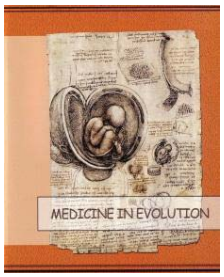
Diagnosis of laboratory in the infections produced by *Clostridium Difficile*



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Abstract

Aim and objectives: Regarding the diagnosis of laboratory in the infections produced by the gram-positive bacillus, anaerobe, *Clostridium Difficile* has as target the bacteriological examination, following the basic examination of the microbiological diagnosis of the infectious diarrheic syndrome, being represented by the cultivation of the fecal matters on specific culture mediums.

Material and methods: We accomplished a prospective and retrospective study, on eighty of clinical isolated samples of *C. Difficile* to identify and underline the genotypic and/or phenotypic characteristics between the isolated strains from cases of unique infections and those isolated from recurrent cases, based on the microbiologic diagnosis registered in the bacteriological register of the laboratory of medical analysis, S.C. Diaser, Oradea.

Results and discussions: The most important factor of virulence is the release of multiple toxins, and namely large glycosylated exotoxins A and B.

Conclusions: The placing of the diagnosis of laboratory in regard to *Clostridium Difficile*, is based on the culture and the detection of toxins in fecal samples.

Keywords: *Clostridium Difficile,* anaerobe, exotoxins, glycosylated

INTRODUCTION

In regard to the germination of the spores of *C. Difficile* it is supported by modifications of the composition of biliary acids. A small number of bacteria, producing hydrolase enzymes, has as result a reduction of the secondary biliary acids, that inhibits normally the growth of the vegetative cells and a simultaneous growth of the primary biliary acids as the cholate or the taurocholic acid that stimulates the germination of the spores [1-4]. It is observed that, while the cholate and the wistaria can promote the formation of spores of *C. Difficile*, the chenodeoxycholate acts as an inhibitor of the spores formation.

The bacteriocins are ribosomal synthetized antimicrobial peptides with activity of narrow or wide spectrum against other bacterial species [5].

C. Difficile is most frequently met in old age, presenting also a more severe result in this population. For the explaining of this phenomenon it is presupposed the existence of many mechanisms. Thus, a first mechanism if that of an innate or a humoral inadequate immune answer that can lead to a larger incidence and, also, to the severity of *C. Difficile* [6,7]. The second mechanism can be associated with the change of the intestinal microbial composition, for example, the loss of the bacterial diversity during the ageing, that could promote the colonizing of *C. Difficile* [8].

Aim and objectives

The purpose of this study regarding the diagnosis of laboratory in the infections produced by *C. Difficile* has as target the bacteriological examination, following the basic examination of the microbiological diagnosis of the infectious diarrheic syndrome, being represented by the cultivation of the fecal matters on specific culture mediums. This presupposes the isolation of the bacterial etiologic agent on adequate mediums and it identification based on the morphological characteristics, of culture, of exoenzymes and antigenic.

Objectives: Isolation without enriching or direct isolation; Identification corresponding to each methodology of investigation; Prominence of the utilization of enriching mediums for the isolation of the aerobe enteritis pathogens.

MATERIAL AND METHODS

We accomplished a prospective and retrospective study, on eighty of clinical isolated samples of *C. Difficile* to identify and underline the genotypic and/or phenotypic characteristics between the isolated strains from cases of unique infections and those isolated from recurrent cases, based on the microbiologic diagnosis registered in the bacteriological register of the laboratory of medical analysis, S.C. Diaser, Oradea.

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 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 will reduce the chances of isolating the pathogen bacteria.

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

The collection from the stool spontaneous emitted – is preferred and is indicated in all the forms of acute diarrhea when the fecal matter emitting is frequent.

For the bacterial examinations, the collection is made with the collection recipient „spoon", concerning the liquid portions and, especially, those mucous and bloody portions, if there are ones. The volume of the collection has to be of minimum 5 ml or 3-5 cm3, if the stool is formed.

RESULTS AND DISCUSSIONS

There are performed in the suspension of fecal matters, directly, dispersions on two selective mediums. It is preferred added with 5% of ram defibrinated blood, agar phenethyl alcohol, that allows the growth of clostridia and other anaerobe gram positive in the intestinal content.

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

The isolation with enriching, consists of the procedure of enriching that was recommended and is used currently for a suite of enteric pathogens that are dispersed in a small number on the unit of volume of fecal matters [8-10]. Following the pathogen process that was developed, the excreted bacteria are dispersed in a fecaloid mass becoming abundant by the inhaling of intestinal hydro-electrolytic liquids. As a consequence the reduces density of pathogens has determined the introducing of a process of enriching of the etiologic agent in salmonellosis, yersiniosis, cholera [12-14].

Regarding the low diarrheic syndromes, recto-sigmoidal and in post antibiotics therapy intestinal dysbacteriosis, the etiologic agent eliminated at a larger density doesn't need enriching that would modify the reports between the groups of bacteria composing the fecal matter.

The phases of the bacteriological examination by cultivation are presented below (Figure 1), after the initial phase, respectively the collection, the methodological lines regarding the isolation and identification corresponding to each methodology of investigation are: aerobe, microaerophilic and anaerobe.

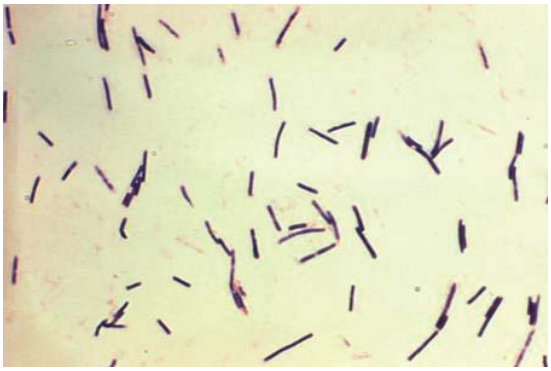

Figure 1. *Difficile*, gram-positive bacillus, anaerobe

From the registered data, regarding the isolation of the aerobe bacteria we are entitled to say that the aerobe bacterial etiology represents more than half of the known etiology of the diarrheic syndrome [15,16]. In part, this "dominant" is determined also by the possibilities of investigation, accessible to the most of the laboratories from the hospitals and anti-epidemic centers, that allow the specification of the etiology more frequently than in the case of other groups of bacterial etiologic or viral agents. Being known the unsuccessful isolation caused by the reduced number of etiologic agents on the unit of volume of the investigated sample, in some Enterobacteriaceae illness is recommended the "enriching of the inoculum by

subcultivation on medium that favor preferably the multiplication of the enteritis pathogens (Figures 2,3).

Figure 2. Colonies of *C. Difficile*, medium of blood agar

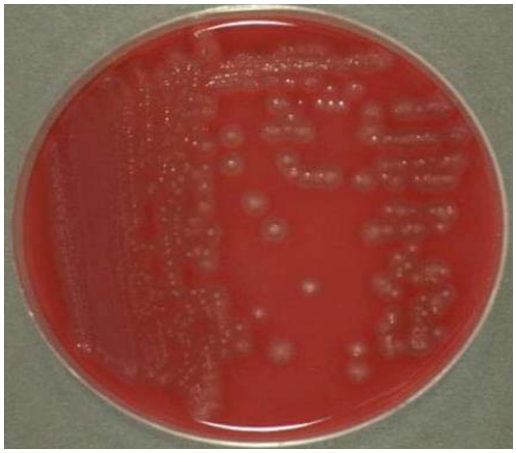

Figure 3. Colonies of *C. Perfringens*, medium of blood agar

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

The incidence of *Clostridium Perfringens* in difference food in USA was studies by Strang and the collaborators. They isolated this microorganism from 16,4% of the samples of raw beef, of chicken and of fish; from the condiments 5%; from fruits and vegetables 3,8%; from refrigerated food from the market 2,7% from the food prepared in housework 1,8%. In the minced beef, *C. Perfringens* in quantity of 100 or less per gram was found in 87% of the 95 of samples, while 45 of the 95 (47%) of the samples contained this microorganism in levels < 1000/g. In a study performed in USA, in the period 2001-2002 on 445 samples of totally minced muscle and emulsified of raw pork, of beef and of chicken, it was found that the spores of *C. Perfringens* did not cross 2,0 log_{10} being an average of 1,56 log_{10} ufc/g.

CONCLUSIONS

The placing of the diagnosis of laboratory in regard to *Clostridium Difficile*, is based on the culture and the detection of toxins in fecal samples. The culture is accomplished on a selective medium available on the market.

The morphology of the *C. Difficile* colony is typical also when it is examined at an optical microscope.

The definitive identification is best obtained by the chromatography of the liquid with gas.

The culture is very sensitive, but, when is used alone without testing the toxin, it leads to the low specificity and the wrong diagnosis when there are no symptoms.

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

With all these, this approach has no sensitivity and was not detected but only in 30% of the patients. Many immune-enzymatic tests (EIA) were introduced by different producers for the detection of toxin A alone or for both toxins A and B.

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