.25		0.25 to 2	ND	
A. awamori (2)	SYO	0.06	0.06	100	0.25	0.25	100	0.125 to 0.25	ND	100
	CLSI	≤0.03 to 0.125	ND		0.125 to 0.25	ND		0.25 to 0.5	ND	

[a]Posaconazole (PSC), voriconazole (VRC), and itraconazole (ITC) MICs were defined as the antifungal concentrations at which complete (100%) inhibition of growth of the *Aspergillus* species isolates was observed and are reported as the range and mode(s) (i.e., most frequent MIC[s] for each species). ND, not determined. For each species, the essential agreement (EA) between MIC values (± 2 2-fold dilutions) was calculated by comparison of MIC results obtained with the SYO method to those obtained with the CLSI method.

[b]Except for *A. nidulans*, all of the less common or cryptic *Aspergillus* species listed belonged to the following *Aspergillus* sections, per molecular and/or proteomic-based identification: *Fumigati* (*A. lentulus* and *Aspergillus* [*Neosartorya*] spp.), *Flavi* (*A. oryzae*), and *Nigri* (*A. tubingensis*, *A. foetidus*, and *A. awamori*).

[c]Data include 1 isolate each of *A.* (*Neosartorya*) *hiratsukae*, *A. thermomutatus* (*Neosartorya pseudofischeri*), and *A.* (*Neosartorya*) *udagawae*. In accordance with the recent taxonomists' recommendations for species for which a single-name nomenclature (i.e., keeping the name *Aspergillus* for all species of this genus) must be applied (17), the old teleomorphic name is indicated in parentheses.

isolates with TR_{46}/Y121F T289A mutations were found to have non-WT phenotypes for both posaconazole and voriconazole that were determined by both the SYO method (MICs of 1 and ≥8 μg/ml, respectively) and the CLSI method (MICs of ≥1 and >8 μg/ml, respectively). Once again, for 4 of these isolates, a non-WT phenotype for itraconazole was determined by the CLSI method (MICs, 2 μg/ml) but by not the SYO method (MICs, 0.5 to 1 μg/ml). Not surprisingly, MIC results showing discrepancies between commercial antifungal susceptibility methods (i.e., Etest and SYO) and the reference antifungal susceptibility method (i.e., EUCAST) (23), as well as between Etest and SYO methods (15), have been reported in previous evaluation studies. As outlined by Arendrup et al. (9), this issue can be related to the relatively low numbers of *Aspergillus* isolates with acquired resistance mechanisms that were tested in the single studies.

In conclusion, data originating from the present study support the claim that the SYO method is equivalent to the CLSI reference method for the identification of triazole resistance or decreased susceptibility (non-WT; MIC > ECV) in most *Aspergillus* species. Posaconazole MIC values of ≤0.5 μg/ml provided separation between WT strains of *A.*

TABLE 3 Triazole MICs for *Aspergillus* species isolates carrying a mutated *cyp51* gene, as determined by SYO and CLSI methods[a]

Organism (designation)	Type of mutation detected[b]	SYO MIC (μg/ml)			CLSI MIC (μg/ml)			Non-wild-type phenotype for each indicated triazole, according to the ECV[c]
		PSC	VRC	ITC	PSC	VRC	ITC	
A. fumigatus (v075-77)	TR_{34}/L98H	**0.5**	1	>16	**1**	1	>16	PSC (by CLSI only), ITC
A. fumigatus (v082-04)	TR_{34}/L98H	1	4	>16	1	2	>16	PSC, VRC, ITC
A. fumigatus (v085-79)	TR_{34}/L98H	1	8	>16	2	4	>16	PSC, VRC, ITC
A. fumigatus (v110-25)	TR_{34}/L98H	1	4	>16	1	4	>16	PSC, VRC, ITC
A. fumigatus (v128-51)	TR_{34}/L98H	**0.5**	**2**	2	**1**	**1**	2	PSC (by CLSI only), VRC (by SYO only), ITC
A. fumigatus (v099-47)	TR_{46}/Y121F T289A	1	>8	1	1	>8	1	PSC, VRC
A. fumigatus (v115-49)	TR_{46}/Y121F T289A	1	>8	**1**	2	>8	**2**	PSC, VRC, ITC (by CLSI only)
A. fumigatus (v116-78)	TR_{46}/Y121F T289A	1	>8	**1**	1	>8	**2**	PSC, VRC, ITC (by CLSI only)
A. fumigatus (v134-70)	TR_{46}/Y121F T289A	1	>8	**1**	1	>8	**2**	PSC, VRC, ITC (by CLSI only)
A. fumigatus (v135-16)	TR_{46}/Y121F T289A	1	8	**0.5**	1	>8	**2**	PSC, VRC, ITC (by CLSI only)
A. oryzae (UCSC-943)	T788G	0.5	>8	0.5	1	2	1	ND

[a]MICs of the triazoles posaconazole (PSC), voriconazole (VRC), and itraconazole (ITC) were determined as specified in the text (also see Tables 1 and 2 for details). MIC values of >16 μg/ml for itraconazole obtained by both methods were reported unchanged, whereas MIC values of ≥16 μg/ml for voriconazole obtained with the CLSI method were reported as >8 μg/ml, according to that specified in the text.

[b]Mutations occurring in the *cyp51a* gene of *A. fumigatus* and in the *cyp51c* gene (the homologue of *cyp51a*) of *A. oryzae*, which encode azole target enzyme, are indicated. The T788G missense mutation has been described, for the first time, in a clinical isolate of *A. flavus* (a species closely related to *A. oryzae*), with the data showing reduced *in vitro* susceptibility to voriconazole (MIC, 8 μg/ml) and itraconazole (MIC, 2 μg/ml) (22).

[c]ECVs were those published by Espinel-Ingroff et al. (10). Accordingly, PSC ECVs were used to identify non-wild-type (non-WT) isolates of *A. fumigatus*, *A. terreus*, and *A. niger* (ECV = >0.5 μg/ml), *A. flavus* (ECV = >0.25 μg/ml), and *A. nidulans* (ECV = >1 μg/ml); VRC ECVs were used to identify non-WT isolates of *A. fumigatus*, *A. flavus*, and *A. terreus* (ECV = >1 μg/ml) and of *A. niger* and *A. nidulans* (ECV = >2 μg/ml); and ITC ECVs were used to identify non-WT isolates of *A. fumigatus*, *A. flavus*, *A. terreus*, and *A. nidulans* (ECV = >1 μg/ml) and of *A. niger* (ECV = >2 μg/ml). Gray-shaded zones highlight those *A. fumigatus* isolates for which non-WT phenotypes were determined by only one of two methods (i.e., SYO or CLSI), where boldface denotes the MIC values that gave rise to the discrepancies between the methods. ND, not determined (because ECVs are lacking for the indicated species).

fumigatus (and *A. flavus*) species complexes and those harboring mutations in the *cyp51* gene, as tested here (Table 1) and by others (26). However, the simultaneous testing of voriconazole and itraconazole—as allowed through use of the SYO antifungal panel—against these species was shown to enhance the identification of *A. fumigatus* strains with *cyp51a* gene alterations, especially the TR_{34}/L98H and TR_{46}/Y121F T289A mutations (the latter being associated with particularly high [≥16 μg/ml] voriconazole MICs; see reference 27) which confer triazole cross-resistance. Future studies are expected to clarify the clinical relevance of *Aspergillus* (or other mold) testing in the absence of CLSI CBPs for licensed triazoles, as well as the mechanisms of resistance in less-common non-*A. fumigatus* species.

Ultimately, while we agree that the SYO microdilution panel offers a practical alternative to the reference (CLSI or EUCAST) method for antifungal susceptibility testing of molds (16), clinical microbiologists who use SYO in the routine setting, as we do, are required to compare the MIC mode and range data for each mold species tested in their own laboratory with the MIC distributions freely available on line or in the published literature (9). This would guarantee that MIC endpoints generated in the laboratory for each mold species would mirror those of the reference antifungal susceptibility testing methods and thus would be able to be correctly used for clinical purposes. However, as variation between laboratories that use reference methods may occur, it is desirable that a quality control standard for MIC values should also be part of the CE marking of the SYO method.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00168-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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