MEDIA FOR THE ISOLATION OF CLOSTRIDIA FROM FECAL MATERIAL

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RESEARCH ARTICLE

Abstract

The Gram-positive bacilli, most of which exhibit peritrichous flagella, are anaerobic microorganisms, with very few species being aerotolerant. They are spore-forming bacilli, with oval or round spores located centrally, subterminally, or terminally. The spores are heat-resistant and larger than the bacterial cell, causing deformation. They are catalase-negative, with some species fermenting sugars while others do not. They are Gram-positive or Gram-variable bacilli with rounded ends, and their bacterial body is distorted by the larger endospore. Some species may have capsules. They can be cultivated on standard media and incubated at 37°C in anaerobic conditions. They grow slowly, forming colonies. On blood agar, most species exhibit beta hemolysis. Identification of Clostridium species is based on biochemical and metabolic characteristics.

Keywords: bacilli, microorganism, catalase-negative

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INTRODUCTION

The vegetative forms of Clostridia are not very resistant in the external environment, while the spores have significant resistance. They are strictly anaerobic, spore-forming microorganisms, non-capsulated, motile, with peritrichous flagella, and can be found in the soil. Clostridium tetani represents a pathogenic species due to its elaboration of neurotropic exotoxins.

In their vegetative state, they are present in the human and animal intestines and are excreted into the external environment through fecal matter, where they sporulate. The spores are highly resistant. In a hospital environment, they can contaminate bandages, surgical thread, talcum powder, and linens.

They are large bacilli with rounded ends, Gram-positive, and peritrichous flagella. In the presence of oxygen, they form a terminal spherical spore larger than the diameter of the bacterial cell, giving the microorganism the appearance of a matchstick, drumstick, or sewing pin.

They produce hemolysin, a neurotropic exotoxin, proteases, and gelatinases. The vegetative forms are destroyed by exposure to temperatures of 55-60°C for 15-20 minutes and by disinfectant substances.

Spores resist temperatures of 100°C, but they are destroyed by autoclaving at 120°C and 1 atm for 30 minutes, as well as by the Poupinel method for 1 hour at 180°C. In an external environment, under dry conditions, spores remain viable for decades and even hundreds of years. They exhibit flagellar H antigens, somatic O antigen in the bacterial wall, and a protein-structured exotoxin. All tetanus bacilli produce a single type of toxin, encoded plasmidically. The immune response involves the production of antitoxin antibodies, which are not useful for protection or diagnostic purposes. Clostridium tetani spores enter the body through so-called "tetanigenic" wounds: deep wounds that provide anaerobic conditions with crushed tissue. Spores enter these wounds from soil and objects contaminated with spores.

MATERIAL AND METHODS

I conducted a prospective study based on microbiological diagnoses recorded in the bacteriological registry of the S.C. Diaser medical analysis laboratory in Oradea. To conduct the study, I also accessed the archive registered in the specific computer program of the S.C. Diaser laboratory in

Oradea, specifically within the unit's computerized database.

Materials required for the examination:

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

For a stool culture, a fecal sample of 5-10g should be collected and placed in the fecal collector 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 exceeding 10g, as they may reduce the chances of isolating pathogenic bacteria.

RESULTS AND DISCUSSIONS

The pathological product consists of fecal matter. Stained smears are prepared using methylene blue or Gram staining. Cultivation is carried out under strict anaerobic conditions. Bacteria are identified based on biochemical characteristics and the demonstration of toxigenicity.

They can be cultivated on standard media provided that they are incubated at 37°C under strict anaerobic conditions. After 1-2 days, they form large, fimbriated, transparent S-type colonies surrounded by a fluffy zone. On blood agar media, colonies are surrounded by beta hemolysis.

They produce enzymes that break down glucose with the release of acid, produce lipase, gelatinase, and lecithinase. Differentiation of C. botulinum types is based on reactions involving maltose and sucrose decomposition, esculin hydrolysis, and protein breakdown with the release of indole.

Clostridium perfringens is involved in foodborne illnesses caused by the consumption of partially cooked poultry meat that has been refrigerated and then reheated before serving. The exotoxin produced by C. perfringens is a heat-resistant protein enterotoxin that is a component of the spore and is released during germination.

Table 1

Averages for the isolation of *Clostridia* from fecal specimens

The medium	Nutrient content	Selective composition."	The appearance of the colonies		Observe
			Cl. Perfringens	*Cl. Difficile*	
Gellulose Blood	Infusion of ox heart Triptose, agar Defibrinated sheep blood"		Hemolysis with incomplete lysis halo (hemolysin X) inhibited by anti-pergtingens A serum	4 mm non-hemolytic Translucent Gray Flat Iridescence	Hemolysis diphasic warm/cold (24 hours at 37°C/24 hours at 4°C)
Phenylethyl agar with sheep blood	Casein peptic digestion Soy papain digestion"	Phenethyl alcohol	Large (3-5 mm) Hemolytic Cl. perfr. Type C. Warm-cold hemolysi	4mmm Nehemolitice Gri translucide Plate Iridiscente	

In this study, the culture media used for the detection of clostridia in fecal samples included blood agar and phenylethyl alcohol agar with sheep blood. Regarding the nutritional composition, it consisted of bovine heart infusion, tryptose, agar,

defibrinated sheep blood, trypsin digest of casein, and papain digest of soy. The selective composition included phenylethyl alcohol. The appearance of colonies for Clostridium perfringens was characterized by hemolysis with an incomplete lytic halo, which was inhibited by anti-perfringens A serum. For Clostridium difficile, the colonies were non-hemolytic, grayish-translucent, and flat. It was observed that a biphasic hemolysis occurred, both warm and cold, after 24 hours at 37°C and 24 hours at 4°C, respectively.

In this study, titled "Comparison of Five Cultural Procedures for Isolating the Bacterium Clostridium difficile from Fecal Samples," several procedures for culturing the bacterium Clostridium difficile from fecal samples were described. The objective was to determine the effectiveness of five of these methods for isolating C. difficile from the feces of patients suspected of having a C. difficile-associated disease. A total of 564 fecal samples were cultured using heat shock, ethanol treatment (ET), and direct seeding on Carr-Scarborough agar with cefoxitin-fructose (CCFA) with horse blood (C/S medium), BBL CCFA medium, and Remel C. difficile agar. Cytotoxicity tests were conducted for all samples. A total of 113 samples (20%) tested positive for C. difficile using one or more methods. The number of positive cultures using heat shock, ET, and direct seeding on C/S medium, BBL CCFA medium, and Remel C. difficile agar was 79 (70%), 89 (79%), 91 (81%), 79 (70%), and 52 (46%), respectively. It was concluded that ethanol treatment and direct seeding on the C/S medium were the most effective procedures for isolating C. difficile from fecal samples, and there was significant variation in the performance of modified CCFA from different manufacturers.

CONCLUSIONS

C. perfringens is a heat-resistant protein enterotoxin that is a component of the spore and is released upon germination.

Culture media used for the detection of clostridia in fecal specimens include gelose-blood agar and phenylethyl alcohol agar with sheep blood.

The nutritional composition consists of beef heart infusion, tryptose, agar, defibrinated sheep blood, trypsinized casein digestion, and soy papain digestion.

Colonies of Cl. perfringens have a hemolytic appearance with an incomplete lysis halo, which is inhibited by antiperfringens A serum.

For Cl. difficile, colonies appear non-hemolytic, grayish-translucent, and flat.

Differentiation of C. botulinum types is based on maltose and sucrose fermentation reactions, esculin hydrolysis, and protein decomposition with indole release.

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