The sensitivity of some Candida Spp strains isolated from animals to natural extracts

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Abstract. The researches were made during February – May 2013 within the Microbiology Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca. A total number of 18 Candida spp. strains isolated from different animal species and humans, and 2 reference strains were tested regarding their sensitivity to polyphenols and natural extracts using the diffusimetric technique. Candida spp. strains were isolated from parrot faeces, dog otitis, mastitis cow milk and human tonsillitis. Two Candida albicans control strains were also used within the study (ATCC® 90028™ and ATCC® 10231™). The polyphenols and natural extracts taken into study were represented by grape seed extracts, α-pinene, β-pinene, carvacrol, tea tree, fir and nystatin as standard antimycotic. Most of the strains were resistant to α-pinene, β-pinene and grape seed extract, good sensitivity was registered for tea tree, fir and nystatin, but the best overall results with an average of 38.5 mm inhibition area was observed for carvacrol.

Key words: Candida spp., polyphenols, natural extracts, sensitivity.

INTRODUCTION

Candida spp. yeast is normally present in mucous membranes of humans and animals. Their pathogenicity mostly develop in case of immunosupressed and antibiotic treated individuals. The last decades registered an increased in number of infected subjects, one can also consider candida involvement in cancer development. Constant interest of different research team in the identification of possible alternative treatments in candidiasis was revealed by publishing high quality articles in the field. A recent study of a research team in comparing the in vivo and in vitro efficiency of the essential oil extracted from dill seeds (Anethum graveolens L) on 10 Candida spp. strain using in vitro MIC method followed by in vivo administration of the strain in female mice previously immunosupressed. The sensitivity to dill seeds was compared to a standard antymycotic – fluconazole in this case (Hong Z. et al., 2011).

A different article published in 2009 is testing the efficiency of polyphenols (catechins and teaflavins) extracted from black tea on Candida spp. strains. The method chosen for this study was also MIC, preparing four concentrations of each extract. The results demonstrated the sensitivity of candida to polyphenols used as antifungal drugs (Sitheeque MA. et al., 2009). Another study tested the sensitivity of Candida spp. to catechins extracted from green tea leaves at 24 and 48h using two methods – MIC and MFC (minimum fungal concentration). Candida albicans strains resistant to fluconazole were sensitive to catechines extracted from green tea leaves (Yedegari M. et al., 2009).

Lately antymycotics were used frequently and for long periods of time without prior susceptibility testing. This approach has led to the emergence of resistant strains, hence the need for alternative therapies. This study is aiming to test in vitro susceptibility of Candida spp. strains isolated from humans and animals to the inhibitory action of polyphenols (grape seed extracts, α-pinene, β-pinene, carvacrol, tea tree, fir and nystatin).
MATERIALS AND METHODS

The investigations took place during February – May 2013 within the Microbiology Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca. A total number of 18 Candida spp. strains were isolated from both healthy and diseased animals, as well as samples collected from human. The samples were represented by: mastitis cow milk, dogs suffering from otitis, cats and dogs suffering from tonsillitis and parrot faeces. The samples of human origin were represented by pharyngeal exudates and urine. Two control Candida albicans strains were also used within the study (ATCC® 90028™ and ATCC® 10231™).

The samples were initially streaked on Sabouraud glucose agar (SGA) and incubated for 48 hours. The colonies were then identified using classical microbiological methods (microscopic and cultural). The determination was based on the diffusimetric method, each strain being suspended in saline to a turbidity similar to 0,5 McFarland scale (150 x 10^6 CFU/ml). Glucose agar Petri plates were flooded with the suspension, the excess was removed. After drying the plates, the solutions were added in the wells. In case of tea tree and fir, filter paper disk were used for the determination. The plates were incubated at 37°C for 24 hours and at 22°C for another 24 hours. For the interpretation of results the inhibition area diameters were measured for each product.

RESULTS AND DISCUSSIONS

The best diameter of the inhibition area was observed for carvacrol with an average of 38.5 mm, followed by tea tree extract with the value of 21.07 mm, fir with 16.92 mm, α-pinene with 5.15 mm, β-pinene and grape seed extract with 3.07 mm. For nystatin as standard antimycotic, the average of the inhibition areas was 18.3 mm.

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Fig. 1. Inhibition areas for Candida spp. strain isolated from mastitic cow milk
Fig. 2. Inhibition areas for Candida spp. strain isolated from parrot faeces

The use of natural extracts for the treatment of different infections including candidiasis is of major importance in the last decades. Previous researches mostly aimed to
reveal the sensitivity to black or green tea leaves (2, 3). Many such extracts were tested using disk diffusion test or minimum inhibitory concentration (MIC).

Our findings demonstrated that carvacrol had the best efficiency of the tested products, in the undiluted concentration even better than the standard antimycotic product (nystatin). Similar studies also demonstrated good efficiency of natural extracts such as dill (1), black tea leaves (2) and green tea leaves (3). Disk diffusion test is a simple reliable technique recommended for the determination of the sensitivity of Candida strains to natural extracts.

Further researches will aim to compare the sensitivity tested using disk diffusion test with the more precise MIC test.

CONCLUSIONS

Most of the tested strains were resistant to α-pinene, β-pinene and grape seed extract. A good sensitivity was observed for tea tree extract, fir and nystatin.
The widest inhibition areas were obtained for carvacrol with an average of 38.5 mm diameter. For yeast infections treatment and prevention, food sources with high content of carvacrol are strongly recommended.

ACKNOWLEDGMENTS

This article was supported by UASVM RU-ISI project number 1353/8.02.2013.

REFERENCES