ENRICHMENT MEDIA FOR THE ISOLATION OF AEROBIC ENTERIC PATHOGENS

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RESEARCH ARTICLE

Abstract

The bacteria in the Shigella group are strictly adapted to humans and are always pathogenic. They are found in the intestines and fecal matter of patients, convalescents, as well as apparently healthy carriers. Species in the Yersinia genus have reservoirs in rodents, birds, domestic mammals, from where they spread into the surrounding environment, contaminating soil and food. They are present in the bodies of vectors such as rat fleas and human fleas. There are 36 known species of Vibrio, of which at least 12 are pathogenic or potentially pathogenic to humans. Bacteria from this genus most commonly cause gastrointestinal diseases, but also invasive infections.

Keywords: bacteria, pathogenic, reservoir

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INTRODUCTION

Enterobacteriaceae have been identified in the normal intestinal flora, some in the upper respiratory tract flora, water, soil, plants, etc. Enterobacteriaceae are responsible for a significant portion of nosocomial infections. Members of the Enterobacteriaceae family have a complex antigenic structure.

The somatic antigen O (endotoxin) represents the repetitive terminal polysaccharide units of the lipopolysaccharide (LPS) in the cell wall of Gram-negative bacteria. LPS is also composed of a core, which is similar in all Gram-negative bacteria, and lipid A, present in all Enterobacteriaceae, responsible for their toxic activity in the host organism. The somatic antigen "O" is thermostable and alcohol-resistant. It can be detected through agglutination reactions and leads to the formation of IgM antibodies in the host organism.

The antigen H (flagellar) is of a proteinaceous nature and, in the host organism, leads to the formation of IgG antibodies that immobilize the bacteria, thereby reducing their virulence. It is found in the flagella. It is denatured by 50°C alcohol and a temperature of 70°C. It can be detected through agglutination reactions.

The antigen K (capsular) is of a polysaccharide nature and is present in encapsulated bacteria. It provides resistance to phagocytosis and invasiveness to the bacteria. Its presence results in non-agglutinability in reactions for detecting the somatic antigen O.

In addition to these antigens, we also mention the exotoxin represented by the Shiga toxin.

Vibrios are aquatic microorganisms that can survive in marine or freshwater environments and form a native microflora in willow waters, salt lakes, estuaries, and marine and oceanic waters near temperate and warm coastal areas.

In freshwater, non-halophilic species like V. cholerae can be found, but their presence is transient. In marine and estuarine waters, halophilic species that tolerate high NaCl concentrations persist in multiple forms: the free form, the epibiotic form, and the dormant form.

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 relied on the archived data stored in the laboratory's

specific computer program and the unit's computerized database.

Materials required for sample collection:

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

For fecal culture, a sample of fecal matter weighing 5-10 grams should be collected and placed in the feces collector with transport medium. If the stool is liquid, 5 ml should be collected. It is recommended to select a portion that is liquid, mucous, or bloody, if present. Do not collect quantities exceeding 10 grams, as this may reduce the chances of isolating pathogenic bacteria.

Isolation of aerobic bacteria:

- The sample is inoculated on two culture media, one being weakly selective (MacConkey) and the other moderately selective (Hektoen). It is incubated for 24 hours at 35-37°C, and cultures are monitored at 24 and 48 hours for the appearance of characteristic colonies. For the Vibrio genus, the recommended selective medium is BSA (bile salt 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 (sodium acid selenite broth for Salmonella spp., alkaline peptone water or broth with taurocholate and peptone at pH 8.0-9.0 for Vibrio). After incubation, smears and cultures can be made from the upper part of the medium. It is then incubated for 24 hours at 35-37°C, followed by transfers to culture media.

RESULTS AND DISCUSSIONS

Based on the recorded data, as far as the isolation of aerobic bacteria is concerned, I am justified in stating that the aerobic bacterial etiology represents more than half of the known etiology of the diarrheal syndrome. In part, this "dominance" is also determined by the investigational possibilities accessible to most laboratories in hospitals and epidemiological centers, which allow for the determination of etiology much more frequently than in the case of other groups of bacterial or viral etiological agents. Given the known difficulties in isolation due to the low number of etiological agents per unit volume of the investigated sample, in some enterobacteriosis cases, "enrichment of the inoculum through subculturing on media that preferentially promote the multiplication of enteric pathogens" is recommended.

Antibodies detected by the Western blot method are directed against the three Yersinia species: enterocolitica, pseudotuberculosis, and pestis. The test uses secretory antigens derived from Yersinia (Yop: Yersinia outer proteins) that are serologically relevant and are separated based on molecular weight through polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE), and subsequently electrophoretically transferred to a nitrocellulose membrane (Western blotting). Free binding sites on the membrane are saturated with a protein solution, after which the matrix is washed and cut into strips. To detect specific anti-Yersinia antibodies, the antigen-loaded strip is incubated with the patient's diluted serum. If specific antibodies are present in the serum, they will bind to the corresponding antigens on the strip. After a washing step, the strip is incubated, depending on the class of antibodies being tested, with a labeled human anti-IgG or anti-IgA conjugate marked with alkaline phosphatase. The conjugate will bind to the antigen-antibody complex formed. After removing the unbound conjugate through washing, a chromogenic substrate is added. If the bound conjugate is present, the enzymatic reaction will produce a violet-colored product on the bands occupied by specific antibodies. Thus, the bands visualized on the strip are the result of the binding of specific antibodies to individual antigens. Each strip contains a control band at the top, representing the reaction control necessary to confirm the correct performance of the test. The test is validated if the YopD (35 kDa) band of the IgA and IgG control is visible. The intensity of the bands that appear in the patients' serum is evaluated using the control band as a

reference. Bands with an intensity equal to or greater than that of the control are marked with an X in the working protocol. Very intense bands will be marked with XX, while bands with lower intensity than the control will not be considered. IgG antibodies are produced in the case of chronic yersiniosis, reactive arthritis associated with Yersinia, and acute yersiniosis. In the early stage of yersiniosis, IgG antibodies are rarely detectable. IgG antibodies persist for a minimum of 5 months from the onset of the disease, but most often for an extended period (over 5 years). IgG antibodies are directed against all proteins secreted by Yersinia, but most often against YopE (23 kD), YopD (35 kD), YopB (41 kD), and YopH (51 kD). IgA antibodies appear prominently in the early phase of acute yersiniosis. In cases of reactive arthritis associated with Yersinia, the IgA response is directed against the YopD antigen (35 kD) in 90% of cases. In the case of chronic yersiniosis, IgA antibodies are detectably directed against YopE (23 kD), YopD (35 kD), and YopB (41 kD). In complicated yersiniosis cases, IgA antibodies persist in most cases for several years, while in uncomplicated yersiniosis, typically only a few months. The enrichment media commonly used for the isolation of aerobic enteric pathogens from the Salmonella, Yersinia, Vibrio groups are presented in Table 1.

Table 1.

Enrichment Media for Isolation of Enteric Aerobic Pathogens

The medium	Inhibitors for associated flora	Incubation temperature	Incubation period	
			Salm.	Yersinia
Alkaline Peptone Water	Ph 9,0-9,2	35℃/37℃	__	__
Nutrient Broth for Gram-Negative Bacilli."	Sodium deoxycholate	35℃/37℃ 22℃	18-24 hours __	__ 24-48 hours
Selenite sodium acid broth	Sodium selenite	35/40℃	18-24 hours	__
Tetrathionate broth	Bile salts Brilliant green Iodine	35℃/37℃	18-24 hours	__
Rappaport broth	Malachite green	37℃	18-24 hours	__
Phosphate buffer solution	__	3-5℃	__	2-3 weeks

Figure 1. ***Salmonella – Shigella*, colonii lactozo – pozitiv, mediul de cultură SS.**
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For Yersinia, a current enrichment procedure is to keep the peeled buffer in phosphate buffer solution for 2-3 weeks at 4-5°C, after which it is seeded according to selective media. Since bacteria of the genus Yersinia grow preferentially at 22-29°C, simple incubation at this temperature achieves enrichment of the broth for gram-negative bacilli.

For Vibrio alkaline peptone water (pH 9.0-9.2) is the most effective enrichment mode. Both V.cholerae and V.parahaemolyticus grow quickly, so that after 6-12 hours of incubation at 35-37°C, subculture can be carried out on the selective media for the isolation of vibrios.

CONCLUSIONS

1. The first isolation for Shigella is done on weakly or moderately selective and differential media (Istrati-Meitert, ADCL) that semi-incubate at 37°C, 18-24 hours.
2. The genus Yersinia grows preferentially at 22-29°C, simple incubation at this temperature achieves the enrichment of the broth for gram-negative bacilli.
3. For Vibrio, alkaline peptonated water (pH 9.0-9.2) is the most efficient way of enrichment, they grow quickly, so after 6-12 hours of incubation at 35-37°C, subduction can be carried out on the selective media specific to the isolation of vibrios.
4. Transparent, lactose-negative colonies are seeded on TSI, MIU, Simmons differential media for resumptive biochemical identification of the isolated strain.
5. Confirmation is carried out by agglutination tests with specific group and type sera.

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