In Vitro Assessment of Antifungal Drugs and Sulfamethoxazole-Trimethoprim against Clinical Isolates of *Conidiobolus lamprauges*

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Conidiobolomycosis is an infection for which there are no sufficient clinical or *in vitro* data to support a consensus about the optimal treatment, and consequently, cases of therapeutic success and failure have been described for all forms of therapy (1–3). We have determined the *in vitro* activities of sulfamethoxazole, trimethoprim, and several antifungal drugs alone and in combination against *Conidiobolus lamprauges*, a pathogenic agent of nasopharyngeal conidiobolomycosis in animals (4, 5) and in one case of disseminated human disease (3).

Seven nonduplicate *C. lamprauges* isolates obtained from cases of sheep rhinoconidiobolomycosis from central-western Brazil, previously identified based on their macro- and microscopic characteristics (6) as well as the nucleotide sequences of their 18S ribosomal DNA (rDNA) genes (7), were used. The sequences obtained were analyzed, and multiple-sequence alignment was done with *Conidiobolus coronatus*, *C. lamprauges*, and *Conidiobolus incongruus* reference strains. The antimicrobials used in the susceptibility tests are described in Table 1. Broth microdilution susceptibility tests were performed by following the CLSI M38-A2 protocol (8, 9), except with the final inocula, which were diluted 1:10 to produce more consistent mycelial growth for the determination of MIC endpoints. The interactions between drugs were evaluated using a checkerboard test (10) and by a colorimetric 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT) time-kill assay (11).

The MICs and minimum effective concentrations (MECs) of the antimicrobial drugs evaluated against *C. lamprauges* are presented in Table 1. Amphotericin B (MIC₁₀₀ range, 1 to 2 µg/ml), terbinafine (MIC₁₀₀ range, 1 to 4, and MIC₈₀, 0.06 to 2 µg/ml), and miconazole (MIC₁₀₀ range, 4 to 16 µg/ml) were considered the most effective drugs because they required the lowest concentrations for the *in vitro* inhibition of *C. lamprauges*. Checkerboard results (Table 1) showed synergistic interactions of 100% for the sulfamethoxazole-trimethoprim combination, 71% for the terbinafine-azole antifungal combinations, and 29% for the terbinafine-

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TABLE 1 *In vitro* susceptibilities of sulfamethoxazole, trimethoprim, and antifungal drugs alone and in combination against seven *Conidiobolus lamprauges* isolates^a

Drugs used alone		Drugs used in combination					
Drug ^e	MIC or MEC range (GM) (μg/ml)	Drug combination (drug[s] 1 + drug 2)	MIC range (GMs) (μg/ml)		FICI range (GM) ^d	FICI interpretation (%)	
			Drug(s) 1	Drug 2		Syn	Ind
		SMX + TMP	4 to 8 (5.94)	8 to 32 (23.78)	0.18 to 0.37 (0.31)	100	0
ANI ^b	>512	SMX-TMP ^r + AMB	0.25, 1 to 8, 32 (0.41, 1.64)	1 to 2 (1.22)	1.03 to 3.00 (1.21)	0	100
CAS ^b	256 to >512 (840)	SMX-TMP ^r + ANI	8, 32 to 16, 64 (9.75, 39)	0.06 to 4 (0.11)	1.00 to 2.00 (1.64)	0	100
CTX ^b	32 to 256 (141.32)	SMX-TMP ^r + CAS	4, 16 to 16, 64 (9.75, 39)	0.125 to 16 (0.45)	1.01 to 2.01 (1.64)	0	100
MFG ^b	64 to 256 (141.32)	SMX-TMP ^r + MFG	0.25, 1 to 16, 64 (2.44, 9.76)	0.06 to 64 (8.73)	0.53 to 2.50 (1.24)	0	100
MCZ ^b	4 to 16 (7.25)	SMX-TMP ^r + MCZ	0.25, 1 to 0.5, 2 (0.28, 1.12)	4 to 16 (7.25)	0.56 to 2.03 (1.06)	0	100
POS ^b	>32	SMX-TMP ^r + POS	4, 16 to 8, 32 (5.38, 21.52)	0.003 to 0.03 (0.01)	0.51 to 1.00 (0.91)	0	100
SMX ^b	128 to 1024 (724.08)	SMX-TMP ^r + VOR	4, 16 to 8, 32 (7.25, 29)	0.015 to 4 (0.15)	0.51 to 2.00 (1.22)	0	100
TMP ^b	256 to 512 (380.41)	SMX-TMP ^r + TRB	0.25, 1 to 4, 16 (0.37, 1.48)	0.25 to 4 (1.49)	0.53 to 1.25 (0.89)	0	100
TRB ^b	1 to 4 (2.21)						
VOR ^b	>512	TRB + AMB	0.06 to 4 (0.3)	0.003 to 2 (0.09)	1.00 to 1.03 (1.02)	0	100
		TRB + ANI	0.5 to 4 (1.81)	0.06 to 1 (0.13)	0.51 to 1.00 (0.82)	0	100
ANI ^c	>8	TRB + CAS	1 to 2 (1.81)	0.125 to 4 (0.25)	0.51 to 1.01 (0.82)	0	100
CAS ^c	>8	TRB + MFG	0.06 to 2 (0.55)	8 to 64 (26.25)	0.37 to 0.75 (0.56)	29	71
CTX ^d	16 to 32 (26.25)	TRB + MCZ	0.06 to 1 (0.33)	0.06 to 8 (0.9)	0.15 to 1.03 (0.42)	71	29
MFG ^c	>8	TRB + POS	0.06 to 4 (0.5)	0.003 to 4 (0.01)	0.07 to 1.00 (0.29)	71	29
SMX ^d	16 to 64 (26.25)	TRB + VOR	0.25 to 2 (0.61)	0.015 to 0.5 (0.07)	0.12 to 1.00 (0.28)	71	29
TMP ^d	128 to 512 (282.65)						
TRB ^d	0.06 to 2 (0.74)						

^aAbbreviations: MEC, minimum effective concentration; GM, geometric mean; FICI, fractional inhibitory concentration; FICI, FICI index; Syn, synergism; Ind, indifference; SMX, sulfamethoxazole; TMP, trimethoprim; AMB, amphotericin B; ANI, anidulafungin; CAS, caspofungin; MFG, micafungin; MCZ, miconazole; POS, posaconazole; VOR, voriconazole; TRB, terbinafine; CTX, co-trimoxazole (SMX-TMP at a ratio of 5:1).

^bMIC (100% growth inhibition).

^cMEC.

^dMIC (80% growth inhibition).

^eDrugs were purchased from Sigma Aldrich (St. Louis, MO, USA), except for anidulafungin (Pfizer, New York, NY, USA) and micafungin (Astellas, Tokyo, Japan).

^fThe association of SMX and TMP at a proportion of 1:4 was the combination for which the lowest FICs and 100% of synergistic interactions were observed. For this reason, this proportion was used to assess the interactions of SMX-TMP with all of the antifungal drugs evaluated.

^gThe FICI was determined from the nonturbid wells along with the turbidity/nonturbidity growth interface (MICs of 100% growth inhibition when AMB was associated with other drugs and at least 80% inhibition of growth compared with the growth of control wells for the all other combinations) after 48 h of incubation at 37°C and interpreted as follows: FICI of ≤ 0.5 , synergism; FICI of >0.5 to ≤ 4 , indifference; and FICI of >4 , antagonism.

FIG 1 XTT reduction assay. Percentages of viable hyphae of *C. lamprauges* exposed to sulfamethoxazole (SMX), trimethoprim (TMP), terbinafine (TRB), miconazole (MCZ), posaconazole (POS), and voriconazole (VOR) at different concentrations (micrograms per milliliter) alone and in combination. Data represent the means $\pm$ standard errors of the means from seven strains in triplicate. Hyphal viability was determined at 12, 24, and 48 h of incubation at 37°C. Data were subjected to logarithmic transformation to obtain homogeneity between groups and were analyzed by one-way analysis of variance with the Holm-Sidak test for statistical comparisons between drug combination groups versus groups in which the drug was used alone. The statistics program SigmaPlot 12.5 was used for data analysis. An asterisk indicates a significant difference ($P \leq 0.01$), and a hashtag indicates a significant difference ($P < 0.001$) from the groups in which a drug was used alone.

micafungin combination. All other interactions were indifferent. An XTT reduction assay showed significantly greater hyphal damage for the sulfamethoxazole-trimethoprim combination than for drugs used alone (Fig. 1A and B), but levels of hyphal viability after terbinafine treatment and after treatment with azole antifungals did not differ significantly (Fig. 1C).

Susceptibility tests for *C. lamprauges* have previously been described in only a few reports (3, 9, 12), and these presented results similar to those obtained through this study. Previous works demonstrate the preventive and therapeutic potential of sulfamethoxazole-trimethoprim against *Pneumocystis*, *Aspergillus*, and *Cryptococcus* species (13, 14), suggesting that these drugs may also have potentially been used for the treatment of conidiobolomycosis.

In conclusion, the *in vitro* data reported here suggest that the combinations of sulfamethoxazole-trimethoprim and terbinafine alone or associated with azole antifungal drugs deserve attention as candidates for the treatment of conidiobolomycosis infections. Additional *in vitro* and *in vivo* studies with a large number of clinical isolates and antimicrobial combinations are needed to better understand the susceptibility of *C. lamprauges* to the antimicrobials described in this study.

Accession number(s). Nucleotide data for the 3 isolates were deposited in GenBank under accession numbers [GQ221848](https://doi.org/10.1101/111111), [GQ221849](https://doi.org/10.1101/111111), and [GQ217491](https://doi.org/10.1101/111111). All strains were deposited in the Culture Collection of Filamentous Fungi (Fiocruz, RJ, Brazil) with additional internal transcribed spacer sequence analysis (data not shown).

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