IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING OF SOME *CANDIDA* SPP. STRAINS USING MODERN PHENOTYPIC METHODS

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Abstract

The aim of this study was to identify Candida isolates from animals and humans using VITEK 2 card system, a modern phenotypic method testing 64 biochemical characters and the susceptibility testing using MIC method on the same Vitek 2, for 6 modern antifungals. The research was conducted during January-June 2016 in the Department of Microbiology, Faculty of Veterinary Medicine in Cluj-Napoca. Identification was made from 48 hrs isolated colonies, preparing a suspension of 1.8-2.2 optical density, and 280µl were substracted and transferred over in saline for susceptibility testing. After a period of 18-19 hours for analysis of 64 biochemical characters for each tested strain, the results were saved/printed as Laboratory report in PDF format. From the total of 20 tested strains, six were represented by Candida albicans, five strains were identified as Candida krusei, 3 were Candida tropicalis 2 Candida catenulata 2 Candida parapsilosis and other two Cryptococcus laurentii. Most identified species are susceptible to micafungin with MIC ranging from 0.06 and 0.12 depending on the species, except for Candida parapsilosis. Voriconazole was also very efficient, with MIC value of 0.12 for almost all identified species. Caspofungin efficiency is specie-related, so for C. albicans and C. tropicalis MIC is 0.25, for C. krusei is 0.5 and for C. catenulata and C. parapsilosis MIC equals 1. Flucytosine has a constant value of 1 in all identified species, except for C. krusei with MIC value of 4 or 8 - resistant strains.

Keywords: *Candida spp., identification, Vitek 2, antifungals.*

Introduction

Candida yeast is a commensal micro-organisms normally present in the gastrointestinal tract and an opportunistic causative agent of infections in humans and animals. It is a medically important fungus, candidiasis and candidemia which induces in patients with compromised immune system, accompanied by increasing levels of pro-inflammatory cytokines (IL-17) (Kumamoto, 2011).

Candidiasis most commonly affects the mucous membranes, where this yeast is found normally found, with possible lesions at te level of the digestive tract, from the mouth to the stomach. Usually it remains limited to areas with squamous epithelium. Genital tract, skin and nails may also be involved. Respiratory or intestinal infections may occasionally occur. Epithelial surfaces forms of candidiasis appears as off-white plates with a yellowish tint or gray areas of ulceration show a different degree of inflammation. Diphtheria membranes can form in the gut and respiratory tract, may lead to the formation of visceral abscesses. Granulomatous lesions are rare and the inflammatory response of neutrophils is predominantly (Pohlman, 2013).

In recent years they research to identify methods of treatment and antifungal compounds with specific strains of *Candida albicans* have been performed. As eukaryotes fungi have a similar structure to the human cells, and it is difficult to identify the lethal dose of a substance for a specific microorganism, so that does not affect the patient. Pathogenicity of *Candida albicans* species depends on the complexity of virulence factors. These include the transition from yeast form to hyphae, antigenic variability, phenotypic changes, adherence to host tissue, cell surface hydrophobicity, and the production of extracellular enzymes. Pathogenicity and virulence factors involved in biological structure are derived from the fungal cell wall. The structure of the proteins of the microorganism are involved in adhesion to host cells.

Identification of Candida species isolated from animals and humans is an important research area since yeast infections are diagnosed rather frequent in the past decades, and more frequently strains resistant to usual antimycotics are isolated. In addition to the main specie

Candida albicans, species such as *C. famatra, C. krusei, C. parapsilosis, C. tropicalis*, etc. are involved in candidiasis ethiology in both animals and humans.

The study was conducted on Candida isolates from animals and humans, and major objectives were the identification of Candida isolates from animals and humans using cards VITEK 2 system, a modern phenotypic method testing 64 biochemical characters and susceptibility testing method based on the same MIC Vitek 2 system, for a number of 6 modern antifungals.

Materials and methods

The researches of this study were conducted during January-June 2016 in the Laboratory of Microbiology, Faculty of Veterinary Medicine in Cluj-Napoca. A total of 34 Candida specimens isolated from animals with different pathologies (otitis, pharyngitis, mastitis), humans, and strains that contaminated diverse culture media, were included in this study. In the end, identification and susceptibility testing was performed for a total of 20 strains. 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.

These samples were initially inoculated on Saboudaud dextrosis agar, and incubated for 48 hours at 25° C in aerobic conditions. The isolated colonies were subsequently used for the identification and susceptibility testing, in a Vitek® 2 compact 15 machine. The method for testing was represented by the introduction by means of automatic dispenser of 3 ml saline into the tubes and then using a plastic pipette, a fragment of an isolated colony was used to prepare a suspension of 1,8 to 2.2 optical density. Optical density evaluation was performed using an automatic device.

After preparing the inoculum, each tube was used to fill an identification card (YST-21 343), based on a phenotypic method that analyzes biochemical 64 characters. Identification cards/sensitivity test tubes were placed in suspension with the plastic tube attached to the tube containing the suspension, which then will be automatically transfer the card. These cards provide quick and accurate identification of a wide range of yeasts, up to 50 species.

The 20 samples were also tested regarding antifungal susceptibility to 6 modern antifungals, using minimum inhibitory concentration (MIC) method. The original amount of 3 ml suspension 1.8-2.2 optical density was used to collect 280µl and transfer over 3 ml of saline. Then specific cards (AST-YS07-414967) are attached to the tubes and the machine automatically fills the cards with the specimen suspension. The antifungals tested were amphotericin B, caspofungin, fluconazole, flucytosine, micafungin and voriconazole.

Results and discussions

Out of the 20 strains tested, six were represented by *Candida albicans*, five strains were identified as *Candida krusei*, 3 strains were identified as *Candida tropicalis* and 2 strains identified for each *Candida catenulate*, *Candida parapsilosis* and *Cryptococcus laurentii*.

The susceptibility testing of 6 antifungals agains 20 strains of Candida, showed that most species resulted in very good sensitivity levels to micafungin, MIC for this antifungal ranging from to 0.12 depending on the species, except for *Candida parapsilosis* strains.

Voriconazole also showed good results, with MIC value less or equal to 0.12 for almost all species identified. Caspofungin efficiency was specie related, so for *C. albicans* and *C. tropicalis* MIC was less or equal to 0.25, for *C. krusei* is equal to 0.5 and for *C. catenulata* and *C. parapsilosis* MIC equals 1. Flucytosine had a constant value less or equal to 1 for all species identified, except for *C. krusei*, with MIC value of 4 or 8, depending on the strain, results the show resistance of this specie.

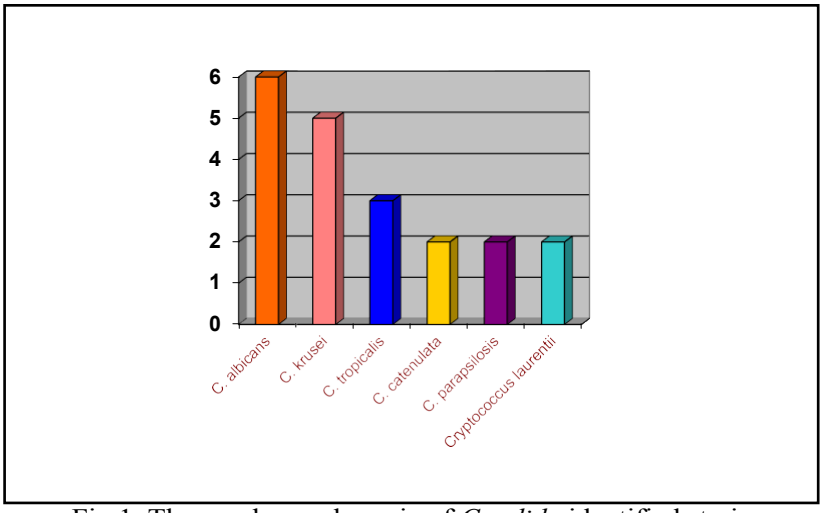

Fig 1. The number and specie of *Candida* identified strain

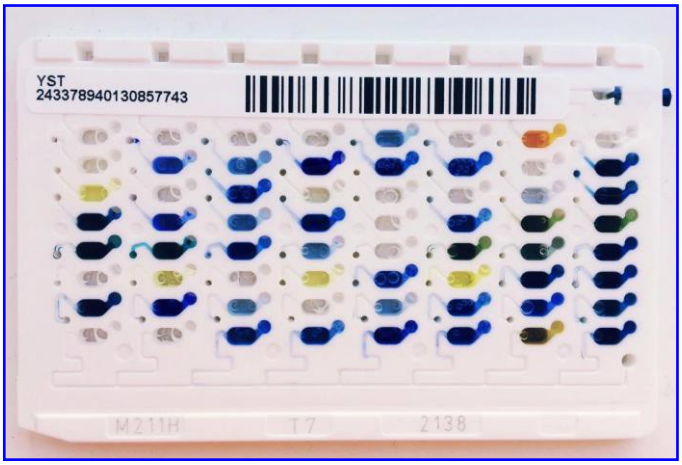

Fig. 2. Card for Candida species identification

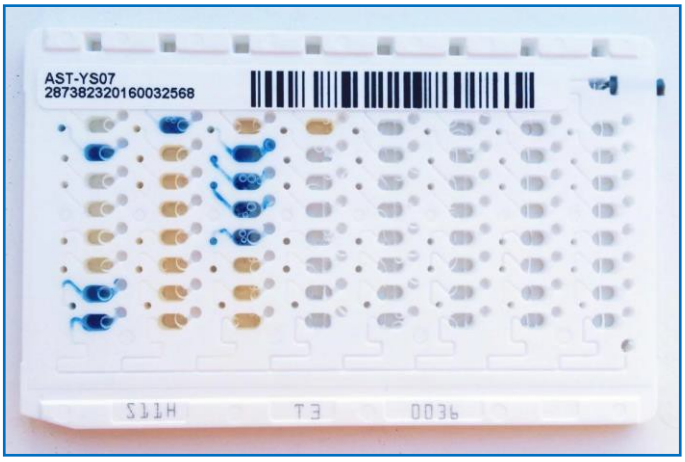

Fig. 3. Card for susceptibility testing

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:

- In the area of Cluj-Napoca, isolation of yeasts from genus Candida is very common, and card identification system is a precise method, with simultaneous identification and susceptibility testing and initiation of therapy is poaaible in a relatively short interval.
- Identified strains were diverse, with a major representation for *C. albicans*, while 2 strains of *Cryptococcus laurentii* were isolated from cosmetic creams, species which are morphologically identical to Candida strains, except that pink pigmented colonies are revealed on SDA.
- Modern antifungal sensitivity test for Candida species revealed generally good and very good results, the most effective being micafungin and voriconazole.
- Antifungals less effective were Fluconazole and Flucytosine, especially against strains isolated from mastitic cow milk (*Candida krusei*), which showed resistance for these products.

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