



# Canine sporotrichosis: polyphasic taxonomy and antifungal susceptibility profiles of *Sporothrix* species in an endemic area in Brazil

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## Abstract

Sporotrichosis, a mycosis caused by pathogenic species of the genus *Sporothrix*, affects diverse species of mammals. Until 2007, *Sporothrix schenckii* was considered the unique etiologic agent of sporotrichosis. Canine sporotrichosis is a poorly reported disease, and the majority of cases are from Rio de Janeiro, Brazil. There are scarce studies on the characterization of canine isolates of *Sporothrix schenckii* complex, as well as few antifungal susceptibility data available. The aim of this study was to characterize the clinical isolates of *Sporothrix* from dogs from Brazil at species level and evaluate their antifungal susceptibility profile. Polyphasic taxonomy was used to characterization at species level (morphological, phenotypical characteristics, and molecular identification). Antifungal susceptibility profiles (amphotericin B, itraconazole, ketoconazole, posaconazole, and terbinafine) were determined using the Clinical and Laboratory Standards Institute broth microdilution method (M38-A2). According to phenotypic identification and molecular analysis, 46 isolates included in this study were identified as *S. brasiliensis* and one as *S. schenckii*. Amphotericin B presented the highest minimum inhibitory concentration values, and the other drugs showed effective in vitro antifungal activity. This is the first report of *S. schenckii* in dogs from Brazil, since *S. brasiliensis* is the only species that has been described in canine isolates from Rio de Janeiro to date. Nevertheless, no differences were observed in the antifungal susceptibility profiles between the *S. brasiliensis* and *S. schenckii* isolates, and it is important to continuously study new canine clinical isolates from Rio de Janeiro, Brazil.

**Keywords** *Sporothrix* species · Sporotrichosis · Dogs · Antifungal susceptibility · Molecular characterization

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## Introduction

Sporotrichosis is a subcutaneous mycosis caused by pathogenic species of the genus *Sporothrix* that affects humans and animal species, mainly cats [1, 2]. Canine sporotrichosis is an uncommon disease, and current knowledge is derived from a few case reports and case series [3]. Some canine cases have been documented in Italy [4], the USA [5], and Brazil [6–8], which has the highest number of reports at Rio de Janeiro since the late 1990s [9–12].

In dogs, the infection can be acquired during hunting activities when *Sporothrix* spp. are introduced through thorn injuries or wood splinters [13]. Another type of transmission of this fungus to dogs is through close contact with sick cats, which is the most frequent form of infection of *Sporothrix* spp. in the metropolitan region of Rio de Janeiro [6]. More

importantly, despite the significant increase in case reports of zoonotic transmission involving sick cats in Brazil in the last two decades, especially in Rio de Janeiro [9–11, 14, 15], there is no evidence of dogs facilitating transmission of *Sporothrix* spp. to humans and other animals. Sporotrichosis in dogs is usually characterized by cutaneous lesions on the head, especially on the nasal region and thorax. Osteoarticular involvement and disseminated forms are rarely observed [16]. The most frequently observed lesions are nodules and ulcers [6]. In dogs, three clinical presentations have been reported: localized cutaneous or fixed cutaneous, cutaneous lymphatic, and disseminated forms [5, 13, 17]. In dogs, the lesions are neither painful nor pruritic, the fungus is difficult to find in the cytopathological examination of exudate from skin lesions, and generally, the animals are in good clinical condition [17].

Until 2007, *Sporothrix schenckii* was considered the unique etiologic agent of sporotrichosis in humans and animals [18]. Molecular studies performed since 2007 culminated in the description of eight pathogenic species within the genus *Sporothrix*. *Sporothrix schenckii* sensu stricto, *S. brasiliensis*, *S. globosa*, and *S. luriei* belong to the *Sporothrix schenckii* complex, while *S. mexicana*, *S. pallida*, *S. humicola*, and *S. chilensis* are members of *Sporothrix pallida* complex [19–24]. Currently, *S. schenckii*, *S. brasiliensis*, and *S. globosa* are considered to be the main species of clinical interest in humans [25–27], and *S. brasiliensis* and *S. schenckii* have been reported in cats and dogs [8, 28–32].

*Sporothrix brasiliensis* has been described as an emerging species that is highly pathogenic among humans and cats with a predominant geographic distribution in Brazil, although it was recently described in Argentina as well [19, 22, 28, 32–39]. Few studies have characterized canine isolates of the genus *Sporothrix*. In Brazil, 18 isolates from the southern region, 2 isolates from Rio de Janeiro, and 1 canine isolate from São Paulo were previously characterized as *S. brasiliensis* [8, 28, 40]. *Sporothrix schenckii* is considered the second most pathogenic species of the genus *Sporothrix* in murine experimental models of infection [33]; however, this species has not yet been reported in dogs [19, 41]. *Sporothrix luriei* has rarely appeared in cases of human and animal sporotrichosis, with only two human cases, including one case in Africa [20] and another case in India [42]; interestingly, this species has been characterized in a Brazilian canine case [34].

The most common drugs used in the treatment of canine sporotrichosis are itraconazole, ketoconazole, and potassium iodide [6, 43]. Due to the emergence of resistant species and the occurrence of therapeutic failures, *in vitro* antifungal susceptibility tests were developed. The determination of the minimal inhibitory concentration (MIC) is important for the treatment orientation and the evaluation of new antifungal agents [44]. There are studies of the antifungal susceptibility of *Sporothrix* isolates derived from human cases [45–48].

However, few studies on the antifungal susceptibility profiles of canine *Sporothrix* sp. isolates have been performed [8–34].

Only one report described the characterization of two isolates of *Sporothrix* from canine origin in Rio de Janeiro, Brazil [8], which is the region that has the highest number of animal sporotrichosis cases described in the world [12]. The aims of this study were to characterize at the species level the isolates of *Sporothrix schenckii* complex, obtained from canine cases of sporotrichosis in Rio de Janeiro, Brazil, to evaluate their antifungal susceptibility profile and to correlate them with the clinical and epidemiological characteristics.

## Methodology

**Isolates** Forty-seven isolates of *Sporothrix* spp. from dogs assisted at the Laboratory of Clinical Research on Dermatозoonoses in Domestic Animals—Evandro Chagas National Institute of Infectious Diseases (INI)/Fiocruz were included in this study. *Sporothrix* spp. were isolated from skin and mucosal lesions of dogs before they had received an antifungal treatment. The isolates were subcultured in potato dextrose agar (PDA) and then stored in 10% skimmed milk (Fluka Analytical, Sigma-Aldrich, Switzerland) at  $-20\text{ }^{\circ}\text{C}$  at the Mycology Laboratory—INI/Fiocruz. Clinical and epidemiological data were collected from the dog's medical records.

**Morphological and physiological studies** The polyphasic taxonomy was used to identify the fungal isolates at the species level, which included morphological, physiological, and molecular studies, with the filamentous form of the fungus used for all identification stages. For the phenotypic tests, the isolates were subcultured in PDA (Difco™; Becton, Dickinson and Company, Sparks, MD, USA) and visually examined for dihydroxynaphthalene melanin (DHN melanin) production [49]. To study conidiogenesis, these isolates were subcultured on corn meal agar (BBL™; BD, Franklin Lakes, NJ, USA) and incubated at  $30\text{ }^{\circ}\text{C}$  in a dark environment. After 10 days, the microscopic characteristics were evaluated [19].

The growth rate of colonies after 21 days of incubation at  $30\text{ }^{\circ}\text{C}$  and  $37\text{ }^{\circ}\text{C}$  in PDA medium was evaluated in triplicate at different times [19, 27]. Carbohydrate assimilation tests were performed in 96-well racks containing yeast nitrogen base (YNB) culture medium (Difco™; Becton, Dickinson and Company, Sparks, MD, USA) supplemented with sucrose or raffinose at a 0.5% concentration [19, 22, 32]. Cultures on YNB supplemented with glucose were used as positive controls for growth, and cultures on YNB without carbohydrates were used as negative controls. The results of morphological and physiological tests were interpreted based on a previously proposed taxonomic key [19, 20].

For the classification of the isolates regarding thermotolerance, the percent inhibition of growth was calculated as

previously reported [50]. Isolates whose growth at 37 °C was reduced by 50% or more, and were classified as having low thermotolerance, and the isolates whose growth at 37 °C was reduced by less than 50% were classified as having high thermotolerance.

**DNA extraction and analysis** For the identification through genotypic testing, the genomic DNA was extracted from the filamentous form using chloroform/isoamyl alcohol (24:1) [27]. For PCR, fingerprinting was used the universal T3B primer (5'-AGGTCGCGGGTTTCGAATCC-3') to distinguish between the species of the genus *Sporothrix* [32, 51]. Strains of species of the genus *Sporothrix* associated with cases found in humans and animals, *S. brasiliensis* (IPEC16490), *S. globosa* (IPEC27135), *S. mexicana* (MUM11.02), and *S. schenckii* (IPEC27722), were used as controls for molecular identification. Succinctly, we used the PCR reaction mix 10× buffer with KCl, dNTP mix 0.2 mM, MgCl<sub>2</sub> 50 mM, and platinum *Taq* DNA polymerase 1.0 U and 10 mM T3B primer [32, 51]. The T3B fingerprinting profiles obtained were analyzed with Bionumerics (version 5.1; Applied Maths BVBA, Sint-Martens-Latem, Belgium). Similarity coefficients were calculated using the Dice algorithm, and cluster analysis was performed by means of the unweighted paired group method using arithmetic averages (UPGMA).

**Antifungal susceptibility testing** The antifungal susceptibility tests were performed in 11 isolates selected after polyphasic taxonomy. The inocula were prepared in sterile saline solution after incubation of each strain in PDA for 7 days at 37 °C. The antifungal susceptibility tests were conducted according to the M38-A2 protocol of the Clinical and Laboratory Standards Institute [52], using RPMI 1640 medium buffered to pH 7.0 with 0.165 mol/L morpholinepropanesulfonic acid (Sigma-Aldrich). The different working concentrations of antifungal drugs were distributed into wells of round-bottom 96-well microplates. Wells containing RPMI-1640 medium with DMSO and the fungal inoculum and without any antifungal agent were used as growth controls or, when only medium with DMSO was added, as sterility controls. Quality controls with the reference strains *Aspergillus fumigatus* ATCC 204305 and *A. flavus* ATCC 204304 were included. Tests were validated only if MIC values for these strains were within the range described in the M38-A2 reference document. MICs were determined by visual inspection after 48–72 h of incubation at 35 °C for better visualization of the fungal growth, as described [53, 54]. Amphotericin B (AMB), terbinafine (TRB), posaconazole (POS), ketoconazole (KTZ), and itraconazole (ITZ) (Sigma Chemical Corporation, St. Louis, MO, USA) were tested. All the tests were performed in triplicate for each isolate. According to the recent proposal of epidemiological cutoff values (ECV) for *Sporothrix* spp. [55], it is possible to classify the isolates of

*Sporothrix* spp. as wild type (WT) and non-wild type (non-WT). Isolates with MIC values equal or less than the ECVs are defined as wild-type (WT) isolates, and isolates with MICs higher than the ECVs have elevated odds to present resistance mechanisms to the tested drug [53, 55]. According to the study, the MIC values proposed as ECV are 4 µg/ml for AMB, 2 µg/ml for ITZ, 2 µg/ml for POS, 2 µg/ml for KTZ, and 0.12 µg/ml for TRB [55]. It is possible to classify the isolates of *S. brasiliensis* and *S. schenckii* as wild type (WT) and non-wild type (non-WT); however, the study data were insufficient for the calculation of ECV of the *S. schenckii* isolates for KTZ and TRB.

**Statistics** An exploratory analysis of the data was performed by applying the simple frequencies for each isolate to the species level of the genus *Sporothrix* as well as the clinical and epidemiological variables for the dogs, which were described in the evaluated individual medical records. For determining the association between related samples, the Wilcoxon test was used. *p* values < 0.05 indicated significant associations in the statistical tests. The obtained data were stored and analyzed in a database using the Statistical Package for Social Science (SPSS) software version 16.0.

## Results

The clinical and epidemiological characteristics of the 47 dogs included in this study are reported in Table 1. Nineteen dogs presented only cutaneous lesions, 9 presented only mucosal lesions, and 19 presented mucosal and cutaneous lesions (Fig. 1).

The results of phenotypic tests are reported in Online Resource 1. The median diameter of the colonies incubated at 30 °C after 21 days was 30 mm (range 13–54 mm), whereas the median for colonies at 37 °C was 10.5 mm (range 5–20 mm). It was possible to observe that the growth of colonies at 30 °C was better than at 37 °C (*p* < 0.05). In the carbohydrate assimilation tests, glucose was assimilated by all isolates, including the controls, after 10 days. All isolates from this study showed growth at 37 °C, and they were then classified as isolates with low and high thermotolerances, but only six isolates were classified as having high thermotolerance. With the results of the morphological and physiological tests, it was possible to compare the results of each isolate to a previously proposed taxonomic key [19]. Two isolates presented compatible phenotypic characteristics with *S. schenckii* and one with *S. brasiliensis*. However, the remaining 44 isolates did not presented compatible phenotypic characteristics that allowed to define to which species they belonged and were classified as *Sporothrix* spp. These isolates showed conclusive results at the species level using T3B PCR fingerprinting, 46 isolates

**Table 1** Clinical and epidemiological aspects of 47 dogs with sporotrichosis assisted at INI/Fiocruz, Rio de Janeiro, Brazil, as well as phenotypic and molecular characterization of their isolates

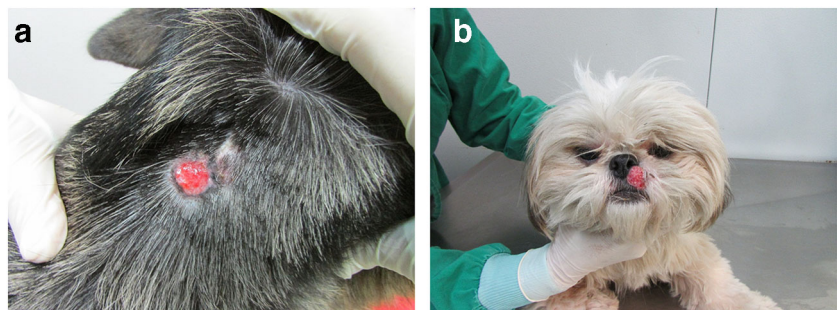
Clinical and epidemiological variables	Number (%)
Species determined by phenotypic tests	<i>Sporothrix</i> spp.—44 (93.62%) <i>S. schenckii</i> —2 (4.26%) <i>S. brasiliensis</i> - 1 (2.12%)
Species determined by molecular tests	<i>S. brasiliensis</i> —46 (97.88%) <i>S. schenckii</i> —1 (2.12%)
Gender	Male—30 (63.82%) Female—17 (36.18%)
Route of transmission	Unknown—19 (40.42%) Scratch/bite of cats—18 (38.30%) Contact with cats—9 (19.15%) Contact with soil/plants—1 (2.13%)
Clinical form	Fixed cutaneous—26 (55.32%) Cutaneous disseminated—11 (23.40%) Mucosal—9 (19.15%) Lymphocutaneous—1 (2.13%)
Respiratory signs	Yes—36 (76.60%) No—11 (23.40%)
Skin lesions	Yes—38 (80.85%) No—9 (19.15%)
Mucosal lesions	Yes—28 (59.57%) No—19 (40.43%)
Outcome	Clinical cure—22 (46.81%) Treatment abandonment—13 (27.65%) Therapeutic failure—7 (14.90%) Death by another cause—3 (6.39%) Death by sporotrichosis—2 (4.25%)

were characterized as *S. brasiliensis*, and one isolate (IPEC24C) was characterized as *S. schenckii* (Fig. 2). In addition, eight isolates demonstrated a small intraspecific variability when compared with the *S. brasiliensis* type strain.

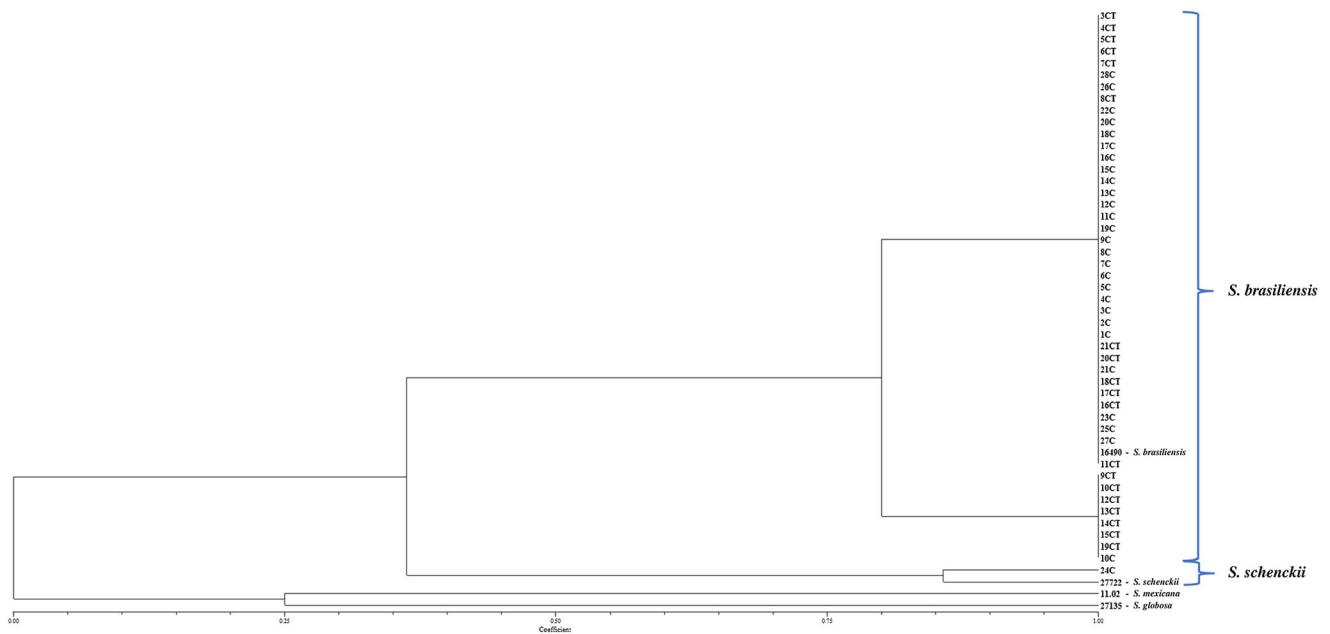
Antifungal susceptibility tests were performed in the isolate characterized as *S. schenckii* and in isolates characterized as *S. brasiliensis*. The antifungal susceptibility profiles of the *S. brasiliensis* and *S. schenckii* isolates are reported in Table 2. AMB presented the highest MIC values for the canine isolates, ranging from 1.0 to 8.0 µg/ml. KTZ, ITZ, POS,

and TRB showed good antifungal activity in the test (range 0.06–0.50 µg/ml, 0.12–0.50 µg/ml, 0.25–1.0 µg/ml, and 0.03–0.50 µg/ml, respectively) with no differences between species. For the antifungals that were tested, canine isolates of *S. brasiliensis* were all classified as WT for ITZ and POS, isolate IPEC13C was classified as non-WT for AMB, and IPEC22C was classified as non-WT for TRB. The ECV of the *S. schenckii* isolate (IPEC24C) was not evaluated for KTZ and TRB, but it was classified as WT for ITZ and POS as well as non-WT for AMB.

**Fig. 1** **a** Dog with ulcerated cutaneous lesion on the neck caused by *S. schenckii* (IPEC24C). **b** Dog with ulcerated mucocutaneous lesion on the nasal region caused by *S. brasiliensis* (IPEC17C)







**Fig. 2** Phylogenetic tree showing species identification and the degree of similarity between T3B fingerprinting profiles among the *Sporothrix* isolates obtained of the 47 dogs at the Laboratory of Clinical Research

on Dermatozoonoses in Domestic Animals (INI)/Fiocruz, Rio de Janeiro, Brazil, 2013 to 2018 using the UPGMA cluster method

**Discussion**

After the description of new species from the genus *Sporothrix*, the identification of clinical isolates has been performed worldwide, especially in regions where a large number of sporotrichosis cases occurs [20, 22, 24, 28, 32, 37, 38, 56, 57].

Sporotrichosis in dogs is a poorly described disease compared to humans and cats; therefore, this study has an impact on the knowledge of different aspects of the disease in dogs,

including the species of *Sporothrix* sp. that affect the canine population and their antifungal susceptibility profile. Few studies carried out in the southern and southeastern regions of Brazil have characterized canine isolates for which the predominant species was *S. brasiliensis* [8, 28, 40]. *Sporothrix luriei* has also been reported once in a study carried out in Rio Grande do Sul [34]. In our study, after molecular characterization using T3B PCR fingerprinting, the species *S. brasiliensis* was found in most of the isolates, which

**Table 2** Antifungal treatment in 11 dogs with sporotrichosis assisted at INI/Fiocruz, Rio de Janeiro, Brazil, and the corresponding MIC values according to the antifungal susceptibility tests from their isolates

Isolate code	Species determined by molecular tests	Initial therapeutic scheme	Change of therapeutic scheme	Treatment time to clinical cure (weeks)	MIC (µg/ml)				
					AMB	KTZ	ITZ	TRB	POS
24C	<i>S. schenckii</i>	KTZ 200 mg/dog q24h	ITZ 250 mg/dog q24h	24	8.00	0.50	0.50	0.06	0.50
10C	<i>S. brasiliensis</i>	ITZ 100 mg/dog q24h	–	32	4.00	0.25	0.50	0.06	0.50
12C	<i>S. brasiliensis</i>	ITZ 200 mg/dog q24h	–	–	2.00	0.50	0.50	0.03	0.50
13C	<i>S. brasiliensis</i>	ITZ 200 mg/dog q24h	ITZ 300 mg/dog q24h	44	8.00	0.50	0.50	0.12	1.00
14C	<i>S. brasiliensis</i>	ITZ 300 mg/dog q24h	–	32	2.00	0.06	0.25	0.06	0.25
16C	<i>S. brasiliensis</i>	ITZ 100 mg/dog q24h	–	56	2.00	0.25	0.25	0.06	1.00
17C	<i>S. brasiliensis</i>	–	–	–	2.00	0.50	0.25	0.12	0.50
18C	<i>S. brasiliensis</i>	ITZ 200 mg/dog q24h	–	20	1.00	0.25	0.25	0.06	0.50
21C	<i>S. brasiliensis</i>	KTZ 100 mg/dog q24h	ITZ 150 mg/dog q24h	–	2.00	0.50	0.25	0.06	0.50
22C	<i>S. brasiliensis</i>	ITZ 200 mg/dog q24h	ITZ 300 mg/dog q24h	–	2.00	0.12	0.12	0.50	0.25
23C	<i>S. brasiliensis</i>	ITZ 150 mg/dog q24h	–	76	2.00	0.50	0.50	0.06	0.50

MIC minimum inhibitory concentration, AMB amphotericin B, KTZ ketoconazole, ITZ itraconazole, POS posaconazole, TRB terbinafine, – not applicable

corroborates the findings of previous studies in animals from Brazil [8, 28, 32, 34, 37, 38, 58]. We documented here one isolate identified as *S. schenckii*. This study is the first report of this species affecting dogs in Brazil and the first report of another species besides *S. brasiliensis* affecting domestic animals from the metropolitan region of Rio de Janeiro, which is the major area of occurrence of animal sporotrichosis in the world. This finding highlights the possibility that *S. schenckii* may also circulate among cats in the same region as a causal agent since these animals are the main source of infection for dogs in this scenario [6]. To date, studies with isolates obtained from cats in Rio de Janeiro have only described *S. brasiliensis* [28, 32, 37, 38]. Other species, in addition to *S. brasiliensis*, have been identified in isolates from Rio de Janeiro that were obtained from human patients with sporotrichosis, such as *S. schenckii* and *S. globosa* [27, 56], which reinforces the hypothesis that other species may also circulate among animals.

The majority of dogs affected by sporotrichosis in this study acquired the infection through contact with cats that had sporotrichosis since they have an important role in the transmission of the fungus due the high fungal burden in their lesions [59]. Specifically, it was possible that the dog with sporotrichosis caused by *S. schenckii* (IPEC24C) was infected through contact with a stray cat that had sporotrichosis and frequented the backyard of the dog/owner's home. This dog and its owner acquired the disease, but it was not possible to characterize the isolate from the owner. Also, it was not possible to isolate the fungus from the feline host. However, we cannot reject the possibility of *S. schenckii* transmission through the classical route. A previous study reported that a common route of infection for *S. schenckii* could occur in an environmental manner or in transmission through infected cats [25].

It was not possible to determine a taxonomic classification at the species level only with phenotypic characterization to classify the *Sporothrix* spp. in the majority of isolates tested. In most isolates, only glucose and sucrose were assimilated in the carbohydrate assimilation test, which suggested the species *S. pallida* or *S. globosa*. In fact, the isolates could not be classified as *S. pallida* because of the presence of dematiaceous conidia or as *S. globosa* because the isolates were thermotolerant at 37 °C [19], which did not allow the identification of the species by this method. One isolate (IPEC22C) had a hyaline colony without pigment production in PDA, but we observed the presence of dematiaceous and hyaline conidia in micromorphology that corroborates with the results of previous studies in humans and cats [22, 32]. However, most of the canine isolates had similar phenotypic profile regardless of the species identified by molecular technique, which differed from the results in previous studies of human and feline isolates [22, 32] which suggested a variability phenotypic in our isolates.

Unlike studies with feline and human isolates, it was not possible the identification at the species level only using a taxonomic key. Due to this diversity of results in the phenotypic tests, we suggest that mycologists should be cautious when identifying species of the genus *Sporothrix* in canine isolates through only morphological and physiological tests.

Despite the inconclusive results of the phenotypic tests in the most of studied isolates, the molecular technique has successfully identified all the isolates, and there was a disagreement between the results of phenotypic and molecular techniques, since only one isolate was correctly identified in both techniques as *S. brasiliensis*. The other two isolates characterized as *S. schenckii* in phenotypic tests were identified as *S. brasiliensis* in molecular identification. Other studies that performed the characterization of canine isolates using phenotypic and molecular techniques did not show such disagreement, which does not corroborate with our findings [28, 34]. The isolates used in other studies was from other regions of Brazil; in addition, the number used was lower compared with our study, which could justify the disagreement. However, our results were similar to others reported previously in studies with human isolates [22], as well as characterization of feline isolates [32, 60, 61].

There was a small intraspecific variability among our isolates classified as *S. brasiliensis* as observed in human and feline cases caused by *S. brasiliensis* described in a previous study [32, 51], and by correlating these findings with the clinical and epidemiological data of dogs, it was possible to observe that five of these animals presented therapeutic failure as an outcome. A larger number of phenotypic and genotypic studies in clinical isolates of *Sporothrix* sp. from dogs and cats are necessary, in areas where animal sporotrichosis occurs, for a more accurate evaluation of detected intraspecific variabilities and the possibility of association with the clinical and epidemiological characteristics of patients.

Few studies that evaluated antifungal susceptibility profile of canine isolates have been performed when compared with studies on human and feline isolates [8, 22, 45, 46, 48, 62, 63]. The identification of a new species in dogs may have implications in disease epidemiology, which emphasizes the importance of the study of the antifungal susceptibility profile of clinical isolates of these animals. From in vitro studies with clinical isolates, it is possible to choose a therapeutic alternative that is more effective and carries out detection of resistant strains [44].

In our study, the MIC values for AMB ranged from 1.0 to 8.0 µg/ml, which are values that have been previously observed in other studies with feline and canine isolates [8, 30, 48, 53]. Regarding AMB, only two isolates were classified as non-WT, according to classification proposed by Espinel-Ingroff and coworkers [55]; however, this drug was not used in any of the dogs in this study.

Of the 11 dogs that had the antifungal susceptibility from their isolates evaluated included the dog with sporotrichosis caused by *S. schenckii* and the other 10 dogs with sporotrichosis caused by *S. brasiliensis*, which were selected randomly. Of these dogs, six achieved the clinical cure outcome after ITZ treatment, including the dog with sporotrichosis caused by *S. schenckii* (IPEC24C). In this case series evaluated, the results of in vitro susceptibility tests for ITZ coincided, in most cases (6/11), with the favorable outcome of treatment with this drug. The MIC values for ITZ did not exceed 0.5 µg/ml, which indicates that these isolates were WT strains, and thus, there was a high probability of them being susceptible to ITZ. However, two dogs (IPEC21C and IPEC24C) started treatment with KTZ and needed to change to ITZ due to insufficient clinical response, despite the low MIC values for KTZ observed ( $\leq 1.0$ ) and classification of isolates as WT strains. One dog obtained the clinical cure (IPEC24C), and in the other case, there was loss to follow-up. More importantly, other factors related to patients should be considered, such as their immune response or the use of adjuvants in the treatment might interfere with correlating in vitro and in vivo findings [55].

The MIC values found in our study corroborate the results previous described using POS [8, 45, 64]. The use of this drug in vivo was described in a murine model of sporotrichosis caused by *S. brasiliensis*, which showed a reduction of the fungal load after treatment [65]; however, it has a high commercial cost, which could limit the use of this drug as a therapeutic alternative.

Since the first studies of antifungal susceptibility, TRB has been a drug that constantly presents low MIC values against different species of *Sporothrix* [45, 48, 53, 62]. Recently, the clinical effectiveness of TRB was observed in two canine cases [8]. The results of antifungal susceptibility tests for TRB in our study remain promising, although we found a non-WT isolate (IPEC22C), with a MIC value of 0.50 µg/ml. Nevertheless, therapeutic studies with a larger number of animals are needed to define the role of this drug in the treatment of canine sporotrichosis.

Our study suggests that *S. brasiliensis* is the main etiologic agent associated with canine sporotrichosis in the metropolitan region of Rio de Janeiro. However, the genotypic characterization of *S. schenckii* from a dog that was infected through contact with a sick cat confirms the circulation of this pathogenic species in an animal population for the first time in the endemic region of Rio de Janeiro after the taxonomic changes made to the genus *Sporothrix*. Therefore, we believe that other species can also circulate among cats in this region, which play an important role in zoonotic transmission of sporotrichosis, which play an important role in zoonotic transmission of sporotrichosis. Characterization studies of canine and feline isolates of *Sporothrix* sp. (in endemic regions or where new cases are occurring) are strongly suggested to determine the circulating

species among the animal population, which may present different modes of transmission, pathogenicity, and susceptibility to antifungal agents. This is the first report of *S. schenckii* in dog from Brazil and native of Rio de Janeiro. Nevertheless, no differences were observed in the clinical presentation of patients and antifungal susceptibility profiles between the *S. brasiliensis* and *S. schenckii* isolates.

The high prevalence of *S. brasiliensis* in the canine population and the identification of *S. schenckii* in the main endemic area of animal sporotrichosis in the world reaffirm the importance of studies on the identification of species and the antifungal susceptibility profile of clinical isolates from animals and their associations with clinical, epidemiological, and therapeutic aspects.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest.

**Ethical approval** The study was approved by the Ethics Committee on Animal Use of the Oswaldo Cruz Foundation (CEUA/Fiocruz; number: LW-17/17).

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