Evaluation of antifungal susceptibility of some *Candida* spp. strains using *Multodisc* antifungal disk system

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Abstract
The aim of this study was to evaluate the antifungal susceptibility of 33 Candida spp. isolates to classic antifungals included in Multodisc® system kits (Liofilchem – Italy). The research was conducted during January-June 2014 in the Department of Microbiology, Faculty of Veterinary Medicine, Cluj-Napoca, Romania. Identification was made from 48 hrs isolated colonies, using microscopic and biochemical methods (API Candida). From the total of 33 tested strains, 13 were represented by Candida albicans, 9 strains were identified as Candida krusei, 6 were Candida tropicalis 3 Candida catenulata and 2 were Candida parapsilosis. The antifungals included in the disk were represented by Econazole, Nystatin, Griseofulvin, Amphotericin B, Flucytosine, Miconazole, Metronidazole and Ketoconazole. The susceptibility test principle was based on the disk diffusion test, while the interpretation was performed determining the inhibition area diameter. The most efficient antifungal was represented by Ketoconazole with an average of the inhibition area of 26.4 mm followed by Miconazole with 23.4 mm and Econazole with 22.7 mm. The least efficient antifungals were represented by Metronidazole, Griseofulvin and Nystatin.
Keywords: *Candida spp., antifungals, susceptibility, Multodisc.*

Introduction

Candida yeast is a commensal micro-organism normally present in the gastrointestinal tract and an opportunistic causative agent of infections in humans and animals. The need for reproducible and clinically relevant antifungal susceptibility testing has been prompted by the increasing number of invasive fungal infections (IFIs), the expanding use of antifungal agents, and the recognition of antifungal resistance as an important clinical problem (Johnson, 2008).

In recent years, along with an increase in the incidence of candidiasis, there has been an important shift away from *Candida albicans* towards non albicans spp. The change in the epidemiology of Candida infections can be attributed to various factors like severe immunocompromised status of the host, exposure to broad spectrum antibacterial agents and empirical use of antimycotics. However, the clinical manifestations of infections caused by different non albicans Candida (NAC) spp. are usually indistinguishable from those by *C. albicans* (Deorukhkar, 2014).

In vitro antifungal susceptibility testing is now standardized internationally and is becoming essential in patient management and resistance surveillance; it remains less utilized than antibacterial testing (Deorukhkar, 2014).

Clearly, the disc diffusion method has the potential to provide a simple means of performing in vitro tests, but not all antifungal agents are available in discs. Furthermore, the discs are very expensive and their acquisition in developing countries is sometimes difficult (Magaldi, 2004).

The study was conducted on Candida isolates from animals and humans, and major objectives were to evaluate the antifungal susceptibility of 33 Candida spp. isolates to classic antifungals included in Multodisc® system kits (Liofilchem – Italy) and to recommend the best antifungal for the specific treatment.

Materials and methods

The researches of this study were conducted during January-June 2014 in the Laboratory of Microbiology, Faculty of Veterinary Medicine in Cluj-Napoca. A total of 33 Candida specimens isolated from animals with different pathologies (otitis, pharyngitis, mastitis), humans, and strains that contaminated diverse culture media, were included in this study. Candida tested strains were isolated from samples of mastitic cow milk, ear discharge from dogs with otitis, throat swabs from human subjects tonsillitis, urine samples from women with cystitis, faecal samples from hens and pigeons, ruminal fluid samples from cows, various strains that contaminated the culture media, throat swab samples from dogs and cosmetic products. Identification was made from 48 hrs isolated colonies, using microscopic and biochemical methods (API Candida).

Pure culture 48 hrs old well isolated yeast colonies were suspended in 5 ml of a sterile physiological solution until 0.5 McFarland turbidity is reached. A sterile swab was immersed in the suspension broth and then squeezed on the wall of the test tube to eliminate excess fluid. The swab was dragged along the surface of a SDA agar plate as to produce even growth. Antifungals included in the ring were represented by Econazole (ECN - 10µg), Nystatin (NY – 100 I.U.), Griseofulvin (GF - 10µg), Amphotericin B (AMB - 20µg), Flucytosine (FY - 1µg), Miconazole (MCL - 10µg), Metronidazole (MTZ - 10µg) and Ketoconazole (KCA - 10µg). The Multodisc® ring was positioned within 15 minutes from inoculation of the plates, pressing them with sterile pliers on the surface of the agar and then incubated at 35°C +/- 2° for 20-24 hours.

After incubation, the plates are examined; the inhibition halos around each disc are examined and compared with the standard inhibition haloes: in this way, the microorganisms are defined as being susceptible, intermediate or resistant to the tested antifungal agents.

Results and discussions

From the total of 33 tested strains, 13 were represented by *Candida albicans*, 9 strains were identified as *Candida krusei*, 6 were *Candida tropicalis* 3 *Candida catenulata* and 2 were *Candida parapsilosis*.

Susceptibility test principle was based on the disk diffusion test, while the interpretation was performed determining the inhibition area diameter. The interpretation was performed individually for each antifungal and yeast strain, and then the average of inhibition area was determined for each antifungal. Where total resistance was observed, the value considered for the calculation was zero. The most efficient antifungal was represented by Ketoconazole with an average of the inhibition area of 26.4 mm, followed by Miconazole with 23.4 mm and Econazole with 22.7 mm. The least efficient antifungals were represented by Metronidazole with 12.3 mm, Griseofulvin with 9.11 and Nystatin with 8.32 mm.

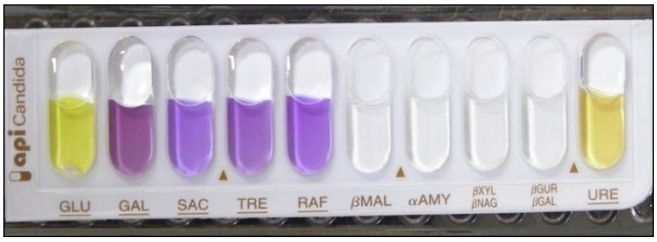

Fig. 1. Candida krusei strain identification – API Candida gallery

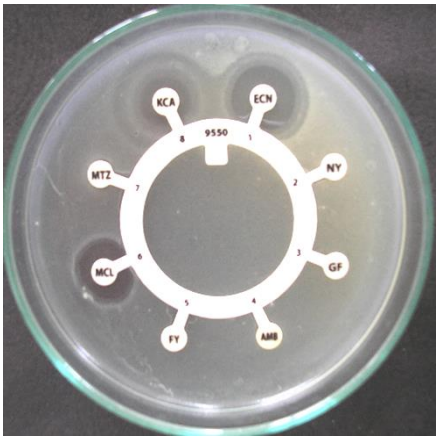

Fig. 2. Susceptibility testing for strain 23 A - *Candida albicans*

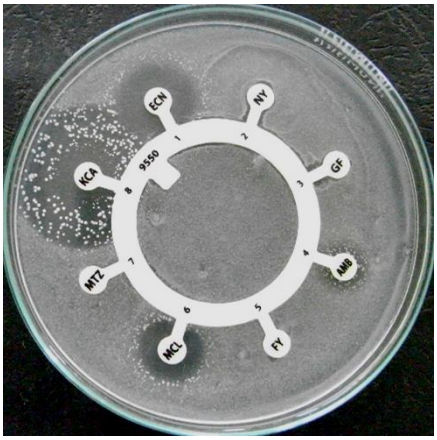

Fig. 3. Susceptibility testing for strain LM - 2 - *Candida krusei*

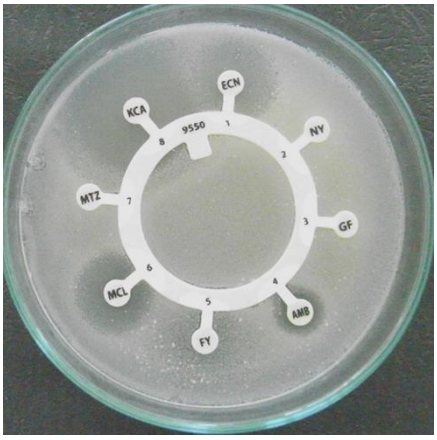

Fig. 4. Susceptibility testing for strain 171 S - *Candida tropicalis*

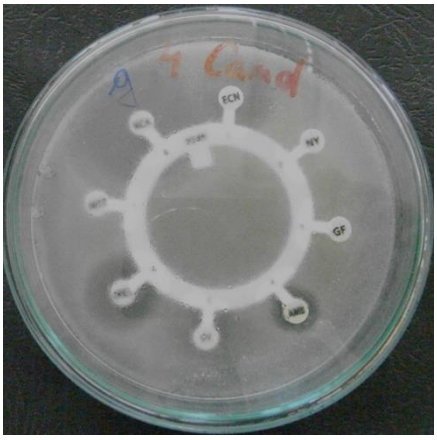

Fig. 5. Susceptibility testing for strain 4 Cand. - *Candida parapsilosis*

The results are in agreement to the studies of Giri, 2014, that on 39 Candida isolated obrained resistence patterns for fluconazole, ketoconazole and amphotericin B. The disk-diffusion method can offer a good level of sensitivity if the technique is performed by CLSI standards.

Comparing the results of Multodisc® test with the results of Etest, is observed that fluconazole is generally recommended as a therapeutic choice for systemic candidiasis, but amphotericin B does not have the same efficiency against Candida isolated in Cluj-Napoca compared to human patients with oral candidiasis (Song, 2015).

Conclusions

The researches on identification of *Candida spp*. isolates from animals and humans in Cluj county area and the sensitivity of these strains to antifungal resulted in the following conclusions:

- *Api Candida* system is a good method, easy to use and represents a relatively quick and inexpensive tool for Candida specie identification.
- *C. albicans* predominates in Cluj-Napoca area, followed by *C. krusei, C. tropicalis, C. catenulata* and *C. parapsilosis*.
- Classic antifungals tend to be less efficient in *in vitro* susceptibility testing of Candida species, due to their frequent use, except for Ketoconazole and Miconazole.
- The use of antifungals against Candida infections requires prior susceptibility testing in order to prevent antifungal resistance to these products.

References

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