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SELECTIVE MEDIA FOR THE ISOLATION OF THE STOOL YEASTS

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Abstract

The fungal infections and especially those determined by yeasts have grown significantly in the last years. The appearance of the conflict between the yeasts and the host is favored by a series of endogen or hexogen factors that either induce the host an immunosuppression condition or modifies the quantity of the existent report between the yeasts and the other categories of microorganisms. The microscopic exam follows the presence, the form and the dimensions of the yeast formations: blastopores, pseudohyphae, hyphae, arthrospores eventually. Details like the presence or absence of the capsule, the method of sprouting will be apprehended as important aspects for the future identification. For the patients under antifungal treatment it is possible the highlighting of the yeast in the microscopic exam in the absence of their growth on culture media. The cultivation of the pathologic products is made on agar Sabouraud with Chloramphenicol and Gentamicin, medium that inhibits the multiplying of the contaminated bacteria and allows a good development of the yeasts.

Keywords: fungi, endogen, capsule

INTRODUCTION

Fungi are a wide group of microorganisms, differentiated enough of other forms of life in order to be considered as a different universe. They are eukaryote cells that possess a nucleus with a nuclear membrane and a cell wall made of polysaccharides, polypeptides and chitin. The have heterotroph nutrition and they live as saprophytes, parasites or commensals, for a wide variety of organic substrates. Their structure can be unicellular, as is the case of yeasts, or multicellular, observed in cells that are elongated in order to form filaments or hyphae. They are divided through a transversal wall, called septum. Some fungi have a very tight septum for this reason they are called asepted. The filaments create a structure called mycelium. Some fungi present both forms of growth and as a consequence they can exist either under the form of yeasts, or in the form of filaments, depending the temperature. The phase of yeast is created in the host tissue and in the culture developed at 37°C, and the phase of mould is observed in the

cultures developed at temperatures between 25-28°C. These fungi are called dimorphs. There are still microorganisms that produce also yeasts and filaments so that the two forms can coexist and their appearance is not necessarily determined by the temperature. These fungi are called polymorphs.

Nystatin, discovered in 1951 was the first polienic antifungal used with success under topical and oral form in the treatment of candidiasis. Nytamicin and amphotericin B, used in the treatment of systemic mycoses, are also part of the category of polienic antifungals. Griseofulvin, antifungal administered orally and produced in 1959 from Penicillium griseofulvum proved to be efficient for the dermatophytes. It is used in the treatment of superficial mycoses, especially in the dermatophytes of the hair. The introducing of the asolic antifungals has marked the development of the dermal therapy. The asolic derivatives are distinguished by their wide range of action, being active on dermatophytes, yeasts and a large part of the pathogen mould for human. In 1969 it appeared under the denomination of imidazolic derivatives: clotrimazole and miconazole, followed by econazole and ketoconazole used in the local treatment of cutaneous mycoses. Ketoconazole was the first systemic antimycotic with wide range from this group. The systemic treatment of mycoses had an important ascension once the fluconazole and itraconazole were discovered, antifungal from the triazolic derivatives group.

MATERIAL ŞI METHOD

We accomplished a prospective study, based on the microbiologic diagnosis registered in the bacteriologic register of the laboratory of medical analysis, S.C. Diaser, Oradea.

For the performing of the study we used also the archive, registered in the specific program of the computer from the laboratory of S.C. Diaser, Oradea, in the computerized data base of the unit, respectively.

Necessary materials for the performing of the examination:

■ A recipient of collection (collection recipient with collecting spoon) with transport medium
■ Wooden spatula
■ Latex gloves

For the collection of fecal matter it has to be collected a sample of fecal matter of 5-10g introduced in the collection recipient of fecal matter with transport medium. If the stool is liquid, it will be collected 5 ml. It is recommended to be chosen a liquid, mucous and bloody portion, if there is one. Don't collect quantities larger than 10g because it will reduce the chances of isolating the pathogen bacteria.

Considerations of pre-collection:

■ 8 days before the collection of the sample, don't take antibiotics or antiparasitary medicine.

■ The diet is not necessary.

Collection and transport of the samples

In regard to the collection, it has to be done as close to the beginning of the disease and before the initiation of any antimicrobial treatment.

• Collection from the stool emitted spontaneously – is preferable and is indicated in all the forms of acute diarrhea when the emission of fecal matters is frequent.

• For bacterial and parasitary examinations, the collection is made with the "spoon" of the collection recipient, concerning the liquid parts and especially, the mucous and/or bloody one, if there are. The volume of the collection has to be of minimum 5 ml or 3-5 cm^3, if the stool is formed.

• For isolations or virusologic exams is collected 5-10 cm^3 **fecal matters or minimum 5 ml, if the stool is not formed.**

• Rectum collection – is recommended in:

- Chronic shigellosis where the curettage of the rectal mucous with the probe or with the tampon offers greater chances to isolation;
- The investigation of the bearers of Shigella and Salmonella, with the exception of those of S. Typhi.

For this type of collection are used Nelaton probes (nr.14-16) or adequate tampons, thus: with the tampon, soaked in isotone saline solution (not to use lubricant gels) is penetrated the anal sphincter by slow rotation, introducing intra rectum approximately 15 cm. It will be proceeded identically also with the Nelaton probe, to which is adapted a syringe (10 ml) with which are made 1-2 aspirations. After collection, the probes and tampons are introduced in sterile recipients that contain preservation medium, are labeled correspondingly and are sent to the laboratory.

The **transport** of the samples and their processing is made in maximum 1 h, if they were collected in recipients without transport medium (with transport at room temperature), or they can be kept up to 24 h at room temperature, if they were collected in recipients that contain Cary-Blair transport medium that assures a good durability of the bacterial intestinal pathogens. An exception to these rules is the samples collected in the suspicion of infection with Shigella spp, very sensitive bacteria that need seeding from the media of culture immediately after collection.

For the viral etiology the samples that are not processed imediately have to be kept at – 70°C.

Isolation of the yeasts

Microscopy

• on colored gram smears is followed the presence of yeasts in large and predominant quantity compared to the diminished fecaloid bacterial flora.

• it is decisive for the cultivation of fecal matters for the isolation and quantification of yeasts.

The cultivation on Sabouraud medium with Chloramphenicol, with observance at 48–72 h.

In order to establish the mycotic etiology of the diarrheic syndrome it will be performed the quantitative exam of the yeasts, the determination of the units forming mycotic colonies per g or ml of fecal matters, respectively, significant quantity being a number of $>10^9$UFC/g or ml of fecal matters.

RESULTS AND DISCUSSIONS

On the culture media the yeasts of medical interest are developed in 49-72 hours of incubation, in some suspicious diagnoses the period of observance being prolonged up to 7 days. The temperature of incubation after the seeding is in congruence with the anatomic area of provenience of the sample: 36-37°C for the internal and deep samples or of 30°C for the superficial samples. After the obtaining of primo culture it is necessarily verified its purity, because the bacterial contamination compromises the further phase of identification of the species. For this purpose are performed gram colored smears that will be examined under the microscope with the immersion objective. In case of the presence of bacteria there are replications made for the purification of the culture.

Usually it is made with the help of a standard biochemical system based on the fermenting of 7 sugars and an enzymatic test. With the help of this system you can identify the following species: Candida spp., Cryptococcus spp., Trichosporon spp., Saccharomyces cerevisiae, Rhodotorula.

The microscopic aspect is decisive for the cultivation of the stool in order to isolate and quantify the yeasts. Always colored intensely gram-positive, depending on the taxonomic group, they have different forms, of spherical or oval yeasts disposes in moniliform piles or chains most often branched. The disposing and morphology suggests the species, for this reason the microscopy of the sample and afterwards of the isolates is essential in the mycological examination.

The isolation of the yeasts was made by cultivation on agar media that include inhibitors for bacteria. The previous phases of the cultivation are identical to those for the bacteriological investigation, with the mention that the collection is made always from a stool emitted spontaneously.

Although there were considered lots of media, in the literature there are a few specifications to the isolation of the fungi from fecal matter.

In table 1. are presented two selective media recommended for the isolation of the yeasts from products with bacterial flora of rich association. Both are versions of the Sabouraud medium supplemented with mixture of antibiotics inhibitor for the aerobe bacteria from fecal matter.

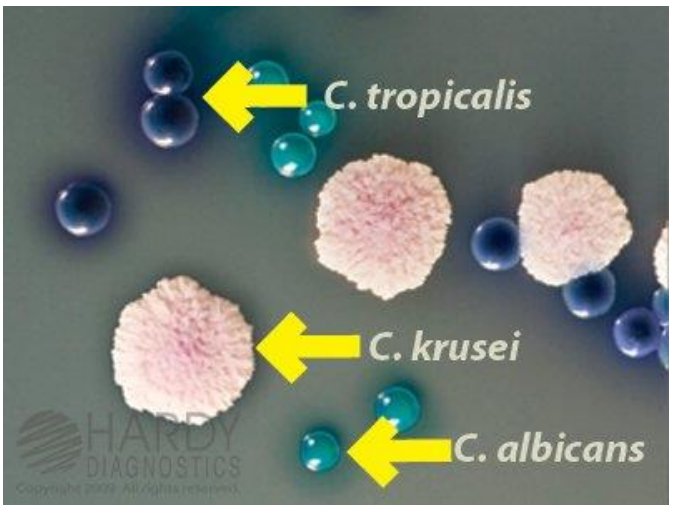

Fig.1. Specific colonies for *C. Albicans, C. Krusei, C. Tropicalis.*

Table 1.

Selective media for the isolation of the stool yeasts

Medium	Abbrev.	Nutritive constituency	Selective constituency	Incubation at 25℃ or 35℃
Sabouraud	Sabouraud CCG	Glucose Bactopeptone NaCl Agar	Chloramphenicol Cycloheximid Gentamicin	Selective for *C. albicans*
Sabourand with dextrose Chloramphenicol	Sabouraud DC	Glucose Neopeptone Agar	Chloramphenicol	Selective for fungi

The Sabouraud CCG medium is highly selective allowing the development of only *C.albicans.* The version Sabouraud DC is less inhibiting, allowing the development also of other yeasts, so it has a wide utilization.

In order to incriminate the mycotic etiology in an diarrheic syndrome it is necessary a quantitative examination of the yeasts, the determination of the number of units forming mycotic colonies (UFC)/g or ml of fecal matter, respectively, and identification of the respective yeasts.

From the initial suspension of fecal matter, in isolated saline solution, are made dilutions 10^{-2} and 10^{-3} (respectively 10^{-3} and 10^{-4} from the product), of which, 0,2 ml is seeded on a plate of Sabouraud DC by flooding. Also from the initial suspension are made dispersions with the loop on Sobouraud CCG.

The preliminary identification is based on the characteristics of colonies and on the microscopic characteristics (form, dimensions, presence of capsule).

Candida albicans has a major role in mycotic etiology of the diarrheic syndrome. The presence especially in immunodeficiency represents one of the frequent intercurrent infections in AIDS. Aspects of cultivability of *C. albicans* are characteristic on agar Sabouraud CCG, being the only yeast that is developed on such a degree of selectivity.

The study regarding the "Immobilization of the filamentous mushrooms. A new frontier in the production of organic acids" highlights some fungal metabolites, primary or secondary, and enzymes and other products of biotransformation of fungal origin that play a strategic role in many technologies and especially in processing the food. Among these, the organic acids seem to be of major interest. The citric acid is already produced in more than 3 tons / year and the demand on the market for other acids, as it would be the gluconic, lactic and malic one, is in continuous increase. This revision is dedicated mainly to the most recent progresses in the production of organic acids from the systems of immobilized fungal cells. The production of fumaric acid by immobilized *Rhizopus* arrhizus is reported as case study. In fact, the fermentative production of this acid, which was for a long time abandoned from economic reasons and replaced with the direct chemical synthesis, would benefit largely by the utilization of a technology reliant on immobilized cells.

CONCLUSIONS

1. The microscopic exam follows the presence, the form and the dimensions of the yeast formations: blastopores, pseudohyphae, hyphae, arthrospores eventually. Details like the presence or absence of the capsule, the method of sprouting will be apprehended as important aspects for the future identification.
2. On the culture media the yeasts of medical interest are developing in 48-72 hours of incubation, in certain diagnosis suspicions the period of observance being prolonged up to 7 days.
3. The temperature of incubation after seeding is in concordance with the anatomic area of provenience of the sample: 36-37°C for the internal and deep samples or of 30°C for the superficial samples.
4. The Sabouraud CCG medium is highly selective allowing the developing only of *C.albicans.* The Sabouraud DC version is less inhibiting, allowing the development also of other yeasts, having a wide utilization.

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