UNIVERSIDADE FEDERAL DE MINAS GERAIS

Instituto de Ciências Biológicas

Programa de Pós-Graduação em Microbiologia

Isabela Lima de Miranda

DO AMBIENTE AO HOSPEDEIRO: abordagem Saúde Única sobre a susceptibilidade e mecanismos de resistência em leveduras do gênero *Trichosporon* frente a antifúngicos clínicos, agroquímicos e sanitizantes *in vitro* e *in vivo*

Belo Horizonte

2025

Isabela Lima de Miranda

DO AMBIENTE AO HOSPEDEIRO: abordagem Saúde Única sobre a susceptibilidade e mecanismos de resistência em leveduras do gênero *Trichosporon* frente a antifúngicos clínicos, agroquímicos e sanitizantes *in vitro* e *in vivo*

Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Microbiologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito para aprovação no Mestrado em Ciências Biológicas com ênfase em Microbiologia.

Orientador: Prof. Dr. Daniel de Assis Santos

Coorientador: Dr. Victor Augusto Teixeira Leocádio

Belo Horizonte

2025

043 Miranda, Isabela Lima de.
Do ambiente ao hospedeiro: abordagem saúde única sobre a susceptibilidade e mecanismos de resistência em leveduras do gênero Trichosporon frente a antifúngicos clínicos, agroquímicos e sanitizantes in vitro e in vivo [manuscrito] / Isabela Lima de Miranda. – 2025.
68 f. : il. ; 29,5 cm.

Orientador: Prof. Dr. Daniel de Assis Santos. Coorientador: Dr. Victor Augusto Teixeira Leocádio.
Dissertação (mestrado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Microbiologia.

1. Microbiologia. 2. Saúde Única. 3. Tricosporonose. 4. Farmacorresistência Fúngica. I. Santos, Daniel de Assis. II. Leocádio, Victor Augusto Teixeira. III. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas. IV. Título.

CDU: 579

Ficha Catalográfica elaborada pela bibliotecária Fabiane C M Reis CRB 6 2680

UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
CURSO DE PÓS-GRADUAÇÃO EM MICROBIOLOGIA

ATA DE DEFESA DE DISSERTAÇÃO

ATA DA DEFESA DE DISSERTAÇÃO DE **ISABELA LIMA DE MIRANDA**

Nº REGISTRO: 2024653892

Às **14:00** horas do dia **24 de novembro de 2025**, reuniu-se no Instituto de Ciências Biológicas da UFMG, a Comissão Examinadora composta pelos Drs. Gustavo José Cota de Freitas (Residente Pós-doutoral do Departamento de Microbiologia/ICB/UFMG), Estefânia Mara do Nascimento Martins (CDTN) e o Prof. Dr. Daniel de Assis Santos (Orientador), para julgar o trabalho final **"Do ambiente ao hospedeiro: abordagem Saúde Única sobre a susceptibilidade e mecanismos de resistência em leveduras do gênero *Trichosporon* frente a antifúngicos clínicos, agroquímicos e sanitizantes *in vitro* e *in vivo"***, da aluna **Isabela Lima de Miranda,** requisito final para a obtenção do Grau de **MESTRA EM CIÊNCIAS BIOLÓGICAS: MICROBIOLOGIA**. Abrindo a sessão, o Presidente da Comissão, Prof. Dr. Daniel de Assis Santos - Orientador, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra à candidata, para a apresentação de seu trabalho. Seguiu-se a arguição pelos Examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu, sem a presença da candidata e do público, para julgamento e expedição de resultado final. A candidata foi considerada **APROVADA**. O resultado final foi comunicado publicamente à candidata pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora. A candidata tem 60 (sessenta) dias, a partir desta data, para entregar a versão final da dissertação ao Programa de Pós-graduação em Microbiologia da UFMG e requerer seu diploma.

Belo Horizonte, 24 de novembro de 2025

Membros da banca:

Dr. Gustavo José Cota de Freitas

Profa. Dra. Estefânia Mara do Nascimento Martins

De acordo:

Prof. Dr. Daniel de Assis Santos

(Orientador)

Prof. Dr. Caio Tavares Fagundes

(Coordenador do Programa de Pós-graduação

em Microbiologia)

Documento assinado eletronicamente por **Daniel de Assis Santos**, **Professor do Magistério Superior**, em 25/11/2025, às 09:38, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Caio Tavares Fagundes**, **Coordenador(a) de curso de pós-graduação**, em 25/11/2025, às 09:50, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Gustavo José Cota de Freitas**, **Usuário Externo**, em 26/11/2025, às 08:52, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Estefânia Mara do Nascimento Martins**, **Usuário Externo**, em 26/11/2025, às 18:01, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

A autenticidade deste documento pode ser conferida no site https://sei.ufmg.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **4579648** e o código CRC **55CE4426**.

Referência: Processo nº 23072.279061/2023-54 SEI nº 4579648

Agradecimentos

A jornada até aqui foi repleta de desafios, aprendizados e conquistas que não seriam possíveis sem o apoio de muitas pessoas e instituições, às quais sou imensamente grata. Em primeiro lugar, agradeço ao Laboratório de Micologia da UFMG, que foi não apenas um espaço de trabalho, mas também um ambiente de crescimento, amadurecimento e companheirismo. Além disso, agradeço a todos os meus colegas, que contribuíram muito com este trabalho. Aos meus orientadores (e coorientadores) nesta caminhada, meu profundo agradecimento por sua dedicação, paciência, incentivo e por compartilhar comigo não apenas seu conhecimento, mas também sua paixão pela ciência. Obrigado por acreditarem no meu potencial mesmo nos momentos de incerteza. Sou igualmente grata às agências de fomento que financiaram esta pesquisa, em especial a CAPES, cujo apoio foi essencial para a realização deste trabalho e para minha formação acadêmica. À minha família, agradeço o amor incondicional, todos os conselhos, palavras de encorajamento e por estarem sempre ao meu lado, mesmo nos momentos mais difíceis. Agradeço em especial a minha mãe, ao meu pai e ao meu irmão. As minhas melhores amigas que me acompanharam ao longo desta caminhada, dentro e fora da universidade, obrigado por cada conversa, cada riso, cada gesto de apoio. A amizade de vocês tornou tudo mais leve. Por fim, ao meu parceiro, agradeço por ser presença constante, pelo carinho, pela compreensão e o apoio incondicional todos os dias. Te amo mais que o infinito. A todos vocês, meu mais sincero obrigada. Esta conquista é nossa!

Resumo

O gênero *Trichosporon* inclui leveduras oportunistas amplamente distribuídas no ambiente e presentes na microbiota de humanos e animais, capazes de causar diferentes tipos de infecção. A tricosporonose invasiva é uma doença emergente que afeta principalmente indivíduos imunocomprometidos, com manifestações que variam de fungemia a meningite. Dentro desse gênero, a resistência antifúngica tem se tornado cada vez mais comum. No entanto, a variabilidade entre espécies e a ausência de métodos padronizados de teste dificultam o diagnóstico e o tratamento eficaz, sobretudo no contexto da Saúde Única. Diante disso, este estudo investigou a resistência antifúngica em *Trichosporon* spp. por meio de uma revisão da literatura e da análise e padronização metodológica, utilizando uma coleção de amostras isoladas de fontes clínicas, animais e ambientais. Inicialmente, foi realizada uma revisão bibliográfica sobre a resistência intrínseca e adquirida em *Trichosporon* spp., incluindo os mecanismos envolvidos frente aos principais grupos de antifúngicos clínicos — polienos, azóis, equinocandinas e flucitosina. Os resultados evidenciaram que essas leveduras são intrinsecamente resistentes às equinocandinas e apresentam baixa sensibilidade à anfotericina B e aos azóis, sendo os triazóis — especialmente o voriconazol — os fármacos mais eficazes. A flucitosina, por sua vez, não é recomendada para o tratamento da tricosporonose. De forma geral, a resistência está associada principalmente a mecanismos como bombas de efluxo, formação de biofilme e mudanças na constituição da parede celular e membrana lipídica. Em seguida, a metodologia de microdiluição em caldo aplicada a *Trichosporon* spp. foi investigada e padronizada para anfotericina B e fluconazol. A partir da padronização metodológica, foram estabelecidas condições otimizadas para a determinação da concentração inibitória mínima (CIM), utilizadas no estudo experimental deste trabalho. Posteriormente, a susceptibilidade das 33 amostras foi avaliada frente a antifúngicos clínicos, desinfetantes e agroquímicos, utilizando o método previamente padronizado. Com base em ensaios *in vitro*, doze amostras foram inicialmente classificadas como resistentes a antifúngicos clínicos e possivelmente resistentes cruzadas a agroquímicos e desinfetantes. Ensaios adicionais, incluindo avaliação do crescimento e da produção de biofilme, permitiram refinar essa seleção, resultando em quatro amostras com perfis de resistência mais evidentes. Essas linhagens foram então testadas em modelo murino de infecção, confirmando a resistência *in vivo*. Animais infectados com as amostras resistentes e tratados com anfotericina B ou fluconazol apresentaram maior carga fúngica, recuperação comprometida, e maior colonização do sistema nervoso central, sugerindo uma possível associação entre resistência, virulência e ineficácia do tratamento. De forma geral, este trabalho elucidou de maneira mais clara a resistência e seus mecanismos dentro do gênero *Trichosporon*, destacando a possibilidade do surgimento e da influência da resistência e da resistência cruzada no contexto de Saúde Única, relacionando também esses achados ao tratamento ineficaz e ao aumento da virulência.

Palavras-chave: *Trichosporon* spp.; antifúngicos clínicos; agroquímicos; desinfetantes; resistência; virulência.

Abstract

The *Trichosporon* genus comprises opportunistic yeasts widely distributed in the environment and present in the microbiota of humans and animals, capable of causing different types of infection. Invasive trichosporonosis is an emerging disease that primarily affects immunocompromised individuals, with clinical manifestations ranging from fungemia to meningitis. Within this genus, antifungal resistance has become increasingly common. However, the variability among species and the lack of standardized testing methods hinder accurate diagnosis and effective treatment, especially within the One Health context. In this study, antifungal resistance in *Trichosporon* spp. was investigated through a literature review combined with methodological analysis and standardization, using a collection of isolates obtained from clinical, animal, and environmental sources. First, a comprehensive review was conducted on intrinsic and acquired resistance in *Trichosporon* spp., including the mechanisms involved in resistance to major classes of clinical antifungals—polyenes, azoles, echinocandins, and flucytosine. The findings showed that these yeasts are intrinsically resistant to echinocandins and display low susceptibility to amphotericin B and azoles, with triazoles—particularly voriconazole—being the most effective drugs. Flucytosine, in turn, is not recommended for the treatment of trichosporonosis. Overall, resistance is primarily associated with mechanisms such as efflux pump activity, biofilm formation, and alterations in the cell wall and lipid membrane composition. Next, the broth microdilution methodology for *Trichosporon* spp. was investigated and standardized for amphotericin B and fluconazole. Based on this standardization, optimized conditions for determining the minimum inhibitory concentration (MIC) were established and applied in the experimental component of this study. Subsequently, susceptibility of the 33 isolates was assessed against clinical antifungals, disinfectants, and agrochemicals using the standardized method. Based on *in vitro* assays, twelve isolates were initially classified as resistant to clinical antifungals and potentially cross-resistant to agrochemicals and disinfectants. Additional assays, including growth evaluation and biofilm production, refined this selection, resulting in four isolates with more pronounced resistance profiles. These selected strains were then tested in a murine infection model, confirming resistance *in vivo*. Animals infected with the resistant isolates and treated with amphotericin B or fluconazole exhibited higher fungal burden, impaired recovery, and increased colonization of the central nervous system, suggesting a possible association between resistance, virulence, and treatment failure. Overall, this study provides a clearer understanding of resistance and its mechanisms within the *Trichosporon* genus, highlighting the emergence and impact of resistance and cross-resistance in the One Health context, and linking these findings with ineffective treatment and enhanced virulence.

Keywords: *Trichosporon* spp.; clinical antifungals; agrochemicals; disinfectants; resistance; virulence.

Sumário

1. Introdução e Justificativa

O gênero *Trichosporon* compreende fungos ubíquos presentes no ambiente e associados a animais, incluindo seres humanos, onde integram a microbiota normal da pele, trato vaginal e gastrointestinal, bem como de aves. Apesar de geralmente comensais, algumas espécies podem atuar como patógenos oportunistas, causando infecções superficiais ou invasivas graves, especialmente em indivíduos imunocomprometidos (MEHTA et al., 2021; LARA et al., 2021; LO et al., 2021; BOBREK et al., 2021; CHANDER, 2018; COLOMBO et al., 2011). O potencial patogênico depende de fatores de virulência, flexibilidade metabólica e capacidade de invasão tecidual, podendo ocorrer por via exógena (cateteres) ou endógena (translocação intestinal) (MEHTA et al., 2021; MARINÉ et al., 2015).

Recentemente, a taxonomia desse gênero foi revisada, e algumas das espécies usadas neste trabalho agora pertencem ao gênero *Cutaneotrichosporon* (*Cutaneotrichosporon jirovecii* e *Cutaneotrichosporon mucoides*) (TAKASHIMA et al., 2022). Entretanto, neste estudo, devido à identificação realizada por MALDI-TOF no início do trabalho e ao seu reconhecimento na micologia médica, citaremos e discutiremos essas espécies sob a perspectiva do antigo gênero *Trichosporon*.

O teste de susceptibilidade antifúngica normalmente segue o método de microdiluição em caldo do CLSI, embora ainda não haja padronização específica para este gênero (CLSI, 2017; MEHTA et al., 2021). De modo geral, isolados de *T. asahii* apresentam maior sensibilidade a triazóis (voriconazol (VCZ), itraconazol (ITR) e fluconazol (FCZ)) em comparação aos polienos (anfotericina B (AMB)) e resistência intrínseca às equinocandinas (micafungina (MCF)) (MEHTA et al., 2021; SINGH et al., 2019; COLOMBO et al., 2011).

Nesse contexto, a resistência em *Trichosporon*, assim como em outros gêneros ubíquos, pode ser influenciada por agentes ambientais, como agroquímicos (tebuconazol (TEB), carbendazim (CBZ), piraclostrobina (PIR) e mancozeb (MZB)) e desinfetantes (cloreto de benzalcônio (BZK) e cloreto de didecildimetilamônio (DDA)), além de antimicrobianos clínicos (como os citados acima) utilizados de forma inadequada. Esses compostos atuam em diferentes alvos celulares e metabólicos, podendo favorecer e até mesmo induzir o desenvolvimento de mecanismos de resistência (BASTOS et al., 2017/2019/2025; CARNEIRO et al., 2020).

Dessa forma, no contexto de Saúde Única, que integra saúde humana, animal e ambiental, essa resistência aos antifúngicos representa uma ameaça crescente, reconhecida por organizações como CDC e OMS (ADISASMITO t al., 2022; WOODS et al., 2023). Por isso, é

fundamental aprofundar o conhecimento sobre a resistência e seus mecanismos de ação, padronizar as metodologias de avaliação da susceptibilidade dessas leveduras e monitorar linhagens de *Trichosporon* isoladas de pacientes, animais e ambientes, de forma *in vitro* e *in vivo*. Visto que, essa resistência intrínseca ou adquirida pode comprometer o tratamento e favorecer a disseminação da tricosporonose invasiva (VELAZQUEZ-MEZA et al., 2022; MEHTA et al., 2021).

Portanto, essa dissertação abordará esses tópicos e será estruturada em formato de compilação de artigos, construída a partir dos trabalhos publicados e dos manuscritos produzidos ao longo do período do mestrado. Inicialmente, serão apresentados os objetivos gerais e específicos, que nortearam o desenvolvimento da pesquisa e orientaram as principais perguntas científicas do estudo. Em seguida, cada capítulo corresponderá a um artigo científico, organizado de forma a responder progressivamente às questões propostas nos objetivos. Essa estrutura permitirá uma abordagem integrada, em que cada artigo representa uma etapa complementar da investigação — desde a revisão bibliográfica até a padronização da metodologia central e a realização dos ensaios experimentais — culminando em uma discussão ampla e consolidada dos resultados à luz da perspectiva da Saúde Única.

No âmbito deste mestrado, realizei a investigação mais abrangente já conduzida no Brasil sobre o gênero *Trichosporon*, integrando amostras de origem humana, animal e ambiental. Ao longo do trabalho, padronizei e executei testes de susceptibilidade antifúngica, avaliei o potencial de resistência e de resistência cruzada frente a antifúngicos clínicos, agroquímicos e desinfetantes, e explorei possíveis mecanismos envolvidos nesses fenótipos. Esse conjunto de abordagens permitiu elucidar, de maneira inédita, como diferentes pressões seletivas podem moldar a resistência em *Trichosporon*, contribuindo para o enfrentamento de falhas terapêuticas e riscos emergentes no âmbito da Saúde Única.

2. Objetivos Geral e Específicos

2.1 Objetivo Geral

- Investigar a resistência e seus mecanismos; padronizar e testar a susceptibilidade a antifúngicos clínicos, agroquímicos e desinfetantes; e avaliar a resistência *in vitro* e *in vivo* no gênero *Trichosporon*, utilizando uma coleção de amostras de diferentes origens.

2.2 Objetivos Específicos

- Realizar uma revisão bibliográfica sobre a resistência e os mecanismos de resistência no gênero *Trichosporon*;
- Coletar, isolar e identificar amostras do gênero *Trichosporon* provenientes de animais de criatório, pacientes e ambientes associados (criatório e hospitalar) de diferentes estados do Brasil para montar um banco de leveduras para o estudo em questão;
- Padronizar a metodologia de microdiluição em caldo para avaliar a susceptibilidade de leveduras do gênero *Trichosporon*;
- Avaliar a susceptibilidade das leveduras isoladas frente aos diferentes antifúngicos clínicos (fluconazol, itraconazol, voriconazol, anfotericina B e micafungina), agroquímicos (tebuconazol, carbendazim, piraclostrobina e mancozeb) e desinfetantes (cloreto de benzalcônio e cloreto de didecildimetilamônio);
- Investigar a resistência *in vitro* e selecionar as amostras por meio da avaliação do crescimento e da formação de biofilme;
- Detectar e investigar a resistência *in vivo* das amostras selecionadas.

3. Capítulo 1

Com objetivo de realizar uma revisão bibliográfica sobre resistência e os mecanismos de resistência no gênero *Trichosporon*, foi elaborado e publicado o artigo de revisão *Trichosporon* and Antifungal Resistance: Current Knowledge and Gaps na revista Mycopathologia em 04/07/2025 (DOI: 10.1007/s11046-025-00969-z). O estudo aborda principalmente os perfis de susceptibilidade e os mecanismos de resistência do fungo frente aos principais antifúngicos clínicos atualmente utilizados — azóis, anfotericina B, equinocandinas e flucitosina. Além disso, a revisão destaca lacunas significativas no conhecimento sobre os mecanismos de resistência e a possível ocorrência de resistência cruzada entre compostos clínicos, agrícolas e desinfetantes, no contexto da Saúde Única. Entre os principais achados, o artigo ressalta a necessidade de padronização das metodologias de susceptibilidade, a relevância de estudos aprofundados para elucidar os mecanismos de resistência e a importância de uma abordagem integrada sob a perspectiva da Saúde Única, considerando a interação entre ambiente, animais e seres humanos na disseminação de cepas resistentes.

Mycopathologia (2025) 190:59
https://doi.org/10.1007/s11046-025-00969-z

REVIEW

Trichosporon and Antifungal Resistance: Current Knowledge and Gaps

Isabela Lima Miranda · Nalu Teixeira Aguiar Peres · Rafael Wesley Bastos · Luana Rossato · Florent Morio · Daniel Assis Santos

Received: 15 February 2025 / Accepted: 12 June 2025 / Published online: 4 July 2025

Abstract *Trichosporon* spp. are ubiquitous environmental and emerging opportunistic fungi that can be part of the normal microbiota of the skin, vagina, and gastrointestinal tract in humans and other animals. In a state of microbiota imbalance, both immunocompromised and non-immunocompromised patients are susceptible to trichosporonosis, which can present as either superficial infections, such as White Piedra, or deep-seated infections, such as fungemia. Due to intrinsic resistance or limited efficacy reported with fluconazole, polyenes, echinocandins, and flucytosine, voriconazole is currently recommended in clinical guidelines. Biofilm formation and the overexpression of efflux pumps may contribute to this limited efficacy, but other resistance mechanisms have also been reported. The high antifungal resistance levels present in this genus highlight the urgent need for new therapeutic approaches and a better understanding of *Trichosporon* pathogenesis and the molecular mechanisms underlying this resistance. In this review, we discuss antifungal resistance and the mechanisms of this resistance in *Trichosporon* spp., addressing key points that require further investigation.

Keywords Review · *Trichosporon* spp. · Antifungal resistance · Mechanism resistance

Handling Editor: Marcia de Souza Carvalho Melhem.

I. L. Miranda · N. T. A. Peres · D. A. Santos (✉)
Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais 31270-901, Brazil
e-mail: das@ufmg.br; dasufmg@gmail.com

N. T. A. Peres · R. W. Bastos · D. A. Santos
National Institute of Science and Technology in Human Pathogenic Fungi, Ribeirão Preto, Brazil

R. W. Bastos
Departamento de Microbiologia e Parasitologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, 59078-970, Brazil

L. Rossato
Laboratório de Pesquisa em Ciências da Saúde, Universidade Federal da Grande Dourados, Mato Grosso do Sul, 79804-970, Brazil

F. Morio
Nantes Université, CHU Nantes, Cibles et Médicaments des Infections et de l'Immunité, IICiMed, UR 1155, 44000 Nantes, France

Background on *Trichosporon* spp. and Trichosporonosis

The *Trichosporon* genus belongs to the phylum Basidiomycota, class Tremellomycetes, order Trichosporonales, and family Trichosporonaceae. Morphologically, the *Trichosporon* genus is characterized by the production of true hyphae, pseudohyphae, arthroconidia, and blastoconidia. It is known to contain at least 15 clinically relevant species, but new, normally clinically relevant, species are regularly being described [1, 2].

Until 1992, nearly all infections caused by this genus were linked to *Trichosporon beigelii* [1]. However, taxonomy was gradually updated based on biochemical and molecular data, allowing a more accurate differentiation between species previously grouped under *T. beigelii* and *T. cutaneum* [3]. Furthermore, a recent taxonomic revision led to the reclassification of some species previously grouped within the *Trichosporon* genus, now described as part of the *Cutaneotrichosporon* genus (e.g., *Cutaneotrichosporon cutaneum* and *Cutaneotrichosporon mucoides*) [1, 3].

According to NCBI taxonomy, there are currently 15 known *Trichosporon* species: *T. aquatile*, *T. asahii*, *T. asteroides*, *T. beigelii*, *T. caseorum*, *T. coremiiforme*, *T. dohaense*, *T. faecale*, *T. infestans*, *T. inkin*, *T. insectorum*, *T. japonicum*, *T. lactis*, *T. austroamericanum*, and *T. ovoides*. Although predominantly associated with the human microbiota and clinical manifestations, most of these species have also been isolated from environmental sources [2, 5–7].

Currently, *T. asahii* is considered the main species of the genus, causing invasive trichosporonosis in clinical settings [1, 4]. Besides *T. asahii*, *T. inkin* and the recently described *T. austroamericanum* are also important agents in invasive infections [1, 2].

This genus comprises ubiquitous fungi that can be found both in the environment and in association with humans and other animals [5, 6]. It can be part of the normal microbiota of the skin, vagina, and gastrointestinal tract of various animals, such as birds and humans [5–7]. However, changes in the microenvironment can trigger their pathogenic potential, leading to trichosporonosis. This ability depends on host factors, virulence factors, metabolic flexibility, and the fungi's ability to invade tissue [8].

Infections caused by these fungi can be broadly classified as superficial, which can occur in both immunosuppressed and non-immunosuppressed individuals, or life-threatening invasive diseases, which occur mainly in immunosuppressed patients [2, 5, 6].

Clinical Manifestations and Diagnosis of Trichosporonosis

Benign, irregular nodules on the hair shaft, known as White Piedra, represent superficial infections typically caused by *T. inkin* and *T. ovoides*, and are usually observed in immunocompetent individuals [9, 10]. These nodules range in color from white to light brown, possess a soft texture, and are loosely attached to hair on the beard, mustache, eyebrows, axillae, and genital areas. Diagnosis is primarily clinical and confirmed through direct microscopic examination and fungal culture [9–11].

In contrast, invasive trichosporonosis is more frequently associated with immunocompromised patients, including individuals living with HIV/AIDS, cancer patients, transplant recipients, and those exposed to specific risk factors such as major surgery or severe burns [5, 6]. In such cases, *T. asahii* is the most frequently isolated species, affecting multiple organs with a broad spectrum of clinical manifestations. These include fungemia (in over 70% of cases), pneumonia, and cutaneous lesions, and are associated with high mortality rates ranging from 50 to 70% [5, 6]. These clinical manifestations are often nonspecific and may include persistent fever unresponsive to broad-spectrum antibiotics, pulmonary involvement, skin manifestations (including papules, nodules, or necrotic lesions), and signs of disseminated infection involving the lungs, liver, spleen, kidneys, skin, and central nervous system. Diagnosis is challenging due to the overlap of symptoms and signs with those of other fungal and bacterial infections. Definitive diagnosis requires the isolation of *Trichosporon* spp. from sterile sites such as blood or tissue cultures, often supported by direct microscopy and histopathological examination [5, 6, 8, 16–18, 21]. Early identification and prompt initiation of appropriate antifungal therapy are critical.

Notably, during the COVID-19 pandemic, severe and fatal opportunistic infections caused by *T. asahii* were reported, either as co-infections or secondary infections following COVID-19 [12–15]. Moreover, dissemination to the central nervous system has been documented, leading to cases of meningitis [16–18]. Although invasive trichosporonosis is uncommon in immunocompetent individuals, it can still occur under specific circumstances, such as in post-surgical patients or those in intensive care units, including cases of mediastinitis [2]. The clinical presentation in these cases remains nonspecific, and diagnosis continues to pose a significant challenge.

Given the growing incidence of antifungal resistance, particularly in invasive infections, it is essential to investigate resistance mechanisms and evaluate the

effectiveness of current antifungal therapies. The following sections will examine the efficacy of different antifungal classes (azoles, polyenes, echinocandins, and flucytosine), highlighting key studies and resistance mechanisms specific to *Trichosporon* species.

What do We Know About Antifungal Resistance to Common Antifungals?

Azoles

Azoles are a class of synthetic antifungal agents widely used to treat a range of fungal infections, including those caused by *Trichosporon* species [22]. Their primary mechanism of action involves the disruption of ergosterol biosynthesis (a critical component of the fungal cell membrane) through the inhibition of lanosterol-14-α demethylase, encoded by the *ERG11* gene [23]. However, high minimum inhibitory concentrations (MICs) values to these antifungals in vitro are commonly observed in this genus. *T. asahii* typically displays low MICs values to voriconazole and posaconazole, but higher MICs values for fluconazole [22]. For this reason, within this class, triazoles (especially voriconazole) are considered the most effective agents for managing trichosporonosis and are recommended as first-line therapy for patients with invasive disease (including central nervous system infections), although fluconazole is still widely used in clinical practice in some countries [5, 6, 10, 16, 20]. Overall, susceptibility to azoles varies among *Trichosporon* isolates, with some reports of high MIC values for azoles in strains capable of biofilm formation [25, 26].

Despite the absence of interpretative breakpoints or epidemiological cutoff values (ECV) for *Trichosporon* and antifungals, several studies have used adapted CLSI (*Clinical and Laboratory Standards Institute*—Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard: Wayne, Pennsylvania, USA, 2012) and EUCAST (EUCAST guidance on Interpretation of MICs for rare yeast without breakpoints, 2024) guidelines to generate in vitro susceptibility data. Among these studies, the CLSI method remains the most used, as summarized in Table 1.

Francisco et al. [4] analyzed antifungal susceptibility in several clinical isolates of the *Trichosporon* genus, focusing on 273 clinical isolates of *T. asahii*.

Table 1 Timeline of significant MIC studies within the genus *Trichosporon* used in this review

Study	Tokunaga et al. [48] – 1992	Guo et al. [27] – 2019	Francisco et al. [4] – 2019	Kuo et al. [29] – 2021	Wongsuk et al. [28] – 2022
Number of isolates tested	52 clinical isolates	133 clinical isolates	358 clinical isolates	115 clinical isolates	53 clinical isolates
MIC method	CLSI	CLSI	CLSI	CLSI	CLSI
MIC range values	Flucytosine: MIC range of 3.12–6.25 µg/mL	Amphotericin B: MIC range of 0.125–4 µg/mL Caspofungin and Micafungin: MIC of > 8 µg/ml 5-FC: MIC range of 0.25–64 µg/mL Fluconazole: MIC range of 0.125- > 512 µg/mL Voriconazole: MIC range of 0.008–16 µg/mL Itraconazole: MIC range of 0.125–32 µg/mL	Fluconazole: MIC range of 0.25–64 µg/mL Voriconazole: MIC range of 0.03–2 µg/mL Posaconazole: MIC range of 0.03–2 µg/mL Amphotericin B: MIC range of 0.25- > 16 µg/mL	Amphotericin B: MIC range of 0.12–2 µg/mL Caspofungin and Micafungin: MIC of > 8 µg/ml 5-FC: MIC range of 0.06- > 64 µg/mL Fluconazole: MIC range of 0.12–16 µg/mL Voriconazole: MIC range of 0.008–8 µg/mL Posaconazole: MIC range of 0.008–1 µg/mL Itraconazole: MIC range of 0.015–1 µg/mL	Voriconazole: MIC range of 0.01–0.25 µg/mL Fluconazole: MIC range of 0.5–8 µg/mL Amphotericin B: MIC range of 0.25- > 16 µg/mL

Fluconazole was the least effective antifungal (suggestive ECV set at 8 µg/mL), indicating reduced susceptibility. In contrast, voriconazole demonstrated superior in vitro activity among the azoles tested, with a suggestive ECV of 0.25 µg/mL. Guo et al. [27] also reported elevated fluconazole MICs for 25% of *T. asahii* isolates (MIC ≥ 8 µg/mL), while voriconazole MICs remained low (geometric mean MIC of 0.09 µg/mL) (Table 1). However, different *Trichosporon* species may have distinct antifungal resistance profiles [4, 27].

More recent studies corroborate the potent in vitro efficacy of voriconazole against *Trichosporon* clinical isolates. For example, Wongsuk et al. [28], in a study on antifungal susceptibility, genome sequencing, and biofilm formation of clinical samples, demonstrated that voriconazole remains the most effective antifungal agent for treating *Trichosporon* infections (MIC range of 0.01–0.25 µg/mL). In addition, posaconazole also exhibits significant antifungal activity (Table 1). In a study involving 115 clinical isolates, Kuo et al. [29] reported a mean MIC of 0.251 µg/mL for posaconazole, compared to 0.111 µg/mL for voriconazole. Furthermore, some studies suggest that combining voriconazole with amphotericin B enhances therapeutic outcomes, particularly in cases of severe or refractory infections [20] (Table 1).

Although still poorly investigated, *Trichosporon* spp. resistance to azoles may be linked to mechanisms such as biofilm formation, *ERG11* gene mutations, and the overexpression of efflux pumps, as observed in other pathogenic yeasts like *Candida* spp. and *Cryptococcus* spp. [5, 25, 26]. Additionally, biofilm formation has been associated with increased tolerance and antifungal resistance, linked to extracellular DNA and persistent cells in *Trichosporon* [25, 30, 31] (Table 2 and Fig. 1).

In many opportunistic fungal species, including *Candida* spp. and *Aspergillus* spp., mutations leading to amino acid substitutions in the *ERG11* gene reduce azole binding to the target enzyme [32, 33]. Some studies have evidenced the role of this mechanism in azole resistance in *Trichosporon* [34, 35]. Hisako et al. [35] described that long-term fluconazole use may select for the G453R amino acid substitution in Erg11p, leading to azole resistance in *T. asahii*. Abbes et al. [34] also reported amino acid substitutions potentially involved in azole resistance. *ERG11* sequencing revealed two mutations, H380G and S381A, in TN325U11 (MIC for fluconazole of 8 µg/mL) and H437R in TN114U09 (MIC for fluconazole of 256 µg/mL), occurring in highly conserved regions (close to the heme-binding domain). However, further functional validation is necessary (Table 2 and Fig. 1).

Despite the discussion on point mutations, the primary reason for variations in MIC values for azoles appears to be active efflux pumps. Overexpression of these pumps leads to enhanced drug efflux, reducing intracellular azole concentrations and contributing to treatment failure in *Trichosporon* and other opportunistic fungi [36]. Padovan et al. [23] used Rhodamine 6G, a substrate of ATP-binding cassette transporters, to demonstrate that efflux pump overexpression significantly contributes to azole resistance. In 2021, Abbes et al. [34] used RT-PCR targeting *Pdr11* and *Mdr* genes to show that efflux pump activity in *T. asahii* is similar to that in *Candida* species and contributes to fluconazole resistance (Table 2 and Fig. 1). Furthermore, Aguiar et al. in 2023 [24] provided indirect evidence that inhibiting these efflux pumps with promethazine could be a strategy to control *T. asahii* and *T. inkin*.

Subsequent studies [25] explored the *MDR* and esterase superfamilies in *T. asahii*, offering more insights into *TaMDR* and *TaPLA2* genes associated with azole resistance and active efflux. This study compared *TaPLA2*-overexpressing strains with their parental strains, analyzing the role of *TaPLA2* in azole resistance. The overexpression of efflux pumps (such as *TaMDR*) and increased biofilm formation suggest that *T. asahii PLA2* enhances drug resistance to azoles by improving drug efflux and biofilm formation. Additionally, the upregulation of membrane lipid metabolism has been associated with azole resistance [25] (Table 2 and Fig. 1).

Other hypotheses are also under investigation. For instance, Ma et al. [37] demonstrated that farnesol (a quorum-sensing molecule) increases azole tolerance in fluconazole-resistant *Trichosporon* strains. Additionally, azole exposure induces significant transcriptional changes in the *T. asahii* genome. These transcriptional alterations may contribute to resistance development, as the fungus adapts its gene expression in response to antifungal pressure [4].

Table 2 Timeline of significant discoveries and insights into resistance and resistance mechanisms to clinical antifungals within the genus *Trichosporon*

Date	2011–2019	2019	2019–2021	2021	2022	2022–2024
Authors	Iturrieta-González et al. [26] Colombo et al. [6] Hisako et al. [35] Singh et al. [10] Guo et al. [27] Xia et al. [47] Pfaller MA [40]	Francisco et al. [4] Guo et al. [27]	Padovan et al. [23] Abbes et al. [34] Kuo et al. [29] Mehta et al. [5]	Cordeiro et al. [31] Hoenigl et al. [49]	Pereira et al. [30]	Nobrega et al. [36] Ma et al. [25, 37] Lino et al. [16] Agrawal S [20]
Susceptibility data/ contribution to the state of art	More sensitive to triazoles, more resistant to polyenes and intrinsically resistant to echinocandins; 5-flucytosine is not an effective drug	Voriconazole presents the best in vitro activity among the antifungals tested	Resistance to azoles	Increased tolerance and antifungal resistance New antifungals	Increased tolerance and antifungal resistance	Resistance to azoles Voriconazole (also in combination with amphotericin B) is the most recommended treatment
Resistance mechanisms	Increased tolerance and antifungal resistance could be involved with biofilm, mutation, efflux pumps and membrane lipids; lower β-1,3-glucan may be involved with echinocandins resistance	Exposure to azoles causes profound transcriptional changes in the genome which can lead to antifungal resistance	High activity of efflux pumps; mutations in *ERG11* could be important as well	Biofilm and persistent cells	Biofilm and extracellular DNA	Mutations in *ERG11* are not the main mechanism of resistance; overexpression of efflux pumps and membrane lipids are the main ones involved; biofilm formation is also important

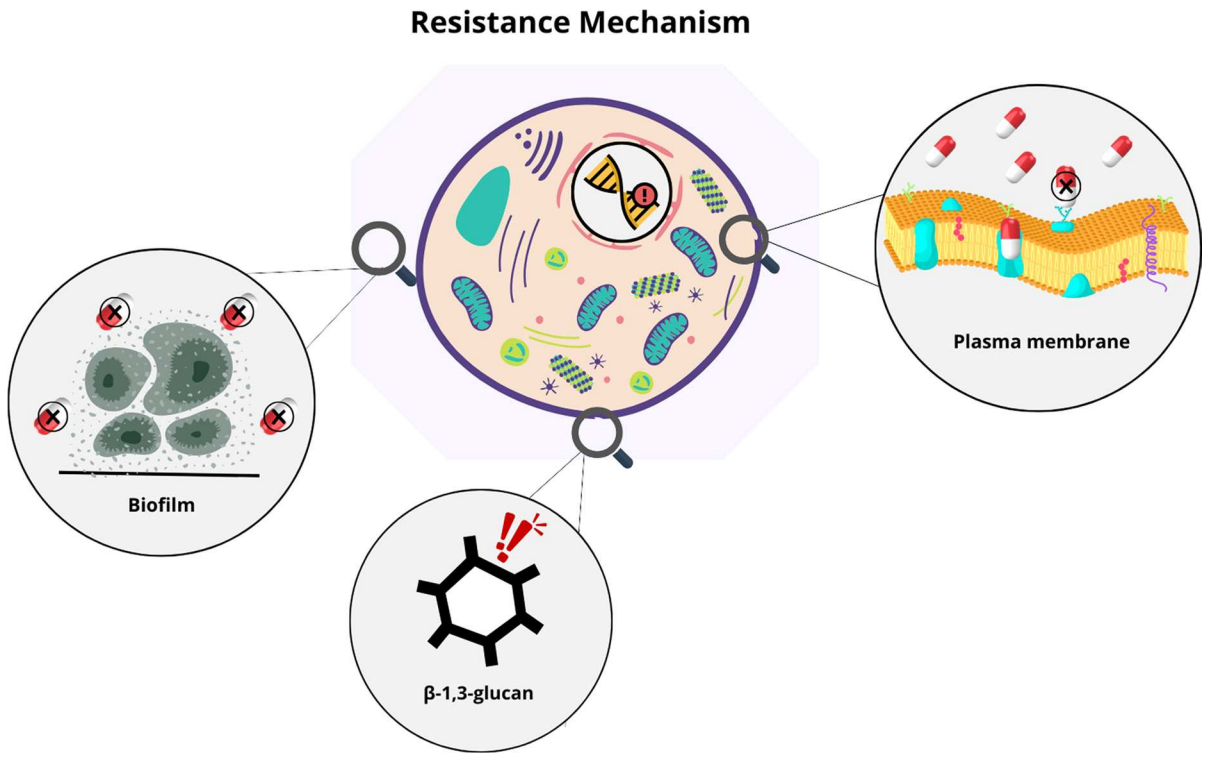


Fig. 1 Main resistance mechanisms of *Trichosporon* genus: modification of the drug target and overexpression of efflux pumps in plasma membrane; low frequency of the drug target in the cell wall (β-1,3-glucan); mutation and modification in genome/trascriptome targets; and biofilm formation

Polyenes

Polyenes are natural products (produced by *Streptomyces* spp.) and a class of antifungal drugs characterized by multiple conjugated double bonds and a heavily hydroxylated region on the ring opposite to the conjugated system. They primarily target the fungal plasma membrane by binding to ergosterol, leading to cell membrane disruption [38]. However, some studies on yeasts, such as *Cryptococcus* spp., suggest that these drugs also play a role in inducing endogenous reactive oxygen species (ROS) and peroxynitrite in the cell [39]. Amphotericin B is the only polyene clinically approved for invasive fungal diseases but has shown poor efficacy in treating trichosporonosis [5].

High MIC values for amphotericin B, with an epidemiological cutoff value (suggestive ECV) at 48 h set at 4 µg/mL for *T. asahii*, were found by Francisco et al. [4]. Similarly, Guo et al. [27] reported high amphotericin B MIC values (ranging from 0.125 to 4 µg/mL) for *T. asahii* isolates. Other studies reinforce these findings, showing amphotericin B MIC values varying from 2 to 16 µg/mL [28, 29]. This evidence highlights the role of microbiological resistance to amphotericin B (MIC ≥ 2 µg/mL) in treatment failure, as high values are generally incompatible with standard treatment due to the drug's toxicity. However, susceptibility may vary among other species, since lower MIC values have been detected for *T. inkin* [4, 27] (Table 1).

Despite these findings, the mechanisms underlying *Trichosporon* resistance to amphotericin B are not yet fully understood. Some authors suggest that biofilm formation, associated with extracellular DNA and persistent cells, may contribute to resistance [26, 30, 31]. Resistance could also be linked to active efflux mechanisms, lipid membrane alterations, and metabolic adaptations, considering the primary target and mode of action of this drug, similar to azole resistance [25, 37]. However, these hypotheses are only

briefly suggested by some authors [5, 6] (Table 2 and Fig. 1).

Echinocandins

Echinocandins, a class of lipopeptide molecules, primarily target cell wall biosynthesis by inhibiting the synthesis of β-1,3-glucan, a key component encoded by the *FKS* genes [40]. However, their efficacy against *Trichosporon* species has been consistently questioned, as these species may exhibit intrinsic resistance to echinocandins, such as micafungin and caspofungin [5, 6, 10]. This resistance is supported by other studies, including those by Guo et al. [27], Kuo et al. [29], and Matsumoto et al. [41], which collectively conclude that echinocandins, such as micafungin and caspofungin, are ineffective in treating trichosporonosis (Table 1 and Fig. 1).

As shown in Table 1, susceptibility studies have reported high echinocandin MIC values (greater than 8 µg/mL) against *Trichosporon* [27, 29]. This innate resistance may explain the occurrence of *Trichosporon* fungemia in patients receiving echinocandin prophylaxis or empirically [43, 44].

Despite these consistent findings, the precise mechanisms underlying echinocandin resistance in *Trichosporon* species remain poorly understood. One study suggested that intrinsic resistance in this genus is not linked to natural amino acid changes in the *FKS* gene [45]. Other studies proposed a lower proportion of β-1,3-glucan in the cell wall of this genus [40]. Further research is needed to better elucidate the molecular basis of this resistance and to explore alternative therapeutic strategies for managing trichosporonosis, particularly in patients with invasive infections (Table 2 and Fig. 1).

Flucytosine

Flucytosine, an antimetabolite drug, is typically used to treat systemic and severe infections caused by *Cryptococcus* spp. and other specific cases, always in combination with another antifungal. It interferes with DNA, RNA, and protein synthesis [46]. However, its efficacy against *Trichosporon* species, particularly *T. asahii*, remains controversial [47]. Recent studies have also tested flucytosine (5-FU), but the MIC range is consistently large, reaching > 64 µg/mL [27, 29] (Table 1).

Furthermore, an earlier study by Tokunaga et al. [48] demonstrated that the MIC values for flucytosine against *T. beigelii* strains ranged between 3.12 and 6.25 µg/mL, proposing it as a good antifungal. However, these values are significantly higher than those observed in other genera. This suggests a potential resistance of *Trichosporon* species to flucytosine, but more comprehensive studies are required to fully assess its clinical relevance (Table 1).

Given the variable response rates and observed resistance, flucytosine is not widely recommended as monotherapy for *Trichosporon* infections, particularly in the case of biofilm-associated infections where resistance tends to be more pronounced [5, 26] (Table 1 and 2) (Fig. 1).

Treatment Recommendations to Trichosporonosis

Topical or oral azoles, particularly imidazoles (mainly ketoconazole), in conjunction with appropriate personal hygiene to prevent recurrence, have demonstrated promising outcomes in the treatment of superficial infections, including White Piedra. The standard dosage of oral ketoconazole ranges from 200 to 400 mg per day, administered either as a single oral dose or divided into two doses (morning and evening). However, the most recommended formulation is topical ketoconazole, in the form of a 2% shampoo or cream [11, 19].

For invasive infections, triazoles have shown superior efficacy, particularly when used in combination with other antifungal agents such as amphotericin B. This therapeutic strategy generally involves the use of oral or intravenous triazoles in conjunction with intravenous amphotericin B, tailored to the severity of the infection and the pharmacokinetic profiles of the antifungal agents [5, 10, 20]. In this context, voriconazole is currently recommended as the first-line treatment for invasive trichosporonosis. The initial recommended dosage is 6 mg/kg intravenously every 12 h for the first 24 to 48 h, followed by 4 mg/kg every 12 h, or transitioning to oral administration (100 mg to 200 mg every 12 h), for maintenance therapy. Treatment may be discontinued once clinical resolution is achieved, laboratory results confirm infection control, and radiologic imaging no longer indicates signs of invasive fungal disease. However, ongoing monitoring of the patient's clinical status and

potential antifungal resistance remains essential [5, 10, 16, 20, 21].

May Newer Antifungals Help in Treating Trichosporonosis?

Some new drugs are currently being evaluated for the treatment of opportunistic yeasts, including Ibrexafungerp, Rezafungin, Olorofim, Opelconazole, and Fosmanogepix [49] (Table 2). However, there are no in-depth studies on Rezafungin, Olorofim, and Opelconazole indicating their effectiveness against trichosporonosis.

Ibrexafungerp (formerly SCY-078 or MK-3118) is a first-in-class triterpenoid antifungal that inhibits the biosynthesis of β-(1,3)-D-glucan in the fungal cell wall, a mechanism similar to echinocandins. The in vitro activity was tested in some *Trichosporon* species, but the activity of ibrexafungerp was variable, making it difficult to predict the use of this drug in trichosporonosis [50].

Fosmanogepix is a potent new antifungal agent targeting the fungal Gwt1 enzyme. It has previously been demonstrated to have good in vitro activity against clinical isolates of *Candida* and *Aspergillus* species. A recent study demonstrated the potential of fosmanogepix against some yeasts, including *Trichosporon* [51].

Some other groups are testing natural compounds in this context. For instance, Yang et al. [52] analyzed the antifungal characteristics of allicin against *T. asahii*. Allicin is the main biologically active component with broad-spectrum antimicrobial activity in garlic, and these findings shed new light on the potential of allicin as an alternative treatment strategy for trichosporonosis [52].

Trichosporon spp. Resistance from the One Health Context

The One Health approach, published by the tripartite strategic partnership of FAO/OIE/WHO, recognizes that human health, animal health, and the environment are interconnected, with antimicrobial resistance being one of the priorities of this alliance [53] (Fig. 2). Of particular concern, resistance to antifungals has significantly increased in yeasts, putting millions of people affected annually by yeast-like fungi at risk [53]. Thus, the CDC and WHO have already considered antifungal resistance in yeasts to be a serious threat to global public health (2019 CDC's Antibiotic Resistance Threats in the United States Report).

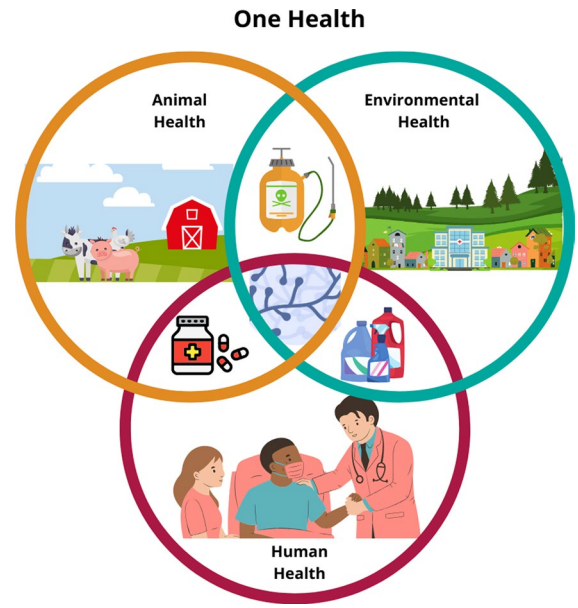


Fig. 2 *Trichosporon* genus in the One Health context: antifungal resistance in yeasts, driven by environmental factors such as agrochemicals, disinfectants and antimicrobials, poses a growing threat to global health. Ubiquitous and opportunistic pathogens like *Trichosporon* exposed to these substances may develop cross-resistance, linking human, animal and environmental health, and challenging the antifungal resistance and treatment perspective

Studies have pointed out that exposure to agricultural fungicides, such as azoles, select for resistant genotypes in *Aspergillus fumigatus*, which may develop cross-resistance to the medications used in humans, potentially leading to treatment failure [54, 55]. From this point of view, it is now clear that environmental yeasts may also develop resistance to clinical antifungals, which could be transmitted to human and animal populations. There is growing evidence that pathogenic yeasts, such as *Candida* spp. and *Cryptococcus* spp., can develop resistance to azoles due to pesticide exposure [54, 56–58].

In this context, *Trichosporon* is widely distributed in nature, encompassing species that inhabit different ecological niches and can be found in water or soil, as well as interacting with animals (such as birds, as

well as bird droppings) and humans [3, 5, 59–61]. Since the *Trichosporon* genus is composed of ubiquitous emerging yeasts found both in the environment and in animal host, it is important to consider that the exposure to antimicrobials, agrochemicals, and disinfectants may influence the development of resistance. These substances may contribute to the development of resistance in these yeasts, potentially leading to the occurrence of cross-resistance, as has already been demonstrated for other species [56–58] (Fig. 2). Therefore, the potential transmission routes (from the environment or animals to humans) could also influence the efficacy of the treatment, in this context of One Health.

Perspectives and Current Gaps

Trichosporonosis poses significant clinical challenges and urgently needs further scientific investigation, particularly concerning antifungal therapy and potential multidrug resistance (resistance to at least two different classes of drugs). Without new therapeutic strategies and a deeper understanding of its pathogenicity, trichosporonosis will continue to pose a substantial threat to vulnerable patient populations.

Although there is still limited data to support susceptibility-driven antifungal therapy, in vitro susceptibility testing using reference methods is recommended for epidemiological purposes. Furthermore, the establishment of ECV/clinical breakpoints is also important to facilitate the interpretation of MICs in clinical labs. Some recommendations are now available (2024) from EUCAST (EUCAST guidance on Interpretation of MICs for rare yeasts without breakpoints), and some studies are already contributing to this scenario [4], but this is just the beginning. This will further allow us to identify isolates, showing evidence of acquired resistance. Moreover, some studies continue to highlight the differences between species within this genus when it comes to susceptibility to antifungal agents. Therefore, further in-depth studies on this topic would also be important.

Additionally, more in-depth studies on the applicability and effectiveness of new antifungals for the *Trichosporon* genus are necessary. In addition to clinical trials, which will be crucial for introducing these new forms of treatment for trichosporonosis.

Significant advancements are still needed to gain a deeper understanding of trichosporonosis susceptibility and treatment. There is also a pressing need for more precise diagnosis, prescriptions, and dosage guidelines. Furthermore, *Trichosporon* spp. is becoming increasingly relevant within the One Health framework, as environmental exposure to fungicidal substances may be contributing to its growing resistance.

Acknowledgements We thank the following agencies for their support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Brazilian Ministry of Health (408540/2022-2 and 444501/2023-1), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-88887.966527/2024-00) and CAPES-COFECUB program (Código de Financiamento 001), and the National Institute of Science and Technology INCT Funvir from Brazil (CNPq). D.A.S. (303762/2020-9) is a research fellow of CNPq.

Author contribution All authors contributed equally to this work (writing and revision).

Funding This work was supported by the following funding agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Brazilian Ministry of Health (408540/2022-2 and 444501/2023-1), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-88887.966527/2024-00) and CAPES-COFECUB program (Código de Financiamento 001), and the National Institute of Science and Technology INCT Funvir from Brazil (CNPq). D.A.S. (303762/2020-9) is a research fellow of CNPq.

Declarations

Conflict of interest There is no conflict of interest in this work.

Ethical approval This work is a review; therefore, an ethical approval statement is not required.

References

1. Takashima M, Sugita T. Taxonomy of Pathogenic Yeasts *Candida*, *Cryptococcus*, *Malassezia*, and *Trichosporon*. Med Mycol J. 2022;63(4):119–32. https://doi.org/10.3314/mmj.22.004.
2. Francisco EC, Desnos-Ollivier M, Dieleman C, Boekhout T, Santos DWCL, Medina-Pestana JO, Colombo AL, Hagen F. Unveiling *Trichosporon austroamericanum* sp. nov.: A Novel Emerging Opportunistic Basidiomycetous Yeast Species. Mycopathologia. 2024 May 6;189(3):43. https://doi.org/10.1007/s11046-024-00851-4.
3. Aude Commenges, Françoise Coucheney, Marie-Hélène Lessard, Djamel Drider, Steve Labrie. *Trichosporon asahii*: Taxonomy, health hazards, and biotechnological

applications. Fungal Biology Reviews, Volume 49, 2024, 100369, ISSN 1749–4613. https://doi.org/10.1016/j.fbr.2024.100369.
4. EC Francisco, JN de Almeida Junior, F de Queiroz Telles, VR Aquino, AVA Mendes, MGM de Andrade Barberino, P de Tarso, O Castro, T Guimarães, RC Hahn, ACB Padovan, GM Chaves, AL Colombo. Species distribution and antifungal susceptibility of 358 *Trichosporon* clinical isolates collected in 24 medical centres. Clinical Microbiology and Infection. Volume 25, Issue 7, 2019, Pages 909.e1–909.e5. 2019 Jul. https://doi.org/10.1016/j.cmi.2019.03.026.
5. Mehta V, Nayyar C, Gulati N, Singla N, Rai S, Chandar J. A Comprehensive Review of *Trichosporon* spp.: An Invasive and Emerging Fungus. Cureus. 2021 Aug 21;13(8):e17345. https://doi.org/10.7759/cureus.17345.
6. Colombo AL, Padovan AC, Chaves GM. Current knowledge of *Trichosporon* spp. and Trichosporonosis. Clin Microbiol Rev. 2011 Oct;24(4):682–700. https://doi.org/10.1128/CMR.00003-11.
7. Cafarchia C, Camarda A, Romito D, Campolo M, Quaglia NC, Tullio D, Otranto D. Occurrence of yeasts in cloacae of migratory birds. Mycopathologia. 2006[a] Apr; 161(4): 229–234. https://doi.org/10.1007/s11046-005-0194-z.
8. Mariné M, Brown NA, Riaño-Pachón DM, Goldman GH. On and Under the Skin: Emerging Basidiomycetous Yeast Infections Caused by *Trichosporon* Species. PLoS Pathog. 2015;11(7): e1004982. https://doi.org/10.1371/journal.ppat.1004982.
9. Chander J. Textbook of medical mycology. Chander J (ed): Jaypee Publishers, New Delhi. 2018. https://books.google.com.au/books/about/Textbook_of_Medical_Mycology.html?id=OLpEDwAAQBAJ&redir_esc=y.
10. Singh S, Capoor MR, Varshney S, Gupta DK, Verma PK, Ramesh V. Epidemiology and Antifungal Susceptibility of Infections Caused by *Trichosporon* Species: An Emerging Non-Candida and Non-Cryptococcus Yeast Worldwide. Indian J Med Microbiol. 2019 Oct-Dec;37, 536–541. https://doi.org/10.4103/ijmm.IJMM_19_146.
11. Guerrero-Ponce AE, Araiza J, Tirado-Sánchez A, Bonifaz A. Review Article White Piedra: Review of 131 cases. Mycoses. 2024;67(1): e13668. https://doi.org/10.1111/myc.13668.
12. Pumeesat P, Wongsuk T. Genetic analysis of emerging fungal pathogens: *Trichosporon asahii*. Diagn Microbiol Infect Dis. 2023;107(3): 116057. https://doi.org/10.1016/j.diagmicrobio.2023.116057.
13. Chang DSJ, Harfouch CW, Melendez ML. *Trichosporon asahii* opportunistic pneumonia after a severe *COVID-19* infection. IDCases. 2023;31: e01701. https://doi.org/10.1016/j.idcr.2023.e01701.
14. Benelli JL, Basso RP, Grafulha TW, Poester VR, Munhoz LS, Martins KB, Zogbi HE, Von Groll A, Severo CB, Stevens DA, Xavier MO. Fungal Bloodstream Coinfection by *Trichosporon asahii* in a COVID-19 Critical Patient: Case Report and Literature Review. Mycopathologia. 2022;187(4):397–404. https://doi.org/10.1007/s11046-022-00637-6.
15. Cronyn V, Howard J, Chiang L, Le L, Tims-Cook Z, Gertz AM. *Trichosporon asahii* Urinary Tract Infection in a Patient with Severe COVID-19. Case Rep Infect Dis. 2021;17(2021):6841393. https://doi.org/10.1155/2021/6841393.
16. Lino R, Guimarães AR, Sousa E, Azevedo M, Santos L. Emerging Fungal Infections of the Central Nervous System in the Past Decade: A Literature Review. Infect Dis Rep. 2024;16:952–76. https://doi.org/10.3390/idr16050076.
17. Dong DV, Boutin S, Sang VV, Manh ND, Hoan NX, Quang HX, Lien TT, Trang VD, The NT, Linh LTK, Schmauder K, Ueltzhöffer V, Hafza N, Hauswaldt S, Rupp J, Kremsner PG, Song LH, Nurjadi D, Peter S, Velavan TP. Optimization of the Diagnosis of Central Nervous System Infections in Vietnamese Hospitals: Results From a Retrospective Multicenter Study. Open Forum Infect Dis. 2024 Sep 13;11(9):ofae531. https://doi.org/10.1093/ofid/ofae531.
18. Milan EP, Silva-Rocha WP, de Almeida JJS, Fernandes TUG, de Araújo Prudente AL, de Azevedo MF, Francisco EC, de Azevedo Melo AS, Colombo AL, Chaves GM. *Trichosporon inkin* meningitis in Northeast Brazil: first case report and review of the literature. BMC Infect Dis. 2018;18(1):470. https://doi.org/10.1186/s12879-018-3363-7.
19. Robles-Tenorio A, Lepe-Moreno KY, Mayorga-Rodríguez J. White Piedra, a Rare Superficial Mycosis: an Update. Curr Fungal Infect Rep. 2020;14:197–202. https://doi.org/10.1007/s12281-020-00389-0.
20. Agrawal S. Spectrum of signs, symptoms, and treatment in amphotericin B-resistant *Trichosporon* endophthalmitis: A series of ten cases of post-cataract surgery cluster endophthalmitis. Indian J Ophthalmol. 2022;70(11):4004–9. https://doi.org/10.4103/ijo.IJO_1938_22.
21. Chen SC, Perfect J, Colombo AL, Cornely OA, Groll AH, Seidel D, Albus K, de Almedia JN, Gilroy G-E, Lass-Flörl C, Ostrosky-Zeichner L, Pagano L, Papp T, Rautemaa-Richardson R, Salmanton-García J, Spec A, Steinmann J, Arikan-Akdagli S, Arenz DE, Sprute R, Duran-Graeff L, Freiberger T, Girmenia C, Harris M, Kanj SS, Roudbary M, Lortholary O, Meletiadis J, Segal E, Tuon FF, Wiederhold N, Bicanic T, Chander J, Chen YC, Hsueh PR, Ip M, Munoz P, Spriet I, Temfack E, Thompson L, Tortorano AM, Velegraki A, Govender NP. Global guideline for the diagnosis and management of rare yeast infections: an initiative of the ECMM in cooperation with ISHAM and ASM. Lancet Infect Dis. 2021;21(12):e375–86. https://doi.org/10.1016/S1473-3099(21)00203-6.
22. Desnos-Ollivier M, Lortholary O, Bretagne S, Dromer F. Azole Susceptibility Profiles of More than 9,000 Clinical Yeast Isolates Belonging to 40 Common and Rare Species. Antimicrob Agents Chemother. 2021;65(6):e02615-e2620. https://doi.org/10.1128/AAC.02615-20.
23. Padovan ACB, Rocha WPDS, Toti ACM, Freitas de Jesus DF, Chaves GM, Colombo AL. Exploring the resistance mechanisms in *Trichosporon asahii*: Triazoles as the last defense for invasive trichosporonosis. Fungal Genet Biol. 2019 Dec;133:103267. https://doi.org/10.1016/j.fgb.2019.103267.
24. Aguiar ALR, Silva BND, Fiallos NM, Pereira LMG, Silva ML, Souza PFSM, Portela FVM, Sidrim JJC, Rocha

MFG, Castelo-Branco DSCM, Cordeiro RA. Promethazine inhibits efflux, enhances antifungal susceptibility and disrupts biofilm structure and functioning in *Trichosporon*. Biofouling. 2023;39(2):218–30. https://doi.org/10.1080/08927014.2023.2202315.

25. Ma X, Liu H, Liu Z, Wang Y, Zhong Z, Peng G, Gu Y. *Trichosporon asahii PLA2* Gene Enhances Drug Resistance to Azoles by Improving Drug Efflux and Biofilm Formation. Int J Mol Sci. 2023;24(10):8855. https://doi.org/10.3390/ijms24108855.
26. Iturrieta-González IA, Padovan AC, Bizerra FC, Hahn RC, Colombo AL. Multiple species of *Trichosporon* produce biofilms highly resistant to triazoles and amphotericin B. PLoS ONE. 2014;9(10): e109553. https://doi.org/10.1371/journal.pone.0109553.
27. Guo Lyu S, Hsueh P, Al-Hatmi AMS, Meis JF, Hagen F, Xiao M, Wang H, Barresi C, Zhou MDE, Hoog GS, Xu Y 2019. Invasive Infections Due to *Trichosporon*: Species Distribution, Genotyping, and Antifungal Susceptibilities from a Multicenter Study in China. J Clin Microbiol 57:https://doi.org/10.1128/jcm.01505-18.
28. Wongsuk T, Boonsilp S, Pumeesat P, Homkaew A, Sangsri T, Chongtrakool P. Genotyping, antifungal susceptibility testing, and biofilm formation of *Trichosporon* spp. isolated from urine samples in a University Hospital in Bangkok, Thailand. Acta Microbiol Immunol Hung. 2022 Aug 16;69(3):247–257. https://doi.org/10.1556/030.2022.01797.
29. Kuo SH, Lu PL, Chen YC, Ho MW, Lee CH, Chou CH, Lin SY. The epidemiology, genotypes, antifungal susceptibility of *Trichosporon* species, and the impact of voriconazole on *Trichosporon* fungemia patients. J Formos Med Assoc. 2021;120(9):1686–94. https://doi.org/10.1016/j.jfma.2020.12.007.
30. Pereira LMG, Andrade ARC, Portela FVM, Aguiar ALR, Silva BND, Moura SGB, Pergentino MLM, Rocha MFG, Sidrim JJDC, Castelo Branco DSCM, Cordeiro RA. Heterologous extracellular DNA facilitates the development of *Trichosporon asahii* and *T. inkin* biofilms and enhances their tolerance to antifungals. Biofouling. 2022 Sep;38(8):778–785. https://doi.org/10.1080/08927014.2022.2130788.
31. Cordeiro RA, Aguiar ALR, da Silva BN, Pereira LMG, Portela FVM, de Camargo ZP, de Lima-Neto RG, Castelo-Branco DSCM, Rocha MFG, Sidrim JJC. *Trichosporon asahii* and *Trichosporon inkin* Biofilms Produce Antifungal-Tolerant Persister Cells. Front Cell Infect Microbiol. 2021;22(11): 645812. https://doi.org/10.3389/fcimb.2021.645812.
32. Flowers SA, Colón B, Whaley SG, Schuler MA, Rogers PD. 2015. Contribution of Clinically Derived Mutations in *ERG11* to Azole Resistance in *Candida albicans*. Antimicrob Agents Chemother 59. https://doi.org/10.1128/aac.03470-14.
33. He X, Li S, Kaminskyj SGW. 2014. Using *Aspergillus nidulans* To Identify Antifungal Drug Resistance Mutations. Eukaryot Cell 13. https://doi.org/10.1128/ec.00334-13.
34. Abbes S, Sellami H, Neji S, Trabelsi H, Makni F, Ayadi A. Implication of efflux pumps and *ERG11* genes in resistance of clinical *Trichosporon asahii* isolates to fluconazole. J Med Microbiol. 2021 Mar 10;70(3). https://doi.org/10.1099/jmm.0.001236.
35. Hisako K, Tokimatsu I, Ishii H, Kawano R, Shirai R, Kishi K, Hiramatsu K, Kadota J. Cloning of the lanosterol 14-α-demethylase (*ERG11*) gene in *Trichosporon asahii*: a possible association between G453R amino acid substitution and azole resistance in *T. asahii*. FEMS Yeast Res. 2012 Sep;12(6):662–7. https://doi.org/10.1111/j.1567-1364.2012.00816.x.
36. Nobrega de Almeida J Jr, Jimenez-Ortigosa C, Francisco EC, Colombo AL, Perlin DS. *ERG11* Analysis among Clinical Isolates of *Trichosporon asahii* with Different Azole Susceptibility Profiles. Antimicrob Agents Chemother. 2022 Dec 20;66(12):e0110122. https://doi.org/10.1128/aac.01101-22.
37. Ma X, Yang W, Yang A, Chen D, Wang C, Ling S, Cao S, Zuo Z, Wang Y, Zhong Z, Peng G, He M, Gu Y. Metabolome and Transcriptome Combinatory Profiling Reveals Fluconazole Resistance Mechanisms of *Trichosporon asahii* and the Role of Farnesol in Fluconazole Tolerance. Microorganisms. 2023;11(11):2798. https://doi.org/10.3390/microorganisms11112798.
38. Carolus H, Pierson S, Lagrou K, Van Dijck P. Amphotericin B and Other Polyenes-Discovery, Clinical Use, Mode of Action and Drug Resistance. J Fungi (Basel). 2020;6(4):321. https://doi.org/10.3390/jof6040321.
39. Gabriella Freitas Ferreira. Ludmila de Matos Baltazar, Julliana Ribeiro Alves Santos, Andrea Souza Monteiro, Lucia Alves de Oliveira Fraga, Maria Aparecida Resende-Stoianoff, Daniel Assis Santos, The role of oxidative and nitrosative bursts caused by azoles and amphotericin B against the fungal pathogen *Cryptococcus gattii*. J Antimicrob Chemother. 2013;68(8):1801–11. https://doi.org/10.1093/jac/dkt114.
40. Pfaller MA. Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. Am J Med. 2012;125(1 Suppl):S3-13. https://doi.org/10.1016/j.amjmed.2011.11.001.
41. Matsumoto Y, Arakawa S, Sadahira K, Sato T, Yamazaki K, Nishimoto S. Skin Manifestations of Micafungin Breakthrough Disseminated Trichosporonosis in Acute Megakaryoblastic Leukemia. Med Mycol J. 2024;65(1):17–21. https://doi.org/10.3314/mmj.23-00009.
42. Matsumoto Y, Sugiyama Y, Nagamachi T, Yoshikawa A, Sugita T. Hog1-mediated stress tolerance in the pathogenic fungus *Trichosporon asahii*. Sci Rep. 2023;13(1):13539. https://doi.org/10.1038/s41598-023-40825-y.
43. Kimura M, Asano-Mori Y, Sakoh T, Abe M, Ueno K, Hoshino Y, Nakamura S, Umeyama T, Yamagoe S, Miyazaki Y, Baba M, Okada C, Ogura S, Mitsuki T, Yamaguchi K, Yuasa M, Kaji D, Kageyama K, Nishida A, Taya Y, Ishiwata K, Takagi S, Yamamoto H, Yamamoto G, Uchida N, Wake A, Taniguchi S, Araoka H. Factors Associated with Breakthrough Fungemia Caused by *Candida*, *Trichosporon*, or *Fusarium* Species in Patients with Hematological Disorders. Antimicrob Agents Chemother. 2022;66(3): e0208121. https://doi.org/10.1128/AAC.02081-21.
44. Itoh K, Iwasaki H, Negoro E, Shigemi H, Tokimatsu I, Tsutani H, Yamauchi T. Successful Treatment of Breakthrough *Trichosporon asahii* Fungemia by the

Combination Therapy of Fluconazole and Liposomal Amphotericin B in a Patient with Follicular Lymphoma. Mycopathologia. 2021;186(1):113–7. https://doi.org/10.1007/s11046-020-00525-x.
45. Hori Y, Shibuya K. Role of FKS Gene in the Susceptibility of Pathogenic Fungi to Echinocandins. Med Mycol J. 2018;59(2):E31–40. https://doi.org/10.3314/mmj.18.004. Erratum.In:MedMycolJ.2018;59(4):J72.doi:10.3314/mmj.18.004E.
46. Loyse A, Dromer F, Day J, Lortholary O, Harrison TS. Flucytosine and cryptococcosis: time to urgently address the worldwide accessibility of a 50-year-old antifungal. J Antimicrob Chemother. 2013;68(11):2435–44. https://doi.org/10.1093/jac/dkt221.
47. Xia Z, Yang R, Wang W, Cong L. Genotyping and antifungal drug susceptibility of *Trichosporon asahii* isolated from Chinese patients. Mycopathologia. 2012;173(2–3):127–33. https://doi.org/10.1007/s11046-011-9478-7.
48. Tokunaga S, Ohkawa M, Nakashima T, Yamaguchi K, Nishikawa T, Matsushita T, Mikawa I, Hirano S, Kawaguchi S, Miyagi T, et al. Clinical evaluation of flucytosine in patients with urinary fungal infections. Jpn J Antibiot. 1992 Aug;45(8):1060–4. Japanese. PMID: 1433895.
49. Hoenigl M, Sprute R, Egger M, et al. The Antifungal Pipeline: Fosmanogepix, Ibrexafungerp, Olorofim, Opelconazole, and Rezafungin. Drugs. 2021;81:1703–29. https://doi.org/10.1007/s40265-021-01611-0.
50. Jallow S, Govender NP. Ibrexafungerp: A First-in-Class Oral Triterpenoid Glucan Synthase Inhibitor. J Fungi (Basel). 2021;7(3):163. https://doi.org/10.3390/jof7030163.
51. Pfaller M, Huband M, Bien PA, Carvalhaes CG, Klauer A, Castanheira M. In vitro activity of manogepix and comparators against infrequently encountered yeast and mold isolates from the SENTRY Surveillance Program (2017–2022). Antimicrob Agents Chemother. 2024;68(2):e0113223. https://doi.org/10.1128/aac.01132-23.
52. Yang X, Bai S, Wu J, Fan Y, Zou Y, Xia Z, Ao J, Chen T, Zhang M, Yang R. Antifungal Activity and Potential Action Mechanism of Allicin against *Trichosporon asahii*. Microbiol Spectr. 2023;11(3): e0090723. https://doi.org/10.1128/spectrum.00907-23.
53. Adisasmito WB, Almuhairi S, Behravesh CB, Bilivogui P, Bukachi SA, Casas N, Cediel Becerra N, Charron DF, Chaudhary A, Ciacci Zanella JR, Cunningham AA, Dar O, Debnath N, Dungu B, Farag E, Gao GF, Hayman DTS, Khaitsa M, Koopmans MPG, Machalaba C, Mackenzie JS, Markotter W, Mettenleiter TC, Morand S, Smolenskiy V, Zhou L. One Health: A new definition for a sustainable and healthy future. PLoS Pathog. 2022;18(6): e1010537. https://doi.org/10.1371/journal.ppat.1010537.
54. Castelo-Branco D, Lockhart SR, Chen YC, Santos DA, Hagen F, Hawkins NJ, Lavergne RA, Meis JF, Le Pape P, Rocha MFG, Sidrim JJC, Arendrup M, Morio F. Collateral consequences of agricultural fungicides on pathogenic yeasts: A One Health perspective to tackle azole resistance. Mycoses. 2022;65(3):303–11. https://doi.org/10.1111/myc.13404.
55. Ren J, Jin X, Zhang Q, Zheng Y, Lin D, Yu Y. Fungicides induced triazole-resistance in *Aspergillus fumigatus* associated with mutations of TR46/Y121F/T289A and its appearance in agricultural fields. J Hazard Mater. 2017;15(326):54–60. https://doi.org/10.1016/j.jhazmat.2016.12.013.
56. Carneiro HCS, Ribeiro NQ, Bastos RW, Santos DA. Effect of non-antifungal agrochemicals on the pathogenic fungus *Cryptococcus gattii*. Med Mycol. 2020;58(1):47–53. https://doi.org/10.1093/mmy/myz018.
57. Bastos RW, Freitas GJC, Carneiro HCS, Oliveira LVN, Gouveia-Eufrasio L, Santos APN, Moyrand F, Maufrais C, Janbon G, Santos DA. From the environment to the host: How non-azole agrochemical exposure affects the antifungal susceptibility and virulence of *Cryptococcus gattii*. Sci Total Environ. 2019;1(681):516–23. https://doi.org/10.1016/j.scitotenv.2019.05.094.
58. Bastos RW, Carneiro HCS, Oliveira LVN, Rocha KM, Freitas GJC, Costa MC, Magalhães TFF, Carvalho VSD, Rocha CE, Ferreira GF, Paixão TA, Moyrand F, Janbon G, Santos DA. Environmental Triazole Induces Cross-Resistance to Clinical Drugs and Affects Morphophysiology and Virulence of *Cryptococcus gattii* and *C. neoformans*. Antimicrob Agents Chemother. 2017 Dec 21;62(1):e01179–17. https://doi.org/10.1128/AAC.01179-17.
59. Lara BR, de Camargo BB, Paula CR, Junior DPL, Garces HG, Arnoni MV, Silveira M, Gimenes VMF, Siqueira LPM, Takahashi JPF, Melhem MSC, Richini-Pereira VB, Anversa L, Ruiz LDS. Comparing the phenotypic, genotypic, and proteomic identification of *Trichosporon* species: A globally emerging yeast of medical importance. Med Mycol. 2021;59(12):1181–90. https://doi.org/10.1093/mmy/myab050.
60. Lo C, Kang CL, Sun PL, Yu PH, Li WT. Disseminated Fungal Infection and Fungemia Caused by *Trichosporon asahii* in a Captive Plumed Basilisk (*Basiliscus plumifrons*). J Fungi (Basel). 2021;7(12):1003. https://doi.org/10.3390/jof7121003.
61. Bobrek K, Sokół I, Gaweł A. Uncommon Non-*Candida* Yeasts in Healthy Turkeys-Antimicrobial Susceptibility and Biochemical Characteristic of *Trichosporon* Isolates. Pathogens. 2021;10(5):538. https://doi.org/10.3390/pathogens10050538.

4. Capítulo 2

Com objetivo de realizar a padronização da metodologia usada neste trabalho para a avaliação da susceptibilidade no gênero *Trichosporon*, foi elaborado e publicado o artigo de metodologia Optimized Conditions for Broth Microdilution Susceptibility Testing of *Trichosporon* spp. to Clinical Antifungals na revista Brazilian Journal of Microbiology em 22/08/2025 (DOI: 10.1007/s42770-025-01766-y). O estudo apresenta o desenvolvimento de um protocolo otimizado para testes de microdiluição em caldo, com a finalidade de avaliar a concentração inibitória mínima dentro do gênero *Trichosporon*, contemplando adaptações nas condições de incubação e nos critérios de leitura. Essa padronização permitiu resultados mais reprodutíveis e precisos para diferentes espécies de *Trichosporon* frente aos antifúngicos de uso clínico fluconazol e anfotericina B. Entre os principais resultados, foram determinadas as condições ideais de temperatura, período de incubação e critérios de leitura da inibição fúngica, o que facilitou comparações entre estudos e contribuiu para o estabelecimento de valores de referência e futuras diretrizes de teste de susceptibilidade para o gênero.

Brazilian Journal of Microbiology
https://doi.org/10.1007/s42770-025-01766-y

MEDICINE AND PUBLIC HEALTH - RESEARCH PAPER

Optimized conditions for broth microdilution susceptibility testing of *Trichosporon* spp. to clinical antifungals

Isabela Lima Miranda[1] · Nalu Teixeira Aguiar Peres[1,2] · Rafael Wesley Bastos[2,3] · Luana Rossato[4] · Gabriela Silva Cruz[5] · Maria Isabel Azevedo[6] · Kássia Jéssica Galdino Silva[3] · Débora S. C. M. Castelo-Branco[5] · Fabíola Lucini[4] · Daniel Assis Santos[1,2]

Received: 12 May 2025 / Accepted: 7 August 2025

Abstract

The resistance in *Trichosporon* species poses a significant challenge in clinical treatment, limiting the efficacy of commonly used antifungal drugs. In this context, *T. asahii* poses substantial risks as an opportunistic pathogen, especially in immunocompromised patients, where the effective antifungal treatment is also challenged by the absence of standardized testing methods. In this scenario, the present study evaluated and discussed the broth microdilution susceptibility testing of *Trichosporon* species to fluconazole and amphotericin B. A total of 33 *Trichosporon* spp. strains, isolated from different sources, along with reference strains, were tested. Different adaptations of the CLSI guidelines were applied to investigate optimal conditions for minimum inhibitory concentration (MIC) determination in this genus. The results revealed that fluconazole showed less variation between the tested incubation periods, with reading at 50% inhibition, while amphotericin B demonstrated more accurate results with extended incubation (48 h) and reading at 100% inhibition. Additionally, fluconazole exhibited higher MICs when isolates were incubated at 30 °C, with a range of 1–32 µg/mL, where amphotericin B showed higher MICs at 37 °C, with a range of 0.5–4 µg/mL. This work also reveals significant variability in susceptibility results, underscoring the necessity for standardized testing protocols. Based on the results, the study recommends for the optimal susceptibility testing a 48 h incubation period at 37 °C, with reading breakpoints of 50% inhibition for fluconazole and 100% inhibition for amphotericin B. Yet, this study highlights the urgent need for standardized testing methods and better understanding of antifungal resistance in *Trichosporon* infections.

Keywords *Trichosporon* · Standardization · Broth microdilution · Susceptibility test · Clinical antifungals

Communicated by Rosana Puccia

✉ Daniel Assis Santos
dasufmg@gmail.com

[1] Department of Microbiology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte 31270-901, MG, Brazil

[2] National Institute of Science and Technology in Human Pathogenic Fungi, Ribeirão Preto, Brazil

[3] Department of Microbiology and Parasitology, Biosciences Center, Federal University of Rio Grande do Norte, Natal 59078-970, RN, Brazil

[4] Health Sciences Research Laboratory, Federal University of Grande Dourados, Dourados 79804-970, MS, Brazil

[5] Department of Pathology and Forensic Medicine, Center of Biomedicine, Federal University of Ceará, Fortaleza 60430-275, CE, Brazil

[6] Department of Preventive Veterinary Medicine, Veterinary School, Federal University of Minas Gerais, Belo Horizonte 31270-901, MG, Brazil

Published online: 22 August 2025

Introduction

Over the past few decades, *Trichosporon* species have been increasingly recognized as opportunistic pathogens capable of causing life-threatening invasive diseases, particularly in immunosuppressed patients [1–3]. Most species of this genus are opportunistic pathogens, normally residing as commensals on the skin and gastrointestinal tract of healthy individuals (both humans and other animals). However, under an imbalanced environment, *Trichosporon* spp. may cause trichosporonosis, also depending on specific virulence factors and metabolic flexibility [2, 4, 5].

Trichosporonosis is primarily caused by six species: *T. asahii* and *T. mucoides*, which are the main agents of invasive infections, and *T. cutaneum*, *T. asteroides*, *T. ovoides*, and *T. inkin*, which are primarily associated with superficial infections [2, 3]. Recently, the taxonomy of this genus was revised, and some of the species tested and cited in this work now belong to the genus *Cutaneotrichosporon* (*Cutaneotrichosporon jirovecii*, *Cutaneotrichosporon mucoides*, and *Cutaneotrichosporon cutaneum*) [6]. However, in this study, we will discuss these species from the perspective of the former genus *Trichosporon*.

Benign irregular nodules on the hair shaft, known as White Piedra, represent the most common clinical presentation of superficial infection [7]. In cases of invasive infection, trichosporonosis is predominantly observed in immunocompromised patients, such as those with advanced HIV infection, cancer, or transplant recipients. In these cases, *T. asahii* is the most common agent, and the infection can affect a wide range of organs with highly variable clinical manifestations, such as fungemia (> 70%), pneumonia, and skin lesions, with lethality rates of 50–70% [2, 3]. Notably, during the COVID-19 pandemic, severe cases of co-infection and opportunistic infections after COVID-19 caused by *T. asahii* were described [8–11].

These life-threatening invasive diseases have become increasingly frequent and are often associated with ineffective antifungal treatments or antifungal resistance [1, 8, 9]. Furthermore, some studies have reported that a few *Trichosporon* isolates exhibit reduced susceptibility to certain azoles and polyenes, highlighting the potential for antifungal resistance in this genus to agents commonly used in clinical practice for treating fungal infections. Notably, this genus displays intrinsic resistance to echinocandins [2, 3].

In this context, antifungal susceptibility testing would be useful to foresee the best treatment for each patient. However, the methodology for determining the Minimum Inhibitory Concentrations (MIC) for antifungals is not yet well standardized for *Trichosporon* spp. The Clinical and Laboratory Standards Institute (CLSI) guidelines are the most used for determining yeast susceptibility and do not include the genus *Trichosporon* [12]. Consequently, researchers use different parameters (i.e., culture medium, inoculum concentration, temperature, and incubation period) to study antifungal susceptibility, leading to difficulties in reproducing data and interpreting the results [2, 3].

In this context, the establishment of a standardized broth microdilution susceptibility test for *Trichosporon* spp. is crucial due to its increasing clinical significance, particularly in immunocompromised patients. Therefore, this study aims to establish reproducible conditions for performing broth microdilution antifungal susceptibility testing of *Trichosporon* spp. against fluconazole and amphotericin B (the most used antifungals for treating fungal infections), including incubation period, temperature, and reading breakpoints. The goal is also to minimize the impact of potential antifungal resistance on treatment efficacy. These factors are critical when working with opportunistic environmental yeasts that can adapt to and thrive in animal hosts, potentially exhibiting reduced susceptibility to common treatments.

Table 1 Number of isolates per species

Species	Number of Isolates
Trichosporon asahii	17
Cutaneotrichosporon mucoides (*Trichosporon mucoides*)	8
Trichosporon japonicum	4
Cutaneotrichosporon jirovecii (*Trichosporon jirovecii*)	3
Trichosporon coremiforme	1

Materials and methods

Samples

In this study, we performed the susceptibility of 33 *Trichosporon* spp. isolates (Table 1) to the antifungals amphotericin B and fluconazole. Additionally, we used four reference strains as controls (chosen for their clinical relevance): *Cryptococcus neoformans* H99, *Cryptococcus gattii* R265, *Candida glabrata* ATCC 2001, and *Candida albicans* SC5314 (ATCC MYA-2876D-5) [13–15]. The isolates were collected from environmental and human sources, isolated on CHROMagar, identified using MALDI-TOF, and stored in a BHI medium supplemented with 20% glycerol collection at -80 °C. The collection is deposited in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN code AA7A6D7). The CLSI protocol [12] was used as a reference, but adaptations were made to suit our laboratory protocol.

Inoculum preparation

The inoculum was prepared following the methodology described in previous studies [13–15]. The samples were cultured on Sabouraud Dextrose Agar (SDA) at 37 °C for 48 h [13–15]. After incubation, a portion of the colony was transferred into 3 mL of 0.9% saline in a glass tube and homogenized [13–15]. Due to the tendency of the yeast to form clumps, the suspensions were left to decant for 5 min. After decantation, 2.5 mL of the supernatant was carefully collected for further processing. The transmittance of the inoculum was then adjusted to 75–77% at 530 nm, corresponding to a concentration of 1–5 × 10^6 cells/mL [13–15]. This stock inoculum was subsequently diluted in RPMI 1640 medium with MOPS (0.165 M) in two steps: a 1:50 dilution, followed by a 1:20 dilution, yielding a final concentration of 1–5 × 10^3 cells/mL [13–15]. The inoculum was transferred into 96-well flat-bottomed plates. The CLSI protocol [12] was used as a reference, but adaptations were made to suit our laboratory protocol.

Antifungals

The antifungals fluconazole and amphotericin B were obtained from Sigma-Aldrich and solubilized in sterilized distilled water and DMSO, respectively, to prepare stock solutions at a concentration of 5000 µg/mL [13–15]. They were prepared in stock solutions in DMSO, followed by serial dilutions in RPMI. The final concentration ranges were 0.03–16 µg/mL for amphotericin B and 0.125–64 µg/mL for fluconazole [13–15]. The CLSI protocol [12] was used as a reference, but adaptations were made to suit our laboratory protocol.

Susceptibility test conditions

All experiments were performed to test different incubation conditions: temperatures of 30 °C and 37 °C, and periods of 24 h and 48 h. Plates were read visually, and reading breakpoints of 50% growth inhibition compared to the drug-free growth control, as well as 100% growth inhibition, were used to determine the antifungal minimum inhibitory concentrations (MIC). Additionally, minimum inhibitory concentrations (MIC_{50} and MIC_{90}) were calculated, allowing the determination of the minimum concentration required to inhibit 50% and 90% of the isolate's growth, respectively [13–15]. The CLSI protocol [12] was used as a reference, but adaptations were made to suit our laboratory protocol.

Results

The results for *Cryptococcus neoformans* H99, *Cryptococcus gattii* R265, *Candida glabrata* ATCC 2001, and *Candida albicans* SC5314 (ATCC MYA-2876D-5) were consistent with the established standards, reinforcing the reproducibility of the protocol [12]. For *Trichosporon* spp., the results are summarized in Table 2. All the strains were evaluated under all the conditions tested, with the aim of standardizing.

Observing the growth pattern of the samples during the experiment, we noticed differences related to temperature and incubation period. All isolates showed growth within 24 h; however, in most cases, the best metabolic rate seemed to occur after 48 h of growth, where they demonstrated greater multiplication in the wells. Regarding temperature, some isolates exhibited preferential growth at one of the tested temperatures, where they demonstrated greater multiplication in the wells, while others seemed to grow equally under both conditions.

All isolates showed some growth within 24 h, regardless of the drug (Table 2). However, for most isolates, the 48-hour incubation period appeared more promising, allowing the yeast to grow and develop sufficiently to provide the best MIC determination. This was particularly noticeable for amphotericin B, where many of the isolates (24/33) showed a doubled MIC value after 48 h of incubation. Thus, 48 h of incubation might be the most reliable period to more accurately determine the antifungal susceptibility of this genus.

Temperature appeared to influence isolate growth, regardless of the drug (Table 2). Some isolates did not show sufficient growth to analyze inhibition depending on the temperature. One *T. asahii* isolate did not exhibit enough growth to analyze inhibition at 30 °C, while three isolates of *Cutaneotrichosporon jirovecii* (formerly *T. jirovecii*) did not exhibit sufficient growth to analyze inhibition at 37 °C.

When observing the percentage of growth inhibition, readings were taken at 50% and 100% inhibition (Table 2), as the drugs can be either fungicidal (amphotericin B) or fungistatic (fluconazole). Moreover, it is important to note that this is visual reading, which can be influenced by technical expertise in performing the test. We observed that for fluconazole, the 100% reading was not accurate, as expected for a fungistatic drug, since it can reach values higher than the highest concentrations tested. However, for amphotericin B, the 100% reading was more accurate, as expected for a fungicidal drug, as it completely inhibits the growth of this yeast.

As shown in Table 2, for fluconazole, the MIC values ranged from 0.125 to >64 µg/mL. In contrast, for amphotericin B, the MIC range was 0.06–4 µg/mL. Notably, the

Table 2 MIC (Minimum Inhibitory Concentration) results for fluconazole and amphotericin B against *Trichosporon* samples. Samples labeled as "N" demonstrated enough growth at only one of the tested temperatures, failing to grow at the other temperature, which made it impossible to assess susceptibility under that specific condition. Each color represents a species: blue (*Cutaneotrichosporon mucoides* / *T. mucoides*), orange (*Cutaneotrichosporon jirovecii* / *T. jirovecii*), red (*T. coremiforme*), green (*T. japonicum*) and yellow (*T. asahii*). Range represents the interval of values, while MIC_{50} and MIC_{90} refer to the minimum concentrations required to inhibit the growth of 50% and 90% of the samples, respectively

Strain Number	MIC (µg/mL)															
	Fluconazole								Amphotericin B							
	24 hours				48 hours				24 hours				48 hours			
	30°C		37°C		30°C		37°C		30°C		37°C		30°C		37°C	
	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%
1	4	16	1	2	4	16	1	4	0.5	1	0.5	1	0.5	2	0.5	1
2	8	16	4	16	8	16	4	16	0.25	0.5	0.25	0.5	0.5	1	0.25	0.5
3	8	16	1	4	8	16	1	4	0.25	0.5	0.5	0.5	0.25	1	0.25	1
4	32	64	4	>64	32	>64	4	>64	0.5	1	0.5	0.5	0.5	1	0.5	1
5	8	16	1	16	8	16	2	16	0.25	1	0.25	0.5	0.5	1	0.5	1
6	8	64	4	32	8	64	8	64	0.5	1	0.25	0.5	0.5	1	0.5	1
7	8	16	2	4	8	16	2	8	0.5	1	0.25	0.5	0.5	1	0.25	1
8	8	>64	0.5	4	8	>64	1	4	0.5	1	0.25	0.5	0.5	1	0.25	1
9	1	2	N		2	4	N		0.5	1	N		0.5	1	N	
10	1	2	N		1	2	N		0.25	0.5	N		0.5	1	N	
11	1	2	N		1	2	N		0.25	1	N		0.5	1	N	
12	2	8	1	4	2	8	1	4	0.25	1	0.5	1	0.25	1	0.5	1
13	2	8	0.5	1	2	8	0.25	1	0.5	1	0.5	1	0.5	1	0.5	1
14	1	4	0.5	1	1	4	8	>64	0.5	1	0.5	1	0.5	1	0.5	1
15	4	8	0.125	4	8	16	0.5	4	0.5	1	0.5	1	0.5	2	1	2
16	2	8	0.125	4	8	16	2	16	0.25	1	0.5	1	0.5	2	1	2
17	4	8	1	4	4	16	1	4	0.125	0.5	0.125	0.5	0.25	1	0.5	1
18	32	64	8	64	32	>64	8	>64	0.5	1	0.5	1	0.5	1	0.5	1
19	4	8	1	2	4	16	1	8	0.25	1	0.125	0.5	0.25	1	0.5	1
20	2	8	0.5	1	4	16	0.5	2	0.25	0.5	0.5	1	0.25	1	0.5	1
21	8	16	1	2	8	16	1	4	0.25	0.5	0.5	2	0.5	1	1	2
22	4	16	1	2	4	16	1	4	0.06	0.125	0.25	0.5	0.25	0.5	0.25	1
23	8	16	1	2	8	16	1	4	0.125	0.25	0.25	0.5	0.25	0.5	0.25	1
24	8	32	4	8	8	32	4	8	0.5	1	0.5	1	0.5	1	1	2
25	8	32	4	8	8	32	4	8	0.5	1	0.5	1	0.5	1	1	2
26	4	16	1	4	4	16	1	4	0.5	1	0.5	1	0.5	1	0.5	1
27	4	16	1	2	4	16	2	4	0.25	1	0.5	1	0.5	1	0.5	1
28	4	8	1	4	8	16	1	4	0.5	1	0.5	1	1	1	0.5	1
29	4	8	1	2	8	16	1	8	0.5	1	0.5	1	1	1	1	2
30	4	8	1	8	8	16	1	8	0.5	1	0.5	1	1	2	1	2
31	N		0.5	1	N		0.5	2	N		0.25	0.5	N		0.5	1
32	8	16	1	4	8	16	1	4	0.06	0.125	0.25	1	0.25	1	1	4
33	8	16	0.5	4	8	16	1	4	0.5	1	0.5	1	0.5	2	0.5	2
Range	**1-32**	**2->64**	**0.125-8**	**1->64**	**1-32**	**2->64**	**0.25-8**	**2->64**	**0.06-0.5**	**0.125-1**	**0.125-0.5**	**0.5-2**	**0.25-1**	**0.5-2**	**0.25-1**	**0.5-4**
MIC_{50}	**4**	**16**	**1**	**4**	**8**	**16**	**1**	**4**	**0.5**	**1**	**0.5**	**1**	**0.5**	**1**	**0.5**	**1**
MIC_{90}	**8**	**64**	**4**	**16**	**8**	**64**	**4**	**64**	**0.5**	**1**	**0.5**	**1**	**1**	**2**	**1**	**2**

highest MIC values (32 µg/mL for fluconazole and 4 µg/mL for amphotericin B) were predominantly observed against *Cutaneotrichosporon mucoides* (formerly *T. mucoides*) and *T. asahii* at 30 °C / 50% reading / 48 h for fluconazole, and for *T. asahii* at 37 °C / 100% reading / 48 h for amphotericin B (Table 2).

Considering both antifungal agents under the tested conditions, several interesting results were observed (Table 2). For fluconazole, the highest MIC_{50} and MIC_{90}, following the previously defined parameters, were 8 µg/mL at 48 h, 30 °C, and 50% inhibition as the reading breakpoint. For amphotericin B, the highest MIC_{50} and MIC_{90}, following the previously defined parameters, were 1 µg/mL and 2 µg/mL, respectively, at 48 h, 37 °C, and 100% inhibition as the reading breakpoint.

Table 3 provides a summary of these results, showing the specific conditions under which the best MIC values (as observed in this study) were recorded for both fluconazole and amphotericin B. Despite the significant variation in the results, this study aimed to discuss the optimal conditions

Table 3 Comparison of MIC results for clinical antifungals fluconazole and amphotericin B

Fluconazole	Amphotericin B
Reading breakpoint of 50% growth inhibition	Reading breakpoint of 100% growth inhibition
Reading at 24–48 h of growth	Reading at 48 h of growth
Highest MIC values at 30 °C	Highest MIC values at 37 °C

Table 4 Distribution of MIC range, MIC_{50} and MIC_{90} values for fluconazole and amphotericin B among species, considering only the optimal conditions established in this study—37°C, 48 hours, with 50% growth inhibition for fluconazole and 100% inhibition for amphotericin B. Samples labeled as "-" did not exhibit sufficient growth at the tested temperature, preventing susceptibility assessment under these specific conditions

Species	MIC Range	MIC_{50}	MIC_{90}
Fluconazole			
Trichosporon asahii	0.5–8 µg/mL	1 µg/mL	4 µg/mL
Cutaneotrichosporon mucoides (*Trichosporon mucoides*)	1–8 µg/mL	2 µg/mL	4 µg/mL
Trichosporon japonicum	0.25–8 µg/mL	0.5 µg/mL	2 µg/mL
Cutaneotrichosporon jirovecii (*Trichosporon jirovecii*)	-	-	-
Trichosporon coremiforme	1 µg/mL	1 µg/mL	1 µg/mL
Amphotericin B			
Trichosporon asahii	1–4 µg/mL	1 µg/mL	2 µg/mL
Cutaneotrichosporon mucoides (*Trichosporon mucoides*)	0.5–1 µg/mL	1 µg/mL	1 µg/mL
Trichosporon japonicum	1–2 µg/mL	1 µg/mL	2 µg/mL
Cutaneotrichosporon jirovecii (*Trichosporon jirovecii*)	-	-	-
Trichosporon coremiforme	1 µg/mL	1 µg/mL	1 µg/mL

for evaluating the antifungal susceptibility of *Trichosporon* spp.

Based on the results, this study discussed and established the optimal conditions for evaluating the antifungal susceptibility of *Trichosporon* spp. Under these conditions, Table 4 presents the MIC Range, MIC_{50}, and MIC_{90} values for fluconazole and amphotericin B among species. The values were determined at 37 °C for 48 h, using 50% growth inhibition for fluconazole and 100% inhibition for amphotericin B. Among the species, *Cutaneotrichosporon mucoides* (*Trichosporon mucoides*) showed the highest MIC values for voriconazole, with a range of 1–8 µg/mL, an MIC_{50} of 2 µg/mL, and an MIC_{90} of 4 µg/mL. In contrast, *Trichosporon asahii* exhibited the highest MIC values for amphotericin B, with a range of 1–4 µg/mL, an MIC_{50} of 1 µg/mL, and an MIC_{90} of 2 µg/mL.

Discussion

In the review by Mehta et al. [2] and other referenced studies, a significant variation was observed in the parameters used for susceptibility assays with *Trichosporon* spp. This variation highlights the lack of standardization in susceptibility testing for this genus, including the inconsistency in tests conducted with the substances commonly used for the treatment of fungal infections, such as fluconazole and amphotericin B [3, 13, 16, 17].

In this study, the CLSI methodology was employed with certain modifications applicable to this genus [12]. Despite the observed variation, the MIC_{50} and MIC_{90} values in this study were generally consistent with those described in the literature so far, demonstrating some effectiveness. However, the results of this study also highlight some elevated values that may indicate potential resistance, as previously reported in the literature (32 µg/mL for fluconazole and 4 µg/mL for amphotericin B). These are important factors to consider when discussing precise and effective treatment for a life-threatening invasive disease [2, 3, 13, 16, 17].

In the absence of interpretative breakpoints or epidemiological cutoff values (ECVs) for *Trichosporon* and antifungal agents, several studies have also applied adapted CLSI guidelines [12] to generate in vitro susceptibility data. Among these, Guo et al. [18], Wongsuk et al. [19], and Kuo et al. [20] reported consistently elevated fluconazole MICs (≥8 µg/mL) and high amphotericin B MIC values (ranging from 4 to >16 µg/mL), which are in line with the findings of the present study. In a more recent study (2025), de Souza et al. [21] reported fluconazole MIC values ranging from 0.5 to 8 µg/mL and amphotericin B MICs from 0.125 to >16 µg/mL, also consistent with the data presented here. However, it is important to emphasize that these studies did not employ fully standardized protocols, which limits the direct comparability of the evaluated conditions and the resulting data. This reinforces the relevance of the present study and underscores the urgent need to standardize antifungal susceptibility testing for *Trichosporon* spp.

Therefore, in this study, we will now discuss the optimal conditions for this susceptibility test. The isolates exhibited higher MIC values against fluconazole when incubated at 30 °C. As a fungistatic agent, the reading breakpoint to determine the MIC is 50% growth inhibition, although both 50% and 100% inhibition were observed visually. Importantly, no significant difference was found between the MIC results at 24 h and 48 h of fluconazole incubation, suggesting that extended incubation does not affect the antifungal susceptibility results for *Trichosporon* spp. under these conditions. As previously demonstrated for other fungi [12].

Conversely, for amphotericin B, the highest MIC values were recorded at 37 °C. As a fungicidal compound, the reading breakpoint to establish the MIC should be 100% growth inhibition, despite visual readings showing both 50% and 100% inhibition. A significant difference was observed between the MIC results at 24 h and 48 h of incubation, with most isolates doubling the MIC value after 48 h. This suggests that extended incubation may be necessary for more accurate susceptibility testing of *Trichosporon* species when using amphotericin B.

These findings are consistent with existing literature, which indicates that at physiological temperature (37 °C), *Trichosporon* species are generally more susceptible to azoles than to polyenes (observed during the susceptibility test and treatment of trichosporonosis). Moreover, different MIC values are observed in literature depending on the species. In this study, aligning with what has been previously described, we observed that some species (such as *T. mucoides* and *T. asahii*) present higher MIC values [2, 17]. Additionally, we present the distribution of key MIC values among the species, highlighting that the MIC values for fluconazole and amphotericin B were higher in *T. mucoides* and *T. asahii* compared to the other species.

Furthermore, as established in standardized protocols for other yeasts, it is important to apply a 50% growth inhibition threshold for fungistatic drugs and a 100% threshold for fungicidal drugs, in accordance with the CLSI guidelines [12]. In this context, although this study discusses various experimental conditions, the results obtained for fluconazole and amphotericin B align with the standardized approach recommended by the CLSI for other yeasts.

Also, as environmentally opportunistic yeasts capable of adapting, *Trichosporon* species can exhibit different metabolic rates depending on the situation or environmental conditions. In this way, variability in growth rates observed within the standard incubation period (also temperature) can affect susceptibility test outcomes. Some isolates, or even entire species, may show different rates of growth in different conditions, potentially influencing MIC readings and results [2].

Regarding the time differences during the incubation period, it is important to highlight that the variations were not only observed in the presence of the drug, but also in the growth controls of the experiment. This suggests that the observed variations are not solely attributable to the effects of the antifungal agents, but that longer incubation periods influence the fungal fitness.

Some isolates did not grow adequately at 37 °C, which hindered the determination of MICs under this condition. As 37 °C corresponds to the physiological temperature of the human body, this finding is particularly noteworthy. The reduced and delayed growth at this temperature may have taxonomic implications related to the specific species involved, could represent an artifact of in vitro conditions, or may reflect the predominantly environmental origin of the samples involved. However, none of these hypotheses can be confirmed at this stage, as no studies addressing this phenomenon are currently described. Notably, *Trichosporon jirovecii* has been reported both as part of the human microbiota and as a opportunistic pathogen, demonstrating its ability to grow well at 37 °C [22–24]. Therefore, based on this available data, the most plausible explanation for the limited growth in our study is the influence of in vitro conditions, possibly combined with the environmental origin of the isolates. It is also worth mentioning that, to date, *Trichosporon austroamericanum* is the only species reported in the literature to have been exclusively isolated from a single source (or temperature in some way)—clinical samples—whereas most *Trichosporon* species are generally ubiquitous [25].

However, based on the evaluation of these results, this study identified optimal conditions for performing susceptibility assays with *Trichosporon* spp. We recommend performing susceptibility assays using 48 h of incubation at 37 °C (clinical context), with 50% and 100% inhibition reading breakpoints for fluconazole and amphotericin B, respectively. Still, these conclusions may also be applicable to other antifungal agents within these drug classes (azoles and polyenes). Nevertheless, it is important to note that these parameters are already described in the CLSI documents for other yeasts [12]. It is an interesting observation, since the method will not require huge adaptation for clinical laboratories.

Furthermore, the discussion in this work is of utmost importance, especially considering that there are currently two main issues related to the lack of a standardized susceptibility test: the absence of interpretive breakpoints for establishing susceptibility/resistance and the lack of clinical application of susceptibility assays to guide the prescription of antifungal treatments for patients. Therefore, there is a need for standardized testing methods (CLSI) and a better

understanding of antifungal susceptibility to improve the treatment of *Trichosporon* infections.

Based on our data, the parameters recommended by CLSI for other yeasts—such as incubation time, temperature, and inhibition reading—are also applicable to *Trichosporon* spp. This finding supports the notion that only minimal adaptations are needed for applying current standardized methods to *Trichosporon* in clinical laboratories. However, it is important to highlight that the establishment of interpretative breakpoints or epidemiological cutoff values (ECVs) for *Trichosporon* and antifungal agents remains an urgent need.

Conclusion

In conclusion, the variations observed in our susceptibility assays underscore the need for a standardized approach to evaluate *Trichosporon* susceptibility. The findings of this study suggest optimal conditions for susceptibility testing in the clinical context, which appear to align well with the parameters already established by CLSI for other yeasts. In addition, our data underscore the need for further studies to establish a standard method for evaluating the antifungal susceptibility of *Trichosporon* spp., which will enable the calculation of antifungal epidemiological cutoff values and, possibly, the determination of interpretive breakpoints for susceptibility/resistance.

Author contributions All the authors contributed equally.

Funding Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Brazilian Ministry of Health (408540/2022-2 and 444501/2023-1), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-88887.966527/2024-00) and CAPES-COFECUB program (Código de Financiamento 001), and the National Institute of Science and Technology INCT Funvir from Brazil (CNPq). D.A.S. (303762/2020-9) is a research fellow of CNPq.

Data availability This work includes all the data used in this research.

Declarations

Conflict of interest No conflict of interest in this work.

References

1. Matsumoto Y, Arakawa S, Sadahira K, Sato T, Yamazaki K, Nishimoto S (2024) Skin manifestations of Micafungin breakthrough disseminated trichosporonosis in acute megakaryoblastic leukemia. Med Mycol J 65(1):17–21. https://doi.org/10.3314/mmj.23-00009
2. Mehta V, Nayyar C, Gulati N et al (2021) A comprehensive review of *Trichosporon* spp.: an invasive and emerging fungus. Cureus 13(8):e17345. https://doi.org/10.7759/cureus.17345
3. Colombo AL, Padovan AC, Chaves GM (2011) Current knowledge of *Trichosporon* spp. And trichosporonosis. Clin Microbiol Rev 24:682–700. https://doi.org/10.1128/CMR.00003-11
4. Mariné M, Brown NA, Riaño-Pachón DM, Goldman GH (2015) On and under the skin: emerging basidiomycetous yeast infections caused by *Trichosporon* species. PLoS Pathog 11(7):e1004982. https://doi.org/10.1371/journal.ppat.1004982
5. Cafarchia C, Camarda A, Romito D, Campolo M, Quaglia NC, Tullio D, Otranto D Occurrence of yeasts in cloacae of migratory birds. Mycopathologia 2006ª Apr; 161(4): 229–234. https://doi.org/10.1007/s11046-005-0194-z
6. Takashima M, Sugita T (2022) Taxonomy of pathogenic yeasts *Candida*, *Cryptococcus*, *Malassezia*, and *Trichosporon*. Med Mycol J 63(4):119–132. https://doi.org/10.3314/mmj.22.004
7. Chander J. Textbook of medical mycology. Chander J (ed) (2018) : Jaypee Publishers, New Delhi. Available at: https://books.google.com.au/books/about/Textbook_of_Medical_Mycology.html?
8. Pumeesat P, Wongsuk T (2023) Genetic analysis of emerging fungal pathogens: *Trichosporon Asahii*. Diagn Microbiol Infect Dis 107(3):116057. https://doi.org/10.1016/j.diagmicrobio.2023.116057
9. Chang DSJ, Harfouch CW, Melendez ML (2023) *Trichosporon Asahii* opportunistic pneumonia after a severe COVID-19 infection. IDCases 31:e01701. https://doi.org/10.1016/j.idcr.2023.e01701
10. Benelli JL, Basso RP, Grafulha TW, Poester VR, Munhoz LS, Martins KB, Zogbi HE, Von Groll A, Severo CB, Stevens DA, Xavier MO (2022) Fungal bloodstream Co-infection by *Trichosporon Asahii* in a COVID-19 critical patient: case report and literature review. Mycopathologia 187(4):397–404. https://doi.org/10.1007/s11046-022-00637-6
11. Cronyn V, Howard J, Chiang L, Le L, Tims-Cook Z, Gertz AM (2021) *Trichosporon Asahii* urinary tract infection in a patient with severe COVID-19. Case Rep Infect Dis 2021:6841393. https://doi.org/10.1155/2021/6841393
12. CLSI. M27-Ed4 and M60-Ed1: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Clinical and Laboratory Standards Institute (2017) Available at: https://clsi.org/about/news/clsi-publishes-new-and-updated-microbiology-documents/. Accessed on: July 14, 2025
13. Lemes RM, Lyon JP, Moreira LM, de Resende MA (2010) Antifungal susceptibility profile of *Trichosporon* isolates: correlation between CLSI and etest methodologies. Braz J Microbiol 41(2):310–315. https://doi.org/10.1590/S1517-83822010000200008
14. Singulani JL, Silva DL, Lima CM et al (2023) COVID-19 and candiduria: an investigation of the risk factors and immunological aspects. Braz J Microbiol 54:1783–1793. https://doi.org/10.1007/s42770-023-01042-x
15. Freitas GJC, Ribeiro NQ, Gouveia-Eufrasio L, Emidio ECP, Guimarães GM, César IC, Paixão TA, Oliveira JBS, Caza M, Kronstad JW, Santos DA (2023) Antimalarials and amphotericin B interact synergistically and are new options to treat cryptococcosis. Int J Antimicrob Agents 62(1):106807. https://doi.org/10.1016/j.ijantimicag.2023.106807
16. Singh S, Capoor MR, Varshney S, Gupta DK, Verma PK, Ramesh V Epidemiology and antifungal susceptibility of infections caused by *Trichosporon* species: an emerging Non-*Candida* and Non-*Cryptococcus* yeast worldwide. Indian J Med Microbiol 2019 Oct-Dec ;37, 536–541. https://doi.org/10.4103/ijmm.IJMM_19_146
17. Francisco -EC, de Almeida Junior JN, de Queiroz Telles F, Aquino VR, Mendes AVA, de Andrade Barberino MGM, de Tarso P, Castro O, Guimarães T, Hahn RC, Padovan ACB, Chaves GM, Colombo AL (2019) Jul Species distribution and antifungal susceptibility of 358 *Trichosporon* clinical isolates collected in 24

medical centres. Clinical Microbiology and Infection. Volume 25, Issue 7, 2019, Pages 909.e1-909.e5. https://doi.org/10.1016/j.cmi.2019.03.02

18. Guo Lyu S, Hsueh P, Al-Hatmi AMS, Meis JF, Hagen F, Xiao M, Wang H, Barresi C, Zhou MDE, Hoog GS, Xu Y (2019) Invasive infections due to *Trichosporon*: species distribution, genotyping, and antifungal susceptibilities from a multicenter study in China. J Clin Microbiol 57. https://doi.org/10.1128/jcm.01505-18
19. Wongsuk T, Boonsilp S, Pumeesat P, Homkaew A, Sangsri T, Chongtrakool P (2022) Genotyping, antifungal susceptibility testing, and biofilm formation of *Trichosporon* spp. Isolated from urine samples in a university hospital in bangkok, Thailand. Acta Microbiol Immunol Hung 69(3):247–257. https://doi.org/10.1556/030.2022.01797
20. Kuo SH, Lu PL, Chen YC, Ho MW, Lee CH, Chou CH, Lin SY (2021) The epidemiology, genotypes, antifungal susceptibility of *Trichosporon* species, and the impact of voriconazole on *Trichosporon* fungemia patients. J Formos Med Assoc 120(9):1686–1694. https://doi.org/10.1016/j.jfma.2020.12.007
21. de Souza Jimenez MG, de Azevedo MF, Francisco EC, de Andrade Oliveira Boczar AM, Padovan ACB, Milan EP, da Silva Rocha WP, Chaves GM (2025) Characterization of virulence factors, cellular stress response, and antifungal susceptibility testing of *Trichosporon* spp. Isolated from Northeast Brazilian patients. J Fungi (Basel) 11(4):255. https://doi.org/10.3390/jof11040255
22. Martínez-Herrera E, Duarte-Escalante E, Reyes-Montes MDR, Arenas R, Acosta-Altamirano G, Moreno-Coutiño G, Vite-Garín TM, Meza-Robles A, Frías-De-León MG (2021 Jul-Sep) Molecular identification of yeasts from the order trichosporonales causing superficial infections. Rev Iberoam Micol 38(3):119–124. https://doi.org/10.1016/j.riam.2021.01.004
23. Lu JJ, Lo HJ, Lee CH, Chen MJ, Lin CC, Chen YZ, Tsai MH, Wang SH (2021) The use of MALDI-TOF mass spectrometry to analyze commensal oral yeasts in nursing home residents. Microorganisms 9(1):142. https://doi.org/10.3390/microorganisms9010142
24. Oliveira FA, Bernardes-Engemann AR, Almeida-Silva F, Motta BDS, Almeida MA, Teixeira MM, d'Avila Freitas A, Geraldo KM, Veloso VG, Grinsztejn B, de Souza Borges Quintana M, Almeida-Paes R, Zancopé-Oliveira RM (2024 Oct-Dec) Isolation of fungi from the Trichosporonaceae family in urine samples from COVID-19 patients: should we worry about it? Rev Iberoam Micol. 41(4):58–67. https://doi.org/10.1016/j.riam.2024.10.003
25. Francisco EC, Desnos-Ollivier M, Dieleman C, Boekhout T, Santos DWCL, Medina-Pestana JO, Colombo AL, Hagen F (2024) Unveiling *Trichosporon austroamericanum* sp. nov.: A novel emerging opportunistic basidiomycetous yeast species. Mycopathologia 189(3):43. https://doi.org/10.1007/s11046-024-00851-4

5. Capítulo 3

Com objetivo de avaliar e investigar a resistência *in vitro* e *in vivo* do gênero *Trichosporon* sob a luz da Saúde Única, foi elaborado e será submetido o artigo A One Health Approach to Study *Trichosporon* spp. Resistance to Antifungals na revista Science of the Total Environment ainda no ano 2025. Para finalizar, este artigo representa a integração dos dois anteriores, explorando de forma aplicada a resistência do gênero *Trichosporon* abordada no primeiro capítulo, por meio da metodologia padronizada no segundo. Com o objetivo de avaliar e investigar a resistência *in vitro* e *in vivo* sob a perspectiva da Saúde Única, foram conduzidos ensaios experimentais que permitiram compreender o comportamento do fungo frente aos principais antifúngicos clínicos. Entre os principais resultados, foi detectada resistência *in vitro*, incluindo uma possível resistência cruzada a agroquímicos e desinfetantes, a qual também se manteve nos ensaios *in vivo* realizados em camundongos. Além disso, os experimentos em modelo animal evidenciaram uma relação entre resistência, virulência e falha terapêutica, sugerindo paralelos com infecções humanas. Esses achados reforçam a importância da abordagem integrada da Saúde Única neste estudo e destacam a relevância do gênero *Trichosporon* no contexto das infecções fúngicas emergentes e resistentes.

A One Health Approach to Study *Trichosporon* spp. Resistance to Antifungals

Isabela Lima Miranda[1], Victor Augusto Teixeira Leocádio[1], César Moura[1], Nalu Teixeira Aguiar Peres[1,2], Rafael Wesley Bastos[2,3], Kássia Jéssica Galdino Silva[3], Luana Rossato[4], Fabíola Lucini[4], Débora S. C. M. Castelo-Branco[5], Gabriela Silva Cruz[5], Maria Isabel Azevedo[6], Florent Morio[7], José Rivaldo Lima[7], Isabelle Ourliac-Garnier[7], Daniel Assis Santos[1,2,*]

[1] Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG 31270-901, Brazil.

[2] Instituto Nacional de Ciência e Tecnologia em Fungos Patogênicos Humanos, INCT, Ribeirão Preto, Brazil.

[3] Departamento de Microbiologia e Parasitologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, RN 59078-970, Brazil.

[4] Laboratório de Pesquisa em Ciências da Saúde, LPCS, Universidade Federal da Grande Dourados, Dourados, MS 79804-970, Brazil.

[5] Departamento de Patologia e Medicina Legal, Centro de Biomedicina, Universidade Federal do Ceará, Fortaleza, CE 60430-275, Brazil.

[6] Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG 31270-901, Brazil.

[7] Nantes Université, CHU Nantes, Cibles et Médicaments des Infections et de l'Immunité, IICiMed, UR 1155, F-44000 Nantes, France.

***Corresponding author:** Daniel de Assis Santos − Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, Minas Gerais, Brazil; orcid.org/0000-0002-1108-5666; Email: dasufmg@gmail.com

Abstract: The One Health concept emphasizes the interconnectedness of human, animal, and environmental health, highlighting the importance of integrated approaches to address antimicrobial resistance. Opportunistic yeasts, particularly *Trichosporon* species, have gained attention for their ability to cause invasive infections and exhibit antifungal resistance, especially in immunocompromised patients. These yeasts, widely distributed in the environment and commensally in animals, may develop resistance after exposure to antifungals, agrochemicals, or disinfectants, affecting trichosporonosis treatment. In this context, we evaluated the *in vitro* and *in vivo* resistance in a collection of 33 *Trichosporon* isolates obtained from patients, animals, and related environments in Brazil within a One Health framework. *T. asahii* was the most prevalent species, while others displayed more restricted ecological niches. Minimal inhibitory concentration testing revealed that several isolates, predominantly *T. asahii*, were resistant to clinical antifungals and suggested potential cross-resistance with agrochemicals and disinfectants. This resistant phenotype was also confirmed in a murine trichosporonosis model, where mice infected with some resistant strains exhibited higher fungal burdens and increased dissemination into the central nervous system, linking antifungal resistance to enhanced virulence. Moreover, these selected resistant isolates displayed higher growth and biofilm formation at host temperature, whereas other isolates showed lower proliferation consistent with susceptibility assay results. Overall, these findings highlight the multifactorial nature of antifungal resistance in *Trichosporon* and its clinical and public health implications. They reinforce the urgent need to standardize susceptibility testing and cutoff values, implement integrated surveillance systems, and promote responsible use of antifungals, agrochemicals, and disinfectants under a One Health framework.

Keywords: *Trichosporon* spp., antifungal resistance, cross-resistance, One Health, *in vitro*, *in vivo*, Brazil.

1. Introduction

The One Health concept has gained increasing relevance in recent years as an integrated approach to human, animal, and environmental health [38, 40–42]. In this context, emerging diseases involving opportunistic yeasts and antifungal resistance underscore the urgent need for coordinated strategies that bridge disciplines and sectors [35–42]. Among these pathogens, yeasts of the ubiquitous genus *Trichosporon* have gained growing attention over the last decade due to their association with antifungal resistance and invasive or disseminated infections, particularly in immunocompromised patients [1–6, 17, 40].

By fostering collaboration among medical, veterinary, and environmental sciences, the One Health framework enables a comprehensive investigation into how exposure of opportunistic yeasts to antimicrobials, agrochemicals, and disinfectants may contribute to the development of clinical antifungal resistance. Such exposures can precondition yeasts and activate stress-response mechanisms, potentially inducing resistance and cross-resistance, as observed in *Aspergillus*, *Candida*, and *Cryptococcus* [35–40]. Considering that *Trichosporon* species are ubiquitous in environment and occur as commensals in animals, this perspective is particularly pertinent, as resistance in this genus may be influenced by substances routinely used in human medicine, veterinary, and agriculture [17, 40].

Although most *Trichosporon* species are harmless commensals colonizing the skin and gastrointestinal tract of humans, as well as other mammals and birds, they can become pathogenic under conditions of host imbalance, particularly *T. asahii* [1–6]. Pathogenicity is driven by specific virulence factors and metabolic adaptability, with infections occurring both as primary opportunistic infections, for example, in transplant or chemotherapy patients, and as secondary or co-infections associated with conditions such as HIV and COVID-19 [1-7, 10–13]. Superficial infections, such as white piedra, are generally common in immunocompetent individuals and have a favorable prognosis, whereas invasive and disseminated *Trichosporon* infections, such as fungemia, carry high mortality rates (40–60%) in immunocompromised patients, particularly when inappropriate therapy is administered in the context of antifungal resistance [14, 15].

In this scenario, antifungal susceptibility testing for *Trichosporon* is typically performed using the broth microdilution method according to Clinical and Laboratory Standards Institute guidelines [16]. These tests aim to evaluate the effectiveness of various antifungals as potential therapeutic agents [2, 17]. However, standardized susceptibility testing for this genus remains incomplete, and the response of many *Trichosporon* species to commonly used antifungals is still poorly characterized. Available studies indicate that *Trichosporon* isolates are generally more susceptible to triazoles, particularly voriconazole, than to polyenes, exhibit intrinsic resistance to

echinocandins, while flucytosine is not recommended. Susceptibility profiles can vary depending on the species [2, 17–23]. Collectively, these findings support voriconazole as the current first-line therapy for trichosporonosis, especially in severe infections such as those involving the central nervous system [24]. Combination therapy with amphotericin B may improve outcomes in resistant cases [25]. Despite these therapeutic recommendations, the mechanisms underlying resistance to azoles and polyenes in *Trichosporon* remain incompletely understood. Proposed mechanisms include efflux pump activity, biofilm formation, mutations, alterations in membrane lipids, and metabolic adaptations, all of which may contribute directly to therapeutic failure [17, 26–34].

Overall, the study of *Trichosporon* through a One Health lens highlights the complex interplay between environmental exposure, antifungal resistance, and clinical outcomes, emphasizing the need for integrated surveillance and intervention strategies across human, animal, and environmental health sectors. In this study, *Trichosporon* isolates collected from patients, animals, and related environments in Brazil were analyzed, and their susceptibility was assessed to investigate both *in vitro* and *in vivo* resistance. Given the high degree of intrinsic and acquired resistance in this genus, particularly in the context of therapeutic failure, this research is of critical importance, especially considering the alarming scenario of invasive trichosporonosis within a One Health framework.

2 Materials and Methods

2.1. Samples

In this study, we evaluated the susceptibility of 33 *Trichosporon* spp. isolates to clinical antifungals, agrochemicals, and disinfectants. In addition, four reference strains were used as controls due to their clinical relevance and CLSI validation: *Cryptococcus neoformans* H99, *Cryptococcus gattii* R265, *Candida glabrata* ATCC 2001, and *Candida albicans* SC5314 (ATCC MYA-2876D-5) [16, 40, 43, 44]. The isolates were obtained from human patients, hospital environment, animals (poultry and swine, including oral, cloacal, and rectal samples), and their environmental sources across various states in Brazil (MG, RN, MS, and CE), following approval from the respective ethics committees (CEUA protocol 7/2023 and COEP protocol CAAE 70201223.6.3001.5124). Samples were cultured and isolated on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol and CHROMagar, identified by MALDI-TOF MS, and stored in Brain Heart Infusion (BHI) medium supplemented with 20% glycerol at −80 °C [16, 21, 40] (Table 1). The collection is deposited in the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN, code AA7A6D7).

A recent taxonomic revision reassigned some species previously isolated in this study to the genus *Cutaneotrichosporon* (*C. jirovecii* and *C. mucoides*) [8, 9]. However, based on the MALDI-TOF identification results obtained in this study, these isolates will continue to be included in the analysis, while also being referred to by their updated genus names (Table 1).

Table 1: Identification and description of the samples.

Sample Code	MALDI-TOF Identification	Origin	Source	State
A1-2	*Trichosporon asahii*	Animal	Poultry oral / cloacal swab	MG
A1-5	*Trichosporon asahii*	Animal	Poultry oral / cloacal swab	MG
A1-7	*Trichosporon mucoides* (*C. mucoides*)	Animal	Poultry oral / cloacal swab	MG
A1-9	*Trichosporon mucoides* (*C. mucoides*)	Animal	Poultry oral / cloacal swab	MG
A1-14	*Trichosporon mucoides* (*C. mucoides*)	Environmental	Poultry waterer / feeder	MG
A1-22	*Trichosporon mucoides* (*C. mucoides*)	Environmental	Poultry waterer / feeder	MG
A1-23	*Trichosporon mucoides* (*C. mucoides*)	Environmental	Poultry waterer / feeder	MG
A1-24	*Trichosporon jirovecii* (*C. jirovecii*)	Environmental	Poultry waterer / feeder	MG
A2-5	*Trichosporon mucoides* (*C. mucoides*)	Environmental	Poultry waterer / feeder	MG
A2-7	*Trichosporon mucoides* (*C. mucoides*)	Environmental	Poultry waterer / feeder	MG
A3-10	*Trichosporon japonicum*	Environmental	Poultry waterer / feeder	MG
A3-17	*Trichosporon coremiiforme*	Animal	Swine oral / rectal swab	MG
A3-28	*Trichosporon japonicum*	Animal	Swine oral / rectal swab	MG
346B	*Trichosporon asahii*	Animal	Swine oral / rectal swab	CE
362B	*Trichosporon asahii*	Animal	Poultry oral / cloacal swab	CE
C226	*Trichosporon asahii*	Animal	Swine oral / rectal swab	CE
PH8	*Trichosporon asahii*	Human	Auricular	CE

7L	*Trichosporon mucoides* (*C. mucoides*)	Human	Blood	RN
314L	*Trichosporon asahii*	Environmental	Poultry excrement	RN
356L	*Trichosporon asahii*	Environmental	Swine waterer / feeder	RN
359L	*Trichosporon asahii*	Environmental	Swine waterer / feeder	RN
361L	*Trichosporon jirovecii* (*C. jirovecii*)	Environmental	Poultry waterer / feeder	RN
368L	*Trichosporon jirovecii* (*C. jirovecii*)	Animal	Swine oral / rectal swab	RN
10F	*Trichosporon asahii*	Animal	Poultry oral / cloacal swab	MS
18F	*Trichosporon asahii*	Animal	Poultry oral / cloacal swab	MS
196	*Trichosporon asahii*	Human	Urine	MS
6A4	*Trichosporon asahii*	Animal	Poultry oral / cloacal swab	MS
Ta9	*Trichosporon asahii*	Human	Urine	MS
Ta14F	*Trichosporon asahii*	Environmental	Poultry waterer / feeder	MS
Ta57F	*Trichosporon asahii*	Animal	Poultry oral / cloacal swab	MS
Ta92	*Trichosporon asahii*	Human	Urine	MS
105[a]	*Trichosporon japonicum*	Environmental	Hospital environment	MS
105B	*Trichosporon japonicum*	Environmental	Hospital environment	MS

2.2. Susceptibility testing against clinical antifungals, agrochemicals, and disinfectants

2.2.1. Antifungals

For the determination of the minimum inhibitory concentration (MIC), yeast susceptibility was assessed using the broth microdilution method, following the CLSI guidelines [16] with adaptations [52]. The clinical antifungals fluconazole, voriconazole, itraconazole, micafungin, and amphotericin B; the agrochemicals tebuconazole, pyraclostrobin, mancozeb, and carbendazim; and the disinfectants didecyldimethylammonium (DDA) chloride and benzalkonium (BZK) chloride were obtained from Sigma-Aldrich. Stock solutions (5.000 µg/mL) were prepared in DMSO, followed by serial dilutions in RPMI 1640 medium buffered with MOPS. The final concentration ranges were tested as follows: voriconazole (0.008–4 µg/mL), micafungin (0.015–8 µg/mL), amphotericin B and itraconazole (0.03–16 µg/mL), DDA chloride, BZK chloride, pyraclostrobin, carbendazim, and mancozeb (0.06–32 µg/mL), and fluconazole and tebuconazole (0.125–64 µg/mL) [16, 35-37, 40].

2.2.2. Inoculum preparation

The inoculum was first prepared by culturing isolates on SDA at 37 °C for 48 h. Colonies were then suspended in 3 mL of 0.9% saline, homogenized, and left to decant for 5 min to reduce clumping. From this, 2.5 mL of the supernatant was collected, and the transmittance was adjusted to 75–77% at 530 nm, corresponding to 1–5 × 10^6 cells/mL. This suspension was subsequently diluted in RPMI, yielding a final inoculum concentration of 1–5 × 10^3 cells/mL. The inoculum was then dispensed into 96-well flat-bottom microplates containing the serially diluted compounds [16, 21, 40, 43, 44, 52].

2.2.3. Susceptibility test conditions

Plates were incubated at two temperature conditions (30 °C and 37 °C) for 48 h. MICs were determined visually using two criteria: 50% growth inhibition for azoles and complete (100%) growth inhibition for the other compounds. In addition, MIC ranges, MIC_{50}, and MIC_{90} values were calculated, defined respectively as the interval of observed MICs, and the minimum concentrations required to inhibit the growth of 50% and 90% of the isolates [16, 21, 40, 43, 44, 52]. Furthermore, for the analyses and identification of samples considered resistant in this study, specific cutoff values were used. These values were established based on literature references and on the mean MIC values multiplied by a factor of two to four for each compound considering both temperatures values [2, 3, 17–25, 35, 36, 40, 54-56] (Table 2).

Table 2: Cutoff values considered in this study.

	µg/Ml
Voriconazole	>0.125
Fluconazole	>8
Itraconazole	>1
Amphotericin B	>1
Micafungin	8
Tebuconazole	>2
Pyraclostrobin	>8
Mancozeb	>16
Carbendazim	>16
DDA chloride	>8
BZK chloride	>16

2.3. *In vitro* growth curve

Cell proliferation was evaluated using a growth curve assay by measuring absorbance at 600 nm after incubation in RPMI medium for 24 to 72 h for the 33 strains. Sterile flat-bottom 96-well plates were prepared with RPMI in each well, including five replicates per strain and one sterility control containing only RPMI. Inoculum for all 33 isolates was prepared as described in section 2.2.2, diluted in RPMI to 1–5 × 10^4 cells/mL, and then added to the appropriate wells. Plates were incubated in a humidified chamber at 30 °C and 37 °C for 72 h, with optical density readings taken at 0, 24, 48, and 72 h to assess cell proliferation and growth [51]. All samples were analyzed, and the results are presented as the growth at 48h of protocol.

2.4. *In vitro* biofilm formation

Cell viability and metabolic activity were evaluated using a biofilm formation assay by measuring absorbance at 492 nm after adding XTT and menadione to biofilms formed over 48 h. Sterile flat-bottom 96-well plates were prepared with RPMI in each well, including three replicates per strain and one sterility control containing only RPMI. Inoculum for all 33 isolates was prepared as described in section 2.2.2, and 1–5 × 10^6 cells/mL were then added to the designated wells. Plates were incubated at 30 °C and 37 °C for 1 h without agitation to allow cell adhesion. After removing the supernatant and washing with saline, RPMI was added again, and plates were incubated without agitation for 24 h to promote biofilm formation. The washing and medium renewal steps were repeated, followed by an additional 24 h incubation. After 48 h, wells were washed three times with saline, and XTT with menadione was added to assess cell viability and metabolic activity. Plates were incubated in the dark at 30 °C and 37 °C for 2 h, and optical density readings were measured to indirectly quantify biofilm formation based on cellular metabolic activity [30].

2.5. Antifungal resistance in murine model

Male C57BL/6 mice (6–8 weeks old, 20-25 g) were obtained from the Central Animal Facility of the Federal University of Minas Gerais (CEUA Protocol 323/2024). Animals were immunosuppressed with intraperitoneal injections of 5-fluorouracil (75 mg/kg/day) for three consecutive days [46]. On day four, mice were intravenously infected via the lateral tail vein with 1 × 10^6 viable yeast cells (5 µL suspension) using a microsyringe. This assay was performed only with selected isolates identified throughout the study, considering

all characteristics associated with resistance evaluated here, and including both susceptible and resistant controls. The susceptible group comprised *T. asahii* Ta57F, *C. mucoides* (*Trichosporon mucoides*) A1-9, and *T. japonicum* A3-10, while the resistant group included *T. asahii* Ta9, *T. asahii* A1-2, *C. mucoides* (*Trichosporon mucoides*) A1-14, and *T. japonicum* 105B. All resistant isolates were further divided into untreated and treated subgroups. Treated groups received fluconazole (10 mg/kg/day) or amphotericin B (0.5 mg/kg/day) 24 h post-infection, depending on their previously detected resistance profile. Mice were monitored daily, and body weight was recorded until euthanasia (at the 10^{th} day of the protocol). Spleen, kidney, liver, and brain were collected for colony-forming unit (CFU/g) counting [44-50].

2.6. Statistical analysis and Data visualization

Statistical analyses and data visualization were performed using GraphPad Prism (GraphPad Inc., San Diego, CA, USA), RStudio (Posit Software, PBC, Boston, MA, USA), and BioRender (BioRender.com). Maps, pie charts, and column or curve graphs were generated in GraphPad Prism and RStudio, depending on the type of data and visualization required. Heatmaps were created in RStudio using appropriate packages to illustrate variations in quantitative data, such as MIC values and cutoff points across isolates, compounds, and conditions. Figures and schematic representations were prepared in BioRender. All data were analyzed using Student's t-test or analysis of variance (ANOVA), with statistical significance defined as $p < 0.05$.

3. Results

3.1. Epidemiologic data of the *Trichosporon* spp. isolates

Figure 1 illustrates the epidemiology of the isolates collected from four different Brazilian states. Figure 1A shows the geographic distribution of isolates in the states of Minas Gerais (MG), Mato Grosso do Sul (MS), Rio Grande do Norte (RN), and Ceará (CE). Figure 1B depicts the distribution of isolates from different sample sources in relation to each species.

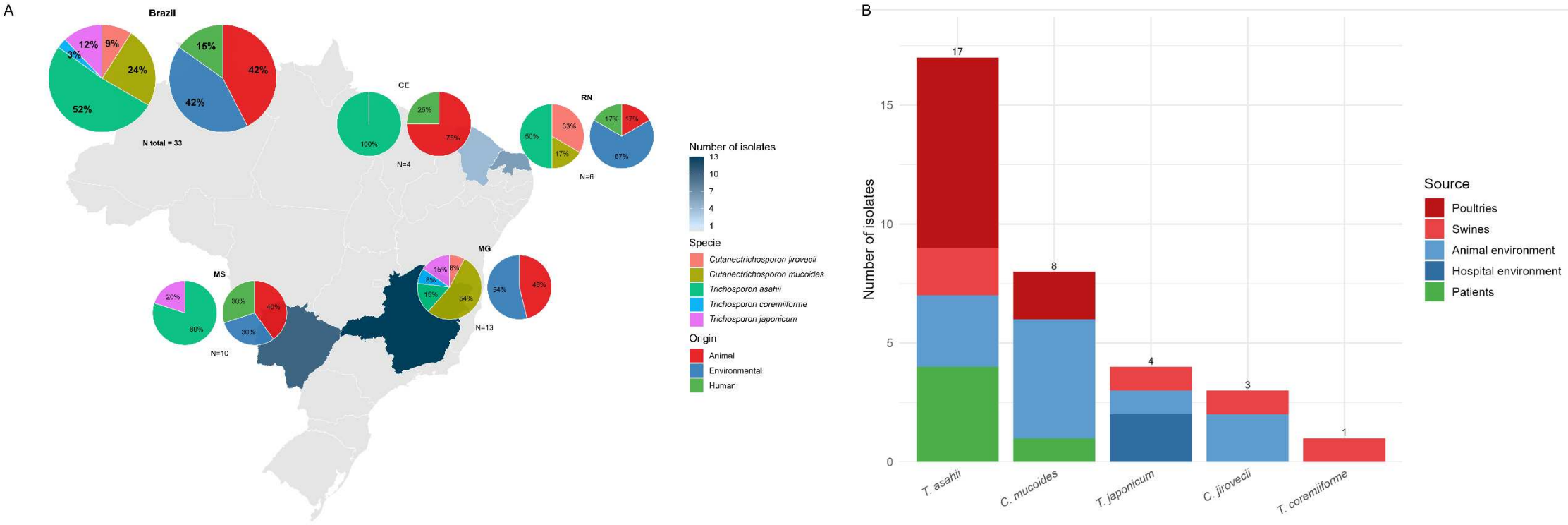


Figure 1: Geographical distribution and epidemiology of *Trichosporon* isolates. (A) Geographic distribution and number of isolates in each state (MG, MS, RN, and CE), including the percentage of isolation by species and sample origin in each state, as well as the overall distribution across Brazil. (B) Representation of the number of isolates from different sample sources in relation to each isolated species. Produced in RStudio.

Overall, *Trichosporon asahii* was the most frequent species (52%), followed by *C. mucoides* (*Trichosporon mucoides*) (24%), *T. japonicum* (12%), *C. jirovecii* (*Trichosporon jirovecii*) (9%), and *T. coremiiforme* (3%). Regarding origin, 42% of isolates were obtained from animals, 42% from environmental sources (animal and hospital), and 15% from humans. In Minas Gerais (N = 13), the most frequent species was *C. mucoides* (*Trichosporon mucoides*) (54%), followed by *T. asahii* (15%), *T. japonicum* (15%), *T. coremiiforme* (8%), and *C. jirovecii* (*Trichosporon jirovecii*) (8%). Most isolates were from environmental sources related to animals (54%), followed by those from animals (46%). In Mato Grosso do Sul (N = 10), *T. asahii* predominated (80%), with *T. japonicum* representing 20%. Isolates were obtained from animals (40%), environmental sources related to animals and hospital (30%), and humans (30%). In Rio Grande do Norte (N = 6), *T. asahii* was the most frequent species (50%), followed by *C. jirovecii* (*Trichosporon jirovecii*) (33%) and *C. mucoides* (*Trichosporon mucoides*) (17%) obtained from the animal environment (67%), animals (17%), and patients (17%). In Ceará (N = 4), *T. asahii* was the only species recovered (100%), with isolates originated from animals (75%) and human patients (25%).

As depicted in Figure 1B, *T. asahii* was the most frequent species (n = 17), recovered from all sources except hospital environments. *C. mucoides* (*Trichosporon mucoides*) accounted for 8 isolates, mainly from poultry and their environments, as well as from human patients. *T. japonicum* was represented by 4 isolates, distributed among swine, their environments, and hospital settings. *C. jirovecii* (*Trichosporon jirovecii*) comprised 3 isolates, all obtained from swine and related environments. Finally, *T. coremiiforme* was the least frequent species, with only one isolate obtained from swine. Overall, the data highlight *T. asahii* as the dominant species across multiple ecological niches, whereas other species exhibited a narrower and more niche-specific distribution.

3.2. Susceptibility analysis and Resistance screening

Following fungal identification, all isolates were subjected to broth microdilution assays to determine their susceptibility to clinical antifungals (voriconazole, fluconazole, itraconazole, amphotericin B, and micafungin), agrochemicals (mancozeb, carbendazim, tebuconazole, and pyraclostrobin), and disinfectants (DDA chloride and BZK chloride). These results are displayed as heatmaps (Figure 2A–C) of MIC values for the 33 isolates at 30 °C and 37 °C against each group of compounds, as well as the MIC range, MIC_{50}, and MIC_{90} values.

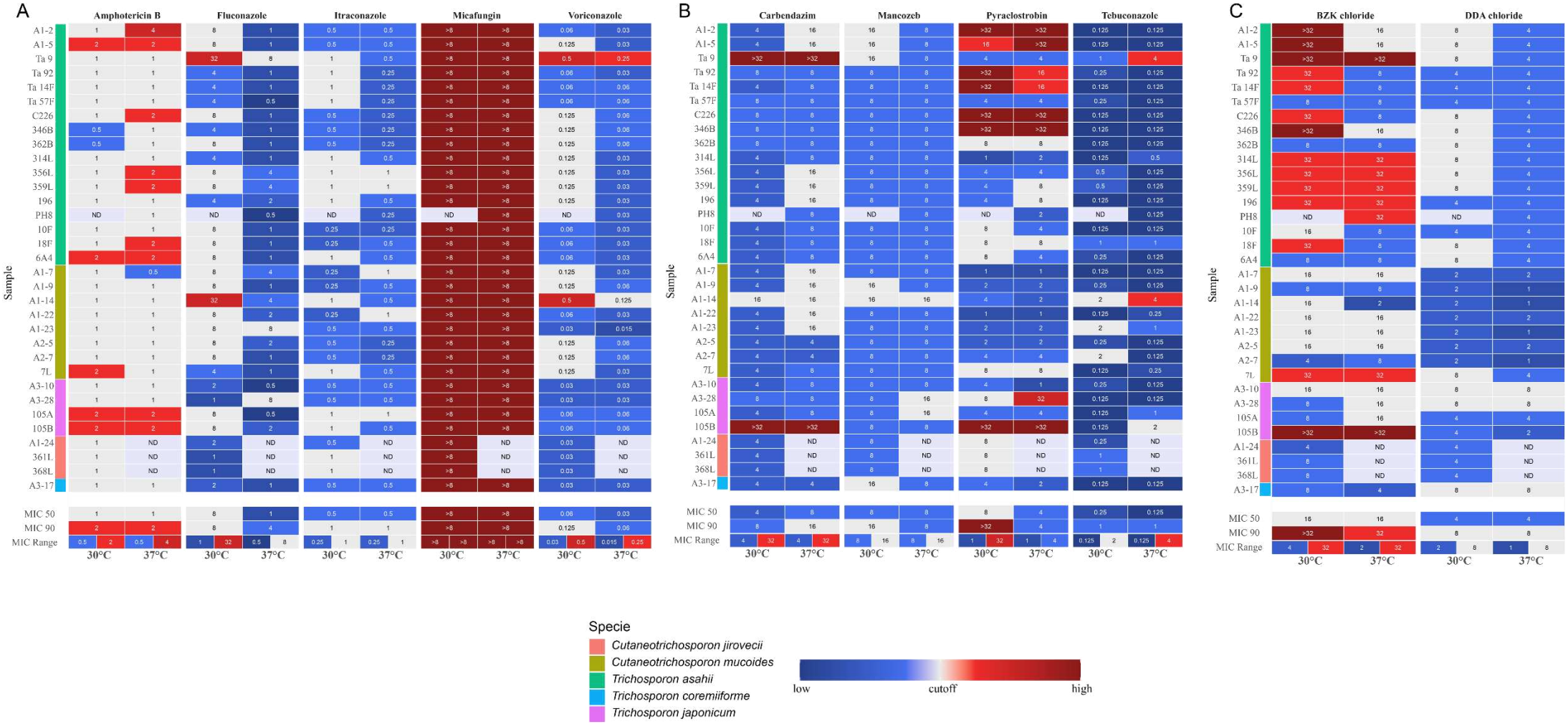


Figure 2: Heatmap of minimum inhibitory concentration (MIC) values of clinical antifungals, agrochemicals, and disinfectants for 33 *Trichosporon* isolates at 30 °C and 37 °C. (A) Clinical antifungals, (B) agrochemicals, and (C) disinfectants. In the heatmap, blue indicates low MIC values, grey represents cutoff points, and red indicates high values. Species are represented by the colors in the legend. "ND" denotes isolates that did not show sufficient growth for MIC determination at one of the tested temperatures. The MIC range, MIC_{50}, and MIC_{90} values are also presented, where the MIC range indicates the lowest and highest minimal inhibitory concentrations observed among the isolates, MIC_{50} represents the concentration at which 50% of the isolates are inhibited, and MIC_{90} corresponds to the concentration that inhibits 90% of the isolates. Produced in RStudio.

Overall, when comparing the five antifungals (Figure 2A), echinocandins were uniformly ineffective, polyenes exhibited partial activity with frequent intermediate to high MIC values, and azoles were the most effective, particularly voriconazole which consistently showed low MICs, and itraconazole, which displayed low to intermediate values. For amphotericin B (AMB), values ranged from 0.5 to 2 µg/mL, with most isolates represented in light blue and grey tones (0.5–1 µg/mL). In contrast, high MIC values were found for isolates 6A4, 7L, 18F, 105A, 105B, 356L, 359L, A1-5, and C226 (2 µg/mL - red). The highest value was observed for isolate A1-2 (4 µg/mL - intense red). In contrast, the micafungin MIC of 8 µg/mL at both temperatures (intense red) were obtained for all the strains, indicating intrinsic resistance. Furthermore, itraconazole MIC ranged from 0.25 to 1 µg/mL, mostly in intermediate to light blue and grey tones, indicative of low to intermediate inhibitory concentrations, with no isolate exceeding the cutoff. In the case of fluconazole (FCZ), 0.5–8 µg/mL (dark blue to grey tones) was found for most of the isolates, while high values (32 µg/mL - red) were found for A1-14 and Ta9 strains. Among all compounds, voriconazole (VCZ) provided the lowest MIC values (0.015–0.5 µg/mL), predominantly in dark to light blue, with some grey (0.015–0.125 µg/mL), indicating high susceptibility. However, MIC values of 0.5 µg/mL (highlighted in red) were found for isolates A1-14 and Ta9.

Figure 2B shows the heatmap for the agrochemicals MIC values. For carbendazim (CBZ), values ranged between 4 and 16 µg/mL (intermediate to light blue and grey tones). However, high carbendazim MICs (>32 µg/mL) were obtained for the strains 105B and Ta9. Pyraclostrobin (PYR) exhibited low to intermediate MICs for most isolates (1–8 µg/mL - dark blue to grey tones), whereas values >32 µg/mL (intense red) were found for isolates 105B, 346B, A1-2, A1-5, C226, Ta14F, and Ta92. For mancozeb, MIC values were relatively homogeneous, mostly 8–16 µg/mL (light blue and grey). Otherwise, tebuconazole (TEB) presented the lowest MICs among the agrochemicals (0.125–4 µg/mL), predominantly within the dark to light blue range (0.125–1 µg/mL), indicating high activity, except for MIC values of 4 µg/mL (red), which were found for two isolates (Ta9 and A1-14). Interestingly, this same phenomenon of reduced susceptibility found in these two isolates was also observed for fluconazole and voriconazole, suggesting a cross-resistance phenotype. Similarly, pyraclostrobin and carbendazim displayed elevated MIC values for isolates such as 105B, A1-2, A1-5, and C226, which also demonstrated this same phenomenon of reduced susceptibility towards amphotericin B.

Regarding the disinfectants (Figure 2C), BZK chloride MIC values ranged from 4 to >32 µg/mL, with marked variability among isolates. High concentrations (>32 µg/mL - red) were found for 105B, 346B, A1-2, A1-5, and Ta9 strains. For DDA chloride, values were much narrower, most values within 1–8 µg/mL (dark blue to grey tones), and none exceeded 8 µg/mL (cutoff), indicating higher susceptibility compared to BZK chloride.

Overall, while some isolates displayed marked resistance to BZK chloride, all remained susceptible to DDA chloride. These results also point to the cross-resistance of BZK chloride with antifungals and agrochemicals, particularly in isolates 105B, A1-2, A1-5, Ta9, 7L, 356L, 359L, 18F, and C226.

Figure 2 also highlights temperature-dependent effects on both fungal growth and compound activity. Three isolates (A1-24, 361L, and 368L) failed to grow sufficiently at 37 °C, while the isolate PH8 showed insufficient growth at 30 °C. Some compounds also displayed temperature-dependent activity: MICs were higher at 30 °C for fluconazole, voriconazole, and BZK chloride, but higher at 37 °C for amphotericin B and tebuconazole. These findings indicate that both fungal growth and compound activity against *Trichosporon* are temperature dependent, which has important implications for environmental persistence, host adaptation, and isolate origin.

Finally, Figure 2 also shows the cutoffs values (grey) considered in this study (Table 2). Using these criteria, twelve isolates (A1-2, 105B, Ta9, A1-14, A1-5, 105A, C226, 7L, 356L, 359L, 6A4, and 18F) provided high MICs for clinical antifungal (AMB, VCZ, or FCZ) and subsequently for an agrochemical (PYR, CBZ, or TEB) and/or a disinfectant (BZK chloride). This approach was designed to illustrate the potential cross-resistance of clinical antifungals, agrochemicals and/or disinfectants. For polyenes (AMB), potential cross-resistance was detected with PYR (MIC > 8 µg/mL), CBZ and BZK chloride (MIC > 16 µg/mL). For azoles, potential cross-resistance was observed with TEB (MIC = 4 µg/mL), CBZ and BZK chloride (MIC > 16 µg/mL). Therefore, in this study, these 12 strains were considered as resistant.

These findings suggest that uncontrolled environmental or animal exposure to these compounds may induce acquired resistance in these yeasts, potentially reducing their susceptibility to therapeutic drugs during infection—particularly through the induction of shared resistance mechanisms under stress.

In addition, three susceptible controls were included, representing the same species as the resistant isolates: *T. asahii* Ta57F, *C. mucoides* (*Trichosporon mucoides*) A1-9, and *T. japonicum* A3-10.

3.3. Growth curves and biofilm formation

To further characterize the isolates, we performed additional assays to evaluate traits potentially associated with antifungal tolerance and resistance. Overall, growth and biofilm formation did not show a direct correlation with resistance; however, some resistant isolates—particularly *T. asahii* Ta9, *T. asahii* A1-2, *C. mucoides* (*Trichosporon mucoides*) A1-14, and *T. japonicum* 105B —exhibited enhanced growth and biofilm production at 37 °C, suggesting improved adaptation to host conditions.

Figure 3 summarizes the results of growth and biofilm formation at 37 °C for these four resistant isolates and the three susceptible controls (Ta57F, A1-9, and A3-10). In general, all resistant isolates displayed superior growth (Figure 3A) and biofilm formation (Figure 3B) compared with susceptible controls. Among all samples, isolate 105B showed the highest growth rate (Figure 3A, *), while A1-14 exhibited the greatest biofilm-forming capacity (Figure 3B, *). As these phenotypic traits may reflect mechanisms contributing to antifungal resistance, the four resistant isolates were selected for subsequent *in vivo* investigations under host-like conditions.

The selection followed a funneling strategy, beginning with isolate distribution by species and sources, followed by narrowing to those exhibiting high MIC values and evidence of potential cross-resistance, and finally prioritizing isolates showing enhanced growth and robust biofilm formation at 37 °C. By integrating these criteria, the selected isolates represent phenotypes most relevant for studying antifungal resistance, stress adaptation, and pathogenicity in *Trichosporon*, thereby ensuring that subsequent *in vivo* analyses focus on strains with the highest clinical and biological relevance.

Figure 3 also presents the mean absorbance values (indicated by dotted lines) obtained from all 33 isolates tested (represented in Supplementary Figure 1), serving as a reference for overall growth (~0.4 Abs) and biofilm formation (~1.5 Abs) at 37 °C. This reference allows direct comparison between the selected isolates and the population mean, highlighting their distinct phenotypic profiles that may contribute to their increased tolerance to antifungal agents in host conditions.

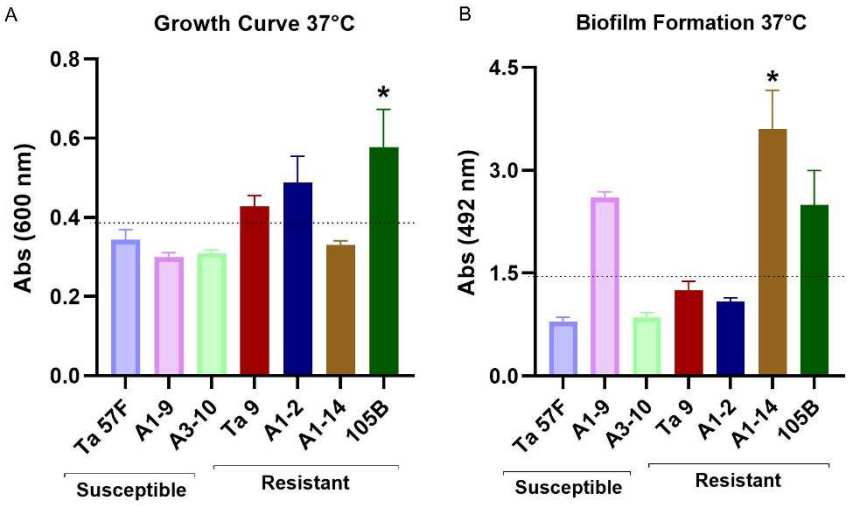


Figure 3: *In vitro* evaluation of growth curve and biofilm formation for the selected samples at host temperature. (A) Growth capacity determined by measuring absorbance at 600 nm using a spectrophotometer after 48 h of incubation at 37 °C. (B) Biofilm formation assessed by measuring absorbance at 492 nm using a spectrophotometer after 48 h of incubation at 37 °C. The selected isolates are organized into two groups according to antifungal susceptibility: susceptible (Ta57F, A1-9, and A3-10) and resistant (Ta9, A1-2, A1-14, and 105B). All resistant isolates showed significantly higher values compared with the susceptible controls ($p < 0.05$). Furthermore, isolates 105B and A1-14 exhibited the highest overall values among all samples (*). The dotted line represents the mean value of the 33 samples. Data were analyzed and plotted using GraphPad Prism with Student's *t*-test and one-way ANOVA.

Furthermore, supplementary Figure 1A shows that most isolates grew well at both 30 °C and 37 °C, except for PH8, A1-24, 361L, and 368L, which exhibited reduced growth at one of the temperatures, consistent with their lower proliferation observed in susceptibility assays. Supplementary Figure 1B indicates that all isolates were capable of forming biofilms at both temperatures, with no apparent correlation to MIC values. Overall, although no direct association was found between resistance, growth, or biofilm formation, the previously mentioned resistant isolates exhibited enhanced performance at 37 °C, reinforcing their potential adaptation to host conditions, as previously reported.

3.4. *In vivo* analysis of antifungal resistance in a murine model of *Trichosporon* infection

Based on previous *in vitro* data, four resistant isolates (Ta9, A1-2, A1-14, and 105B) and three susceptible ones (Ta57F, A1-9, and A3-10) were selected for murine infection assays. Mice were immunosuppressed with 5-fluorouracil (5-FU), as confirmed by a marked reduction in leukocyte counts (Figure 4A). On day 4 of the experimental protocol, animals were inoculated with either resistant or susceptible isolates. Antifungal treatment with fluconazole (FCZ) or amphotericin B (AMB) was initiated one day later in selected resistant groups.

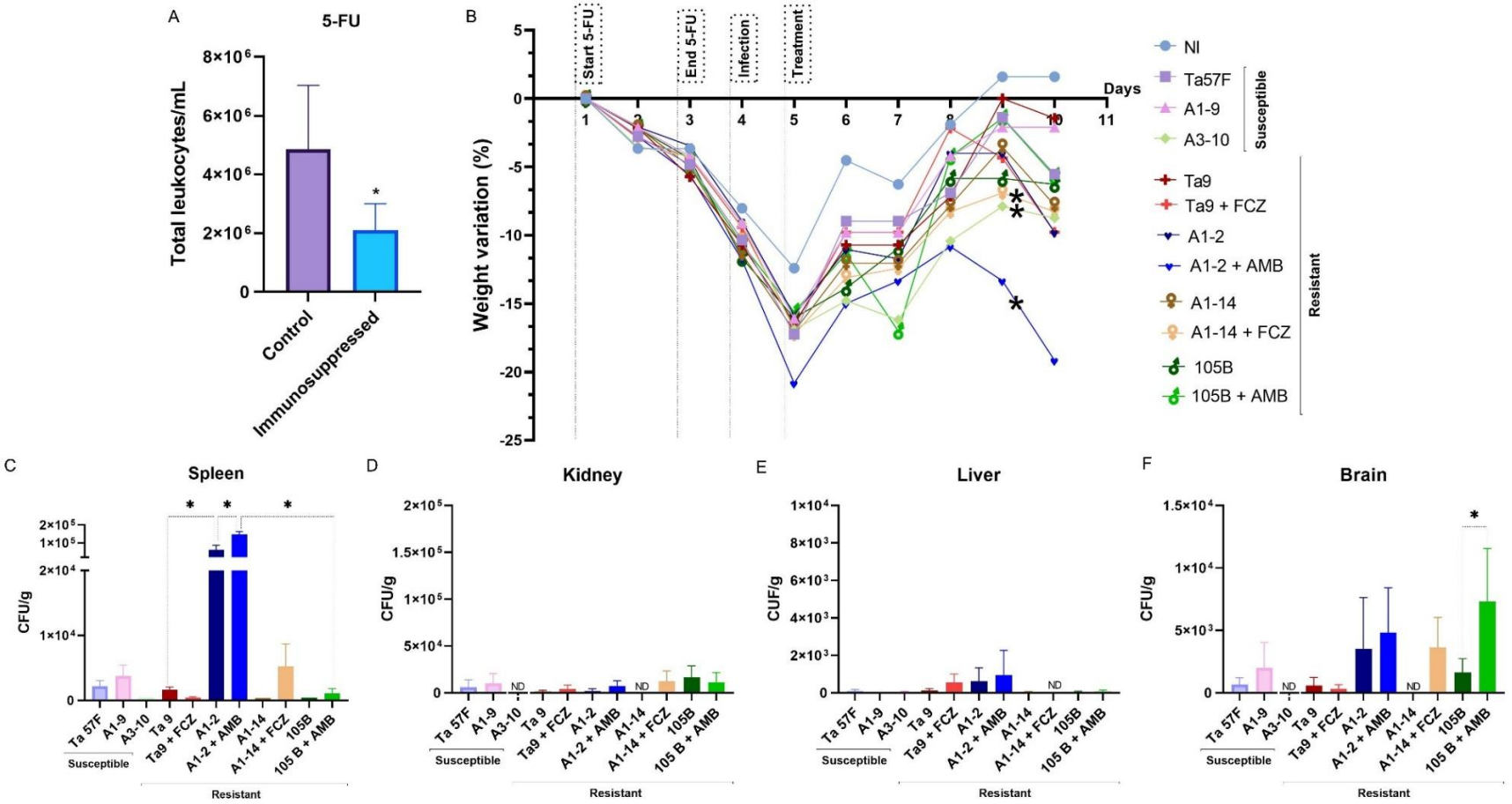


Figure 4: *In vivo* evaluation of immunosuppression, body weight variation, and fungal burden in a murine infection model. (A) Efficacy of immunosuppression achieved by intraperitoneal injection of 5-fluorouracil (75 mg/kg) for three consecutive days ($p < 0.05$). (B) Body weight variation (%) throughout the 10-day experimental period in mice infected with susceptible (Ta57F, A1-9, and A3-10) or resistant isolates (Ta9, A1-2, A1-14, and 105B), and in resistant groups treated with amphotericin B (0.5 mg/kg/day) or fluconazole (10 mg/kg/day). Asterisks in the line graphs indicate significant differences compared with non-infected (NI) controls ($p < 0.05$). The experimental timeline is represented by the dotted line: three days of immunosuppression, infection on day 4, and treatment starting on day 5 and continuing until euthanasia (day 10). (C–F) Fungal burden in the spleen, kidney, liver, and brain, respectively, expressed as colony-forming units (CFU/g). Asterisks and dotted lines in panels (C) and (F) indicate statistically significant differences between the respective groups ($p < 0.05$). Each group had 6 mice. ND indicates no detectable growth. Data was analyzed and plotted using GraphPad Prism, applying Student's *t*-test and one-way ANOVA.

All animals exhibited progressive weight loss from days 1 to 4, which intensified following infection; however, gradual recovery began around day 6 (Figure 4B). Only the groups infected with A3-10, A1-14 + FCZ, and A1-2 + AMB displayed significantly delayed recovery compared to non-infected (NI) controls (Figure 4B, *), whereas the remaining groups recovered similarly to NI.

Susceptible isolates (Ta57F and A1-9) caused mild infections characterized by rapid weight recovery and low fungal burdens (Figure 4B–F). In contrast, mice infected with A3-10 exhibited slower recovery despite a low fungal load, suggesting the involvement of additional host–pathogen interactions (Figure 4B–F). Overall, lower virulence appeared to correlate with antifungal susceptibility.

Fungal burden analysis among resistant isolates revealed marked inter-isolate variability. Notably, *T. asahii* A1-2 showed a higher spleen CFU count than *T. asahii* Ta9 (Figure 4C, *), indicating variability in virulence potential among resistant strains of the same species, likely associated with strain-specific traits.

Mice infected with resistant A1-14 and treated with FCZ, or with resistant A1-2 and treated with AMB, exhibited impaired body weight recovery (Figure 4B, *), consistent with the elevated fungal burdens detected in multiple organs (Figure 4C–F). In some instances, antifungal therapy appeared to exacerbate infection, as observed for A1-2 versus A1-2 + AMB (Figure 4C, *) and 105B versus 105B + AMB (Figure 4F, *), the latter showing pronounced CNS involvement and extensive fungal dissemination. Notably, the resistant isolate A1-2 (MIC = 4 μg/mL) displayed greater virulence than 105B (MIC = 2 μg/mL) under AMB treatment (Figure 4C, *), also underscoring the influence of resistance level on disease outcome. Overall, fluconazole treatment was associated with a more favorable prognosis than amphotericin B.

Collectively, these findings demonstrate that antifungal resistance affects not only therapeutic efficacy but also fungal virulence. Strain-specific traits, antifungal resistance, and resistance levels collectively modulate infection dynamics and therapeutic efficacy. Altogether, these results highlight the clinical and environmental importance of continuous surveillance of antifungal resistance within *Trichosporon* species.

4. Discussion

In this study, we investigated the antifungal resistance profiles of opportunistic *Trichosporon* isolates obtained from clinical, animal, and environmental sources, both *in vitro* and *in vivo*. By assembling and characterizing this diverse collection, we provide a comprehensive overview of resistance patterns across the

genus. The inclusion of isolates and compounds from multiple origins highlights the potential for environmental and animal reservoirs to contribute to the dissemination and development of resistant strains, reinforcing the relevance of a One Health perspective. This integrative approach represents one of the few comprehensive assessments of *Trichosporon* resistance conducted in South America. Overall, this work aimed to detect, understand, and investigate the causes and consequences of resistance and cross-resistance in *Trichosporon* spp. from a One Health perspective (Figure 5).

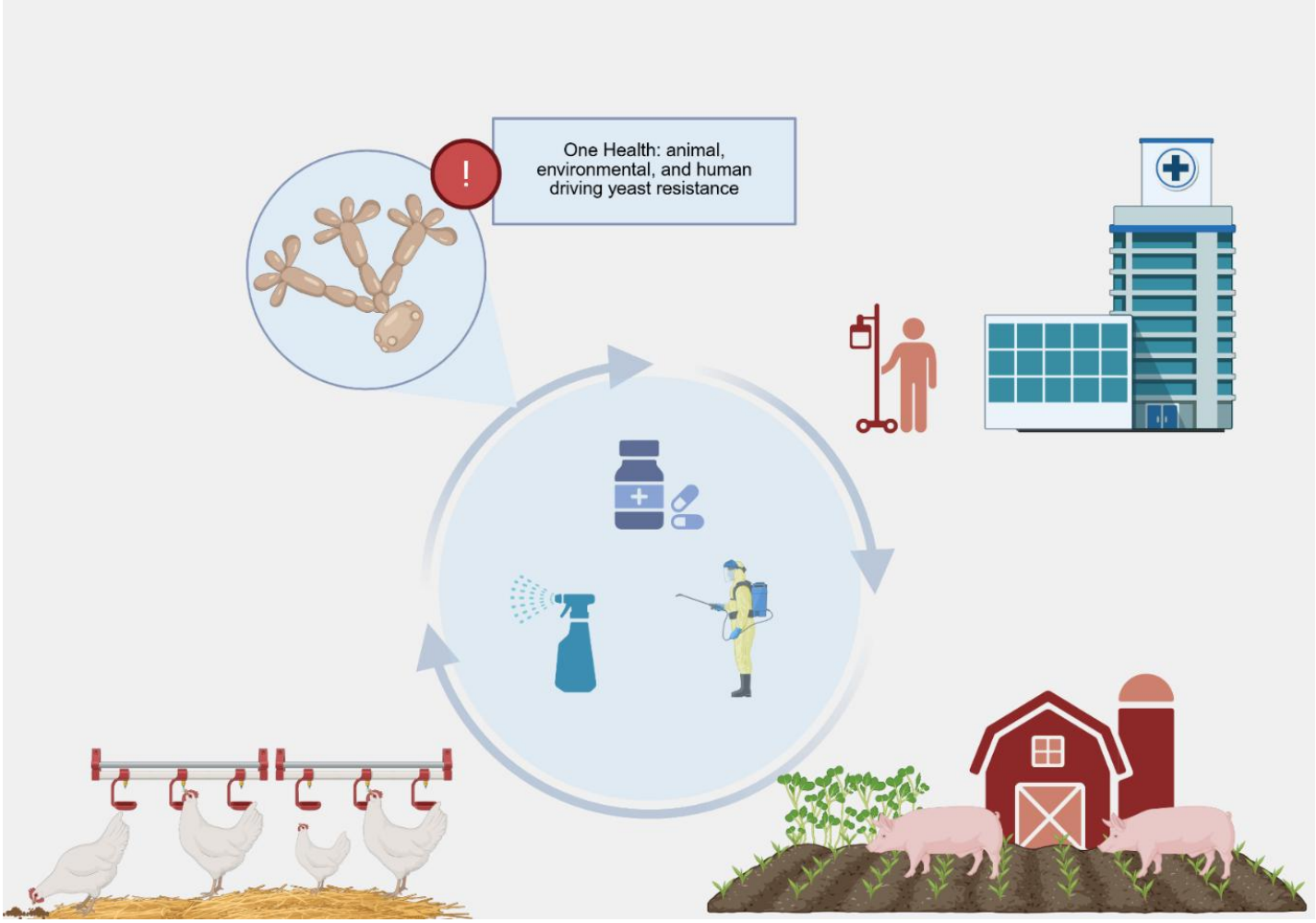


Figure 5: One Health perspective of antifungal resistance. Samples were collected from farm animals and their environments, as well as from patients and hospital settings. Continuous exposure of *Trichosporon*, ubiquitous opportunistic yeasts, to clinical antifungals, agrochemicals, and disinfectants may drive resistance and cross-resistance, thereby compromising the treatment of trichosporonosis. Produced in BioRender (BEWJPEJ8-0003).

The distribution of *Trichosporon* isolates across different Brazilian states reveals important ecological and epidemiological patterns. *T. asahii* emerges as the dominant species, reflecting its broad distribution and adaptability to diverse hosts and environments, whereas species such as *C. mucoides* (*Trichosporon mucoides*), *T. japonicum*, and *C. jirovecii* (*Trichosporon jirovecii*) show more restricted distributions linked to specific

ecological niches such as poultry or swine farming or clinical settings. *T. coremiiforme* was detected only once, indicating a narrower ecological presence. These patterns highlight potential reservoirs and transmission routes, particularly for *T. asahii* and *C. mucoides*, which are among the most virulent species implicated in invasive trichosporonosis [1–7]. The balance between animal and environmental sources underscores their close interplay, while the lower frequency of human isolates suggests a selective but clinically relevant emergence. Furthermore, the widespread use of antifungals, agrochemicals, and disinfectants in hospitals, agriculture, and the environment may drive adaptive responses that contribute to resistance and cross-resistance, as observed in other yeasts such as *Aspergillus*, *Candida*, and *Cryptococcus* [35–40]. These findings underline the ecological plasticity of *T. asahii*, the niche specialization of other species, and the importance of a One Health perspective to understand the epidemiology and resistance dynamics of *Trichosporon*, with direct implications for the treatment of trichosporonosis.

In this context, this study investigated potential resistance of *Trichosporon* isolates to commonly used clinical antifungals, agrochemicals, and disinfectants using the CLSI methodology with specific modifications for this genus [16, 52]. Despite some variation, MIC values generally aligned with those reported in the literature for *Trichosporon* spp., with fluconazole typically ≤8 μg/mL (though higher values occur), voriconazole and itraconazole ranging 0.06–1 μg/mL, amphotericin B ranging 0.125 to >16 μg/mL (>2 μg/mL often considered resistant), and micafungin generally ineffective (>8 μg/mL). MIC patterns for other compounds varied by genus and isolate, with no standardized breakpoints for *Trichosporon*. Species-specific differences were observed, but most MICs fell within previously reported ranges and previously described effectiveness [2, 3, 16–25, 35, 36, 40, 54-56]. Notably, for several isolates, were detected elevated MIC values for amphotericin B (2–4 μg/mL), fluconazole (>8 μg/mL), voriconazole (>0.125 μg/mL), pyraclostrobin (>8 μg/mL), carbendazim and BZK chloride (>16 μg/mL), and tebuconazole (>2 μg/mL), suggesting possible resistance and cross-resistance. These results, along with the lack of standardized testing protocols, highlight the relevance of this work and the urgent need to standardize antifungal susceptibility testing for *Trichosporon* spp., particularly within a One Health framework amid rising antifungal resistance concern.

Therefore, this study identified 12 isolates with resistance patterns meeting defined MIC thresholds, representing three species: *T. asahii* (most prevalent), *C. mucoides* (*Trichosporon mucoides*), and *T. japonicum*. The presence of resistant isolates across these species suggests that environmental and animal exposure to antifungals, agrochemicals, or disinfectants may drive the emergence of resistance phenotypes, potentially facilitating cross-resistance through shared stress-response mechanisms within a One Health context [35–42].

While no direct correlation was found between resistance and phenotypic traits such as growth capacity or biofilm formation, certain resistant isolates with the highest MICs—105B, A1-2, A1-14, and Ta9—exhibited enhanced growth and biofilm-forming ability at host temperature (37 °C), indicating greater adaptability and persistence under antifungal pressure [17, 26–34]. These combined characteristics of species identity, MIC profile, and phenotypic performance justify their selection as representative models for further *in vivo* studies of antifungal resistance and pathogenicity in animal infection contexts.

The animal model provided key insights into the interplay between antifungal resistance, therapeutic failure, and virulence in *Trichosporon* infections. Resistance detected *in vitro* was largely maintained *in vivo*, compromising treatment efficacy and clinical outcomes. Treated resistant isolates exhibited higher fungal burdens, delayed recovery, and increased dissemination to the central nervous system (CNS), intensifying disease severity and worsening prognosis. Notably, fluconazole and amphotericin B—typically effective in murine trichosporonosis models—exacerbated infection under resistance conditions, although azoles still outperformed polyenes [53]. The combination of therapeutic failure, resistance, virulence, and CNS involvement in certain isolates underscores the clinical challenges posed by these infections, particularly in the current scenario where CNS involvement is associated with poorer outcomes [24].

Furthermore, the results demonstrate that temperature critically affects both isolate growth and compound activity. Several isolates showed slower growth depending on incubation temperature, suggesting temperature-dependent metabolic adaptation. Notably, *C. jirovecii* (*Trichosporon jirovecii*) exhibited reduced growth at 37 °C, a phenomenon not yet described in the literature. Compound activity was also temperature sensitive: higher MICs for fluconazole, voriconazole, and BZK chloride were observed at 30 °C, whereas amphotericin B and tebuconazole were less effective at 37 °C. These findings indicate that temperature shapes both *Trichosporon* physiology and antifungal susceptibility, with implications for infection dynamics, environmental persistence, and resistance development.

From a One Health perspective, these results highlight that environmental and animal reservoirs can serve as sources of clinically relevant resistant strains, driven by the widespread use of antifungals in agriculture, veterinary medicine, and clinical practice. Such cross-domain selective pressures may sustain and amplify resistance traits, representing a growing threat to human and animal health. These findings emphasize the urgent need for integrated surveillance, responsible antifungal stewardship, and the development of novel therapeutic strategies within a unified One Health framework.

5. Conclusion

This study shows that ecological diversity, metabolic plasticity, and resistance profiles in *Trichosporon* are interrelated and shape its epidemiology and pathogenicity. The predominance of *T. asahii* and the presence of resistant isolates highlight its clinical importance and the emergence of less susceptible phenotypes, driven by antifungal, agrochemical, and disinfectant use. Resistance was associated withtreatment failure and higher virulence *in vivo*, underscoring its persistence and multifactorial nature. These findings call for standardized susceptibility testing, stronger surveillance, and responsible antifungal stewardship under a One Health perspective.

6. Statements and Declarations

CRediT authorship contribution statement: Isabela Lima Miranda: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing – review & editing, Writing – original draft. Daniel Assis Santos: Conceptualization, Project administration, Supervision, Funding acquisition, Writing – review & editing. Nalu Teixeira Aguiar Peres: Writing – review & editing, Methodology, Investigation, Resources. Rafael Wesley Bastos: Writing – review & editing, Methodology, Investigation, Resources. Maria Isabel Azevedo: Writing – review & editing, Methodology, Investigation, Resources. Debora S.C.M. Castelo-Branco: Writing – review & editing, Methodology, Investigation, Resources. Luana Rossato: Writing – review & editing, Methodology, Investigation, Resources. Florent Morio: Writing – review & editing. Jose Rivaldo Lima: Writing – review & editing. Isabelle Ourliac-Garnier: Writing – review & editing. Kassia Jessica Galdino Silva: Methodology, Investigation. Gabriela Silva Cruz: Methodology, Investigation. Fabiola Lucini: Methodology, Investigation. Victor Augusto Teixeira Leocadio: Methodology, Investigation. Cesar Moura: Methodology, Investigation.

Declaration of Generative AI and AI-assisted technologies in the writing process: During the preparation of this work the authors used ChatGPT OpenAI to improve the language. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Funding: This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (grant number 408540/2022-2), Brazilian Ministry of Health (grant 444501/2023-1), National Institute of Science and Technology (INCT) Funvir from Brazil (grant number 405934/2022), Coordenação de Aperfeiçoamento de

Pessoal de Nível Superior (CAPES) (grant number 88887.966527/2024-00), and CAPES-COFECUB program (financing Code 001). D.A.S. (303762/2020- 9) is a research fellow of the CNPq.

Declaration of competing interest: The authors declare that they have no conflicts of interest to disclose.

Data Availability: All data is available in the article.

7. References

1- Francisco EC, Desnos-Ollivier M, Dieleman C, *et al*. Unveiling *Trichosporon austroamericanum* sp. nov.: A Novel Emerging Opportunistic Basidiomycetous Yeast Species. Mycopathologia. 2024 May 6; 189(3):43. doi: 10.1007/s11046-024-00851-4.

2- Mehta V, Nayyar C, Gulati N, *et al*. A Comprehensive Review of *Trichosporon* spp.: An Invasive and Emerging Fungus. Cureus. 2021 Aug 21; 13(8):e17345. doi: 10.7759/cureus.17345.

3- Colombo AL, Padovan AC, Chaves GM. Current knowledge of *Trichosporon* spp. and Trichosporonosis. Clin Microbiol Rev. 2011 Oct; 24(4):682-700. doi: 10.1128/CMR.00003-11.

4- Lara BR, de Camargo BB, Paula CR, *et al*. Comparing the phenotypic, genotypic, and proteomic identification of *Trichosporon* species: A globally emerging yeast of medical importance. Med Mycol. 2021 Dec 3; 59(12):1181-1190. doi: 10.1093/mmy/myab050.

5- Lo C, Kang CL, Sun PL, *et al*. Disseminated Fungal Infection and Fungemia Caused by *Trichosporon asahii* in a Captive Plumed Basilisk (*Basiliscus plumifrons*). J Fungi (Basel). 2021 Nov 24; 7(12):1003. doi: 10.3390/jof7121003.

6- Bobrek K, Sokół I, Gaweł A. Uncommon Non-*Candida* Yeasts in Healthy Turkeys-Antimicrobial Susceptibility and Biochemical Characteristic of *Trichosporon* Isolates. Pathogens. 2021 Apr 30; 10(5):538. doi: 10.3390/pathogens10050538.

7- Mariné M, Brown NA, Riaño-Pachón DM, *et al*. On and Under the Skin: Emerging Basidiomycetous Yeast Infections Caused by *Trichosporon* Species. PLoS Pathog. 2015 Nov 7; e1004982. doi: 10.1371/journal.ppat.1004982.

8- Takashima M, Sugita T. Taxonomy of Pathogenic Yeasts *Candida*, *Cryptococcus*, *Malassezia*, and *Trichosporon*. Med Mycol J. 2022; 63(4):119-132. doi: 10.3314/mmj.22.004.

9- Commenges A, Coucheney F, Lessard MH, *et al*. *Trichosporon asahii*: Taxonomy, health hazards, and biotechnological applications. Fungal Biology Reviews. 2024; Volume 49, 100369. doi: 10.1016/j.fbr.2024.100369.

10- Pumeesat P, Wongsuk T. Genetic analysis of emerging fungal pathogens: *Trichosporon asahii*. Diagn Microbiol Infect Dis. 2023 Nov; 107(3):116057. doi: 10.1016/j.diagmicrobio.2023.116057.

11- Chang DSJ, Harfouch CW, Melendez ML. *Trichosporon asahii* opportunistic pneumonia after a severe COVID-19 infection. IDCases. 2023; 31:e01701. doi: 10.1016/j.idcr.2023.e01701.

12- Benelli JL, Basso RP, Grafulha TW *et al*. Fungal Bloodstream Co-infection by *Trichosporon asahii* in a COVID-19 Critical Patient: Case Report and Literature Review. Mycopathologia. 2022 Aug; 187(4):397-404. doi: 10.1007/s11046-022-00637-6.

13- Cronyn V, Howard J, Chiang L, *et al*. *Trichosporon asahii* Urinary Tract Infection in a Patient with Severe COVID-19. Case Rep Infect Dis. 2021 Dec 17; 2021:6841393. doi: 10.1155/2021/6841393.

14- Castano G, Yarrarapu SNS, Mada PK. Trichosporonosis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2024 Jan–; PMID: 29494084.

15- Matsumoto Y, Arakawa S, Sadahira K, *et al*. Skin Manifestations of Micafungin Breakthrough Disseminated Trichosporonosis in Acute Megakaryoblastic Leukemia. Med Mycol J. 2024; 65(1):17-21. doi: 10.3314/mmj.23-00009.

16- CLSI. M27-Ed4 and M60-Ed1: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Clinical and Laboratory Standards Institute; 2017. Available at: https://clsi.org/about/news/clsi-publishes-new-and-updated-microbiology-documents/. Accessed on: July 14, 2025.

17- Miranda IL, Peres NTA, Bastos RW, *et al*. *Trichosporon* and Antifungal Resistance: Current Knowledge and Gaps. Mycopathologia. 2025 Jul 4; 190(4):59. doi: 10.1007/s11046-025-00969-z.

18- Francisco EC, JN de Almeida Junior, F de Queiroz Telles, *et al*. Species distribution and antifungal susceptibility of 358 *Trichosporon* clinical isolates collected in 24 medical centers. Clinical Microbiology and Infection. 2019 Jul; Volume 25, Issue 7, Pages 909.e1-909.e5. doi: 10.1016/j.cmi.2019.03.026.

19- Guo Lyu S, Hsueh P, Al-Hatmi AMS, *et al*. Invasive Infections Due to *Trichosporon*: Species Distribution, Genotyping, and Antifungal Susceptibilities from a Multicenter Study in China. J Clin Microbiol. 2019; 57:10.1128/jcm.01505-18. doi: 10.1128/jcm.01505-18.

20- Kuo SH, Lu PL, Chen YC, *et al*. The epidemiology, genotypes, antifungal susceptibility of *Trichosporon* species, and the impact of voriconazole on *Trichosporon* fungemia patients. J Formos Med Assoc. 2021 Sep; 120(9):1686-1694. doi: 10.1016/j.jfma.2020.12.007.

21- Lemes RM, Lyon JP, Moreira LM, *et al*. Antifungal susceptibility profile of *Trichosporon* isolates: correlation between CLSI and etest methodologies. Braz J Microbiol. 2010 Apr; 41(2):310-5. doi: 10.1590/S1517-83822010000200008.

22- Singh S, Capoor MR, Varshney S, *et al*. Epidemiology and Antifungal Susceptibility of Infections Caused by *Trichosporon* Species: An Emerging Non-*Candida* and Non-*Cryptococcus* Yeast Worldwide. Indian J Med Microbiol. 2019 Oct-Dec; 37, 536-541. doi: 10.4103/ijmm.IJMM_19_146.

23- Wongsuk T, Boonsilp S, Pumeesat P, *et al*. Genotyping, antifungal susceptibility testing, and biofilm formation of *Trichosporon* spp. isolated from urine samples in a University Hospital in Bangkok, Thailand. Acta Microbiol Immunol Hung. 2022 Aug 16; 69(3):247-257. doi: 10.1556/030.2022.01797.

24- Lino R, Guimarães AR, Sousa E, *et al*. Emerging Fungal Infections of the Central Nervous System in the Past Decade: A Literature Review. Infect. Dis. 2024; *16*, 952-976. doi: 10.3390/idr16050076.

25- Agrawal S. Spectrum of signs, symptoms, and treatment in amphotericin B-resistant *Trichosporon* endophthalmitis: A series of ten cases of post-cataract surgery cluster endophthalmitis. Indian J Ophthalmol. 2022 Nov; 70(11):4004-4009. doi: 10.4103/ijo.IJO_1938_22.

26- Abbes S, Sellami H, Neji S, *et al*. Implication of efflux pumps and ERG11 genes in resistance of clinical *Trichosporon asahii* isolates to fluconazole. J Med Microbiol. 2021 Mar 10; 70(3). doi: 10.1099/jmm.0.001236.

27- Aguiar ALR, Silva BND, Fiallos NM, *et al*. Promethazine inhibits efflux, enhances antifungal susceptibility and disrupts biofilm structure and functioning in *Trichosporon*. Biofouling. 2023 Feb; 39(2):218-230. doi: 10.1080/08927014.2023.2202315.

28- De Andrade IB, Figueiredo-Carvalho MHG, Chaves ALDS, *et al*. Metabolic and phenotypic plasticity may contribute for the higher virulence of *Trichosporon asahii* over other Trichosporonaceae members. Mycoses. 2023 May; 66(5):430-440. doi: 10.1111/myc.13562.

29- Iturrieta-González IA, Padovan AC, Bizerra FC, *et al*. Multiple species of *Trichosporon* produce biofilms highly resistant to triazoles and amphotericin B. PLoS One. 2014 Oct 31; 9(10):e109553. doi: 10.1371/journal.pone.0109553.

30- Ma X, Liu H, Liu Z, *et al*. *Trichosporon asahii* PLA2 Gene Enhances Drug Resistance to Azoles by Improving Drug Efflux and Biofilm Formation. Int J Mol Sci. 2023 May 16; 24(10):8855. doi: 10.3390/ijms24108855.

31- Ma X, Liu Z, Zeng X, *et al*. Genome-Wide Identification and Characterization of the Medium-Chain Dehydrogenase/Reductase Superfamily of *Trichosporon asahii* and Its Involvement in the Regulation of Fluconazole Resistance. J Fungi (Basel). 2024 Feb 1; 10(2):123. doi: 10.3390/jof10020123.

32- Ma X, Yang W, Yang A, *et al*. Metabolome and Transcriptome Combinatory Profiling Reveals Fluconazole Resistance Mechanisms of *Trichosporon asahii* and the Role of Farnesol in Fluconazole Tolerance. Microorganisms. 2023 Nov 17; 11(11):2798. doi: 10.3390/microorganisms11112798.

33- Nobrega de Almeida J Jr, Jimenez-Ortigosa C, Francisco EC, *et al*. ERG11 Analysis among Clinical Isolates of *Trichosporon asahii* with Different Azole Susceptibility Profiles. Antimicrob Agents Chemother. 2022 Dec 20; 66(12):e0110122. doi: 10.1128/aac.01101-22.

34- Padovan ACB, Rocha WPDS, Toti ACM, *et al*. Exploring the resistance mechanisms in *Trichosporon asahii*: Triazoles as the last defense for invasive trichosporonosis. Fungal Genet Biol. 2019 Dec; 133:103267. doi: 10.1016/j.fgb.2019.103267.

35- Bastos RW, Freitas GJC, Carneiro HCS, *et al*. From the environment to the host: How non-azole agrochemical exposure affects the antifungal susceptibility and virulence of *Cryptococcus gattii*. Sci Total Environ. 2019 Sep 1; 681:516-523. doi: 10.1016/j.scitotenv.2019.05.094.

36- Bastos RW, Carneiro HCS, Oliveira LVN, *et al*. Environmental Triazole Induces Cross-Resistance to Clinical Drugs and Affects Morphophysiology and Virulence of *Cryptococcus gattii* and *C. neoformans*. Antimicrob Agents Chemother. 2017 Dec 21; 62(1):e01179-17. doi: 10.1128/AAC.01179-17.

37- Carneiro HCS, Ribeiro NQ, Bastos RW, *et al*. Effect of non-antifungal agrochemicals on the pathogenic fungus *Cryptococcus gattii*. Med Mycol. 2020 Jan 1; 58(1):47-53. doi: 10.1093/mmy/myz018.

38- Castelo-Branco D, Lockhart SR, Chen YC, *et al*. Collateral consequences of agricultural fungicides on pathogenic yeasts: A One Health perspective to tackle azole resistance. Mycoses. 2022 Mar; 65(3):303-311. doi: 10.1111/myc.13404.

39- Ren J, Jin X, Zhang Q, *et al*. Fungicides induced triazole-resistance in *Aspergillus fumigatus* associated with mutations of TR46/Y121F/T289A and its appearance in agricultural fields. J Hazard Mater. 2017 Mar 15; 326:54-60. doi: 10.1016/j.jhazmat.2016.12.013.

40- Bastos RW, Peres NTA, Santos DA, *et al*. Antifungal resistance in yeasts from One Health perspective: A Brazilian study. Science of The Total Environment. 2025; Volume 973, 179139. doi: 10.1016/j.scitotenv.2025.179139.

41- Woods M, McAlister JA, Geddes-McAlister J. A One Health approach to overcoming fungal disease and antifungal resistance. WIREs Mech Dis. 2023 Jul-Aug; 15(4):e1610. doi: 10.1002/wsbm.1610.

42- Adisasmito WB, Almuhairi S, Behravesh CB, *et al*. One Health: A new definition for a sustainable and healthy future. PLoS Pathog. 2022 Jun 23; 18(6):e1010537. doi: 10.1371/journal.ppat.1010537.

43- Singulani JL, Silva DL, Lima CM, *et al.* COVID-19 and candiduria: an investigation of the risk factors and immunological aspects. *Braz J Microbiol* **54**. 2023 Jul 5; 1783–1793. doi: 10.1007/s42770-023-01042-x.

44- Freitas GJC, Ribeiro NQ, Gouveia-Eufrasio L, *et al*. Antimalarials and amphotericin B interact synergistically and are new options to treat cryptococcosis. Int J Antimicrob Agents. 2023 Jul; 62(1):106807. doi: 10.1016/j.ijantimicag.2023.106807.

45- Yang X, Bai S, Wu J, *et al*. Antifungal Activity and Potential Action Mechanism of Allicin against *Trichosporon asahii*. Microbiol Spectr. 2023 Jun 15; 11(3):e0090723. doi: 10.1128/spectrum.00907-23.

46- Santana AB, Souto BS, Santos NCM, *et al*. Murine response to the opportunistic bacterium *Pseudomonas aeruginosa* infection in gut dysbiosis caused by 5-fluorouracil chemotherapy-induced mucositis. Life Sciences. 2022; Volume 307. doi: 10.1016/j.lfs.2022.120890.

47- Alexandra M, Gloria M, Azalia M, *et al*. Cytokines profile in immunocompetent mice during *Trichosporon asahii* infection, *Medical Mycology*. 2018 Jan; Volume 56, Issue 1, Pages 103–109. doi: 10.1093/mmy/myx018.

48- Mariné M, Bom VL, de Castro PA, *et al*. The development of animal infection models and antifungal efficacy assays against clinical isolates of *Trichosporon asahii*, *T. asteroides* and *T. inkin*. Virulence. 2015; 6(5):476-86. doi: 10.1080/21505594.2015.1020273.

49- Yamagata E, Kamberi P, Yamakami Y, *et al*. Experimental Model of Progressive Disseminated Trichosporonosis in Mice with Latent Trichosporonemia. J Clin Microbiol Rev 38. 2000. doi: 10.1128/jcm.38.9.3260-3266.2000.

50- Berenbaum MC. Role of mitosis and mitotic inhibition in the immunosuppressive action of thioguanine. *Nature (London)*. 1966; 210: 41–43.

51- Meletiadis J, Meis JF, Mouton JW, *et al*. Analysis of growth characteristics of filamentous fungi in different nutrient media. J Clin Microbiol. 2001 Feb; 39(2):478-84. doi: 10.1128/JCM.39.2.478-484.2001.

52- Miranda IL, Peres NTA, Bastos RW, *et al.* Optimized conditions for broth microdilution susceptibility testing of *Trichosporon* spp. to clinical antifungals. *Braz J Microbiol*. 2025 Aug; Epub ahead of print, PMID: 40844555. doi: 10.1007/s42770-025-01766-y.

53- Yamamoto K, Makimura K, Sudo T, *et al.* Experimental disseminated trichosporonosis in mice: tissue distribution and therapy with antifungal agents. *Journal of Medical and Veterinary Mycology*. 1997 Jan; Volume 35, Issue 6, Pages 411–418. doi: 10.1080/02681219780001511.

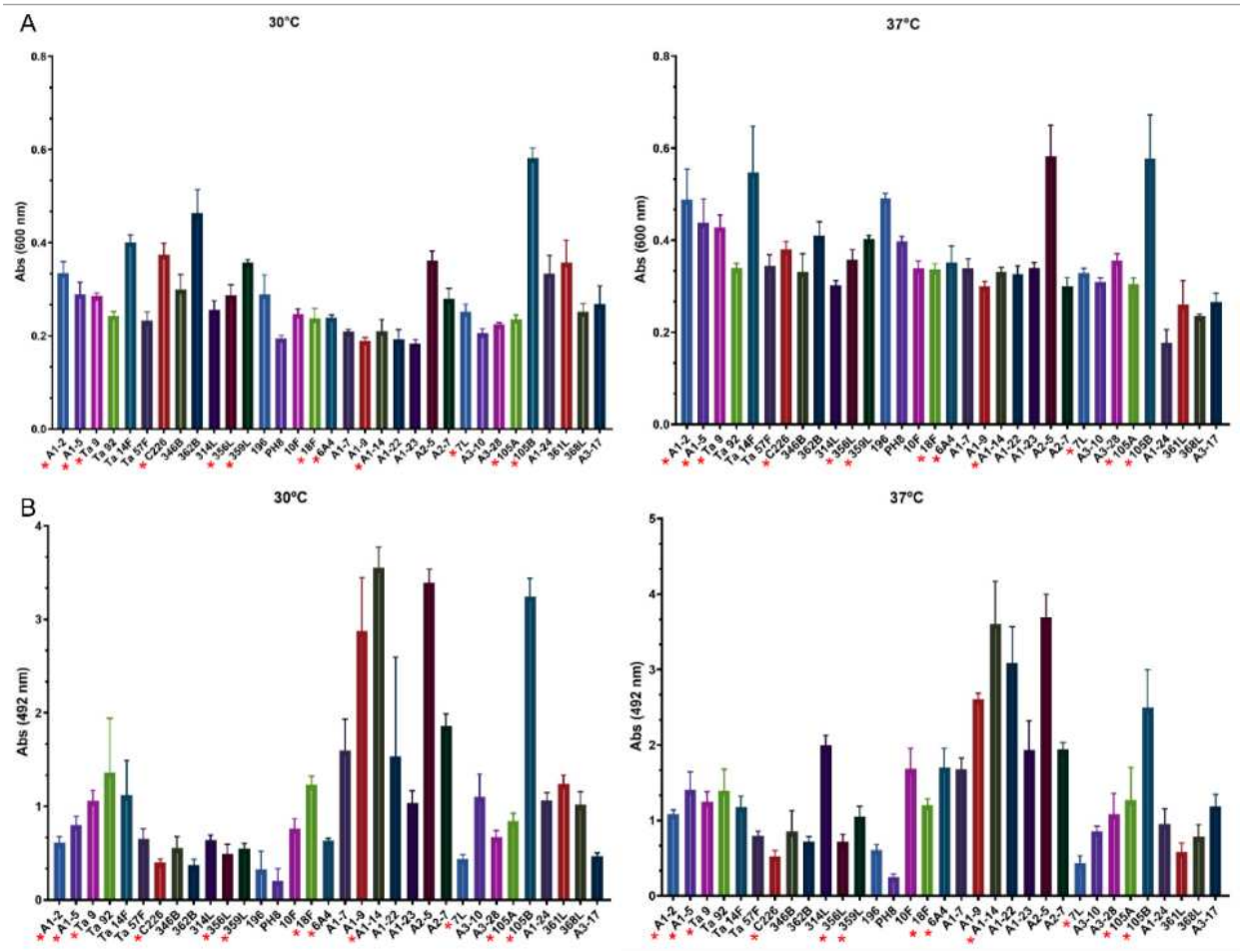
54- Moen B, Rudi K, Bore E, *et al*. Subminimal inhibitory concentrations of the disinfectant benzalkonium chloride select for a tolerant subpopulation of *Escherichia coli* with inheritable characteristics. Int J Mol Sci. 2012;13(4):4101-4123. doi: 10.3390/ijms13044101.

55- Korbecka-Paczkowska M, Karpiński TM. In Vitro Assessment of Antifungal and Antibiofilm Efficacy of Commercial Mouthwashes against *Candida albicans*. Antibiotics (Basel). 2024 Jan 25;13(2):117. doi: 10.3390/antibiotics13020117.

56- Passos Lima Y, de Paiva Macedo J, Barbosa Ferreira Machado A, *et al*. Biocide Tolerance, Biofilm Formation, and Efflux Pump Activity in Clinical Isolates of *Trichosporon asahii*. Infect Dis Rep. 2025 Aug 6;17(4):97. doi: 10.3390/idr17040097.

8. Supplementary Material

Supplementary Figure 1: *In vitro* evaluation of growth curve and biofilm formation for all the 33 samples in this study. (A) Growth capacity determined by measuring absorbance at 600 nm using a spectrophotometer after 48 h incubation at 30 °C and 37 °C. (B) Biofilm production activity assessed by measuring absorbance at 492 nm using a spectrophotometer after 48 h of pre-formed biofilms at 30 °C and 37 °C. The * indicates the resistant isolates, and the samples are organized by species, grouped in the same order as Figure 2. Produced in GraphPad Prism.



6. Discussão

A resistência antifúngica no gênero *Trichosporon* tem se consolidado como um desafio na micologia médica, tanto pelo aumento da incidência de infecções invasivas quanto pelas elevadas taxas de mortalidade em pacientes imunocomprometidos. A revisão bibliográfica realizada neste trabalho evidenciou que a maioria dos casos graves e daqueles associados à resistência é causada por *T. asahii*, espécie considerada o principal agente da tricosporonose invasiva, embora outras espécies também apresentem relevância clínica. O panorama atual indica que as opções terapêuticas disponíveis são limitadas, uma vez que equinocandinas e flucitosina são ineficazes, os polienos frequentemente apresentam CIMs elevados, e os azóis demonstram atividade variável — com o fluconazol exibindo os maiores valores de CIM e o voriconazol os mais baixos. Nesse contexto, o voriconazol permanece como a principal recomendação terapêutica. Entretanto, relatos cada vez mais frequentes de resistência têm sido associados a mecanismos como formação de biofilme, superexpressão de bombas de efluxo, mutações em genes como *ERG11*, alterações na composição lipídica da membrana, adaptações metabólicas, entre outros. Esses aspectos ressaltam a necessidade urgente de padronização metodológica e de investigações experimentais que permitam compreender e manejar de forma mais precisa a resistência e o tratamento de infecções causadas por esse gênero, especialmente no contexto de Saúde Única, também abordado na revisão (BASTOS et al., 2017/2019/2025; MEHTA et al., 2021; CARNEIRO et al., 2020; SINGH et al., 2019; COLOMBO et al., 2011).

Nesse sentido, a padronização das condições de microdiluição em caldo para ensaios de susceptibilidade representou um avanço significativo no presente estudo. Foram testadas diferentes condições de temperatura, tempo de incubação e critérios de leitura da inibição, sendo observado que a leitura após 48 horas de incubação a 37 °C é a mais adequada, com leitura de 50% de inibição para fluconazol e 100% para anfotericina B. Essa padronização foi focada nesses dois compostos antifúngicos mais comuns, mas pode ser extrapolada para diversos polienos e azóis utilizados atualmente no tratamento. Além disso, este trabalho ressaltou a importância dessa padronização pelo CLSI para reduzir a variabilidade entre diferentes estudos, fornecendo uma base sólida para futuras comparações interlaboratoriais e favorecendo a definição de valores de corte epidemiológicos (ECVs) e clínicos mais consistentes de internacionalmente comparáveis (MEHTA et al., 2021; SINGH et al., 2019; COLOMBO et al., 2011).

Com base nos resultados da revisão e a padronização metodológica, o estudo experimental proposto neste trabalho ampliou e aprofundou a discussão ao investigar essa resistência *in vitro* e *in vivo* sob a perspectiva de Saúde Única, incluindo isolados provenientes de pacientes humanos, animais de criação e ambientes correlacionados em diferentes regiões do Brasil, bem como diversos compostos antimicrobianos (antifúngicos clínicos, agroquímicos e desinfetantes).

Essa abordagem evidenciou a predominância epidemiológica de *T. asahii*, bem como a circulação e relevância de outras espécies na resistência, demonstrando a importância de uma vigilância integrada. Além disso, a partir do teste de microdiluição em caldo para a determinação do CIM, observou-se resistência não apenas a antifúngicos clínicos (anfotericina B, fluconazol e voriconazol), mas também a agroquímicos e desinfetantes, como tebuconazol, carbendazim, piraclostrobina e cloreto de benzalcônio, sugerindo a ocorrência de resistência cruzada decorrente da exposição ambiental. Essa constatação amplia o problema da resistência antifúngica para além do ambiente hospitalar, relacionando-o ao uso indiscriminado de substâncias antimicrobianas em diferentes setores. Adicionalmente, a análise *in vivo* confirmou que isolados resistentes tratados exibem pior recuperação e maior carga fúngica, associadas a maior disseminação para o sistema nervoso central em modelo murino, além de estarem correlacionadas a desfechos clínicos mais desfavoráveis. Esses achados são particularmente relevantes, pois indicam que a resistência antifúngica pode não apenas comprometer a eficácia do tratamento, mas também potencializar a virulência das cepas.

Em conjunto, os três estudos demonstram que a resistência em *Trichosporon* é um fenômeno multifatorial e ecossistêmico, que deve ser abordado sob a perspectiva integrada da Saúde Única. A revisão da literatura reforçou a gravidade do problema e a limitação das opções terapêuticas disponíveis, associadas aos mecanismos de resistência desse gênero; a padronização metodológica forneceu ferramentas essenciais para a obtenção de dados reprodutíveis e comparáveis; e o estudo da resistência dentro do contexto de Saúde Única estabeleceu a relação entre epidemiologia, resistência e virulência, evidenciando que o problema ultrapassa os limites do ambiente hospitalar, alcançando dimensões ambientais e veterinárias. Assim, a presente dissertação não apenas descreve o estado atual da resistência no gênero *Trichosporon*, mas também propõe soluções práticas e estratégicas, como a padronização de testes de susceptibilidade, a implementação de sistemas integrados de vigilância e a promoção do uso racional de antifúngicos, agroquímicos e desinfetantes. Tais

medidas são fundamentais para mitigar o impacto da resistência antifúngica, melhorar o manejo clínico da tricosporonose e orientar pesquisas futuras, visando novas abordagens terapêuticas e políticas de saúde pública sob uma perspectiva integrada e global de Saúde Única.

7. Conclusão

Os resultados deste estudo demonstram que *Trichosporon* spp. apresenta tanto resistência intrínseca quanto adquirida a múltiplas classes de antifúngicos clínicos, incluindo azóis, polienos, equinocandinas e flucitosina. Essa resistência está associada a diversos mecanismos moleculares e celulares, como o aumento da atividade de bombas de efluxo, a formação de biofilmes, mutações genéticas, alterações na composição lipídica da membrana e adaptações metabólicas que favorecem a sobrevivência em condições de estresse antifúngico. No âmbito metodológico, foi padronizada a técnica de microdiluição em caldo para a avaliação da susceptibilidade à anfotericina B e ao fluconazol no gênero *Trichosporon*, resultando em maior confiabilidade e reprodutibilidade das análises *in vitro*. Em seguida, essa metodologia foi aplicada para testar a coleção de 33 leveduras isoladas e identificadas a partir de fontes clínicas, animais e ambientais, frente a antifúngicos clínicos, agroquímicos e desinfetantes. Entre essas, *T. asahii* foi a espécie mais frequentemente isolada e apresentou os maiores níveis de resistência. A partir desses ensaios, 12 isolados resistentes foram selecionados e, com base nos perfis fenotípicos e nas análises epidemiológicas, quatro deles foram escolhidos para investigação da resistência em modelo *in vivo*. Os ensaios *in vivo* confirmaram falhas terapêuticas e evidenciaram, em alguns casos, a capacidade de colonização do sistema nervoso central (SNC), além de maior virulência associada aos fenótipos resistentes. Ademais, observou-se que a exposição prévia a desinfetantes e agroquímicos pode favorecer o desenvolvimento de resistência cruzada, destacando a importância das pressões seletivas ambientais no contexto da Saúde Única. Assim, reforça-se que a realização de testes de susceptibilidade e o uso racional de antifúngicos são essenciais para o enfrentamento da resistência antifúngica e para a prevenção de agravamentos clínicos em diferentes setores da saúde humana, animal e ambiental.

8. Perspectivas

Já estão em andamento os experimentos para a investigação dos mecanismos genéticos e moleculares da resistência no gênero *Trichosporon*, com a perspectiva de me aprofundar neste tópico no doutorado. Espero ao final do mestrado e doutorado contribuir para o maior

conhecimento dos mecanismos de resistência deste fungo, a partir da genômica e transcriptômica.

9. Referências

ADISASMITO, W. B. et al. One Health: a new definition for a sustainable and healthy future. *PLoS Pathogens*, v. 18, n. 6, e1010537, 2022. DOI: https://doi.org/10.1371/journal.ppat.1010537.

BASTOS, R. W. et al. Antifungal resistance in yeasts from One Health perspective: a Brazilian study. *Science of The Total Environment*, v. 973, p. 179139, 2025. DOI: https://doi.org/10.1016/j.scitotenv.2025.179139.

BASTOS, R. W. et al. Environmental triazole induces cross-resistance to clinical drugs and affects morphophysiology and virulence of *Cryptococcus gattii* and *C. neoformans*. *Antimicrobial Agents and Chemotherapy*, v. 62, n. 1, e01179-17, 2017. DOI: https://doi.org/10.1128/AAC.01179-17.

BASTOS, R. W. et al. From the environment to the host: how non-azole agrochemical exposure affects the antifungal susceptibility and virulence of *Cryptococcus gattii*. *Science of The Total Environment*, v. 681, p. 516-523, 2019. DOI: https://doi.org/10.1016/j.scitotenv.2019.05.094.

BOBREK, K. et al. Uncommon non-*Candida* yeasts in healthy turkeys – antimicrobial susceptibility and biochemical characteristic of *Trichosporon* isolates. *Pathogens*, v. 10, n. 5, p. 538, 2021. DOI: https://doi.org/10.3390/pathogens10050538.

CARNEIRO, H. C. S. et al. Effect of non-antifungal agrochemicals on the pathogenic fungus *Cryptococcus gattii*. *Medical Mycology*, v. 58, n. 1, p. 47-53, 2020. DOI: https://doi.org/10.1093/mmy/myz018.

CHANDER, J. (ed.). *Textbook of medical mycology*. New Delhi: Jaypee Publishers, 2018. Disponível em: https://books.google.com.au/books/about/Textbook_of_Medical_Mycology.html. Acesso em: 14 jul. 2025.

CLSI – Clinical and Laboratory Standards Institute. *M27-Ed4 and M60-Ed1: Reference method for broth dilution antifungal susceptibility testing of yeasts*. Wayne, PA: CLSI, 2017. Disponível em: https://clsi.org/about/news/clsi-publishes-new-and-updated-microbiology-documents/. Acesso em: 14 jul. 2025.

COLOMBO, A. L. et al. Current knowledge of *Trichosporon* spp. and trichosporonosis. *Clinical Microbiology Reviews*, v. 24, n. 4, p. 682-700, 2011. DOI: https://doi.org/10.1128/CMR.00003-11.

LARA, B. R. et al. Comparing the phenotypic, genotypic, and proteomic identification of *Trichosporon* species: a globally emerging yeast of medical importance. *Medical Mycology*, v. 59, n. 12, p. 1181-1190, 2021. DOI: https://doi.org/10.1093/mmy/myab050.

LO, C. et al. Disseminated fungal infection and fungemia caused by *Trichosporon asahii* in a captive plumed basilisk (*Basiliscus plumifrons*). *Journal of Fungi (Basel)*, v. 7, n. 12, p. 1003, 2021. DOI: https://doi.org/10.3390/jof7121003.

MARINÉ, M. et al. On and under the skin: emerging basidiomycetous yeast infections caused by *Trichosporon* species. *PLoS Pathogens*, v. 11, n. 7, e1004982, 2015. DOI: https://doi.org/10.1371/journal.ppat.1004982.

MEHTA, V. et al. A comprehensive review of *Trichosporon* spp.: an invasive and emerging fungus. *Cureus*, v. 13, n. 8, e17345, 2021. DOI: https://doi.org/10.7759/cureus.17345.

SINGH, S. et al. Epidemiology and antifungal susceptibility of infections caused by *Trichosporon* species: an emerging non-*Candida* and non-*Cryptococcus* yeast worldwide. *Indian Journal of Medical Microbiology*, v. 37, n. 4, p. 536-541, 2019. DOI: https://doi.org/10.4103/ijmm.IJMM_19_146.

TAKASHIMA, M. et al. Taxonomy of pathogenic yeasts *Candida, Cryptococcus, Malassezia* and *Trichosporon*. *Medical Mycology Journal*, v. 63, n. 4, p. 119-132, 2022. DOI: https://doi.org/10.3314/mmj.22.004.

VELAZQUEZ-MEZA, M. E. et al. Antimicrobial resistance: One Health approach. *Veterinary World*, v. 15, n. 3, p. 743-749, 2022. DOI: https://doi.org/10.14202/vetworld.2022.743-749.

WOODS, M. et al. A One Health approach to overcoming fungal disease and antifungal resistance. *WIREs Mechanisms of Disease*, v. 15, n. 4, e1610, 2023. DOI: https://doi.org/10.1002/wsbm.1610.

Curriculum vitae

Nome: Isabela Lima de Miranda

Lattes: https://lattes.cnpq.br/0115506996961611

Formação Acadêmica

- Bacharel em Ciências Biológicas

Atuação e Linha de Pesquisa

- Micologia Médica e Ambiental

Atividade Profissional

- Mestranda em Microbiologia – Laboratório de Micologia, Universidade Federal de Minas Gerais (UFMG)

Estágios

- Iniciação Científica (3 anos) no Laboratório de Micologia, Universidade Federal de Minas Gerais (UFMG)

Bolsas Obtidas

- CNPq – Bolsa de Iniciação Científica (3 anos)
- CAPES – Bolsa de Mestrado (2 anos)

Participação em Eventos

- Simpósios de Microbiologia da UFMG: 2021, 2022 e 2024
- X Congresso Brasileiro de Micologia (2024)

Participação em Minicursos

- Laboratório de Ensino em Experimentação Animal (2021)
- Ciências Forenses (2023)
- Identificação de Fungos Patogênicos – X Congresso Brasileiro de Micologia (2024)

Apresentações em Eventos (pôster)

- Semanas do Conhecimento da UFMG: 2021, 2022 e 2023
- Simpósios de Microbiologia da UFMG: 2021, 2022 e 2024
- X Congresso Brasileiro de Micologia (2024)

Publicações em Anais de Eventos

- Semanas do Conhecimento da UFMG: 2021, 2022 e 2023
- Simpósios de Microbiologia da UFMG: 2021, 2022 e 2024
- X Congresso Brasileiro de Micologia (2024)

Publicações em Revistas Especializadas

- 4 artigos publicados:
    - 2 como primeira autora (DOI: 10.1007/s11046-025-00969-z e DOI: 10.1007/s42770-025-01766-y)
    - 2 como coautora (DOI: 10.1021/acsinfecdis.4c00732 e DOI: 10.1016/j.scitotenv.2025.179139)

Resumo Profissional

Desde a graduação em Ciências Biológicas, desenvolvi forte motivação pela pesquisa científica. Durante três anos de iniciação científica, aprofundei meus conhecimentos em microbiologia médica e ambiental, adquirindo habilidades técnicas, pensamento crítico e paixão pela investigação. Nesse período, defini minha trajetória acadêmica, optando pela continuidade imediata no mestrado em Microbiologia.

No mestrado, a pesquisa tornou-se mais estruturada e aprofundada. Atualmente, atuo no Laboratório de Micologia da UFMG, investigando a magnitude, o impacto e os mecanismos de resistência antifúngica em *Trichosporon* spp., patógeno oportunista de relevância clínica e ambiental. Esse estudo integra abordagens da microbiologia médica e ambiental sob a perspectiva de Saúde Única (*One Health*), reconhecendo a interconexão entre saúde humana, animal e ambiental.

Minha trajetória científica reforçou a convicção de que a pesquisa é uma jornada contínua de descoberta. Por isso, considero o doutorado o próximo passo natural, permitindo aprofundar a compreensão dos mecanismos de resistência fúngica e contribuir para soluções inovadoras com impacto positivo na saúde humana, animal e ambiental.