

# MICROSPORUM CANIS STRAINS SENSITIVITY TO ANTIFUNGAL DRUGS

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## Abstract:

Researches presented in this study underlines results obtained in sensitivity of *Microsporum canis* strains from dogs and cats on different antifungal drugs. It was found that of the five antifungal substances tested by the diffusimetric method, in terms of efficacy on isolated *M. canis* strains (n = 116), Clotrimazole showed special activity, with the inhibition zones diameter ranging from 21 mm to 40 mm, with an average of  $31.28 \pm 5.18$  mm. With lower efficacy, causing areas of inhibition smaller than Clotrimazole, from intermediate to sensitive, Miconazole and Nystatin acted. There were strains sensitive to Miconazole, but intermediate sensitive to Nystatin, suggesting the need to test the sensitivity to the antifungal substance before starting a treatment.

**Key words:** dermatophytosis, *Microsporum canis*, antifungal substances, sensitivity test

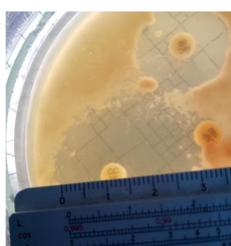
## Introduction

- Dermatophytoses are of all fungal infections the most common forms of infection in humans, affecting more than 20% - 25% of the world's population (7). It is estimated that in the human population, 30% - 70% of adults are asymptomatic carriers of these pathogens (17). From studies conducted so far, it is estimated that zoophilic species are responsible for about 30% of human dermatophytosis and usually cause acute inflammatory conditions. Anthropophilic species account for approximately 70% of infections in these hosts, causing a chronic infection with slow progression, suggesting that the fungus has adapted to the human host (19). Compared with the importance given to fungal infections in humans, in animals fungal diseases are relatively neglected, even if they are a source of up to 80% of human skin problems in rural areas and 20% of infections in urban areas (21).
- Another aspect that must be reported in fungal infections, both in humans and animals is the manifestation of resistance to certain antifungals used in treatment. The most important element that can induce the appearance of antifungal resistance seems to be the improper prescription of systemic antifungal agents and their indiscriminate use (9). The incidence of fungal infections, including resistant infections, has increased during the last few years, and may be due to inadequate or irregular use of drugs or increased incidence of immunodeficiency states (23). There is no clear evidence of the dosing strategy to be used during treatment and prophylaxis to avoid the best resistance, especially for animals (23).
- There are medical studies that have suggested measures to prevent and suppress the occurrence of antifungal resistance, which specify the prudent use of antifungals and their appropriate dosage, treatment with an antifungal appropriate to the identified etiological agent, after establishing its sensitivity to antifungal substances (5).
- In veterinary medicine for the treatment of dermatophytosis in pets there are a limited number of drugs, specially conditioned for dogs and cats. For this are used medical antifungal ointments recommended for the treatment of human dermatosis. Also, for pets systemic treatments of this type of disease, commercially veterinary products are limited, and as result, drugs used for human dermatophytosis treatment are used, but these are difficult to dose.
- In the view of these aspects, the aim of this study was to test the sensitivity of strains of *Microsporum canis*, isolated from different cases encountered in Timisoara veterinary practices, in order to assess the efficacy of some products that are frequently recommended for the treatment of this infection and to observe the antifungal resistance in some strains of this dermatophyte specie.

## Material and method

- A total of 116 samples composed of fur, nail and skin scraping specimens were collected from 40 (25%) dogs and 76 (62.5%) cats with clinical suspicion of dermatophytosis, in veterinary clinics from Timisoara. Sabouraud's dextrose agar (SDA - 1% peptone, 2% dextrose, and 2% agar) with the addition of chloramphenicol, previously poured into sterile Petri dishes, was used for cultivation. Incubation of the plates was performed at 27 - 28 ° C for 7-10 days. The identification of the species of *Microsporum canis* was made on the basis of cultural and microscopic characteristics.
- Microsporum canis* strains antifungal sensitivity testing was performed by diffusimetric method. For this, antifungal substances frequently used in this type of dermatophytosis therapy, both in animals and in humans, in topical applications, baths, but also in systemic treatment were chosen. The antifungal substances used for this study are: Ketoconazole, Clotrimazole, Miconazole, Amphotericin B and Nystatin (HI Media Laboratories).
- For the interpretation was taken into account the diameter of the inhibition zone, measured in mm with the ruler, in two - three directions, including the disk and the criteria specified by the discs manufacturer (HI Media Laboratories) that present the corresponding values in qualitative attributes: resistant strains, intermediate resistance or sensitive strains

Interpretation	Sensitive	Sensitive intermediary	Resistant
Diameter of inhibition zone (mm)	≥ 20	10 -19,9	5-9

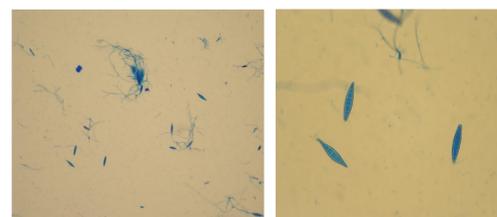


Measurement of the *M. canis* inhibition zone

**Acknowledgement:** Thanks to the clinicians for their involvement in this study



**Microsporum canis - macroscopic characters: obverse and reverse**



**Microsporum canis - microscopic aspect**

**A-** Hyphae and macroconidia *M. canis* - 10x  
**B-** Macroconidia of *M. canis* - 40x

## Results and discussions

- The results regarding the testing of the sensitivity to certain antifungals of some strains of *Microsporum canis* isolated from dogs and cats collected from veterinary clinics from Timisoara are presented in table

No of samples	Diameter of inhibition zone ( $\bar{x} \pm sdx$ - mm )				
	Miconazole	Ketoconazole	Clotrimazole	Amphotericin B	Nystatin
116	19.2±1.7	6.4±1.8	31.8±5.1	5.3±0.9	14.78±3.4

- Overall, it was found that of all the five antifungal substances tested by the diffusimetric method, in terms of efficacy on isolated *M. canis* strains (n = 116), Clotrimazole showed outstanding activity. Compared to all strains tested (116 strains) this substance showed remarkable efficacy, with the diameter of the induced inhibition zones ranging from 21 mm to 40 mm, with an average of  $31.28 \pm 5.18$  mm. A good antifungal action was found in both Miconazole and Nystatin.
- Miconazole determined an inhibition area with an average of  $19.21 \pm 1.79$  mm, with diameters ranging from 16 mm to 22 mm. Out of a total of 116 strains, 70 were sensitive to this substance (60.34%) and 46 strains were intermediate sensitive (39.65%).
- Nystatin exhibit an inhibition zone average of  $14.78 \pm 3.47$  mm, but out of a total of 116 strains, only 30 (25,86) were sensitive to this substance. The other 86 (74,13%) strains of *M. canis* proved to be intermediate sensitive.
- However, analyzing in detail, the sensitivity of each strain was found that all 116 were sensitive to Clotrimazole, but the sensitivity to the other two antifungals (Miconazole and Nystatin) was different, in the sense that there were strains that were sensitive to Miconazole (70 strains), but intermediate sensitive to Nystatin (86 strains).
- Compared to Ketoconazole and Amphotericin B all tested strains can be considered resistant. The diameter of the zones of inhibition induced by the two antifungals was non-existent (microcompet diameter = 5 mm) or extremely small, averaging  $6.4 \pm 1.81$  mm for Ketoconazole and  $5.36 \pm 0.94$  for Amphotericin B

## Conclusions

- The present study revealed that of the five substances tested for antifungal efficacy on some strains of *Microsporum canis*, Clotrimazole showed outstanding activity, causing areas of inhibition that were well above the sensitivity limit given by the microcub manufacturer.
- Miconazole and Nystatin acted with lower efficacy, given smaller inhibition areas smaller than Clotrimazole, from intermediate to sensitive.
- Of the total strains tested, all presented sensitivity to Clotrimazole, but sensitivity to the other two antifungals, was different, being observed strains sensitive to Miconazole but intermediate to Nystatin.
- In Ketoconazole and Amphotericin B, all tested strains were considered resistant, the area of inhibition being non-existent or very small.
- Sensitivity tests for antifungal substances for dermatophytes should become common in veterinary and human practice both to ensure the effectiveness of treatment and to prevent the onset of resistance.