Molecular identification of *Sporothrix* species involved in the first familial outbreak of sporotrichosis in the state of Espírito Santo, southeastern Brazil

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Sporotrichosis is a widespread subcutaneous mycosis caused by the dimorphic fungi now known as the Sporothrix schenckii *complex. This complex is comprised of at least six species, including* Sporothrix albicans, Sporothrix brasiliensis, Sporothrix globosa, Sporothrix luriei, Sporothrix mexicana *and* S. schenckii. *Cases of sporotrichosis have significantly increased in Brazil over the past decade, especially in the state of Rio de Janeiro (RJ), where an epidemic among cat owners has been observed. The zoonotic transmission from cats to humans suggests a common source of infection and indicates that animals can act as vectors. We performed a molecular characterisation of samples collected during the first outbreak of familial sporotrichosis caused by* S. brasiliensis *in the state of Espírito Santo, Brazil. These results represent the first description of such an outbreak outside the endemic area of zoonotic sporotrichosis in RJ.*

Key words: *Sporothrix brasiliensis* - sporotrichosis - Espírito Santo - outbreak - calmodulin gene - taxonomy

Sporotrichosis is a widespread subcutaneous mycosis with high endemicity in Latin America, South Africa, India and Japan (Lopez-Romero et al. 2011). The infection is caused by dimorphic fungi of the *Sporothrix schenckii* species complex (Marimon et al. 2007), which is comprised of at least six species, including *Sporothrix albicans, Sporothrix brasiliensis*, *Sporothrix globosa*, *Sporothrix luriei, Sporothrix mexicana and Sporothrix schenckii* (Marimon et al. 2007, 2008). In humans, sporotrichosis is classically known as gardener's disease and is generally associated with soil transmission. In the sporotrichosis epidemic that occurred in the state of Rio de Janeiro (RJ), Brazil, transmission of the disease was associated with scratches or bites from cats infected with *S. schenckii* (Schubach et al. 2008).

A species-level classification of the *Sporothrix* complex has now been proposed (Marimon et al. 2007). In a previous study, Oliveira et al. (2011) disagreed with earlier data published by Marimon et al. (2007) that assumed that the differentiation of species within the *Sporothrix* complex could be easily accomplished without molecular methods. Our group has reported that the correlation between molecular data and phenotypic characteristics is crucial for the identification of species in the *Sporothrix* complex. In the current study, we characterised four *Sporothrix* strains at the species level using a polyphasic analysis (Oliveira et al. 2011). The strains were obtained from sporotrichosis cases that occurred in one cat (ES213) and three family members (ES210, ES211 and ES212; ages 30, 14 and 10 years, respectively) from a rural area in the state of Espírito Santo (ES). The patients developed skin ulcers with irregular borders and satellite microabscesses located in the lower buttocks, thighs and neck as clinically and epidemiologically described by Falqueto et al. (2012). The isolated strains were compared with the following three control strains: *S. brasiliensis* (CBS 120339; formerly IPEC 16490) (Marimon et al. 2007), *S. schenckii* (IPEC 27722) (Oliveira et al. 2011) and *S. globosa* (IPEC 27135) (Oliveira et al. 2011).

The phenotypic characteristics of the isolates are shown in the Table. Overall, the sympodial conidia of the isolates were hyaline or slightly pigmented. The greatest fungal growth was observed at 30ºC with a minimum colony diameter of 37 mm and a maximum colony diameter of 45 mm. At 37ºC, a colony diameter minimum of 9 mm and a colony diameter maximum of 13 mm were observed.

Assimilation tests using dextrose, sucrose and raffinose were performed in triplicate in yeast nitrogen base medium. After 10 days, the four isolates assimilated only sucrose and glucose (Table). All isolates showed a biochemical pattern of carbohydrate assimilation typical of *S. globosa* or *S. albicans* according to Marimon's key. However, the average colony diameter at 30ºC (diameter of the colonies not exceeding 50 mm) excluded *S. albicans* in this cases; in addition the strains produce dematiaceous conidia. The isolates also could not be classified as *S. globosa* because they were thermotolerant. The isolates were therefore presumptively identified as *Sporothrix* spp based on phenotypic characteristics.

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TABLE

Phenotypic and genotypic characteristics of *Sporothrix* species complex from the state of Espírito Santo, Brazil

Species	Conidia (%)			Colony diameter					Assimilation test			Genotypic sequencing
	Dematiaceous				Range		Average ± SD					
	Globose	Triangular	Elongated	Hyaline	30ºC	37ºC	30ºC	37ºC	Glucose	Sucrose	Raffinose	
Sporothrix brasiliensis (type)	0	1	0	0	35	13	35 ± 0	13 ± 0	+	-	-	*S. brasiliensis*
Sporothrix spp (4)[a]	4	0	0	0	37-45	9-13	41 ± 3.4	10.7 ± 1.7	+	+	-	*S. brasiliensis*
Sporothrix schenckii (control)	0	1	0	0	51	3	51 ± 0	3 ± 0	+	+	+	*S. schenckii*
Sporothrix globosa (control)	1	0	0	0	34	7	34 ± 0	7 ± 0	+	+	-	*S. globosa*
Total	5	2	0	0								

a: sampling not identified to species level by phenotypic tests; SD: standard deviation; +: positive; -: negative.

Consensus tree of *Sporothrix* based on partial calmodulin gene sequences of four strains and the National Center for Biotechnology Information public GenBank sequences AM398393.1 (*Sporothrix mexicana*), AM398382.1 (*Sporothrix albicans*), AM116899.1 (*Sporothrix brasiliensis*), HQ426961.1 (*Sporothrix schenckii*) and GU456632.1 (*Sporothrix globosa*) that was constructed with MEGA version 4.0.2 and 1,000 bootstrap replicates.

Genomic DNA was obtained from the isolates in mould phase and sequencing of the partial calmodulin-encoding gene (CAL) was performed as previously described (Oliveira et al. 2010, 2011) using the Genomic Platform-DNA Sequencing at Oswaldo Cruz Foundation (RPT01A) in Brazil. The similarity of the partial CAL gene sequences to those obtained from the National Center for Biotechnology Information GenBank database confirmed with high bootstrap support that all isolates were *S. brasiliensis* (Figure). All sequences were deposited in the GenBank database under accessions JQ915210 through JQ915213.

During the past decade, cases of sporotrichosis have significantly increased in Brazil. The occurrence of these cases has been particularly evident in RJ; here, an epidemic resulting from zoonotic transmission from cats to humans has been observed, leading to small outbreaks among cats owners and veterinary professionals. Such an outbreak suggests a common source of infection between humans and cats and indicates that animals can act as vectors for the transmission of this fungal disease (Schubach et al. 2008, Reis et al. 2009).

All isolates were obtained from individuals living in a rural area in ES. ES borders RJ, which is endemic to zoonotic sporotrichosis (Falqueto et al. 2012). The outbreak in ES showed similarities to cases of epidemic sporotrichosis reported in RJ, including the fact that most individuals affected were women that were engaged in domestic duties, in contact with sick cats and were from low-income areas (Freitas et al. 2010). These similarities suggest that this emerging disease could be spreading in Brazil.

To our knowledge, this is the first molecular characterisation of a familial outbreak of zoonotic sporotrichosis caused by *S. brasiliensis* outside of the endemic area in RJ.

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