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IDENTIFICATION OF THE FUNGI THROUGH CANDIFAST TEST

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Abstract

The most frequently met species of the Candida Species is Candida Albicans, having a commensal role, but also pathogen for the immune-compromised persons or with different degrees of immune-depression. From the point of view of the morphology of yeasts from Candida species, it can be seen the fact that this fungus is pleiomorphic and that the change of form is in fact an adapting to the specific conditions of medium and to the exploitation to the maximum of the available nutrients. The blastospores are adapted to the fluid medium and the forms of hyphae and pseudohyphae are adapted to the solid or semisolid sublayer. The different forms are related to the virulence and thus to the capacity to invade the tissues.

Keywords: Candifast, hyphae, *Candida Albicans*

INTRODUCTION

The infections with *Candida* are very different as manifestation and aspect, for this reason they are divided depending on the place of appearance of the infection and the intensity and aspect of infection.

Candida holds a genetic equipment made of chromosomes, the set being diploid. So it is represented by a number of chromosomes numbered from 1 to 7 and the eight is named chromosome R.

Candida krusei , this species being isolated of the milk products, from different food with traditional nature as is beer and pickles. This species forms white to cream colonies, smooth faced, and microscopically are presented under the form of cells of yeast, small, long, oval, with blastoconidias, they don't have a capsule. Also, they form abundant pseudhyphae with oval blastoconidias in branches placed verticil.

The test of germinative tubes is negative, the hydrolysis of the urea is variable, the growth on medium with negative cyclohexomide, and the growth at 37 ℃ is positive.

Candida parapsilosis is a species that grows well on the Sabouraud medium forming white or cream colonies, smooth, and microscopically has the aspect of some normal cells of small yeasts, of globular or oval forms, it presents blastoconidias. The grow as abundant pseudohyphae branched, as some trees with groups of 2-3 blastoconidias. From the point of view of the biochemical properties it doesn't hydrolyses the urea, the growth at 37°C, and the test of the germinative tubes is negative.

Candida tropicalis is another species that is part of the human commensal flora and has the capacity to ferment the glucose and maltose but doesn't ferment the lactose and urea, and for sucrose, galactose and trehalose the tests are variable. They are used also in some biotechnologies for example the production of biodiesel. The form white to cream colonies on Sabouraud agar medium, (as for *Candida albicans*), smooth and microscopically are presented as cells of spherical or subspherical yeasts with blastoconidias. On the medium of corn extract and with Tween 80, appear pseudohyphae that are branched, with oval blastoconidias without forming terminal vesicles. The test of the tubes of germination is negative, the growth at 37 ℃ is positive, the hydrolysis of the urea is negative, and the growth on the medium with cycloheximide is positive.

MATERIAL AND METHODS

In the case of the studies regarding the infections with yeasts from this study, were collected only nasal and/or pharyngeal secretions, being resumed to the performing of the sensitivity of the fungi to the Candifast test. The samples were taken from the specialized employees appointed and the analysis was made in the laboratory.

The method by which was determined the sensitivity of the fungi is Candifast.

For the determination of the sensitivity of the fungi were followed the next steps:

1. It were inoculated some young colonies in the reagent R1, homogenizing well. Then R1 is compared with the control of turbidity.
2. From R1 are poured with the pipet 100 microliters in the reagent R2.

3. Then from reagent R1 are poured with the pipet in the 8 buckets, beginning from ACT (acditiona) to LAC (lactose), pouring with the pipet 2 drops of paraffin oil.
4. From R2 are poured with the pipet in the other 8 buckets, provided with antifungal
5. (Amphotericin B, Nistatin, Flucytosine, Econazol, Ketoconazol, Myconazol, Fluconazol), followed also by the pouring with the pipet of 2 drops of paraffin oil.
6. Finally, is replaced the foil over the sample, where is written above, the code of the patient, respectively his name.
7. The sample is incubated at 37 ℃, for a period of 24 – 48 hours.

RESULTS AND DISCUSSIONS

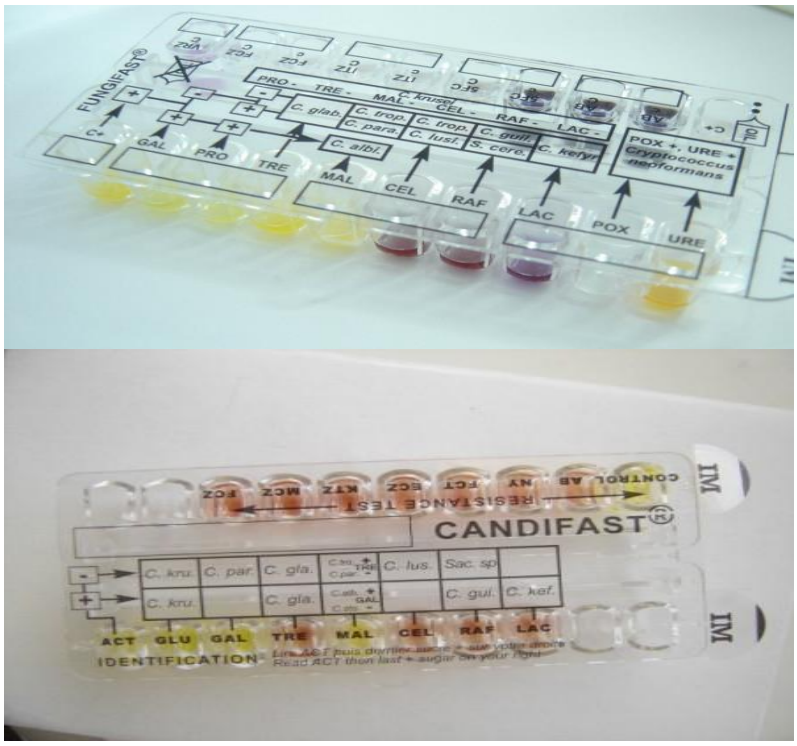

Fig. no. 1. Determination of the fungal sensitivity to Candifast

The samples from each patient were labeled and then processed in the laboratory by cultivating on culture medium, the microscopic analysis, respectively the submitting to the Candifast test.

The cultivation was made mainly on the culture medium Sabouraud dextrose agar without and with chloramphenicol.

From the culture mediums were samples and discharged on the slide an isolated colony that was examined under the microscope and submitted then to different other biochemical tests.

The microscopic aspect of the cultures is diverse. Thus it were observed levuric cells, blastopores, filaments, filamenting being characteristic for Candida and also chlamydospores, which appear only at Candida albicans Spheric cells of 20 – 22 μm in diameter, refringent, with double outline, that are found at the extremity of some pseudomycelium branches. It were used for the determination of the species of Candida chromogenic mediums.

Regarding the test of identification of the fungal sensitivity, it was accomplished according to the standard procedures CCLS or CLSI from USA for the reference laboratories. The determination is made according to the Candifast tests, determining the species and also the resistance to antifungal. Thus, the biochemical tests concern the ACT(acditiona) as a standard test, GLU (Glucose), GAL (Galactose), TRE (Trehalose), MAL (Maltose), CEL(Cellobiose), RAF(Rafinose), LAC(Lactose) added together with the red indicator of phenol in each bucket of the test.

The antifungals are Amphotericin B (4 μg/ml); Nistatin (200UI/ml); Fluconazole (16μg/ml), Flucytosine (35μg/ml); Econazole (16μg/ml); Ketoconazole (16μg/ml); Miconazole (16 μg/ml). Each series of buckets contain also a control one.

The results are visualized by a simple spontaneous colorimetric reaction, without instrumental or expert interpretation. The table of identification and the diagram are available for the interpretation of the results. It is possible to be used also the markings from the adhesive band for the testing tray.

It is followed the sensitivity of the fungi to antifungals. Thus in the case when it turned to yellow, orange or pink it means that the result is resistant, and if it turns to red, then the result is sensitive.

The general precision of this test was of 90% after 24 h and 95% after 48 h.

CONCLUSIONS

Once the number of invasive fundal infections caused by the species of Candida and the resistance to the antifungal therapy increases, the in vitro testing of the antifungal sensitivity becomes an important part of the laboratories of clinical microbiology.

Together with the method of micro dilution of the brodies and the method of diffusion on disk, are used more and more commercial methods for the testing of the antifungal sensitivity, as is the Candifast test.

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