Annals of the University of Oradea, Fascicle: Ecotoxicology, Animal Husbandry and Food Science and Technology, Vol. XIX/A 2020
Analele Universităţii din Oradea, Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară, Vol. XIX/A 2020

IDENTIFICATION OF C.DIFFICILE IN FOOD TOXINFECTION

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Abstract

The microorganisms represent the object of study of the microbiology. They compose a wide and heterogeneous group as a biological activity, having as common characters the microscopic dimensions that make them invisible to the naked eye.

In this group are included the bacteria, microscopic fungi (yeasts, molds), viruses. The microbiology reunites the biological sciences that have as object the study of microorganisms. Kircher intuits the existence of the microorganisms.

The theory of the spontaneous generation was pretty fast explained. If the food is staying more time in unfavorable conditions of environment, they enter in putrefaction. If they are examined under the microscope it is observed the presence of a very large number of bacteria. Where do these bacteria come from if they are not found in fresh food. Some researchers say that from the air, others spontaneous from inert matter.

Keywords: microorganism, molds, yeast

INTRODUCTION

The numerous microorganisms are saprophytes and some of them, including here the bacteria, viruses, yeasts, molds and protozoans occupy an important place in the food industry.

The bacteria, organisms present in almost all the natural ecosystems, is well defined biologically by the prokaryotic type of organization.

The viruses are microorganism extremely small, which in order to multiply utilize the resources of the host cell of vegetal type, animal or bacterial.

The yeast and molds are fungi that contain chlorophyll. They have variable dimensions, from unicellular organisms to large fungi.

The protozoans are unicellular organisms considered in the category of the animal kingdom due to the different forms in which they live. These can produce diseases in human beings and animals.

By the determining of the sequence there is information achieved, being supplied new data necessary for the establishing of the evolutional

relations. As any other technique of the molecular biology, the determination of the DNA or RNA sequence needs time and large costs.

Some members of the Eubacteria gender are identified frequently by the appealing to method of special colors and various biochemical tests.

The analysis of ribosomal RNA is made by analyzing the ribonucleic acid from the ribosomes and is possible the obtaining of information that can be used in taxonomy, the collected data being able to be used for the identification of the similarities of the RNA sequences.

From the structural point of view these organelle are made of ribosomal RNA and ribosomal proteins.

The analysis of DNA consists of the fact that the hybridizations of DNA-DNA or DNA-RNA were used a period of time and continue to be extremely valuable in the bacterial systematization. It was found that the ideal reference system for the bacterial taxonomy is the complete sequencing of the DNA of an organism.

MATERIAL AND METHODS

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

The period for which was extended the study is of 5 years, including the period 01.01.2014-31.12.2019.

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

The necessary materials for the performing of the examination:

- A recipient of collection (collection recipient of fecal matter with collecting spoon) with transport medium
- Wood 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 harvested 5 ml. It is recommended to be chosen a liquid, mucous and bloody portion, if there is one. Don’t collect larger quantities than 10 g because will reduce the chances to isolate the pathogen bacteria.

RESULTS AND DISCUSSIONS

From the suspension of fecal matters are made directly dispersions on to selective mediums.

Agar phenethyl alcohol, preferably spiked with 5% of defribinated ram blood, that allows the growth of clostridias and other anaerobe gram positive present in the intestine content.

Agar with egg yolk, fructose and antibiotics (cefoxitin and D – cycloserine). This medium with high selective capacitive inhibits the other clostridias and anaerobe gram-positive cocci: they don't inhibit the *C.difficille*. Both mediums are incubated anaerobe 48 hours at 37°C.

The isolation with enriching, consists of the procedure of enriching, that was recommended and is used currently for a wide range of enteric pathogen that are found dispersed in a small number on the unit volume of fecal matters. The pathogen process being developed, the excreted bacteria are dispersed in a fecaloid mass becoming abundant by inhaling the intestine hydro-electrolytic. As a consequence the reduced density of the pathogens determined the introducing of a process of enriching of the etiologic agent in salmonellosis, yersiniosis, cholera.

In the low diarrhea syndromes, rectal-sigmodian and in the postantibiotics therapy intestinal disbacteriosis, the etiologic agent eliminated at a large density doesn't need enriching that could modify the reports between the groups of bacteria included in the fecal matters.

The phases of the bacteriologic examination by cultivation are presented below, after the initial phase, respectively the sampling, the methodological lines regarding the isolation and identification corresponding to each methodology of investigation are: aerobe, microaerophile and anaerobe.

From the registered data, in regard to the isolation of aerobe bacteria I am entitled to affirm that the aerobe bacterial etiology represents more than half of the known etiology of the diarrhea syndrome. In part, this "dominant" is caused also by the possibilities of investigation, accessible to the most laboratories from the hospitals and antiepidemic centers, that allow the specifying of the etiology more frequently than in the case of other groups of bacterial or viral etiologic agents. Being known the unsuccessful isolations due to the reduced number of etiologic agents on the unit of volume of the investigated sample, in some enterobacteriosis is recommended the "enriching of the inoculum by sub cultivation on mediums that favor by preference of the multiplication of the enteritis pathogens."

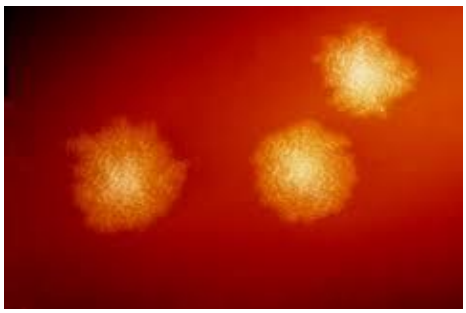

Fig.1. *C.difficille. medium of culture agar - blood. www.marlerblog.com*

The study regarding the "Detecting of *Clostridium difficile* and of the toxins in test samples of minced meat and cube of chopped red meat in modified athmosphere" underlines the prevalence of *Clostridium difficile* in the packed samples (MAP) chopped (n: 50) and the samples of red meat (n:50); It was determined the toxin from isolated and was detected the sensitivity to antibiotics, to metronidazole, vancomycin and clindamycin. *C. difficile* was isolated 4%, of the 50 samples of chopped red meat and 2%, of the 50 samples of cube chopped red meat. All the three isolated were confirmed by PCR as being *C. difficile* by detecting the gene. Three of the isolated, from the 5 of *C. difficile* presented a toxigenic nature, two of them bore genes of toxin type B (tcdB), one of them toxin gene type A (tcdA). When the profile of resistance to antibiotics was analyzed phetotipically, only *C. difficile* type A (tcdA) was resistant to clindamycin. All the isolated were sensitive to vancomycin and metronidazole. The result of this study demonstrated that the strains of *C. difficile* detected in the test samples of red meat packed in modified atmosphere (MAP) can be a possible problem for the public health.

CONCLUSIONS

Giving the diagnosis of laboratory in regard to the *Clostridium difficile,* is based on the culture and detection of toxins on the fecal samples. The culture is accomplished on selective medium available commercially.

The morphology of the *Clostridium difficile* colony is typical when it is examined under the optical microscope. The definitive identification is obtained the best by the chromatography of the liquid with gas.

The culture is very sensitive, but, when it is used alone without testing the toxin, lead to the low specificity and wrong diagnostic when there are not symptoms.

The detection of the toxin by an analysis of cytotoxin in the culture of the tissue followed by the neutralization with specific antiserum is often considered standard.

Beside all these, this approach has no sensitivity and did not detect but up to 30% of the patients. More immuno-enzymatic tests (EIA) were introduced by different manufacturers for the detecting of the toxin A alone or for both toxin A and B.

Some of them are conceive to give results in less than 1 hour. The comparative studies of the immune-enzymatic kits have reported that the sensitivity and specificity are a bit smaller than the cytotoxin tests.

The isolations in regard to *C. difficile* for the production of toxins, the colonies isolated on selective mediums are tested for the production of toxins in vitro either by a test of cytotoxicity or by direct immune-enzymatic tests. It has a higher sensitivity than the test of cytotoxicity and equivalent specificity. In the normal laboratory, the detection of the culture and of the toxins have to be performed on each test sample and, in the cases of toxic-positive and fecal culture, the toxigenic cultures have to be performed on isolated colonies.

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