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Emergence of azole-resistant *Candida albicans* in small ruminants

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Abstract Small ruminant production is a common agricultural activity worldwide. However, studies on the fungal microbiota of these animals are scarce. Therefore, this study aimed at isolating yeasts from goats and sheep and evaluating the antifungal susceptibility of the recovered *Candida albicans*. A total of 120 animals from farms in Ceará State, Brazil, were assessed in this study. The samples were collected from nasal, oral and rectal cavities with sterile swabs. *Candida* spp., *Trichosporon* spp. and *Rhodotorula* spp. were isolated from small ruminants. Resistance to three azole drugs was observed in *C. albicans*. In summary, *Candida* spp. were predominantly observed as part of the microbiota of the nasal, oral and rectal cavities of small ruminants, including azole-resistant strains of *C. albicans*.

Keywords *Candida albicans* · Yeast · Antifungal resistance · Small ruminants

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Introduction

Small ruminant production is an agricultural activity practiced around the globe. In Brazil, this activity has expanded and it contributes for the social economic development of the country, especially in the Northeast [1, 2]. However, studies on the fungal microbiota of small ruminants are scarce. Commensal microorganisms may become pathogenic when favorable host-associated conditions are met [3]. *Candida* is the most important yeast genus causing infections, with *Candida albicans* as the most frequent disease-associated species [4]. Therefore, this study aimed at isolating yeasts from the nasopharynx, oropharynx and rectal cavity of small ruminants and evaluating the antifungal susceptibility of the recovered *C. albicans*.

Materials and Methods

Isolation and Fungal Identification

Clinical samples were collected from 60 goats and 60 sheep from Ceará State, Brazil. This study was approved by the Animal Research Ethics Committee of the Ceará State University (protocol—12641465-6). Samples were collected from the nasopharynx, oropharynx and rectal cavity with sterile swabs. Swabs were inoculated on Petri dishes containing 2 % Sabouraud dextrose agar with chloramphenicol (0.5 g/L), and the plates were incubated at 25 °C for up to 10 days, with daily evaluation for the presence of microbiological growth. Macromorphological, micromorphological and biochemical features were assessed in order to identify the species. In cases of dubious identification, the strains were identified through Vitek 2 [5–7].

Antifungal Susceptibility Assay

Antifungal susceptibility test was performed through broth microdilution as standardized by the Clinical Laboratory Standards Institute [8]. At first, the susceptibility of *C. albicans* ($n = 21$) to amphotericin B (AMB), fluconazole (FLC) and itraconazole (ITC) was tested. In addition, 11 *C. albicans* were tested against voriconazole (VRC) and caspofungin (CAS), which are more commonly used in medical practice, and the agricultural drug tetraconazole (TRC). All samples were tested in duplicate. The minimum inhibitory concentration (MIC) for AMB was defined as the lowest concentration capable of inhibiting 100 % of the yeast growth, while for azole derivatives and CAS, MICs were defined as the lowest concentration capable of inhibiting 50 % of yeast growth, when compared to the control well [8]. MICs ≥ 8, ≥ 1, ≥ 1 and ≥ 1 μg/mL indicated resistance to FLC, ITC, VRC and CAS, respectively [8, 9].

Results and Discussion

A total of 160 *Candida* spp. isolates were obtained, while only 16 *Rhodotorula* spp. and 17 *Trichosporon* spp. were recovered from different sites (Table 1). *C. albicans* (21/160 isolates) was the third most isolated species, and the oropharynx was the anatomical site that was most frequently colonized by this yeast species. This finding supports the idea that animals are the primary ecological niche of this yeast species, not environmental sources [10].

The results obtained for the antifungal susceptibility are presented in Table 2. Resistance to CAS was not observed, similar to what was observed in other studies with healthy animals [6, 7]. However, azole resistance rates were high, with 3/21 *C. albicans*

Table 1 Yeasts isolated from nasopharynx, oropharynx and rectal cavity of goats and sheep

Species	Goats			Sheep			Total
	Nasopharynx	Oropharynx	Rectum	Nasopharynx	Oropharynx	Rectum	
Candida albicans	–	7	2	–	9	3	21
C. tropicalis	25	21	4	2	12	4	68
C. famata	7	2	6	5	4	–	24
C. parapsilosis Complex	1	3	3	2	4	2	15
C. krusei	4	3	4	1	1	–	13
C. guilliermondii	3	7	2	1	2	1	16
C. glabrata	–	–	1	1	1	–	3
Rhodotorula glutinis	–	1	–	7	3	–	11
R. mucilaginosa	–	–	–	–	1	1	2
R. minuta	–	1	1	–	1	–	3
Trichosporon sp.	1	1	–	5	4	6	17
Total	41	46	23	24	42	17	193

Table 2 In vitro antifungal susceptibility of *C. albicans* strains recovered from goats and sheep

Antifungal agent	Isolates (n)	Range (μg/mL)	GM	Resistant (*n*)
AMB	21	0.03–1.0	0.305	0
FLC	21	0.5–64.0	8.979	10
ITC	21	0.03–16.0	0.876	9
VRC	11	0.03–2.0	0.389	6
CAS	11	0.03–0.25	0.086	0
TRC	11	0.06–8	1.655	NA

MB amphotericin B; *FLC* fluconazole; *ITC* itraconazole; *VRC* voriconazole; *CAS* caspofungin; *TRC* tetraconazole; *GM* geometric mean; resistance breakpoints: AMB > 1; FLC ≥ 8; ITC ≥ 1; VRC ≥ 1 and CAS ≥ 1 μg/mL. *NA* not applicable

simultaneously resistant to the three tested azoles, 6/21 resistant to FLC and ITC, and 3/11 resistant to VRC. These findings are relevant because the investigated animals were healthy and did not have a history of antifungal treatment.

As for tetraconazole, there are no resistance breakpoints established, but it presented a high MIC range, when compared to ITC and VRC. Resistance to azole is usually a consequence of changes in the gene sequences or their expression due to selective processes caused by previous exposure to these drugs, since they are commonly used in clinical or agricultural practices [7, 11, 12]. Thus, knowledge on products that are used to treat and control diseases in crop destined to animal feeding is important.

The high rate of azole resistance among *C. albicans* strains observed in this study is of concern. Small ruminant production is an important activity in Northeastern Brazil, especially in small family farms [13, 14], where farming activities are usually more promiscuous, with different animal species being raised together under poor sanitary conditions.

This study suggests that small ruminants should be monitored for the emergence of potentially pathogenic azole-resistant *C. albicans* because animals may serve as sources of human infections with this yeast [15, 16]. In addition, it is important to highlight that over 60 % of emerging pathogens are zoonotic and agricultural practices represent a risk activity for the emergence of resistant microorganisms [17]. In conclusion, this study showed that drug-resistant *C. albicans* strains are part of the microbiota of goats and sheep and may represent a potential threat to human and animal health.

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