



# In vitro antifungal susceptibility of *Sporothrix globosa* isolates from Jilin Province, northeastern China: comparison of yeast and mycelial phases

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## Abstract

The dimorphic fungus *Sporothrix globosa* is the predominant etiologic agent causing sporotrichosis in China, particularly in the northeast. It has been demonstrated that the incubation temperature and growth phase can influence in vitro antifungal susceptibility profiles of *S. schenckii* sensu stricto and *S. brasiliensis* (sibling species of *S. globosa*). Few studies have reported on the antifungal susceptibility of *S. globosa*, especially using large numbers of isolates. In this study, we assessed the susceptibility of 80 isolates of *S. globosa* originating from Jilin Province, northeastern China, to six antifungal agents (itraconazole, terbinafine, voriconazole, posaconazole, fluconazole, and amphotericin B), at varying incubation temperatures and in different fungal growth phases. The isolates were most sensitive to terbinafine (geometric mean [GM] of the minimum inhibitory concentration [MIC]: 0.0356 µg/ml for the mycelial phase at 30 °C, 0.0332 µg/ml for the mycelial phase at 35 °C, and 0.031 µg/ml for the yeast phase, respectively), followed by posaconazole (GM of the MIC: 4.2501 µg/ml for the mycelial phase at 30 °C, 1.4142 µg/ml for the mycelial phase at 35 °C, and 0.7195 µg/ml for the yeast phase, respectively) and itraconazole (GM of the MIC: 6.8448 µg/ml for the mycelial phase at 30 °C, 3.1383 µg/ml for the mycelial phase at 35 °C, and 1.0263 µg/ml for the yeast phase, respectively). The isolates were relatively resistant to fluconazole (GM of the MIC: 76.7716 µg/ml for the mycelial phase at 30 °C, 66.2570 µg/ml for the mycelial phase at 35 °C, and 24.4625 µg/ml for the yeast phase, respectively) and voriconazole (GM of the MIC: 26.2183 µg/ml for the mycelial phase at 30 °C, 13.6895 µg/ml for the mycelial phase at 35 °C, and 1.3899 µg/ml for the yeast phase, respectively). For all the tested azole drugs, the MICs at 30 °C were significantly higher than those at 35 °C ( $P < 0.001$ ); for all agents except terbinafine, the MICs of *S. globosa* in the yeast phase were significantly lower than those of the strains in the mycelial phase ( $P < 0.001$ ). These results show that the sensitivities of *S. globosa* to antifungal compounds are dependent on incubation temperature and growth phase. To the best of our knowledge, this is the largest study of antifungal susceptibility of *S. globosa* isolates reported to date. To establish epidemiological cutoff values for *S. globosa*, further antifungal susceptibility testing studies by independent laboratories located in different regions and using uniform conditions are required.

**Keywords** Growth phase · *Sporothrix globosa* · *Sporothrix schenckii* · Antifungal susceptibility · Incubation temperature

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## Introduction

Sporotrichosis is a chronic subcutaneous mycosis affecting humans and animals caused by species of the dimorphic fungi of the genus *Sporothrix*, such as *S. brasiliensis*, *S. schenckii* sensu stricto, and *S. globosa* [1, 2]. *S. globosa* is distributed worldwide, with isolates reported from Latin America, Spain, Japan, Madagascar, Iran, India, and China [3–5]. In Jilin Province, northeastern China, where the largest number of sporotrichosis cases are reported in the world, almost all the patients are from underdeveloped rural areas [6]. *S. globosa* is the only etiologic agent of sporotrichosis reported in this area [7]. The predominant types of infection are fixed and

lymphocutaneous sporotrichosis; these conditions are not life-threatening, but require long-term, regular chemotherapy. Therefore, the length and cost of treatment are important factors affecting patient compliance, and consequently influence the effect of treatment. Clinical resistance to antifungals in sporotrichosis patients has also been reported recently [8]. Research findings from antifungal susceptibility studies are important in guiding clinical therapy.

An incubation temperature of 35 °C is recommended for in vitro antifungal susceptibility testing using the broth microdilution method for filamentous fungi (nondermatophyte molds) of the Clinical and Laboratory Standards Institute (CLSI; reference method M38); 30 °C can also be used for certain fungi [9]. When compared with *S. schenckii* and *S. brasiliensis*, the poor thermotolerance of *S. globosa* limits growth at 35 °C [7, 10]. Therefore, an incubation time of > 72 h is needed for *S. globosa*, compared with 48–72 h for the *S. schenckii* complex, to guarantee the quality of the growth control. As a result, the susceptibility testing parameters for *S. schenckii* and *S. brasiliensis* are not consistent with those for *S. globosa*. There are also significant differences of antifungal susceptibility between the mycelial and yeast growth phases of *S. schenckii* and *S. brasiliensis* [11, 12]. However, there has been no assessment of the influence of incubation temperature and growth phase on the susceptibility of *S. globosa* using a large number of isolates.

This study investigated the in vitro antifungal susceptibility of 80 isolates of *S. globosa* originating from Jilin Province, northeastern China, to six antifungal agents at different incubation temperatures and by growth phase.

## Materials and methods

### Fungal strains and culture conditions

Eighty isolates of *S. globosa* from human sporotrichosis patients were obtained from different regions of Jilin Province, northeastern China (Fig. 1). All the isolates were identified as *S. globosa* by morphology and molecular identification (amplification and sequencing of the calmodulin gene, *CAL*; see [supplementary material](#)). The origins of isolates and GenBank accession numbers are shown in Table 1.

Strains of *Candida parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) were used as quality control strains, and were provided by Dr. Yu Zhang (Second Hospital of Jilin University, Changchun, China). *S. globosa* ATCC MYA-4912 was used as the reference strain. Mycelial-phase strains were obtained by growth on potato-dextrose-agar (PDA; BD, Franklin Lakes, NJ, USA) at 30 °C for 10–14 days. To convert strains into the yeast phase, mycelial cultures were subcultured on brain heart infusion (BHI; BD)-agar at 35 °C

for 7 days. Three successive passages were performed to obtain the yeast phase.

### Antifungal drugs

Six antifungal compounds were used: itraconazole (ITZ; Tokyo Chemical Industry Co., Ltd. Tokyo, Japan), terbinafine (TRB; Tokyo Chemical Industry Co., Ltd.), voriconazole (VCZ; Tokyo Chemical Industry Co., Ltd.), posaconazole (PCZ; Toronto Research Chemicals, Toronto, ON, Canada), and amphotericin B (AMB; Sigma-Aldrich, St. Louis, MO, USA), which were dissolved in dimethyl sulfoxide (Bio Basic Inc., Toronto, ON, Canada) at 1.6 mg/ml as a stock solution, and fluconazole (FCZ; Tokyo Chemical Industry Co., Ltd.), which was dissolved in water at 2.56 mg/ml. The final concentrations used in susceptibility tests were 0.06–32 µg/ml for AMB and VCZ, 0.03–16 µg/ml for ITZ and PCZ, 0.0078–4 µg/ml for TRB, and 128–0.25 µg/ml for FCZ.

### Mycelial and yeast inoculum preparation

Antifungal susceptibility tests were conducted according to CLSI protocols M27 and M38 for yeast and mycelial phases respectively with some modifications [9, 13]. Subculture of *S. globosa* mycelial phase was incubated on PDA at 30 °C for 7 days, and then 1 ml of sterile 0.85% saline solution was added. The conidia were resuspended by scraping the culture surface with a sterile tip. The suspension was transferred to a 2-ml sterile tube and allowed to settle for 5 min. The upper homogeneous suspension was transferred to a sterile tube and mixed by vortexing for 15 s. The density of the suspension was adjusted to an optical density (OD) of 0.09–0.13 based on the absorbance at 530 nm. This suspension was diluted 1:50 in RPMI 1640 culture medium (Gibco, Life Technologies, NY, USA) with 3-(N-morpholino) propanesulfonic acid (Genview, Beijing, China) buffer (pH 7.0). The concentration of this suspension was approximately  $0.4 \times 10^4$  to  $5 \times 10^4$  colony-forming units (CFU)/ml.

Subculture of *S. globosa* yeast phase was followed by three passages of growth on BHI-agar at 35 °C for 7 days. A suspension was produced as described above for the mycelial phase and adjusted to  $1 \times 10^6$  to  $5 \times 10^6$  CFU/ml with sterile 0.85% saline by counting the yeast cells using a hemocytometer. This suspension was then diluted 1:50 in sterile 0.85% saline and subsequently 1:20 in RPMI 1640 culture medium, resulting in a concentration of  $1 \times 10^3$  to  $5 \times 10^3$  CFU/ml. The concentration of the inoculum was checked by determination of colony counts on PDA.

### Antifungal susceptibility test

Inocula (yeast or mycelial) were used to fill each well of 96-well round-bottom microplates (Greiner Bio-One,

**Fig. 1** Maps showing the origin of *Sporothrix globosa* isolates in Jilin Province, northeastern China



Frickenhäusen, Germany) with 0.1 ml of suspension. The cell suspension was then diluted with 0.1 ml of RPMI 1640 culture medium containing serial dilutions of antifungal compounds to obtain the final concentration. Cell suspensions without antifungal compounds and RPMI 1640 culture medium were used as growth controls and blank controls, respectively. The microplates were incubated at 35 °C (yeast phase) for 72 h, or at 30 °C and 35 °C (mycelial phase) for 48–72 h. The results were detected by visual observation. For the mycelial phase, the minimum inhibitory concentration (MIC) of FCZ was defined as the minimum concentration that inhibited 50% of growth, the MIC of TRB was defined as the minimum concentration that inhibited 80% of growth, and the MICs of all other drugs were defined as the minimum concentration that inhibited 100% of growth compared with the growth controls. For the yeast phase, the MIC of AMB was defined as the minimum concentration that inhibited 100% of growth, the MIC of TRB was defined as the minimum concentration that inhibited 80% of growth, and the MICs of triazoles were defined as the minimum concentration that inhibited 50% of growth compared with the growth controls.

To determine minimal fungicidal concentrations (MFCs), 10- $\mu$ l aliquots from wells showing 100% growth inhibition were spread on Sabouraud dextrose agar (BD) plates in

duplicate and incubated at 30 °C for 7 days. The MFC was defined as the lowest concentration of antifungal that killed approximately 99% to 99.5% of the fungi (i.e., such that < 3 colonies grew) [14, 15]. All tests were conducted in duplicate [11].

### Statistical analysis

The Mann–Whitney *U* test was used for comparisons between results from the yeast and mycelial phases and between results for the antifungal agents (SPSS software v18.0, IBM, New York, NY, USA). A *P* value < 0.05 was considered statistically significant. For each antifungal tested, values of MIC<sub>50</sub> and MIC<sub>90</sub> (i.e., the lowest antifungal concentrations able to inhibit 50% and 90% of the fungal growth of the isolates, respectively), as well as MFC<sub>50</sub> and MFC<sub>90</sub> (i.e., the lowest concentrations able to kill 50% and 90%, respectively, of the fungal isolates), were calculated.

### Results

The antifungal susceptibility profiles of 80 isolates of *S. globosa* in the mycelial and yeast phases are presented

**Table 1** The 80 isolates of *Sporothrix globosa* used in this study

Origin	Isolate ID	Gender (M/F)	Age (year)	Clinical form	GenBank accession code
Baicheng City ( <i>n</i> = 5)	FHJU11051803	M	0.25	F	KY350125
	FHJU11053102	F	57	D	KY350126
	FHJU11110301	F	43	F	KY349940
	FHJU11122805	M	34	F	KY349945
	FHJU12061704	F	44	F	KY349939
Baishan City ( <i>n</i> = 3)	FHJU09042601	F	54	F	KY349965
	FHJU10122702	F	24	F	KY350091
	FHJU13031801	F	51	F	KY349978
Changchun city ( <i>n</i> = 15)	FHJU11061001	M	9	L	KY350041
	FHJU12013003	F	68	L	KY350077
	FHJU12020401	F	42	F	KY350082
	FHJU12021201	F	51	L	KY350084
	FHJU12021506	F	54	L	KY350093
	FHJU12031903	M	33	F	KY350042
	FHJU12032401	F	60	L	KY350062
	FHJU12050403	F	9	L	KY350039
	FHJU12052202	F	79	F	KY350063
	FHJU12052302	F	62	L	KY350079
	FHJU12061503	F	47	F	KY350067
	FHJU12061601	F	37	F	KY350083
	FHJU12062301	F	6	F	KY350045
	FHJU12082002	F	64	F	KY350070
	FHJU13032302	M	17	F	KY350001
Jilin City ( <i>n</i> = 10)	FHJU11030803	F	75	L	KY350129
	FHJU11052001	M	4	F	KY349993
	FHJU11102001	M	4	F	KY349972
	FHJU12021602	F	44	L	KY349987
	FHJU12030604	F	5	F	KY349988
	FHJU12031206	M	60	F	KY349983
	FHJU12032601	M	6	F	KY349992
	FHJU12040304	M	12	F	KY349990
	FHJU12062602	F	54	L	KY349984
	FHJU12091101	F	59	L	KY349973
Liaoyuan City ( <i>n</i> = 7)	FHJU11021301	F	46	F	KY349979
	FHJU11021806	M	48	D	KY349976
	FHJU11061302	F	70	L	KY350006
	FHJU11070404	M	59	L	KY350005
	FHJU11120503	M	1.5	F	KY349994
	FHJU12061702	F	28	L	KY350004
	FHJU13041102	F	42	F	KY349975
Siping City ( <i>n</i> = 15)	FHJU11011004	M	3	F	KY349996
	FHJU11011202	F	47	L	KY350034
	FHJU11021107	F	6	L	KY350012
	FHJU11022201	M	48	L	KY349998
	FHJU11022802	F	67	L	KY350018
	FHJU12020201	F	55	F	KY350023
	FHJU12022803	M	2	F	KY350037
	FHJU12033001	M	82	L	KY350009
	FHJU12040302	F	70	F	KY349995

**Table 1** (continued)

Origin	Isolate ID	Gender (M/F)	Age (year)	Clinical form	GenBank accession code
Songyuan City (n = 15)	FHJU12041004	F	44	F	KY350130
	FHJU12050903	M	55	L	KY350022
	FHJU12051605	F	24	F	KY350010
	FHJU12062901	F	4	F	KY350000
	FHJU12080901	F	54	F	KY349997
	FHJU13032301	M	14	L	KY350019
	FHJU11062006	F	50	F	KY350028
	FHJU11081502	M	25	F	KY349970
	FHJU11090602	F	58	F	KY349957
	FHJU11112801	M	55	F	KY349969
	FHJU11120502	F	75	F	KY350132
	FHJU12021402	F	62	L	KY350029
	FHJU12022102	F	54	L	KY349971
	FHJU12030901	M	43	F	KY350035
	FHJU12032602	F	58	F	KY350031
	FHJU12032804	F	5	F	KY349935
	FHJU12041902	M	55	F	KY350033
	FHJU12051002	F	64	F	KY349966
	FHJU12082201	F	55	L	KY349937
	Tonghua City (n = 5)	FHJU13031803	F	46	F
FHJU13040501		M	10	F	KY349961
FHJU11111102		M	44	L	KY350050
FHJU11122402		F	50	F	KY350027
FHJU12053001		F	49	F	KY350025
Yanbian Korean Autonomous Prefecture (n = 5)	FHJU13022601	F	58	L	KY350003
	FHJU13051103	F	7	F	KY350002
	FHJU11102401	F	3	F	KY350047
	FHJU11121301	F	77	L	KY350049
	FHJU11122602	M	7	L	KY350118
	FHJU12031204	F	68	L	KY350119
	FHJU12040702	M	65	F	KY350115

F, female; M, male; FHJU, First Hospital of Jilin University

Clinical form: F, fixed cutaneous; L, lymphocutaneous; D, disseminated cutaneous

in Table 2. The MICs for the quality control strains were consistent with CLSI guidelines, and the results for the reference strain *S. globosa* ATCC MYA-4912 are shown in the [supplementary material](#). First, the effects of six antifungal drugs were compared. Regardless of the growth phase or incubation temperature, TRB showed the lowest MICs, significantly lower than for all the other tested antifungals (all *P* values < 0.001). Meanwhile, the geometric means (GMs) of the MICs of TRB were 0.0356 µg/ml for the mycelial phase at 30 °C, 0.0332 µg/ml for the mycelial phase at 35 °C, and 0.031 µg/ml for the yeast phase, respectively. FCZ was the antifungal with the highest MICs and the GMs were 76.7716 µg/ml for the mycelial phase at 30 °C,

66.2570 µg/ml for the mycelial phase at 35 °C, and 24.4625 µg/ml for the yeast phase, respectively. The MICs of PCZ obtained with the mycelial phase at 30 °C and 35 °C and the yeast phase were significantly lower than those of all the other antifungal drugs except TRB (all *P* values < 0.05). In both the mycelial (30 °C and 35 °C) and yeast phases, > 60% of the *S. globosa* isolates tested had MICs within the range of three serial dilutions of the antifungal concentration (Fig. 2). The ranges of the MICs for AMB and PCZ for the mycelial phase at 35 °C were wider than those for the other agents evaluated, ranging from 0.25–> 32 to 0.0313–16 µg/ml, respectively.

The MICs of *S. globosa* in the yeast phase were significantly lower than the MICs for the mycelial phase at both



**Table 2** MICs of six antifungal agents against the mycelial and yeast growth phases at different incubation temperatures of 80 isolates of *S. globosa*

Growth phase/temp (°C)	Parameter	MIC (µg/ml)					
		ITZ	AMB	VCZ	PCZ	TRB	FCZ
M/30	Range	0.25–> 16 <sup>A,a,b</sup>	1–> 32 <sup>A,a,b</sup>	4–> 32 <sup>A,a,b</sup>	0.5–> 16 <sup>A,a</sup>	0.078–1	8–> 218 <sup>A,a,b</sup>
	GM	6.8448	13.2232	26.2183	4.2501	0.0356	76.7716
	MIC <sub>50</sub>	8	16	32	4	0.0313	128
	MIC <sub>90</sub>	16	32	> 32	16	0.125	> 128
M/35	Range	0.125–> 16 <sup>B,a,b</sup>	0.25–> 32 <sup>a,b</sup>	1–32 <sup>B,a,b</sup>	0.0313–16 <sup>B,a</sup>	0.0078–1	4–> 218 <sup>B,a,b</sup>
	GM	3.1383	12.6626	13.6895	1.4142	0.0332	66.2570
	MIC <sub>50</sub>	4	16	16	2	0.0313	64
	MIC <sub>90</sub>	16	32	32	4	0.0625	> 218
Y/35	Range	0.0313–4 <sup>C,a,b</sup>	0.125–32 <sup>C,a,b</sup>	0.0625–8 <sup>C,a,b</sup>	0.0313–4 <sup>C,a</sup>	< 0.0078–3	2–128 <sup>C,a,b</sup>
	GM	1.0263	6.1156	1.3899	0.7195	0.031	24.4625
	MIC <sub>50</sub>	1	8	2	1	0.0313	16
	MIC <sub>90</sub>	2	16	4	2	0.125	64

MIC, minimum inhibitory concentration; GM, geometric mean; MIC<sub>50</sub>, MIC at which 50% of the isolates were inhibited; MIC<sub>90</sub>, MIC at which 90% of the isolates were inhibited; ITZ, itraconazole; AMB, amphotericin B; VCZ, voriconazole; PCZ, posaconazole; TRB, terbinafine; FCZ, fluconazole

A indicates a significant difference ( $P < 0.001$ ) compared with the MICs in the yeast phase (Mann–Whitney  $U$  test)

B indicates a significant difference ( $P < 0.001$ ) compared with the MICs in the mycelial phase at 30 °C (Mann–Whitney  $U$  test)

C indicates a significant difference ( $P < 0.001$ ) compared with the MICs in the mycelial phase at 35 °C (Mann–Whitney  $U$  test)

a indicates a significant difference ( $P < 0.05$ ) in the MICs compared with TRB in the same conditions (Mann–Whitney  $U$  test)

b indicates a significant difference ( $P < 0.05$ ) in the MICs compared with PCZ in the same conditions (Mann–Whitney  $U$  test)

temperatures for AMB, VCZ, ITZ, PCZ, and FCZ ( $P < 0.001$ ); the only exception was for TRB. The MICs obtained for the mycelial phase at 30 °C were significantly higher than those obtained for the same phase at 35 °C for VCZ, ITZ, PCZ, and FCZ ( $P < 0.001$ ), but not AMB and TRB. The susceptibility to VCZ was the most significantly different between the two phases; the GMs of the MICs for the mycelial phase at 30 °C and 35 °C were nearly 26 and 10 times higher, respectively, than that for the yeast phase. The GM of the MIC for AMB at 30 °C (13.2232 µg/ml) was higher than that at 35 °C (12.6626 µg/ml), although this difference was not significant ( $P = 0.902$ ).

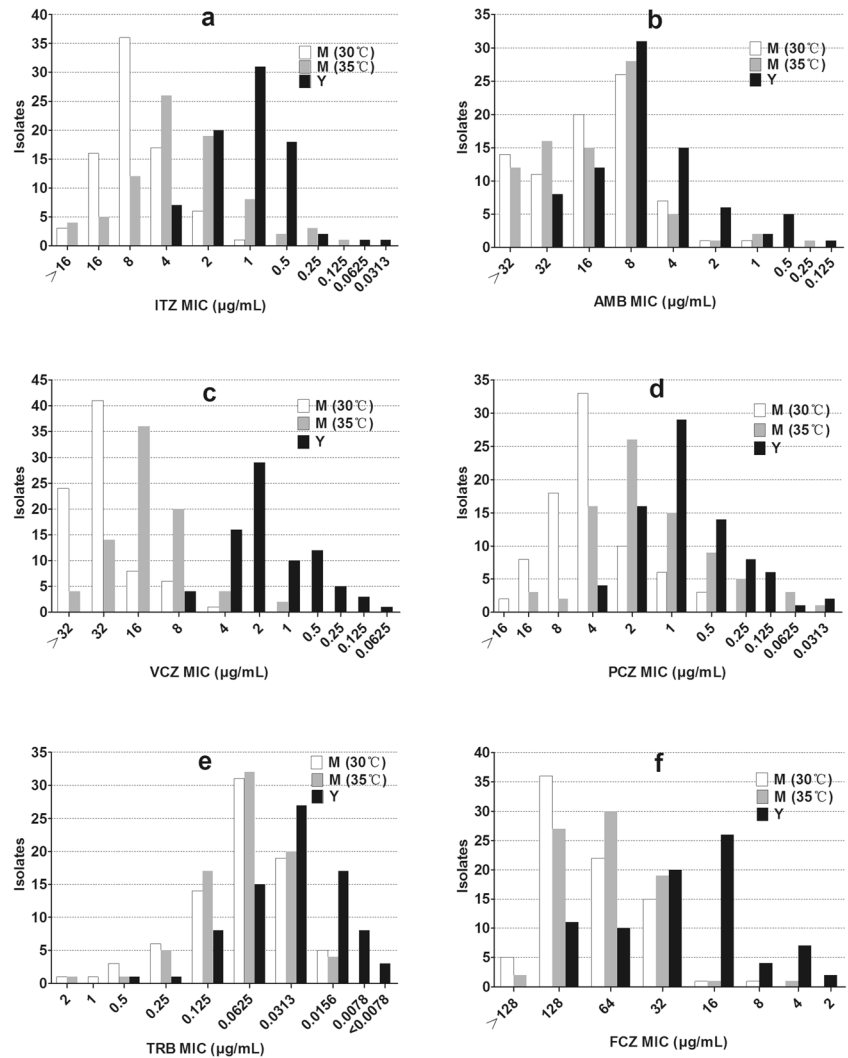
As the MICs for most isolates in the mycelial phase at 30 °C were near or above the maximal drug concentrations, the MFCs for the mycelial phase at 30 °C were not compared. The MFC values for TRB were significantly lower than those of the other compounds ( $P < 0.001$ ), and the GMs of the MFC measured for the mycelial phase at 35 °C and the yeast phase were 0.1272 µg/ml and 0.0989 µg/ml, respectively. The MFC<sub>90</sub> values for the antifungals tested against both phases were higher than the maximum concentrations of the antifungal agents in the susceptibility testing, except for TRB and PCZ. For the azoles used against the yeast phase, the MFCs were at least eightfold higher than the MICs. Meanwhile, the MFCs for AMB and TRB were fourfold higher than the MICs (Table 3).

## Discussion

The conditions used for in vitro susceptibility testing, such as incubation temperature and growth phase, were closely related to the sensitivity to antifungal agents of *S. schenckii* sensu stricto and *S. brasiliensis* [11, 16]. However, there are few reports for *S. globosa*, especially studies assessing large numbers of isolates. Virulence profiles indicate that *S. globosa* is less pathogenic than *S. brasiliensis* and *S. schenckii*, which might explain why fewer sporotrichosis patients are identified as infected with *S. globosa* compared with the other species [10]. The thermotolerance of *S. globosa* is also lower than that of the other two species; at a culture temperature of 35 °C, *S. globosa* grows slowly or is growth limited, whereas *S. schenckii* and *S. brasiliensis* grow rapidly in the same conditions [10, 17]. Therefore, the conditions used for in vitro susceptibility testing of the *S. schenckii* complex (CLSI recommend 35 °C for 48–72 h) are probably not suitable for *S. globosa*. However, there is no previous report on the sensitivity to antifungals of *S. globosa* at different temperatures.

Our in vitro susceptibility profiles demonstrated that as the incubation temperature of the microplates increased, the MICs of the antifungals tested against *S. globosa* were significantly decreased, except for AMB and TRB. Trilles et al. [12] found that the incubation temperature exerted a significant influence on the MICs of drugs toward *S. schenckii* sensu lato. However, their study showed the changing trends of the

**Fig. 2** Distribution of itraconazole (a), amphotericin B (b), voriconazole (c), posaconazole (d), terbinafine (e), and fluconazole (f) minimum inhibitory concentrations, tested at 30 or 35 °C against the mycelial (M) and yeast (Y) phases of *S. globosa* isolates (*n* = 80) from Jilin Province, northeastern China



**Table 3** MFCs of six antifungal agents against the mycelial and yeast growth phases of 80 isolates of *S. globosa*

Growth phase/temp (°C)	Parameter	MFC (µg/ml)					
		ITZ	AMB	VCZ	PCZ	TRB	FCZ
M/35	Range	2–> 16 <sup>A,a,b</sup>	1–> 32 <sup>A,a,b</sup>	> 32 <sup>A,a,b</sup>	0.25–> 16 <sup>A,a</sup>	0.0156–4	64–> 128 <sup>a,b</sup>
	GM	> 16	> 32	> 32	10.3747	0.1271	> 128
	MFC <sub>50</sub>	> 16	32	> 32	16	0.125	> 128
	MFC <sub>90</sub>	> 16	> 32	> 32	> 16	0.25	> 128
Y/35	Range	0.5–> 16 <sup>a,b</sup>	0.5–> 32 <sup>a,b</sup>	1–> 32 <sup>a,b</sup>	0.25–> 16 <sup>a</sup>	0.0078–2	32–> 128 <sup>a,b</sup>
	GM	13.5714	22.82432	19.0273	8.4268	0.0989	> 128
	MFC <sub>50</sub>	16	32	32	8	0.25	> 128
	MFC <sub>90</sub>	> 16	> 32	> 32	16	2	> 128

MFC, minimum fungicidal concentration; GM, geometric mean; MFC<sub>50</sub>, MFC at which 50% of the isolates were killed; MFC<sub>90</sub>, MFC at which 90% of the isolates were killed; ITZ, itraconazole; AMB, amphotericin B; VCZ, voriconazole; PCZ, posaconazole; TRB, terbinafine; FCZ, fluconazole

A indicates a significant difference (*P* < 0.005) in the MFCs compared with the yeast phase (Mann–Whitney *U* test)

a indicates a significant difference (*P* < 0.05) in the MFCs compared with TRB in the same conditions (Mann–Whitney *U* test)

b indicates a significant difference (*P* < 0.05) in the MFCs compared with PCZ in the same conditions (Mann–Whitney *U* test)

MICs were independent of the classification of the antifungal agents in their study, e.g., the GMs of the MICs for ITZ were 4.08  $\mu\text{g/ml}$  at 30 °C and 1.47  $\mu\text{g/ml}$  at 35 °C, while the GMs of the MICs for VCZ were 9.81 and > 16  $\mu\text{g/ml}$ , respectively. Such results are mostly related to the species of the isolates tested. For *S. globosa*, the higher incubation temperature would be accompanied by prolonged incubation time (> 72 h), which can be attributed to the lower thermotolerance of this strain. Our study showed, in contrast to triazoles, that the GM of the MICs for AMB exhibited no difference between incubation at 35 °C and 30 °C (13.2232  $\mu\text{g/ml}$  at 30 °C and 12.6626  $\mu\text{g/ml}$  at 35 °C). Similar results were also found for *Paracoccidioides brasiliensis* [18], another dimorphic fungus. This difference between drug types is associated with the mechanisms by which they inhibit fungal growth. The triazoles competitively inhibit the activity of intracellular cytochrome P450 [19] and lanosterol 14 alpha-demethylase in fungi [20], while AMB inhibits the synthesis of the fungal membrane by binding to ergosterol [21]. The process of AMB binding with ergosterol could be more effective at higher temperatures [22].

In our study, the MICs of the antifungals for *S. globosa* in the yeast phase were significantly lower than those for the fungus in the mycelial phase. These results were consistent with previous reports [4, 23]. Mahmoudi et al. assayed the antifungal susceptibilities of four *S. globosa* isolates, collected from Iran, to five antifungal agents and the results showed that the MICs of ITZ, VCZ, and FCZ were lower for the fungus in the yeast phase than in the mycelial phase [4]. Two isolates from Argentina also showed differences between phases with respect to AMB, FCZ, and ITZ [23]. It is possible that the structure of the cell wall changes when *S. globosa* transitions to the yeast phase, making its cell wall more permeable than that in the mycelial phase.

Since *S. schenckii* was reclassified as a complex [10, 17], several in vitro antifungal susceptibilities have been reassessed to verify the differences among different strains. Marimon tested 92 isolates belonging to five species of the *S. schenckii* complex in vitro using 12 antifungal agents, and the results showed significant differences in their responses [24]; Stopiglia tested the susceptibility of 85 isolates identified as *S. schenckii*, *S. brasiliensis*, and *S. globosa* to six antifungal agents and found no difference among different strains [25]. However, because of a lack of sufficient isolates, it is difficult to assess whether the antifungal susceptibility is different among different species of the *S. schenckii* complex. Recently, epidemiologic cutoff values (ECVs) for antifungal agents against *S. schenckii sensu stricto* and *S. brasiliensis* have been proposed, based on data obtained from 17 independent laboratories for a large number of isolates [26]. The experimental conditions for establishing the MICs were an inoculum of conidial suspension and an incubation temperature of 35 °C. Meanwhile, the MICs of five antifungal agents against

*S. globosa* were also tested. The most frequently obtained MICs against *S. schenckii sensu stricto*, *S. brasiliensis*, and *S. globosa* were 1, 1, and 2  $\mu\text{g/ml}$ , respectively, for AMB; 0.5, 1, and 0.5  $\mu\text{g/ml}$ , respectively, for ITZ; 1, 1, and 0.5  $\mu\text{g/ml}$ , respectively, for PCZ; 16, 8, and 8  $\mu\text{g/ml}$ , respectively, for VCZ; and 0.5, 0.06, and 0.06  $\mu\text{g/ml}$ , respectively, for TRB. The antifungal susceptibilities of these fungi showed differences, and *S. globosa* was the most sensitive to the antifungal agents. The GMs of the MICs of *S. globosa* were 1.35  $\mu\text{g/ml}$  for AMB, 0.61  $\mu\text{g/ml}$  for ITZ, 0.75  $\mu\text{g/ml}$  for PCZ, 6.21  $\mu\text{g/ml}$  for VCZ, and 0.57  $\mu\text{g/ml}$  for TRB [26]. The isolates tested in the present study were sensitive to TRB and resistant to VCZ. However, the GM of the MIC of most of the isolates tested with AMB in a previous study [24] was significantly lower than that observed in the present study (12.6626  $\mu\text{g/ml}$ ), which might be related to the origin of the isolates. We found no significant association between the clinical characteristics of sporotrichosis (e.g., the clinical form, patient gender, patient age) and the antifungal susceptibility of *S. globosa*. Hence, the clinical manifestation of the disease is most likely related to the immune status of the patient, rather than the susceptibility of the infecting strain.

Our in vitro susceptibility profiles showed that TRB was the most active agent against the 80 isolates of *S. globosa* tested regardless of incubation temperature or growth phase, which was consistent with previous reports [27, 28]. Our previous survey showed that the average clinical treatment with TRB was for 2.21 months with a dose of 250 mg/day for cutaneous or lymphocutaneous sporotrichosis, compared with 1.86 months (200 mg/day) for treatment with ITZ [29]. A similar observation was reported in Brazil, where the cure rates for TRB (250 mg/day, 11.5 weeks) and ITZ (100 mg/day, 11.8 weeks) were 92.7% and 92%, respectively [30]. Although TRB showed the potential to treatment systemic sporotrichosis when evaluated in an experimental model [31], the clinical application of TRB is less effective, which might be related to distribution of the pathogen in the host [21]. Furthermore, the optimal dose and duration of treatment by TRB is a subject of debate among clinical practitioners [32]. TRB is still not recommended as a first-line treatment for cutaneous sporotrichosis. It is also not recommended for disseminated sporotrichosis [31]. This emphasizes that discordance may exist between in vitro and in vivo conditions, which indicates caution is needed when interpreting in vitro antifungal susceptibility results.

In our study, PCZ showed higher activity against *S. globosa* than ITZ; the latter is the first-line therapy in treatment of sporotrichosis [33]. Nonetheless, PCZ is not the first choice for treatment of normal cutaneous or lymphocutaneous sporotrichosis because of its price and limited availability. PCZ can be administered in difficult treatment of disseminated sporotrichosis or after failure of treatment with ITZ [34].



To the best of our knowledge, this is the largest study of *in vitro* antifungal susceptibility of *S. globosa* isolates reported to date. We have found that the sensitivities of *S. globosa* to antifungal compounds are dependent on the incubation temperature and growth phase. To establish ECVs, it is necessary to conduct studies in uniform conditions and obtain more data from independent laboratories located in different regions.

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## Compliance with ethical standards

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

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