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NOTES

In Vitro Susceptibilities of 217 Clinical Isolates of Zygomycetes to Conventional and New Antifungal Agents▽

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We evaluated the in vitro susceptibilities of 217 zygomycetes to amphotericin B, ketoconazole, fluconazole, itraconazole, voriconazole, posaconazole, caspofungin, and flucytosine. The significant in vitro activity of posaconazole against several species appears to support its reported clinical efficacy. Decreased susceptibility to amphotericin B was noted with *Cunninghamella bertholletiae*.

Invasive zygomycosis is a devastating disease in immunocompromised individuals, inciting significant morbidity and mortality (1, 9, 12, 14, 15, 21, 26, 27). Amphotericin B, with its lipid formulations, has been the mainstay of treatment for several decades; however, the newer triazole posaconazole exhibits promising activity and is currently undergoing extensive clinical investigation (13, 30). The interest in this compound has prompted a retrospective review of our in vitro susceptibility data for this and other conventional antifungal agents against agents of zygomycosis.

A total of 217 clinical isolates in the order *Mucorales* were forwarded to the Fungus Testing Laboratory from January 2001 to February 2007. These isolates were submitted for fungal identification and/or antifungal susceptibility testing and were received from numerous large tertiary-care centers across the United States. While it was not possible to accurately categorize the type of zygomycosis for these strains from the information available, it was evident that the majority of isolates were associated with rhinoorbital or rhinocerebral disease, pulmonary zygomycosis, or various cutaneous manifestations. Tables 1 and 2 show the sources and species distribution.

The antifungal agents tested included amphotericin B deoxycholate (E.R. Squibb, Princeton, NJ), ketoconazole and itraconazole (Janssen Pharmaceutica, Beerse, Belgium), fluconazole and voriconazole (Prizer, Groton, CT), posaconazole (Schering-Plough, Kenilworth, NJ), caspofungin (Merck, Rahway, NJ), and flucytosine (Hoffman-LaRoche, Nutley, NJ). Analytical powders were obtained from the respective manufacturers. Testing was in a microtiter format in essential agreement with CLSI (formerly NCCLS) method M38-A for filamentous fungi as previously described (17). End points for caspofungin corresponded to the minimal effective concentration (MEC) as defined by Kurtz et al. (16) in 1994 and by Arikan et al. (2) in 2001. Amphotericin B and caspofungin were tested in antibiotic medium 3 (Difco [Becton Dickinson], Sparks, MD), while the other agents were evaluated in RPMI 1640 (Hardy Diagnostics, Santa Monica, CA). End points were determined by visual inspection after 24 h of incubation at 35°C. The number of isolates that exceeded the suggested MIC or MEC breakpoints was determined for each antifungal agent.

In vitro antifungal susceptibility data are presented in Table 2. For the *Mucorales* as a whole, amphotericin B was the most active antifungal agent, with the majority of strains displaying MICs near the suggested breakpoint of ≤1 μg/ml. This is in

TABLE 1. Sources of clinical isolates tested

Source	*n*
Nasal sinus	51
Hard palate	3
Cutaneous tissue	34
Lung/bronchoalveolar lavage	42
Pleural fluid	4
Sputum	12
Wound	8
Tissue[a]	40
Bone	2
Blood	5
Abscess[b]	6
Peritoneal fluid	2
Urine	1
Bladder	1
Catheter line	3
Eye	1
Unknown	2
Total	217

[a] Tissue type not specified.
[b] Liver abscess, 3; brain abscess, 1.

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TABLE 2. In vitro antifungal susceptibility data for 217 clinical isolates of zygomycetous fungi

Organism (no. of isolates tested)	Antifungal agent (breakpoint, μg/ml)[a]	MIC (μg/ml)			% Susceptible (no. of isolates tested)[b]
		Range	50%	90%	
Rhizopus sp. (101)	AMB (≤1)	0.03–0.5	0.25	0.5	**100** (86)
	5-FC (≤16)	>64	NA[c]	NA	0 (4)
	KTC (≤4)	1–16	1	NA	83 (6)
	ITC (≤0.5)	0.03–>8	0.5	4	62 (50)
	FLC (≤32)	>64	>64	>64	0 (14)
	VRC (≤2)	2–>8	8	>8	5 (54)
	POS (≤0.5)	0.06–4	0.25	1	**80** (66)
	CAS (≤2)	>16	>16	>16	0 (44)
Rhizopus arrhizus (20)	AMB (≤1)	0.06–0.5	0.25	0.25	**100** (15)
	5-FC (≤16)	>64	NA	NA	0 (1)
	KTC (≤4)	8	NA	NA	0 (1)
	ITC (≤0.5)	0.25–2	0.5	NA	50 (6)
	FLC (≤32)	>64	NA	NA	0 (3)
	VRC (≤2)	8–>8	>8	NA	0 (5)
	POS (≤0.5)	0.03–1	0.25	1	**64** (14)
	CAS (≤2)	>16	>16	NA	0 (5)
Rhizopus microsporus var. *rhizopodiformis* (12)	AMB (≤1)	0.03–0.5	0.25	0.25	**100** (10)
	5-FC (≤16)	>64	NA	NA	0 (1)
	KTC (≤4)	2	NA	NA	0 (1)
	ITC (≤0.5)	0.25–1	0.5	NA	60 (5)
	FLC (≤32)	>64	NA	NA	0 (4)
	VRC (≤2)	4–>8	NA	NA	0 (4)
	POS (≤0.5)	0.25–2	0.25	NA	**78** (9)
	CAS (≤2)	>16	>16	NA	0 (7)
Rhizopus microsporus var. *microsporus* (1)	AMB (≤1)	0.25	NA	NA	**100** (1)
	5-FC (≤16)	NT[d]	NA	NA	NT
	KTC (≤4)	NT	NA	NA	NT
	ITC (≤0.5)	1	NA	NA	0 (1)
	FLC (≤32)	NT	NA	NA	NT
	VRC (≤2)	NT	NA	NA	NT
	POS (≤0.5)	NT	NA	NA	NT
	CAS (≤2)	NT	NA	NA	NT
Mucor sp. (41)	AMB (≤1)	0.125–4	0.25	0.5	**94** (36)
	5-FC (≤16)	>64	>64	NA	0 (5)
	KTC (≤4)	2–16	NA	NA	33 (3)
	ITC (≤0.5)	0.25–>8	0.5	>8	57 (14)
	FLC (≤32)	>64	>64	NA	0 (6)
	VRC (≤2)	4–>8	>8	>8	0 (20)
	POS (≤0.5)	0.06–2	0.5	2	**70** (20)
	CAS (≤2)	>16	>16	>16	0 (15)
Mucor circinelloides group (6)	AMB (≤1)	0.06–0.5	0.25	NA	**100** (5)
	5-FC (≤16)	>64	NA	NA	0 (1)
	KTC (≤4)	16	NA	NA	0 (1)
	ITC (≤0.5)	2–>8	NA	NA	0 (4)
	FLC (≤32)	>64	NA	NA	0 (2)
	VRC (≤2)	>8	NA	NA	0 (3)
	POS (≤0.5)	1–2	NA	NA	0 (3)
	CAS (≤2)	>16	NA	NA	0 (3)
Rhizomucor sp. (5)	AMB (≤1)	0.125–0.25	0.125	NA	**100** (5)
	5-FC (≤16)	NT	NA	NA	NT
	KTC (≤4)	NT	NA	NA	NT
	ITC (≤0.5)	0.125–1	NA	NA	67 (3)
	FLC (≤32)	>64	NA	NA	0 (2)
	VRC (≤2)	8–>8	NA	NA	0 (3)
	POS (≤0.5)	0.06–1	NA	NA	**67** (3)
	CAS (≤2)	>16	NA	NA	0 (1)
Absidia sp. (3)	AMB (≤1)	0.25–0.5	NA	NA	**100** (2)
	5-FC (≤16)	NT	NA	NA	NT

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TABLE 2—*Continued*

Organism (no. of isolates tested)	Antifungal agent (breakpoint, μg/ml)[a]	MIC (μg/ml)			% Susceptible (no. of isolates tested)[b]
		Range	50%	90%	
	KTC (≤4)	NT	NA	NA	NT
	ITC (≤0.5)	0.5–1	NA	NA	50 (2)
	FLC (≤32)	NT	NA	NA	NT
	VRC (≤2)	>8	NA	NA	0 (3)
	POS (≤0.5)	0.125	NA	NA	**100** (1)
	CAS (≤2)	>16	NA	NA	0 (3)
Absidia corymbifera (9)	AMB (≤1)	0.25–0.5	0.25	NA	**100** (6)
	5-FC (≤16)	NT	NA	NA	NT
	KTC (≤4)	NT	NA	NA	MT
	ITC (≤0.5)	0.125–0.5	NA	NA	100 (4)
	FLC (≤32)	NT	NA	NA	NT
	VRC (≤2)	8–>8	NA	NA	0 (3)
	POS (≤0.5)	0.06–0.25	NA	NA	**100** (4)
	CAS (≤2)	>16	NA	NA	0 (3)
Cunninghamella sp. (13)	AMB (≤1)	0.25–2	1	NA	**63** (8)
	5-FC (≤16)	>64	NA	NA	0 (1)
	KTC (≤4)	>16	NA	NA	0 (1)
	ITC (≤0.5)	0.125–4	1	NA	29 (7)
	FLC (≤32)	>64	NA	NA	0 (1)
	VRC (≤2)	0.5–>8	>8	>8	10 (10)
	POS (≤0.5)	0.06–1	0.5	NA	**75** (8)
	CAS (≤2)	>16	NA	NA	0 (5)
Apophysomyces elegans (6)	AMB (≤1)	0.03–1	0.125	NA	**100** (6)
	5-FC (≤16)	NT	NA	NA	NT
	KTC (≤4)	NT	NA	NA	NT
	ITC (≤0.5)	0.03–4	0.125	NA	80 (5)
	FLC (≤32)	>64	NA	NA	0 (1)
	VRC (≤2)	0.125	NA	NA	100 (1)
	POS (≤0.5)	≤0.016–1	0.03	NA	**83** (6)
	CAS (≤2)	>16	NA	NA	0 (1)

[a] AMB, amphotericin B; 5-FC,flucytosine; KTC, ketoconazole; ITC, itraconazole; FLC, fluconazole; VRC, voriconazole; POS, posaconazole; CAS, caspofungin. For all except caspofungin, suggested 24-h MIC breakpoints for polyene and azole susceptibility are shown. For caspofungin, the suggested 24-h MEC breakpoint for echinocandin susceptibility is shown. There are no CLSI established MIC/MEC breakpoints for any of the organism-drug combinations.

[b] Boldface indicates in vitro activity for amphotericin B and posaconazole.

[c] NA, not applicable.

[d] NT, not tested.

accordance with previous reports (4, 8, 11, 18, 20, 23, 24). Posaconazole appeared to be the second most active agent against various genera and species and is an exception among the azoles. This corroborates other reports (4, 11, 23, 28). Studies in animal models (7, 29) and clinical investigations (13, 30) indicate promising in vivo efficacy. Other than in some anecdotal case reports, the azoles/triazoles have traditionally not been efficacious (5, 23, 24, 28), and this seems to be supported by in vitro data. Itraconazole demonstrated limited activity in only a minority of isolates. A wide range of MICs for itraconazole has been reported by other investigators (5, 10, 24, 28). Fluconazole, along with the newer triazole antifungal voriconazole, demonstrated poor or no activity. Caspofungin, an echinocandin targeting the cell wall, also lacks in vitro activity against zygomycetes (8, 19, 24), although improved survival in an animal model was recently demonstrated in combination with amphotericin B lipid complex (25).

Data for individual genera were similar, with some exceptions. *Rhizopus* and *Mucor* represent the most frequently encountered genera in clinical practice. Most species of *Rhizopus* ($n = 134$) and *Mucor* ($n = 47$), appeared to be highly susceptible to amphotericin B (100% and 94 to 100%, respectively) and demonstrated variable susceptibility to posaconazole (0 to 80%). *Cunninghamella bertholletiae* was the single most resistant species tested in this cohort. Literature on *Cunninghamella bertholletiae* is limited, but all studies indicate higher MICs (5, 24, 28). Although 37% of the isolates in our study, had MICs of 2 μg/ml for amphotericin B, the use of liposomal preparations generally provides levels of drug well exceeding this concentration (3). While *Cunninghamella bertholletiae* is a rare clinical isolate, it has been associated with more aggressive disease and consequently with higher mortality (22). Approximately 75% of the isolates appeared to be susceptible to posaconazole. Although the number of *Apophysomyces elegans* isolates tested was small, most strains were susceptible to amphotericin B ($n = 6$), 83% were susceptible to posaconazole ($n = 6$), and 80% appeared to be susceptible to itraconazole. All *Absidia* isolates tested against amphotericin B ($n = 8$) and posaconazole ($n = 5$) were susceptible. *Absidia* species may be the most susceptible species, as shown in other studies as well (6, 18, 28).

Our study corroborates the findings of other authors that

zygomycetes consist of a heterogeneous group of fungi with variable susceptibilities to antifungals (5). This variability is intrinsic, occurs among and within different genera and species, and further supports the genus level identification of etiologic agents for the appropriate management of refractory infections.

The above findings should be interpreted with caution, as the clinically relevant MIC breakpoints for zygomycetes are lacking. Moreover, the lipid formulations of amphotericin B may achieve concentrations far exceeding the MIC (3). The angioinvasive properties of zygomycetes render surgical debridement an essential part of management, as are correction of immune defects and elimination of predisposing conditions.

To our knowledge, this is the largest collection of in vitro susceptibility data for clinical isolates. Amphotericin B demonstrated the most favorable activity. Although azoles have traditionally been inactive against zygomycetes, the newer agent, posaconazole, demonstrated promising in vitro activity. *Rhizopus* and *Mucor* species demonstrated variable but overall favorable susceptibilities, with *Cunninghamella bertholletiae* appearing to be the most resistant species.

REFERENCES

1. **Almyroudis, N. G., D. A. Sutton, P. Linden, M. G. Rinaldi, J. Fung, and S. Kusne.** 2006. Zygomycosis in solid organ transplant recipients in a tertiary transplant center and review of the literature. Am. J. Transplant. **6:**2365–2374.
2. **Arikan, S., M. Lozano-Chiu, V. Paetznick, and J. H. Rex.** 2001. In vitro susceptibility testing methods for caspofungin against *Aspergillus* and *Fusarium* isolates. Antimicrob. Agents Chemother. **45:**327–330.
3. **Bekersky, I., R. M. Fielding, D. E. Dressler, J. W. Lee, D. N. Buell, and T. J. Walsh.** 2002. Pharmacokinetics, excretion, and mass balance of liposomal amphotericin B (AmBisome) and amphotericin B deoxycholate in humans. Antimicrob. Agents Chemother. **46:**828–833.
4. **Cuenca-Estrella, M., A. Gomez-Lopez, E. Mellado, M. J. Buitrago, A. Monzon, and J. L. Rodriguez-Tudela.** 2006. Head-to-head comparison of the activities of currently available antifungal agents against 3,378 Spanish clinical isolates of yeasts and filamentous fungi. Antimicrob. Agents Chemother. **50:**917–921.
5. **Dannaoui, E., J. Meletiadis, J. W. Mouton, J. F. G. M. Meis, P. E. Verweij, and the Eurofong Network.** 2003. In vitro susceptibilities of zygomycetes to conventional and new antifungals. J. Antimicrob. Chemother. **51:**45–52.
6. **Dannaoui, E., J. W. Mouton, J. F. G. M. Meis, P. E. Verweij, and Eurofung Network.** 2002. Efficacy of antifungal therapy in a nonneutropenic murine model of zygomycosis. Antimicrob. Agents Chemother. **46:**1953–1959.
7. **Dannaoui, E., J. F. Meis, D. Loebenberg, and P. E. Verweij.** 2003. Activity of posaconazole in treatment of experimental disseminated zygomycosis. Antimicrob. Agents Chemother. **47:**3647–3650.
8. **Diekema, D. J., S. A. Messer, R. J. Hollis, R. N. Jones, and M. A. Pfaller.** 2003. Activities of caspofungin, itraconazole, posaconazole, ravuconazole, voriconazole, and amphotericin B against 448 recent clinical isolates of filamentous fungi. J. Clin. Microbiol. **41:**3623–3626.
9. **Dromer, F., and M. R. McGinnis.** 2003. Zygomycosis, p. 297–308. *In* E. J. Anaissie, M. R. McGinnis, and M. A. Pfaller (ed.), Clinical mycology, Churchill Livingstone, Philadelphia, PA.
10. **Eisen, D. P., and J. Robson.** 2004. Complete resolution of pulmonary *Rhizopus oryzae* infection with itraconazole treatment: more evidence of the utility of azoles for zygomycosis. Antimicrob. Agents Chemother. **47:**159–162.
11. **Espinel-Ingroff, A.** 2006. Comparison of three commercial assays and a modified disk diffusion assay with two broth microdilution reference assays for testing zygomycetes, *Aspergillus* spp., *Candida* spp., and *Cryptococcus neoformans* with posaconazole and amphotericin B. J. Clin. Microbiol. **44:**3616–3622.
12. **Gonzalez, C. E., M. G. Rinaldi, and A. M. Sugar.** 2002. Zygomycosis, p. 895–914. *In* R. C. Moellering, Jr., T. J. Walsh, and J. H. Rex (ed.), Infectious disease clinics of North America, W. B. Saunders Company, Philadelphia, PA.
13. **Greenberg, R. N., K. Mullane, J.–A. H. van Burik, I. Raad, M. J. Abzug, G. Anstead, R. Herbrecht, A. Longston, K. A. Marr, G. Schiller, M. Schuster, J. R. Wingard, C. E. Gonzalez, S. G. Revankar, G. Corcoran, R. J. Kryscio, and R. Hare.** 2006. Posaconazole as salvage therapy for zygomycosis. Antimicrob. Agents Chemother. **50:**126–133.
14. **Kontoyiannis, D. P., V. C. Wessel, G. P. Bodey, and K. V. I. Rolston.** 2000. Zygomycosis in the 1990s in a tertiary-care cancer center. Clin. Infect. Dis. **30:**851–856.
15. **Kontoyiannis, D. P., M. S. Lionakis, R. E. Lewis, G. Chamilos, M. Healy, C. Perego, A. Safdar, H. Kantarjian, R. Champlin, T. J. Walsh, and I. I. Raad.** 2005. Zygomycosis in a tertiary-care center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. J. Infect. Dis. **191:**1350–1360.
16. **Kurtz, M. B., I. B. Heath, J. Marrinan, S. Dreikorn, J. Onishi, and C. Douglas.** 1994. Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)-beta-D-glucan synthase. Antimicrob. Agents Chemother. **38:**1480–1489.
17. **NCCLS/CLSI.** 2002. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard. Document M38-A. National Committee for Clinical Laboratory Standards, Wayne, PA.
18. **Otcenasek, M., and V. Buchta.** 1994. In vitro susceptibility to 9 antifungal agents of 14 strains of zygomycetes isolated from clinical specimens. Mycopathologia **128:**135–137.
19. **Pfaller, M. A., F. Marco, S. A. Messer, and R. N. Jones.** 1998. In vitro activity of two echinocandin derivatives, LY303366 and MK-0991 (L-743,792) against clinical isolates of *Aspergillus*, *Fusarium*, *Rhizopus*, and other filamentous fungi. Diagn. Microbiol. Infect. Dis. **30:**251–255.
20. **Pfaller, M. A., S. A. Messer, R. J. Hollis, R. N. Jones, and the Sentry Participants Group.** 2002. Antifungal activities of posaconazole, ravuconazole, and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: report from SENTRY antimicrobial surveillance program, 2000. Antimicrob. Agents Chemother. **46:**1032–1037.
21. **Ribes, J. A., C. L. Vanover-Sams, and D. J. Baker.** 2000. Zygomycosis in human disease. Clin. Microbiol. Rev. **13:**236–301.
22. **Roden, M. M., T. E. Zaoutis, W. L. Buchanan, T. A. Knudsen, T. A. Sarkisova, R. L. Schaufele, M. Sein, T. Sein, C. C. Chiou, J. H. Chu, D. P. Kontoyiannis, and T. J. Walsh.** 2005. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin. Infect. Dis. **41:**634–653.
23. **Sabatelli, F., R. Patel, P. A. Mann, C. A. Mendrick, C. C. Norris, R. Hare, D. Loebenberg, T. A. Black, and P. M. McNicholas.** 2006. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. Antimicrob. Agents Chemother. **50:**2009–2015.
24. **Singh, J., D. Rimek, and R. Kappe.** 2005. In vitro susceptibility of 15 strains of zygomycetes to nine antifungal agents as determined by the NCCLS M38-A microdilution method. Mycoses **48:**246–250.
25. **Spellberg, B., Y. Fu, J. E. Edwards, Jr., and A. S. Ibrahim.** 2005. Combined therapy with amphotericin B lipid complex and caspofungin acetate of disseminated zygomycosis in diabetic ketoacidotic mice. Antimicrob. Agents Chemother. **49:**830–832.
26. **Sugar, A. M.** 1992. Mucormycosis. Clin. Infect. Dis. **14**(Suppl. 1)**:**S126–S129.
27. **Sugar, A. M.** 2005. Agents of mucormycosis and related species, p. 2973–2984. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Principles and practice of infectious diseases, 6th ed. Churchill Livingstone, Philadelphia, Pa.
28. **Sun, Q. N., A. W. Fothergill, D. I. McCarthy, M. G. Rinaldi, and J. R. Graybill.** 2002. In vitro activities of posaconazole, itraconazole, voriconazole, amphotericin B, and fluconazole against 37 clinical isolates of zygomycetes. Antimicrob. Agents Chemother. **46:**1581–1582.
29. **Sun, N. Q., L. K. Najvar, R. Bocanegra, D. Loebenberg, and J. R. Graybill.** 2002. In vivo activity of posaconazole against *Mucor* spp. in an immunosuppressed-mouse model. Antimicrob. Agents Chemother. **46:**2310–2312.
30. **Van Burik, J.-A. H., R. S. Hare, H. F. Solomon, M. L. Corrado, and D. P. Kontoyiannis.** 2006. Posaconazole is effective salvage therapy in zygomycosis: a retrospective summary of 91 cases. Clin. Infect. Dis. **42:**e61–e65.