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The Effect of some Physical and Chemical Factors on *Candida spp.* Strains Isolated from Animals

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Abstract. The researches were made during October 2009 – June 2010 within the Microbiology Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca. A total number of 24 *Candida spp.* strains isolated from both healthy and diseased animals were tested regarding the sensitivity to physical factors (temperature, ultraviolet radiations and microwaves) and chemical products (alcohol 70% and sodium hydroxide 1,2,3 and 4% concentrations). The tested strains proved to be sensitive to physical and factors, mostly to ultraviolet radiations, microwaves and alcohol.

Key words: *Candida spp.*, sensitivity, physical-chemical factors.

INTRODUCTION

Fungi are eukaryotic organisms with approximately 300 000 different species. Of these, about 200 are potential parasites, with only a few of these affecting animals (1). Fungal diseases of mammals, mycoses, range from the common mild cutaneous or subcutaneous skin infections, to the potentially lethal acute or chronic infection of deep tissues that are typically caused by *Candida* species. Of the *Candida* species affecting animals, *Candida albicans* is by far the most common. *Candida albicans* belongs to the class Ascomycetes and the family Saccharomycetaceae. This yeast can live as harmless commensal in many different body locations, and is carried in almost half of the population (5). However, in response to a change in the host environment, *Candida* can convert from a benign commensal into a disease-causing pathogen, causing infections in the oral, gastrointestinal, skin and genital tracts (1,).

Ultraviolet radiations adversely affect the development of fungi, particularly when in liquid medium. Dry, dead yeast exposed to light only after 14-20 days (2). A high frequency ultrasound causes cell membrane rupture, releasing protoplasmatic content. Chemical agents with antifungal action, very numerous and varied in composition have the feature of being highly active in vitro, may not be used in vivo due to their high toxicity and their low penetration capacity (4,5). For these reasons, most of them are only used as disinfectants or substances for external use in dermatomycosis (6).

The researches aimed to evaluate “in vitro” efficiency of physical and chemical factors on 24 *Candida spp.* strains isolated from both healthy and diseased animals. The factors tested within this study can be used mostly in the disinfection of the surfaces.

MATERIALS AND METHODS

The investigations took place during October 2009 – June 2010 within the Microbiology Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca. A total number

of 24 *Candida spp.* strains were isolated from both healthy and diseased animals. The samples collected from diseased animals and their type of lesions were represented by: mastitis cow milk, dogs suffering from otitis, cats and dogs suffering from tonsillitis and parrot faeces. The strains isolated were characterized morphologically – round or ovoid cells, grouped as bunches, different in size, and cultural – aspects in liquid and solid mediums.

The strains identified as *Candida spp.* were tested regarding the sensitivity/resistance to the following physical factors:

Temperature – for each tested strain 5 tubes (5 ml/tube) of 48 hours culture in glucose nutrient broth were prepared at the density of 2 McFarland scale. Culture tubes were placed in a water bath heated to 65°C, the temperature being maintained for 10, 15, 20, 30 and 45minutes. After this exposure, from each tube was removed 1 ml, which was dispersed on nutrient agar in Petri plates that were incubated at 37°C and then at room temperature. The plates were examined at 48, 72 and 96 hours, considering the development and number of colony forming units (CFU/ml).

Ultraviolet radiations - from 24h culture broth of the tested strains, sowings on solid medium were performed in Petri dishes (three plates for each exposure time and strain). They were introduced in thermostat for 20 minutes for drying the surface, after which half of the Petri dish was exposed while half was covered with an opaque ceramic plate. The first half was exposed to UV radiation for 5 minutes, 15 minutes and 30 minutes.

The plates were further incubated at 37°C for 24 hours and subsequently at room temperature. For the interpretation it was evaluated the development or development inhibition of the culture in the exposed area compared to covered area.

Microwaves. For each strain tested were prepared three tubes of glucose nutrient broth culture of 24 hours, (5 ml/tube), the density of 2 McFarland scale. The tubes were placed in a microwave oven at power 100 W and exposure times of 1 minute (tube 1), 2 minutes (tube 2) and 3 minutes (tube 3). After exposures of each tube were performed sowings on culture medium to verify the effect induced.

The chemical factors tested considered the possibility of their use mostly in the disinfection of surfaces and were represented by 70% alcohol and sodium hydroxide solution.

Materials prepared: 70% alcohol (commercial product) and sodium hydroxide solution, in concentration of 1,2,3 and 4%; *Candida spp.* glucose broth suspension prepared from colonies growth on solid agar at 5 McFarland scale optical density (approximately 1.5 x 109 germs / ml); glucose nutrient broth tubes; Petri dishes with nutrient agar glucose.

Working technique: *Candida spp.* suspensions of the tested strains were assigned to the quantity of 0.1 ml in each tube. The contact time for each substance and concentration was 5, 10, 15 and 30 minutes. After this contact, in each tube, were performed sowing in liquid and solid medium to verify the viability of these strains. The tubes were incubated in thermostat at 37°C for 24 hours and then up to one week at room temperature. Of the tubes remained clear sowings were performed on nutrient broth and agar to evaluate the effect: bactericidal or bacteriostatic. These tubes were kept in observation for 10 days.

RESULTS AND DISCUTION

Temperature: At 65°C *Candida spp.* strains tested showed variability regarding the inactivation, depending on the time of exposure. After 10 minutes of exposure 13 of the 24 tested strains were inactivated, with a percentage of 54.16%. After 15 minutes the number of inactivated strains reached 19, with the percentage of 79.16, while at 20 minutes, 22 strains

were inactivated, with the percentage of 91.66. At the exposure times of 30 and 45 minutes all the culture media sowed remained sterile.

Adverse effect is achieved by coagulation or distortion of various parts of the parietal or cytoplasmic structures, changes that are incompatible with survival, something demonstrated by the fact that cells were no longer present in the culture media.

Ultraviolet radiation (UV) is an invisible component of solar radiation, the wavelength lies between infrared radiation and X rays. There are three types of UV light (UV-A, UV-B and UB-C), which differ in wavelengths and have different effects on living organisms. UV-C radiation has a wavelength between 100 nm and 280 nm having a destructive effect on living cells, causing severe cellular damage. UV-C lamps generate radiation with a wavelength of 253.7 which has anti-yeast activity (4). To kill microorganisms, UV rays penetrate the cell membrane, crossing the cell contents and destroy him, causing injuries that prevent yeast activity and its ability to reproduce. Yeasticidal spectrum of UV-C lamps is large; they are able to destroy the organism that contains nucleic acids (DNA or RNA). Radiation doses are expressed in mWs/cm^2, efficiency is high (90-99%) (2).

In our working conditions, irradiation was produced by a lamp power of 60W and at a distance of about 40-50 cm, inside a laminar flow hood. Ultraviolet radiation proved very active, having "cid" effect which was expressed by inhibiting the development of culture plate area exposed to radiation (medium remains transparent). Minimum time which led to inactivation was 5 minutes.

Effectiveness of antibacterial action of UV devices depends on achieving optimum working conditions. Exposure time depends on the type of microorganisms. In case of surface disinfection, exposure time depends on the distance from the radiation source. An optimal size of irradiated area and exposure time is obtained for a distance of 1 m. The distance from the radiation source must not exceed 3.5m.

Microwaves are a form of electromagnetic energy generated by electronic devices having wavelength between 30 cm and 1 mm while frequency ranges 10^9-$3x10^{11}$Hz. Electromagnetic field of microwaves interactions with biological structures at the cellular level causing direct and indirect effects. Following penetration of microwaves in the watery medium, there is a strong agitation of water molecules, which results in sudden temperature increase leading to yeast inactivation. As mentioned mechanisms are membrane potential changes irreversibly altered transmembrane transport processes, membrane irreversible electroporation, and formation of free radicals with high oxidation capacity. It interacts with components of cell membrane leading to block sites on the membrane, process specific to microorganisms with active multiplication (5).

In our working conditions, *Candida* suspensions after 1 minute of exposure to microwaves were inactivated and did not developed in culture, aspect also observed for the exposure times of 2 and 3 minutes. In all cases the tubes sowed remained sterile.

Chemical substances used in this experiment are utilized routinely in various activities of disinfection being considered efficient on yeasts and accessible on the cost price.

Alcohol has antibacterial properties in concentrations of 50-70%. In the presence of alcohol at an appropriate concentration in microbial cells, processes of dehydration occur, precipitation of proteins and albumins, changes which are incompatible with survival. A higher concentration has a lower activity, because following precipitation of albumin inside the cell membrane, alcohol cannot penetrate inside the cell. Below 50° antimycotic action also decreases due to the reduction of albumin coagulation. Due to the avidity to water, alcohol

dehydrates the cytoplasm and may precipitate albumin. Many bacteria are killed in vitro very fast by alcohol in concentration of 40-100° (6).

In case of 70% alcohol all the tested strains were already inactivated after 5 minutes of contact, the medium sowed remained sterile. The same situation was registered for contact times of 10, 15 and 30 minutes.

Sodium hydroxide is an alkaline substance with strong disinfectant properties, having a wide use in the disinfection of shelters. Use of hot solutions improves antilevuric activity (6). The results obtained in our studies are presented in table 1.

Tab 1.

Sensitivity of Candida spp. strains to sodium hydroxide

	NaOH solutions concentration	Contact times			
		5 minutes	10 minutes	15 minutes	30 minutes
Number of resistant strains	1%	21	13	6	2
	2%	14	6	3	0
	3%	5	1	0	0
	4%	0	0	0	0

The results reveal that the solutions of NaOH presented a lower inhibitory effect compared with alcohol efficiency. At 5 minutes of contact for the concentration of 1%, 21 strains of 24 (87.5%) resisted maintaining their viability in cultures. After 10 minutes of contact 13 of 24 (54.16%) strains were not sensitive to sodium hydroxide, while after 15 minutes of contact 6 strains (25%) resisted. The number of resistant strains after 30 minutes of contact at the concentration of 1% was 2 (8.33%). For the concentration of 2% the number of resistant strains was reduced for each contact time compared to 1% concentration. At 3% concentration after 15 minutes of contact none of the tested strains resisted, situation similar to 4% concentration at all contact times. We recommend that disinfections in case of *Candida spp.* to use a minimum concentration of 3% and a minimum 15 minutes of contact.

CONCLUSIONS

- The temperature of 65°C used for inactivation proved efficient only after 20 minutes of contact, while after 10 respectively 15 minutes many of the tested strains resisted.
- Ultraviolet radiations had a very good destructive effect, after 5 minutes of exposure, all tested strains were inactivated.
- In case of microwaves the power used (100 W) lead to the inactivation of all strains after 1 minute of exposure.
- 70% alcohol used as current disinfectant is very efficient even after 5 minutes of contact.
- Sodium hydroxide proved efficient only in concentrations of 3% and a contact time of minimum 15 minutes.

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