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In vitro Susceptibility of *Prototheca* Species to Antifungal Agents

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Abstract: *Prototheca* sp. can assume high economic significance in the dairy industry and pose a potential risk for the public health. Studies on the susceptibility of *Prototheca* spp. to antimicrobials have demonstrated its high level of resistance. We investigated the *in vitro* susceptibility of ten *Prototheca zopfii* isolates retrieved from mastitic milk and one *P. wickerhamii* referent strain, from American Type Collection using MIC Test Strip. *P. zopfii* isolats showed susceptibility to MIC between 1,5-4 µg/mL for ketoconazole, and between 1,5-3 µg/mL for amphotericin B. In contrast *P. wickerhamii* strain prouved to be efficient to a MIC of 2 µg/mL in case of ketoconazole and 0,75 µg/mL for amphotericin B. Both species tested showed to be resistant to itraconazole.This study demonstrates different in vitro susceptibility patterns of *P. wickerhamii* and *P. zopfii*, reinforcing the necessity for more investigation into drugs that can be used with clinical efficacy.

Keywords: *Prototheca*, susceptibility, antifungal agent

INTRODUCTION

Prototheca spp. are unicellular, achlorophylic, nonphotosynthetic algae, globally ubiquitous, and readily isolated from rivers, lakes, ponds, and soil (Huerre 1993). Typical sources of *Prototheca* species are the slime flux of trees, grass, fresh and salt water, wastewater, animals such as cattle, deer, and dogs, stables, animal buildings, excrement (Pore *et al*., 1983; Pore , 1986), and food such as butter, potato peels, cow's milk, soil, and bananas (Pore, 1985; Pore, 1986).Currently *P. zopfii*, *P. wickerhamii*, *P. stagnora*, *P. ulmea*, *P. blaschkeae* have been recognized.

They are opportunistic pathogen for humans and animals. Although canine and bovine protothecosis have been reported more widely, infections in humans are rare, particularly in patients with an intact immune system (Woolrich *et al*, 1994). The majority of protothecal infections in humans is associated with *Prototheca wickerhamii*, while in animals with *P. zopfii* (Casal, 1981; Follador *et al*, 2001; Jensen *et al*., 1998; Lass-Flörl et al., 2004; Leimann *et al*, 2004).

Treatment of protothecal infections remains controversal, and various treatment regimens have been attempted, but there has been no consistency in the clinical responses (Lass-Flörl et al., 2004)

This paper aimed to investigated the antimicrobial activity of ketoconazole, itraconazole and amphotericin B against two *Prototheca* species, as well as to found the most efficient antifungal agent used in treating of protothecosis.

MATERIALS AND METHODS

In vitro antimicrobial susceptibility of ten *Prototheca zopfii*. isolates and one *P. wickerhamii* referent strain was measured using minimal inhibitory concentration (MIC) Test Strip. The isolates were identified on the basis of the presence of daughter cells and on biochemical features (assimilation of glucose, galactose, glycerol, sucrose and trehalose, and growth at 28 and 37ºC). Three antifungal were evaluated: ketoconazole, itraconazole and amphotericin B.

MIC Test Strips is a quantitative assay for determining the Minimum Inhibitory Concentration of antimicrobial agents against microorganisms to indicate appropriate patient treatment and for identifying resistance patterns. When the MIC Test Strip is applied onto an inoculated agar surface, the preformed exponential gradient of antimicrobial agent was transferred into the agar matrix. After 24-48 hours incubation or longer, a symmetrical inhibition ellipse centered along the strip was formed. The MIC was read directly from the scale in terms of µg/mL, at the point where the edge of the inhibition ellipse intersects with the MIC Test Strip.

RESULTS AND DISCUSSION

Tests carried out in order to establishing the antimcrobials effect of antifungal agents showed different aspects according to the drugs and to the species of *Prototheca* tested

Table 1 summarizes the *in vitro* susceptibility profile of *Prototheca* species to antifungal egent evaluated.

Tab. 1

In vitro susceptibility profile of two *Prototheca* species to antifungal tested

ISOLATES TESTED		ANTIFUNGAL TESTED		
		Ketoconazole MIC (µg/ml)	Itraconazole MIC (µg/ml)	Amphotericin B MIC (µg/ml)
P. zopfii	1	2	R	1,5
	2	3	R	3
	3	4	R	2
	7	4	R	1,5
	11	4	R	2
	14	2	R	1,5
	16	1,5	R	1,5
	17	4	R	2
	18	4	R	3
	20	3	R	2
P. wickerhamii		2	R	0,75

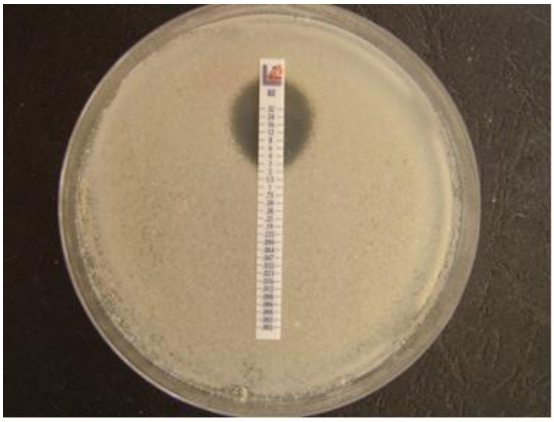

Fig. 1, *P. zopfii* (2 isolates) - the efficiency of ketoconazole to a MIC of 3

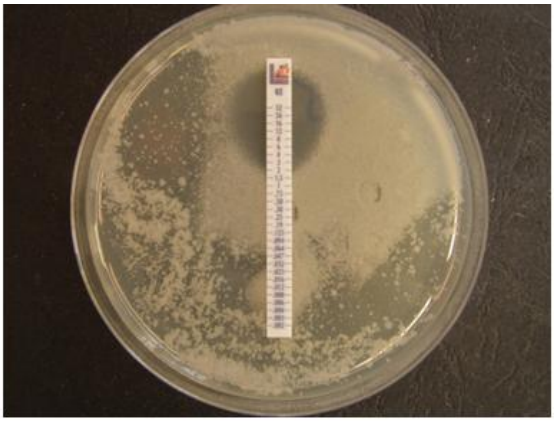

Fig. 2, *P. wickerhamii* - the efficiency of ketoconazole to a MIC of 2

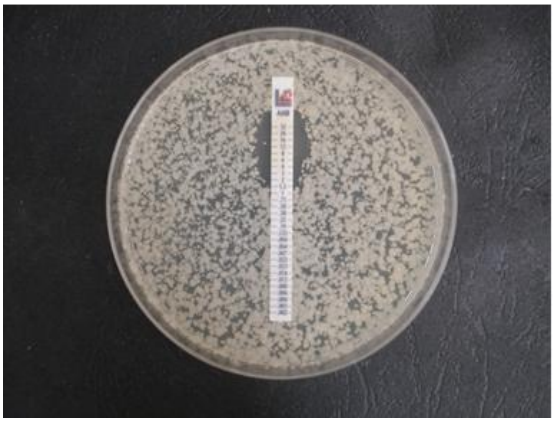

Fig. 3, *P. zopfii* (1 isolates) - the efficiency of Amphotricin B to a MIC of 1,5 µg/ml

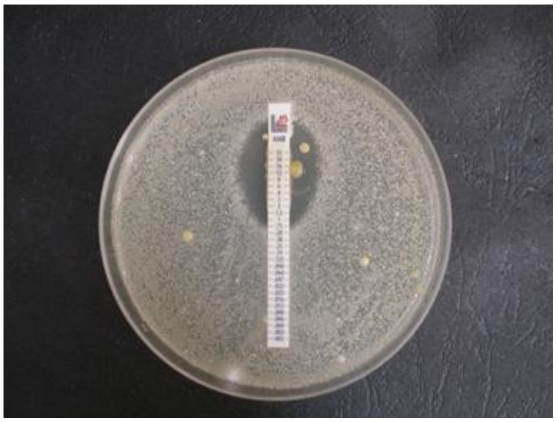

Fig. 4, *P. wickerhamii* - the efficiency of amphotericin B to a MIC of 0,75 µg/ml

All the *P. zopfii* tested isolates prouved to be susceptible to ketoconazole (with the MIC between 1,5 µg/ml – 4 µg/ml) and to amphotericin B (MIC between 1,5 µg/ml – 4 µg/ml). In case of *P. wickerhamii* ketoconazole prouved to be efficient to a MIC of 2 µg/ml, while amphotericin B to a MIC of 0,75 µg/ml. Both species tested were resistant to itraconazole.

A graphic representation of the MIC for antifungal agent used, for both *Prototheca* species tested is depicted in the fig. no. 5.

Our research performed in order to test the *in vitro* eficiency of some antifungal products demonstrated that ketoconazole and amphotericin B can be used in protothecosis therapy. The main feature of minimim inhibitory concentration for the antifungal tested, is their low value, that could to be an advantage if the use of products in protothecosis therapy.

Although our date reveal the in vitro effectiveness of antifungal tested, in vivo studies should be carried out.

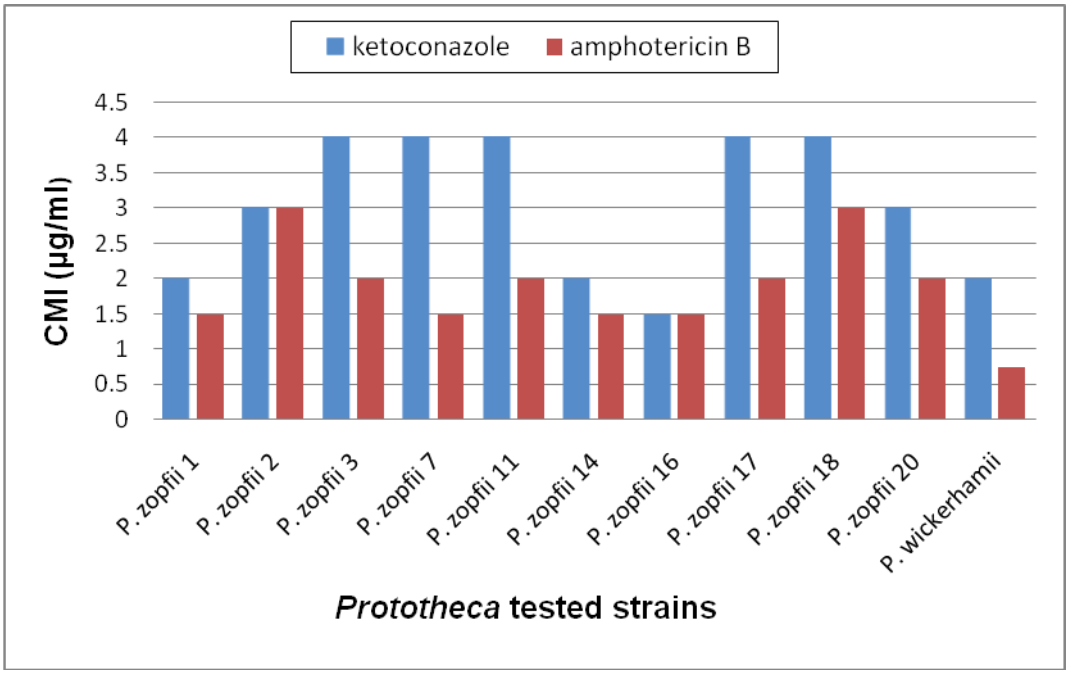

Fig. 5. The efficiency of antifungal agent tested (MIC) form both *Prototheca* species tested

CONCLUSIONS

- From all the products tested ketoconazole (MIC 1,5 µg/ml – 4 µg/ml) and amphotericin B (MIC 0,75 µg/ml - 4 µg/ml) were efficient for both Protoheca species tested.
- Both Prototheca species were resistant to itraconazole.
- Although our study reveal the efficiency of ketoconazole and amphotericin B, furthere research will be done in order to evaluated in vivo efficiency of those products.

ACKNOLEGEMENTS

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