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Short communication

In vitro susceptibility of *Conidiobolus lamprauges* recovered from sheep to antifungal agents

Juliana Simoni Moraes Tondolo [a], Érico Silva de Loreto [a], Valéria Dutra [b], Luciano Nakazato [b], Daphine Ariadne Jesus de Paula [b], Régis Adriel Zanette [a], Sydney Hartz Alves [c], Janio Morais Santurio [a,*]

[a] *Programa de Pós-Graduação em Farmacologia, Centro de Ciências da Saúde, Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria, Brazil*
[b] *Programa de Pós-graduação em Ciências Veterinárias, Universidade Federal de Mato Grosso, Cuiabá, Brazil*
[c] *Programa de Pós-Graduação em Ciências Farmacêuticas, Centro de Ciências da Saúde, Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria, Brazil*

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ABSTRACT

Data regarding the susceptibility of *Conidiobolus lamprauges* is limited and there is no consensus about the optimal treatment for infections caused by *Conidiobolus* spp. In this context, the objective of this study was to evaluate the *in vitro* susceptibility of six *C. lamprauges* strains isolated from sheep conidiobolomycosis to amphotericin B, ketoconazole, fluconazole, itraconazole, posaconazole, voriconazole, anidulafungin, caspofungin, micafungin, flucytosine, and terbinafine using the CLSI M38-A2 microdilution technique. Terbinafine was the most active (MIC range <0.06–0.5 μg/mL). Resistance or reduced susceptibility was observed for amphotericin B and azole and echinocandin antifungals. Additional studies are necessary to determine the therapeutic potential of terbinafine as monotherapy or in combination therapy with other antifungals.
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1. Introduction

Entomophthoramycosis caused by *Conidiobolus lamprauges* is a rare and chronic, pyogranulomatous, subcutaneous disease of humans and other mammals, such as horses and sheep (de Paula et al., 2010; Furlan et al., 2010; Humber et al., 1989; Kimura et al., 2011; Vilela et al., 2010). The disease presents clinically with involvement of the nasopharyngeal region, affecting the ethmoidal region, turbinate bones, and occasionally, the central nervous system, lymph nodes, and pleura (de Paula et al., 2010; Furlan et al., 2010; Humber et al., 1989; Vilela et al., 2010). However, the recent first description of this species causing a fatal disseminated human fungal infection (Kimura et al., 2011) highlights the need for a better clinical and laboratory understanding of this species.

There is no consensus about the optimal treatment for infections caused by *Conidiobolus* spp. A saturated solutions of potassium iodide (SSKI), azole and echinocandin antifungals, terbinafine, amphotericin B, and flucytosine administered as monotherapy or in combinations have been described in cases of therapeutic success and failure (Bento et al., 2010; Choon et al., 2012; Chowdhary et al., 2010; Isa-Isa et al., 2012; Kimura et al., 2011; Reiss et al., 2012; Walker et al., 1992; Walsh et al., 1994), and reports evaluating the susceptibility of *Conidiobolus* spp. have shown decreased susceptibility or resistance to most antifungal agents (Bento et al., 2010; Fischer et al., 2008; Guarro et al., 1999; Hawkins et al., 2006; Kimura et al., 2011; Walsh et al., 1994). In this context, the aim of

* Corresponding author at: Universidade Federal de Santa Maria (UFSM), Av. Roraima n° 1000, Prédio 20, sala 4139, Santa Maria CEP 97105-900, RS, Brazil. Tel.: +55 55 3220 8906; fax: +55 55 3220 8906.
E-mail address: janio.santurio@gmail.com (J.M. Santurio).

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this study was to evaluate the *in vitro* susceptibility of *C. lamprauges* to antifungal agents using the CLSI M38-A2 microdilution technique.

2. Materials and methods

We evaluated the susceptibility of six *C. lamprauges* strains isolated from cases of sheep conidiobolomycosis that were previously identified by molecular phylogenetic analyses based on 18S ribosomal DNA (de Paula et al., 2010). All sheep had a granulomatous reaction in the nasopharyngeal region that extended to the turbinates, nasal sinuses, orbit, and cribriform plate, with frequent invasion of the brain.

Amphotericin B (AMB, Sigma®), azole antifungals [ketoconazole (KTZ, Janssen-Cilag Pharmaceutica®), fluconazole (FLZ, Sigma®), itraconazole (ITZ, Janssen Pharmaceutica®), posaconazole (POS, Schering-Plough®), and voriconazole (VRZ, Pfizer®], echinocandins [anidulafungin (AND, Pfizer®), caspofungin (CAS, Merck®), and micafungin (MYC, Astellas®)], flucytosine (5FC, Sigma®), and terbinafine (TRB, Novartis®) were obtained commercially and were diluted in dimethylsulfoxide or distilled water to generate stock solutions.

The susceptibility tests were performed following the CLSI M38-A2 protocol (Clinical and Laboratory Standards Institute, 2008). Briefly, all isolates were grown on potato dextrose agar for seven days at 35 °C. The colonies were covered with approximately 1 mL of sterile 0.85% saline, and a suspension was obtained by gently probing the colonies with the tip of a transfer pipette. This suspension was transferred to a sterile tube and after allowing hyphal fragments to settle for 5 min, the upper homogeneous suspension was transferred to a new sterile tube and mixed with a vortex mixer for 15 s. The densities of the conidial suspensions were adjusted to an optical density at 530 nm that ranged from 0.15 to 0.17 and then diluted 1:50 in RPMI 1640 broth (with L-glutamine and glucose, buffered to pH 7.0 with 0.165 M MOPS) to obtain inocula twofold more concentrated than the density required (0.4×10^4 to 5×10^4 CFU/mL). The tests were performed in microdilution trays and the minimum inhibitory concentrations (MICs) were scaled as MIC-1 (100% visible-growth inhibition for AMB, ITZ, POS, and VRZ) and MIC-2 (50% (KTZ, FLZ, and 5FC) or 80% (TRB) visible-growth inhibition). Minimum effective concentration (MEC) was defined as the minimal drug concentration producing short, stubby, highly branched hyphae for AND, CAS, and MYC. MICs and MECs were determined at 24 and 48 h and performed in duplicate.

The minimum fungicidal concentrations (MFCs) were determined by subculturing 100 μL from each well without visual detectable growth into 1 mL of sterile Sabouraud broth and incubating the mixture at 35 °C for 7 days. The geometric means (GMs) of off-scale MICs were converted to the next lowest or next highest concentration.

3. Results

The results are summarized in Table 1. TRB was considered the most effective drug tested for the *in vitro* inhibition of *C. lamprauges*, with MIC-2 (GM) values ranging from <0.06 to 0.5 (0.12) μg/mL at both 24 and 48 h. MIC-1 (GM) values ranged from 0.5 to 1 (0.56 [24 h]; 1.0 [48 h]) μg/mL for AMB, 1 to 32 (4) μg/mL for ITZ, 2 to >32 (8) μg/mL for POS, and >16 μg/mL for VRZ. MIC-2 (GM) values ranged from 8 to 32 (14.25) μg/mL for KTZ and >64 μg/mL for both FLZ and 5FC. The AND, CAS, and MYC MEC were >8 μg/mL. The MFCs (GM) ranged from 0.25 to 2 (0.79) μg/mL for TRB, 2–4 (3.17) μg/mL for AMB, 2 to >32 (8) μg/mL for ITZ and 2 to >32 (12.7) μg/mL for POS.

4. Discussion

Conidiobolus spp. are saprophytes that are distributed widely and are found in soil and decaying organic material. Among the more than 25 described species, *C. coronatus*, *C. incongruus*, and *C. lamprauges* are known to cause diseases

Table 1
Antifungal activities of antifungal drugs against six isolates of *Conidiobolus lamprauges*.

Conidiobolus lamprauges isolates ($n = 6$)	MIC/MEC range (GM)		MFC range (GM
	24 h	48 h	
MIC-1			
Amphotericin B	0.5–1 (0.56)	1 (1)	2–4 (3.17)
Itraconazole	1–32 (4)	1–32 (4)	2–>32 (8)
Posaconazole	2–>32 (8)	2–>32 (8)	2–>32 (12.7)
Voriconazole	>16	>16	>16
MIC-2			
Terbinafine	<0.06–0.5 (0.12)	<0.06–0.5 (0.12)	0.25–2 (0.79)
Ketoconazole	8–32 (14.25)	8–32 (14.25)	32–>32 (> 32)
Fluconazole	>64	>64	>64
Flucytosine	>64	>64	>64
MEC			
Anidulafungin	>8	>8	>8
Caspofungin	>8	>8	>8
Micafungin	>8	>8	>8

MIC, minimum inhibitory concentrations; MFC, minimum fungicidal concentrations; GM, geometric mean; MIC-1, 100% visible-growth inhibition; MIC-2, 50% (ketoconazole, fluconazole, flucytosine) or 80% (terbinafine) visible-growth inhibition; MEC, minimum effective concentration.

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