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In vitro susceptibility of antifungal drugs against *Sporothrix brasiliensis* recovered from cats with sporotrichosis in Brazil

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Abstract

Sporotrichosis is an important subcutaneous mycosis of humans and animals. Classically, the disease is acquired upon traumatic inoculation of *Sporothrix* propagules from contaminated soil and plant debris. In addition, the direct horizontal transmission of *Sporothrix* among animals and the resulting zoonotic infection in humans highlight an alternative and efficient rout of transmission through biting and scratching. *Sporothrix brasiliensis* is the most virulent species of the *Sporothrix schenckii* complex and is responsible for the long-lasting outbreak of feline sporotrichosis in Brazil. However, antifungal susceptibility data of animal-borne isolates is scarce. Therefore, this study evaluated the *in vitro* activity of amphotericin B, caspofungin, itraconazole, voriconazole, fluconazole, and ketoconazole against animal-borne isolates of *S. brasiliensis*. The susceptibility tests were performed through broth microdilution (M38-A2). The results show the relevant activity of itraconazole, amphotericin B, and ketoconazole against *S. brasiliensis*, with the following MIC ranges: 0.125–2, 0.125–4 and 0.0312–2 μg/ml, respectively. Caspofungin was moderately effective, displaying higher variation in MIC values (0.25–64 μg/ml). Voriconazole (2–64 μg/ml) and fluconazole (62.5–500 μg/ml) showed low activity against *S. brasiliensis* strains. This study contributed to the characterization of the *in vitro* antifungal susceptibility of strains of *S. brasiliensis* recovered from cats with sporotrichosis, which have recently been considered the main source of human infections.

Key words: *Sporothrix brasiliensis*, cats, antifungal susceptibility, sporotrichosis, *Sporothrix schenckii* complex.

Introduction

Sporotrichosis is a worldwide subcutaneous mycosis, with high endemicity in tropical and subtropical areas;[1] nevertheless, etiological agents are not evenly distributed.[2] This infection is caused by the dimorphic fungus, previously described by B. R. Schenck, in 1898, as the single species *Sporothrix schenckii*. Phylogenetic inferences based on protein coding loci supports a high level of speciation in *Sporothrix*, what lead to the description of cryptic agents in the clinical practice,[3,4] including *Sporothrix brasiliensis* (clade I), *S. schenckii sensu stricto* (clade II), *Sporothrix globosa* (clade III), and *Sporothrix luriei* (clade VI). The species embedded in the *S. schenckii* complex cause sporotrichosis in humans and other mammals, presenting fundamental differences between routes of transmission, frequency, and pathogenicity. The occurrence of sporotrichosis in animals, especially in cats,[5,6] and its transmission to humans has been described in many countries.[1,7]

In Brazil, large epidemics of sporotrichosis occurred due to zoonotic transmission, and cats are currently pointed as the main source of infection.[8–12] Over the last two decades, there has been a major outbreak of sporotrichosis in cats in southeastern Brazil, with an overwhelming occurrence of *S. brasiliensis*.[6,9,13] *S. brasiliensis* is the most virulent species of *S. schenckii* complex, being associated with high host mortality and dissemination after challenge in murine models.[14,15] It is a species with low genetic diversity and identical genotypes of *S. brasiliensis* from cats and humans have been described.[9,13] Despite the increasing incidence of sporotrichosis, there are only few studies on the antifungal susceptibility of *S. brasiliensis* from human infections[16–18] and, even less, on the antifungal susceptibility of strains recovered from cats.[19,20]

Thus, the objective of this study was to evaluate the *in vitro* antifungal activity of amphotericin B, caspofungin and azoles (itraconazole, voriconazole, fluconazole, and ketoconazole) against strains of *S. brasiliensis* from cases of feline sporotrichosis recovered during the long-lasting outbreak of this mycosis in Brazil.

Materials and methods

Strains and antifungal agents

The present study included a total of 48 Brazilian strains of *S. brasiliensis*. These strains were identified through the partial sequencing of the calmodulin-encoding gene[9] and by RFLP-PCR.[16] The antifungal agents were obtained as pure powders. The drugs tested included amphotericin B (AMB) (Sigma Chemical Corporation, USA), itraconazole (ITC) (Janssen Pharmaceutica, Belgium), voriconazole (VRC) (Pfizer Pharmaceuticals, USA), fluconazole (FLC) (Pfizer, Brazil), ketoconazole (KTC) (Sigma Chemical Corporation, USA) and caspofungin (CAS) (Merck Sharp & Dohme, Brazil).

For the preparation of stock solutions, AMB, ITC, VRC, and KTC were diluted in 100% DMSO, while FLC and CAS were dissolved in sterile distilled water. The material was homogenized with magnetic stirrer until complete dissolution of the drug and then transferred to sterile microtubes. Subsequently, all solutions were diluted with RPMI 1640 medium with L-glutamine, without sodium bicarbonate (Sigma), buffered to pH 7.0 with 0.165 M 3 - [N-morpholino] propanesulfonic acid (MOPS, Sigma) and stored at −20°C until use.

In vitro susceptibility tests

The antifungal susceptibility studies were carried out through the use of *Sporothrix* spp. conidia. Thus, for the preparation of fungal inocula, the strains of *Sporothrix* spp. were subcultured on brain heart infusion agar (BHI) and incubated for 5 to 6 days at 25–30°C.[21] Afterward, 1 ml of 0.9% sterile saline was added to the glass slant containing the fungal colony, which was then gently scraped with sterile cotton swabs. The suspension was transferred to a sterile test tube containing 4 ml of 0.9% saline solution, and it was manually homogenized and allowed to settle for 5 minutes. Subsequently, the suspension was diluted 1:10 with RPMI 1640 and it was quantified (CFU ml^{-1}) on BHI agar plates to obtain a final concentration of 1×10^5 at 5×10^5 CFU.ml^{-1}.

The minimum inhibitory concentrations (MICs) were determined by the microdilution method as described in the M-38A2 protocol, standardized by the Clinical and Laboratory Standards Institute (CLSI).[22] All tests were performed in duplicate for each strain. The results were obtained visually after 3 days of incubation at 35°C. The MIC of the drugs FLC, KTC, and CAS was defined as the lowest drug concentration able to inhibit 50% of visible fungal growth when compared to the drug-free growth control well. For AMB, ITC, and VRC the MIC was defined as the lowest concentration capable of preventing 100% fungal growth.[23] For quality control of antifungal susceptibility testing, *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 strains were used. The MIC results were analyzed and the geometric mean, MIC50 and MIC90 for each tested antifungal drug were calculated.

Results

The results obtained in this study against 48 *S. brasiliensis* isolated from feline cases of sporotrichosis are described in Table 1. The azole ITC demonstrated good activity against the tested strains of *S. brasiliensis*, showing MIC range of

Table 1. Antifungal susceptibility of 48 strains of *Sporothrix brasiliensis* from cats.

	MIC μg ml^{-1} (No. of strains)					
Species	AMB	ITC	VRC	FLC	KTC	CAS
S. brasiliensis					0.0312 (1)	0.25 (1)
	0.125 (1)		2 (1)			
(n = 48)		0.125 (1)			0.0625 (3)	2 (3)
	0.25 (3)		4 (1)	62.5 (3)		
		0.25 (3)			0.125 (12)	4 (4)
	0.5 (7)		8 (10)	125 (20)		
		0.5 (13)			0.25 (12)	8 (15)
	1 (29)		16 (12)	250 (21)		
		1 (27)			0.5 (14)	16 (18)
	2 (7)		32 (15)	500 (4)		
		2 (4)			1 (5)	32 (6)
	4 (1)		64 (9)			
					2 (1)	64 (1)
Geometric mean	0.90	0.77	20.16	182	0.27	10.37
MIC50	1	1	16	250	0.25	16
MIC90	2	2	64	500	1	32
C. parapsilosis						
ATCC 22019	1	0.5	0.0312	2	0.125	0.5
C. krusei						
ATCC 6258	1	0.5	0.125	32	0.25	0.25

Note: AMB: Amphotericin B; ITC: Itraconazole; VRC: Voriconazole; FLC: Fluconazole; KTC: Ketoconazole; CAS: Caspofungin.

0.125–2 μg/ml. Similarly, AMB and KTC showed MIC intervals of 0.125–4 μg/ml and 0.0312–2 μg/ml, respectively. The echinocandin CAS showed higher variation in MIC values, exhibiting MIC range of 0.25–64 μg/ml. On the other hand, VRC and FLC showed low activity against all tested strains, with MIC ranges of 2–64 μg/ml and 62.5–500 μg/ml, respectively. The MICs for the control strains are within the expected range, as described by the CLSI guidelines.[22]

Discussion

In vitro antifungal susceptibility tests are of great interest for epidemiological surveillance to monitor susceptibility profiles and the global emergence of resistance.[24] Despite the enormous health burden of zoonotic sporotrichosis, for which cats currently represent the major source of human infections,[12] there are few studies indicating the antifungal susceptibility of the different species of *S. schenckii* complex isolated from cats, especially *S. brasiliensis*. Recently, phylogenetic analysis revealed a high prevalence of this species in feline sporotrichosis outbreaks.[6,9] Considering the different routes of transmission, public health policies should change according to the type of disease, whether it is a sapronosis or a zoonosis. In this scenario, cat treatment with antifungals constitutes an important aid to control the high levels of *Sporothrix* transmission between animals and cross-species transmission to humans. In the present study, clinical isolates of *S. brasiliensis* obtained from feline sporotrichosis were evaluated for their *in vitro* response to antifungals.

Cats may experience severe forms of sporotrichosis, with multiple cutaneous lesions or disseminated systemic form.[25] ITC, KTC, potassium iodide (KI), and AMB are the most common drugs described in the treatment of feline sporotrichosis.[26–28] KTC (50–100 mg/cat/day) may be used to treat feline sporotrichosis, but it is associated with a high occurrence of adverse effects in cats. ITC (50–100 mg/cat/day) is the drug of choice due to its effectiveness and safety.[26] Currently, KI (2.5–20 mg/kg/day) capsules are also an effective option for feline sporotrichosis treatment.[28] In cases of feline sporotrichosis refractory to ITC, KI capsules as monotherapy or associated with the triazole, as well as AMB, might be considered as alternatives.[29]

Reports on the administration of AMB for treatment of feline sporotrichosis are scarce. Intravenous administration (IV) of the drug in cats is limited because of serious adverse effects and because there are no reports of clinical cure through the use of this drug in cats with sporotrichosis. Intralesional (IL) and subcutaneous (SC) administration of AMB, rather than IV administration, were successful used in combination with oral ITC in cats with refractory sporotrichosis.[29] Ishida et al.[30] using a murine model of infection, showed the efficacy of AMB–d, alone or followed by maintenance treatment with ITC, as the drug of choice for the treatment of disseminated *S. brasiliensis* infection.

Clinical cure is observed regardless the initial clinical findings or therapeutic regimen, despite the cases with fatal outcomes.[25–28] Treatment may take a few weeks to several months and must be continued for at least one month after the complete healing of the lesion. Exogenous reinfection and recurrence after cure may occur.[29] Specific biosafety procedures to reduce risks during the handling of cats with potential sporotrichosis are strongly recommended and should be followed by veterinarians and cat owners, such as appropriate personal protective equipment.[11]

Therefore, in this study, higher antifungal activity, shown by the lower MIC geometric means, was found for ITC, KTC, and AMB. These results are in accordance with MIC values found in other studies with *S. brasiliensis* strains.[3,16–18,20] Stopiglia et al.[17] presented similar results for ITC, KTC, and AMB with MIC intervals of 0.06–2 μg/ml, 0.03–1 μg/ml and 0.2–4 μg/ml, respectively. Marimon et al.[3] also demonstrated the *in vitro* activity of these antifungals, presenting MIC intervals of 0.5–2 μg/ml for ITC, 0.06–0.5 μg/ml for KTC and 1–4 μg/ml for AMB. In agreement with good *in vitro* activity depicted by low MICs presented here, successful treatment of feline sporotrichosis is achieved using amphotericin B,[27,31] itraconazole,[26,32] and ketoconazole.[26] However adverse effects may occur.

MIC breakpoints have not been well established yet for the *S. schenckii* complex, although the M38-A2 document[22] suggests that, for analytical purposes, an MIC of $\geq$4.0 μg/ml for ITC may be considered resistant for some filamentous fungi. According to this observation, Oliveira et al.[20] reported a greater proportion of itraconazole-resistant species in animal sporotrichosis than in human sporotrichosis. Borba-Santos et al.[18] observed that *S. brasiliensis* isolates recently recovered (from 2011–2012) during the Rio de Janeiro epidemic were less susceptible to AMB, ITC, PSC, VRC, and terbinafine than isolates recovered before 2004 (from 1998 to 2004). Rodrigues et al.[16] using haplotype networks, demonstrated that screening for antifungal susceptibility in a genetically diverse population could help to uncover itraconazole-resistant *S. brasiliensis* isolates and therefore improve our ability to adjust therapeutic regimens and reduce relapse. In the present study, none of the tested strains was classified as itraconazole-resistant, what may be related to temporal sampling and low genetic diversity of the evaluated population. It is noteworthy, that the present study reports for the first time the susceptibility of a great number of strains of *S. brasiliensis* to CAS, with an MIC geometric mean of 11.23 μg/ml. On the other hand, a large variation in MIC values was observed, suggesting that the susceptibility of *S. brasiliensis* to CAS is strain-dependent. Oliveira et al.[20] analyzed the activity of this antifungal agent against one isolate of *S. brasiliensis* from a cat with sporotrichosis and showed an MIC value of 16 μg/ml. Rodrigues et al.[16] also reported high MICs for CAS ($\geq$16 μg/ml) using isolates recovered from human patients with sporotrichosis.

As observed by other authors,[3,16–18,20] the azoles FLC and VRC showed low activity against *S. brasiliensis*, presenting high MIC values against all isolates included in this study. Marimon et al.[3] demonstrated MICs of 128 μg/ml for FLC against all tested strains and MIC intervals of 0.5–16 μg/ml for VRC, with a geometric mean of 3.88 μg/ml. Stopiglia et al.[17] showed an MIC range of 16–128 μg/ml for FLC and 1–16 μg/ml for VRC. Oliveira et al.[20] found MIC values of 64 and 8 μg/ml, for FLC and VRC, respectively.

In general, clinical isolates of strains of the *S. schenckii* complex show a broad *in vitro* resistance to fluconazole.[16,17] Stopiglia et al.[17] suggest that this observation indicates an intrinsic resistance to this drug. Fluconazole is only modestly effective for treatment of sporotrichosis and should be considered as a second-line therapy, with clinical efficacy estimated in 31% for osteoarticular or visceral sporotrichosis and in 71% for cases of lymphocutaneous infection.[33] The poor *in vitro* activity of VRC has also been demonstrated for other species of the *S. schenckii* complex.[16–18] According to Fernández-Silva et al.,[34] VRC shows just a modest activity in a murine model of disseminated sporotrichosis. The drug even showed some efficacy in prolonging survival and reducing the fungal load in organs of mice infected with *S. schenckii*, but in animals infected with *S. brasiliensis* this drug had no activity.

This study emphasizes the importance of epidemiological monitoring of strains of *Sporothrix* spp. from felines and contributes to the characterization of the antifungal susceptibility of *S. brasiliensis*, an important pathogen responsible for causing large outbreaks in humans and animals.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Barros MBDL, Paes RA, Schubach AO. *Sporothrix schenckii* and Sporotrichosis. *Clin Microbiol Rev* 2011; **24**: 633–654.
2. Zhou X, Rodrigues AM, Feng P et al. Global ITS diversity in the *Sporothrix schenckii* complex. *Fungal Divers* 2014; **66**: 153–165.
3. Marimon R, Serena C, Gené J et al. *In vitro* antifungal susceptibilities of five species of *Sporothrix*. *Antimicrob Agents Chemother* 2008; **52**: 732–734.
4. Marimon R, Cano J, Gené J et al. *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. *J Clin Microbiol* 2007; **45**: 3198–3206.
5. Kovarik CL, Neyra E, Bustamante B. Evaluation of cats as the source of endemic sporotrichosis in Peru. *Med Mycol* 2008; **46**: 53–56.
6. Montenegro H, Rodrigues A, Dias M et al. Feline sporotrichosis due to *Sporothrix brasiliensis*: an emerging animal infection in São Paulo, Brazil. *BMC Vet Res* 2014; **10**: 269.
7. Rees RK, Swartzberg JE. Feline-transmitted sporotrichosis: A case study from California. *Dermatol Online* 2011; **17**: 2.
8. Oliveira MME, Almeida-Paes R, Muniz MM et al. Phenotypic and molecular identification of *Sporothrix* isolates from an epidemic area of sporotrichosis in Brazil. *Mycopathologia* 2011; **172**: 257–267.
9. Rodrigues AM, de Melo Teixeira M, de Hoog GS et al. Phylogenetic analysis reveals a high prevalence of *Sporothrix brasiliensis* in feline sporotrichosis outbreaks. *PLoS Negl Trop Dis* 2013; **7**: e2281.
10. Cordeiro FN, Déa C, Paula R, Bruno CB. Familial occurrence of zoonotic sporotrichosis. *An Bras Dermatol* 2011; **86**: 2009–2012.
11. Silva MB, Costa MM, Torres CC et al. Brasil Urban sporotrichosis: a neglected epidemic in Rio de Janeiro, Brazil. *Cad Saude Publica* 2012; **28**: 1867–1880.
12. Pereira SA, Dib I, Gremião F et al. The epidemiological scenario of feline sporotrichosis in Rio de Janeiro, State of Rio de Janeiro, Brazil. *Rev Soc Bras Med Trop* 2014; **47**: 392–393.
13. Rodrigues MA, de Hoog GS, Pires DC et al. Genetic diversity and antifungal susceptibility profiles in causative agents of sporotrichosis. *BMC Infect Dis* 2014; **14**: 219.
14. Arrillaga-Moncrieff I, Capilla J, Mayayo E et al. Different virulence levels of the species of *Sporothrix* in a murine model. *Clin Microbiol Infect* 2009; **15**: 651–655.
15. Fernandes GF, Santos PO, Rodrigues AM et al. Characterization of virulence profile, protein secretion and immunogenicity of different *Sporothrix schenckii sensu stricto* isolates compared with *S. globosa* and *S. brasiliensis* species. *Virulence* 2013; **4**: 241–249.
16. Rodrigues AM, de Hoog GS, de Camargo ZP. Genotyping species of the *Sporothrix schenckii* complex by PCR-RFLP of calmodulin. *Diagn Microbiol Infect Dis* 2014; **78**: 383–387.
17. Stopiglia CDO, Magagnin CM, Castrillón MR et al. Antifungal susceptibilities and identification of species of the *Sporothrix schenckii* complex isolated in Brazil. *Med Mycol* 2014; **52**: 56–64.
18. Borba-Santos LP, Rodrigues MA, Gagini TB et al. Susceptibility of *Sporothrix brasiliensis* isolates to amphotericin B, azoles, and terbinafine. *Med Mycol* 2014; **53**: 178–188.
19. Meinerz ARM, Nascente PS, Schuch LFD et al. *In vitro* susceptibility of isolates of *Sporothrix schenckii* to terbinafine and itraconazole. *Rev Soc Bras Med Trop* 2007; **40**: 60–62.
20. Oliveira DC, Lopes PGM, Spader TB et al. Antifungal susceptibilities of *Sporothrix albicans*, *S. brasiliensis*, and *S. luriei* of the *S. schenckii* complex identified in Brazil. *J Clin Microbiol* 2011; **49**: 3047–3049.
21. Brilhante RSN, Silva NF, Marques FJF et al. *In vitro* inhibitory activity of terpenic derivatives against clinical and environmental strains of the *Sporothrix schenkii* Complex. *Med Mycol* 2014; **53**: 93–98.
22. CLSI 2008 *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*; Approved Standard — Second Edition.
23. Alvarado-Ramírez E, Torres-Rodríguez JM. *In vitro* susceptibility of *Sporothrix schenckii* to six antifungal agents determined using three different methods. *Antimicrob Agents Chemother* 2007; **51**: 2420–2423.
24. Cuenca-Estrella M, Rodriguez-Tudela JL. The current role of the reference procedures by CLSI and EUCAST in the detection of resistance to antifungal agents *in vitro*. *Expert Rev Anti Infect* 2010; **8**: 267–276.
25. Schubach TM, Schubach A, Okamoto T et al. Evaluation of an epidemic of sporotrichosis in cats: 347 cases (1998–2001). *J Am Vet Med Assoc* 2004; **224**: 1623–1629.
26. Pereira SA, Passos SRL, Silva JN et al. Response to azolic antifungal agents for treating feline sporotrichosis. *Vet Rec* 2010; **166**: 290–294.
27. Gremião I, Schubach T, Pereira S et al. Treatment of refractory feline sporotrichosis with a combination of intralesional amphotericin B and oral itraconazole. *Aust Vet J* 2011; **89**: 346–351.
28. Reis EG, Gremião ID, Kitada AA et al. Potassium iodide capsule in the treatment of feline sporotrichosis. *J Fel Med Surg* 2012; **14**: 399–404.
29. Gremião ID, Menezes RC, Schubach TM et al. Feline sporotrichosis: epidemiological and clinical aspects. *Med Mycol* 2015; **53**: 15–21.
30. Ishida K, de Castro RA, Borba Dos Santos LP et al. Amphotericin B, alone or followed by itraconazole therapy, is effective in the control of experimental disseminated sporotrichosis by *Sporothrix brasiliensis*. *Med Mycol* 2015; **53**: 34–41.
31. Gremião IDF, Schubach TMP, Pereira SA et al. Intralesional amphotericin B in a cat with refractory localised sporotrichosis. *J Feline Med Surg* 2009; **11**: 720–723.
32. Madrid IM, Mattei A, Martins A et al. Feline sporotrichosis in the southern region of Rio Grande do Sul, Brazil: clinical, zoonotic and therapeutic aspects. *Zoonoses Public Health* 2010; **57**: 151–154.
33. Kauffman CA, Pappas PG, McKinsey DS et al. Treatment of lymphocutaneous and visceral sporotrichosis with fluconazole. *Clin Infect Dis* 1996; **22**: 46–50.
34. Fernández-Silva F, Capilla J, Mayayo E et al. Modest efficacy of voriconazole against murine infections by *Sporothrix schenckii* and lack of efficacy against *Sporothrix brasiliensis*. *Mycoses* 2014; **57**: 121–124.