The Efficiency of Some Antifungal Products on Genus *Malassezia* Fungi

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Abstract: The researches were made during 2006-2007 within the Microbiology Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca. A number of 23 *Malassezia pachydermatis* strains were tested regarding the sensibility to antifungal products currently used in therapy. The antymycotic products were represented by ketoconazole, itraconazole, nystatin and acetic acid. The best efficiency was registered for itraconazole, followed by ketoconazole, nystatin and acetic acid.

Key words: *Malassezia pachydermatis*, dogs, treatment, antifungal, efficiency;

INTRODUCTION

The genus *Malassezia* is known to include thirteen species of yeasts, many of which have been associated with various diseases in warm blooded vertebrates. *Malassezia pachydermatis* is a lipophilic budding yeast that colonizes the skin and mucosal sites of healthy dogs (1,2). Despite being part of the normal cutaneous micro flora, it is known that the yeast may become pathogen under certain circumstances (1,3). *Malassezia* dermatitis, an inflammatory dermatitis associated with elevated populations of *M. pachydermatis* on the skin of dogs, has been recognized with increased frequency (2,3).

The microscopic appearance of *Malassezia pachydermatis* is oval to peanut-shaped and is approximately 2-3 µm in width and 4-5 µm in length. Colonies are medium sized, round and convex shaped, white-yellow colored, developed on solid environments in 5-7 days. Reproduction is asexually achieved by unipolar or sympodial (*M. sympodialis*) budding (1,4).

Unlike many bacteria or other fungi, *Malassezia* yeasts are rarely found in the environment. Their habitat is primarily the skin and mucosa of mammals and birds. In healthy dogs, *M. pachydermatis* can be isolated from the ear channel, anus, rectum, oral cavity and, less commonly, the nose and vagina. On the normal canine skin, carriage of the yeast is most common in the interdigital areas and around the mouth but uncommon on the axilla or dorsum (1,5). In other species, *Malassezia* organisms have been recovered from the skin of healthy cats, ferrets, foxes, bears, pigs, horses, birds and rhinoceroses.

The researches aimed to evaluate “in vitro” efficiency of azole and polyene agents (ketoconazole, itraconazole), antifungal (nystatin) and acetic acid on 23 *M. pachydermatis* strains isolated from healthy dogs and dogs with ear and cutaneous lesions.

MATERIAL AND METHODS

The investigations took place during 2006-2007 within the Microbiology Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca. The 23 *Malassezia pachydermatis* strains
tested in the study were isolated from dogs in Cluj-Napoca (14), Bari – Italy (7) and Bucharest (2). Each strain was initially suspended in Salina to a density of 0.5 McFarland scale, and a 9 cm Petri dish containing Sabouraud agar was flooded. The excess was removed and the agar surface dried. Buckets of 3 mm diameter were performed, and the products to be tested were placed similar to the antibiograma model.

The products tested were the following:

1. **Ketoconazole** – is a synthetic antifungal drug to prevent and treat skin and fungal infections having wide spectrum for dermatophites (*Microsporum, Tricophyton, Epidermophyton*), yeasts (*Candida, Pityrosporum Malassezia, Torulopsis, Cryptococcus*), unicellular algae (*Prototheca*), gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*) and anaerobes. The mechanism of action is cell membrane destruction increasing its permeability due to the direct action and ergosterol biosynthesis inhibition.

2. **Itraconazole** – a triasolc derivate active on dermatophytes, yeasts, fungi and unicellular algae. In vitro studies proved that itraconazole is preventing ergosterol synthesis in fungi cells, a vital yeast cell membrane component. It is indicated in treating candidosis, pityriazis versicolor, dermatomycosis, criptococcosia and other systemic mycosis.

3. **Nistatin** – is a polienic macrolide having active mechanism on fungi cell membrane, forming nystatin channel complex – membrane cholesterol, the loss of intracellular ionic constituents and cell death. Is indicated in Candida infections, ringworm and pityriazis.

4. **Acetic acid** – a product recommended in the treatment of the otitis produced by genus *Malassezia* yeast, the concentration of the solution being of 2%. The recommendations are to be used in association with boric acid.

After the products were placed, the plates were incubated for 5 days at 32°C.

**RESULTS AND DISCUSSIONS**

From the products tested, the best efficiency was registered for itraconazole, followed by ketoconazole and nystatin. Acetic acid determined the appearance of inhibition areas just in case of 4 strains isolated from dogs in Cluj-Napoca (strains 7, 12, 25 and 26) and 3 strains isolated from Bari (7, 15, and 25).

For Itraconazole, sensitive proved to be the strains isolated from Bari and Bucharest followed by the strains isolated in Cluj-Napoca. The same situation was registered for Ketoconazole and Nystatin.

The most sensitive strain to Itraconazole was strain 1 isolated from Bucharest with a diameter of the inhibition area of 36 mm. A good sensibility was also registered for the strains isolated in Bari, the average of the inhibition area being 31.5, while the strains isolated from Cluj-Napoca were less sensitive, with the inhibition area of 17 mm.

Existed situations when none of the utilized products has any efficiency on the tested strains (strain 4 – Cluj-Napoca). In table 1 are presented the number of the strain, geographical area of provenience and the diameter of the inhibition areas for each strain and product.
The results obtained for the tested products revealed a very good efficiency of Itraconazole, Ketoconazole and Nystatin, while acetic acid had a reduced capacity of inhibiting the development of the yeasts from genus *Malassezia*.

The two strains from Bucharest tested with antimycotic substances proved to be very sensitive to Ketoconazole, with diameters of inhibition areas of 30 and respectively 28 mm, for Itraconazole of 36 and 31 mm, for Nystatin of 14 respective 13 mm, while acetic acid had no efficiency in case of the strains isolated from Bucharest.

The representation of the average area diameter for the strains isolated from Cluj-Napoca, Bucharest and Bari for the tests of the efficiency of the products is represented in chart 1:

![Chart 1](image_url)

Chart 1. Comparative representation of the average efficacy of the products tested in three geographical areas
According to the data published by Pinchbeck and col., 2002, itraconazole (azole group), has the best efficiency in the treatment of the otitis and dermatitis caused by *Malassezia pachydermatis*, data similar to the results obtained in this study (4).

Brito and col., 2007, are mentioning that from 34 *M. pachydermatis* tested strains, all presented sensitivity to Itraconazole while 29 were sensitive to Ketoconazole (1).

**CONCLUSIONS**

1. From the antimycotic products tested, the highest efficiency was registered for itraconazole, followed by ketoconazole, nystatin and acetic acid.
2. Reported to the geographical area of strains provenience, the order of strains sensitivity was Bari, Bucharest and respectively Cluj-Napoca, area where the 14 tested strains presented the lowest sensitivity to the products tested.
3. In case of two strains isolated from Cluj-Napoca, strain 4 proved to be resistant to all the products tested, while strain number 10 presented moderate sensitivity to nystatin (8 mm).
4. The average of the inhibition area registered for the antimycotic products in case of the strains isolated from Cluj-Napoca registered the lowest values, being of 12,28 mm for ketoconazole, 17,14 mm for itraconazole, 5,42 mm for nystatin, respectively 2,07 mm for acetic acid.

**REFERENCES**