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Microdilution Susceptibility Testing of Amphotericin B, Itraconazole, and Voriconazole against Clinical Isolates of *Aspergillus* and *Fusarium* Species

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We compared the activities of amphotericin B, itraconazole, and voriconazole against clinical *Aspergillus* ($n = 82$) and *Fusarium* ($n = 22$) isolates by a microdilution method adopted from the National Committee for Clinical Laboratory Standards (NCCLS-M27A). RPMI 1640 (RPMI), RPMI 1640 supplemented to 2% glucose (RPMI-2), and antibiotic medium 3 supplemented to 2% glucose (AM3) were used as test media. MICs were determined after 24, 48, and 72 h. A narrow range of amphotericin B MICs was observed for *Aspergillus* isolates, with minor variations among species. MICs for *Fusarium* isolates were higher than those for *Aspergillus* isolates. MICs of itraconazole were prominently high for two previously defined itraconazole-resistant *Aspergillus fumigatus* isolates and *Fusarium solani*. Voriconazole showed good in vitro activity against itraconazole-resistant isolates, but the MICs of voriconazole for *F. solani* were high. RPMI was the most efficient medium for detection of itraconazole-resistant isolates, followed by RPMI-2. While the significance remains unclear, AM3 lowered the MICs, particularly those of amphotericin B.

Treatment modalities for systemic mold infections in immunocompromised patients are limited. Amphotericin B has long been the primary drug for the treatment of serious antifungal infections, and encouraging results with itraconazole for the treatment of *Aspergillus* infections have also been reported (4). Preliminary studies with the new triazole derivatives voriconazole (5, 15, 21) and SCH-56592 (19) as well as the echinocandins LY-303366 (23, 25) and MK-0991 (1) have also demonstrated that these drugs have favorable activity against some mold isolates. However, treatment of systemic mold infections is still troublesome (2, 3).

Great progress toward antifungal susceptibility testing of yeasts has been made so far (16). Parallel work on relevant test parameters for molds (method, test medium, inoculum size, incubation temperature, and time) is under way (6–11, 20, 24), and a proposed standard has recently been published by the National Committee for Clinical Laboratory Standards (NCCLS) for susceptibility testing of molds that cause invasive infections (17). However, the clinical significance of this methodology remains uncertain, and interpretive breakpoints have not yet been proposed.

In the study described in this report, we have investigated the in vitro activities of amphotericin B, itraconazole, and voriconazole against clinical *Aspergillus* and *Fusarium* isolates. The NCCLS M27-A microdilution method for yeasts (16) is the basis for current efforts to develop a suitable procedure for molds (17), and we used several variations of this method in an effort to determine suitable assay conditions.

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MATERIALS AND METHODS

Isolates. Eighty-two isolates of *Aspergillus* (*A. flavus* [$n = 27$], *A. fumigatus* [$n = 26$], *A. niger* [$n = 17$], *A. terreus* [$n = 9$], and *A. nidulans* [$n = 3$]) and 22 isolates of *Fusarium* (*F. solani* [$n = 18$] and *F. oxysporum* ($n = 4$]) were tested. Two itraconazole-resistant isolates of *A. fumigatus* (isolates NCPF 7099 and NCPF 7100) were kindly provided by D. W. Denning and were included in the study. The organisms were grown on Sabouraud dextrose agar (SDA) and were maintained on slants at −70°C until they were tested. Identification was based on the colony morphology and microscopic features (12). Prior to testing, the isolates were subcultured onto SDA slants and were incubated at 35°C. The incubation period was 72 h for *Aspergillus* species other than *A. nidulans* and 7 to 10 days for *A. nidulans*. *Fusarium* isolates were grown at 35°C for 72 h and then at room temperature until day 7. One of the *A. fumigatus* isolates (isolate 2-160) was included in each run as a quality control strain.

Antifungal agents. Amphotericin B (Squibb), itraconazole (Janssen Pharmaceuticals), and voriconazole (Pfizer) were kindly provided by their respective manufacturers as standard powders. All three drugs were dissolved in dimethyl sulfoxide, and stock solutions were stored at −70°C.

Antifungal susceptibility testing. A broth microdilution method adopted from the M27-A microdilution methodology for yeasts (5, 7, 16, 17) was used. Briefly, 10 twofold serial dilutions of the antifungal agents were prepared in microdilution plates at concentrations between 0.03125 and 16 μg/ml for itraconazole and voriconazole and 0.0078 and 4 μg/ml for amphotericin B. Plates were prepared in bulk and were stored at −70°C until the day of testing.

Three different test media were used: RPMI 1640 (Sigma Chemical Co., St. Louis, Mo.) with L-glutamine but without sodium bicarbonate and with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (RPMI); RPMI 1640 supplemented in the same fashion but also containing 20 g of glucose per liter (RPMI-2); and antibiotic medium 3 (lot JD4ZSG; BBL, Becton Dickinson), buffered by adding 1 g of Na_2HPO_4 and 1 g of NaH_2PO_4 to each liter of medium and then supplemented to 20 g of glucose per liter (AM3). All media were adjusted to pH 7.0.

The mold suspension to be tested was prepared by covering the SDA slant culture with sterile 0.85% saline and gently probing the colonies with the tip of a Pasteur pipette. For *A. nidulans*, the clumped colonies were dispersed with a wooden stick and were homogenized in saline to obtain the harvest. The resulting mixture of conidia and hyphae was transferred to a sterile tube and was vortexed for 1 min. Heavy particles of the suspension were allowed to settle for 3 to 5 min, and the upper homogeneous suspension was collected for inoculum adjustment. The inoculum density was adjusted with a spectrophotometer at a 530-nm wavelength. The target percentages of transmittance were 80 to 82% for *Aspergillus* spp. and 68 to 70% for *Fusarium* spp. The prepared inocula were stored at 4°C

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TABLE 1. Geometric mean MICs and ranges of MICs of amphotericin B after 24, 48, and 72 h

Species	No. of isolates	Medium	GM[a] (range) MIC (μg/ml)		
			24 h	48 h	72 h
Aspergillus					
A. flavus	27	RPMI	0.9 (0.5–2)	1.17 (1–4)	1.63 (1–4)
		RPMI-2	0.81 (0.25–2)	1.14 (1–4)	1.43 (1–4)
		AM3	0.57 (0.5–1)	1.03 (0.5–4)	1.33 (0.5–4)
A. fumigatus	26				
General isolates	24	RPMI	0.87 (0.5–1)	1.33 (1–2)	2.18 (1–4)
		RPMI-2	0.73 (0.5–1)	1.3 (1–4)	1.94 (1–4)
		AM3	0.36 (0.25–1)	0.65 (0.25–1)	1.09 (0.5–2)
Itraconazole-resistant isolates	2	RPMI	1 (1)	1.41 (1–2)	1.41 (1–2)
		RPMI-2	1 (1)	1.41 (1–2)	2.83 (2–4)
		AM3	0.25 (0.25)	0.71 (0.5–1)	1 (1)
A. niger	17	RPMI	0.33 (0.125–1)	0.54 (0.25–1)	0.75 (0.25–2)
		RPMI-2	0.26 (0.06–0.5)	0.32 (0.125–1)	0.54 (0.25–1)
		AM3	0.09 (0.03–0.25)	0.29 (0.125–0.5)	0.44 (0.125–1)
A. terreus	9	RPMI	0.63 (0.25–1)	2.33 (0.5–>4)	4.32 (1–>4)
		RPMI-2	0.63 (0.25–1)	1.85 (0.5–>4)	3.17 (0.5–>4)
		AM3	0.68 (0.125–1)	2.94 (0.5–>4)	4.67 (0.5–>4)
A. nidulans[b]	3	RPMI		1 (1)	2 (2)
		RPMI-2		2 (2)	2 (2)
		AM3		0.5 (0.5)	1 (1)
Fusarium					
F. solani	18	RPMI	1.85 (1–4)	3.3 (1–>4)	4.39 (2–>4)
		RPMI-2	1.53 (0.5–4)	2.83 (1–>4)	3.17 (1–>4)
		AM3	2 (0.5–>4)	2.83 (1–>4)	3.56 (1–>4)
F. oxysporum	4	RPMI	2.38 (2–4)	3.36 (2–4)	4 (4)
		RPMI-2	2 (2)	2.83 (2–4)	4 (4)
		AM3	2 (1–2)	5.66 (4–>4)	6.73 (4–>4)

[a] GM, Geometric mean.
[b] No growth at 24 h.

for up to a week prior to testing. The adjusted inoculum was diluted 1:50 before it was dispensed and yielded a double-strength inoculum containing 0.9×10^4 to 4.7×10^4 conidia/ml (5). The inoculum size was verified by determination of the number of viable CFU after plating of appropriate dilutions of the inoculum onto SDA.

The plates were incubated at 35°C and the results were read visually at 24, 48, and 72 h. For amphotericin B, the MIC was the lowest concentration of drug which produced an optically clear well. For itraconazole and voriconazole, the MIC was the lowest concentration of drug which produced a prominent decrease in turbidity (approximately 50% reduction in growth) (5, 16, 17). For purposes of computation, high off-scale MICs were converted to the twofold concentration just above the highest drug concentration tested.

RESULTS

The geometric mean MIC and the MIC range for all test media at 24, 48, and 72 h are shown in Tables 1, 2, and 3 for amphotericin B, itraconazole, and voriconazole, respectively. At 24 h, sufficient growth was consistently obtained for isolates of all species except *A. nidulans*. MICs tended to remain the same or increase only one- to twofold at 48 and 72 h compared with the MICs at 24 h for most isolates and antifungal agents. The exception to this rule was that itraconazole MICs for *F. oxysporum* in RPMI and RPMI-2 increased dramatically at 72 h.

Amphotericin B MICs spanned a relatively narrow range for both *Aspergillus* and *Fusarium*. *A. fumigatus* and *A. flavus* MICs were the highest, followed by those for *A. terreus*. MICs for isolates of *A. niger* were the lowest. Amphotericin B MICs were higher for *Fusarium* isolates than for *Aspergillus* isolates, with slightly higher MICs for *F. oxysporum* than for *F. solani*. The amphotericin B susceptibilities of the two itraconazole-resistant isolates did not significantly differ from those of the other *Aspergillus* isolates.

Itraconazole MICs were distributed in a narrow range, with similar low values for *Aspergillus* except for the two known itraconazole-resistant isolates. At 24 h, the MIC for one of the itraconazole-resistant isolates was significantly higher (MIC, >16 μg/ml) only in RPMI, whereas the MICs for the other itraconazole-resistant isolate were low in RPMI and RPMI-2 (MICs, 0.25 and 0.5 μg/ml, respectively). MICs rose to >16 μg/ml at 48 h in both media for both isolates. Testing in AM3 yielded only modestly increased MICs for these isolates at 48 h. Since the MICs for the resistant isolates were previously defined by using no growth or virtually no growth as the endpoint (2), we attempted to determine the MICs by using this endpoint as well. This enabled us to obtain MICs of >16 μg/ml for both isolates at 24 h in RPMI and RPMI-2 and for one of the isolates in AM3 (data not shown in Table 2). The lowest

TABLE 2. Geometric mean MICs and ranges of MICs of itraconazole after 24, 48, and 72 h

Species	No. of isolates	Medium	GM[a] (range) MIC (μg/ml)		
			24 h	48 h	72 h
Aspergillus					
A. flavus	27	RPMI	0.17 (0.03–0.25)	0.19 (0.03–0.25)	0.25 (0.06–0.5)
		RPMI-2	0.26 (0.06–0.5)	0.29 (0.06–0.5)	0.32 (0.125–0.5)
		AM3	0.17 (0.03–0.25)	0.22 (0.03–0.5)	0.25 (0.125–0.5)
A. fumigatus	26				
General isolates	24	RPMI	0.28 (0.06–0.5)	0.42 (0.25–1)	0.47 (0.25–1)
		RPMI-2	0.3 (0.06–0.5)	0.4 (0.125–2)	0.49 (0.25–1)
		AM3	0.13 (0.03–0.25)	0.31 (0.125–0.5)	0.31 (0.25–0.5)
Itraconazole-resistant isolates	2	RPMI	2.83 (0.25–>16)	>16 (>16)	>16 (>16)
		RPMI-2	1.41 (0.5–4)	>16 (>16)	>16 (>16)
		AM3	0.35 (0.25–0.5)	2 (2)	2 (2)
A. niger	17	RPMI	0.24 (0.03–1)	0.42 (0.06–2)	0.59 (0.06–2)
		RPMI-2	0.29 (0.06–1)	0.44 (0.06–1)	0.52 (0.06–1)
		AM3	0.16 (0.03–0.5)	0.25 (0.03–1)	0.38 (0.06–1)
A. terreus	9	RPMI	0.1 (0.03–0.25)	0.17 (0.125–0.25)	0.17 (0.125–0.25)
		RPMI-2	0.15 (0.03–0.5)	0.2 (0.03–0.5)	0.25 (0.06–0.5)
		AM3	0.15 (0.03–0.25)	0.16 (0.03–0.25)	0.18 (0.03–0.25)
A. nidulans[b]	3	RPMI		0.5 (0.5)	0.5 (0.5)
		RPMI-2		0.5 (0.5)	0.5 (0.5)
		AM3		0.25 (0.25)	0.25 (0.25)
Fusarium					
F. solani	18	RPMI	>16 (>16)	>16 (>16)	>16 (>16)
		RPMI-2	>16 (>16)	>16 (>16)	>16 (>16)
		AM3	>16 (>16)	>16 (>16)	>16 (>16)
F. oxysporum	4	RPMI	0.11 (0.03–0.25)	0.5 (0.5)	22.6 (8–>16)
		RPMI-2	0.42 (0.25–0.5)	0.5 (0.5)	19 (4–>16)
		AM3	0.15 (0.03–0.25)	0.35 (0.25–0.5)	1.41 (1–2)

[a] GM, geometric mean.
[b] No growth at 24 h.

itraconazole MICs were obtained for *A. terreus* isolates, followed by those for *A. niger*. Itraconazole MICs were >16 μg/ml for all *F. solani* isolates, whereas itraconazole MICs were relatively lower for *F. oxysporum* isolates.

The voriconazole MICs for *Aspergillus* isolates covered a narrow range, with no prominent difference among species. Of great interest was the low voriconazole MICs for itraconazole-resistant isolates. Voriconazole MICs for isolates of *Fusarium* were higher than those for most isolates of *Aspergillus*, with the greatest MICs seen for *F. solani*.

To compare the MICs obtained in RPMI-2 with those obtained in RPMI, the results for 79 *Aspergillus* and 22 *Fusarium* isolates for which MICs were readable at 24 h were analyzed (Table 4). As seen in Table 4, RPMI-2 generated the same or slightly decreased amphotericin B MICs compared to the MICs generated by the reference medium, RPMI. On the contrary, RPMI-2 generated slightly increased itraconazole and voriconazole MICs for *Aspergillus* isolates. On the other hand, voriconazole MICs in RPMI-2 were the same or slightly lower than those in RPMI for the *Fusarium* isolates. In addition, testing in RPMI-2 promoted somewhat more dense growth, which made the MIC more apparent, especially with the azoles.

The MICs obtained in AM3 also compared with those obtained in RPMI (Table 5). AM3 lowered the MICs of amphotericin B more predominantly than RPMI-2 did. The effect of AM3 on the itraconazole MICs was variable and was hard to predict. Since *F. solani* isolates, which constituted the majority of *Fusarium* isolates tested, did not give on-scale itraconazole MICs, the precise effect of the media on the MICs for *Fusarium* was hard to quantitate. The effect of AM3 was variable and unpredictable for the voriconazole MICs for *Aspergillus* isolates, whereas it tended to slightly decrease the voriconazole MICs for *Fusarium*.

DISCUSSION

In this study, we have (i) surveyed the in vitro activities of amphotericin B, itraconazole, and voriconazole for a large collection of *Aspergillus* and *Fusarium* isolates, (ii) evaluated the effects of different test media, and (iii) explored the utility of 24 h of incubation by comparing it to the 48- and 72-h incubation periods used more often for molds; and finally, on the basis of those results, (iv) we can suggest possible variations to test parameters for in vitro antifungal susceptibility tests of molds. In an effort to confirm that our testing methods could discriminate between susceptible and resistant isolates, we included two *A. fumigatus* strains that are known from other

TABLE 3. Geometric mean MICs and ranges of MICs of voriconazole after 24, 48, and 72 h

Species	No. of isolates	Medium	GM[a] (range) MIC (μg/ml)		
			24 h	48 h	72 h
Aspergillus					
A. flavus	27	RPMI	0.16 (0.03–0.5)	0.25 (0.03–0.5)	0.36 (0.06–1)
		RPMI-2	0.19 (0.06–0.5)	0.29 (0.125–1)	0.35 (0.125–1)
		AM3	0.22 (0.06–0.5)	0.35 (0.125–0.5)	0.42 (0.125–0.5)
A. fumigatus	26				
General isolates	24	RPMI	0.17 (0.125–0.5)	0.24 (0.125–0.5)	0.27 (0.125–0.5)
		RPMI-2	0.3 (0.125–0.5)	0.3 (0.125–0.5)	0.32 (0.125–1)
		AM3	0.18 (0.06–0.5)	0.26 (0.125–0.5)	0.34 (0.25–0.5)
Itraconazole-resistant isolates	2	RPMI	0.125 (0.125)	0.18 (0.125–0.25)	0.18 (0.125–0.25)
		RPMI-2	0.18 (0.125–0.25)	0.25 (0.125–0.5)	0.35 (0.25–0.5)
		AM3	0.09 (0.06–0.125)	0.25 (0.125–0.5)	0.25 (0.125–0.5)
A. niger	17	RPMI	0.11 (0.03–0.5)	0.24 (0.06–0.5)	0.32 (0.06–1)
		RPMI-2	0.15 (0.06–0.5)	0.35 (0.06–1)	0.42 (0.06–1)
		AM3	0.09 (0.03–0.5)	0.18 (0.125–0.5)	0.28 (0.125–1)
A. terreus	9	RPMI	0.16 (0.06–1)	0.25 (0.125–2)	0.37 (0.125–2)
		RPMI-2	0.17 (0.06–1)	0.32 (0.25–1)	0.4 (0.25–2)
		AM3	0.17 (0.125–0.25)	0.29 (0.125–0.5)	0.29 (0.125–0.5)
A. nidulans[b]	3	RPMI		0.5 (0.5)	0.5 (0.5)
		RPMI-2		0.5 (0.5)	0.5 (0.5)
		AM3		0.25 (0.25)	0.25 (0.25)
Fusarium					
F. solani	18	RPMI	1.92 (0.25–4)	3.43 (1–8)	4.16 (1–8)
		RPMI-2	1.78 (0.25–4)	4 (1–16)	5.04 (1–16)
		AM3	0.76 (0.25–2)	1.78 (1–8)	3.05 (1–8)
F. oxysporum	4	RPMI	0.5 (0.25–1)	1.41 (1–2)	2 (1–4)
		RPMI-2	0.5 (0.5)	1.68 (1–4)	1.68 (1–4)
		AM3	0.84 (0.5–1)	1 (0.5–2)	1.41 (1–4)

[a] GM, geometric mean.
[b] No growth at 24 h.

work to be resistant to itraconazole in vivo. Unfortunately, the lack of well-characterized isolates with defined resistance to amphotericin B or voriconazole prevented us from extending this approach to the other drugs.

The amphotericin B MICs for the isolates tested spanned a relatively narrow range. Although the MICs for the *A. fumigatus* and *A. flavus* strains were the highest and the MICs for *A. niger* were the lowest among those for the *Aspergillus* isolates, the differences were not striking and classification of these isolates as either potentially susceptible or putatively resistant does not seem to be warranted. By comparison with its activity against *Aspergillus* spp., amphotericin B had limited activity against both *F. solani* and *F. oxysporum*.

Amphotericin B susceptibility has previously been tested by several investigators. Dupont and Drouhet (4) tested 16 *A. fumigatus* isolates in Casitone complex broth medium at 30°C by a microdilution method and observed an MIC range of <0.09 to 0.36 at 24 h. The results of more recent studies carried out by Espinel-Ingroff and colleagues (5, 6), Gehrt et al. (8), and Pujol et al. (20) on amphotericin B susceptibility by the proposed NCCLS method were comparable to our results for both *Aspergillus* and *Fusarium*. Murphy et al. (15) also

TABLE 4. Evaluation of MICs in RPMI-2 compared to those in RPMI for *Aspergillus* and *Fusarium*[a]

Drug and species	No. (%) of isolates for which MIC in RPMI-2 is as follows compared to MIC in RPMI[b]:				
	≥−2	−1	0	+1	≥+2
Amphotericin B					
Aspergillus		18 (22.8)	60 (76)	1 (1.3)	
Fusarium	1 (4.6)	6 (27.3)	13 (59.1)	2 (9.1)	
Itraconazole					
Aspergillus	1 (1.3)	7 (8.9)	37 (46.8)	33 (41.8)	1 (1.3)
Fusarium			19 (86.4)	1 (4.6)	2 (9.1)
Voriconazole					
Aspergillus		4 (5.1)	45 (57)	26 (33)	4 (5.1)
Fusarium		5 (22.7)	14 (63.6)	3 (13.6)	

[a] The MICs for 79 *Aspergillus* isolates and 22 *Fusarium* isolates which were readable after 24 h were analyzed.
[b] ≥−2, MIC in RPMI-2 is twofold or more than twofold lower; −1, MIC in RPMI-2 is onefold lower; 0, MIC in RPMI-2 is identical to MIC in RPMI; +1, MIC in RPMI-2 is onefold higher; ≥+2, MIC in RPMI-2 is twofold or more than twofold higher.

TABLE 5. Evaluation of MICs in AM3 compared to those in RPMI for *Aspergillus* and *Fusarium*[a]

Drug and species	No. (%) of isolates for which MIC in AM3 is as follows compared to MIC in RPMI[b]:				
	≥−2	−1	0	+1	≥+2
Amphotericin B					
Aspergillus	24 (30.4)	35 (44.3)	16 (20.3)	4 (5.1)	
Fusarium		7 (31.8)	8 (36.4)	7 (31.8)	
Itraconazole					
Aspergillus	13 (16.4)	20 (25.3)	35 (44.3)	10 (12.7)	1 (1.3)
Fusarium			21 (95.5)		1 (4.6)
Voriconazole					
Aspergillus	1 (1.3)	13 (16.6)	44 (55.7)	20 (25.3)	1 (1.3)
Fusarium	8 (36.4)	7 (31.8)	5 (22.7)	1 (4.6)	1 (4.6)

[a] The MICs for 79 *Aspergillus* isolates and 22 *Fusarium* isolates which were readable after 24 h were analyzed.

[b] ≥−2, MIC in AM3 is twofold or more than twofold lower; −1, MIC in AM3 is onefold lower; 0, MIC in AM3 is identical to MIC in RPMI; +1, MIC in AM3 is onefold higher; ≥+2, MIC in AM3 is twofold or more than twofold higher.

reported MIC ranges similar to ours for *A. fumigatus*, *A. flavus*, and *A. niger* (0.5 to 2, 1 to 4, and 0.5 to 1 μg/ml, respectively, at 48 h). These results indicate that the in vitro susceptibility of *Aspergillus* isolates to amphotericin B exhibits a similar pattern within each species, with minor variations between isolates. The frequency of isolates for which MICs were predominently high seems to be low.

In our studies with itraconazole, we were able to make use of two known itraconazole-resistant isolates. These isolates behaved quite distinctly, and the MICs for none of our other *Aspergillus* isolates were as high as those for those two isolates. However, identification of the resistant isolates was not readily seen under all conditions. When tested in AM3, the MICs for the resistant isolates were only slightly higher than those for the other isolates. In previous tests with these isolates with 48 and 72 h of incubation, 10^6 conidia/ml as the inoculum (compared to 10^4 conidia/ml as the inoculum in our study), and no growth or virtually no growth as the endpoint (compared to a 50% reduction in growth in our study), RPMI-2 was found to most reliably identify these two resistant isolates (2). Determination of MICs by using the endpoint criterion of a 100% reduction in growth instead of a 50% reduction in growth enabled us to obtain MICs of >16 μg/ml for both resistant isolates in RPMI and RPMI-2 and one of the resistant isolates in AM3. These results raise the possibility that a 100% reduction endpoint might be more useful for identifying itraconazole-resistant *Aspergillus* isolates. The other parameter which differed between our work and the previous study was the inoculum density. We did not use 10^6 conidia/ml but, rather, used 10^4 conidia/ml, which is also the inoculum used in the proposed NCCLS method (17). The effects of variations in endpoint and inoculum density appear to require further study.

Whereas our findings for itraconazole and *Aspergillus* isolates were similar to those from prior work (4, 15), somewhat different results were seen for *F. solani* and *F. oxysporum* isolates. Espinel-Ingroff (5) reported MIC ranges of 1 to >16 μg/ml for both *F. solani* and *F. oxysporum*. Modal MICs of >16 μg/ml were determined for both *Fusarium* species in another study (6). We observed essentially no in vitro activity of itraconazole (MIC, >16 μg/ml) against *F. solani*. Against *F. oxysporum*, we observed itraconazole activity similar to that against *Aspergillus* species at 24 and 48 h but high itraconazole MICs at 72 h in RPMI and RPMI-2. Whether these findings are due to the relatively slow growth of *F. oxysporum* remains unclear.

Of significant interest, we observed that voriconazole has good activity against the itraconazole-resistant *Aspergillus* isolates. This observation correlates well with the previously reported voriconazole MICs for these isolates (18). Our findings also suggest that voriconazole is active in vitro against the *Fusarium* isolates. The MICs reported for *Fusarium* in previous studies (5) with a 100% reduction endpoint criterion were similar to or slightly higher than ours (MICs, 4 and 8 to 16 μg/ml for *F. oxysporum* and *F. solani*, respectively). These slightly higher MICs were probably attributable to the use of a 100% growth reduction endpoint rather than the 50% growth reduction endpoint that we used. Radford et al. (21) obtained voriconazole MIC ranges of 1 to 4 μg/ml for *F. solani* and 0.5 to 2 μg/ml for *F. oxysporum* by an agar dilution method. In that study, the MIC was defined as the lowest concentration at which there was no visible growth on the agar.

Investigation of the effect of different test media was the other major aim of our study. Since AM3 was shown in previous studies to highlight amphotericin B-resistant isolates of *Candida* species (13, 14), we examined the effect of AM3 on the MICs for *Aspergillus* and *Fusarium* isolates. Our isolates did not include any that were known to be resistant to amphotericin B. In general, AM3 had a tendency to slightly decrease the MICs for some isolates, particularly when testing was done with amphotericin B, but the significance of this effect is unclear. Importantly, however, testing on AM3 failed to detect the itraconazole-resistant isolates. Furthermore, this medium generated relatively low itraconazole MICs for *F. oxysporum* at 72 h. While the clinical significance of this finding for *F. oxysporum* remains unclear, the itraconazole-resistant isolates have been proven to be clinically resistant to itraconazole. AM3 thus offers no obvious advantages for amphotericin B and is less effective for the azoles. RPMI-2 generated slightly lower amphotericin B MICs and slightly higher azole MICs for some isolates. These changes in MICs were not significant in either case. RPMI proved to be more successful in detecting itraconazole-resistant isolates. These results suggest that RPMI-2, except for providing good growth and ease of determination of MICs, particularly for azoles, does not seem to be superior to RPMI in terms of detection of resistance.

The inoculum size used in this study provided sufficient growth at 24 h for all isolates except *A. nidulans* isolates, indicating that MICs can be read as early as 24 h without delay. A slight to moderate increase in the MIC with an extended incubation period was observed in previous investigations (2, 8), as well as our study. The increase in MIC was more prominent for *F. oxysporum* when incubation was extended to 72 h. Although reading at an early time point may improve the correlation of the MIC with the clinical outcome for *Candida* (22), the validity and clinical implication of MICs for molds obtained at 24 h need to be clarified by in vivo studies.

In conclusion, our data show that RPMI-2 and AM3 do not seem to offer advantages in identification of mold isolates resistant to the antifungal agents tested. An incubation period of 24 h is sufficient for most of the isolates, which is a promising finding for the quick retrieval of antifungal susceptibility test results. On the basis of the methods that we have used, in vitro resistance of *Aspergillus* isolates to amphotericin B and voriconazole appears to be infrequent. *Fusarium* isolates have limited susceptibility to itraconazole and voriconazole. The significance of these in vitro data needs to be clarified by further testing of clinically resistant isolates and in experiments with animals.

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