

Antifungal Susceptibility of 44 Clinical Isolates of *Fusarium* Species Determined by Using a Broth Microdilution Method

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The MICs and minimum fungicidal concentrations of amphotericin B, natamycin, miconazole, itraconazole, and flucytosine against 17 isolates of *Fusarium solani*, 14 isolates of *Fusarium moniliforme*, 10 isolates of *Fusarium oxysporum*, and 3 isolates of *Fusarium semitectum* were determined by a broth microdilution method. Amphotericin B and natamycin were the most active agents tested and failed to show any inoculum size effect. In contrast, miconazole and itraconazole showed poor inhibitory and fungicidal activities, and the inoculum size had a major effect on the results. Flucytosine had no activity against any of the isolates tested.

Fusarium spp. have been implicated in an increasing number of opportunistic infections in immunocompromised patients (1-3, 5-7, 9, 11, 13, 15, 16). Currently, few data are available from in vitro and in vivo studies on the susceptibility of these organisms to various antifungal agents, and as a result, the treatment of choice for *Fusarium* infections remains to be determined. To evaluate the potential value of various antifungal agents, we compared the in vitro inhibitory and fungicidal activities of amphotericin B, natamycin, miconazole, itraconazole, and flucytosine against 44 clinical isolates of *Fusarium* spp.

Natamycin was obtained from Geist-Brocades, Delft, The Netherlands. Amphotericin B was obtained from E. R. Squibb and Sons, Princeton, N.J.; miconazole and itraconazole were from Janssen Pharmaceutical, Inc., Piscataway, N.J.; and flucytosine was from Hoffmann-La Roche, Inc., Nutley, N.J. Powders of amphotericin B, natamycin, itraconazole, and flucytosine were initially diluted in dimethyl sulfoxide at a starting concentration of less than or equal to 4% dimethyl sulfoxide. Monistat, the commercial preparation of miconazole, was used because of the poor solubility of miconazole in dimethyl sulfoxide. Twofold serial dilutions of all drugs were made in Eagle minimum essential medium (EMEM) (Life Technologies, Inc., Grand Island, N.Y.). The ranges of dilutions were 8 to 0.0075 $\mu\text{g/ml}$ for amphotericin B, 32 to 0.03 $\mu\text{g/ml}$ for natamycin, miconazole, and itraconazole, and 512 to 0.5 $\mu\text{g/ml}$ for flucytosine. EMEM was buffered with MOPS (morpholinepropanesulfonic acid). Its final pH was 7.00 (E. Anaissie, A. Espinel-Ingroff, and T. Kerkerling, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C-305, p. 444).

The *Fusarium* strains tested were provided by the Fusarium Research Center, Pennsylvania State University, University Park, and represented clinical isolates from various institutions across the United States, including seven isolates from the M. D. Anderson Cancer Center. The fungal species were distributed as follows: *F. solani*, 17; *F. moniliforme*, 14; *F. oxysporum*, 10; and *F. semitectum*, 3. Organisms were subcultured in sterile flasks containing sterile water supplemented with 2.5% glucose, 2.5% normal saline solution, 0.1% KCl, and 0.01% Tween 80. The flasks were covered with aluminum foil and shaken continuously at 22°C

for 48 h. Suspensions were then filtered twice through sterile gauze to remove hyphae, and the resulting spore suspension was manually counted undiluted in a hemacytometer. The spores were diluted or concentrated in EMEM to produce the inoculum, which was either 10^6 (high inoculum) or 10^4 (low inoculum) spores per ml.

Portions (0.01 ml) of each spore suspension containing either the high or low inoculum were added to all wells of microdilution plates which contained 100 μl of either amphotericin B, natamycin, miconazole, itraconazole, flucytosine, or no drug (control) in EMEM. Thus, the final inoculum was either 10^5 (high) or 10^3 (low) spores per ml. All plates were incubated at 35°C for 48 h. Susceptibility testing was performed in duplicate. The MIC was considered the lowest concentration of the drug that prevented visible growth. With a calibrated loop, 10 μl from wells without visible growth or with very minimal growth was subcultured on Sabouraud dextrose agar at 35°C for 48 h. The minimum fungicidal concentration (MFC) was defined as the concentration causing a 99.9% reduction from the original inoculum size, which meant the growth of less than two colonies on subculturing.

On the basis of the MICs for 90% of the strains (MIC_{90}) and the MFCs for 90% of the strains (MFC_{90}), amphotericin B was active within clinically achievable peak concentrations in serum (1 to 2 $\mu\text{g/ml}$) (Tables 1 and 2). Trough concentrations of the drug in serum were significantly lower than both MIC_{90} s and MFC_{90} s for *Fusarium* spp. The significance of amphotericin B levels in serum remains to be determined.

Natamycin is a tetraene polyene with good in vitro activity against various molds (10). Preliminary pharmacokinetic data from dogs, sheep, and pigs indicate that after a single intravenous dose of 7.5 mg of natamycin per kg, a peak concentration in serum of 40 $\mu\text{g/ml}$ is reached (8). However, no pharmacokinetic data for humans are available. These animal pharmacokinetic data coupled with the MIC_{90} and MFC_{90} of natamycin suggest that this drug may have a role in the treatment of fusariosis (Tables 1 and 2).

Both amphotericin B and natamycin had distinct endpoints which were visible after 48 h of inoculation. No significant inoculum size effect was observed with either drug.

The remaining drugs had poor activity. In addition, the

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TABLE 1. MICs of antifungal agents against *Fusarium* species

Agent	MIC ($\mu\text{g/ml}$) ^a at inoculum size:					
	10 ⁵ Spores per ml			10 ³ Spores per ml		
	50%	90%	Range	50%	90%	Range
Amphotericin B	1	2	1-2	1	1	1-2
Natamycin	2	4	2-4	2	4	2-4
Miconazole	>32	>32	8->32	>32	32	1->32
Itraconazole	>32	>32	>32	>32	>32	8->32
Flucytosine	>512	>512	>512	>512	>512	>512

^a 50% and 90%, MICs for 50 and 90% of isolates, respectively.

inoculum size significantly altered the susceptibility to both miconazole and itraconazole. While seven isolates were inhibited by $\leq 8 \mu\text{g}$ of miconazole per ml at the low inoculum size (range, 1 to 8 $\mu\text{g/ml}$), only two of those isolates remained susceptible at 8 $\mu\text{g/ml}$ when the high inoculum was used. Similarly, the MIC of itraconazole for the same seven isolates was $\leq 32 \mu\text{g/ml}$ (range, 8 to 32 $\mu\text{g/ml}$) at the low inoculum and increased to $>32 \mu\text{g/ml}$ for all seven isolates at the high inoculum. Flucytosine did not show any significant inhibitory activity against any of these *Fusarium* isolates.

There were no significant differences in the susceptibility of the four species of *Fusarium* to the antifungal drugs tested in the study. In addition, results did not vary by more than one twofold dilution between duplicate tests.

Previously published data on the in vitro susceptibility of *Fusarium* isolates to antifungal agents are limited to a total of 32 strains, collected from 12 reports and tested by 12 separate laboratories. The majority of these studies tested a single organism. Only one study tested 10 organisms (14). Furthermore, there were significant methodological differences among the only three laboratories which provided detailed information about their testing technique. These differences include the method, the medium composition, the medium pH, the incubation time, the nature of the inoculum, and the physical state of the drugs (7, 11, 15).

A summary of the reported results is shown in Table 3. In agreement with our findings, all organisms were highly resistant to flucytosine and susceptible to natamycin. Miconazole showed some activity, while ketoconazole was poorly active. Various susceptibilities to amphotericin B were noted with some strains reported to be highly resistant (11, 12, 15), which is in contrast to our findings. This discrepancy may be accounted for by the methodological differences. Higher amphotericin B MICs against *Fusarium* spp. can be seen if certain media (such as synthetic amino acid medium fungus) are used (Anaissie et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1989; M. Pfaller and J. N. Galgiani, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C-304, p. 444).

While amphotericin B and natamycin appeared to be the most active drugs tested, these findings do not necessarily

TABLE 2. MFCs of antifungal agents against *Fusarium* species^a

Agent	MFC ($\mu\text{g/ml}$) ^b		
	50%	90%	Range
Amphotericin B	1	2	1-4
Natamycin	2	4	2-8
Miconazole	>32	>32	16->32
Itraconazole	>32	>32	>32
Flucytosine	>512	>512	>512

^a Final inoculum size, 10⁵ spores per ml.

^b 50% and 90%, MFCs for 50 and 90% of the isolates, respectively.

TABLE 3. Literature summary of in vitro susceptibility of *Fusarium* spp. to antifungal agents

Drug	No. of isolates susceptible/no. tested	MIC range ($\mu\text{g/ml}$)
Amphotericin B	5 ^a /32	0.25-50
Natamycin	10/10	3.12-6.25
Flucytosine	0/21	>40->323
Ketoconazole	1 ^b /9	3.2->200
Miconazole	9/12	0.39->100

^a One organism resistant when a different medium was used.

^b Organism resistant with a higher inoculum.

imply that these antibiotics are active in the setting of invasive fusariosis. It could also be argued that an MFC₉₀ of 2 $\mu\text{g/ml}$ is indicative of relative resistance to amphotericin B. Furthermore, 8 of 10 isolates whose MICs were also read at 72 h showed twofold-higher values when read at 72 instead of 48 h. It may very well be that the MIC reading at 72 h correlates better with in vivo response of *Fusarium* spp. to antifungal agents. In that case, our tested organisms would indeed be relatively resistant to amphotericin B, and results by others showing resistance to the drug would then be correct. In addition, the in vitro activity of azoles has been shown to correlate poorly with their in vivo efficacy. Only in vivo tests would be predictive of the activity of itraconazole in fusariosis (4).

In this study, a practical and economical microtiter method was used to determine the in vitro susceptibility of *Fusarium* spp. to various antifungal agents. The results suggest that both polyenes, amphotericin B and natamycin, have significant in vitro activity without any inoculum size effect, while flucytosine, miconazole, and itraconazole showed poor activity with a significant inoculum size effect. No differences were observed in the susceptibility of the four *Fusarium* spp. to antifungal agents. A murine model of systemic fusariosis is currently being used in an attempt to validate these in vitro findings (C. Legrand, E. Anaissie, and J. Roe, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, B-289, p. 78).

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