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CLINICAL MICROBIOLOGY - REVIEW

Antifungal resistance on *Sporothrix* species: an overview

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Abstract

Introduction The treatment of human and animal sporotrichosis is often performed with antifungal agents; however, the emergence of antifungal-resistant strains of *Sporothrix* species has been reported. We aimed to discuss the ability of *Sporothrix* species in developing resistance to the conventional antifungals and mechanisms for this.

Methodology Published data on databases (PubMed, Science Direct, Google Scholar) were investigated using a combination of keywords from 2008 to 2019 by the StArt tool.

Results The minimal inhibitory concentrations values based on the Clinical and Laboratory Standards Institute (CLSI) from eight references were classified according to the epidemiological cutoff values in wild-type or non-wild-type strains. In this way, non-wild-type *S. schenckii* and, mainly, *S. brasiliensis* isolates were recognized on itraconazole, amphotericin B, terbinafine, and voriconazole, which are strains that deserve more attention toward antifungal control, with a probable risk of mutation to antifungal resistance. Among the few reviewed studied on antifungal resistance, the melanin production capacity (DHN-melanin, L-DOPA melanin, and pyomelanin), the low genetic diversity due to the abnormal number of chromosomes, and the mutation in cytochrome P450 are some of the factors for developing resistance mechanism.

Conclusions The emergence of *Sporothrix* species with in vitro antifungal resistance was evidenced and the possible mechanisms for resistance development may be due to the melanin production capacity, genetic diversity and mutations in cytochrome P450. Further studies should be carried out targeting gene expression for the development of antifungal resistance on *Sporothrix* species in order to prospect new therapeutic targets for human and veterinary use.

Keywords Sporotrichosis · *Sporothrix* species · Antifungal resistance · Melanin · Genetic diversity · Mutation · Gene expression

Introduction

Sporotrichosis is a fungal disease caused by the dimorphic species of the *Sporothrix* genus with a worldwide distribution, affecting both humans [1, 2] and animals [3, 4]. Among the species that most infect mammals, the clinical (pathogenic) clade refers to *Sporothrix schenckii* sensu stricto (*s. str.*), *S. brasiliensis*, *S. globosa*, and *S. luriei*, whereas *Sporothrix mexicana* belongs to the environmental clade [5].

Once a traumatic injury occurs with inoculation of *Sporothrix* conidia inside the skin, the clinical signs vary from fixed and multiple cutaneous to lympho-cutaneous and systemic forms [6], the worst presentations of which are often related to poor immunological conditions [7]. These factors can affect the healing using conventional therapy, which is recommended by performing the antifungal susceptibility assay for the choice of the antifungal agent and the effectiveness of the therapy. However, refractory cases to conventional therapies, such as itraconazole, have been reported, for example, in humans by

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S. schenckii [2] and by *S. globosa* [1], as well as in felines [8] by *S. schenckii* [9], and canines by *S. brasiliensis* [4]. This mini-review aimed to discuss the ability of *Sporothrix* species in developing resistance to antifungal agents.

Research strategy

Scientific studies were investigated by four independent examiners in the databases of PubMed, Science Direct, and Google Scholar from 2008 to 2019. For the research, the following keywords were used in two groups of combined keywords as a strategy: ["*Sporothrix schenckii* complex" or "sporotrichosis" or "*Sporothrix*"] and ["antifungal" or "antifungal susceptibility testing" or "antifungal resistance" or "resistance mechanism" or "melanin" or "mutation" or "gene expression"]. Studies on *Sporothrix* species from both human and animal sporotrichosis cases were included. No restriction of language was applied.

Reviews, meeting abstracts, chapter of books, as well as studies without in vitro antifungal susceptibility testing, such as molecular identification, the activity of plant extracts, or no antifungal resistance of *Sporothrix* species, were excluded (Fig. 1). The classification criteria of inclusion and exclusion were performed using the StArt (State of the Art through Systematic Review, version 2.3.4.2) tool. Regarding the antifungal susceptibility testing, only those that described lower minimal inhibitory concentration able to inhibit the fungal growth by 50% (MIC_{50}) and 90% (MIC_{90}) of the total isolates were included.

In vitro antifungal resistance on *Sporothrix* species

Standard guidelines of microdilution testing by Clinical and Laboratory Standard Institute (CLSI) [10] and European Committee for Antimicrobial Susceptibility Testing (EUCAST) [11] have shown the antifungal susceptibility profile of fungal species. In the last years, increasing reports of antifungal resistance have been related to *Sporothrix* species.

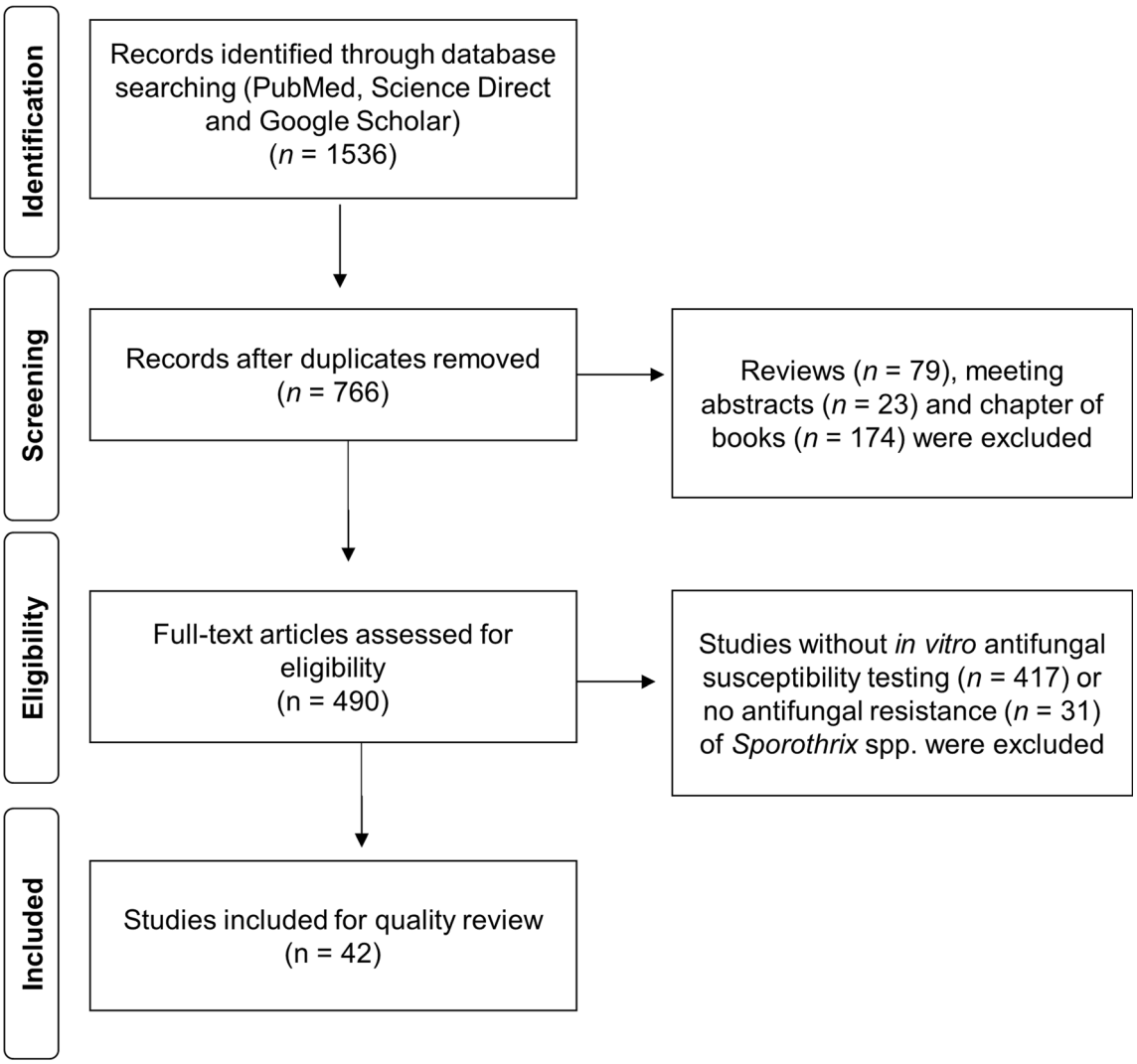

Fig. 1 Flowchart of research evidence and criteria for inclusion and exclusion of studies

Studies have classified the antifungal susceptibility of *S. schenckii*, in which the minimal inhibitory concentrations (MICs) values equal to or higher than 8 μg/mL may be considered resistance. In this way, *S. brasiliensis* from humans [1, 2, 12, 13] and animal [13–15] cases has been described as itraconazole-resistant isolates.

Recently, Espinel-Ingroff et al. [3] characterized the epidemiological cutoff values (ECVs) based on CLSI MICs data of antifungal agents available from worldwide laboratories. The ECV allows identifying the non-wild-type (non-WT) *Sporothrix* species with reduced susceptibility to the antifungal agent due to acquired mutational or resistance mechanism from the wild-type (WT) isolates, which are the isolates with no acquired resistance mechanism. For *S. schenckii* and *S. brasiliensis*, the following ECVs to identify the WT strains and non-WT strains are presented in Table 1. In Table 2, we compiled the MIC values of *S. schenckii* and *S. brasiliensis* tested by eight manuscripts and classified them as non-WT and WT, based on the ECVs proposed by Espinel-Ingroff et al. [3].

According to Table 2, *S. schenckii* from worldwide and Brazil were classified as non-WT isolates when tested with itraconazole, due to the high MIC_{50} [15] and MIC_{90} [12, 15], as well as with amphotericin B [12]. A similar finding was shown on the MIC_{90} of posaconazole against *S. schenckii* [16]. In turn, no *S. schenckii* isolates from Argentina were classified as non-WT among the tested antifungals [17].

Table 1 Epidemiological cutoff values (ECVs) of different antifungal agents for identifying the wild-type (WT) and non-wild-type (non-WT) strains of *Sporothrix schenckii* and *Sporothrix brasiliensis*, as proposed by Espinel-Ingroff et al. [3]*

Sporothrix species	Antifungal agents†	ECVs*	
		WT (μg/mL)	Non-WT (μg/mL) §
S. schenckii	AMB	≤ 4	> 4
	ITR	≤ 2	> 2
	POS	≤ 2	> 2
	VOR	≤ 64	> 64
S. brasiliensis	AMB	≤ 4	> 4
	ITR	≤ 2	> 2
	POS	≤ 2	> 2
	VOR	≤ 32	> 32
	KET	≤ 2	> 2
	TERB	≤ 0.12	>0.12

*ECVs values were based on minimal inhibitory concentrations (MIC) by microdilution test (M38-A2 guideline of the Clinical and Laboratory Standards Institute, CLSI [10], as proposed by Espinel-Ingroff et al. [3])

† *AMB*, amphotericin B; *ITR*, itraconazole; *POS*, posaconazole; *VOR*, voriconazole; *KTZ*, ketoconazole; *TRB*, terbinafine

§ Non-WT strains are recognized as *Sporothrix* spp. strains with reduced susceptibility to antifungals and, therefore, less likely to respond to the antifungal therapy

Among the studies that tested *S. brasiliensis* (Table 2), we observed non-WT isolates when tested with terbinafine [14, 16, 17], voriconazole [18], and itraconazole [14, 19]. On the other hand, WT *S. brasiliensis* isolates were recognized to several antifungals [20, 21]. Although itraconazole showed better activity against *S. brasiliensis* compared with *S. schenckii* [12, 16], high MIC values of this antifungal agent were recognized on animal-borne *S. brasiliensis* isolates from Southern Brazil [14, 19], which both MIC_{90} values classified these isolates as non-WT. Relating to terbinafine, high activity was shown against all *Sporothrix* species tested by Marimon et al. [16]. However, according to the ECVs proposed by Espinel-Ingroff et al. [3], the MIC_{90} values of this antifungal against *S. brasiliensis* were high, as well as those found on the MIC_{50}/MIC_{90} values of terbinafine against *S. brasiliensis* isolates from Southern Brazil [14] and Argentina [17]. These *S. brasiliensis* were all classified as non-WT strains and, therefore, are also potential terbinafine-resistant isolates. Besides that, MIC_{90} of voriconazole against *S. brasiliensis* [18] also was classified as non-WT.

Few studies using microdilution tests by the EUCAST were found on *Sporothrix* species. A study tested 91 Brazilian and Spanish *S. schenckii* strains by this guideline [22], showing an antifungal variability in this fungal species. Although this reference was not included in our study due to the lack of MIC_{50} and MIC_{90} values, Gutierrez-Galhardo et al. [22] showed the emergence of antifungal-resistant isolates of *S. schenckii* to fluconazole, itraconazole, voriconazole, posaconazole, and terbinafine. Considering the breakpoint [3], non-WT strains of *S. schenckii* to itraconazole (MIC range of 0.12 to > 8 μg/mL) and posaconazole (MIC range of 0.06 to > 8 μg/mL) are suggested, according to the susceptibility testing results using the EUCAST guideline and performed by Gutierrez-Galhardo et al. [22]. Besides that, an *S. schenckii* isolate from a cutaneous case of sporotrichosis in an immunocompetent patient showed high MIC values (> 8 μg/mL) for itraconazole, voriconazole, fluconazole, isavuconazole, and anidulafungin by EUCAST testing, suggesting in vitro resistance [23] and, therefore, a possibly non-WT strain with acquired resistance mechanism.

In this way, it is noteworthy that the emergence of *S. schenckii* and *S. brasiliensis*, classified as non-WT strains to itraconazole, amphotericin B, terbinafine, and voriconazole, is possibly less likely to respond the antifungal therapy and, therefore, potential-resistant isolates to these antifungal agents.

Mechanisms for resistance development

Melanin production capacity

Sporothrix species can produce three types of melanin [24]. DHN-melanin (I) is a synthesized substance by acetyl coenzyme A by polyketide standards, and L-DOPA-melanin (II) is synthesized by the L-3,4-dihydroxyphenylalanine (L-DOPA) pathway, wherein tyrosinases or laccases hydroxylate tyrosine

Table 2 Susceptibility profile of *Sporothrix schenckii* and *Sporothrix brasiliensis* from different geographic origins by microdilution test and classified as wild-type (WT) and non-wild-type (non-WT) *Sporothrix* species

Origin of the *Sporothrix* spp. (*n*), according to the cited reference	Antifungal agents *	MIC (μg/mL)			Profile § (MIC_{50}/ MIC_{90})
		Range	MIC_{50}	MIC_{90}	
Sporothrix schenckii					
Worldwide (34) [16]	AMB	0.5–4	4	4	WT/WT
	ITR	1–32	4	32	non-WT/non-WT
	POS	0.5–16	1	8	WT/non-WT
	VOR	2–32	32	32	WT/WT
Brazil (39) [12]	AMB	1–> 16	8	> 16	non-WT/non-WT
	ITR	0.25–> 16	1	4	WT/non-WT
	POS	0.06–> 16	1	2	WT/WT
	VOR	4–> 16	16	> 16	WT/n.s.
Argentina (13) [17]	AMB	0.5–4	2	2	WT/WT
	ITR	0.13–2	2	2	WT/WT
	POS	0.13–1	0.5	1	WT/WT
	VOR	8–16	8	16	WT/WT
Sporothrix brasiliensis					
Worldwide (23) [16]	AMB	1–4	2	4	WT/WT
	ITR	0.5–2	0.5	1	WT/WT
	POS	0.25–1	0.5	1	WT/WT
	VOR	0.5–16	4	8	WT/WT
	KET	0.06–0.5	0.125	0.25	WT/WT
	TERB	0.06–0.25	0.06	0.25	WT/non-WT
Brazil (48) [18]	AMB	0.125–4	1	2	WT/WT
	ITR	0.125–2	1	2	WT/WT
	VOR	2–64	16	64	WT/non-WT
	KET	0.03–2	0.25	1	WT/WT
Brazil (22) [12]	AMB	1–8	4	4	WT/WT
	ITR	0.25–4	1	2	WT/WT
	POS	0.5–2	1	2	WT/WT
	VOR	2–> 16	16	> 16	WT/n.s.
Brazil (35) [14]	AMB	0.125–8	1	4	WT/WT
	ITR	0.25–8	0.5	4	WT/non-WT
	TRB	0.0156–4	0.25	2	non-WT/non-WT
Brazil (29) [19]	ITR	0.125–> 16	1	> 16	WT/non-WT
Brazil (23) [20] †	AMB	0.25–4	1	2	WT/WT
	ITR	0.06–2	0.5	0.5	WT/WT
	VOR	1–16	8	16	WT/WT
	KET	0.03–1	0.12	0.5	WT/WT
	TRB	0.01–0.5	0.06	0.12	WT/WT
Brazil (25) [21]	AMB	0.5–4	2	4	WT/WT
	ITR	1–2	1	2	WT/WT
	POS	0.5–1	1	1	WT/WT
	VOR	2–> 16	8	> 16	WT/n.s.
	KET	0.5–2	1	2	WT/WT
	TRB	0.03–0.12	0.06	0.06	WT/WT
Argentina (8) [17]	AMB	0.5–4	2	4	WT/WT
	ITR	0.25–2	2	2	WT/WT

Table 2 (continued)

Origin of the *Sporothrix* spp. (*n*), according to the cited reference	Antifungal agents *	MIC (μg/mL)			Profile [§] (MIC_{50}/ MIC_{90})
		Range	MIC_{50}	MIC_{90}	
	POS	0.25–2	1	2	WT/WT
	VOR	8–16	8	16	WT/WT
	KET	0.13–2	1	2	WT/WT
	TRB	0.13–0.5	0.25	0.5	non-WT/non-WT

**MIC*, minimal inhibitory concentration; *AMB*, amphotericin B; *ITR*, itraconazole; *POS*, posaconazole; *VOR*, voriconazole; *KTZ*, ketoconazole; *TRB*, terbinafine; *n.s.*, not specific

[§] *Sporothrix* species tested by M38-A2 guideline of the Clinical and Laboratory Standards Institute [10] and classified as WT (susceptible isolates) and non-WT (potentially resistant isolates) and less likely to respond to the antifungal therapy, according to the epidemiological cutoff values (ECVs) proposed by Espinel-Ingroff et al. [3]; all MIC values of *Sporothrix* species were considered on mycelial phase and from papers in which the MIC range, MIC_{50} and MIC_{90} values were performed

[†] MIC_{50} and MIC_{90} values found by Stopiglia et al. [20] were calculated by Almeida-Paes et al. [21]

form dopaquinone via DOPA, which then self-oxidizes and polymerizes, resulting in a polyphenolic heteropolymer that is black. Finally, pyomelanin (III) is synthesized by L-tyrosine catabolism through p-hydroxyphenylpyruvate and homogentisic acid (HGA). These pigments are secondary metabolites consisting of phenolic and indolic monomers.

DHN-melanin synthesis does not require specific precursors, while the synthesis of L-DOPA and pyomelanin is increased in the presence of L-DOPA and L-tyrosine, respectively [24]. In the host, *Sporothrix* spp. synthetizes DHN and L-DOPA, which can protect fungi against antifungal and immune response, inhibiting phagocytosis and macrophage death [25] and suppress inflammation. Melanin biosynthesis in different species of *Sporothrix* spp. is not fully elucidated, but in general, the production of this pigment is higher and faster on *S. brasiliensis* than in *S. schenckii*.

The production of L-DOPA-melanin, pyomelanin, or DHN-melanin is associated with lower susceptibility to amphotericin B [26], with pyomelanin being more protective than L-DOPA-melanin. Besides, melanin protects *Sporothrix* species against the effect of terbinafine, an alternative antifungal to amphotericin B on sporotrichosis therapy. Although the biosynthesis pathways of DHN-melanin, L-DOPA-melanin, and pyomelanin are not fully elucidated on *Sporothrix* spp., the pathways are well established in *Aspergillus fumigatus*. In this fungal species, the DHN-melanin biosynthesis involves six genes (abr1, abr2, ayg1, arp1, arp2, and pks/alb1) located on the second chromosome [27]. Signals for expression of this cluster are unknown; however, it is believed to be related to conidiation. The synthesis of pyomelanin involves a cluster of six genes (hppD, hmgX, hmgA, fahA, maiA, and hmgR), also located on chromosome 2.

Unlike DHN-melanin synthesis, the pyomelanin synthesis is related to conidial germination, which is regulated by surface sensors, because some detect L-tyrosine or L-phenylalanine and others detect signs of stress on cell wall integrity, such as Wsc1, Wsc3, and MidA. These signals are transmitted via Rho GTPases and mitogen-activated protein (MAP) kinases, which in *A. fumigatus* is composed of BcK1, Mkk2, and MpkA [27]. In another study [28], the genome analysis of *S. schenckii* and *S. brasiliensis* by homology showed that the enzymes pigment-biosynthesis-protein yellowish-green 1, polyketide-synthase I and III, tetrahydroxyphenylpyruvate-trihydroxinaphtale-redutase, scytalone dehydratase, laccase, tyrosinase, and 4-hydroxyphenylpyruvate dioxygenase were involved in the melanin metabolism.

For a better understanding of the effect of melanin production on *Sporothrix* species against the conventional antifungals, further in vitro, in vivo, and in silico studies should be performed, focusing on how DHN-melanin, L-DOPA-melanin, and pyomelanin synthesis occur. It is important to study the pathogenic *Sporothrix* species in different cell phases (yeast and mycelial phases), allowing the prospecting of new therapeutic targets.

Genetic diversity

Genetic diversity may have important ecological consequences at the population, community, and ecosystem levels [29], since it is related to a better adaptive capacity under selection pressure on *Sporothrix* species with high genetic diversity [30, 31]. High degrees of antifungal resistance were recognized among clinical clades of *Sporothrix*, which showed multidrug-resistant phenotypes [12]. In this way, genetic diversity seems to play a role in the acquisition of variability in the antifungal susceptibility. Phylogenetic and population genetic analyses showed that there is a purifying selection process in the recent evolutionary past of *S. brasiliensis* and *S. globosa*, which are the species with the least genetic polymorphism [16, 32–35]. In turn, *S. schenckii s. str.* showed high polymorphism in size and number of chromosomes [32], which is related to the higher variability in the antifungal susceptibility [12, 16], virulence [36], and genomic organization [32].

The genus *Sporothrix* has high intrinsic antifungal resistance [12]; therefore, studies have sought to understand the role of genetic diversity in the variability of antifungal susceptibility and virulence in *Sporothrix* sensu latu *(s. l.)*. In vitro studies of antifungal susceptibility showed that *S. brasiliensis* and *S. schenckii s. str.* are more susceptible to antifungals than *S. globosa* and *S. mexicana* [16]. Among the clinical clades, *S. brasiliensis* and *S. schenckii s. str.* have been shown more in vitro susceptible to antifungals than *S. globosa* and *S. mexicana* [16]. In most clinical cases, *S. brasiliensis* responds well to antifungal drugs and shows lower MICs values [3, 12]. However, due to selective pressure, some fungal isolates may be induced to show polymorphism in the number and size of chromosomes, which seem to play a role in the development of clones with antifungal resistance genes.

However, it has recently been shown that isolates of *S. brasiliensis* recovered from cats and dogs living in the extreme south of Brazil showed low in vitro susceptibility to branded and compounded itraconazole formulations [37], characterizing them as itraconazole-resistant isolates. Considering that *S. brasiliensis* has at least two distinct sources in Brazil [34], the emergence of *S. brasiliensis* in Southern Brazil with antifungal resistance seems to be related to the genetic diversity. Phylogenetic analysis showed that *S. brasiliensis* strains from the Rio Grande do Sul differed from the strains recovered from cases in São Paulo, Minas Gerais, and Paraná, which share the same genotype of the Rio de Janeiro outbreak [34]. Moreover, genetic diversity is lower in *S. brasiliensis* in comparison with *S. schenckii s. str.* [33].

It is known that the prolonged exposure to antifungals and the host immunity may exert selective pressure on pathogenic fungi [30]. Although this phenomenon can occur spontaneously, it has also been observed in fungi exposed to antifungal agents, and this finding supports the involvement of chromosomal polymorphism in *Sporothrix* species for the acquisition of variability of antifungal susceptibility profiles [32, 34]. Changes in the genomic architecture and the emergence of an abnormal number of chromosomes were noted in triazoles-resistant *Candida albicans* and *Cryptococcus neoformans* [38–40]. The abnormal number of chromosomes is called aneuploidy, and offer additional copies of resistance genes, leading to the manifestation of a phenotype with antifungal resistance [38, 39] and quick adaptation [40]. These findings seem to play a role in the acquisition of antifungal resistance among *Sporothrix* species.

The molecular mechanisms involved in antifungal resistance are complex and still poorly understood. One of the tools suggested to better understand the mechanisms involved in the intrinsic and acquired resistance in the pathogenic species of *Sporothrix* species is the study of the differential expression of genes through transcriptomics and proteomics, which are still scarce. Further studies must be carried out to understand the mechanisms involved in the resistance of *Sporothrix* species under pressure by antifungals.

Mutations in cytochrome P450 monooxygenase

It is known that the azole antifungal agents act by inhibiting cytochrome P450 monooxygenases, particularly the P450 CYP51 that is involved in the ergosterol biosynthesis [41, 42]. Itraconazole is the first-choice antifungal for sporotrichosis treatment, whereas ketoconazole was found to be inefficient. Therefore, Matowane et al. [42] performed an in silico analysis with the focus on CYP51 to better elucidate the ketoconazole-resistance of *S. schenckii*. In this study, the presence of CYP51 in *S. schenckii* coupled with effective treatment with itraconazole suggests that there is inhibition of CYP51, the target of itraconazole. In silico analysis of CYP51 also revealed that mutations at the itraconazole-binding site, especially T230N, are related to increased azole resistance, as demonstrated with T229A equivalent mutation in *C. albicans*. The amino acid located at the entrance of the channel to the active site plays a key role related to resistance to ketoconazole. This study revealed new perspectives for a better understanding of the resistance of *S. schenckii* to azole class [42].

Concluding remarks

Sporothrix species from human and animal cases have shown ability in developing antifungal resistance to conventional antifungal, and *S. brasiliensis* is the fungal species with a high ability to acquire mutational or resistance mechanism. From the reviewed studies, few studies have focused on the mechanism of development of antifungals resistance. Although this ability is not fully elucidated, the resistance development on *Sporothrix* species was related to the melanin production capacity (DHN-melanin, L-DOPA-melanin, and pyomelanin), to the low genetic diversity possibly due to the abnormal number of chromosomes, and to the mutations in cytochrome P450. In this sense, further studies should be carried out targeting gene expression for the development of antifungal resistance to prospect new therapeutic targets.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Not applicable. This paper does not contain any studies with experimental animals.

Informed consent Not applicable. This paper does not contain any studies with human participants performed by any of the authors.

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