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Large-Scale Evaluation of *In Vitro* Amphotericin B, Triazole, and Echinocandin Activity against *Coccidioides* Species from U.S. Institutions

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ABSTRACT Large-scale testing of *Coccidioides* isolates has not been performed, and the frequency of clinical isolates with elevated amphotericin B or triazole MICs has not been evaluated. *Coccidioides* isolates ($n = 581$) underwent antifungal susceptibility testing. Elevated MIC values were observed for fluconazole (≥16 μg/ml, 37.3% of isolates; ≥32 μg/ml, 7.9% of isolates), itraconazole (≥2 μg/ml, 1.0% of isolates), posaconazole (≥1 μg/ml, 1.0% of isolates), and voriconazole (≥2 μg/ml, 1.2% of isolates). However, mold-active triazoles exhibited low MICs for the majority of isolates tested. Additional correlation with patient outcomes to determine the relevance of elevated MICs in *Coccidioides* isolates is needed.

KEYWORDS *Coccidioides*, coccidioidomycosis, fluconazole, itraconazole, posaconazole, voriconazole, amphotericin B, flucytosine, caspofungin, antifungal susceptibility testing, endemic mycoses

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Coccidioidomycosis is an invasive fungal infection caused by the dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii*. Clinical manifestations vary depending on the extent of infection and the immune status of the host. Treatment of patients with coccidioidomycosis has largely consisted of therapy with amphotericin B formulations for those with severe disease or a triazole, most commonly fluconazole, for those with mild to moderate disease (1).

(This work was presented in part at ASM Microbe, Boston, MA, 2016 [2].)

Despite the significant clinical impact of this infection, large-scale susceptibility studies have not been presented. Previous reports observed fluconazole MICs and minimum fungicidal concentrations (MFCs) that exceeded achievable blood levels with ordinary dosing regimens (3, 4). However, the epidemiology and mechanism(s) of resistance in such isolates, and which alternative agents maintain *in vitro* activity when these isolates are encountered, have not been determined (5).

We assessed the susceptibility profile of a large collection of *Coccidioides* isolates received by the Fungus Testing Laboratory (University of Texas Health Science Center at San Antonio) between 2001 and 2015. During this period, 581 clinical *Coccidioides* isolates were received from disparate geographic origins within North America, and susceptibility testing was performed when they were received. Most of the isolates were from California, Arizona, and Texas. Susceptibility testing was performed by broth macrodilution according to the CLSI M38-A2 reference standard as previously described (6–11). MICs were read as the lowest concentration that resulted in ≥80% inhibition of

TABLE 1 Results of susceptibility testing

Parameter	Susceptibility of isolates to (μg/ml [range])[a]: AMB, ≤0.03 to 4 (n = 397)	FLU, ≤0.12 to ≥64 (n = 581)	ITR, ≤0.03 to >16 (n = 486)	POS, ≤0.03 to >16 (n = 377)	VOR, ≤0.015 to 8 (n = 499)	AFG, ≤0.015 to ≥8 (n = 19)	CFG, ≤0.015 to ≥8 (n = 172)	MFG, <0.015 to 8 (n = 50)
MIC_{50}	0.25	8	0.25	0.125	0.125	0.06	0.125	0.06
MIC_{90}	0.5	16	0.5	0.25	0.25	0.25	8	0.125
GM MIC	0.247	7.710	0.245	0.141	0.107	0.114	0.188	0.089

[a]AMB, amphotericin B; FLU, fluconazole; ITR, itraconazole; POS, posaconazole; VOR, voriconazole; AFG, anidulafungin; CFG, caspofungin; MFG, micafungin.

growth compared with the drug-free control. Differences in the geometric mean (GM) MIC values were assessed for significance by analysis of variance (ANOVA) using Tukey's posttest for multiple comparisons.

Voriconazole was the most potent antifungal agent tested, with a GM MIC of 0.107 μg/ml, which was significantly lower than the GM MIC of fluconazole (7.710 μg/ml), itraconazole (0.245 μg/ml), and amphotericin B (0.247 μg/ml) ($P < 0.05$ for all comparisons) (Table 1). Posaconazole GM MICs (0.141 μg/ml) were similar to those of voriconazole and were significantly lower than those of fluconazole, itraconazole, and amphotericin B ($P < 0.0001$). The differences between posaconazole and voriconazole GM MICs were not significant ($P = 0.98$).

The MIC ranges for each antifungal were wide (Table 2). The MIC_{50} for fluconazole was 8 μg/ml, and the MIC_{90} was 16 μg/ml; more than one-third (215/581, 37.3%) of isolates exhibited fluconazole MICs of ≥16 μg/ml. Furthermore, 22 isolates (22/581, 3.8%) were identified that had fluconazole MICs of ≥64 μg/ml. Elevated MICs for the mold-active triazoles and amphotericin B were uncommon: itraconazole, ≥2 μg/ml (5/486, 1.0%); posaconazole, ≥1 μg/ml (4/377, 1.1%); voriconazole, ≥2 μg/ml (12/499, 1.2%); and amphotericin B, ≥2 μg/ml (11/397, 2.8%).

These results illustrate the relative frequency of elevated fluconazole MICs encountered in clinical *Coccidioides* isolates and the *in vitro* superiority of newer mold-active triazoles. Our results are consistent with those of prior studies of *Coccidioides in vitro* susceptibility (4, 12–14). In all of these smaller studies, fluconazole was less active than comparator antifungals, yet the conclusions of the studies were limited by the relatively low numbers of isolates assessed.

Fluconazole is the most frequently prescribed antifungal medication for the treatment of coccidioidomycosis and is considered first-line therapy for the majority of coccidioidal infections (1). Despite the lack of large-scale susceptibility studies, clinical experience suggests that higher fluconazole doses are often needed for a clinical response to be observed, and doses of 800 mg for primary infection are now routinely recommended by some experts (1).

Together, our results and those of the earlier studies suggest that the *in vitro* MICs of *Coccidioides* are similar to those of *Histoplasma* and *Blastomyces*, mycoses for which fluconazole is not the suggested first-line option (15, 16). Our *in vitro* data provide

TABLE 2 Number of MIC values for each antifungal agent tested at specific concentrations

Agent	Total (n)	No. of values at MIC (μg/ml) of: ≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
AMB	397	–[a]	8	28	81	174	68	27	9	2	0	0	–	–
FLU	581	–	–	–	9	11	13	20	26	58	228	170	24	22
ITR	486	–	34	41	81	120	178	27	1	1	2	1	–	–
POS	377	–	40	59	113	128	33	2	1	0	0	1	–	–
VOR	499	–	40	168	196	67	16	6	3	1	2	0	–	–
AFG	19	1	1	8	6	1	1	0	0	0	1	–	–	–
CFG	172	1	26	24	72	17	2	5	1	4	20	–	–	–
MFG	50	1	5	21	18	4	0	0	0	0	1	–	–	–

[a]–, Not tested.

additional support for the higher fluconazole doses often recommended in the treatment of coccidioidomycosis.

The mold-active triazoles, compared with fluconazole, have favorable *in vitro* activity against *Coccidioides* spp. However, the *in vivo* impact of these findings has yet to be determined. Clinical evaluation of these agents in coccidioidomycosis has consisted almost exclusively of salvage studies (17, 18), with the exception of a single comparative clinical trial (19). In this study, fluconazole (400 mg/day) was directly compared with itraconazole (200 mg twice daily). By 12 months, 57% of patients responded to fluconazole, whereas 72% responded to itraconazole ($P = 0.05$), with relapses reported to be more frequent in the fluconazole-treated group. It remains to be seen if pharmacokinetic and pharmacodynamic parameters are predictive of success in coccidioidomycosis; however, additional investigation is clearly warranted.

Interestingly, we found significant variability in the echinocandin MICs and differences among the three agents. The caspofungin GM MIC (0.188 μg/ml) was higher than those of anidulafungin (0.114 μg/ml) and micafungin (0.089 μg/ml) ($P = 0.008$). The MIC_{90} value for caspofungin (8 μg/ml) was also higher than those found for anidulafungin and micafungin (0.25 and 0.125 μg/ml, respectively). This is in contrast to prior *in vitro* reports suggesting that echinocandins have limited *in vitro* activity against *Coccidioides* spp. (4, 13, 20, 21). These earlier *in vitro* findings are also in contrast to more recent findings of echinocandin *in vivo* activity in murine and human studies of coccidioidomycosis (22–24).

Our study, which evaluated a large number of clinical *Coccidioides* isolates, found a wide range of echinocandin MICs and, combined with more recent clinical reports, suggests that this class may exhibit efficacy in the treatment of coccidioidomycosis. However, given the variable susceptibility, echinocandins should not be used as monotherapy. Additionally, we report for the first time, to our knowledge, the distribution of triazole MICs likely to be encountered during the care of patients with coccidioidomycosis. We commonly found elevated fluconazole MICs, with very high MICs ($\geq$64 μg/ml) in a number of isolates. The majority of these isolates retained comparatively low MICs to other triazoles, suggesting that they may be alternative treatment options, pending further study. The lack of patient data associated with the isolates tested is a significant limitation, and the current data set may be biased in that susceptibility testing was requested only for patients who failed therapy. Additionally, species-specific differences were not examined in this study and should be evaluated. Future work focusing on outcomes in patients with infection from isolates with elevated MICs and the molecular mechanisms responsible for *in vitro* resistance should also be performed.

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