MINISTÉRIO DA EDUCAÇÃO
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ESPOROTRICOSE NO SUL DO BRASIL: DIVERSIDADE GENÉTICA E CLÍNICA, SUSCEPTIBILIDADE ANTIFÚNGICA, DIAGNÓSTICO E BIOPROSPECÇÃO

Mariana Rodrigues Trápaga

Rio Grande, 2025

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Rio Grande, como requisito parcial à obtenção do título de Doutor em Ciências da Saúde.

Orientadora: Profª Dra. Melissa Orzechowski Xavier

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RESUMO

Nas últimas décadas, a esporotricose zoonótica consolidou-se como uma das principais micoses emergentes no Brasil, com crescente expansão para outros países da América Latina. Essa endemia, causada predominantemente por *Sporothrix brasiliensis*, foi inicialmente descrita em surtos epidêmicos no final da década de 1990, de forma independente nos estados do Rio de Janeiro (RJ) e do Rio Grande do Sul (RS). Os gatos domésticos, em razão da elevada carga fúngica, gravidade clínica e hábitos de vida, são os principais transmissores. Com o aumento expressivo de casos, também se observa a ocorrência de formas clínicas atípicas, como as reações de hipersensibilidade, ainda pouco compreendidas. Nesse contexto, o conhecimento epidemiológico, a disponibilidade de métodos diagnósticos acessíveis e o uso racional de antifúngicos constituem pilares fundamentais para o enfrentamento dessa micose, cuja rápida expansão geográfica resultou, recentemente, em sua inclusão na lista de doenças de notificação compulsória em nível nacional. O diagnóstico da doença ainda possui limitações associadas à técnica de cultivo micológico, que demanda tempo e profissionais especializados. Além disso, há a problemática associada ao tratamento, cujo fármaco de escolha, o itraconazol, vem demonstrando limitações quanto à resposta terapêutica, ressaltando a importância da vigilância, bem como da busca por novas alternativas. Fundamentada nessa problemática, a presente tese teve como objetivos: avaliar a diversidade genotípica dos isolados circulantes no RS; determinar o perfil de suscetibilidade antifúngica; descrever o primeiro relato de esporotricose causada por *S. brunneoviolacea*; caracterizar manifestações clínicas de hipersensibilidade em uma área hiperendêmica; validar novos métodos diagnósticos; e explorar a atividade antifúngica de compostos naturais marinhos frente a *S. brasiliensis*. Para contemplar esses objetivos voltados à epidemiologia, vigilância, clínica, diagnóstico e novas alternativas terapêuticas, foram desenvolvidos diferentes experimentos organizados em cinco artigos/manuscritos. **Manuscrito 1**: Foi realizada a análise de 450 isolados de *S. brasiliensis* provenientes de distintas regiões do Rio Grande do Sul, revelando ampla diversidade genética com a identificação de 82 genótipos, distribuídos em três grandes clados. Dois clados inéditos foram evidenciados, sendo um deles o clado majoritariamente associado a região Sul do RS, com maior expansão clonal, e outro associado a região da Serra e Sul, com maior variabilidade genética. Alguns isolados, associados principalmente a região metropolitana, foram agrupados ao clado do RJ previamente descrito. Observou-se ainda uma distribuição bimodal de isolados não selvagens para o itraconazol, indicando uma emergência da resistência na região. Os clados apresentaram diferentes taxas de isolados não-selvagens, variando de cerca de 20% a até 89%. Tais achados evidenciam a circulação de distintas linhagens no estado do RS e com perfis distintos de suscetibilidade, reforçando a importância da vigilância epidemiológica e do monitoramento antifúngico, e o papel da região mais ao sul do Brasil na dinâmica de expansão da doença no cenário nacional. **Manuscrito 2**: Foi relatado o primeiro caso de esporotricose por *S. brunneoviolacea* em um gato doméstico do sul do Brasil. A espécie, previamente considerada ambiental e não patogênica, foi identificada por sequenciamento do gene da calmodulina e análise filogenética, que confirmou seu distanciamento do clado clínico de *Sporothrix*. O caso apresentou evolução clínica rápida e com resposta favorável ao itraconazol. Trata-se da primeira descrição dessa espécie no Brasil e do primeiro registro mundial de infecção felina por *S. brunneoviolacea*, contribuindo para ampliar o entendimento sobre a diversidade etiológica e o potencial zoonótico do gênero *Sporothrix*. **Manuscrito 3:** Foi realizada a primeira descrição detalhada de casos de hipersensibilidade associados à esporotricose no RS, identificando uma frequência de 17,1% entre os pacientes assistidos na rede de referência regional para esporotricose humana do sul do Estado. A manifestação mais comum foi artrite asséptica, frequentemente associada às formas linfocutânea e cutânea fixa. A genotipagem dos isolados provenientes desses pacientes não mostrou distinção em relação aos casos clássicos, indicando que a resposta imunológica do hospedeiro, e não a variabilidade genética do fungo, está na base dessas manifestações imunoalérgicas. **Manuscrito 4**: Foi avaliada e demonstrada a aplicabilidade de uma PCR espécie-específica para o diagnóstico da esporotricose por *S. brasiliensis* em humanos e gatos a partir de amostras não invasivas (swab). A técnica apresentou concordância superior a 86% em relação ao cultivo micológico, configurando-se como uma alternativa viável para o diagnóstico precoce da esporotricose, especialmente em regiões onde há carência de profissionais especializados em micologia. **Manuscrito 5**: Foi explorada pela primeira vez a atividade da microalga *Nannochloropsis oceanica* frente a *S. brasiliensis*. O extrato hexânico não apresentou atividade inibitória direta em concentrações de até 800 µg/mL, e ensaios de combinação revelaram somente efeitos aditivos e sinérgicos fracos com o itraconazol. Apesar dos resultados iniciais não se mostrarem muito promissores, essa avaliação inaugura uma linha de investigação marinha até então inexplorada, e cujas alterações em métodos de extração, solventes e espécies podem revelar potenciais novas moléculas bioativas frente a esporotricose. Assim, a presente tese contribui de forma inédita para a compreensão integrada da diversidade genética e do perfil de suscetibilidade antifúngica de *S. brasiliensis* no RS, evidenciando o papel da região como área de expansão e diversidade da doença no Brasil e descrevendo dois clados inéditos do fungo circulantes no estado. Além disso, descreve o primeiro caso de esporotricose felina por *S. brunneoviolacea* e a primeira ocorrência dessa espécie no país caracteriza manifestações clínicas de hipersensibilidade até então pouco descritas, ampliando o conhecimento sobre a apresentação clínica da esporotricose, valida um método molecular acessível para diagnóstico precoce e abre perspectivas para pesquisas em alternativas terapêuticas a partir da bioprospecção marinha.

Palavras-chave: Genotipagem, Susceptibilidade, Hipersensibilidade, Reação em Cadeia da Polimerase, Bioprospecção

ABSTRACT

Over the past decades, zoonotic sporotrichosis has become one of the main emerging mycoses in Brazil, with a growing expansion to other Latin American countries. This endemic disease, predominantly caused by *Sporothrix brasiliensis*, was first described in outbreaks during the late 1990s, independently in the states of Rio de Janeiro (RJ) and Rio Grande do Sul (RS). Domestic cats, due to their high fungal burden, clinical severity, and behavioral characteristics, are the main transmitters. With the sharp increase in cases, atypical clinical forms, such as hypersensitivity reactions, have also been observed, although they remain poorly understood. In this context, epidemiological knowledge, the availability of accessible diagnostic methods, and the rational use of antifungal agents are fundamental pillars for addressing this mycosis, whose rapid geographic expansion recently led to its inclusion on the list of nationally notifiable diseases in Brazil. Diagnosis of the disease still faces limitations associated with mycological culture, which requires time and trained personnel. Furthermore, treatment remains challenging, as itraconazole, the drug of choice, has shown variable therapeutic responses, emphasizing the importance of surveillance and the search for alternative strategies. Based on this scenario, the present thesis aimed to: evaluate the genotypic diversity of isolates circulating in RS; determine their antifungal susceptibility profiles; describes the first case of sporotrichosis caused by *S. brunneoviolacea*; characterize clinical hypersensitivity manifestations in a hyperendemic area; validate new diagnostic methods; and explore the antifungal activity of natural marine compounds against *S. brasiliensis*. To address these objectives, focused on epidemiology, surveillance, clinical presentation, diagnosis, and therapeutic innovation, different experiments were developed and organized into five manuscripts. **Manuscript 1:** A total of 450 *S. brasiliensis* isolates from different regions of Rio Grande do Sul were analyzed, revealing broad genetic diversity with the identification of 82 genotypes distributed into three major clades. Two novel clades were identified: one predominantly associated with the southern region of the state, showing greater clonal expansion, and another associated with the Serra and southern regions, displaying higher genetic variability. Some isolates, mainly from the metropolitan area, clustered with the previously described RJ clade. A bimodal distribution of non-wild-type isolates to itraconazole was also observed, suggesting emerging resistance in the region. The clades exhibited different rates of non-wild-type isolates, ranging from approximately 20% to 89%. These findings highlight the circulation of distinct *S. brasiliensis* lineages in RS with varying susceptibility profiles, reinforcing the importance of epidemiological surveillance and antifungal monitoring, as well as the role of southern Brazil in the national dynamics of disease expansion. **Manuscript 2:** The first case of sporotrichosis caused by *Sporothrix brunneoviolacea* in a domestic cat from southern Brazil was reported. This species, previously considered environmental and non-pathogenic, was identified by sequencing of the calmodulin gene and phylogenetic analysis, which confirmed its distance from the clinical *Sporothrix* clade. The case exhibited rapid clinical improvement with a favorable response to itraconazole. This represents the first description of this species in Brazil and the first worldwide record of feline infection by *S. brunneoviolacea*, expanding the understanding of the etiological diversity and zoonotic potential of the *Sporothrix* genus. **Manuscript 3:** The first detailed description of hypersensitivity manifestations associated with sporotrichosis in RS was carried out, identifying a frequency of 17.1% among patients assisted by the regional reference network for human sporotrichosis in southern Brazil. The most common manifestation was aseptic arthritis, frequently associated with lymphocutaneous and fixed cutaneous forms. Genotyping of isolates from these patients revealed no distinction from classic cases, suggesting that host immune response, rather than fungal genetic variability, is at the basis of these immunoallergic manifestations. **Manuscript 4:** The applicability of a species-specific PCR assay for the diagnosis of sporotrichosis caused by *S. brasiliensis* in humans and cats using non-invasive samples (swabs) was evaluated and demonstrated. The method showed over 86% concordance with mycological culture, representing a viable alternative for early diagnosis, especially in regions lacking trained mycology professionals. **Manuscript 5:** The antifungal activity of the marine microalga *Nannochloropsis oceanica* against *S. brasiliensis* was explored for the first time. The hexanic extract showed no direct inhibitory activity at concentrations up to 800 µg/mL, and combination assays revealed only weak additive or synergistic effects with itraconazole. Although the initial results were not highly promising, this study inaugurates a previously unexplored marine bioprospecting line, in which variations in extraction methods, solvents, and species may reveal new bioactive molecules with antifungal potential against sporotrichosis. In summary, this thesis provides novel and integrated insights into the genetic diversity and antifungal susceptibility profile of *S. brasiliensis* in Rio Grande do Sul, highlighting the region's role as an area of disease expansion and diversity in Brazil. Moreover, it reports the first case of feline sporotrichosis caused by *S. brunneoviolacea* and the first occurrence of this species in the country, characterizes previously underreported hypersensitivity manifestations, validates an accessible molecular method for early diagnosis, and opens new perspectives for therapeutic research through marine bioprospecting.

Key-words: Genotyping, Susceptibility, Hypersensitivity, Poymerase Chain Reaction, Biopospecting

LISTA DE TABELAS

MANUSCRITO 2

MANUSCRITO 3

MANUSCRITO 4

LISTA DE FIGURAS

MANUSCRITO 1

MANUSCRITO 2

MANUSCRITO 3

MANUSCRITO 4

LISTA DE ABREVIATURAS E SIGLAS

- **AFLP** – Polimorfismo do Comprimento de Fragmento Amplificado (*Amplified Fragment Length Polymorphism*)
- **AMB** – Anfotericina B
- **BHI** – Infusão Cérebro-Coração (*Brain Heart Infusion*)
- **BLAST** – Ferramenta de Alinhamento Local Básico (*Basic Local Alignment Search Tool*)
- **CLSI** – *Clinical and Laboratory Standards Institute*
- **DNA** – Ácido desoxirribonucleico
- **DHN** – Dihidroxinaftaleno
- **DMSO** – Dimetilsulfóxido
- **ELISA** – Ensaio imunoenzimático
- **EMD** – Exame micológico direto
- **EUCAST** – *European Committee on Antimicrobial Susceptibility Testing*
- **ECV** – Valor de Corte Epidemiológico
- **FURG** – Universidade Federal do Rio Grande
- **GMS** – Prata metenamina de Gomori
- **HIV** – Vírus da imunodeficiência humana
- **HU-FURG/EBSERH** – Hospital Universitário da FURG / Empresa Brasileira de Serviços Hospitalares
- **ITC / ITZ** – Itraconazol
- **LAMP** – Amplificação Isotérmica Mediata por Alça (*Loop-mediated isothermal amplification*)
- **MLST** – Tipagem por Sequenciamento Multi-Locus
- **MIC** – Concentração Inibitória Mínima
- **PCR** – Reação em Cadeia da Polimerase
- **qPCR** – PCR Quantitativa
- **RT-qPCR** – PCR em Tempo Real com Transcriptase Reversa
- **RJ** – Rio de Janeiro
- **RS** – Rio Grande do Sul
- **WGS** – Sequenciamento de Genoma Completo
- **EF-1α** – Fator de Elongação 1-alfa
- **MG** – Minas Gerais
- **MST** – Árvore de Mínima Abrangência (*Minimum spanning tree*)
- **DF** – Distrito Federal
- **mg** – miligrama
- **kg** – quilograma
- **CFU** – Unidades Formadoras de Colônia
- **mL** – mililitro
- **µg** – micrograma
- **µM** – micromolar
- **µL** – microlitro
- **RH / HR** – Reação de Hipersensibilidade
- **RPMI 1640** – Meio de Cultura Roswell Park Memorial Institute 1640
- **FICi** – Índice de Concentração Inibitória Fracionada (*Fractional Inhibitory Concentration Index*

SUMÁRIO

1. INTRODUÇÃO

A esporotricose é a principal micose de subcutânea no Brasil e tornou-se emergente nos últimos anos, especialmente devido à espécie *Sporothrix brasiliensis*. Essa espécie está associada a casos graves em gatos domésticos, que são os principais transmissores do fungo e também vítimas da doença, levando a emergência de surtos e hiperendemias em diferentes regiões do Brasil desde a década de 1990 (Poester et al., 2018a; Xavier et al., 2023). Os primeiros casos zoonóticos da esporotricose, que resultaram em endemias, foram descritos concomitantemente no Rio de Janeiro e no Rio Grande do Sul no final dos anos 1990, consolidando esses estados como os epicentros da introdução e emergência da doença (Barros et al., 2004; Nobre et al., 2001a; Schubach et al., 2004; Xavier et al., 2004).

Desde então, múltiplos estudos têm demonstrado a circulação de diferentes genótipos de *S. brasiliensis* relacionados à localização geográfica (De Melo Teixeira et al., 2015; De Souza Rabello et al., 2024; Losada et al., 2023; Spruijtenburg et al., 2023a). Os isolados do RJ e RS já haviam sido diferenciados pela análise filogenética de EF-1α e pela genotipagem de polimorfismos do comprimento de fragmento amplificado (AFLP) (De Carvalho et al., 2022; Rodrigues; De Hoog; De Camargo, 2014; Zhang et al., 2015). Mais recentemente, técnicas moleculares de maior poder discriminatório, como MLST, microssatélites e sequenciamento de genoma total, confirmaram que os clados do RJ e RS pertencem a grupos monofiléticos distintos, ao lado de outros núcleos epidêmicos como Distrito Federal e Minas Gerais (De Souza Rabello et al., 2024; Eudes Filho et al., 2020; Losada et al., 2023), No entanto, os isolados do RS têm se mostrado consistentemente mais diferenciados dos demais, sugerindo uma trajetória epidemiológica própria, em contraste com outras regiões onde há evidências de disseminação a partir do RJ. Esse panorama ressalta a importância de compreender a epidemiologia molecular do RS e seu papel singular na dinâmica nacional e internacional da esporotricose zoonótica.

A genotipagem desempenha papel central na compreensão da dinâmica de expansão da endemia, oferecendo subsídios valiosos para o manejo de surtos epidêmicos e a formulação de políticas públicas (De Carvalho et al., 2022). Em paralelo, o aumento no número de casos tem possibilitado a descrição de apresentações clínicas atípicas (Poester et al., 2024). Embora a esporotricose seja classificada classicamente como uma micose de subcutânea, com predomínio das formas cutâneas (cutânea fixa e linfocutânea), observa-se cada vez mais a ocorrência de formas imunorreativas, associadas a reações de hipersensibilidade do hospedeiro aos propágulos fúngicos (Bittencourt et al., 2022; Orofino-Costa et al., 2022). Nesse contexto, torna-se indispensável o desenvolvimento de estudos integrados de epidemiologia clínica e molecular, capazes de relacionar a variabilidade clínica às diferenças genotípicas, ampliando a compreensão da interação entre fungo, hospedeiro e ambiente.

Outra frente indispensável para o controle da endemia da esporotricose é o diagnóstico. O cultivo micológico é o método "padrão-ouro" de diagnóstico para a esporotricose, porém, *Sporothrix* spp. é um fungo que leva 5-14 dias para crescer, além do tempo, a técnica possui limitações de sensibilidade, associadas principalmente a fatores pré-analíticos (Gremião et al., 2020; Orofino-Costa et al., 2017), e, ainda, o acesso a laboratórios capacitados para realização de diagnóstico micológico é restrito em inúmeras regiões (Hay et al., 2019). Devido ao grave panorama expansivo da doença no Brasil é de extrema importância a busca por métodos diagnósticos de fácil aplicação e interpretação em laboratórios de todo país. Com isso, as técnicas moleculares já vem sendo alvo de estudos para o diagnóstico de esporotricose, e há uma necessidade de mais estudos de padronização para futura aplicação com robustez interlaboratorial (Della Terra et al., 2022; Poester et al., 2021; Zhang et al., 2019).

O tratamento também representa um desafio. O itraconazol continua sendo o fármaco de primeira escolha tanto para humanos quanto para animais (Gremião et al., 2020; Orofino-Costa et al., 2022). Entretanto, nos felinos, a maior gravidade clínica, a necessidade de tratamentos longos (em média quatro meses) e a dependência da adesão do tutor resultam em altas taxas de abandono (30–40%) e de cura relativamente baixas (50–72%) (Gremião et al., 2020; Pereira et al., 2010). Para os humanos já é relatado a necessidade do uso de doses mais altas em isolados do RS quando comparados a isolados do RJ para que se tenha resposta ao tratamento e cura clínica (De Lima Barros; De Almeida Paes; Schubach, 2011; Orofino-Costa et al., 2017; Poester et al., 2022a). Além disso, relatos de resistência clínica e laboratorial de *Sporothrix brasiliensis* têm se tornado cada vez mais frequentes (Ribeiro Dos Santos et al., 2024; Waller et al., 2021c). Esse conjunto de evidências reforça a importância de integrar a vigilância da suscetibilidade antifúngica ao contexto epidemiológico do RS, uma vez que a região concentra cepas com características particulares que podem impactar diretamente a conduta terapêutica.

Tal vigilância é essencial não apenas para antecipar falhas no tratamento, mas também para orientar a formulação de protocolos mais eficazes e adaptados à realidade regional.

Diante dessa problemática, torna-se fundamental o conhecimento do perfil de suscetibilidade dos genótipos circulantes, bem como a busca por novos compostos com atividade antifúngica frente a *S. brasiliensis*, que possam representar alternativas terapêuticas no enfrentamento da doença. Nesse contexto, a bioprospecção de ambientes marinhos tem ganhado destaque nos últimos anos, em razão da ampla diversidade de compostos bioativos disponível, mas ainda pouco explorada em comparação aos recursos terrestres (Cardoso et al., 2020). Entre esses organismos, destaca-se a microalga *Nannochloropsis oceanica*, encontrada na costa da cidade do Rio Grande, local de origem da endemia no RS, que já demonstrou atividade antifúngica contra o fitopatógeno *Fusarium* spp., indicando o potencial de apresentar também compostos ativos frente a *S. brasiliensis* (Scaglioni et al., 2019). A exploração desse recurso inaugura uma linha de investigação inovadora e necessária, voltada à identificação de moléculas que possam auxiliar no enfrentamento dos desafios terapêuticos e na contenção da disseminação da doença no cenário atual.

2. REVISÃO BIBLIOGRÁFICA

2.1 Histórico da esporotricose

O primeiro relato de esporotricose ocorreu em 1896, por Benjamin Robinson Schenk, um estudante de medicina, enquanto trabalhava no Hospital da Universidade de Johns Hopkins, Baltimore, Maryland, Estados Unidos da América. O fungo foi isolado de múltiplos nódulos ulcerativos do antebraço e dedo indicador de um paciente de 36 anos, o qual relatou um trauma perfurante. Erwin Smith, micologista, recebeu a amostra e concluiu que pertencia ao gênero *Sporotrichum* (Schenck, 1898).

O segundo caso foi descrito em 1899 pelo médico Perkins, no qual uma criança de 5 anos foi levada ao consultório médico com uma ferida no dedo ocasionada por um trauma com martelo, que evoluiu e apresentou a forma linfocutânea. Após a evolução da doença, o médico constatou a semelhança e associou ao caso de Schenck, realizando a identificação do fungo da mesma forma feita pelo trabalho anterior, concluindo ser o mesmo patógeno, e identificando-o como *Sporothrix schenckii* (Hektoen; Perkins, 1900).

Na França, em 1903, foi observado o primeiro caso de esporotricose no Hospital Saint Louis por De Beurmann e Ramond, e o fungo foi considerado diferente daquele descrito por Schenck inicialmente, denominado então *Sporotrichum beurmanni* por Metchuchot e Ramond em 1905. Porém, em 1910 Matruchot redescreveu o oganismo como *Sporotrichum schencki.* de Beumann e Gougeret iniciaram um estudo sistemático da esporotricose, e relataram mais de 200 casos humanos na França, descrevendo também as primeiras formas de acometimento de mucosas, formas disseminadas, pulmonares e ósseas (De Beurmann; Gougerot, 1912). Esses autores também descreveram a forma infecciosa como 'pequenas células ovais semelhantes a charutos'. A nomenclatura incorreta, que mantinha o fungo em *Sporotrichum*, gênero que não inclui fungos dimórficos e nem patogênicos para humanos ou outros animais, permaneceu até 1962, quando Carmichael propôs formalmente o gênero *Sporothrix*, com base em diferenças morfológicas e filogenéticas (Carmichel, 1962).

No Brasil, foi relatado o primeiro caso de infecção animal natural, em 1907, por Lutz e Splendore em ratos (Lutz; Splendore, 1907). A possibilidade de transmissão por mordida desses animais foi considerada por Pupo em 1917. O primeiro caso humano no Brasil foi descrito no Rio de Janeiro em 1912 por Terra e Rabelo, no mesmo ano foi registrado caso na Bahia, em 1914 e em 1916 em Minas Gerais e no Rio Grande do Sul, respectivamente (Donadel et al., 1993).

Em 1909 foi demonstrado, experimentalmente, que os gatos possuíam uma maior susceptibilidade a infecção por *S. schenckii* do que outros animais (De Beurmann et al., 1909). O primeiro relato de doença adquirida por gatos foi proveniente de Nova York (Singer; Muncie, 1952). No Brasil o primeiro relato em felinos é registrado em 1956 no estado de Minas Gerais (Freitas et al., 1956). Em 1983 foram descritos os primeiros casos em felinos no Rio de Janeiro (Cruz et al., 1983).

A esporotricose zoonótica foi inicialmente relatada com casos esporádicos associada a arranhões ou mordidas de animais como ratos, tatus, esquilos, cães e gatos (Kauffman, 1999). Entretanto, a primeira epidemia zoonótica associada aos gatos foi detectada no Rio de Janeiro, que de 1998 a 2009 mais de 2.000 casos em humanos e mais de 3.000 casos em gatos foram diagnosticados no instituto de referência do RJ (Barros et al., 2010). No final da década de 90, surto de esporotricose felina com transmissão para humanos foi também registrado na região sul do RS (Nobre et al., 2001a). E, desde então, a endemia cresce em todos os estados brasileiros, e em outros países da América Latina (Xavier et al., 2023).

2.2 Agente etiológico

O gênero *Sporothrix* spp. pertence ao Reino Fungi, Filo Ascomycota, Classe Sordariomycetes, Ordem Ophiostomales, e família Ophiostomataceae. Nesse gênero são descritas mais de 50 espécies válidas, distribuídas em diferentes ecossistemas e com relevância clínica ou ambiental. Dentro dessas, as principais espécies consideradas patógenos de humanos ou animais, incluindo as espécies *S. brasiliensis, S. globosa, S. mexicana, S. luriei, S. pallida,* e *S. schenckii sensu stricto* (Marimon et al., 2007).

Sporothrix spp. é composto por fungos termodimórficos, os quais tem como habitat natural solo rico em matéria orgânica, vegetação em decomposição, palha, madeira, excretas de animais e solos abundantes em celulose. Em estágio saprofítico (25-30ºC) apresenta forma filamentosa, caracterizado por hifas hialinas e septadas, de 1 a 2µm de largura. A formação de conidióforo se dá com conídios unicelulares em formato lacrimal dispostos de forma simpodial na extremidade de conidióforos claviformes. Macroscopicamente, as colônias são coriáceas, inicialmente de cor branca a creme, tornando-se mais escuras com o tempo. Entretanto há cepas que possuem pigmentação desde o início do crescimento, assim como isolados que não melanizam (Almeida-Paes et al., 2016; Gómez-Gaviria; Martínez-Álvarez; Mora-Montes, 2023). Já na sua forma parasitária (35-37ºC) assume a forma de levedura, sendo unicelular, e com células em formato ovalado a alongado, se assemelhando a “charutos” (**Figura 1**).

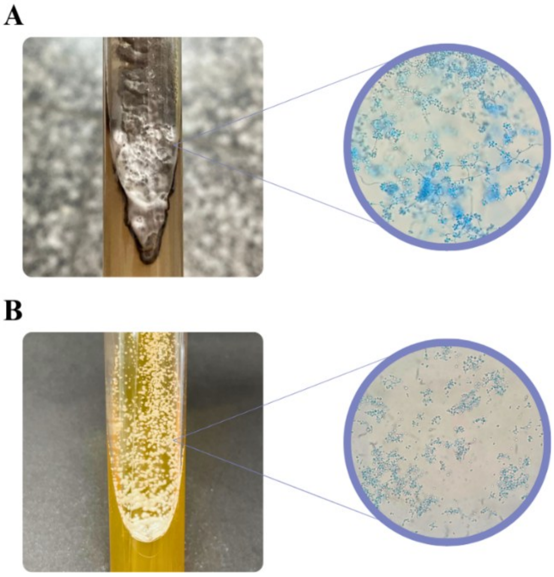

Figura 1: Características morfológicas de *Sporothrix brasiliensis*. (A) *S. brasiliensis* cultivado em ágar batata, a 28 °C por 14 dias, apresentando a forma filamentosa, e sua micromorfologia observada em microscopia (400×) após coloração com lactofenol azul de algodão. (B) *S. brasiliensis* cultivado em ágar infusão cérebro-coração (BHI), a 37 °C por 7 dias, e micromorfologia observada em microscopia (400×) após coloração com lactofenol azul de algodão.

Sugere-se que a espécie *S. brasiliensis* surgiu por uma adaptação da forma saprófita do fungo, visto que a partir de avalições genotípicas se observou a falta de enzimas degradadoras da parede celular vegetal, para a forma parasitária de mamíferos, sendo essa altamente associada a doença na sua forma zoonótica (Teixeira et al., 2014a). Os dados filogenéticos apoiam que essa mudança ocorreu no fim do século XX, e se iniciou no sudeste do Brasil, e concomitantemente no Sul do país (De Souza Rabello et al., 2024; Rodrigues; De Hoog; De Camargo, 2013).

S. brasiliensis possui fatores de virulência fundamentais para sua alta patogenicidade, como a termotolerância, termodimorfismo, produção de melanina, adesinas e formação de biofilme (Gómez-Gaviria; Martínez-Álvarez; Mora-Montes, 2023). A termotolerância e o termodimorfismo são essenciais para permitir o crescimento e a colonização do hospedeiro mamífero, cuja temperatura corporal (~37 °C) é significativamente mais elevada que a ambiental (~25-28ºC) (Almeida-Paes et al., 2015; Queiroz-Telles; Buccheri; Benard, 2019). Além disso, os principais hospedeiros acometidos pela doença, gatos domésticos, apresentam temperatura corporal média de ~39ºC, exigindo maior termotolerância, a qual é encontrada especialmente em *S. brasiliensis* em relação as demais espécies do complexo *S. schenckii* (Marimon et al., 2007).

Em *S. brasiliensis* a melanina é produzida em três formas: melanina DHN (dihidroxinaftaleno), eumelanina e piomelanina, conferindo proteção não apenas contra estresse oxidativo e fagocitose, mas também contra antifúngicos (anfotericina B, terbinafina) (Almeida-Paes et al., 2015, 2016). As adesinas, como a Gp70, desempenham papel importante na adesão ao tecido hospedeiro e estão fortemente associadas à virulência (Castro et al., 2013; Gómez-Gaviria; Martínez-Álvarez; Mora-Montes, 2023). Além disso, *S. brasiliensis* produz diversas enzimas hidrolíticas, como proteinases aspárticas secretadas, lipases, fosfolipases e hemolisinas, que podem auxiliar na invasão tecidual e evasão do sistema imune, embora seu papel específico ainda necessite maior investigação (Gómez-Gaviria; Martínez-Álvarez; Mora-Montes, 2023).

Em *Sporothrix* spp. estão descritos biofilmes estruturalmente complexos, com rede de hifas associadas a matriz polimérica extracelular, que mantem a estrutura do biofilme, e canais de água, os quais permitem o transporte de nutrientes para as células que compõem essa estrutura que permite a adesão celular a superfícies bióticas ou abióticas (Brilhante et al., 2018). Estudos demonstraram a formação de biofilmes maduros e estruturalmente semelhantes aqueles formados *in vitro* em modelos *ex vivo* utilizando unhas de gatos domésticos, sugerindo que esse seja um fator fundamentalmente associado a alta transmissão zoonótica de *S. brasiliensis* (Brilhante et al., 2021; Dos Santos et al., 2022).

2.3 Epidemiologia da Esporotricose Zoonótica por *Sporothrix brasiliensis*

No final do século XX começaram a ser descritos padrões epidemiológicos da esporotricose associados a transmissão por gatos domésticos através de arranhões e mordidas, inicialmente nos estados do Rio de Janeiro e Rio Grande do Sul (Barros et al., 2004; Madrid et al., 2012; Nobre et al., 2001a; Poester et al., 2018a; Schubach et al., 2004). Esse cenário alterou o padrão clássico da esporotricose, que até então, ocorria principalmente por transmissão sapronótica, conhecido como "doença dos jardineiros", com casos esporádicos causados por contato com matéria vegetal contaminada (Marimon et al., 2007).

A esporotricose causada por *S. brasiliensis* mostrou rápida dispersão, consolidando-se como um problema de saúde pública. Os primeiros relatos no fim da década de 1990, no RJ e RS, já apontavam para a gravidade da situação, que hoje se estende a todo território brasileiro, em caráter hiperendêmico em algumas regiões (Rabello et al., 2022; Xavier et al., 2023). Essa rápida expansão está diretamente relacionada ao papel do gato doméstico como principal transmissor, devido à alta carga fúngica presente nas lesões, ao comportamento de arranhar e morder, e ao hábito cultural de acesso irrestrito às ruas, fatores que facilitam a manutenção da cadeia de transmissão (Gremião et al., 2017a). Além disso, casos epizóticos, nos quais gatos transmitem a doença para outros animais, como outros gatos e cães, também são comuns (Madrid et al., 2012).

Nos estados do Rio de Janeiro e Rio Grande do Sul, onde a epidemia iniciou, a hiperendemicidade permanece sendo uma preocupação central. O Rio de Janeiro possui notificação compulsória da doença desde 2013, e no boletim epidemiológico referente aos anos de 2019 e 2020, foram notificados 2.616 casos humanos (Secretaria de Estado de Saúde do Rio de Janeiro, 2021). No RS, Rio Grande e Pelotas são os principais epicentros da doença, a qual só passou a integrar a lista de notificação compulsória do estado em 2024. Porém, estudos demonstram que de 2010 a 2016, 372 casos de esporotricose foram diagnosticados em somente um hospital, sendo os 3 primeiros anos com média de 18 casos ao ano, enquanto os últimos três possuíam 116 casos, um aumento de 635% (Poester et al., 2018a). Nesta mesma região sul do estado, estudo de vigilância e atualização epidemiológica demonstrou um aumento da frequência de casos diagnosticados, com 643 casos entre 2017 e 2021, resultando em média anual de 126 casos (Munhoz et al., 2022).

Outras regiões do RS também tem relatado um aumento significativo de casos de esporotricose com surtos associados, entre o período de 2016-2018 e 2019-2021 houve um aumento de 500% dos casos de esporotricose humana em Porto Alegre (Munhoz et al., 2022). E entre 2020 e 2021, observou-se um aumento de 130% nos casos felinos em cidades da região metropolitana do estado (CRMV-RS, 2021).

Na região Sudeste do país, São Paulo tem se destacado com aumento progressivo dos casos. Entre 2011 e 2014 foram notificados 163 casos em felinos (Montenegro et al., 2014), e no período de 2010 a 2018 foram documentados 260 casos humanos em hospital de referência, com média de 28 casos anuais, variando de 4 a 108 casos/ano (Bittencourt et al., 2022). Em 2025, foi descrita também a dinâmica da esporotricose felina no Espírito

Santo, reforçando a expansão regional da doença e seus impactos na saúde pública (Gomes et al., 2025).

No Nordeste, Pernambuco, Paraíba e Bahia já instituíram notificação compulsória, refletindo o aumento dos casos. Entre 2016 e 2021, Pernambuco registrou 150 casos humanos e 912 animais (SEVS-PE, 2021). Em Salvador, foram notificados 90 casos humanos e 1.016 em animais apenas em 2020, sendo 99% em felinos (SMS, 2021). Mais recentemente, o Piauí entrou no mapa epidemiológico da esporotricose por *S. brasiliensis*, com a emergência de casos humanos e felinos transmitidos por gatos (Pinheiro et al., 2025).

No Centro-Oeste, estados como Goiás e Mato Grosso do Sul também já reportaram surtos e aumento da casuística (Ferreira et al., 2024; Vieira et al., 2025). Além disso, Distrito Federal e Minas Gerais instituíram notificação compulsória, em resposta ao aumento de casos. Na região Norte, o Amazonas vem apresentando expansão significativa, com relatos recentes da predominância de *S. brasiliensis* como agente da esporotricose zoonótica na capital Manaus e municípios próximos (De Souza et al., 2025; Valente et al., 2025).

Como reflexo da expansão nacional, em janeiro de 2025 a esporotricose humana passou a integrar a Lista Nacional de Notificação Compulsória, marcando um avanço para a vigilância epidemiológica no Brasil (Ministério da Saúde, 2025).

Além do território brasileiro, a doença já constitui um desafio regional na América do Sul. A Argentina possui o cenário mais preocupante, demonstrando um aumento no número de casos desde 2011, sendo relatados até então 30 casos em felinos, e 32 em humanos identificados em todo país, incluindo surtos recentes em Puerto Iguazú, na fronteira com Brasil (Córdoba et al., 2018; Etchecopaz et al., 2020, 2021, 2019; Rodriguez et al., 2023). No Chile, em 2023, foram descritos três casos felinos (Thomson et al., 2023). No Paraguai, já há registros de casos autóctones de esporotricose felina e humana por *S. brasiliensis* (Prado et al., 2023; Silvero et al., 2025). Além disso, foi descrito um caso familiar associado à transmissão por gato doméstico, envolvendo dois humanos infectados, representando um dos primeiros relatos da introdução da doença no país a partir de animais provenientes do Brasil (García Duarte et al., 2017).

E fora da América do Sul, casos importados também foram documentados. Nos Estados Unidos, uma paciente com esporotricose relatou viagem ao Brasil e mordida por felino, além disso, outro caso importado de felino brasileiro foi identificado no Reino

Unido, acometendo quatro humanos (Barnacle et al., 2023; Kaadan et al., 2020; Rachman et al., 2022).

2.4 Caracterização molecular e diversidade genética de *Sporothrix brasiliensis*

Até 2006 acreditava-se que a esporotricose possuía apenas um agente etiológico, sendo ele o *Sporothrix schenckii*. Porém, em 2006, foi realizada uma análise filogenética de isolados morfologicamente identificados como *S. schenckii* de diferentes partes do mundo a partir do sequenciamento dos genes de calmodulina (CAL), β-tubulina (BT) e quitina sintase, e neste foi observado que havia uma diferença genotípica que gerou cinco clados, resultando em cinco espécies crípticas, sendo três dessas primariamente descritas (Marimon et al., 2007, 2006). A espécie *S. brasiliensis* foi uma dentre as primariamente descritas, e este clado estava totalmente associado as amostras do Brasil, resultando então no epíteto específico *brasiliensis*. Entre as outras duas estavam *S. globosa* e *S. mexicana*.

A partir desse achado, novos estudos passaram a explorar a diversidade epidemiológica e genotípica das espécies. Em 2014 foi realizado o sequenciamento de genoma completo, comparando a espécie *S. schenckii* e *S. brasiliensis*, sendo demonstrado que o genoma de *S. brasiliensis* é maior que o de *S. schenckii* (33,2Mb vs. 32,4Mb) (Teixeira et al., 2014b). Além disso, *S. brasiliensis* é um organismo haploide, que possui cromossomos que variam de 5 a 7 a depender da cepa, sugerindo ocorrência de rearranjos, como translocações, que explicam essa variação (Sasaki et al., 2014).

Estudos iniciais apontavam que *S. brasiliensis* era monofilético de modo reprodutivo clonal, resultando em uma baixa diversidade haplotípica, e baixa recombinação gênica e números de mutações (De Melo Teixeira et al., 2015; Rangel-Gamboa et al., 2016; Rodrigues; De Hoog; De Camargo, 2014, 2013). Posteriormente, foi identificada a presença de genes de acasalamento *mating type* (MAT) em *S. brasiliensis*, sugerindo potencial de reprodução sexual com distribuição geográfica entre RJ (MAT1-2) e RS (MAT1-1) (De Melo Teixeira et al., 2015).

A diferença genotípica dos isolados que estão no RJ e RS, os epicentros do estabelecimento da epidemia da esporotricose zoonótica, foi confirmada por diversas técnicas, entre elas, análise filogenética de fator de alongamento-1α (EF-1α), e pelo uso de genotipagem do polimorfismo do comprimento de fragmento amplificado (AFLP) (De Carvalho et al., 2020; Rodrigues et al., 2013; Zhang et al., 2015). Nesse contexto, foi demonstrado que o genótipo predominante no RJ, também circula nos estados do Paraná, Minas Gerais e São Paulo, sugerindo a expansão a partir do RJ (Rodrigues et al., 2013).

Mais recentemente, Losada e colaboradores (2023) desenvolveram marcadores microssatélites para genotipagem de *Sporothrix* spp., identificado quatro principais clados de *S. brasiliensis.* O clado do RS mostrou-se o mais distante, enquanto o do RJ apresentou menor diversidade em comparação ao Nordeste, sugerindo migração unidirecional do Sudeste para o Nordeste (Losada et al., 2023). Esses achados reforçam que a diversidade genotípica dos surtos brasileiros está majoritariamente relacionada ao genótipo circulante no RJ.

Spruijetenburg et al. (2023) aplicaram microssatélites direcionada especificamente para avaliação de cepas de *S. brasiliensis*, em combinação com sequenciamento de genoma total, corroborando com a existência de diferentes genótipos que surgiram independentemente de *S. brasiliensis*, dividido principalmente no clado relacionado ao RJ, e genótipos diferentes indicando introdução independente do RS (Spruijtenburg et al., 2023a). Esse estudo vem sendo utilizado como referência para vigilância epidemiológica em outras análises, demonstrando que cepas provenientes do Paraguai e do Paraná se agrupam majoritariamente com o clado do RJ, enquanto cepas do RS se diferenciam desses de forma consistente (Do Prado et al., 2025).

Um estudo de Eudes Filho e colaboradores (2020) por sequenciamento de genoma total evidenciou que isolados do RJ e do Distrito Federal (DF) possuem diferenças genotípicas que os separam em populações monofiléticas. Junto disso, em 2024, Rabello realizou a técnica de tipagem por sequenciamento multi-*loci* (MLST), e nessa descreveu 4 clados, com pelo menos 7 haplótipos diferentes. Neste, a diferenciação das cepas do RJ e RS se mantém como anteriormente relatado, e é concordante com o clado monofilético do DF, além disso, também agrupa as cepas de MG em um grupo monofilético. Junto disso, esse trabalho possui como adicional a avaliação de uma cepa provinda do Paraguai, a qual também se diferencia das encontradas no Brasil (De Souza Rabello et al., 2024). De forma concordante com esses achados, Colombo et al. (2024), utilizando AFLP, demonstraram que cepas de Minas Gerais também tiveram introdução independente, não relacionadas aos genótipos do RJ ou do RS (Colombo et al., 2024).

Com isso, evidencia-se que, apesar das diferentes técnicas utilizadas, não se observa uma expansão clonal única da esporotricose no Brasil, mas sim diferentes introduções independentes. As duas mais bem caracterizadas correspondem às do RJ e do RS, mas novos epicentros, como MG e DF, vêm sendo descritos. Ainda há, entretanto, um longo caminho para compreender plenamente a epidemiologia molecular de *S.*

brasiliensis.

Apesar da disponibilidade de diversas metodologias, ainda não há estudo comparativo abrangente que determine qual técnica apresenta maior poder discriminatório (Spruijtenburg et al., 2024a). Estudos futuros são necessários para essa avaliação e para indicar a ferramenta mais adequada conforme o contexto epidemiológico regional. Com a diferenciação epidemiológica das espécies de *Sporothrix*, a identificação das espécies é de extrema relevância para maior compreensão da doença, visto que há variação quanto as áreas endêmicas, fonte de infecção, virulência e suscetibilidade a antifúngicos (Almeida-Paes et al., 2015; Della Terra et al., 2017). Dado isso, é preconizada a identificação intraespecífica genotipicamente, visto que as características fenotípicas não são de fácil diferenciação (Marimon et al., 2007; Rodrigues; De Hoog; De Camargo, 2013).

Para identificação das espécies são reportados uso de sequenciamento parcial de genes tais como a calmodulina, β-tubulina e EF-1α (De Beer; Duong; Wingfield, 2016; Marimon et al., 2007, 2006, p. 200; Rodrigues; De Hoog; De Camargo, 2013). Além desses, também pode ser utilizado o *barcoding* do espaçador transcrito interno, que possui um poder discriminatório significativo dentro das principais espécies patogênicas (ZHOU et al. 2014). Porém, tem sido descritas técnicas mais rápidas e menos custosas para identificação de espécie nos últimos anos, como por exemplo a descrição de PCR *fingerprint* com primer universal TB3, PCR-RFLP, PCR espécie-específicos, e PCR em tempo real (Oliveira et al., 2013; Rodrigues; De Hoog; De Camargo, 2014, 2015; Zhang et al., 2019).

2.5 Manifestações Clínicas da Esporotricose

A esporotricose é classicamente uma micose subcutânea, que no caso da doença causada por *S. brasiliensis* é transmitida principalmente por mordidas ou arranhões de gatos infectados.

2.5.1 Manifestações em Humanos

Em hospedeiros humanos a doença majoritariamente se manifesta na pele e nos tecidos subcutâneos, sendo a forma linfocutânea a forma mais comum, responsável por 46 a 92% dos casos (Brandolt et al., 2019; Poester et al., 2018b). Clinicamente, inicia-se com uma pequena pústula eritematosa onde houve a inoculação que com o tempo torna-se nodular, e progride para lesões papulonodulares ao longo do cordão linfático da região acometida (Orofino-Costa et al., 2022) (**Figura 2A**). Esse tipo de lesão é mais comum em membros superiores, e em crianças, na face. A forma cutânea fixa é a segunda apresentação clínica mais frequente, contemplando cerca de 25% dos casos, e se caracteriza por uma lesão ulcero-crostosa de bordas irregulares no local de inoculação, sem progressão linfática (**Figura 2B**). A manifestação pode estar associado a capacidade imunológica do hospedeiro, ou a virulência da cepa (Freitas, 2009; Orofino-Costa et al., 2017).

As formas mucosas também têm sido relatadas, quando há contato da propágulos fúngicos direto com mucosa. A esporotricose ocular é a mais comumente descrita (**Figura 2C**), abrangendo 86% dos casos, havendo também descrições em mucosa nasal (11%) e oral (<1%) (Poester et al., 2024; Queiroz-Telles; Buccheri; Benard, 2019). Essas manifestações podem surgir após contato direto com secreções de gatos infectados, já tendo sido demonstrado que a aerossolização de conídios a partir de gatos com acometimento respiratório pelo fungo pode facilitar esse tipo de infecção (Bastos et al., 2022).

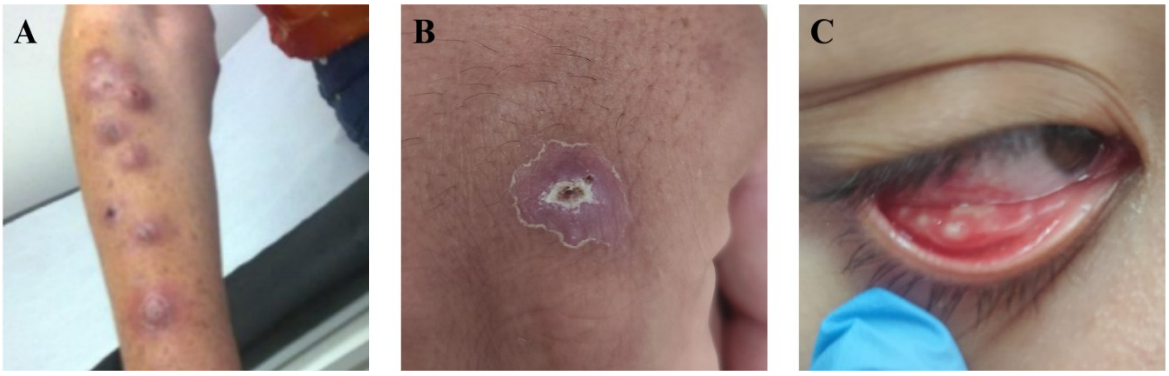

Figura 2: Manifestações clínicas da esporotricose humana. (A) Forma linfocutânea, caracterizada por lesões nodulares ao longo do trajeto linfático; (B) Forma cutânea fixa, com lesão restrita ao local da inoculação; (C) Forma em mucosa ocular, com nódulos na conjuntiva inferior. Imagens: LabMicoFAMED-FURG e SAE, HU-FURG/EBSERH

A forma osteoarticular geralmente decorre da contiguidade de lesões cutâneas profundas, frequentemente associadas a mordidas, e acomete principalmente extremidades pela proximidade com ossos e articulações. Essa forma é mais frequentemente observada em indivíduos imunocomprometidos (Orofino-Costa et al., 2022; Ramos et al., 2021). A forma pulmonar primária ocorre pela inalação de propágulos fúngicos se manifesta principalmente em pacientes que já possuem doença pulmonar prévia. Também pode resultar de disseminação hematogênica, especialmente em pacientes imunossuprimidos (Fichman et al., 2022; Orofino-Costa et al., 2022). E a forma sistêmica disseminada é considerada rara, mas representa a evolução mais grave da doença. Pode ocorrer com ou sem lesão cutânea inicial, e resulta da disseminação hematogênica do fungo, acometendo fígado, baço, rins, ossos e sistema nervoso central, sendo altamente associada à imunossupressão, especialmente em pacientes com HIV/aids (Queiroz-Telles; Buccheri; Benard, 2019). Casos de meningite por *Sporothrix* spp. já foram relatados, configurando manifestação grave e de difícil manejo (Freitas et al., 2012; Lima; Vallier; Silva, 2021; Queiroz-Telles; Buccheri; Benard, 2019).

Além das formas clássicas, manifestações de hipersensibilidade (ou esporotricose imunorreativa) vêm sendo cada vez mais descritas, especialmente em áreas hiperendêmicas. Envolvem eritema nodoso, eritema multiforme, artrite asséptica e síndrome de Sweet, geralmente sem isolamento do fungo (Orofino-Costa et al., 2010; Poester et al., 2024; Procópio-Azevedo et al., 2021). Essas manifestações refletem uma resposta imunológica exacerbada a intensa exposição aos propágulos fúngicos, e representam a forma atípica mais frequentemente relatada.

2.5.2 Manifestações em Animais

Em gatos, hospedeiros mais suscetíveis e que desenvolvem as formas mais graves da doença, predominam lesões cutâneas nodulares e ulceradas em diferentes regiões, mais comumente na cabeça e região nasal (Gremião et al., 2020; Schubach et al., 2004). Essas lesões frequentemente evoluem para úlceras serossanguinolentas ou purulentas, recobertas por crostas. A disseminação é comum e está associada à alta carga fúngica nos felinos **(Figura 3)**.

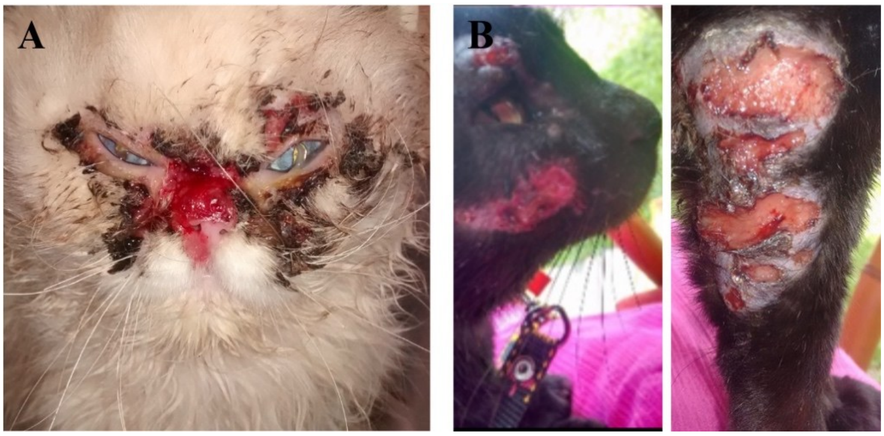

Figura 3: Manifestações clínicas da esporotricose em gatos domésticos. (A) Lesão cutânea localizada, restrita a um único sítio de inoculação; (B) Lesões cutâneas disseminadas, múltiplas e distribuídas em diferentes regiões corporais. Imagens: LabMicoFAMED-FURG e veterinários cadastrados.

Sinais respiratórios (espirros, secreção nasal, dispneia) podem ser observados mesmo na ausência de lesão cutânea, devido ao acometimento da mucosa respiratória (Schubach et al., 2004). Devido à gravidade, a esporotricose felina pode evoluir para forma disseminada, associada a falha terapêutica e óbito (Barros et al., 2004; Gremião et al., 2014).

Embora os gatos domesticos sejam o principal hospedeiro acometido pelo *S. brasiliensis*, outros animais também podem ser infectados. Cães apresentam lesões cutâneas ulceradas, linfadenomegalia e, em alguns casos, sinais respiratórios e disseminados, geralmente após contato próximo com gatos infectados (Dumont-Viollaz; Santos; Thomson, 2025; Gremião et al., 2017a). Além disso, há relatos ocasionais em roedores, tatus, esquilos e até equinos, geralmente em áreas endêmicas, atuando como hospedeiros acidentais (Rossow et al., 2020).

2.6 Diagnóstico da Esporotricose

O diagnóstico da esporotricose é uma união de dados clínicos, epidemiológicos e laboratoriais (De Lima Barros; De Almeida Paes; Schubach, 2011). A doença pode ser considerada possível quando há características clínicas e histórico epidemiológico que suportem a infecção por *Sporothrix* spp. (Queiroz-Telles; Buccheri; Benard, 2019). A confirmação, entretanto, depende da demonstração do agente etiológico por cultivo micológico, citopatologia, histopatologia ou técnicas moleculares.

O cultivo micológico é considerado o padrão-ouro para confirmação diagnóstica. O teste é positivo quando há crescimento de colônias fúngicas com características macro e micromorfológicas típicas de *Sporothrix* spp. As principais amostras utilizadas para cultivo são secreção de lesões coletadas com *swabs*, biópsias de lesão e aspirado de linfonodos. A coleta de secreções a partir de *swab* é a forma menos invasiva, podendo ser utilizada em ambiente ambulatorial e à campo. A sensibilidade do cultivo varia de 66% a 100%, pois depende de fatores pré-analíticos como qualidade da coleta, transporte e armazenamento da amostra. Recomenda-se semear a amostra o mais rápido possível, transportar *swabs* em meio Stuart e conservar biópsias em salina estéril (Civila et al., 2004; Gremião et al., 2020; Orofino-Costa et al., 2017; Schubach et al., 2002).

O cultivo é realizado em ágar Sabouraud com cloranfenicol, podendo incluir cicloheximida, incubado a 25–30 ºC por até 30 dias, dado o possível crescimento tardio (Sanchotene et al., 2015). O crescimento da fase filamentosa ocorre geralmente em 5–7 dias e pode ser confirmado por conversão para a fase leveduriforme em meio BHI (De Lima Barros; De Almeida Paes; Schubach, 2011).

O exame micológico direto (EMD) pode ser realizado por coloração de Gram (células Gram-positivas), ou por técnicas como Prata metenamina de Grocott (GMS), que aumenta a visibilidade das estruturas fúngicas. Em humanos, a sensibilidade do EMD é considerada baixa (<20%), sendo rara a visualização das estruturas fúngicas em secreções ou biópsias cutâneas (De Lima Barros; De Almeida Paes; Schubach, 2011; Orofino-Costa et al., 2017). Já em felinos, devido à elevada carga fúngica, a positividade do exame direto pode ultrapassar 80% em esfregaços de secreções de lesões e aspirados de linfonodos, tornando o método bastante útil no diagnóstico inicial desses animais (Gremião et al., 2017; Silva et al., 2015).

No exame histopatológico, as colorações ácido periódico de Schiff (PAS) e GMS revelam tipicamente um padrão granulomatoso associado a corpos asteroides. Em humanos, a sensibilidade da histopatologia é baixa, mas em felinos pode alcançar valores próximos de 90%, reforçando novamente a diferença de carga fúngica entre espécies (Mahajan, 2014; Zancope-Oliveira et al., 2015).

Testes sorológicos ainda não são amplamente disponíveis comercialmente, mas têm potencial para diagnóstico e monitoramento terapêutico, especialmente nas formas disseminadas e atípicas. Diferentes antígenos vêm sendo avaliados em ensaios ELISA, imunoblot e testes rápidos (Bernardes-Engemann et al., 2014; Cognialli et al., 2022; Fernandes et al., 2011).

As técnicas moleculares vêm ganhando destaque, sobretudo para identificação interespecífica e diagnóstico rápido. Tradicionalmente aplicadas a isolados já cultivados, hoje já se demonstrou a viabilidade de aplicação direta em amostras clínicas. Métodos descritos incluem PCR espécie-específico (Gonsales et al., 2020; Poester et al., 2021; Rodrigues; De Hoog; De Camargo, 2015), *nested*-PCR (Almeida-Silva et al., 2023; Luiz et al., 2022), *rolling circle amplification* (Rodrigues et al., 2015) e qPCR (Della Terra et al., 2022; Marques de Macedo et al., 2023; Zhang et al., 2019).

Rodrigues et al. (2015) aplicaram PCR espécie-específico em biópsias de camundongos experimentalmente infectados, com sensibilidade superior à histolopatogia. Outro estudo, utilizando 15 biópsias de pacientes humanos com esporotricose confirmada, demonstrou que 13 delas (86,7%) foram positivas tanto pelo PCR espécie-específico quanto pelo nested-PCR (De Freitas et al., 2025).

Zhang et al. (2019) relataram qPCR multiplex em biópsias felinas com sensibilidade de 93% versus 87% do cultivo. Três estudos avaliaram swabs: dois com PCR espécie-específico e um com qPCR, evidenciando concordância moderada com o cultivo, mas maior rapidez (Della Terra et al., 2022; Gonsales et al., 2020; Poester et al., 2021). Della-Terra et al. (2021) relataram sensibilidade de 98% do qPCR em swabs, enquanto López et al. (2024) otimizaram o método para tecidos parafinizados (López et al., 2024). Também foi descrito RT-qPCR com sensibilidade de 91,2% em múltiplas amostras clínicas (Marques de Macedo et al., 2023).

Casos atípicos também têm sido alvo das técnicas moleculares: Almeida-Silva et al. (2023) aplicaram PCR em líquido cefalorraquidiano de pacientes com meningite por *Sporothrix* spp., com resultados promissores. Além disso, a técnica de *Loop-mediated isothermal amplification* (LAMP) foi recentemente avaliada: Donadel et al. (2024) relataram especificidade de 100% em amostras de animais experimentais, e outro estudo mais recente aplicou LAMP diretamente em amostras clínicas de felinos, sem necessidade de extração prévia de DNA, alcançando 100% de sensibilidade e especificidade em amostras positivas no cultivo (Araujo et al., 2024; Do Carmo Silva et al., 2025) (**Tabela 1**)

Tabela 1: Resumo de estudos de diagnóstico molecular de *Sporothrix brasiliensis* em amostras clínicas não experimentais, com sensibilidade e especificidade

Técnica	**Hospedeiro**	**Amostra**	**Número de amostras**	**Alvo**	**Sensibilidade**	**Especificidade**	**Autor, ano**
qPCR multiplex	Humano	Biópsia de pele *spiked*	33	Calmodulina	93,90%	100%	Zhang, 2019
PCR	Gato	Secreção de lesão cutânea	70	Calmodulina	65,70%	84,30%	Gonsales et al., 2020
PCR	Gato, Cachorro, Humano	Secreção de lesão cutânea	74	Calmodulina	59%	100%	Poester et al., 2021

qPCR	Gato	Secreção de lesão cutânea	85	β-tubulina	98,57%	100%	Della-Terra et al., 2021
Nested PCR	Gato	Biópsia de pele parafinizado	6	18s rRNA	50%	100%	Luiz et al., 2022
RT-qPCR	Humano	Lesões cutâneas,	61	Subunidade mitocondrial	96,72%	100%	Marques-Macedo, 2023
		Respiratória	3		100%	100%	
		Fluído cefalorraquidiano	4		0%	100%	
qPCR	Humano	Fluído cefalorraquidiano	30	Calmodulina	78,60%	91,70%	Almeida-Silva et al., 2023
Nested PCR				18s rRNA	92,90%	75%	
PCR				Calmodulina	0%	100%	
qPCR multiplex	Gato	Biópsia de pele fresca	10	β-tubulina	100%	100%	López, 2024
		Biópsia de pele parafinizada	9		66,7%	100%	
Nested PCR	Humano	Biópsia de lesão de pele	15	18s rRNA	86,7%	-	Freitas et al., 2025
PCR				Calmodulina	86,7%	-	
LAMP	Gato	Secreção de lesão cutânea	17	Quitina sintase	100%	100%	Silva et al., 2025

Legenda: PCR: Reação em cadeia da polimerase; LAMP: *Loop-mediated isothermal amplification;* qPCR|: Reação em cadeia da polimerase quantitativa; RT: transcriptase reversa

2.7 Tratamento da Esporotricose

A escolha do tratamento para esporotricose depende principalmente da forma clínica, da epidemiologia e da condição imunológica do hospedeiro, além da resposta individual ao antifúngico utilizado. No Brasil, os fármacos atualmente empregados incluem itraconazol, iodeto de potássio, terbinafina e anfotericina B.

O itraconazol é considerado o tratamento de escolha tanto para humanos quanto para felinos nas formas cutâneas e que não são de alto risco (De Lima Barros; De Almeida Paes; Schubach, 2011; Kauffman et al., 2007). Esse fármaco é predominantemente fungistático e atua inibindo a enzima lanosterol 14α-desmetilase, dependente do citocromo P450, bloqueando uma etapa essencial da via de biossíntese do ergosterol, o principal esterol da membrana celular fúngica. Como consequência, ocorre depleção de ergosterol e acúmulo de esteróis tóxicos, comprometendo a integridade da membrana e a viabilidade fúngica (Odds; Brown; Gow, 2003).

Em humanos as doses recomendadas de ITC variam de 100 a 400mg ao dia dependendo do estágio da doença, e resposta do paciente ao tratamento. O tempo de tratamento é geralmente de 3 a 6 meses para formas cutâneas fixas e linfocutâneas, mas pode ultrapassar 12 meses em casos osteoarticulares, pulmonares ou disseminados (Orofino-Costa et al., 2022; Poester et al., 2022a). Apesar de eficaz, o ITC apresenta limitações: hepatotoxicidade, cefaleia, distúrbios gastrointestinais, contraindicações em gestantes e indivíduos com insuficiência hepática/renal, além de interação medicamentosa com mais de 200 fármacos (Kauffman et al., 2007).

Para felinos, o tratamento da esporotricose é considerado desafiador, com dose usual de ITC de 100mg/animal/dia (8-10mg/kg), administrada por no mínimo 4 a 6 meses. O tempo prolongado de tratamento, o custo elevado e a dificuldade de administração oral resultam em taxas de abandono entre 30–40%, e a taxa de sucesso terapêutico varia de ~50-72% conforme a adesão do tutor, a forma clínica e a condição imunológica do animal. Efeitos adversos incluem hepatotoxicidade e anorexia, exigindo monitoramento clínico (De Souza et al., 2018; Gremião et al., 2014, 2020).

O iodeto de potássio é comumente utilizado em associação ao ITC como alternativa eficaz e de baixo custo em casos não responsivos a monoterapia, especialmente nas formas cutâneas fixas e linfocutâneas (Da Rocha et al., 2018; Lyra et al., 2021). Seu mecanismo de ação não está completamente esclarecido, mas acredita-se que atue de forma indireta, modulando a resposta imunológica do hospedeiro, estimulando a atividade dos neutrófilos e a fagocitose (Xue et al., 2009). Contudo, seu uso é limitado pelos efeitos colaterais: distúrbios gastrointestinais e alterações da função tireoidiana, além de contraindicação em gestantes e pacientes com nefropatia (Gremião et al., 2020; Orofino-Costa et al., 2022).

A terbinafina é um antifúngico da classe das alilaminas que inibe seletivamente a enzima esqualeno epoxidase, bloqueando uma etapa inicial da biossíntese do ergosterol. Como consequência, ocorre depleção de ergosterol na membrana celular e acúmulo de esqualeno tóxico, resultando em efeito fungicida (Odds; Brown; Gow, 2003). É um antifúngico utilizado no tratamento da esporotricose como alternativa em pacientes que tem contraindicações para uso do ITC ou iodeto de potássio, dado que possui menor interação medicamentosa (Antunes et al., 2009; Francesconi et al., 2009). Sua eficácia é relatada somente nas formas cutâneas, e a dose recomendada para humanos é de 250mg/dia, podendo ser aumentada para 500mg/dia dependendo da clínica do paciente (Orofino-Costa et al., 2022; Poester et al., 2022a). Em gatos, estudos demonstram ausência de atividade antifúngica do fármaco (Viana et al., 2024).

A anfotericina B (AMB) é um antifúngico da classe dos polienos, administrado por via endovenosa e reservado para casos graves ou refratários. Atua ligando-se ao ergosterol da membrana fúngica e formando poros que aumentam a permeabilidade celular, levando ao vazamento de íons e à morte do fungo. Apesar da alta eficácia, seu uso é limitado pela toxicidade, principalmente nefrotoxicidade e distúrbios eletrolíticos, exigindo monitoramento rigorosos (Kauffman et al., 2007; Odds; Brown; Gow, 2003). Em humanos, a dose cumulativa total varia de 1 a 3g, sendo recomendada a dose de 3-5mg/dia na forma lipídica, e desoxicolato 0,7 – 1mg/dia (Kauffman et al., 2007; Orofino-Costa et al., 2022). Em felinos, a administração é também limitada devido à alta toxicidade, e aos efeitos adversos graves, sendo recomendado o uso somente nas formas mais graves e disseminadas. Já foi descrito o uso da AMB intralesional ou subcutânea associada ao ITC oral como alterativa (Gremião et al., 2011, 2020).

2.7.1 Resistência de *Sporothrix brasiliensis*

Parte da problemática já conhecida dentro das doenças fúngicas em que se possui uma limitada gama de antifúngicos disponíveis devida à similaridade da célula fúngica com a célula dos mamíferos. Além disso, o investimento insuficiente e a negligência histórica em relação a esses patógenos contribuem para a dificuldade no acesso ao tdessas infecções. Para a esporotricose, as opções terapêuticas também são restritas, e já são relatados casos de falha terapêutica com o uso do tratamento de escolha, frequentemente necessitando da associação de medicamentos para resposta clínica (Gremião et al., 2020; Orofino-Costa et al., 2022; Poester et al., 2022a). Além disso, estudos têm demonstrado uma resistência crescente de *Sporothrix brasiliensis* frente aos antifúngicos usuais, particularmente ao itraconazol, mas também envolvendo outros azólicos de uso clínico, como posaconazol, isavuconazol e voriconazol (Nakasu et al., 2021; Ribeiro Dos Santos et al., 2024; Waller et al., 2021c).

Porém, a ainda não há pontos de corte clínicos estabelecidos, que correlacionem diretamente dados laboratoriais e falha terapêutica, tornando desafiadora a interpretação dos resultados *in vitro* de suscetibilidade antifúngica de *Sporothrix* spp. (Espinel-Ingroff et al., 2017). As principais espécies clínicas de *Sporothrix* possuem concentração inibitória mínima (MIC) de antifúngicos relacionada com valores de corte epidemiológicos, sendo considerados selvagens e não selvagens, esses associados a maior associação a aquisição de mecanismos de resistência adicional (Espinel-Ingroff et al., 2017). Para *S. brasiliensis* não são claros os mecanismos de resistência e nem quais genes estão principalmente associados, como já é descrito para outros patógenos fúngicos, incluindo *Candida* spp. e *Aspergillus* spp. (Spruijtenburg et al., 2023b; Verweij et al., 2020). Além disso, um estudo relata que a resistência *in vitro* pode se associar a desfechos clínicos mais graves, especialmente em pacientes imunocomprometidos, indicando que os testes de suscetibilidade podem ter papel orientador na terapia da esporotricose (Bernardes-Engemann et al., 2022).

Estudos sugerem associação com mutações de genes específicos que codificam alvos dos medicamentos azólicos, e as alterações associadas a aneuploidia, que fazem com que os genes e variações fenotípicas de resistência sejam expressos pelo maior número de cópias cromossômicas (Ribeiro Dos Santos et al., 2024; Teixeira et al., 2022). Teixeira et al., (2022) realizou sequenciamento de genoma total (WGS) de cepas selvagens e não selvagens de *S. brasiliensis* para avaliar quais principais mutações estão associadas a essa característica fenotípica, identificando tanto variações estruturais cromossômicas quanto polimorfismos pontuais, incluindo mutações em gene que codifica um ativador transcricional associado à resistência a azóis. Já Bombassaro et al. (2023), também utilizando WGS, não conseguiram associar as mutações detectadas a genes previamente reconhecidos em outros patógenos, sugerindo mecanismos ainda não elucidados.

Portanto, são necessários estudos adicionais para esclarecer quais mutações têm impacto clínico real e se a variabilidade genômica de isolados de diferentes regiões endêmicas pode estar relacionada à maior resistência antifúngica. Além disso, limitações intrínsecas ao uso do ITC, como interações medicamentosas, contraindicações, absorção variável e necessidade de monitoramento de níveis séricos, reforçam a necessidade da busca por alternativas terapêuticas.

A busca por novos compostos pode ocorrer por diferentes estratégias. Uma delas é o reposicionamento de fármacos já disponíveis no mercado, cujas características farmacológicas e perfis de segurança estão bem descritos. Nesse sentido, estudos já demonstraram atividade in vitro frente a *S. brasiliensis* de medicamentos como tacrolimus, miltefosina, ibuprofeno, olorofilm, ácido acetilsalicílico, niclosamida, amlodipina, entre outros (Borba-Santos et al., 2017, 2021, 2022; Poester et al., 2023; Ramos et al., 2024; Seibert et al., 2022; Xavier et al., 2023). Outra estratégia corresponde à síntese e avaliação de novas moléculas químicas, muitas das quais têm mostrado resultados promissores em ensaios laboratoriais, já ultrapassando a marca de 30 compostos testados (Xavier et al., 2023). Por fim, a bioprospecção de compostos bioativos surge como uma vertente complementar, explorando a diversidade natural de micro-organismos e ambientes pouco estudados, o que pode ampliar as opções terapêuticas disponíveis.

2.8 Bioprospecção de compostos com potencial antifúngico frente a *S. brasiliensis*

O histórico da descoberta de medicamentos está diretamente relacionado à exploração da biodiversidade. O primeiro antibiótico, a penicilina, foi descoberto a partir de um fungo em 1929 (Fleming, 1929), e até hoje grande parte dos fármacos de uso clínico deriva de compostos naturais. Entre os antifúngicos, duas das principais classes, os polienos e as equinocandinas, têm origem em microrganismos (Butts; Krysan, 2012). O avanço da resistência aos antifúngicos consolida a necessidade de explorar novas fontes de moléculas bioativas. Nesse cenário, a bioprospecção de ambientes naturais tem se mostrado estratégica na busca por alternativas terapêuticas.

Frente a *Sporothrix brasiliensis,* já foram testados compostos naturais derivados de extratos diretos, óleos essenciais, e produtos secundários. O uso de extratos de *Schinus terebinthifolius* (aroeira-vermelha) demonstrou ação *in vitro* frente a isolados de *S. brasiliensis*, assim como extrato de *Origanum majorana* (orégano), e de *Plinia peruviana* (jabuticaba) (Almeida-Silva et al., 2022; Waller et al., 2021a). E o óleo essencial feito a partir do orégano já teve comprovação de aplicação experimental *in vivo* de esporotricose cutânea (Waller et al., 2021b). Além disso, os metabólitos secundários que são sabidamente produzidos por compostos naturais também são alvos para testes *in vitro* (Brilhante et al., 2015; Waller et al., 2023).

Apesar desses avanços, fontes marinhas são ainda inexploradas frente ao gênero *Sporothrix*. Esse ambiente abriga uma biodiversidade singular, estimada em mais de 250 mil espécies. As condições extremas de pressão, salinidade e temperatura contribuem para a síntese de metabólitos secundários com estruturas e mecanismos distintos dos encontrados em ambientes terrestres (Cardoso et al., 2020). Muitos desses compostos marinhos já apresentaram propriedades farmacológicas relevantes, incluindo atividade antifúngica, além de características farmacocinéticas e farmacodinâmicas favoráveis, que os tornam candidatos promissores para o tratamento de infecções fúngicas invasivas, inclusive por cepas resistentes (Hang; Lu; Jiang, 2024).

O ambiente marinho é reconhecido pela produção de compostos únicos, com estruturas químicas e mecanismos de ação diferenciados, incluindo metabólitos derivados de esponjas, pepinos-do-mar, macroalgas e microrganismos associados (Calabro et al., 2017; Cardoso et al., 2020; Khattab et al., 2018; Martins et al., 2018). Já foram descritos compostos marinhos ativos contra fungos de relevância médica, como *Candida* spp., *Cryptococcus* spp. e *Aspergillus* spp., sugerindo que tais moléculas podem ser alternativas viáveis também frente a *S. brasiliensis* (Cardoso et al., 2020). Notavelmente, glicosídeos triterpênicos isolados de pepinos-do-mar (Holothuroidea) demonstraram atividade antifúngica de amplo espectro, incluindo *Sporothrix schenckii*, espécie próxima a *S. brasiliensis*, com valores de MIC baixos e promissores (Kumar et al., 2007).

Nesse contexto, as microalgas têm se consolidado como um dos grupos de maior interesse biotecnológico. Esses organismos são fontes ricas em lipídios, pigmentos carotenoides, ácidos graxos poli-insaturados, polissacarídeos sulfatados e fenólicos, moléculas já descritas com atividades antimicrobiana, anti-inflamatória e antioxidante (Chiriví-Salomón; García-Huérfano; Giraldo, 2024; Falaise et al., 2016). Ensaios com espécies como *Chlorella* spp. já demonstraram ação antifúngica contra gêneros como *Aspergillus*, *Fusarium* e *Candida*, incluindo a inibição do crescimento micelial (Perveen et al., 2022; Zielinski et al., 2020).

Entre as microalgas, o gênero *Nannochloropsis* tem recebido destaque, principalmente pela abundância de metabólitos bioativos e pela facilidade de cultivo. Estudos com *Nannochloropsis oceanica* apontaram atividade antimicrobiana moderada frente a fungos como *Aspergillus* spp. e *Fusarium* spp., ainda que os resultados sejam preliminares (Aparnadevi; Bragadeeswaran; Ranjithkumar, 2022; Scaglioni et al., 2019). Além disso, revisões recentes ressaltam o potencial de *Nannochloropsis* spp. em virtude da sua potente atividade anti-inflamatória e antioxidante *in vitro*, reforçando seu papel como fonte de moléculas bioativas (Conde et al., 2023). Ademais, a priorização de frações polares e semi-polares de microalgas é recomendada, considerando a maior concentração de metabólitos bioativos nesses extratos e os resultados já descritos frente a outros fungos (Cardoso et al., 2020; Mateos; Pérez-Correa; Domínguez, 2020).

3. JUSTIFICATIVA

As principais frentes para o enfrentamento da endemia associada à esporotricose zoonótica incluem o conhecimento epidemiológico, a vigilância contínua, o diagnóstico precoce e o tratamento adequado. Nesse sentido, o Rio Grande do Sul ocupa um papel estratégico, pois foi um dos epicentros iniciais da doença no Brasil, juntamente ao RJ, sendo ambos caracterizados pela emergência independente de focos epidêmicos (Nobre et al., 2001; Poester et al., 2018; Rabello et al., 2024; Xavier et al., 2004). Diferentemente do que se encontra descrito na literatura científica sobre os isolados fúngicos do RJ, os estudos genotípicos direcionados para o RS incluem uma baixa quantidade de isolados, além de ainda serem escassos.

Assim, torna-se essencial aprofundar o estudo da variação genotípica no RS, compreender sua expansão e avaliar como essas linhagens locais podem contribuir para o aumento da endemicidade da doença no cenário nacional e até mesmo internacional. A análise genética integrada ao contexto regional permite reconhecer não apenas as rotas de disseminação, mas também potenciais diferenças em infectividade, virulência e resistência, informações indispensáveis para subsidiar políticas públicas e o manejo eficaz de surtos epidêmicos.

O aumento do número de casos também tem revelado manifestações atípicas, como as reações de hipersensibilidade, cuja ocorrência ainda é pouco descrita e frequentemente confundida com outras condições (Poester et al., 2024). Investigar essas apresentações no contexto hiperendêmico do RS e compreender o espectro clínico local amplia o entendimento sobre a epidemiologia nacional e sobre a interação entre fungo e hospedeiro, além de contribuir para reduzir erros diagnósticos e terapêuticos.

No campo do diagnóstico, a dependência do cultivo micológico como padrão-ouro representa uma limitação importante, uma vez que o crescimento lento do fungo retarda a confirmação da doença, somando-se à carência de profissionais especializados para realização de exames micológicos clássicos. Nesse sentido, a validação de métodos moleculares espécie-específicos a partir de amostras clínicas não invasivas justifica-se como alternativa capaz de encurtar o tempo até o diagnóstico, ampliar o acesso e favorecer o início precoce do tratamento, impactando diretamente no prognóstico dos pacientes.

O tratamento, por sua vez, permanece desafiador. O itraconazol, fármaco de primeira escolha, apresenta taxas de falha clínica, necessidade de doses elevadas em isolados do RS e eficácia limitada em gatos, além da crescente documentação de resistência em *S. brasiliensis* (Poester et al., 2022; Ribeiro Dos Santos et al., 2024; Waller et al., 2021c). Esse cenário reforça a urgência de integrar a vigilância da suscetibilidade antifúngica ao contexto epidemiológico local, associando-a à variabilidade genética para uma compreensão mais ampla da dinâmica regional da endemia, bem como de seus reflexos no cenário nacional e internacional. Paralelamente, torna-se fundamental a busca por novas alternativas terapêuticas. Nesse contexto, a bioprospecção de compostos bioativos, como microalgas marinhas da região costeira do RS, surge como estratégia complementar na busca por novos compostos com atividade antifúngica.

Assim, esta tese se justifica pela necessidade de integrar estudos de vigilância epidemiológica, clínica, diagnóstico e terapêutica no Rio Grande do Sul, região estratégica para compreender a dinâmica atual da esporotricose no Brasil e na América Latina.

4. OBJETIVOS

4.1 Objetivo geral

Avaliar os genótipos circulantes de *Sporothrix brasiliensis* no Rio Grande do Sul e seu perfil de suscetibilidade antifúngica, descrever o primeiro caso de esporotricose causada por *S. brunneoviolacea*, caracterizar manifestações clínicas de hipersensibilidade em uma região hiperendêmica, validar novos métodos diagnósticos aplicáveis à esporotricose e investigar alternativas terapêuticas a partir de compostos naturais.

4.2 Objetivos específicos

- Caracterizar genotipicamente isolados de *S. brasiliensis* provenientes de casos clínicos humanos e animais em distintas regiões do Rio Grande do Sul;
- Comparar os genótipos identificados no Rio Grande do Sul com aqueles previamente descritos em outros estados brasileiros;
- Avaliar a suscetibilidade antifúngica de isolados de *S. brasiliensis* frente a oito antifúngicos de uso clínico;
- Correlacionar o perfil de suscetibilidade antifúngica do fármaco de escolha com os diferentes genótipos identificados;
- Descrever e caracterizar o primeiro caso de esporotricose felina causada por *Sporothrix brunneoviolacea*, incluindo os aspectos clínicos, micológicos e moleculares do agente etiológico;
- Investigar a epidemiologia das manifestações imunorreativas associadas à esporotricose em área hiperendêmica;
- Comparar o desempenho da PCR com o do cultivo micológico em amostras obtidas por método não invasivo (*swab*) para o diagnóstico de esporotricose humana e felina;
- Avaliar a atividade antifúngica do extrato hexânico da microalga *Nannochloropsis oceanica* frente a *S. brasiliensis*, de forma isolada e em combinação com Itraconazol

5. MANUSCRITO 1

Unique genetic clades and frequent zoonotic transmission of *Sporothrix brasiliensis* with reduced itraconazole susceptibility

Esse artigo está nas normas e com previsão de submissão na *The Lancet Microbe* – Fator de Impacto 20,4.

Este estudo caracterizou a diversidade genética e a suscetibilidade antifúngica de 450 isolados de *Sporothrix brasiliensis* oriundos do Rio Grande do Sul entre 2009 e 2024. Foram identificados três grandes clados genéticos, sendo dois deles inéditos, e diferentes perfis de susceptibilidade, principalmente ao itraconazol. Os achados reforçam a importância da vigilância epidemiológica e do uso criterioso de antifúngicos no controle da esporotricose. A pesquisa foi viabilizada por meio de uma rede sólida de colaboração nacional e internacional. Parte dos experimentos foi realizada durante meu período de Doutorado Sanduíche (novembro de 2024 a abril de 2025) no *Canisius-Wilhelmina Hospital* e no *Radboud University Medical Center* (Holanda), instituições reconhecidas internacionalmente como centros de excelência em micologia médica. Essa parceria possibilitou a integração de metodologias e perspectivas, conectando a epidemiologia local brasileira à expertise europeia. O projeto esteve ainda inserido na Rede Saúde Única FAPERGS/FIOCRUZ, intitulada "Esporotricose e *Sporothrix brasiliensis* no contexto One Health: Promoção da saúde, pesquisa e inovações para melhoria diagnóstica e terapêutica na saúde humana e animal, e para melhor compreensão do impacto da saúde ambiental na interface humano-animal-ecossistema", com coordenação conjunta pelos Laboratórios de Micologia da FAMEDFURG e do INI-Fiocruz, e colaboração de outras IFES, incluindo UFCSPA e UnB. Nesse contexto, o estudo representa o primeiro produto concreto da colaboração dos centros de micologia do Sul do Brasil com o centro Holandês, abrindo caminho para novas pesquisas conjuntas em vigilância, resistência antifúngica e epidemiologia molecular. Assim, este artigo não apenas oferece informações inéditas sobre a epidemiologia e etiologia da esporotricose no sul do Brasil, como também ilustra a trajetória científica que une as demandas locais de saúde pública à cooperação nacional e internacional, reforçando a relevância da tese nos contextos regional e global e sua contribuição com a internacionalização institucional.

Unique genetic clades and frequent zoonotic transmission of *Sporothrix brasiliensis* with reduced itraconazole susceptibility

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Abstract:

Background: *Sporothrix brasiliensis* is driving a major zoonotic sporotrichosis epidemic in Brazil, predominantly via cat-to-human transmission. In the late 1990s a major outbreak was reported in Rio de Janeiro (RJ), while a concomitant independent outbreak emerged in Rio Grande do Sul (RS), the southernmost state of Brazil. We characterized the antifungal susceptibility and genetic diversity of *S. brasiliensis* isolates collected in RS to better understand the regional disease transmission and treatment response.

Methods: We analyzed 450 *S. brasiliensis* isolates from humans, animals, and environment collected across RS between 2009 and 2024. Antifungal susceptibility to itraconazole, amphotericin B, and terbinafine was determined following CLSI guidelines using epidemiological cutoff values (ECV). Genotyping was performed via short tandem repeat (STR) analysis.

Findings: For itraconazole a bimodal distribution was observed with 24% NWT isolates. Amphotericin B and terbinafine MICs were normally distributed with non-wild-type rates in respectively 7% and 54% of isolates, most being one dilution above ECV. STR analysis revealed 82 genotypes clustering into three main clades: the Clade I (related to the RJ strains) and two novel clades. One novel clade formed large transmission clusters with <20% of isolates being NWT for itraconazole, similar to the Clade I. The other clade demonstrated more genetic variation and NWT MICs for 89% of isolates. Notably, from all 11 human patients with available clinical data and elevated itraconazole MICs, strains were isolated prior to azole exposure.

Interpretation: We report the emergence of distinct *S. brasiliensis* clades in RS with different rates of reduced itraconazole susceptibility and evidence of zoonotic transmission. Our findings highlight the urgent need for antifungal stewardship and surveillance in these endemic regions.

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Highlights:

- Largest and most comprehensive molecular and susceptibility study of *Sporothrix brasiliensis* in southern Brazil;
- High proportion of itraconazole non-wild-type (NWT) isolates, with evidence of widespread resistance;
- Multiple genetic clades in Rio Grande do Sul with different NWT rates;
- Zoonotic transmission of NWT isolates from cats to humans;
- Urgent need for surveillance and clinical outcome studies on antifungal resistance.

Research in context

Evidence before this study: Zoonotic sporotrichosis caused by *Sporothrix brasiliensis* is a growing public health concern, mainly in Latin America. Rio de Janeiro and Rio Grande do Sul, were the epicenters of the zoonotic sporotrichosis epidemic, and the first cases have been reported in late 90's. To date, most studies on antifungal resistance and molecular epidemiology include isolates from Rio de Janeiro. Investigations for these aspects on isolates form Rio Grande do Sul remain limited.

Added value of this study: This is the largest genetic and antifungal susceptibility surveillance study of *S. brasiliensis* conducted in Brazil to date. We analyzed 450 isolates collected over 15 years, including human, animal, and environmental sources across Rio Grande do Sul. Our findings reveal a high prevalence of non-wild-type (NWT) isolates for itraconazole. Importantly, we identified a distinct clade of *S. brasiliensis*, characterized by a striking proportion of itraconazole NWT isolates. Furthermore, detection of NWT isolates in humans without previous antifungal exposure provides strong evidence for zoonotic transmission of these strains.

Implications of all the available evidence: This study provides evidence of the emergence of high MICs to itraconazole, the first drug of choice for the treatment of sporotrichosis caused by *S. brasiliensis*. Additionally, it highlights a specific clade circulating in the Rio Grande do Sul with alarmingly high itraconazole MICs, and provides evidence of zoonotic transmission of NWT strains. There is an urgent need for adequate surveillance and targeted management strategies to control the ongoing epidemic in this region.

1. Introduction

Sporotrichosis is the most common type of implantation mycosis worldwide, and is considered a neglected tropical disease by the World Health Organization [1]. In the last decades, there has been a dramatic increase in sporotrichosis cases in Brazil. This is caused by the sudden emergence and spread of *Sporothrix brasiliensis*, a previously unrecognized species[2]. This fungus first emerged in cats, which develop severe disease and became the main vector of transmission. Via inoculation by cats (scratching, biting), *S. brasiliensis* has rapidly spread, currently affecting thousands of felines, dogs and humans every year, with numbers still increasing[3]. This cat-transmitted sporotrichosis also led to outbreaks in other South American countries, with some cases reported outside the continent [4].

Itraconazole is the first-line therapy for all hosts without contraindications (for example pregnancy, drug interaction and pediatric patients) [5,6]. Via oral tablets or suspensions patients are treated daily for a prolonged duration of at least four months. This treatment, especially in cats, poses significant challenges. In domestic cats, the main challenges are the severity of the clinical presentation, the prolonged treatment duration, and the reliance on owner compliance[7]. These factors contribute to a treatment abandonment rate of approximately 30–40% and cure rates ranging from 50–72% [5,8]. In humans, treatment is generally more successful. Nonetheless, there have been reports of reduced *in vitro* susceptibility of *S. brasiliensis*, highlighting the need for ongoing monitoring and studies to investigate if this is associated with clinical failure or a specific genetic background [9,10]. The main strategies to tackle endemic zoonotic sporotrichosis include early diagnosis and effective treatment, but also robust epidemiological surveillance. The latter offers insights into sources of transmission and infection dynamics, allowing public health policies to control and manage outbreaks. In this context, human sporotrichosis became a notifiable disease in Brazil in 2025 [11].

In the late 1990s the first cat-transmitted sporotrichosis cases in Brazil were reported from the non-neighboring states Rio de Janeiro and Rio Grande do Sul, which was followed by enormous outbreaks in both states [12,13]. Although these outbreaks began around the same time, the oldest isolates from each region were genotypically distinct, suggesting independent introductions [14–17]. Isolates highly related to the Rio de Janeiro genotype were also found in many other Brazilian states. The massive dispersal of isolates from this Rio de Janeiro (RJ) clade indicates a huge dissemination potential. As only few isolates from other states were related to isolates from Rio Grande do Sul, it is still unknown whether the outbreak of *S. brasiliensis* in Rio Grande do Sul also involved clonal transmission from a potential Rio Grande do Sul (RS) clade or whether it involved various independent introductions, possibly combined with spread from the RJ clade [14,16]. Remarkably, epidemiological studies in Rio Grande do Sul have shown that a higher dose of itraconazole is needed to achieve clinical cure compared to cases in Rio de Janeiro (200 mg/day vs. 100 mg/day) [6,18], suggesting genetic differences play a role.

This study aimed to understand the unique sporotrichosis situation in Rio Grande do Sul in which massive outbreaks are combined with seemingly reduced antifungal susceptibility. For this purpose we evaluated antifungal susceptibility profiles and genetic diversity of 450 *S. brasiliensis* isolates from Rio Grande do Sul and investigated how these relate to zoonotic transmission of this fungus.

2. Materials and methods

2.1. Isolates

S. brasiliensis strains, collected in 2009 to 2024, were isolated from cats, humans, dogs, cattle and the environment. Isolates were obtained from laboratory culture collections from different Rio Grande do Sul health district regions, including the South (Federal University of Rio Grande, Federal University of Pelotas, Zoonosis Control Center of Pelotas), Metropolitan (Federal University of Health Sciences of Porto Alegre, Porto Alegre Clinical Hospital, State Center for Animal Health Diagnosis and Research), Sierra (University of Caxias do Sul), North (Passo Fundo Clinical Hospital) and Midwest (Federal University of Santa Maria) districts.

2.2. Antifungal susceptibility testing (AFST)

The antifungal susceptibility profiles of *S. brasiliensis* in the mold phase were evaluated by broth microdilutation assay according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 guideline[19]. The isolates were cultured in Potato Dextrose Agar for 7 days at 30ºC and the inoculum was standardized by spectrophotometry in an interval of 0.9×10^4 to 10^5 CFU/mL. The drugs itraconazole (Janssen Cilag, Breda, The Netherlands), posaconazole (Merck, Darmstadt, Germany), isavuconazole (Brasilea Pharmaceutica, Basel, Switzerland), voriconazole (Pfizer Central Research, Sandwich, United Kingdom) and amphotericin B (Bristol Myers Squib, Woerden, The Netherlands) were tested in the final concentrations between 0.016 and 16 µg/mL. Fluconazole (Merck, Darmstadt, Germany), were tested in the final concentrations of 0.063 to 64 µg/mL. Terbinafine (Novartis Pharmaceuticals, Basel, Switzerland) was tested on itraconazole non-wild-type (NWT) isolates between the concentrations 0.004 and 4 µg/mL.

Results for the azoles and amphotericin B were read to visually determine the minimal inhibitory concentration (MIC), defined as the lowest concentration able to inhibit 100% of the fungal growth. For terbinafine, the MIC was defined as the lowest concentration able to inhibit 80% of the fungal growth. The isolates were classified as WT and NWT according to the most recently established epidemiological cut-off values (ECVs) of 8 µg/mL for itraconazole, 4 µg/mL for posaconazole and amphotericin B and 0.125 µg/mL for terbinafine [20].To analyze the antifungal susceptibility profile of the isolates, we used the geometric mean, and MIC 50% and 90%, which indicate the concentration able to inhibit 50% and 90% of the isolates, respectively.

2.3. Genotyping by STR

Genotyping was performed using short tandem repeat (STR) analysis as previously described [21]. In short, three multiplex PCRs, targeting tri- and hexanucleotide repeats (M3-I, M3-II, M6) were used to amplify the microsatellites, followed by capillary electrophoresis on a 3500XL genetic analyzer (Applied Biosystems, Foster City, CA, USA) [21]. Copy numbers were determined using GeneMapper5 (Applied Biosystems, Foster City, CA, USA) and the genetic analysis were done with BioNumerics v7.6.1 (Apllied Maths NV, Sint-Martems-Latem, Belgium). The current found genotypes were compared with all previously genotyped isolates [22].

3. Results

3.1. Epidemiological data

From 2009 to 2024, a total of 450 *S. brasiliensis* isolates were collected from cats (n=299), humans (n=122), dogs (n=26), the environment (n=2) and cattle (n=1) in the state of Rio Grande do Sul, Brazil. The environmental isolates were collected from soil in areas that were affected by cats with sporotrichosis. Isolates were collected from different areas of this state, of which the majority originated from the South health district region (n=395), while other isolates came from Metropolitan (n=34), Sierra (n=16), North (n=3) and Midwest (n=2) (**Figure 1A**). From all isolates 28 were collected in 2009-2014, 109 in 2015-2019, and 313 in 2020-2024 (**Figure 1B**).

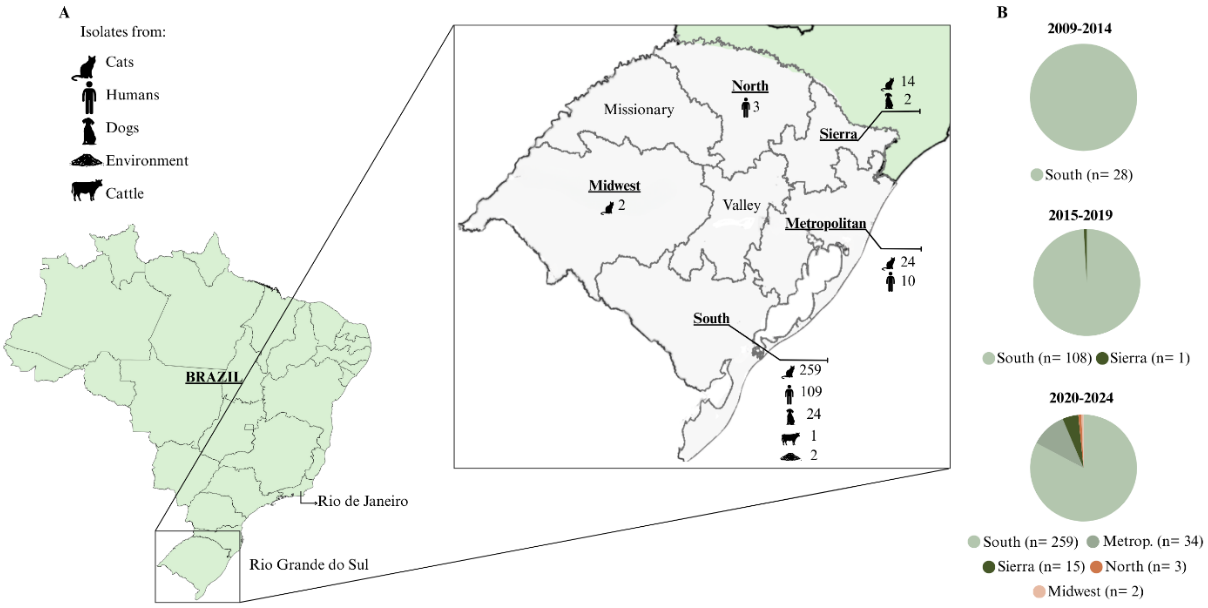

Figure 1: Origin *S. brasiliensis* isolates. (A) Map of Brazil with indicated states Rio Grande do Sul and Rio de Janeiro (left panel) and health districts of Rio Grande do Sul (right panel). Numbers at icons representing cats, humans, dogs, soil, and cattle indicate the number of isolates obtained from each host or source. **(B)** Collection period of isolates from Rio Grande do Sul, categorized by health district regions.

3.2. Antifungal Susceptibility Profiles

AFST according to CLSI microbroth dilution was performed for all isolates in the mycelial phase. For itraconazole, a bimodal distribution was observed with 24% (107/450) of isolates classified as NWT was o (**Figure 2A**). Remarkably, at 16 µg/mL, growth of 26 isolates was comparable to growth control, while 81 isolates demonstrated growth inhibition, estimated at >50% as compared to growth control. For posaconazole, 20% (89/450) of isolates were NWT, which was less as compared to itraconazole (**Supplementary Figure S1**). MICs for voriconazole, isavuconazole and fluconazole were mostly ≥16 µg/mL (**Supplementary Figure S1**). For amphotericin B, 7% (31/450) of isolates were NWT, with two isolates presenting MICs ≥16 µg/mL (**Figure 2B**). Nine isolates were NWT for both itraconazole and amphotericin B.

Next, antifungal susceptibility against terbinafine was determined among the itraconazole NWT isolates. A normal distribution was found with 54% (58/107)) being NWT for terbinafine (**Figure 3**). Two isolates were NWT for all three antifungals tested. MIC results for individual isolates are provided in **Supplementary Material 1**.

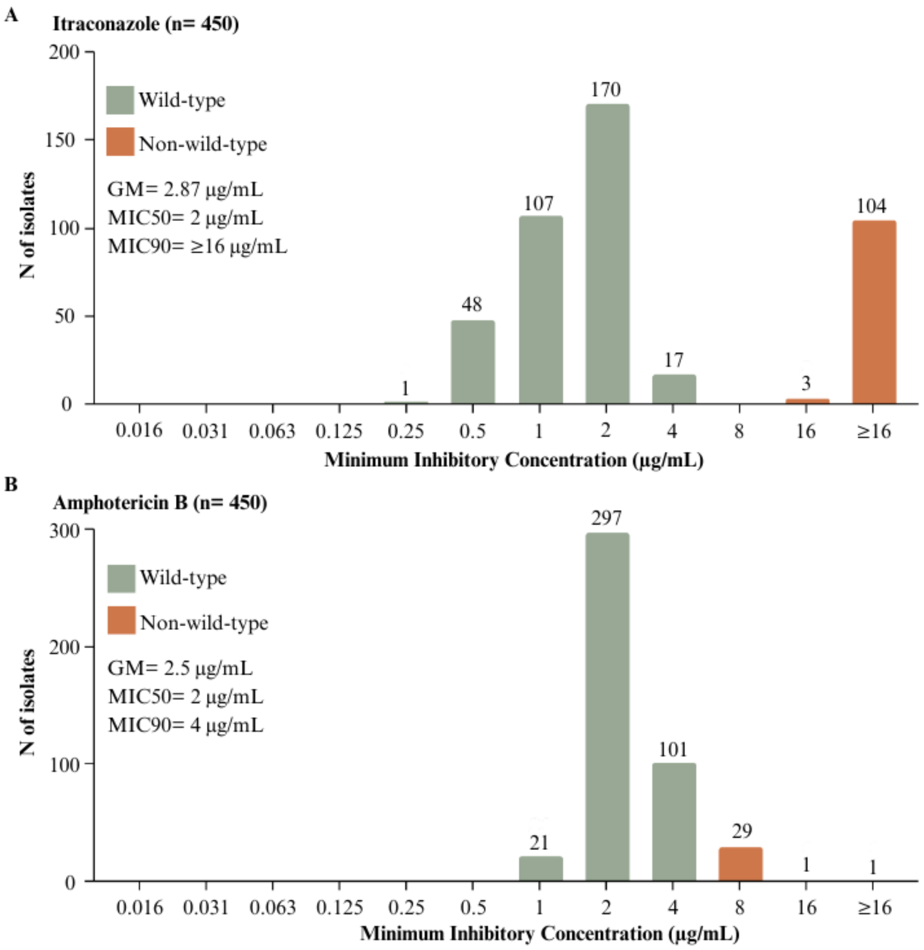

Figure 2: Distribution of minimum inhibitory concentration (MIC) values for itraconazole and amphotericin B. Antifungal susceptibility testing was performed on *Sporothrix brasiliensis* isolates against (**A**) itraconazole and (**B**) amphotericin B according to the Clinical and Laboratory Standards Institute (CLSI) M38-A3 microbroth dilution protocol. Isolates were tested in the mycelial phase, and MICs were determined after 72 hours of incubation at 30 °C. The number of isolates is displayed above the bar.

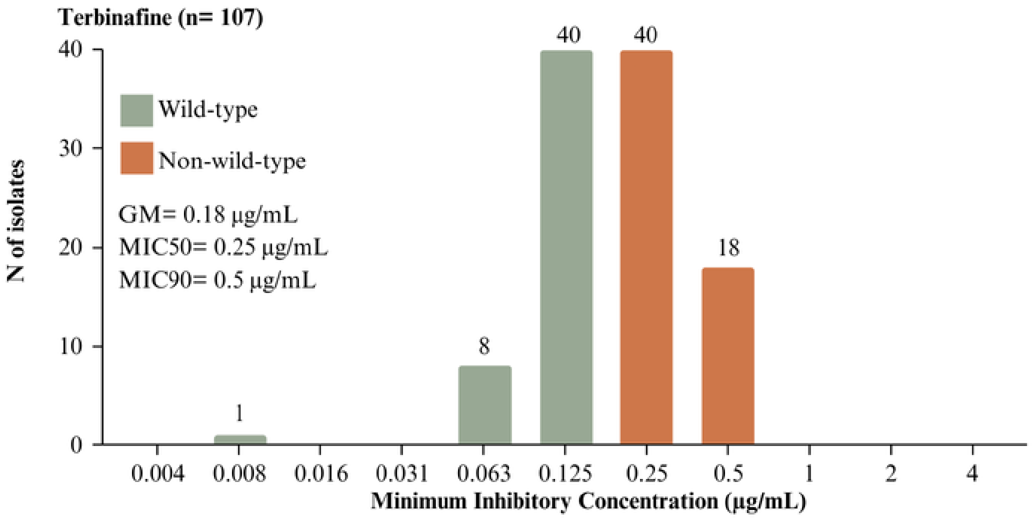

Figure 3: Distribution of minimum inhibitory concentration (MIC) values for terbinafine. Antifungal susceptibility testing was performed on NWT itraconazole *Sporothrix brasiliensis* isolates against terbinafine according to the Clinical and Laboratory Standards Institute (CLSI) M38-A3 microbroth dilution protocol. Isolates were tested in the mycelial phase, and MICs were determined after 72 hours of incubation at 30 °C. The number of isolates is displayed above the bar.

3.3. Epidemiological distribution isolates with reduced itraconazole susceptibility

Next we determined the distribution of WT and NWT isolates for itraconazole, the latter divided in unaffected growth and growth inhibition, for the different health districts, time periods and hosts. In health districts South and Metropolitan, 22.2% (88/395) and 14.7% (5/34) of isolates, respectively, were NWT, while in the Sierra district this was considerably higher with 75.0% (12/16, **Figure 4A**). The Metropolitan district demonstrated the relatively highest percentage of isolates with unaffected growth (40.0%, 2/5) as compared to South and Sierra (22.7% [20/88] and 16.6% [2/12], respectively). The number of isolates from the North and Midwest districts were too low to include in the comparison.

When analyzed by host, rates of NWT isolates for itraconazole were very similar among cats and humans (24.9% and 23.8%, respectively), while also the rate of NWT isolates with unaffected growth was not different (**Figure 4B**). For dogs, 14.3% of isolates showed a reduced itraconazole susceptibility, while none of the NWT isolates showed unaffected growth inhibition. The number of isolates from the environment and cattle were too low to compare. Over time, the percentage of NWT isolates was comparable (**Figure 4C**).

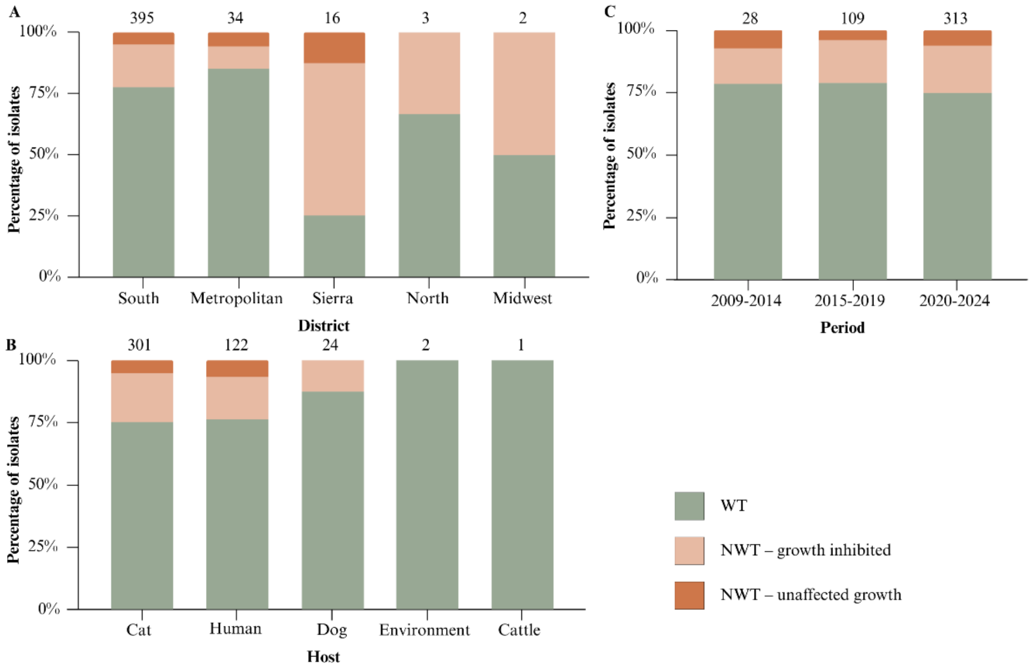

Figure 4: Distribution of WT and NWT isolates per district, host and period. Proportion of itraconazole WT and NWT *Sporothrix brasiliensis* isolates from Rio Grande do Sul by for different (**A**) districts, (**B**) hosts and (**C**) time periods. The number of isolates is displayed above the bar.

3.4. Short tandem repeat genotyping

STR genotyping was performed on all 450 *S. brasiliensis* isolates, which resulted in a total of 82 genotypes. When compared to previously genotyped isolates, the isolates grouped in two main clades with large clusters and highly related genotypes and a third clade with small clusters of less related genotypes (**Figure 5A**). The largest clade, with 378 isolates from this study, mainly harbored isolates from the current study (Rio Grande do Sul) and demonstrated clusters up to 107 isolates per cluster. Besides this Rio Grande do Sul (RS) clade (Clade II), the second main clade, previously identified as the Rio de Janeiro (RJ) clade (Clade I), contained clusters up to 32 isolates per cluster, with at most 9 isolates per cluster from the current study [21]. In total there were 36 isolates from the current study that allocated to the Clade I. The remaining 36 isolates from this study grouped with isolates mostly from Paraná state in southern Brazil with 5 isolates from Paraná state, Brazil at most per cluster (Clade III).

Analyzing the geographic origin of the different isolates from this study only, we found that isolates in the Clade II were mostly found in the southern region of the state, while isolates from the Clade I were predominantly associated with the Metropolitan district (**Figure 5B**). Isolates from the third clade originated from all regions. Furthermore, the genetic variation within the Clades I and II were much smaller with >99% of isolates showing at most one different STR marker with the most closely related isolate with different STR profile, while only 46% of isolates in the third clade were that closely related to other isolates. Next we determined the genotypic distribution of itraconazole WT vs. NWT isolates This distribution was highly different between genetic groups as the relative number of NWT isolates in the Clade II (72/378, 19%) and I (3/36, 8%) clade was much lower than the 32 out of 36 isolates (89%) observed in the Clade III (**Figure 5C, Supplementary Figure S2A**). Moreover, this third clade also harbored relatively more NWT isolates for itraconazole with unaffected growth as compared to the Clade I and II **(Figure 5C, Supplementary Figure S2B).**

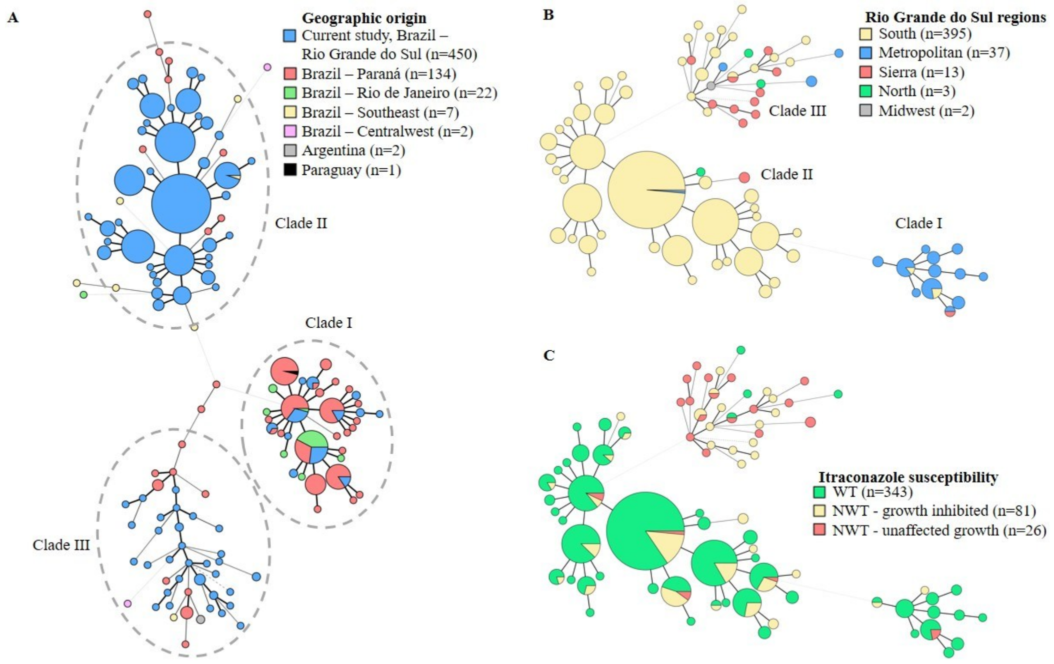

Figure 5: **Genetic variation of *Sporothrix brasiliensis* isolates from different regions and varying itraconazole susceptibility.** Minimum spanning trees of *Sporothrix brasiliensis* isolates from Rio Grande do Sul (RS), demonstrating genetic differences between (**A**) non-RS isolates from previously published studies and all RS isolates from the current study; (**B**) the different Health districts of RS; (**C**) RS isolates grouped by

itraconazole susceptibility profile. Branch lengths of the tree indicate similarity between isolates with thick solid lines (variation in one marker), thin solid lines (variation in two markers), thin dashed lines (variation in three markers) and thin dotted lines (variation in four or more markers).

3.5. Zoonotic transmission of strains with reduced itraconazole susceptibility

To investigate whether the isolates with reduced itraconazole susceptibility might be transmitted from cats to humans, we first determined whether identical genotypes were found for the different hosts. As expected, nearly all clusters consisted of both human and feline isolates, indicating widespread zoonotic transmission (**Figure 6A**). Then from all isolates NWT for itraconazole, we aimed to collect information about the onset of itraconazole treatment in regard to the isolation of the *S. brasiliensis* strain. All 11 isolates with available information, originating from human patients from the public specialised reference service for diagnosis and treatment of sporotrichosis in southern (University Hospital Miguel Riet Corrêa Jr. from Federal University of Rio Grande) [23], were collected prior to antifungal treatment (**Figure 6B**).

When comparing the distribution of clades over time in Rio Grande do Sul, a clear predominance of the Clade II is observed throughout all periods, along with isolates from the residual group, which are also present across all time points. In contrast, isolates from the Clade I appear only in the most recent period (**Figure 7**).

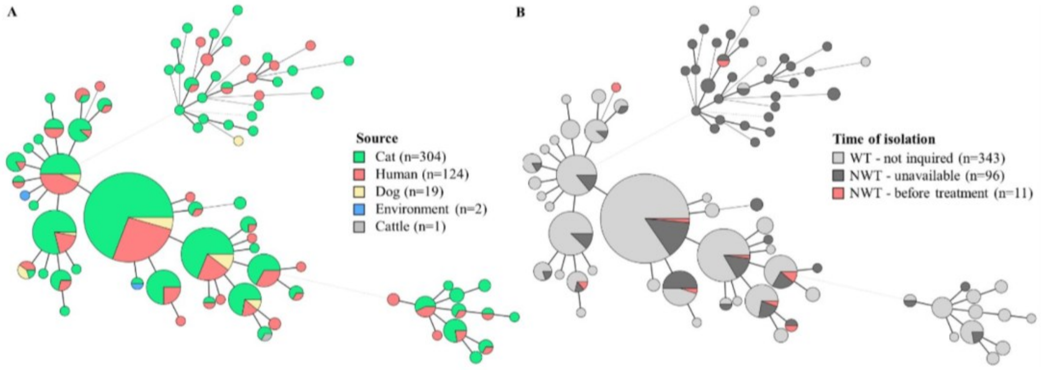

Figure 6: Genetic similarity of *Sporothrix brasiliensis* isolates from different sources and with NWT itraconazole MICs, zoonotically transmitted. Minimum spanning tree of *Sporothrix brasiliensis* isolates from Rio Grande do Sul (RS) indicating genetic similarities for ***(A)*** different host species; ***(B)*** for itraconazole NWT strains, isolated before itraconazole treatment. Onset of treatment in cases with itraconazole-susceptible isolates were not inquired. Branch lengths of the tree indicate similarity between isolates

with thick solid lines (variation in one marker), thin solid lines (variation in two markers), thin dashed lines (variation in three markers) and thin dotted lines (variation in four or more markers).

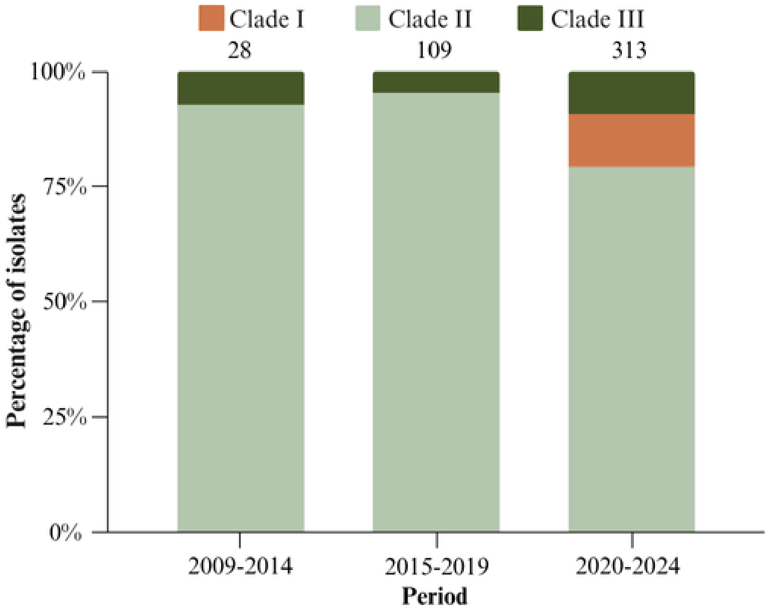

Figure 7: Temporal distribution of *Sporothrix brasiliensis* clades in Rio Grande do Sul state (n= 450).

Discussion

In this study, 450 *S. brasiliensis* isolates from Rio Grande do Sul, the southernmost state of Brazil, were analyzed for their antifungal susceptibility and genetic relatedness. A significant percentage of isolates exhibited high MIC values for itraconazole, the main antifungal agent used to treat sporothrichosis. Moreover, some of these isolates appeared to be collected from humans before the start of itraconazole treatment demonstrating cat-to-human transmission of strains with reduced susceptibility. Finally, two novel clades were identified; the RS clade with large clusters and a clade with less clusters and harboring several isolates from other states.

AFST for itraconazole demonstrated a bimodal distribution with 24% non-wild-type isolates of *S. brasiliensis,* exhibiting high MIC values (≥16 µg/mL). Some of these isolates exhibited no growth reduction at all despite high drug concentrations, whereas others showed a clear reduction in growth. This different growth patterns may be associated with distinct underlying resistance mechanisms. Some isolates may display tolerance, characterized by reduced but sustained growth, whereas others may harbor more pronounced resistance mechanisms that allow growth even at high antifungal concentrations. Although previous studies have also reported similar high MICs for some *S. brasiliensis* isolates, with one study reporting a bimodal distribution, the number of analyzed isolates was relatively small [10,24,25]. Moreover, there has been some debate about the method of ASFT for *Sporothrix* isolates, with some labs not reporting whether the AFST was executed at the pure yeast or filamentous phase. We recently established methods to analyze pure yeast or filamentous phase [26] and included a much larger number of isolates. Thus, we have reinforced and established the presence of reduced itraconazole susceptibility for *S. brasiliensis*. While the impact on clinical outcome in felines and humans remains to be identified, it certainly calls for clinical studies investigating the impact of this reduced itraconazole susceptibility on treatment efficacy.

Besides itraconazole, different other antifungals were tested. Amphotericin B and terbinafine MICs were normally distributed, with a relatively high number of NWT isolates, just one or two dilution factors above the ECV [27]. These findings were likely method-dependent and do not directly suggest an actual reduced susceptibility for these drugs. Then, MICs for voriconazole, isavuconazole and fluconazole were ≥16 µg/mL for most isolates. For posaconazole, a similar bimodal distribution as for itraconazole was observed. The main mechanisms of antifungal azole resistance in *Sporothrix* spp. are only partly understood. Recent studies have reported genetic variations in genes involved in resistance pathways, alterations related to aneuploidy and chromosomal variation,[10,28,29]. Variations in the *CYP51* gene are most often described, suggesting changes in the target enzyme of itraconazole[18]. These genetic and phenotypic variations may help explain the different growth behaviors observed among the resistant isolates.

Next, the epidemiological distribution of isolates with reduced itraconazole susceptibility was investigated. Remarkably, Sierra, a northern region in Rio Grande do Sul, appeared to have more isolates with NWT itraconazole MICs. Subsequent STR genotyping demonstrated that this was due to a different genotype present in this region, a genotype that was also previously found in Parana, a state north of Rio Grande do Sul. Thus regional differences in regard to itraconazole susceptibility were likely due to regional presence of *S. brasiliensis* genotypes. Then, a temporal analysis did not show an increase in the proportion of isolates with high itraconazole MICs over time. While this may be due to the low number of isolates available from earlier years, it is important to emphasize that the absolute number of sporothrichosis cases, and thus also the number of isolates with high itraconazole MICs, has been increasing very quickly over the last years, with yet unknown clinical implications.

With the STR profiling 82 distinct genotypes were identified, which were present in three major clades. While the Clade I and II demonstrated many large clusters, indicating clonal transmission, there was a third clade with more genetic variability and small clusters. At this moment it is not evident whether this clade contains isolates that were independently introduced from the environment or were also clonal transmitted but with much lower transmission potential. The larger variation in STR markers in this clade, as compared to the Clade I and II, support the former hypothesis, but WGS SNP analysis should be performed to confirm this.

From the beginning of the sporotrichosis endemic the Clade II was dominant in Rio Grande do Sul with >90% of isolates exhibiting this genotype from 2009 to 2019. Only from 2020 onwards the Clade I was found in Rio Grande do Sul, mostly in the Metropolitan Health District located along the coast, likely via imported cats or via feline movements and transmission [30]. These findings correspond well with the initial reports of the sporotrichosis endemic suggesting simultaneous but independent emergences in the states of Rio de Janeiro and Rio Grande do Sul via low-resolution genotypic analyses [14,15,17,21]. Our study confirms these earlier studies, with the major RS clade mostly originating from the southern region of the state, where the endemic first emerged alongside the Rio de Janeiro [14,17]. The high and unique transmission potential of both the RS and RJ genotype likely plays an important role in current sporotrichosis endemic.

The presence of isolates with NWT itraconazole MICs was relatively low for the RS and RJ clades with 19% and 8%, respectively, while this was 89% for the third clade. This indicates that both clades with high transmission potential seem to have a lower potential to reduce their susceptibility to itraconazole. Both characteristics likely are due to genetic differences and future studies should identify the genetic factors involved. Then, all large clusters of the RS clade harbored isolates with low and high itraconazole MICs, demonstrating the reduced susceptibility was therapy-acquired. Moreover, from all 11 human zoonotic cases with NWT-itraconazole isolates and known clinical information, all strains were isolated prior to itraconazole exposure, demonstrating cat-to-human transmission of strains with reduced susceptibility. While this shows that in at least some felines itraconazole susceptibility is reduced during treatment, it is not known yet whether this also occurs in human patients treated with itraconazole.

Study limitations include the high number of isolates from the southern region, while some health districts in Rio Grande do Sul were not included at all. In addition, terbinafine was only tested in high MIC itraconazole isolates. Then, the inferred genetic relatedness between isolates should be confirmed with whole genome sequencing (WGS). Finally, limited availability of clinical information only allowed to identify zoonotic transmission of strains with reduced itraconazole susceptibility in 11 human patients.

This study, based on a large and diverse collection of isolates from Rio Grande do Sul, highlights the emergence of *S. brasiliensis* strains with high itraconazole MICs, showing a bimodal distribution. The detection of transmissible NWT isolates raises concerns regarding the spread of these strains. Furthermore, the coexistence of multiple clades, either with low and high genetic diversity, reveals complex transmission dynamics that extend beyond clonal expansion. These novel findings underscore the critical role of the southernmost state of Brazil in the current sporotrichosis scenario and reinforce the urgent need for improved disease management and control strategies to prevent the further dissemination of resistant clades. Additionally, the current findings emphasize the importance of future studies focusing on the clinical impact of reduced itraconazole susceptibility and the underlying mechanisms driving its emergence.

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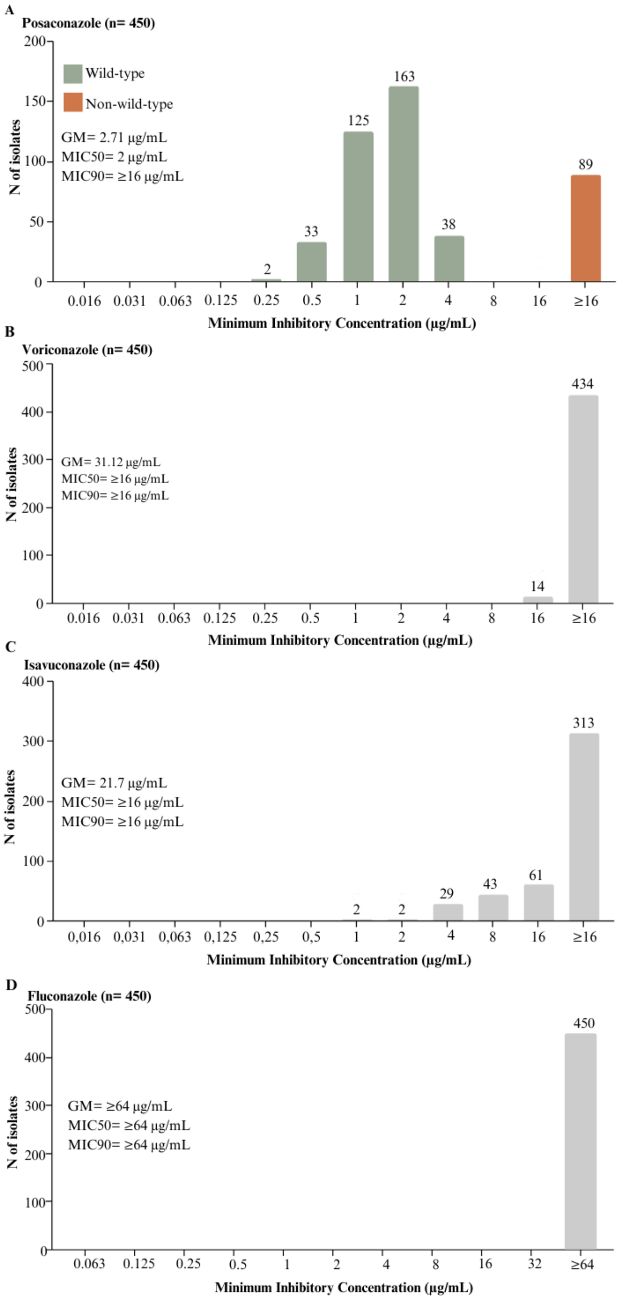
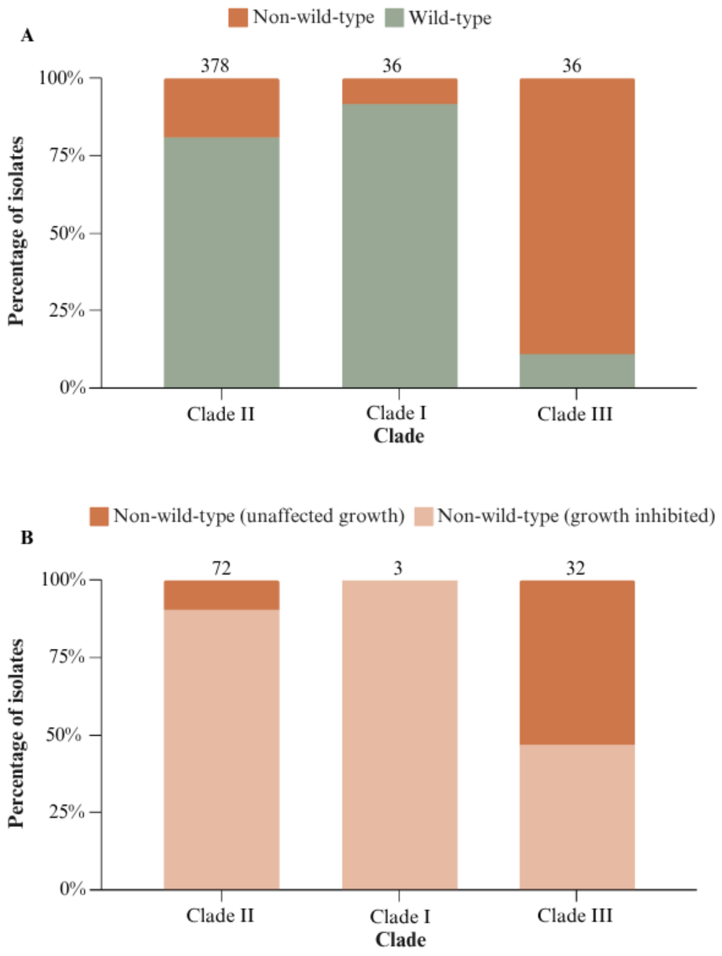


Supplementary Figure S1: Distribution of minimum inhibitory concentrations (MICs) for azoles against 450 *Sporothrix brasiliensis* isolates from Rio Grande do Sul, Brazil. Antifungal susceptibility testing was performed using the CLSI M38-A3 broth microdilution protocol. All isolates were tested in the mycelial phase, and MICs were determined after 72 h of incubation at 30 °C. Geometric mean (GM), MIC_{50}, and MIC_{90} values are presented. (A) Posaconazole (0.016–16 µg/mL); (B) Voriconazole (0.016–16 µg/mL); (C) Isavuconazole (0.016–16 µg/mL); (D) Fluconazole (0.063–64 µg/mL).



Supplementary Figure S2: Distribution of *Sporothrix brasiliensis isolates from Rio Grande do Sul according to* itraconazole susceptibility profiles by genotype clade; **(A)** proportion of WT and NWT isolates (p< 0.001); **(B)** proportion NWT isolates, distribution between unaffected growth and inhibited growth (p< 0.001).

6. MANUSCRITO 2

First report of sporotrichosis by *Sporothrix brunneoviolacea*

Esse artigo está nas normas e com previsão de submissão na *Veterinary Microbiology* – Fator de Impacto 2,7.

Este estudo descreve o primeiro relato de esporotricose causada por *Sporothrix brunneoviolacea* em um gato doméstico no Brasil, ampliando o conhecimento sobre a diversidade etiológica do gênero *Sporothrix* e seu potencial zoonótico. O caso foi identificado no sul do país, em um paciente felino que apresentava lesão ulcerada na região nasal e evolução clínica favorável após curto tratamento com itraconazol. A identificação molecular do agente foi realizada por sequenciamento do gene da calmodulina, que confirmou tratar-se de *S. brunneoviolacea*, espécie até então considerada ambiental e não patogênica. O trabalho foi desenvolvido em colaboração com o grupo de Micologia Médica do Canisius-Wilhelmina Hospital e do Radboud University Medical Center (Holanda), durante meu período de Doutorado Sanduíche, fortalecendo a integração científica entre os dois grupos e ampliando a parceria internacional.

First report of sporotrichosis by *Sporothrix brunneoviolacea*

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Highlights

- First report of sporotrichosis by *Sporothrix brunneoviolacea* in a domestic cat;
- Good clinical response using short course of itraconazole;
- First description of this fungal species in Brazil;
- *In vitro* antifungal susceptibility testing with reference methods did not yield growth.

Abstract

Sporotrichosis is a fungal neglected disease affecting mammals and is primarily caused by species that belong to the *Sporothrix schenckii* complex. Cases caused by species outside this complex are rare and often mild in presentation. Here, we report the first case by *Sporothrix brunneoviolacea* in a domestic cat patient from Southern Brazil.

The patient presented a small ulcerated lesion on the nose at the veterinary clinical. Subsequent relatively short treatment by itraconazole resulted in clinical cure. Moreover, the owner of the cat reported being scratched with subsequent lymphadenopathy development and was also successfully treated with itraconazole. The feline isolate was identified as *Sporothrix brunneoviolacea* by Illumina sequencing of the calmodulin gene, while phylogenetic analysis indicated that this species is not related to other pathogenic *Sporothrix* species. Despite proper growth and sporulation on routinely used media, antifungal susceptibility testing by microbroth dilution according to Clinical & Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) reference guidelines could not be performed due to a lack of growth, also under control conditions. In short, this is the first report of sporotrichosis by *S. brunneoviolacea*, which was originally classified as an environmental and non-pathogenic species and the first description of this species in Brazil.

Keywords: Sporotrichosis; *Sporothrix*; zoonotic transmission; feline

Introduction

Sporotrichosis is a neglected fungal disease reported on all inhabited continents [1]. Originally classified as an implantation mycosis, inhalation of fungal spores or mucosal contact with lesions of infected hosts are also recognized as transmission routes [1]. Humans and domestic cats (*Felis catus*) are most affected by this disease, although infections in other mammals such as dogs (*Canis familiaris*) and rodents are occasionally reported [2]. Sporotrichosis is most severe in cats and is often fatal when not treated with proper antifungal treatment and diagnosed in an early stage of the disease [3]. For humans and other mammals, fixed cutaneous is the most common presentation, followed by lymphocutaneous, disseminated, and ocular forms [2,4,5].

Although over 50 species of the *Sporothrix* genus are described, infections are mainly restricted to species of the clinical clade that comprises *S. schenckii*, *S. globosa*, *S. brasiliensis* and *S. luriei* [6]. Other *Sporothrix* species are usually classified as environmental and are rarely implicated in disease [6]. In South America, *S. brasiliensis* infections are highly prevalent and cases are mainly driven by colonized or infected cats [7]. Conversely, *S. schenckii* and *S. globosa* are the most common agents in Europe and Asia and are often caused by sapronotic transmission [2]. Interestingly, zoonotic transmission of *S. schenckii* by cats has also been reported in Asia, although other *Sporothrix* species are not implicated in this transmission type so far [8]. Regardless of the host, itraconazole is the drug of choice, with terbinafine as an alternative therapeutic option [4,9]. Worryingly, itraconazole resistant *S. brasiliensis* and *S. schenckii* isolates have been reported recently, although a correlation with clinical outcomes remains to be investigated [10]. Here, we report the first case of sporotrichosis by *Sporothrix brunneoviolacea* in a feline patient in Brazil, which was successfully treated with itraconazole.

Case Report

On January of 2024, a 8-months-old, male, non-neutered and antifungal naive cat of not defined breed with access to the street and without known underlying disease was presented to a veterinary clinic in Rio Grande, Rio Grande do Sul, Brazil. A discrete edema with small ulcerated and secretive lesions were present on the nose (**Figure 1**). The veterinarian sent a swab sample in Stuart transport medium for diagnosis to the Mycology Laboratory (MycoLab) of the Faculty of Medicine from Federal University of

Rio Grande. The swab was cultured on plates with Sabouraud dextrose agar with and without cycloheximide (Kasvi ®, São José dos Pinhais, PR, Brazil) at 28°C for 7 days. After 7 days a positive culture was obtained, which was identified as *Sporothrix* sp. by macro and micromorphology. Antifungal treatment by itraconazole (100mg/day; 5mg/kg) capsules mixed with food was initiated for 60 days, resulting in clinical cure. The cat's owner also reported being scratched on the right hand by the cat, and subsequently, developed a small ulcerated lesion with lymphadenopathy. Erroneously she did not consult a clinician and reported cure by self-administration of itraconazole. No culture was obtained from the human patient.

Clinical data were retrieved from the LabMyco database and through a telephone interview with the owner, who assigned a consent form to answer some questions regarding the case. The study adheres to the Declaration of Helsinki, and was approved by the ethics committee of the Federal University of Rio Grande (99284218.5.0000.5324, 2022 and 64230922.8.0000.5324, 2024).

For species identification DNA was extracted with the MagNA Pure and Viral NA Small Volume kit, Pathogen 200SV protocol and the MagNA Pure 96 instrument (all Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturers' instructions. Genomic libraries were prepared and sequenced with the Illumina Novaseq 6000 platform (Illumina, San Diego, CA, USA) with 2- by 150-bp paired-end-read mode. De novo assembly was performed with SPAdes v4.2.0 using default parameters. The calmodulin gene was identified was identified using reference-based mapping and *S. schenckii* (AM490340.1) as a reference. The resulting sequence was aligned with NCBI Nucleotide BLAST https://blast.ncbi.nlm.nih.gov/Blast.cgi indicating the species as *S. brunneoviolacea*. Next, reads were aligned against the *S. brunneoviolacea* reference CBS 124561 genome (GCA_021396205.1) using BWA-MEM v0.7.18, yielding a genomic coverage >99%, confirming accurate species identification.

The resulting *CaM* sequence was compared to all available *Sporothrix* species and additional *Sporothrix brunneoviolacea* isolates present in the National Center for Biotechnology Information Nucleotide database (**Table S1**). Nucleotide alignment was done using MAFFTv7 and the phylogenetic tree was built with IQ-TREE web server as described earlier [11]. Raw data generated in the current study was deposited to the NCBI Sequence Read Archive (SRA) database under BioProject ID PRJNA1252419. The current isolate differed with three nucleotide mismatches to the most closely related *S.*

brunneoviolacea (CBS 124564) (**Figure 2**). Interestingly, the species is only distant related to the clinical clade or other environmental species implicated with disease.

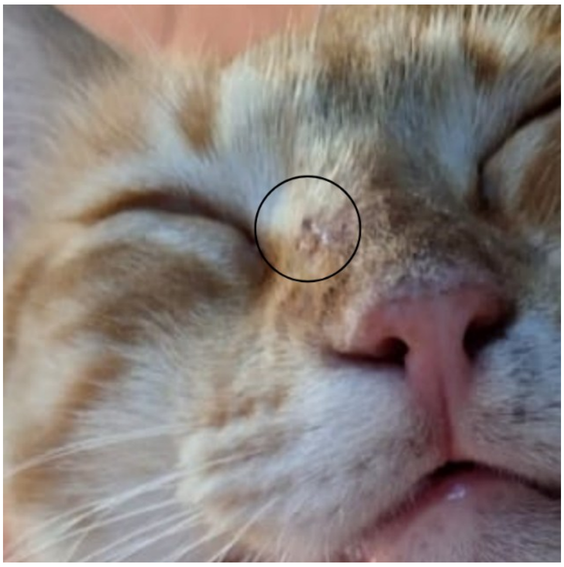

Figure 1: Cutaneous lesion in regressive process after 30 days of itraconazole in the cat infected by *Sporothrix brunneoviolacea*

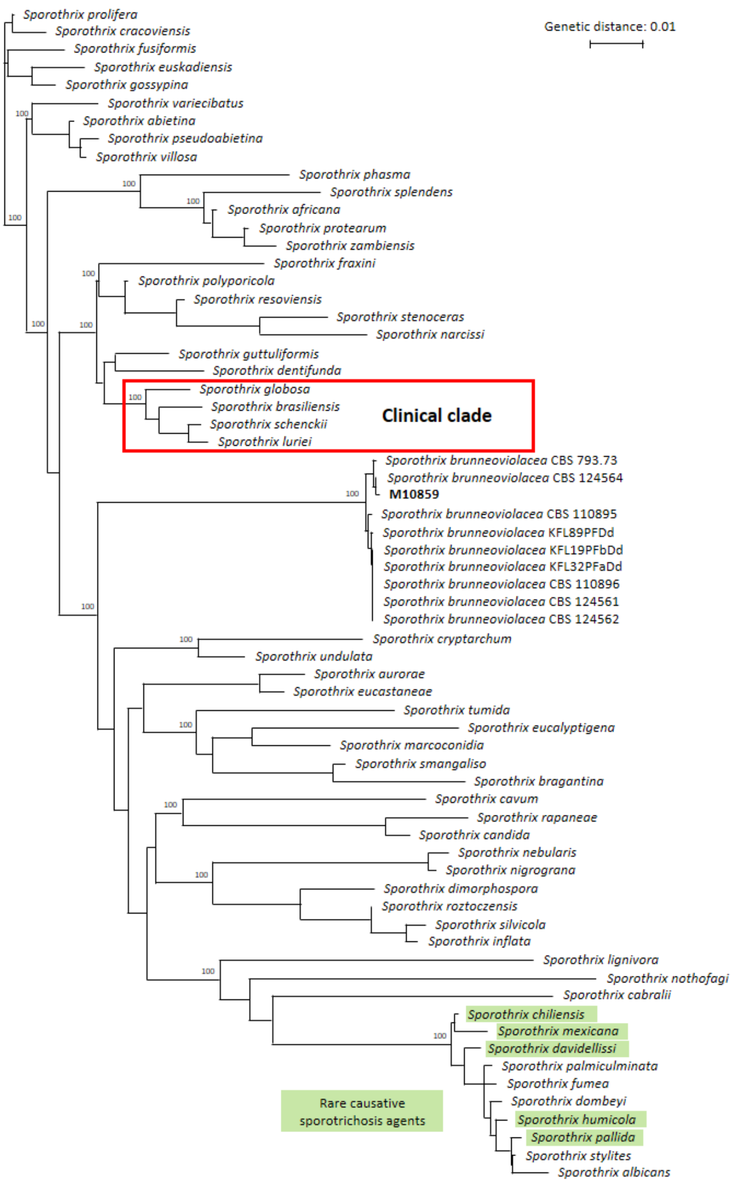

Figure 2: Phylogenetic tree based on calmodulin sequences of *Sporothrix* species, including the current isolate M10859 indicated in bold. Bootstrap values of 100 are shown above the nodes.

Then, the colony characteristics were revised by subculture and slide culture. The colony was flat and velvety, with zonate coloration and grey tones. Microscopic identification revealed the presence of subhyaline, septate hyphae, conidiophores with terminal conidia and small conidia that were globose to oval. Additionally, lateral melanized conidia, a characteristic of *S. brunneoviolacea*, were observed, while diffusible violet-brown pigment, another characteristic of this species [12], was not produced (**Figure 3**).

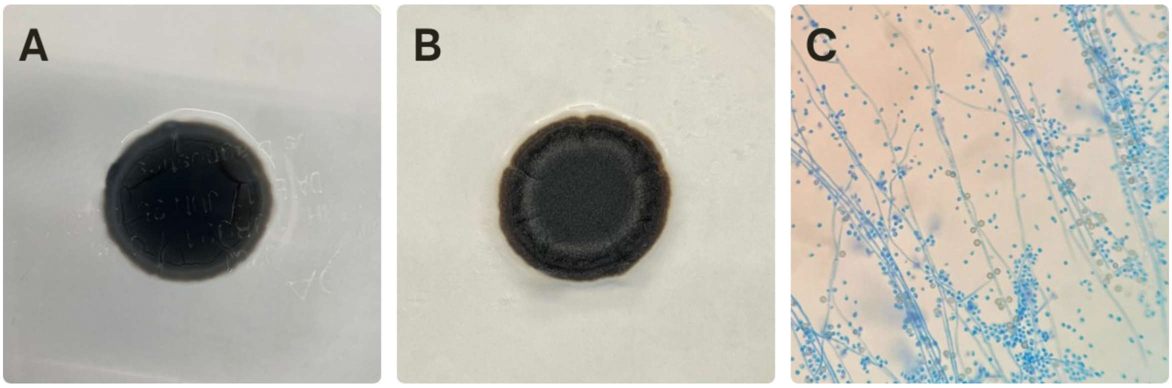

Figure 3: *Sporothrix brunneoviolacea* colony after 14 days of growth on Potato Dextroxe Ágar **(A)** Colony reverse; **(B)** Colony surface morphology; **(C)** Micromorphology showing lateral conidia stained with lactophenol cotton blue (x400).

Next, isolates were grown for seven days on PDA (Difco Laboratories, Detroit, MI, USA) and antifungal susceptibility testing (AFST) was conducted at 30°C with 72 hours of incubation with antifungals amphotericin B (Bristol Myers Squib, Woerden, The Netherlands), itraconazole (Janssen Cilag, Breda, The Netherlands), posaconazole (Merck), following microbroth dilution according to the Clinical & Laboratory Standards Institute (CLSI) reference method M38 and according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) E.def 9.4 [13,14]. Quality control strain *Aspergillus flavus* ATCC 204304 was included during the assays. While the quality control strain yielded minimum inhibitory concentrations (MICs) within the outlined ranges, *S. brunneoviolacea* did not grow at all when incubated according to CLSI and EUCAST microbroth dilution guidelines. Of note, inoculum was repeatedly incubated on different days and incubated for a total of 72 hours, with also the control well without antifungals present showing no growth.

Discussion

The current study identified for the first time a case of sporotrichosis caused by *S. brunneoviolacea*, found in a feline patient from Brazil. Moreover, this is the first description of *S. brunneoviolacea* in Brazil. In the last few decades, an increasing number of rare and novel fungal species have been reported in both animals and humans [15,16]. This is in part due to the increasing burden of fungal infections but also due to the adaptation of fungi to mammal hosts and advancements in species identification [17].

S. brunneoviolacea exhibits discrete distinct morphological characteristics compared to classical pathogenic species of the *Sporothrix* clinical clade [12,15]. Its hyphae are often sub-hyaline, whereas species like *S. schenckii*, *S. brasiliensis* and *S. globosa* are typically fully hyaline. Another notable feature is the abundance of melanized lateral conidia, giving *S. brunneoviolacea* colonies a darker appearance. Texture of the colony typically shows a velvety surface, in contrast to the coriaceous aspect observed in the classical species. This species also grows slower at 30°C than the other clinical species, and shows a limited growth at 37°C, reinforcing its weak thermotolerance, and consequently minor virulence in mammalian hosts. The *S. brunneoviolacea* isolate in this report also exhibited all these characteristics. In contrast, the violet halo, a characteristic pigmentation for this species [12], was not clearly observed in our isolate, even after incubation at 30°C, suggesting intraspecies variability although the influence of differences in culture conditions cannot be excluded.

With *CaM* sequencing, the isolate was identified as *S. brunneoviolacea*, while phylogenetic analysis demonstrated that this species formed a distinct branch within the genus. Earlier investigations found *S. brunneoviolacea* solely in soil from the USA and Poland [12]. The cat likely acquired the fungus from the environment by traumatic inoculation, as is common for most *Sporothrix* species [3]. Given that the owner reported clinical signs of sporotrichosis after being scratched, *S. brunneoviolacea* appears to be capable of zoonotic transmission, which is to date only reported for *S. schenckii* and *S. brasiliensis* [18]. However, it is important to highlight that the potential of zoonotic transmission of this species must be confirmed in future studies since the clinical sporotrichosis signs of the owner were self-reported. Furthermore, the owner lives in a hyperendemic area of cat-transmitted sporotrichosis by *S. brasiliensis*, being previously made aware of the zoonotic transmission of this fungi by local healthcare professionals.

Interestingly, sporotrichosis is predominantly caused by *S. schenckii*, *S. brasiliensis* and *S. globosa*, which are considered highly virulent [1]. Sporotrichosis cases in cats are often disseminated and antifungal treatment takes a prolonged time of usually 2 months or longer in felines [9]. Conversely, there are sporadic cases by species outside the clinical clade (e.g. *Sporothrix chilensis*, *Sporothrix mexicana* and *Sporothrix davidellisii*), which appear to display a lower level of virulence [19]. These cases are overall mild in presentation and antifungal treatment is usually effective within a short period [20]. The feline patient in the current study also displayed a mild form of the disease and was successfully treated with a short course of itraconazole, which is in accordance with earlier reports of 'environmental' *Sporothrix* species. Interestingly, phylogenetics showed there might now be three branches of *Sporothrix* species that cause disease.

For *Sporothrix* species, treatment failure is reported although it remains unclear to which extent antifungal resistance is involved [10,21]. In order to trace and detect elevated MICs, surveillance with reference methods like CLSI or EUCAST microdilution are warranted. Surprisingly, both methods did not work for *S. brunneoviolacea* despite proper sporulation, while these have been extensively applied to species of the clinical clade [22]. To enable sufficient growth in microbroth dilution assays, a more nutrient-rich medium could be worthwhile to investigate. Nonetheless, itraconazole treatment resolved the lesions in both the cat and owner, suggesting antifungal activity.

The main limitation of this study is its retrospective nature that refrained us from collecting isolates from the owner and inspecting tissues to confirm the presence of *Sporothrix* in her lesion. Since no species identification and genotyping could be performed on the human case, zoonotic transmission cannot be confirmed [18].

To conclude, we report the first case of sporotrichosis by *S. brunneoviolacea* that was previously considered an environmental species with no pathogenic potential in mammals. The infected patient was a feline from southern Brazil with a small lesion, which likely infected the owner as well. A short course of itraconazole was sufficient to resolve the lesion. Using the *CaM* sequence, *S. brunneoviolacea* appeared not closely related to other pathogenic *Sporothrix* species. Surprisingly, microbroth dilution by CLSI and EUCAST guidelines did not yield any growth.

Declaration of Competing Interests

EFJM received research grants from Mundipharma and Scynexis, is in the scientific advisory board for Pfizer and has received speaker fees from Gilead Sciences. The other authors declare that they have no competing financial or personal interests which can influence the work reported in this paper.

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Author contributions

All authors contributed to the accomplishment of this manuscript. Conceptualization (MRT, BS). Data Curation (MRT, BS). Formal Analysis (BS, TDG, MRT). Investigation (MRT, BS, TDG, BJFSL, JEDH, BME, KOS, FFB). Methodology (MRT, BS, BJFSL, TDG). Supervision (TDG, EFJM, MOX). Funding acquisition (EFJM, MOX).

Writing—Original Draft (BS), Writing—Review & Editing (MRT, BS, BJFSL, VRP, KOS, FFB, TDG, EFJM, MOX).

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Supplementary

Table S1: Calmodulin control sequences of 65 *Sporothrix* species included in the phylogenetic analysis.

Species	ID	Accession number
Sporothrix abietina	CMW40454	MW579752.1
Sporothrix africana	8.3	MG431430.1
Sporothrix albicans	CBS 302.73	AM398396.1
Sporothrix aurorae	CBS 118837	KX590783.1
Sporothrix bragantina	CBS 474.91	KX590784.1
Sporothrix brasiliensis	CBS 120339	KP101421.1
Sporothrix brunneoviolacea	CBS 793.73	KP017106.1
Sporothrix brunneoviolacea	CBS 110895	KP017104.1
Sporothrix brunneoviolacea	CBS 110896	KP017102.1
Sporothrix brunneoviolacea	CBS 124561	KF574472.1
Sporothrix brunneoviolacea	CBS 124562	KX590803.1
Sporothrix brunneoviolacea	CBS 124564	KP017105.1
Sporothrix brunneoviolacea	KFL19PFbDd	OP589043.1
Sporothrix brunneoviolacea	KFL32PFaDd	OP589037.1
Sporothrix brunneoviolacea	KFL89PFDd	OP589043.1
Sporothrix cabralii	CMW 38098	KX590804.1
Sporothrix candida	CBS 129717	KX590785.1

Sporothrix cavum	KFL42215aDRJ	MW768976.1
Sporothrix chilensis	Ss469	KP711815.1
Sporothrix cracoviensis	KFL2514bRJTD	MH283529.1
Sporothrix cryptarchum	KFL410DB16bRJCU	MH741231.1
Sporothrix davidellissi	CBS 147636	PV092593
Sporothrix dentifunda	CBS 115790	KX590787.1
Sporothrix dimorphospora	CBS 125442	KX590806.1
Sporothrix dombeyi	CBS 455.83	KX590793.1
Sporothrix eucalyptigena	CBS 139899	KX273431.1
Sporothrix eucastaneae	CBS 424.77	KX590781.1
Sporothrix euskadiensis	VPRI43754	MW075144.1
Sporothrix fraxini	KFL21BS16bRJHV	MH283530.1
Sporothrix fumea	CBS 129712	KX590788.1
Sporothrix fusiformis	KFL43916RJSR	MH283518.1
Sporothrix globosa	CBS 120340	KP101459.1
Sporothrix gossypina	ATCC 18999	KX590789.1
Sporothrix guttuliformis	CBS 437.76	KX590807.1
Sporothrix humicola	CBS 118129	KX590808.1
Sporothrix inflata	2 PB-2018	MH283521.1
Sporothrix lignivora	CBS 119147	KP017107.1
Sporothrix luriei	ATCC 18616	AM747302.1
Sporothrix macroconidia	CXY1894	MH592598.1
Sporothrix mexicana	Ss 132	JF811340.1
Sporothrix narcissi	CBS 138.50	KX590791.1
Sporothrix nebularis	CMW27319	OK165572.1
Sporothrix nigrograna	VPRI43755	MW075145.1
Sporothrix nothofagi	CMW 37658	KX590810.1
Sporothrix pallida	CBS 131.56	KX590811.1
Sporothrix palmiculminata	CBS 119590	KX590794.1
Sporothrix phasma	CBS 119721	KX590795.1
Sporothrix polyporicola	CBS 669.88	KX590796.1

Sporothrix prolifera	CBS 251.88	KX590797.1
Sporothrix protearum	CMW:50506	MG431429.1
Sporothrix pseudoabietina	CXY1937	MH592601.1
Sporothrix rapaneae	CMW40369	KU639609.1
Sporothrix resoviensis	KFL204ABRZN16AO	MH741228.1
Sporothrix roztoczensis	KFL96So	OP589055.1
Sporothrix schenckii	UTHSC 04-1064	AM399014.1
Sporothrix silvicola	KFL48So	OP589050.1
Sporothrix smangaliso	CXY1937	MF043586.1
Sporothrix splendens	CMW:50507	MG431431.1
Sporothrix stenoceras	CBS 798.73	KX590782.1
Sporothrix stylites	CBS 118848	KX590812.1
Sporothrix tumida	KFL55RJ	OP589058.1
Sporothrix undulata	KFL398DB16RJEG	MH741239.1
Sporothrix variecibatus	CBS 121961	KX590813.1
Sporothrix villosa	SNM188	MZ019543.1
Sporothrix zambiensis	CMW:29078	MG431432.1

7. MANUSCRITO 3

Sporotrichosis immunoreactive in a hyperendemic area of Southern Brazil

Esse artigo está nas normas e com previsão de submissão na Mycopathologia – Fator de Impacto 2,9

Este trabalho descreve uma série de casos de reações de hipersensibilidade (RH) associadas à esporotricose por *Sporothrix brasiliensis* em uma região hiperendêmica do Rio Grande do Sul. Entre 164 pacientes diagnosticados no periodo estudado, 28 (17,1%) apresentaram RH, principalmente artrite asséptica, relacionadas a formas linfocutâneas ou cutâneas fixas. A genotipagem de isolados não revelou diferenças em relação aos genótipos causadores das formas clássicas, sugerindo que a RH é determinada pela resposta imune do hospedeiro. Os achados indicam um aumento recente na frequência de RH, com predomínio de manifestações clínicas brandas. O estudo foi conduzido no âmbito da Rede de Assistência à Esporotricose, estruturada junto ao Hospital Universitário Dr. Miguel Riet Correa Jr., em parceria com o Serviço de Assistência Especializada em Infectologia e o Laboratório de Micologia da FAMED-FURG. Essa integração entre assistência hospitalar e pesquisa acadêmica possibilitou não apenas o atendimento especializado à população acometida, mas também a coleta sistemática de dados clínicos e microbiológicos, fundamentais para compreender a dinâmica local da doença. Dessa forma, o trabalho representa a primeira descrição detalhada de manifestações de hipersensibilidade associadas à esporotricose no RS, ampliando o entendimento sobre a apresentação clínica dessa micose em áreas hiperendêmicas. Além de contribuir para a caracterização epidemiológica regional, os resultados oferecem subsídios para o reconhecimento precoce e manejo adequado desses casos na prática clínica.

Sporotrichosis immunoreactive in a hyperendemic area of Southern Brazil

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Abstract

Zoonotic sporotrichosis caused by *Sporothrix brasiliensis* is an emerging disease. In recent years, there has been a rise in atypical cases reported in hyperendemic areas, with hypersensitivity reactions (HR) being the most prevalent. However, in Rio Grande do Sul (RS), a hyperendemic region since the 1990s, only a few HR cases have been documented. Thus, we aimed to report a series of HR cases associated with sporotrichosis in this region of Southern Brazil. We conducted a retrospective study (August 2017–December 2024) including all HR cases diagnosed at a tertiary hospital in RS, with genotyping performed on selected isolates. Among 164 patients diagnosed with sporotrichosis, 28 (17.1%) developed HR in association with lymphocutaneous (46%) or fixed cutaneous (36%) lesions, predominantly presenting as aseptic arthritis (82%). All patients with follow-up achieved complete cure within a mean period of 104 days. Nine isolates from proven HR cases were genotyped, showing no genetic distinction from those associated with classical sporotrichosis. Our findings highlight an apparent increase in HR frequency over recent years, suggesting that these reactions are primarily driven by the host immune response rather than by specific antigenic features of distinct *S. brasiliensis* genotypes.

Keywords: *Sporothrix brasiliensis*, aseptic arthritis, polyarthralgia, erythema nodosum, zoonosis.

Introduction

Since the beginning of the zoonotic sporotrichosis endemic in Brazil in the 1990s, the number of reported cases has increased significantly, predominantly associated with *Sporothrix brasiliensis* (1). Cases have been reported in almost all Brazilian states as well as in other Latin American countries (2). Reflecting its growing public health importance, Brazil officially implemented compulsory notification of human sporotrichosis in 2025 (3).

The most common clinical presentation is cutaneous form, which can be fixed or lymphocutaneous (4). However, in hyperendemic areas, reports of atypical forms of sporotrichosis primarily linked to zoonotic transmission have been increasing, represented by immunoreactice cases, leading to hypersensitivity reactions (HR), osteoarticular, mucosal, meningeal, and pulmonary forms (5). The highest frequency of atypical manifestations has been registered in Rio de Janeiro, one of the Brazilian epicenters of sporotrichosis, accounting for 88% of reported cases (5–7). Among these atypical forms, HR is the most prevalent, representing 51% of the cases described in the literature (5).

HR in sporotrichosis is an immunologic reaction to fungal antigens that can result in erythematous lesions (nodosum or multiforme), aseptic arthritis (typically monoarticular and manifests with edema an inflammatory signs) or Sweet's syndrome (erythematous lesions with fever and leukocytosis) (4,8). Although Rio Grande do Sul has reported outbreaks of zoonotic sporotrichosis since its beginning (90s), only a small number of atypical cases (n=7) have been reported (5,9,10). In light of this, we aimed to report a series of HR cases associated with sporotrichosis, updating its data in a hyperendemic area of Rio Grande do Sul, Southern Brazil. In addition, given that the genetic variability of *S. brasiliensis* has been associated with differences in clinical presentation and regional epidemiology, we also explored whether the occurrence of HR could be related to specific genotypes circulating in the state.

Material and Methods

A retrospective study was conducted including all HR sporotrichosis cases diagnosed since the beginning of the specialized reference service for sporotrichosis (SRS) at the University Hospital from the *Universidade Federal do Rio Grande* (FURG) (HU-FURG/EBSERH) in August/2017 to December/2024 in the Southern region of Rio Grande do Sul state (RS), Brazil (11). All patients included were referred to the SRS at the HU-FURG/EBSERH and diagnosed at the Mycology Laboratory of the FURG, Rio Grande City, RS, Brazil.

We included cases of patients with proven and probable sporotrichosis showing HR manifestations. Proven cases were defined as patients who had samples collected that showed *S. brasilienis* growth on culture, and probable cases was defined as patients with clinical-epidemiological data of sporotrichosis (typical lesions and contact with a cat that had confirmation of sporotrichosis) (12). HR cases were classified based on the clinical presentation of the patient according to the description of Sweet's syndrome, erythema nodosum, erythema multiforme, or aseptic arthritis (4,8).

Clinical-epidemiological data of the patients (sex, age, transmission source, clinical presentation of typical sporotrichosis in association with HR, and treatment) were obtained from online medical records and the database of the Mycology Laboratory (FURG). Additionally, during the same study period, we evaluated the total number of sporotrichosis cases (proven and probable, following the same criteria mentioned above) diagnosed in our center. This total was used to calculate the frequency of HR cases within the sporotrichosis cases during the period of the study. Additionally, to compare the frequency of HR cases across the study years, we divided the period into the early years of the SRS center (2017–2020) and the later years (2021–2024). Data were analyzed using descriptive statistics and frequencies. This research was approved by the Ethics Committee of the FURG (process 234/2018).

For a subset of culture-confirmed cases, genotyping of isolates had been previously performed as described by Trapaga et al. (2025). Genotyping was performed using short tandem repeat (STR) analysis, with copy numbers determined in GeneMapper 5 (Applied Biosystems, Foster City, CA, USA), and genetic analyses conducted in BioNumerics v7.6.1 (Applied Maths NV, Sint-Martens-Latem, Belgium). In the present study, these existing genotyping data were incorporated to compare genetic profiles of isolates from patients with HR versus those without HR.

Results

During the 7-years period, 164 patients were diagnosed with sporotrichosis at the SRS, of whom 28 (17.1%) presented hypersensitivity reactions (HR) associated with classical forms of the disease. Between 2017 and 2020, 41 sporotrichosis cases were recorded, including only one HR case (2%). In contrast, from 2021 to 2024, there were 123 cases, with 27 presenting HR (21.9%).

The HR case identified in the first period displayed the lymphocutaneous form, while in the later period most HR cases were lymphocutaneous (13/27), followed by fixed cutaneous (10/27) and mixed forms involving lymphocutaneous and ocular manifestations (4/27). No patients presented pulmonary, meningeal, or osteoarticular involvement.

Most HR patients were women (82.1%; 23/28), with a median age of 44 years (range: 19–70 years). The predominant HR manifestation was aseptic arthritis (82.1%; 23/28), reported clinically as polyarthralgia, followed by erythema nodosum (39.2%; 11/28) (**Figure 1**). All patients reported previous contact or trauma involving infected cats. The median time to diagnosis was 28.5 days, ranging from 7 to over 1000 days.

Regarding treatment, most patients (75%; 21/28) received itraconazole 200 mg/day, with a median duration of 104 days until ambulatory discharge. In two cases, the dose needed to be increased to 400 mg/day, and three patients were treated with terbinafine 250–500 mg/day for at least 90 days (Table 1). Overall, 17 patients achieved clinical cure and were discharged after a median of 106 days of therapy (range: 78–207 days), while 11 were lost during the follow-up.

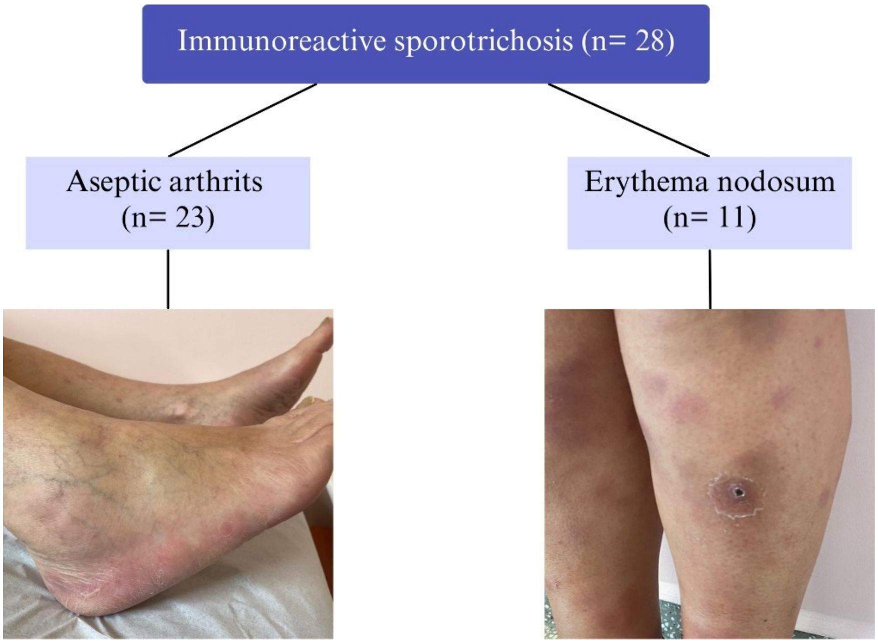

Figure 1: Immunoreactive manifestations of sporotrichosis in patients from a hyperendemic area in Southern Brazil. Aseptic arthritis (A), characterized by joint swelling and pain without evidence of active infection, was identified in 23 patients, whereas erythema nodosum (B), represented by painful erythematous nodules in the lower limbs, was observed in 10 patients.

Of the 28 HR cases, 15 were confirmed by positive fungal culture. From these, nine isolates were successfully recovered and subjected to genotyping analysis. Genotyping identified 24 distinct profiles among all study isolates, five of which were present in HR cases (**Figure 2**). All genotypes belonged to the Rio Grande do Sul clade previously described in the literature (13). No exclusive or divergent genotype was associated with HR, reinforcing the interpretation that these manifestations are primarily driven by host immune factors rather than fungal genetic variability.

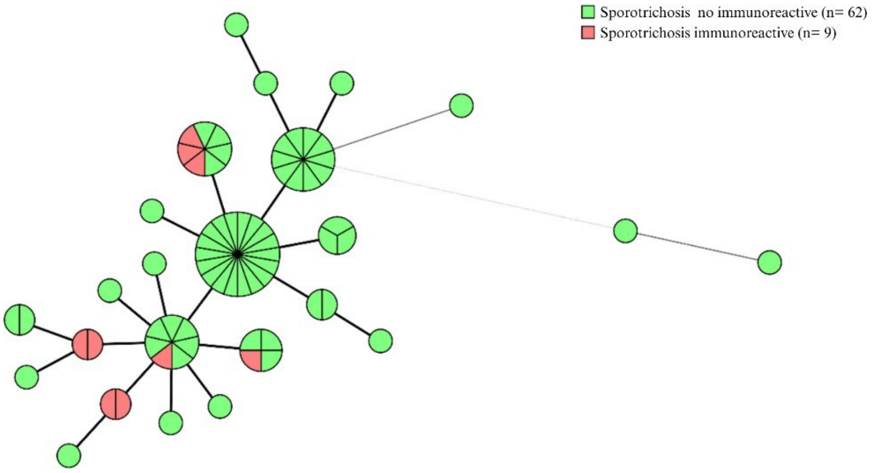

Figure 2: Minimum spanning tree (MST) of *Sporothrix brasiliensis* isolates from Rio Grande, Rio Grande do Sul. Genotypes comparison of the isolates included in our study with and without sporotrichosis hypersensivity reaction.

Table 1: Clinical epidemiological data of patients with hypersensitivity reaction to sporotrichosis in a hyperendemic area from Southern Brazil.

Patient	Sex	Age	Sporothrichosis	Clinical form	Symptoms	Transmission	Lesion Evolution unti Diagnosis	Treatment	Days of treatment	Outcome	Year
1	F	40	Probable	lymphocutaneous	aseptic arthritis, erythematous lesions	Zoonotic	7	ITZ 200mg/day	144	Lost in follow-up	2020
2	F	52	Proven	cutaneous fixed	aseptic arthritis, macular erythematous lesions, fever	Zoonotic	27	ITZ 200mg/day	78	Discharge	2022
3	F	40	Proven	cutaneous fixed	aseptic arthritis,	Zoonotic	>1000	ITZ 200mg/day	207	Discharge	2022
4	F	26	Probable	cutaneous fixed	erythema nodosum cutaneous lesions	Zoonotic	60	ITZ 200mg/day	90	Lost in follow-up	2022
5	M	62	Probable	lymphocutaneous	aseptic arthritis, erythema nodosum cutaneous lesions	Zoonotic	missing	ITZ 200mg/day	90	Discharge	2023
6	F	48	Probable	lymphocutaneous	aseptic arthritis, nodular lesion	Zoonotic	30	TRB 250mg/day	106	Discharge	2023
7	F	63	Probable	cutaneous fixed	aseptic arthritis, erythematous and maculopapular lesions	Zoonotic	150	ITZ 200mg/day	200	Discharge	2023
8	F	58	Proven	lymphocutaneous	aseptic arthritis, nodular lesion	Zoonotic	20	ITZ 200mg/day		Lost in follow-up	2023
9	F	52	Probable	cutaneous fixed	aseptic arthritis, fever, cutaneous lesions	Zoonotic	30	ITZ 200mg/day	112	Discharge	2024
10	F	49	Probable	lymphocutaneous	aseptic arthritis, cutaneous lesios	Zoonotic	missing	ITZ 200mg/day	104	Discharge	2024
11	F	19	Proven	mixed form (lymphocutaneous + ocular)	aseptic arthritis, cutaneous lesions, Parinaud syndrome	Zoonotic	7			Lost in follow-up	2024
12	F	35	Proven	mixed form (cutaneous + lymphocutaneous	aseptic arthritis, erythema nodosum, fever, cutaneous lesions	Zoonotic	18	ITZ 200mg/day	90	Discharge	2024
13	F	42	Proven	cutaneous fixed	aseptic arthritis, erythema nodosum	Zoonotic	7	ITZ 200mg/day	33	Lost in follow-up	2024
14	F	70	Proven	cutaneous fixed	aseptic arthritis	Zoonotic	90	TRB 500mg/day	115	Discharge	2024

15	F	54	Probable	cutaneous fixed	aseptic arthritis, erythematous lesions	Zoonotic	10	ITZ 200mg/day	106	Discharge	2024
16	F	37	Probable	lymphocutaneous	erythema nodosum, arthritis, cutaneous lesions	Zoonotic	missing	ITZ 200mg/day 40d; ITZ 400mg/day 68d	108	Lost in follow-up	2024
17	F	46	Probable	lymphocutaneous	aseptic arthritis, cutaneous lesions	Zoonotic	30	ITZ 200mg/day	136	Discharge	2024
18	M	68	Proven	cutaneous fixed	aseptic arthritis, cutaneous lesions	Zoonotic	missing	TERB 500mg/day	90	Discharge	2024
19	M	24	Proven	lymphocutaneous	erythema nodosum, cutaneous lesions	Zoonotic	10	ITZ 200mg/day	32	Lost in follow-up	2024
20	F	45	Probable	cutaneous fixed	aseptic arthritis, cutaneous lesions	Zoonotic	60	ITZ 200mg/day	163	Discharge	2024
21	F	40	Proven	lymphocutaneous	erythema nodosum, cutaneous lesions	Zoonotic	60	ITZ 200mg/day	66	Lost in follow-up	2024
22	F	20	Proven	mixed form (lymphocutaneous + ocular)	erythema nodosum, cutaneous lesions, Parinaud syndrome	Zoonotic	7	ITZ 200mg/day	60	Lost in follow-up	2024
23	M	40	Proven	lymphocutaneous	aseptic arthrits, cutaneous lesions	Zoonotic	30	ITZ 200mg/day 26d; ITZ 400mg/day 123d	149	Discharge	2024
24	M	27	Proven	lymphocutaneous	aseptic arthrits, cutaneous lesions	Zoonotic	30	ITZ 200mg/day	126	Discharge	2024
25	F	63	Probable	lymphocutaneous	aseptic arthrits, erythema nodosum, cutaneous lesions	Zoonotic	15	ITZ 200 mg/day	171	Discharge	2024
26	F	43	Proven	mixed form (lymphocutaneous + ocular)	aseptic arthrits, erythema nodosum, Parinaud syndrome	Zoonotic	7	ITZ 200mg/day	90	Lost in follow-up	2024
27	F	33	Probable	lymphocutaneous	aseptic arthrits, cutaneous lesions	Zoonotic	Missing	ITZ 100 mg/day (4 months of	19	Lost in follow-up	2024

								previous use); ITC 200mg/day			
28	F	39	Proven	mixed form (lymphocutaneous + ocular)	aseptic arthrits, erythema nodosum, Parinaud syndrome	Zoonotic	Missing	ITZ 200mg/day	182	Discharge	2025

Legend: F: Female; M: Male; HR: Hypersensitivity; ITZ: Itraconazole;. *Classical forms of sporotrichosis associated with hypersensitivity reaction.

Discussion

This study presents the first case series describing hypersensitivity reactions (HR) in sporotrichosis from Southern Brazil. A high prevalence of HR was observed in a relatively short period (seven years), when compared with reports from the other main endemic region: 17.1% of sporotrichosis cases in Southern Brazil (28/164) over seven years, versus 8.1% in Rio de Janeiro over thirteen years (6). Notably, most cases were concentrated in the later years of the study (2021–2024), with a marked increase in both typical and atypical presentations compared to the initial period (21.9%; 27/123 vs. 2.4%; 1/41) (17%; 21/117 vs. 2%; 1/41) (5).

Most of the cases were from women, and the most common lesions were lymphocutaneous and cutaneous fixed, consistent with previously described sporotrichosis epidemiology (4,6). In other centers, the most prevalent HR manifestations reported were erythematous lesions or Sweet syndrome. In contrast, aseptic arthritis was the main presentation in this series, suggesting a distinct regional pattern and contributing novel insights into HR associated with zoonotic sporotrichosis on a global scale (4).

Nevertheless, our patients showed a good prognosis, in which up to 100% evolve to cure without sequelae as already described (5). This good outcome contrasts with other atypical presentations of sporotrichosis, for which deaths and sequelae have been reported, mainly in association with meningeal, osteoarticular, pulmonary, and multifocal disease (5,14). Notably, these more severe forms were absent in our region. The better prognosis of HR is linked to an immunological reaction triggered by contact with fungal propagules, leading to a good therapeutic response, while the other atypical forms are frequently associated with immunosuppressed patients (15).

Patients in this series received a higher dose of itraconazole (200 mg/day) compared with most other studies, which typically report 100 mg/day (7). A previous study from the same region also described higher doses ranging from 200 to 400 mg/day in classical sporotrichosis (11). The regional differences observed, including treatment doses, higher prevalence of HR, predominance of atypical forms with favorable prognosis, and absence of severe cases compared to other epicenters such as Rio de Janeiro, may be related to genotypic variations of *S. brasiliensis*, as documented in the literature (16,17).

However, our genotyping analysis showed no distinction between isolates from HR and classical cases, reinforcing that these reactions are driven by the host immune response rather than by specific fungal genotypes (5,15). As only a limited number of isolates were analyzed, all within the Rio Grande do Sul clade, the potential correlation between genotype, clinical presentation, and epidemiological patterns remains uncertain and deserves further investigation.

This study provides data on atypical presentations of sporotrichosis in Southern Brazil, one of the first regions to report zoonotic outbreaks of this mycosis, where *S. brasiliensis* genotypes differ from those in other Brazilian epicenters. Our findings suggest an increasing frequency of unusual clinical presentations in recent years. Importantly, genotyping analyses indicate that hypersensitivity reactions are not determined by specific fungal isolates, but rather by the host immune response, highlighting the central role of host factors in disease manifestation. Further studies are warranted to explore the interplay between clinical presentations, host immunity, and the virulence of distinct *S. brasiliensis* genotypes.

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8. MANUSCRITO 4

Sporothrix brasiliensis-specific PCR for the diagnosis of cat and human sporotrichosis through non-invasive samples

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Este estudo avaliou um PCR espécie-específico para o diagnóstico de esporotricose causada por *Sporothrix brasiliensis* utilizando amostras não invasivas de humanos e gatos. Foram analisadas 110 amostras de secreção de lesões coletadas por swab, evidenciando uma concordância de 86% em relação ao método padrão-ouro (cultura). O PCR demonstrou ser uma alternativa rápida e eficaz para auxiliar no controle da zoonose, especialmente em locais com escassez de micologistas para a identificação fúngica. Este projeto está inserido na Rede Saúde Única FAPERGS/FIOCRUZ, intitulada "Esporotricose e *Sporothrix brasiliensis* no contexto *One Health*: Promoção da saúde, pesquisa e inovações para melhoria diagnóstica e terapêutica na saúde humana e animal, e para melhor compreensão do impacto da saúde ambiental na interface humano-animal-ecossistema."

Sporothrix brasiliensis-specific PCR for the diagnosis of cat and human sporotrichosis through non-invasive samples

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Keywords: molecular diagnosis; swab; comparison diagnosis methods, zoonosis, *Sporothrix brasiliensis*.

Abstract

Zoonotic sporotrichosis caused by *Sporothrix brasiliensis* is an emerging mycosis in Latin America. One of the problems to quickly treat infected animals and break the transmission chain is associated with the time-consuming gold-standard diagnosis method (culture). We aimed to evaluate a species-specific polymerase chain reaction (PCR) for the diagnosis of sporotrichosis caused by *S. brasiliensis* using non-invasive samples. We performed a retrospective cross-sectional study using samples collected with swabs from humans and cats with clinical suspicion of sporotrichosis. DNA was extracted using a commercial kit, and a species-specific PCR for *S. brasiliensis* detection was performed. One-hundred ten samples were included. PCR showed a good concordance with culture (86% of agreement) for human and cat samples (Kappa coefficient = 0.722, and 0.727, respectively). In conclusion, our data shows that this adapted PCR using non-invasive samples can be applied to sporotrichosis diagnosis, being a good alternative mainly in regions with a lack of mycologists to identify the fungus in culture, contributing to the control of this emergent zoonosis.

Introduction

Currently, zoonotic sporotrichosis is the main emergent implantation mycosis in Latin-America [1,2]. The worrying epidemiological scenario of sporotrichosis is associated with the adaptation of the species *Sporothrix brasiliensis* to domestic cats, the main victims of this pathogen, developing a severe and progressive disease evolving into a systemic form and even death [3,4]. In addition, the high burden of *S. brasiliensis* cells found in the lesions of infected cats facilitates fungal transmission to humans and other animals by scratches and bites of these victims. In addition, mucosal contact of humans with infected secretions of the animals may contribute to sporotrichosis transmission [5,6].

Cat and human diagnosis can be performed with non-invasive samples, especially secretion from ulcerative lesions collected with a swab. Sensitivity rates of 90-95% are report with this biological sample that requires minimal management to be collected, avoiding an invasive procedure to obtain a biopsy sample [7–9]. However, the "gold standard" method for sporotrichosis diagnosis (mycological culture) requires 5 to 21 days for *Sporothrix* spp. growth, and a subsequent polymerase chain reaction (PCR) or sequencing methods for species identification [4,10,11]. Besides, access to laboratories that provide mycological tests is not usually available in many regions of Latin-America, due to the global negligence status of mycoses and lack of investment in this context [12]. These factors can lead to missed and late diagnoses, which impact the control of zoonotic sporotrichosis due to late or absent treatment of infected patients.

On the other hand, molecular assays are more widely known by laboratorian professionals since they are techniques used for a variety of pathogens, without need for specific microbiology knowledge. Thus, these methods could also be applied for the sporotrichosis diagnosis [13,14]. However, few studies evaluated molecular diagnosis using non-invasive samples for this mycosis [15–18]. Considering the importance of an early diagnosis for sporotrichosis control and the promising contribution of molecular approach to this problem, we aimed to evaluate the performance of a species-specific polymerase chain reaction (PCR) for the diagnosis of sporotrichosis in humans and cats through non-invasive samples.

Methods

A retrospective cross-sectional study was conducted in a hyperdendemic region of zoonotic sporotrichosis in southern Rio Grande do Sul, Brazil. We included non-invasive samples from ulcerative cutaneous lesions, collected through sterile swab between July 2020 to August 2023, from humans and cats suspected of sporotrichosis.

Randomized samples were taken from the biorepository of the Mycology Laboratory of Medicine School of Federal University of Rio Grande (MycoLab - FAMED-FURG).

Swab samples were previously eluted in 2 ml of 0.9% saline solution, vortexed for 2 minutes, 100µl were cultured on plates with Sabouraud dextrose agar with and without cycloheximide (Kasvi®, São José dos Pinhais, PR, Brazil), and aliquots were stored at 4 °C (up to 7 days) or at -20 °C (8 days and up to 35 months) at our biorepository. All *Sporothrix* sp. isolated in culture were identified as *S. brasiliensis* by the species-specific PCR described by Rodrigues et al. (2015) [19]. Data regarding the number of colony forming units (CFU) isolated in culture at the diagnosis was collected and categorized in low (<10 CFU), moderate (11-100 CFU) or high (>100 CFU).

We included cases of human sporotrichosis, classified as proven (positive culture for *S. brasiliensis*) or probable (history of contact/trauma with a cat with proven diagnosis but without laboratory confirmation and clinical cure through antifungal treatment), and of cat sporotrichosis defined by a positive culture for *S. brasiliensis* [20].

Aliquots of 200 µl of the samples were used for DNA extraction using the High Pure PCR Template Preparation Kit (Roche®, Mannheim, Germany) following the manufacturer's instructions, with adaptations: overnight incubation with lysis buffer and proteinase K (5 µl – 20 mg/mL) at 37 °C; and second round of lyticase (10µl – 25,000 U) added, incubated for 30 min at 30 ºC. As a control of DNA quality, all samples were subjected to a PCR to detect the mammalian housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using a PCR reaction with a final volume of 25 µl, containing 12.5 µl of Gotaq® G2 Master Mix (2×) (400 µM dNTPs and 3 mM $MgCl_2$) (Promega Corporation, Wisconsin, USA), 0.2 µM of forward (5' CCCATCACCATCTTCCAGG 3') and reverse (5' AGATGATGACCCTTTTGGC 3') GAPDH primers, and 2 µl of DNA. A positive control, using a DNA extract from feline secretion sample was used in all PCR reactions, and RNAse and DNAse-free water was used as a negative control. Electrophoresis was performed on a 1.2% gel by amplifying the amplicons with 148 pb under UV light with a BlueGreen Loading Dye (LCG Biotecnologia, SP, Brasil).

The diagnostic PCR was performed with the primers for *S. brasiliensis* described by Rodrigues et al. (2015). A final volume of 25 µl was used for each PCR reaction, composed by 12.5 µl of Gotaq® G2 Master Mix (2×) (400 µM dNTPs and 3 mM $MgCl_2$) (Promega Corporation, Wisconsin, USA), 0.8 µM of foward (5'

CCCCCGTTTGACGCTTGG 3') and reverse (5' CCCGGATAACCGTGTGTCATAAT 3') *Sbra* primer, and 3 µl of the DNA extracted from the clinical samples. The cycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing temperature by the touchdown method, starting at 70 °C and increasing to 60 °C, extension at 72 °C for 1 min, and extension at 72 °C for 10 min [19]. Electrophoresis was performed on a 1.2% gel by amplifying the amplicons with 469 pb under UV light with a BlueGreen Loading Dye (LCG Biotecnologia, SP, Brasil). Samples with negative results in this first PCR were subjected to a second round PCR using the same amplicons (3 µL) from the previous PCR, under the same cycling conditions, in order to increase the sensitivity of samples with low fungal load, as already described [19]. All reactions had a positive control, using a DNA extracted from a clinical *S. brasiliensis* fungal isolate, and a negative control with RNAse and DNAse-free water.

Agreement assessment between the culture method and species-specific PCR was analyzed using kappa coefficient for human and cat samples. Kappa index was interpreted as follows: poor agreement (0.00-0.20); fair agreement (0.21-0.40); moderate agreement (0.41-0.60); good agreement (0.61-0.80); and very good agreement (0.81-1.00) [21]. The categorization (low, moderate and high) of CFU in culture was compared with the PCR results, using the Chi-square method. P values ≤0.05 were considered significantly. All tests were performed using the SPSS 20.0 statistical program (IBM, Chicago, IL, US).

Results

A total of 110 samples were included, being 36 from humans and 74 from cats. From the 36 human samples, 18 were from sporotrichosis cases (16 proven and 2 probable) and 18 from patients with negative culture but with clinical suspicion of the disease. Regarding the 74 felines samples included, 42 were from sporotrichosis cases and 32 culture-negative. All the human and cat samples were positive for GAPDH.

S. brasiliensis-specific PCR was positive in 13 from the 18 human sporotrichosis cases, and the false-negative results occurred in samples from five proven cases. All 18 negative samples in culture were also negative in the PCR (Table 1), indicating 100% specificity. The concordance between culture and PCR methods for the diagnosis of human sporotrichosis was good (k= 0.722), with 86% agreement.

Regarding cat sporotrichosis cases, PCR was positive in 34 of the 42 samples positive in culture, and in two culture-negative samples. PCR negative results occurred in eight proved cases of feline sporotrichosis and in 30/32 samples from the negative samples in culture (Table 1). The concordance between molecular and culture methods was good (k= 0.727), with 86% agreement.

Table 1. Results of the first and second rounds of the polymerase chain reaction (PCR) for the diagnosis of sporotrichosis from non-invasive samples in comparison with *S. brasiliensis* isolation in culture.

		Culture for *Sporothrix brasiliensis*					
		Humans			**Cats**		
		Positive n (%)	Negative n (%)	Total n	Positive n (%)	Negative n (%)	Total n
PCR 1º round	Positive	5 (28)	0	5(14)	28 (67)	2 (6)	30(41)
	Negative	13 (72)	18 (100)	31(86)	14 (33)	30 (94)	44(59)
	Total	18 (100)	18 (100)	36(100)	42 (100)	32 (100)	74(100)
PCR 2º round*	Positive	8 (61)	0	8(26)	6 (43)	0	6(14)
	Negative	5 (39)	18 (100)	23(74)	8 (57)	30 (100)	38(86)
	Total	13 (100)	18 (100)	31(100)	14 (100)	30 (100)	44(100)

* only negative samples in the first round PCR were submitted to the second round.

Fungal load in positive culture from humans and cats was classified as high in 3 and 15, as moderate in 2 and 8, and as low in 3 and 8 samples, respectively. This data was missing in eight human samples and in 12 cat samples. All human and cat samples with high CFU in culture were positive in the first round of PCR, and the second round of PCR was necessary to increase the positivity rate to 36% (13/36) and 43% (32/74), respectively, of the samples from humans and cats (Figure 1) ($p<0.0\ 01$).

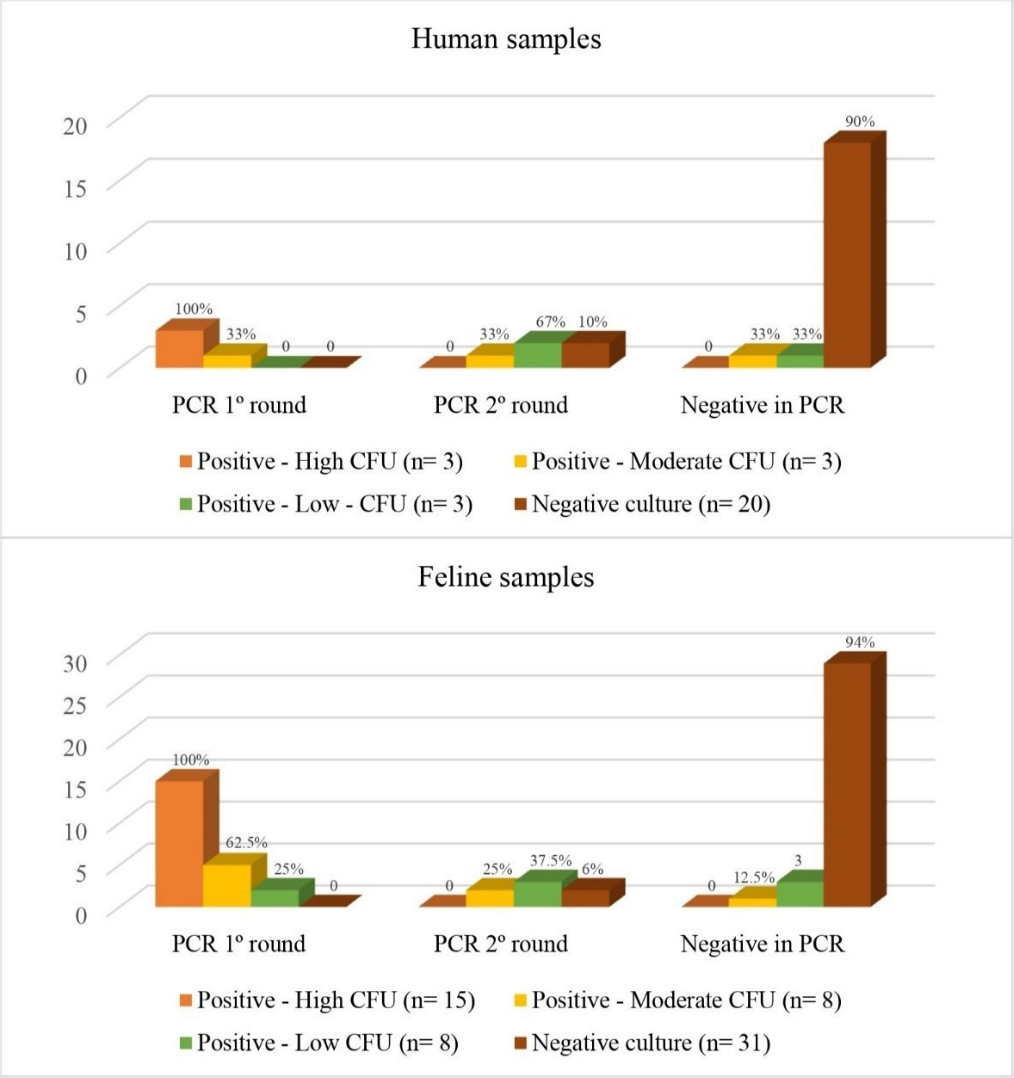

Figure 1. Comparative results of colony forming units (CFU) positivity in the species-specific in first and second round polymerase chain reaction (PCR) for *Sporothrix brasiliensis*, applied to molecular diagnosis of human and feline sporotrichosis, using non-invasive samples.

Discussion

There are few studies using molecular techniques through non-invasive samples to the diagnosis of zoonotic sporotrichosis in the scientific literature [15-18]. Here, we show the potential of a conventional PCR for this purpose, being our study pioneer in including non-invasive samples from human sporotrichosis beside cat samples. A good agreement of PCR assay with the gold standard test for sporotrichosis diagnosis (mycologic culture) described in our results is similar[15] or even better [16] than those previously published regarding cat sporotrichosis using the same conventional PCR and primers of our study, highlighting its potential as an alternative diagnostic method for sporotrichosis due to *S. brasiliensis*.

PCR was able to suggest the diagnosis of two human patients who were previously negative in culture test. Interestingly, both samples were from patients undergoing treatment with itraconazolel, given the suspicion of sporotrichosis, which is a cause of false-negative results in culture due to the antifungal inhibition of the fungi [4,22]. This data was already reported, and reaffirms the benefits in using this molecular technique in patients during antifungal therapy, as well-known in patients with dry lesions [15].

False-negative results in up to 20% of the human and cat samples in our study could be attributed to the low efficacy of fungal DNA extraction from these samples. This hypothesis is supported by the higher positivity of samples after the second round of PCR, and PCR positivity was directly proportional to the fungal load recovered in culture ($p < 0.001$). In samples with moderate CFU, an increase in positivity for humans and cats of 100% and 40%, respectively, was observed in the second round of PCR. The same was observed in low CFU in culture, for humans and cats, with an increase up to 67% and 152%, respectively, in the second round of PCR.

A limitation of our study was the absence of a mechanical lysis step, which improves the efficiency of fungal DNA extraction [23]. This higher load of DNA extracted from non-invasive samples could lead to even better sensitivit, reducing the false-negative rate of PCR in the diagnosis of sporotrichosis, a hypothesis that needs to be confirmed in future studies. Furthermore, future studies are encouraged to optimize this technique using different volumes of DNA to initiate PCR, since quantitative PCR (qPCR) for the diagnosis of sporotrichosis has already shown good results with a volume of 9 μL as well as 2 μL, and this factor may be associated with the availability of products for amplification [18,19].

Quantitative PCR is another molecular method already studied for the diagnosis of sporotrichosis through non-invasive samples, showing >95% of positivity using different targets [17, 18]. Although a higher rate of positivity is described using qPCR in comparison with our results of a conventional PCR (~80%), qPCR has a high initial cost to its implementation and consequently minor access mainly in developing countries such as Brazil and other Latin American countries where *S. brasiliensis* is emerging [13], being the conventional PCR a technique more broadly widespread in laboratories.

Our data contributes to the standardization of a conventional PCR for molecular diagnosis of zoonotic sporotrichosis directly from non-invasive samples. Together with their studies described [15-18] we add an alternative to surpass the scarcity of mycology laboratory support, which occurs even in endemic areas of this mycosis in consequence of the neglected status of fungal infections and the scarcity of mycologists [1,2,24]. In addition, the use of a molecular test for sporotrichosis can reduce the delay of a diagnosis confirmation. In fact, the gold standard methods the final identification of *S. brasiliensis* (culture + PCR for species identification) can reach 30 days, while, this *S. brasiliensis* specific PCR assay can be performed in up to 3 days [13].

In conclusion, our results showed a promising use of a species-specific conventional PCR for *S. brasiliensis* detection from non-invasive clinical samples, which can be applied to the diagnosis of cat and human sporotrichosis. This technique has a high potential to be added especially in areas where there is no mycological diagnosis, or to be added to laboratories that already apply classical methods, decreasing the time for diagnosis, favoring an early treatment of patients and contributting to a better prognosis of sporotrichosis.

Ethical aspects: The study was approved by the Research Ethics Committee in the Health Area of the Federal University of Rio Grande (CEP/FURG), process 6,204,435.

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9. MANUSCRITO 4

Marine microalgal metabolites against *Sporothrix brasiliensis*: *in vitro* evaluation and interaction with itraconazole

Artigo em formato de *short communication* nas normas e com previsão de submissão no periódico *Brazilian Journal of Microbiology* – Fator de Impacto 1,9

Este trabalho avaliou o extrato hexânico de *Nannochloropsis oceanica* frente a isolados clínicos de *Sporothrix brasiliensis*. Embora o extrato sozinho não tenha apresentado atividade inibitória em concentrações de até 800 µg/mL, ensaios de combinação revelaram efeitos benéficos com itraconazol, sem antagonismo. Trata-se do primeiro estudo a explorar compostos de microalgas marinhas contra *S. brasiliensis*, destacando o potencial da bioprospecção como estratégia complementar no tratamento da esporotricose. O estudo foi desenvolvido em parceria com o Laboratório de Bioprospecção de Produtos Naturais Costeiros e Núcleo de Desenvolvimento de Novos Fármacos (NUDEFA) da FURG, que possibilitou o acesso e a disponibilização dos extratos para os testes realizados com os isolados fúngicos. Essa colaboração interdisciplinar aproximou a micologia médica da prospecção de recursos naturais marinhos, ampliando o horizonte de estratégias potenciais para enfrentamento da esporotricose.

Marine microalgal metabolites against *Sporothrix brasiliensis*: *in vitro* evaluation and interaction with itraconazole

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Abstract: Sporotrichosis, a subcutaneous mycosis caused by fungi of the genus *Sporothrix*, has emerged as a major zoonotic disease in Brazil and Latin America, with *Sporothrix brasiliensis* as the primary etiological agent. The increasing reports of isolates with reduced ITZ susceptibility highlight the urgent need for novel therapeutic alternatives or adjuvant strategies. Natural products, including marine-derived metabolites, represent promising sources of antifungal agents. This study investigated the *in vitro* antifungal activity of a hexanic extract of the microalga *Nannochloropsis oceanica* against clinical isolates of *S. brasiliensis* and its interaction with ITZ. Thirteen isolates from humans and cats, as well as a reference strain, were tested using broth microdilution and checkerboard assays. The extract showed no direct inhibitory effect (MIC > 800 µg/mL). Checkerboard assay revealed mostly indifferent interactions, with only one isolate showing additive and another weak synergistic effects, while no antagonism was observed. Although the hexane fraction displayed limited activity, this work represents the first evaluation of *N. oceanica* metabolites against *S. brasiliensis*, pointing to the need for further studies using alternative extraction methods and bioactive fractionation to better assess their therapeutic potential.

Introduction

Sporotrichosis is a neglected tropical implantation mycosis caused by species of the *Sporothrix* genus [1]. Among them, *Sporothrix brasiliensis* stands out as the main virulent and the main agent responsible for ongoing zoonotic outbreaks in Brazil and Latin America, being recently included in the compulsory notification list in Brazil [2–4]. The disease has emerged as a serious public health and veterinary problem. Domestic cats are the main victims and the main transmitter of the fungus, their high fungal burden and frequent progression to disseminated disease facilitate spread to humans and other animals through scratches and bites[3,5]. This unique epidemiological scenario highlights sporotrichosis not only as a veterinary and medical challenge, but also as a model of a One Health issue, demanding innovative diagnostic, therapeutic, and preventive strategies.

Itraconazole (ITZ) remains the treatment of choice for humans and animals, but its limited efficacy in cats (40–77%), given the high cost, and administration difficulties often compromise therapeutic success [5,6]. Reports of isolates with reduced susceptibility to ITZ, and clinical failure highlight the need for alternative or adjunct antifungal strategies [7,8]. In this context, natural products emerge as valuable source of antifungal agents, and several plant-derived compounds have demonstrated activity against *S. brasiliensis*, such as *Schnius terebinthifolius* extracts, *Origanum majorana* extracts, *Plinia peruviana* extracts [9–13].

Marine bioprospecting further expands this potential. The marine environment is recognized as a reservoir of structurally unique metabolites with diverse mechanisms of action, derived from sponges, sea cucumbers, macroalgae, and associated microorganisms [14–16]. Several marine compounds have demonstrated antifungal activity against clinically relevant fungi, including *Candida* spp., *Cryptococcus* spp., and *Aspergillus* spp., suggesting that these molecules could also be effective against S. brasiliensis [15]. Notably, triterpenoid glycosides from sea cucumbers (Holothuroidea) exhibited broad-spectrum antifungal activity, including against *Sporothrix schenckii*, a closely related species, with promising low MIC values [17].

Microalgae of the genus *Nannochloropsis* are a particularly attractive source, producing bioactive metabolites such as lipids, carotenoids, and phenolic compounds with antimicrobial potential [18–20]. Extracts of *N. oceanica* have shown antifungal activity against *Fusarium* spp.[21], phytopathogenic fungi that can occasionally cause opportunistic infections in humans, but their effects against clinically relevant fungi, including *Sporothrix*, remain unexplored.

This study evaluated the *in vitro* antifungal activity of a hexanic extract of *N. oceanica* against clinical isolates of *S. brasiliensis* and investigated its interaction with itraconazole, aiming to explore marine microalgae as a potential source of antifungal agents in the context of sporotrichosis.

Materials and Methods

For the antifungal test, the microalga *Nannochloropsis oceanica* was cultivated in f/2 medium (pH 8) under controlled laboratory conditions: temperature of 23 °C, salinity 28, light intensity of 1000 µmol photons m^{-2} s^{-1}, a 12/12 h light–dark cycle, and constant aeration. Cultures were monitored daily, either directly by cell counting (cells/mL) in a Neubauer chamber or indirectly by measuring optical density at 750 nm using a spectrophotometer, with values converted to cell density by linear regression. After 7 days, at the end of the growth curve, cells were harvested by centrifugation (9000 rpm, 30 min) and dried in an oven at 50 °C for approximately 3 days. Extracts were prepared following Borges et al. (2016), with modifications [22]. Briefly, 1 g of dried biomass was dissolved in 15 mL of hexane and subjected twice to ultrasound treatment (40 Hz, 20 min) with a 20-minute interval between treatments. The extract was filtered through filter paper, centrifuged (3000 rpm, 2 min) to remove residual biomass, and the supernatant was collected. Solvent removal was carried out in a rotary evaporator under reduced pressure (40 °C/4 rpm), followed by drying in an oven (50 °C, 24 h) to eliminate solvent traces. The dried extract was suspended in DMSO (dimethylsulfoxide) to a stock concentration of 1.2 g/mL and stored at 4 °C.

The antifungal activity of the extract was tested *in vitro* against *S. brasiliensis* at concentrations ranging from 12.5 to 800 µg/mL using the broth microdilution method, according to the CLSI M38-A2 guidelines [23]. Thirteen clinical isolates of *S. brasiliensis*, identified by species-specific polymerase chain reaction [24], from humans (n= 8) and cats (n= 5) were obtained from the Medical Mycology Laboratory of FAMED-FURG collection and used in the study, additionally one type strain (CBS 120339) were included. Isolates were cultured on potato dextrose agar (Kasvi®, São José dos Pinhais, Paraná, Brazil) at 25 °C for 7 days, and standardized to a final inoculum of 0.8×10^4 to 1×10^5 CFU/mL. Drug dilutions were prepared in RPMI 1640. Each assay included growth

72 h, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration that completely inhibited fungal growth.

To evaluate interactions between the extract and itraconazole (ITZ; Sporanox®, Janssen), checkerboard assays were performed with seven *S. brasiliensis* isolates. The extract was tested at the concentrations described above, while ITZ was tested at concentrations from 0.031 to 8 µg/mL. Interaction was assessed by calculating the fractional inhibitory concentration index (FICi), interpreted as follows: strong synergism (SS), FICi < 0.5; weak synergism (WS), 0.5 ≤ FICi < 1; additive (AD), 1 ≤ FICi < 2; indifferent (IND), FICi = 2; and antagonism (ANT), FICi > 2 [25].

Results and Discussion

The hexanic extract of *Nannochloropsis oceanica* showed no inhibitory activity against any of the tested *S. brasiliensis* isolates (MIC > 800 µg/mL). In the combination assays with itraconazole, most results indicated indifference (five isolates), while one isolate showed an additive effect and another displayed weak synergism. No antagonism was observed. Taken together, these results indicate that the hexane fraction alone has limited antifungal potential, and the occasional interactions observed with itraconazole were not consistent across isolates.

Notably*, N. oceanica* is a microalga naturally found in Rio Grande, the epicenter of the sporotrichosis epidemic in southern Brazil, which underscores its relevance as a potential local source of antifungal agents [21,26]. However in our study, the extract showed only limited activity against *S. brasiliensis* when tested alone. The lack of inhibitory effect may be associated with the non-polar nature of the hexanic extract, predominantly composed of fatty acids and carotenoids [19], which may not effectively target fungal cell walls or membranes. Nevertheless, previous studies have shown that hexane extracts from other microalgae, such as *Spirulina* sp., as well as from *N. oculata*, can possess significant antimicrobial and anti-inflammatory activities [18,27]. Conversely, polar fractions, particularly those enriched in phenolic compounds, often display stronger antimicrobial properties, suggesting that alternative extraction approaches could yield different results [28,29].

This work represents the first evaluation of marine microalgal compounds against *S. brasiliensis*. The antifungal activity of the hexanic fraction was limited, and only occasional additive or weak synergistic interactions with itraconazole were observed, while no antagonism occurred. These findings indicate that the fraction tested is no sufficient to support antifungal use on its own, but they reinforce the need for further exploration of *N. oceanica* metabolites. Future studies should also consider alternative extraction methods and solvents, since different polarities may yield distinct classes of bioactive molecules with antifungal potential. Expanding the number and genetic diversity of *S. brasiliensis* isolates tested will be essential to determine the consistency of these effects, such as test different extractions or fractions. Further studies should investigate higher concentrations and additional fungal species to better assess the antifungal potential of *N. oceanica* extracts.

Some limitations of this study include the use of only the hexane fraction of the microalga, testing against a single clinically relevant fungal species, and evaluating only a limited concentration range. From a broader perspective, exploring local marine biodiversity, such as microalgae from Rio Grande, RS, Brazil, represents a promising strategy for the discovery of novel antifungal compounds. Even when direct activity is absent, the ability of natural products to enhance the efficacy of existing drugs reinforces their potential role in addressing the challenges of antifungal resistance and treatment limitations in sporotrichosis.

Conclusion

This study demonstrates that hexanic extracts of *N. oceanica* had no direct antifungal effect against *S. brasiliensis*. Only occasional additive or weak synergistic interactions with itraconazole were observed, while no antagonism occurred. As this is the first report evaluating marine microalgae against *S. brasiliensis*, our findings indicate limited activity but reinforce the need for further bioprospecting efforts. Future studies should include alternative extraction methods, testing of additional microalgal species, and a broader and more diverse set of clinical isolates to better assess their antifungal potential.

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10. CONCLUSÕES

Esta tese consolida o Rio Grande do Sul como um cenário-chave para compreender a dinâmica da esporotricose zoonótica no Brasil e na América Latina. A partir da análise de 450 isolados de *Sporothrix brasiliensis*, provenientes de humanos, animais e ambiente, foi possível revelar um panorama inédito da diversidade genética e da suscetibilidade antifúngica do agente. Identificamos a circulação de três clados distintos no estado, incluindo dois inéditos, com padrões de disseminação e adaptação contrastantes: um clado associado principalmente ao sul do estado, onde se iniciaram os primeiros casos concomitantemente ao Rio de Janeiro, marcado por homogeneidade genotípica; e outro, altamente diverso, associado à região da Serra e também ao sul do RS.

A análise temporal demonstrou que os clados vinculados ao RS permanecem em circulação desde 2009, sustentando a endemia por disseminação interna. Já o clado do RJ, foi detectado apenas no período mais recente (2020–2024), e predominou na região metropolitana, refletindo a introdução de novas linhagens e a crescente complexidade epidemiológica da doença. Esse conjunto de achados evidencia que o RS abriga tanto linhagens estáveis quanto perfis mais diversos, reforçando a importância da vigilância molecular contínua.

No campo da suscetibilidade antifúngica, os dados revelaram taxas preocupantes de isolados não-selvagens frente ao itraconazol, em distribuição bimodal sugestiva de resistência emergente. Enquanto o clado predominante no sul do estado apresentou menores taxas de resistência, o terceiro clado descrito pela primeira vez na literatura pelo nosso estudo concentrou impressionantes 89% de isolados não-selvagens. Esses contrastes mostram que o RS reúne linhagens com diferentes estratégias adaptativas, capazes de influenciar de maneira distinta a expansão da endemia podendo impactar diretamente a resposta terapêutica. A detecção de isolados não selvagens em pacientes humanos sem histórico de exposição prévia ao itraconazol confirma a transmissão zoonótica dessas linhagens que, somada à coexistência de isolados suscetíveis e não selvagens dentro de um mesmo genótipo, reforça a urgência de estudos sobre os mecanismos moleculares da resistência e suas implicações clínicas.

Complementando essa perspectiva sobre a diversidade e plasticidade do gênero *Sporothrix*, o relato do primeiro caso de esporotricose felina causada por *S. brunneoviolacea*, espécie até então considerada exclusivamente ambiental, amplia o espectro de agentes associados à doença e reforça a importância da vigilância e da caracterização molecular na detecção de agentes emergentes. Esse achado pioneiro demonstra o potencial zoonótico de outras espécies e reforça que a interface entre ambiente, animais e humanos permanece um eixo central para compreender a dinâmica atual da esporotricose no país.

No campo clínico, a caracterização das reações de hipersensibilidade trouxe dados inéditos para a região, revelando uma frequência superior à observada em outros polos endêmicos e destacando a artrite asséptica como manifestação predominante, em contraste com o eritema nodoso descrito em outros estudos. Além disso, trouxe evidências que essas manifestações estão relacionadas ao perfil imunológico do hospedeiro, e não ao fungo. Essa constatação amplia o espectro clínico reconhecido da esporotricose e oferece subsídios valiosos para reduzir erros diagnósticos e otimizar condutas terapêuticas em áreas hiperendêmicas.

Em relação ao diagnóstico, a validação de uma PCR espécie-específica aplicada a amostras clínicas não invasivas demonstrou acurácia em comparação ao cultivo micológico, com a vantagem de rapidez, acessibilidade e aplicabilidade em diferentes contextos. Essa ferramenta tem potencial para ampliar o acesso ao diagnóstico em locais sem infraestrutura laboratorial especializada em micologia, possibilitar o início precoce do tratamento e contribuir diretamente para o prognóstico dos pacientes e para a vigilância epidemiológica nacional.

Finalmente, a exploração pioneira da bioprospecção de microalgas marinhas como fonte de novos compostos antifúngicos abre uma linha inovadora de investigação terapêutica. Embora os resultados iniciais do extrato hexânico de *Nannochloropsis oceanica* tenham revelado atividade limitada, o estudo estabelece bases para pesquisas futuras com diferentes métodos de extração, frações bioativas e combinações terapêuticas, reforçando o potencial da biodiversidade costeira como recurso estratégico na busca por novos antifúngicos.

Assim, esta tese integra epidemiologia molecular, clínica, diagnóstico e terapêutica para oferecer uma visão abrangente e inédita da esporotricose no Rio Grande do Sul e no Brasil. Seus resultados não apenas aprofundam a compreensão regional da doença, mas também têm implicações diretas para o manejo da endemia em escala nacional e internacional, consolidando o RS como um modelo de estudo da esporotricose zoonótica e um espaço crucial para o desenvolvimento de estratégias de vigilância e controle.

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