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Genotyping and antifungal susceptibility testing of *Sporothrix brasiliensis* isolates from Southern Brazil

Amanda Bombassaro[1,2,3] | Bram Spruijtenburg[1,2] | Fernanda Medeiros[4] | Bruna Jacomel Favoreto de Souza Lima[1,3] | Luana Beatriz Ballardin[5] | Marconi Rodrigues de Farias[5] | Vania Aparecida Vicente[2,6] | Flavio de Queiroz-Telles[3,7] | Jacques F. Meis[1,2,6,8] | Theun de Groot[1,2]

[1]Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands

[2]Center of Expertise in Mycology Radboud University Medical Center/ Canisius-Wilhelmina Hospital and Excellence Center for Medical Mycology, Nijmegen, The Netherlands

[3]Department of Basic Pathology, Graduate Program in Microbiology, Parasitology and Pathology, Biological Sciences, Federal University of Paraná, Curitiba, Brazil

[4]Graduation in Biological Sciences, Federal University of Paraná, Curitiba, Brazil

[5]Graduate Program in Animal Science, Catholic University of Paraná, Curitiba, Brazil

[6]Department of Bioprocess Engineering and Biotechnology, Engineering Bioprocess and Biotechnology Graduate Program, Federal University of Paraná, Curitiba, Brazil

[7]Department of Public Health, Clinical Hospital, Federal University of Paraná, Curitiba, Brazil

[8]Department I of Internal Medicine, University of Cologne, Faculty of Medicine and University Hospital Cologne, Excellence Center for Medical Mycology, Cologne, Germany

Correspondence
Theun de Groot, Department of Medical Microbiology and Infectious Diseases (C70), Canisius Wilhelmina Hospital (CWZ), Postbus 9015, 6532SZ Nijmegen, The Netherlands,
Email: theundegroot7@gmail.com

Abstract

Sporotrichosis is an implantation mycosis caused by the dimorphic fungus *Sporothrix* and mostly involves cutaneous and subcutaneous tissues and the lymphatic vessels. Among more than 50 different species, only *Sporothrix schenckii*, *Sporothrix globosa* and *Sporothrix brasiliensis* are frequently reported to cause infections in humans. *Sporothrix brasiliensis* is remarkably virulent and has been spreading rapidly in Brazil and other Latin American countries. In this study, we aimed to determine the genetic relatedness and antifungal susceptibility of *Sporothrix* strains by analysing 89 isolates from humans and cats in Curitiba, Southern Brazil. Calmodulin sequencing identified 81 *S. brasiliensis* and seven *S. schenckii* isolates. Amplified fragment length polymorphism genotyping analysis showed feline and human isolates clustering together. In vitro susceptibility testing with seven antifungals demonstrated a broad activity against all tested *S. brasiliensis* isolates, with no significant differences in minimal inhibitory concentration (MIC) values between feline and human isolates. Resistance was solely observed in one human isolate against itraconazole and posaconazole, with MICs of ≥16 μg/mL against both antifungals. Whole genome sequencing (WGS) analysis on this isolate and two related susceptible isolates did not reveal any unique substitutions in resistance-associated genes, including *cyp51*, *hmg* and *erg6*, when compared to two related susceptible isolates. The novel antifungal olorofim exhibited excellent activity against this large isolate collection, with all isolates considered as susceptible. Altogether, we indicate zoonotic transmission based on genotyping and revealed a broad activity of seven common antifungals, including olorofim, against a large *S. brasiliensis* isolate collection.

KEYWORDS
antifungal resistance, genotyping, resistance mechanism, *Sporothrix brasiliensis*, sporotrichosis, whole genome sequencing

1 | INTRODUCTION

Sporotrichosis is the most prevalent and globally distributed among the implantation mycoses. This disease is caused by dimorphic fungi from the genus *Sporothrix*, which affects humans and other mammals and commonly involves cutaneous and lymphatic infections.[1] Transmission mostly takes place sapronotically (plant fragments and soil) or zoonotically (several species of domestic and wild animals), according to causal agent.[2] Although sporotrichosis occurs worldwide, most cases are reported in tropical and subtropical zones.[3] Of the 53 validated species in the *Sporothrix* genus, only few are commonly reported to cause infections in humans: *S. schenckii*, *S. globosa* and *S. brasiliensis*.[4,5] Besides lymphocutaneous and skin lesions, these *Sporothrix* species also cause ocular, osteoarticular, pulmonary and neurologic diseases.[6] The systemic or disseminated clinical form is usually found in immunocompromised hosts. Each species presents a different virulence profile: *S. schenckii* mostly causes a benign subacute to chronic mycosis, *S. globosa* mainly causes fixed cutaneous lesions, while *S. brasiliensis* is more virulent with a higher degree of dissemination.[7,8]

In the last two decades after the first epidemic of cat-transmitted sporotrichosis in 1998 in Rio de Janeiro, *S. brasiliensis* became a major public health concern in Brazil with strong increases in the incidence of sporotrichosis in cats, humans and dogs with its unique ability among *Sporothrix* species to be transmitted directly in the yeast form.[6,9] While it was assumed that the observation of *S. brasiliensis* in other regions of the country and even in Argentina and Paraguay was due to clonal spread from Rio de Janeiro, a recent whole genome sequencing (WGS) analysis identified two *S. brasiliensis* isolates from Brasília that were genetically highly distinct, suggesting independent emergence of *S. brasiliensis* in Brazilian regions.[7,10–13] In the last decade, infections in dogs and rats, animals with a close relationship with felines, were also reported, suggesting that *S. brasiliensis* presents an epidemic potential with zoonotic (cat-to-human) and enzootic transmission (cat-to-cat/dog).[9,14]

Despite the differences in virulence, the treatment of human sporotrichosis is the same for all *Sporothrix* species. As such, molecular identification is not necessary to initiate therapy. According to the Infectious Diseases Society of America, itraconazole is first-line therapy for cutaneous, lymphocutaneous and osteoarticular cases, with terbinafine as an alternative option for cutaneous and lymphocutaneous forms.[15] In severe invasive pulmonary or disseminated sporotrichosis, amphotericin B is recommended.[16] Furthermore, cryosurgery and local heat therapy may be applied to reduce the duration of antifungal therapy.[17]

In general *S. schenckii*, *S. globosa* and *S. brasiliensis* isolates exhibit relatively high minimum inhibitory concentrations (MICs) to different antifungal compounds, indicating inherent antifungal resistance.[18] While Clinical and Laboratory Standards Institute (CLSI) breakpoints are not available, epidemiological cut-off values (ECVs) for *S. schenckii*, *S. globosa* and *S. brasiliensis* of different antifungal agents were determined in 2017.[19] The ECVs for these *Sporothrix* species are based on an algorithm involving the MIC distribution of most isolates and allowed the identification of wild-type (WT) and non-WT isolates, with the presumption that the latter have acquired additional resistance mechanisms. Some studies relate the intrinsic resistance to aneuploidy present in this genus, consisting of an abnormal number of chromosomes resulting in low genetic diversity, but with additional copies of resistance genes.[20–22] Other authors suggested a role for melanin, which can protect fungi against antifungal drugs and immune responses.[23,24] Moreover, mutations in specific genes are involved in antifungal resistance, as occurring in the cytochrome P450 family, the target of azoles, or in transporter families or membrane compounds.[25,26]

Therefore, in this study, *S. brasiliensis* strains obtained from human and cat hosts in Curitiba, Brazil, were analysed to better understand their phylogenetic relatedness. In addition, in vitro antifungal susceptibility profiles were evaluated and potential resistance mechanisms were investigated by WGS data.

2 | MATERIALS AND METHODS

2.1 | Isolates and culture conditions

A total of 89 isolates were analysed in this study, 40 isolates from human patients at Clinical Hospital-HC/UFPR and 49 isolates from feline patients at Veterinary Clinic School/PUC, both located in Curitiba, Paraná state, Brazil. Isolates were cultured for 14 days from monosporic colonies onto Sabouraud Dextrose Agar (SDA; Oxoid) at 35°C and stored at −20°C in skim milk. Isolates were deposited at the Microbiological Collections of Paraná Network (CMRP) at the Federal University of Paraná, Brazil (https://www.cmrp-taxonline.com). This study was approved by the HC-UFPR Research Ethics Committee under registration CAAE 12379819.4.0000.0096.

2.2 | *Sporothrix* reference strains

Different strains were used as a reference. The *S. brasiliensis* 5110 (American Type Culture Collection MYA-4823) and *S. schenckii* CBS 130112 were used as controls for molecular identification. For antifungal susceptibility testing, *S. schenckii* CBS 130112 and *S. schenckii* CBS 130113 were used as controls. For amplified fragment length polymorphism (AFLP) fingerprinting, *S. schenckii* CBS 130099, *S. schenckii* CBS 130113, *S. schenckii* CBS 130114, *S. globosa* CBS 130116, *S. globosa* CBS 130117 and *S. mexicana* CBS 132926 were included.

2.3 | DNA extraction and identification

DNA extraction was performed using the MagNA Pure 96 DNA, following the Pathogen 200 SV protocol (Roche Diagnostics, Roche Diagnostics GmbH) as previously described.[27] Briefly, a small portion of each culture was placed in a microtube with lysis solution

(400 μL MagNA Pure Bacteria Lysis Buffer) and ceramic beads (MagNA Lyser Green Beads) after which the cells were lysed by a MagNA Lyser instrument (Roche Diagnostics) for 30 s at 6500 rpm and DNA was extracted and purified with the MagNA Pure 96. A polymerase chain reaction (PCR) amplifying introns two to four and exons two to five of the calmodulin gene was used for species identification using primers Cmd5 5'-CCGAGTACAAGGARGCCTTC-3' and Cmd6 5'-CCGATRGAGGTCATRACGTGG −3' as previously described.[28] Amplicons were purified according to the AmpliClean method (NimaGen), and sequencing PCR was performed using 0.5 μL BrilliantDye premix, 1.75 μL BrilliantDye 5x sequencing buffer (NimaGen), 1 μL Cmd6 primer (5.0 μM), 5.75 μL water and 1 μL purified DNA. Afterwards, products were purified using the D-Pure purification protocol (NimaGen) and sequenced on a 3500 XL genetic analyser (Applied Biosystems). Resulting calmodulin sequences were compared with NCBI GenBank sequences using the BLAST programme (http://www.ncbi.nlm.nih.gov/) to determine their identity. Generated calmodulin sequences were deposited under Genbank accession numbers OQ571231-OQ571318.

2.4 | Genotyping by AFLP fingerprinting

Sporothrix isolates were subjected to amplified fragment length polymorphism (AFLP) genotyping using a method described previously.[29] In short, extracted DNA was submitted to a combined restriction–ligation using 2 U of EcoRI (New England Biolabs), 2 U of MseI (New England Biolabs), 50 pmol of EcoRI adapter (5'-TCGTAGACTGC GTACC-3' and 5'-AATTGGTACGCAGTC-3'), 50 pmol of MseI adapter (5'- GACGATGAGTCCTGAC-3' and 5'-TAGTCAGGACTCAT-3') and 1 U of T4 DNA ligase (Promega). The restriction–ligation products were used in an amplification reaction with EcoRI (5'- FLU-GACTG CGTACCAATTCAC-3') and MseI (5'- GATGAGTCCTGACTAAA-3')-based primers, and amplicons were 50x diluted using water. A mix containing 1 μL of the diluted amplicons, 8.9 μL water and 0.12 μL LIZ600 (Applied Biosystems) was submitted to a heating step at 95°C for 1 min followed by 4°C for 5 min and run onto ABI 3500XL genetic analyser (Applied Biosystems) according to the manufacturer's instructions. Data were analysed using BioNumerics version 7.5 (Applied Maths, Sint-Martens-Latem, Belgium) with Pearson's correlation coefficient and unweighted pair group method with arithmetic mean clustering algorithm.

2.5 | Antifungal susceptibility testing

Antifungal susceptibility profiles of *S. brasiliensis* strains in the yeast phase were evaluated by broth microdilution according to the clinical and laboratory standards institute (CLSI) M27-S4.[30] Broth microdilution test was performed with modifications as described by Marimon et al.[31] Isolates were cultured onto Sabouraud Dextrose Agar plates (Oxoid) at 35°C for 5 days to obtain the yeast phase. After that, cells were diluted in RPMI medium to a transmission of 80%–82% at 530 nm with a Genesys 20 Spectrophotometer (Thermo Fisher Scientific) and subsequently diluted to obtain final concentrations of 1–5 × 10^5 CFU/mL. Antifungal concentrations ranged from 0.016 to 16 μg/mL for amphotericin B (Bristol Myers Squib, Woerden, The Netherlands), isavuconazole (Basilea Pharmaceutica), itraconazole (Janssen Cilag), voriconazole (Pfizer Central Research) and posaconazole (Merck, Sharp & Dome), from 0.002 to 2 μg/mL for terbinafine (Novartis Pharmaceuticals) and from 0.001 to 1 μg/mL for olorofim (F2G).

The microtitre plates were incubated at 35°C and visually interpreted after 72 h. MIC values were read as the lowest antifungal concentration with substantially lower turbidity (50% growth reduction) relative to the growth control, except for amphotericin B (100% growth reduction). Each experiment was performed independently three times. The ECVs suggested by Espinel-Ingroff et al. were implemented as resistant (amphotericin B: ≥4 μg/mL, itraconazole: ≥2 μg/mL, posaconazole: ≥2 μg/mL, terbinafine: ≥0.125 μg/mL and voriconazole: ≥32 μg/mL).[19] To identify isolates with reduced susceptibility for isavuconazole and olorofim, cut-off values of, respectively, 2 and 1 μg/mL were used (expert opinion). AFST metrics and statistical analyses were computed with Excel. A two-sided *t*-test was used to compare MICs of feline and human isolates.

2.6 | Whole genome sequencing and read mapping

Genomic libraries were prepared and sequenced with the MiSeq platform (Illumina) with 2- by 150-bp paired-end-read mode at Eurofins. Reads are publicly available at NCBI under BioProject ID: PRJNA836433. Read data were uploaded to the Galaxy tool, FastQC was used to assess read data quality, and no trimming was performed.[32] Sequenced strains were aligned against the *S. brasiliensis* 5110 reference genome (GCA_000820605.1) using BWA-MEM v0.7.17.1.[33] Read duplicates were removed using RmDup, local realignment was performed using BamLeftAlign, and unpaired reads were removed with BAM Filter. Reads with a MAPQ score < 60 were removed. Potential variations in genes associated with antifungal resistance were analysed by visualisation with JBrowse v1.16.11.[34]

3 | RESULTS

3.1 | Clinical data and molecular characterisation

A total of 89 isolates, 40 human and 49 feline strains of *Sporothrix* spp. were collected between 1989 and 2019 in Curitiba, Brazil (Table S1). Most *Sporothrix* isolates were found after 2010 with a peak in feline sporotrichosis in 2016 followed by an increase in human cases in 2018. In 2016, the Veterinary Clinic School initiated a prevention and treatment project, including limiting animal contact with humans and other cats during treatment, which coincided with a decrease in the number of cases (Figure 1). Isolates with known collection sites were predominantly isolated from cutaneous and

subcutaneous sites ($n = 61$, 68.5%) with a minority of isolates collected from the respiratory system ($n = 7$, 7.9%).

To identify the *Sporothrix* isolates, the calmodulin gene was sequenced. Most of the samples were identified as *S. brasiliensis* ($n = 81$) and a smaller number as *S. schenckii* ($n = 8$). The underlying epidemiological data from these samples indicated that the first *S. brasiliensis* isolates of our study were collected in 2006 from feline cases (CMRP2395 and CMRP2396). The genetic relatedness among isolates was assessed with AFLP genotyping of 81 *S. brasiliensis* and eight *S. schenckii* isolates, while one, two and three reference strains of *S. mexicana*, *S. globosa* and *S. schenckii* were, respectively, included as controls. In total, 95 AFLP profiles were generated with bands in a 10–500 bp range (data not shown) from which a minimum spanning tree (MST) was generated (Figure 2). In general, *S. schenckii* and *S. mexicana* strains clustered as an outgroup with different AFLP patterns compared with *S. brasiliensis* strains. Also, the *S. globosa* strains were more closely related to *S. brasiliensis* strains. Interestingly, feline and human *S. brasiliensis* strains were not distinct from each other or could not be separated with AFLP genotyping.

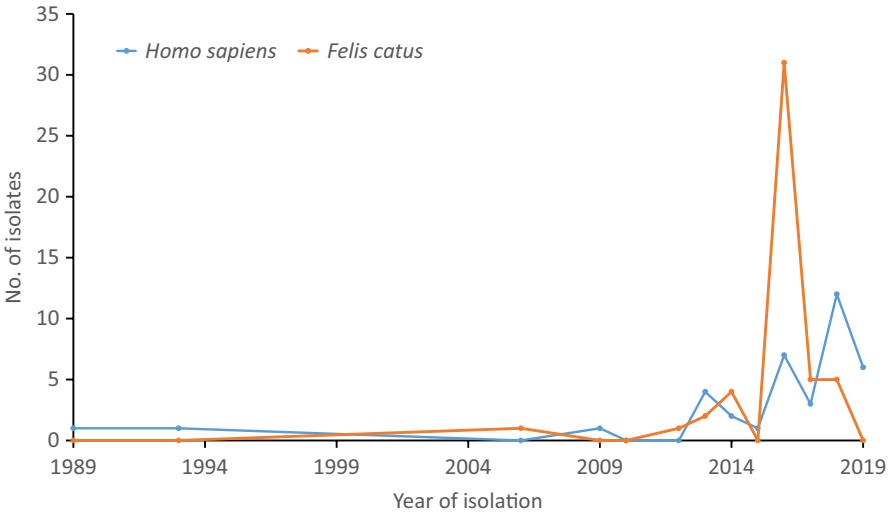

FIGURE 1 Chronological overview of *Sporothrix* spp. isolates collected between 1989 and 2019 in Curitiba, Brazil. Human isolates are depicted in blue and feline isolates in orange.

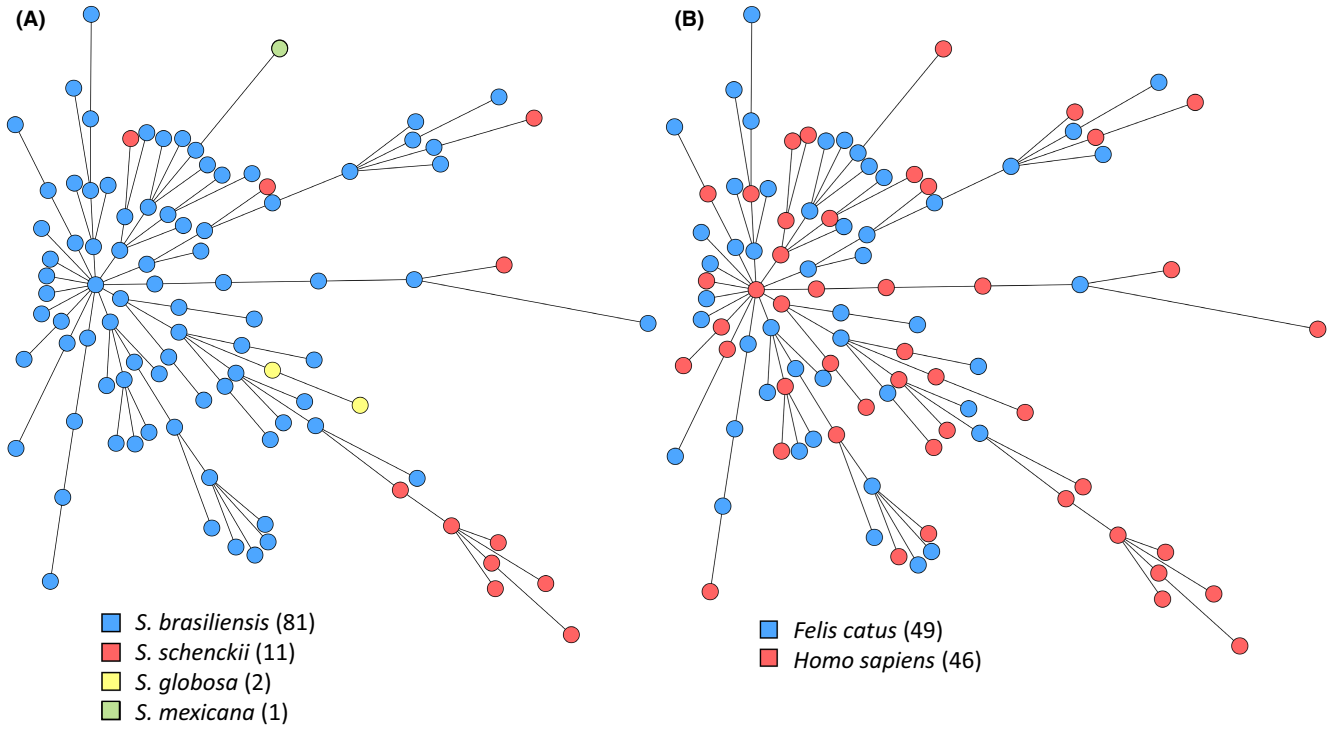

FIGURE 2 Minimum spanning trees of 95 *Sporothrix* spp. based on amplified fragment length polymorphism data. On the left (A) are isolates coloured according to species and on the right (B) are isolates coloured according to the host. Numbers of species and hosts are noted in the colour key between brackets. *S. schenckii* CBS 130099, *S. schenckii* CBS 130113, *S. schenckii* CBS 130114, *S. globosa* CBS 130116, *S. globosa* CBS 130117 and *S. mexicana* CBS 132926 have been included as controls. Branch lengths indicate the similarity between isolates.

3.2 | Antifungal susceptibility testing

Seven antifungal drugs were tested by CLSI broth microdilution against 52 randomly selected *S. brasiliensis* strains. In general, the antifungals exhibited an excellent activity against all *S. brasiliensis* isolates. Only one human isolate (CMRP3984) was found to be resistant against itraconazole and posaconazole with both MICs ≥16 μg/mL (Table 1). Terbinafine and olorofim exhibited the lowest MIC_{50} values of 0.031 μg/mL. The highest MIC_{50} was found for voriconazole at 1 μg/mL. MIC values showed no significant difference between feline and human isolates.

3.3 | Azole resistance genes

In order to investigate the mechanism underlying the resistance against itraconazole and posaconazole, WGS analysis was performed on isolate CMRP3984 and two related susceptible isolates, isolated in the same year, as wild-type controls (Figure S1). In total, 13 genes associated with known azole resistance, including *cyp51A*, *hmg1* and *erg6*, were visually inspected for substitutions. In comparison with the two susceptible isolates, no unique substitutions were identified in the resistant isolate (Table 2).

4 | DISCUSSION

Sporotrichosis occurs worldwide, but most endemic areas are in (sub) tropical regions.[10] The southern region of Brazil has been experiencing a very large sporotrichosis epidemic due to zoonotic transmission over the last decades.[10,35,36] Due to limited diagnostic capabilities, many symptomatic and asymptomatic cases in felines persist for months, leading to transmission of sporotrichosis through cat-human contact.[18] This study provides the first genotypic analysis of both human and feline sporotrichosis from Curitiba, Brazil. The majority of isolates causing sporotrichosis in Curitiba were identified as *S. brasiliensis*. This species is predominant among humans and cats with sporotrichosis in Brazil.[1,9,10,37,38] Transmission routes of *S. brasiliensis* include traumatic inoculation by cat bites and scratches.[10] In addition, we and others also found the presence of *S. brasiliensis* in saliva of cats and in nasal sections[39,40] and, as was hypothesised recently, cat sneezing might be a novel transmission route for the dissemination of *S. brasiliensis*.[41] Furthermore, AFLP genotyping demonstrated that the human and feline isolates did not cluster separately, suggesting probable zoonotic transmission from infected cats. This was also recently shown with microsatellite genotyping (REF).[42] AFLP is an easily executable method with low costs, while drawbacks of this method include reproducibility and discriminatory power. Moreover, it has to be noted that selective bases influence AFLP-generated fingerprints.[43] Other options for selective bases previously reported revealed evolutionary relationships similar to those found for phylogenetic analyses using barcoding genes or ribosomal DNA.[43]

Recent data suggest higher MICs to antifungals for *S. brasiliensis* as compared to other *Sporothrix* spp., in particular against amphotericin B and azoles.[18,26,44–46] These findings have not been confirmed in animal models or in human patients.[16,47–51] In this study, antifungal susceptibility of 52 isolates against seven drugs showed in general an excellent activity. Preceding the AFST, taking place at 35°C, we induced the yeast form by culturing the isolates at 35°C for 5 days, as this constitutes the infective form of *S. brasiliensis*.[48] To the best of our knowledge, there are only two reports regarding AFST on both phases of *S. brasiliensis*.[52,53] Sanchotene and co-workers, and Borba-Santos and co-workers found overall similar MIC_{50} levels for both phases for amphotericin B, itraconazole and terbinafine. For amphotericin B and terbinafine, high MIC values were more often found in the mycelial phase, while the opposite was reported for itraconazole. Similar studies on *S. schenckii* and *S. globosa* demonstrate that for most antifungals, including itraconazole, high MIC levels are more often found for the mycelial phase.[54–56] In our study, itraconazole and terbinafine, reported as the drugs of choice to treat sporotrichosis,[10,57–59] demonstrated low MIC_{50} values, being 0.063 and 0.031 μg/mL, respectively, in addition to low MIC_{90} values of 0.25 and 0.5 μg/mL for itraconazole and terbinafine, respectively. Out of 52 tested isolates, only one was resistant against itraconazole. Since itraconazole is frequently administered to treat sporotrichosis, the resistance in this human isolate might be therapy-induced. In addition to itraconazole, this isolate was also resistant to posaconazole as these two antifungals are chemically highly similar.[26,60,61] Despite the absence of resistant isolates against voriconazole and isavuconazole, these drugs exhibited a four to eightfold higher MIC_{50} and MIC_{90} when compared to itraconazole. Nonetheless, azole MICs found in this study were compatible with those observed in most other studies.[52,59,62]

TABLE 1 MIC ranges, GM, MIC_{50} and MIC_{90} values according to microbroth dilution for 72 h by CLSI M27-S4 standard of 52 *Sporothrix brasiliensis* strains. MIC, minimal inhibitory concentration; GM, geometric mean; $MIC_{50/90}$, MIC values at which 50/90% of isolates are inhibited in their growth.

Antifungal	Range (μg/mL)	MIC mode	GM (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
Amphotericin B	0.031–2	0.125	0.163	0.125	0.5
Voriconazole	≤0.016–4	1	0.841	1	4
Itraconazole	≤0.016 – ≥16	0.063	0.065	0.063	0.25
Isavuconazole	≤0.016–4	0.5	0.254	0.25	1
Posaconazole	≤0.016 – ≥16	0.063	0.083	0.063	0.25
Terbinafine	0.004–0.5	0.031	0.046	0.031	0.5
Olorofim	0.004–0.25	0.016	0.026	0.031	0.125

TABLE 2 Overview of itraconazole and posaconazole resistance-associated substitutions.

Gene	Encoding protein (amino acid length)	Genomic location[a]	Amino acid substitution
abc transport system ATP-binding protein	ABC transport system ATP-binding protein, conjugate transporter-2 family protein (870)	09.1: 2.572.228–2.574.840	None
cdr1	Pleiotropic drug resistance (PDR) family protein (1562)	04.1: 1.301.700–1.297.015	None
cdr4	ABC transporter CDR4 (1554)	06.1: 1.904.177–1.899.513	None
cyp51A	Cytochrome P450 family 51 (526)	11.1: 1.192.826–1.191.169	None
erg2	C-8 sterol isomerase (212)	04.1: 2.629.175–2.628.191	None
erg3	C-5 sterol denaturase (351)	08.1: 3.286.128–3.287.274	None
erg5	C-22 sterol desaturase (534)	09.1: 3.545.423–3.543.497	None
erg6	Sterol 24-C-methyltransferase (342)	10.1: 511.141–509.727	None
erg24	C-14 sterol reductase (505)	09.1: 5.036.722–5.038.315	None
hmg1	3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) (1266)	03.1: 616.367–620.167	None
		10.1: 779.417–781.341	None
mdr1	Mdr1 multidrug resistance transporter (1384)	09.1: 1.265.115–1.269.350	None
mdr2	Mdr2 multidrug resistance transporter (422)	09.1: 2.570.050–2.571.509	None
pdh1	Pyruvate dehydrogenase (559)	10.1: 1.889.214–1.887.462	None

[a]Genomic location in *Sporothrix brasiliensis* 5110 (GCA_000820605.1). Contigs are abbreviated by removal of 'ATWV010000' for each contig.

While low MICs were also obtained with amphotericin B, this antifungal is not commonly used to treat feline sporotrichosis as the method of administration, intravenous, causes serious adverse effects in cats.[59,63] WGS analysis of resistance-associated genes of the single azole-resistant isolate did not reveal any mutations. A point mutation in uninspected transcription factors of efflux pumps, conferring increased expression levels, may be causative for the observed resistance.

To the best of our knowledge, this is the first reported study of olorofim activity to a large collection of *S. brasiliensis* isolates. Olorofim belongs to a new class of antifungals and is currently studied in clinical trials for the treatment of invasive fungal infections. The low MIC values of olorofim in our study are in line with previous reports for *Sporothrix* spp. and in most other filamentous fungi, including *Aspergillus fumigatus*, *Fusarium* spp., *Scedosporium* spp., *Rasamsonia argillacea* species complex, *Penicillium* spp., *Talaromyces* spp., *Trichophyton* spp. and *Lomentospora prolificans*.[57,64–70] Therefore, this drug might be an attractive alternative when resistance against azoles and polyenes will rise.

Since its identification in 2007, *S. brasiliensis* has been a focus of many studies but this disease continues to spread rapidly in Brazil and neighbouring countries.[71] Our findings demonstrate zoonotic transmission and genetic diversity in *S. brasiliensis*. In vitro susceptibility testing against seven antifungals, including olorofim, exhibited excellent activity. Altogether, this study provided novel insights into the transmission and the susceptibility of *S. brasiliensis*.

AUTHOR CONTRIBUTIONS

Amanda Bombassaro: Investigation; writing – original draft; methodology; formal analysis; resources. **Bram Spruijtenburg:** Investigation; visualization; software; validation. **Fernanda Medeiros:** Writing – review and editing; visualization. **Bruna Jacomel Favoreto de Souza Lima:** Writing – review and editing; investigation. **Luana Beatriz Ballardin:** Writing – review and editing; data curation. **Marconi Rodrigues de Farias:** Writing – review and editing; data curation. **Vania Aparecida Vicente:** Writing – review and editing; resources. **Flavio de Queiroz-Telles:** Writing – review and editing; data curation. **Jacques F. Meis:** Resources; conceptualization; supervision; writing – review and editing; funding acquisition. **Theun de Groot:** Writing – review and editing; supervision; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in NCBI Genbank at www.ncbi.nlm.nih.gov/genbank, Genbank accession numbers OQ571231-OQ571318 and in the NCBI SRA database at www.ncbi.nlm.nih.gov/sra, BioProject ID: PRJNA836433.

ORCID

Bram Spruijtenburg https://orcid.org/0000-0002-6734-361X
Jacques F. Meis https://orcid.org/0000-0003-3253-6080
Theun de Groot https://orcid.org/0000-0001-5841-6461

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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