Antifungal susceptibilities of species of the *Sporothrix Schenckii* complex isolated in Italy

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Introduction

Recent molecular studies showed that the dimorphic fungus Sporothrix schenckii is no longer the only species able to cause sporotrichosis, a cutaneous lymphatic or systemic mycosis particularly frequent in certain geographical areas such as Mexico, Brazil, Peru, and India.[1] In fact, *S. schenckii* can now be recognized as a species complex comprising at least six sibling species: *Sporothrix brasiliensis*, *Sporothrix globosa*, *Sporothrix luriei*, *Sporothrix mexicana*, *Sporothrix pallida* (formerly Sporothrix albicans) and *S. schenckii* sensu stricto.[2,3] Like S. schenckii, all these new species have been reported to cause diseases in humans and in other animals[1,4,5] although the extent of their impact on human infections is not yet completely known. However, infections due to *S. schenckii* have also been reported from other parts of the world, including Europe, where sporotrichosis is considered a rare disease.[6] Nevertheless, in recent years, several clinical autochthonous cases have been described in patients and animals that live in European countries, showing that this pathogenic fungus is more widespread than is now believed.[1,6] At present, there are relatively few works that have evaluated the susceptibility of *S. schenckii* sensu lato to antifungal agents and the drugs tested so far have shown, in general, poor activity especially against *S. pallida*, *S. globosa*, and *S. mexicana*. Therefore, in this study we decided to evaluate the activities of a panel of antifungal drugs against all members of the *S. schenckii* complex with particular reference to Italian isolates. To our knowledge this is the first study that evaluates *in vitro* activities of antifungal agents against a number of *Sporothrix* spp. isolates recovered from clinical and environmental samples in Italy.

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Materials and Methods

Fourteen *Sporothrix* spp. were examined in this study (Table 1). Seven of them were environmental *S. pallida* isolates that have already been well characterized in our previous study.[6] The *identity of each isolate was* determined by partial amplification and sequencing of the calmodulin-encoding gene according to recent studies.[2,6] Antifungal activity of seven drugs (Table 1) was evaluated by disk diffusion method according to the procedures reported in the National Committee for Clinical Laboratory Standards (NCCLS) document M44-A.

In this study, a total of 14 clinical and environmental *Sporothrix* spp. were examined to evaluate their susceptibility to a panel of antifungal agents. The resulting values of the in vitro susceptibility of *S. schenckii sensu lato* isolates are shown in Table 1.

All fungal species were resistant to fluconazole, flucytosine and metronidazole whereas were susceptible to nystatin. An excellent broad-spectrum antifungal activity of miconazole was observed against all examined strains. Regarding ketoconazole, different degree of susceptibility were observed. In particular this drug was active against *S. schenckii*, *S. brasiliensis* and *S. mexicana* but for *S. pallida*, *S. globosa* and *S. luriei* was not possible to measure the diameter of the zone of inhibition due to the presence of a high number of resistant colonies.

Discussion and Conclusions

The discovery of genetically different species within the *S. schenckii* population has generated considerable interest on different aspects of their biology including epidemiology, virulence and antifungal susceptibilities. Previous studies have clearly shown that the geographic distribution of members of the *S. schenckii* complex as well as their trends in antifungal susceptibilities are variable[1,2,7] and therefore more attention should be paid in the diagnosis and therapeutic treatment of infections caused by these species. Throughout this work, miconazole, and to a lesser extent nystatin, showed a good activity against all *Sporothrix* species tested while all isolates were resistant to fluconazole, flucytosine and metronidazole which is in agreement with other previous studies.[7] The broad *in vitro* resistance to fluconazole in clinical isolates of the *S. schenckii* complex suggests an intrinsic resistance to this drug. This is an interesting topic for further study, because this drug is considered the second-line treatment for sporotrichosis. One important result of this study is the excellent broad-spectrum antifungal activity displayed by miconazole, an synthetic imidazole antifungal agent which has never been tested against all members of the *S. schenckii* complex so far. Thus, based on our *in vitro* data, we believe that this drug may represent a very promising antifungal agent in the treatment of human and animal sporotrichosis.

Table 1. Fungal strains, species and anitfungal agents used in this study.

Strain	Species	Antifungal agent tested (mean±SD)*:					
		FCN	FY	MZ	MCL	KCA	NY
SPO1	*S. schenckii*	R	R	R	32.50±1.11	29.00±1.22	14.00±2.35
CDM18	*S. schenckii*	R	R	R	22.00±1.35	25.00±1.05	07.00±1.51
SS40	*S. schenckii*	R	R	R	47.00±1,82	49.00±1.87	16.00±1.65
SPA1	*S. pallida*	R	R	R	21.00±1,24	ND	07.00±0.73
SPA2	*S. pallida*	R	R	R	22.00±1.08	ND	09.00±1.04
SPA8	*S. pallida*	R	R	R	19.00±0.86	ND	13.00±1.91
SAM1	*S. pallida*	R	R	R	27.00±1.46	ND	10.00±0.86
BG	*S. pallida*	R	R	R	23.00±1.71	ND	08.00±1.81
BG2	*S. pallida*	R	R	R	21.00±1.38	ND	07.00±1.52
BG6	*S. pallida*	R	R	R	23.00±1.02	ND	11.00±1.31
SS52	*S. brasiliensis*	R	R	R	21.00±1.29	28.70±1.21	09.00±0.50
SS49	*S. globosa*	R	R	R	23.00±1.61	ND	09.00±1.90
FMR9108	*S. mexicana*	R	R	R	22.00±1.10	25.75±2.01	14.00±1.11
KMU2787	*S. luriei*	R	R	R	25.00±1.71	ND	16.00±1.13

*Mean values±standard deviation (SD). FCN, fluconazole; FY, flucytosine; ME, metronidazole; MCL, miconazole; KCA, ketoconazole; NY, nystatin; R, restistant (no alone present); ND, not determined.

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