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In Vitro Effect of Sulfamethoxazole-Trimethoprim against *Histoplasma capsulatum* var. *capsulatum*▽

Raimunda Sâmia Nogueira Brilhante,[1,2]* Maria Auxiliadora Bezerra Fechine,[2] Rossana de Aguiar Cordeiro,[1,2] Marcos Fábio Gadelha Rocha,[1,3] Joyce Fonteles Ribeiro,[1] André Jalles Monteiro,[4] Rita Amanda Chaves de Lima,[1] Jacó Ricarte Lima Mesquita,[6] Zoilo Pires de Camargo,[5] and José Júlio Costa Sidrim[1,2]

Specialized Medical Mycology Center, Postgraduate Program in Medical Microbiology, Federal University of Ceará, Fortaleza, Ceará, Brazil[1]; Postgraduate Program in Medical Sciences, Federal University of Ceará, Fortaleza, Ceará, Brazil[2]; Postgraduate Program in Veterinary Science, State University of Ceará, Fortaleza, Ceará, Brazil[3]; Department of Statistics and Applied Mathematics, Federal University of Ceará, Fortaleza-CE, Brazil[4]; Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo, São Paulo, Brazil[5]; and São José Hospital, Ceará, Brazil[6]

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This study evaluated the *in vitro* effect of sulfamethoxazole-trimethoprim against *Histoplasma capsulatum* var. *capsulatum* isolated from HIV-positive patients. The drugs were tested by microdilution testing in accordance with the CLSI guidelines. All of the strains were inhibited by sulfamethoxazole-trimethoprim, with MIC ranges of 0.039 (sulfamethoxazole)/0.0078 (trimethoprim) mg/ml to 0.625/0.125 mg/ml for mycelial forms and 0.0025/0.0005 to 0.02/0.004 mg/ml for yeast-like forms. However, *in vivo* studies are necessary to evaluate the significance of these results.

Histoplasmosis is an infection caused by the dimorphic fungus *Histoplasma capsulatum.* It is endemic in the Americas (3) and is currently one of the most important systemic infections in Brazil (6). In a retrospective study carried out in Ceará state (northeastern Brazil) from 1995 to 2004, 164 histoplasmosis cases were found in HIV-positive patients (4).

The treatment of histoplasmosis depends on the infection's severity and clinical manifestation, along with individual risk factors (9, 13). Because of the increase in histoplasmosis, particularly in HIV-positive patients, as well as the development of antifungal resistance associated with refractory and repeated infections (13), there is a need for seeking new therapeutic options for this mycosis. Therefore, this study aimed at testing the *in vitro* activity of sulfamethoxazole-trimethoprim (SMX-TMP) against *H. capsulatum* var. *capsulatum* strains isolated from AIDS patients.

A total of 84 clinical strains of *H. capsulatum* isolated from two different biogeographic regions in Brazil were included in this study. Of them, 68 came from Ceará (northeastern semiarid region) and 16 from southeastern states (a subtropical region). The strains were obtained from the collection of the Specialized Medical Mycology Center of Ceará Federal University and were handled in our level 3 biosecurity laboratory.

For each strain, a combined solution of SMX-TMP (Roche, Brazil) was used at a concentration range of 0.0025 mg/ml SMX/0.0005 mg/ml TMP and 20/4 mg/ml. The inocula were prepared as described by Li et al. (10), with some modifications. Briefly, *H. capsulatum* strains were cultured in the mycelial phase onto brain heart infusion (BHI) agar for 7 days at 28°C, and then 2 ml of sterile saline was added to each culture. The surfaces of the mycelia were harvested with a swab. To obtain yeast cells, isolates were cultured on agar BHI with sheep blood (10%) at 35°C and were maintained through weekly passages (7). The supernatant was read in a spectrophotometer at 530 nm, and the transmittance was adjusted to 90 to 95%. The suspensions then were diluted to obtain an inoculum of 0.5×10^3 to 2.5×10^4 CFU/ml, as demonstrated by quantitative colony counts on Sabouraud dextrose agar (10). Susceptibility tests were carried out as described by Nakai et al. (11), except that the readings were performed after 7 or 4 days for mycelial and yeast growth, respectively. For SMX-TMP, the MIC was defined as the lowest concentration at which no visible growth was observed (14). The differences in the MICs between northeastern and southeastern strains were evaluated by Student's *t* test ($P < 0.05$). Susceptibility control tests were performed with amphotericin B (AMB) against 84 and 7 *H. capsulatum* strains in mycelial and yeast-like forms, respectively. SMX-TMP quality control was carried out with *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

All *H. capsulatum* strains were inhibited by SMX-TMP, with MICs ranging from 0.039/0.0078 to 0.625/0.125 mg/ml for the mycelial forms and 0.0025/0.0005 to 0.02/0.004 mg/ml for the yeast-like forms. The SMX-TMP MICs for strains from northeastern Brazil presented geometric means of 0.1544 mg/ml for sulfamethoxazole and 0.0309 mg/ml for trimethoprim for the mycelial forms and 0.0141 mg/ml for sulfamethoxazole and 0.0028 mg/ml for trimethoprim for yeast-like forms. The geometric means for the strains from southeast Brazil were 0.1152 mg/ml for sulfamethoxazole and 0.0230 mg/ml for trimethoprim for the mycelial forms and 0.0079 mg/ml for sulfamethoxazole and 0.0016 mg/ml for trimethoprim for yeast-like forms (Table 1).

* Corresponding author. Mailing address: Rua Barão de Canindé, 210, Montese. CEP: 60.425-540, Fortaleza CE, Brazil. Phone: 55 (85) 3214-2853. Fax: 55 (85) 3295-1736. E-mail: brilhante@ufc.br.

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TABLE 1. MICs of SMX/TMP against strains of *H. capsulatum* var. *capsulatum* in yeast-like and mycelial forms from northeastern and southeastern Brazil[a]

SMX-TMP MICs (mg/ml)	No. of NE strains		No. of SE strains	
	M	Y	M	Y
0.0025/0.0005		1		
0.005/0.001				2
0.01/0.002		1		
0.02/0.004		6		1
0.039/0.0078	2		2	
0.078/0.0156	14		6	
0.156/0.0312	37		5	
0.312/0.0625	13		3	
0.625/0.125	2			
Total	68	8	16	3

[a] Geometric mean MICs for mycelial (M) and yeast-like (Y) forms of strains from northeastern (NE) Brazil were 0.1544/0.0309 and 0.0141/0.0028 mg/ml, respectively. Geometric mean MICs for mycelial and yeast-like forms of strains from southeastern (SE) Brazil were 0.1152/0.0230 and 0.0079/0.0016, respectively.

Susceptibility control tests showed MICs (geometric means) of 0.1168 and 0.0762 μg/ml for AMB against *H. capsulatum* for mycelial and for yeast-like forms, respectively. *E. coli* ATCC 25922 was sensitive and *P. aeruginosa* ATCC 27853 was resistant to SMX-TMP.

Previous studies have described the effect of sulfamethoxazole or SMX-TMP against other pathogenic fungi, such as *Cryptococcus neoformans* (8) and *Aspergillus fumigatus* (1). The results of this study demonstrated for the first time the inhibitory effect of SMX-TMP against *H. capsulatum*. They show that the MICs for strains from northeastern Brazil were higher than those from the southeast. These findings can be related to population profile and/or geoclimatic differences, but further investigation is necessary to explain these hypotheses. The medium composition also may alter the drug effect. *H. capsulatum* strains were inhibited by SMX-TMP diluted in RPMI medium. Yeast nitrogen base (YNB) was not used because no growth of *H. capsulatum* was obtained in this medium (data not shown). If *H. capsulatum* had grown in YNB medium, the MICs of SMX-TMP probably would have been lower than those observed. Afeltra et al. (1) studied the effectiveness of sulfonamides against *Aspergillus* spp. and demonstrated the influence of the composition of the medium in susceptibility tests with SMX-TMP, observing that RPMI, despite being recommended by the CLSI (12), contains four times more *p*-aminobenzoic acid (PABA) than YNB medium according to the manufacturer. Based on Hanafy et al. (8), in a study using sulfamethoxazole against strains of *Cryptococcus* spp., MICs varied from 0.125 to 0.250 mg/ml in RPMI medium, while in media not containing PABA (such as YNB) or compounds related to PABA, the MICs declined to 0.031 to 0.062 mg/ml (8). MICs for *H. capsulatum* yeast-like forms were lower than those for mycelial forms for both northeastern ($P = 0.0000$) and southeastern ($P = 0.0001$) strains.

SMX-TMP has shown good results in the treatment of paracoccidioidomycosis as a supportive therapy after the administration of amphotericin B and when there is an involvement of the central nervous system. The sulfonamides remain the drugs of choice to treat paracoccidioidomycosis in developing countries with substandard socioeconomic conditions. SMX-TMP also is used to treat fungal infections caused by *Pneumocystis jiroveci* in HIV-positive patients in our region, but this combination, with or without antifungals, has never been used to treat histoplasmosis.

Until this study, there had been no reports of the efficacy of SMX-TMP against *H. capsulatum* var. *capsulatum*. However, there are reports of histoplasmosis caused by *Histoplasma capsulatum* var. *duboisii* in Africa being successfully treated with SMX-TMP (2, 5). Therefore, despite the evidence in this study, *in vivo* studies are necessary to evaluate the significance of these results.

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REFERENCES

1. **Afeltra, J., J. F. Meis, R. G. Vitale, J. W. Mouton, and P. E. Verweij.** 2002. *In vitro* activities of pentamidine, pyrimethamine, trimethoprim, and sulfonamides against *Aspergillus* species. Antimicrob. Agents Chemother. **46:**2029–2031.
2. **Ajayi, B. G., B. Osuntokun, O. Olurin, O. O. Kale, and T. A. Junaida.** 1986. Orbital histoplasmosis due to *Histoplasma capsulatum* var. *duboisii*: successful treatment with septrin. J. Trop. Med. Hyg. **89:**179–187.
3. **Baddley, J. W., I. R. Sankara, J. M. Rodriquez, P. G. Pappas, and W. J. Many, Jr.** 2008. Histoplasmosis in HIV-infected patients in a southern regional medical center: poor prognosis in the era of highly active antiretroviral therapy. Diagn. Microbiol. Infect. Dis. **62:**151–156.
4. **Daher, E. F., G. B. Silva, Jr., F. A. Barros, C. F. Takeda, R. M. Mota, M. T. Ferreira, S. A. Oliveira, J. C. Martins, S. M. Araújo, and O. A. Gutiérrez-Adrianzén.** 2007. Clinical and laboratory features of disseminated histoplasmosis in HIV patients from Brazil. Trop. Med. Int. Health **12:**1108–1115.
5. **Egere, J. U., H. C. Gugnani, A. N. Okoro, and A. V. Suseelan.** 1978. African histoplasmosis in Eastern Nigeria: report of two culturally proven cases treated with septrin and amphotericin B. J. Trop. Med. Hyg. **81:**225–229.
6. **Ferreira, M. S., and A. S. Borges.** 2009. Histoplasmose. Rev. Soc. Bras. Med. Trop. **42**:192–198.
7. **Fressatti, R., V. L. Dias-Siqueira, I. E. Svidzinski, F. Herrero, and C. Kemmelmeier.** 1992. A medium for inducing conversion of *Histoplasma capsulatum* var. *capsulatum* into its yeast-like form. Mem. Inst. Oswaldo Cruz **87:**53–58.
8. **Hanafy, A., J. Uno, H. Mitani, Y. Kang, and Y. Mikami.** 2007. *In vitro* antifungal activities of sulfa drugs against clinical isolates of *Aspergillus* and *Cryptococcus* species. Jpn. J. Med. Mycol. **48:**47–50.
9. **Kauffman, C. A.** 2007. Histoplasmosis: a clinical and laboratory update. Clin. Microbiol. Rev. **20:**115–132.
10. **Li, R., M. A. Ciblak, N. Nordoff, L. Pasarell, D. W. Warnock, and M. R. McGinnis.** 2000. *In vitro* activities of voriconazole, itraconazole, and amphotericin B against *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum*. Antimicrob. Agents Chemother. **44:**1734–1736.
11. **Nakai, T., J. Uno, F. Ikeda, S. Tawara, K. Nishimura, and M. Miyaji.** 2003. In vitro antifungal activity of micafungin (FK463) against dimorphic fungi: comparison of yeast-like and mycelial forms. Antimicrob. Agents Chemother. **47:**1376–1381.
12. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard. Document M27-A. National Committee for Clinical Laboratory Standards, Wayne, PA.
13. **Wheat, L. J., A. G. Freifeld, M. B. Kleiman, J. W. Baddley, D. S. McKinsey, J. E. Loyd, and C. A. Kauffman.** 2007. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. Clin. Infect. Dis. **45:**807–825.
14. **Yekutiel, A., I. Shalit, Y. Shadkchan, and N. Osherov.** 2004. In vitro activity of caspofungin combined with sulfamethoxazole against clinical isolates of *Aspergillus* spp. Antimicrob. Agents Chemother. **48:**3279–3283.