R-TYPE COLONIES OF CLOSTRIDIUM DIFFICILE

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RESEARCH ARTICLE

Abstract

R-type colonies (resistance colonies) in C. difficile refer to bacteria that have developed resistance to certain antibiotics. In the case of C. difficile, resistance to antibiotics such as metronidazole, vancomycin, and fluoroquinolones has become an increasing problem in treating infections caused by this bacterium. R-type colonies can survive and multiply in the presence of these antibiotics, making the treatment of infections more difficult. Clostridium difficile (C. difficile) is a gram-positive anaerobic bacterium that can cause intestinal infections, including severe diarrhea and inflammation of the colon. This bacterium can exist in two main forms: the vegetative form and the spore form. When environmental conditions become unfavorable (for example, in the presence of antibiotics or in an environment that does not allow the survival of the bacterium), C. difficile can form spores to survive. Treatment of C. difficile infections can be challenging, and antibiotic resistance can exacerbate the situation. It is important for physicians to carefully monitor the evolution of antibiotic resistance and to try to limit the use of antibiotics responsibly to minimize the risk of selecting R-type colonies and to improve treatment effectiveness. Additionally, new therapeutic strategies are being developed to address this issue, such as microbiome therapies and monoclonal antibody therapies

Keywords: colonies, antibiotics, spore

INTRODUCTION

Clostridium difficile is a Gram-positive anaerobic bacterium, which can cause severe intestinal infections in humans. It is one of the most common causes of nosocomial infections, meaning infections acquired during hospitalization. C. difficile is naturally found in the surrounding environment, but it can become problematic when it overgrows in a person's large intestine.

The main cause of C. difficile infection is the use of antibiotics. When antibiotics destroy the normal intestinal flora, C. difficile can proliferate and cause infection. C. difficile bacteria produce toxins that cause inflammation in the colon, leading to severe diarrhea, abdominal pain, and other symptoms.

Infections with C. difficile range from mild forms of diarrhea to severe infections that can be life-threatening. Individuals at increased risk of C. difficile infections include those who are elderly, have chronic medical conditions, are hospitalized, or have recently been treated with antibiotics.

The diagnosis of C. difficile infection usually involves testing the stool for the presence of toxins produced by the bacterium. Treatment often involves the administration of specific antibiotics such as metronidazole, vancomycin, or fidaxomicin. In some severe cases, surgical intervention may be necessary.

Preventing C. difficile infections involves the judicious use of antibiotics, adherence to hygiene measures such as frequent handwashing, and implementation of infection control practices in healthcare facilities to prevent the spread of the bacterium.

Clostridium difficile can develop on a variety of culture media, and one of the most common media used is blood agar, which is a general bacterial culture medium. This medium contains suitable nutrients for the growth of C. difficile and often includes additives such as oxoid supplements, which promote the growth of this bacterium.

Additionally, a specific culture medium called selective agar for C. difficile can be used for the isolation and identification of this bacterium in clinical samples. This culture medium contains chemicals that inhibit other bacteria and allow preferential growth of C. difficile.

Under the microscope, Clostridium difficile is a Gram-positive bacterium, meaning it will appear violet in Gram staining. It has a characteristic rod shape and forms stress-resistant spores, which can be observed within the bacterial cells. Spores are sometimes responsible for the bacterium's increased resistance to standard treatments such as antibiotics and may play a role in the spread of C. difficile infections.

MATERIAL AND METHODS

I conducted a prospective study based on microbiological diagnoses recorded in the bacteriological registry of the medical analysis laboratory, S.C. Diaser, Oradea. To conduct the

study, I also accessed the archive, recorded in the laboratory's computer program in S.C. Diaser, Oradea, as well as the computerized database of the unit.

Materials required for the examination:

- A collection container (a collection container with a spoon) with transport medium
- Wooden spatulas
- Latex gloves

For a stool culture, a fecal sample of 5-10g should be collected and placed in the collection container with a transport medium. If the stool is liquid, 5ml should be collected. It is recommended to choose a liquid, mucous, or bloody portion, if available. Do not collect quantities greater than 10g, as they may reduce the chances of isolating pathogenic bacteria.

solation of aerobic bacteria. • The sample is inoculated onto two culture media, one weakly selective (MacConkey) and one moderately selective (Hektoen), and incubated for 24 hours at 35-37°C. The cultures are observed at 24 and 48 hours for the appearance of characteristic colonies. For the genus Vibrio, the recommended selective medium is BSA (bile salts agar), and for yeasts, Sabouraud medium with Chloramphenicol is used. • To increase the chances of isolation, the sample is subcultured on enrichment media that promote the multiplication of pathogens (selenite sodium acid broth for Salmonella spp., alkaline peptone water or broth with taurocholate and peptone at pH 8.0-9.0 for Vibrio, from which after incubation, smears and cultures can be performed from the upper part of the medium). Incubation is done for 24 hours at 35-37°C, followed by transfers to culture media.

RESULTS AND DISCUSSIONS

The susceptibility to C. difficile infection is conditioned by the imbalance of the intestinal microflora, leading to "gaps" in the microbiome composition. The impact of C. difficile varies according to age. Although over 50% of infants are colonized with C. difficile, infection is rarely encountered at this age. The majority of symptomatic infections occur after the age of 65, especially in patients with surgical interventions, oncological diseases, and chronic kidney disease.

The risk of community-acquired C. difficile infections is elevated in young individuals, women caring for infants, individuals taking proton pump inhibitors or broad-spectrum antibiotics, and those living near farms or involved in animal husbandry.

Laboratory diagnosis is recommended solely for cases with identifiable risk factors and meeting clinical criteria, ruling out medication-induced diarrhea or alternative causes of diarrheal illness.

Testing for Clostridium difficile is not recommended for formed stools, except in cases of paralytic ileus. The recommended methods for diagnosing C. difficile infection include toxigenic culture, cell cytotoxicity neutralization assay, enzyme immunoassays for toxins A and B, and glutamate dehydrogenase, as well as nucleic acid amplification tests.

Anaerobic culture is performed on selective media such as cycloserine-cefoxitin-fructose agar, following prior decontamination of the stool sample through heat or alcohol treatment. After incubating for several days at temperatures of 20-25°C, yellow, flat colonies with irregular edges and a matte appearance grow. Sensitivity of culture can increase up to 100% through the use of ChromoID medium, enriched with sodium taurocholate, egg yolk, trypticase-soy, and sheep blood.

From the fecal suspension, direct inoculations were performed on two selective media.

Phenylethyl alcohol agar, preferably supplemented with 5% defibrinated sheep blood, which allows for the growth of clostridia and other gram-positive anaerobes present in intestinal contents.

Agar with egg yolk, fructose, and antibiotics (cefoxitin and D-cycloserine). This medium, with high selective capabilities, inhibits other clostridia and gram-positive anaerobic cocci but does not inhibit C. difficile. Both media were anaerobically incubated for 48 hours at 37°C.

From a colony morphology standpoint, C. difficile appears typical when examined under an optical microscope. Definitive identification is best achieved through gas liquid chromatography. While culture is highly sensitive, when used alone without toxin testing, it leads to low specificity and misdiagnosis in asymptomatic cases. Detection of toxin through tissue culture cytotoxin assay followed by neutralization with specific antiserum is often considered the standard. However, this approach lacks sensitivity and has only detected up to 25% of patients. Various enzyme immunoassay (EIA) tests have been introduced by different manufacturers for the detection of toxin A alone or both toxin A and B. Some of these

are designed to yield results in less than 60 minutes. Comparative studies of immunoassay kits have reported slightly lower sensitivity and specificity compared to cytotoxin assays. Isolates regarding C. difficile for toxin production, colonies isolated on selective media are tested for toxin production in vitro either through a cytotoxicity test or through direct enzyme immunoassays. It has higher sensitivity than the cytotoxicity test and equivalent specificity. In routine laboratory, detection of culture and toxins should be performed on each sample, and in cases of toxigenic-positive and fecal culture, toxigenic cultures should be performed on isolated colonies.

The study on "Detection of Clostridium difficile and toxins in ground beef and beef cube samples in modified atmosphere" highlights the prevalence of Clostridium difficile in ground beef packaged samples (MAP) (n: 50) and beef samples (n: 50); Toxin production was determined from isolates, and sensitivity to antibiotics, metronidazole, vancomycin, and clindamycin was detected. C. difficile was isolated in 4% of 50 ground beef samples and 2% of 50 beef cube samples.

All three isolates were confirmed by PCR to be C. difficile by detecting the gene. Three out of the five C. difficile isolates exhibited toxigenic characteristics, two of them carrying the toxin gene type B (tcdB), and one carrying the toxin gene type A (tcdA). When the antibiotic resistance profile was analyzed phenotypically, only C. difficile type A (tcdA) was resistant to clindamycin. All isolates were sensitive to vancomycin and metronidazole. The result of this study demonstrated that the strains of C. difficile detected in modified atmosphere packaged (MAP) beef samples could be a potential public health concern.

In the study "Comparison of the API ZYM system with the API AN-Ident, API 20A, Minitek Anaerobe II, and RapID-ANA systems for the identification of Clostridium difficile," the API ZYM system was compared with four anaerobe identification systems for the definitive identification of Clostridium difficile using 88 cultures of C. difficile grown on Mueller-Hinton blood agar medium. The API ZYM system provided a distinct and consistent enzymatic profile for all test strains, while the sensitivities of the other systems in identifying C. difficile ranged from 78 to 96% (AN-Ident, 77.9%; RapID-ANA, 88.6%; Minitek Anaerobe II, 90.9%; and API 20A, 95.5%). The API ZYM system is highly reliable in identifying C. difficile accurately, it is rapid, and relatively simple to use.

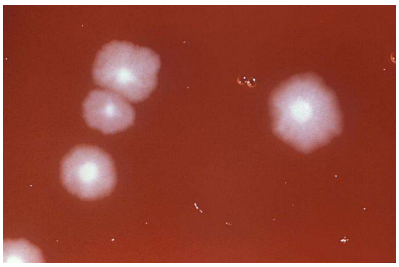

Fig. 1. Colonii de tip R pe mediul geloză – sânge, *C. Difficile*

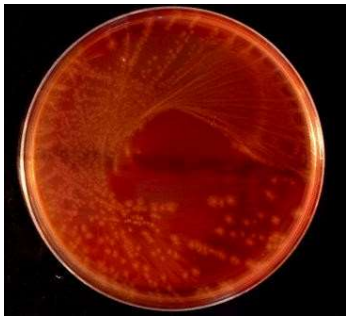

Fig. 2. *C. Difficile, colonii de tip R, mediul geloză – sânge*

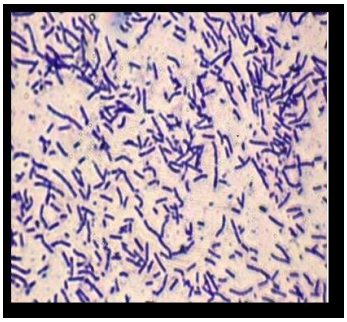

Fig. 3. Bacili Gram – pozitivi, C. Difficile

CONCLUSIONS

On selective media with cycloserine-cefoxitin-fructose agar, yellow, flat colonies with irregular margins and a matte appearance grew.

By using the ChromoID medium, agar enriched with sodium taurocholate, egg yolk, trypticase-soy, and sheep blood, the culture sensitivity increased to 100%.

Definitive identification is best achieved through gas liquid chromatography.

Colonies isolated on selective media are tested for toxin production in vitro either through a cytotoxicity test or through direct enzyme immunoassays.

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