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THE MEDIA OF ENRICHING USED FOR THE ISOLATION OF AEROBE ENTERIC PATHOGENS FROM THE *SALMONELLA, YERSINIA, VIBRIO* GROUPS

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Abstract

All the serotypes of Salmonella enterica subsp. enterica are parasites for human being and mammals, while the other subspecies and Salmonella bongori are met preponderant in birds and animals with cold blood.
On usual media, liquid or solid, they form colonies of S or R type, also common to the other enterobacteriaceae. On agar-blood it usually doesn't determine hemolysis. On selective lactin media they form lactase-negative colonies, some with the black center due to the production of H2S, and on the Wilson-Blair medium a high selective medium for salmonella, they form black colonies with black halo and metallic gloss. Yersinia is gram-negative bacilli or coccobacilli, pleomorphous, non sporulated, with tendency of bipolar coloration. The choleric vibrio is a gram negative bacillus very mobile, with characteristic aspect of comma, due to polar flagella. It grows on selective culture media, supplemented with biliary salts.

Key words: enteric, media, gram negative

INTRODUCTION

Based on the O somatic antigen there were described numerous serologic group noted with the large letters of the alphabet, from the human being are isolated strains belonging especially to the A-E groups. The H antigen, in phase 1 and 2, allows the individualization in the same group of serotypes, over 2000, who received initially the denomination of species. In the present it was accepted the keeping of these denominations, but written without italic letters and with capital letter, as would be Salmonella Typhi, Salmonella Typhimurium, Salmonella Enteritidis. All the serotypes are included in the present in the Kauffmann-White plan. The enteric Salmonellosis are food poisoning and they represent the common form, endemo-epidemic widely spread in all the countries of the world; they are caused most frequently by S. Enteritidis and S. Typhimurium. The symptoms appear 10-24 hours after the water consumption or of food contaminated with non-typhous Salmonella. The characteristic symptoms are: diarrhea, abdominal pain, vomit, fever, that disappear in 2-4 days.

The Yersinia type includes 11 species, among which only 3 are of medical interest: Y. pestis, Y. pseudotuberculosis and Y. enterocolitica. The other species are isolated from the soil, water, from wild mammals, birds and fish and can produce occasional opportunist infections in human being. The denomination of the type was given in honor of the French bacteriologist A. Yersin, who isolated for the first time, in 1894, the etiologic agent of plague, Y. pestis.

The main factors of virulence are: intracellular facultative habitat, the capsule with antiphagogitory role, the secretion of coagulase and fribrinolysis.

The type includes 36 species, among which only 12 species present variable pathogenicity for human being. These microbodies are usually isolated from the aquatic medium, sweet waters, salty, marine waters. The pandemics of cholera produced by V. chorerae have a special historical importance, cholera being today a disease present only in certain regions of the globe, under endemic form. Other species of Vibrio produce extraintestinal infections, from infections of the skin to very severe septicemias. It was isolated from fecal matters of the sick persons and healthy bearers. It was found that it survives for a long time in polluted waters and on contaminated objects.

MATERIAL AND METHODS

We accomplished 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, in the period 01.01.2014-31.12.2019.

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

Necessary materials for the performing of the examination:

■ A recipient of collection (collection recipient with collecting spoon) with transport medium
■ Wooden 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 it will reduce the chances of isolating the pathogen bacteria.

Considerations of pre-collection:

■ 8 days before the collection of the sample, don't take antibiotics or antiparasitary medicine.

■ The diet is not necessary.

Collection and transport of the samples

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

• Collection from the stool emitted spontaneously – is preferable and is indicated in all the forms of acute diarrhea when the emission of fecal matters is frequent.

• For bacterial and parasitary examinations, the collection is made with the "spoon" of the collection recipient, concerning the liquid parts and especially, the mucous and/or bloody one, if there are. The volume of the collection has to be of minimum 5 ml or 3-5 cm^3, if the stool is formed.

• For isolations or virusologic exams is collected 5-10 cm^3 fecal matters or minimum 5 ml, if the stool is not formed.

• Rectum collection – is recommended in:

- Chronic shigellosis where the curettage of the rectal mucous with the probe or with the tampon offers greater chances to isolation;
- The investigation of the bearers of Shigella and Salmonella, with the exception of those of S. Typhi.

For this type of collection are used Nelaton probes (nr.14-16) or adequate tampons, thus: with the tampon, soaked in isotone saline solution (not to use lubricant gels) is penetrated the anal sphincter by slow rotation, introducing intra rectum approximately 15 cm. It will be proceeded identically also with the Nelaton probe, to which is adapted a syringe (10 ml) with which are made 1-2 aspirations. After collection, the probes and tampons are introduced in sterile recipients that contain preservation medium, are labeled correspondingly and are sent to the laboratory.

The transport of the samples and their processing is made in maximum 1 h, if they were collected in recipients without transport medium (with transport at room temperature), or they can be kept up to 24 h at room temperature, if they were collected in recipients that contain Cary-Blair transport medium that assures a good durability of the bacterial intestinal pathogens. An exception to these rules is the samples collected in the suspicion of infection with Shigella spp, very sensitive bacteria that needs seeding from the media of culture immediately after collection.

For the viral etiology the samples that are not processed imediately have to be kept at – 70°C.

RESULTS AND DISCUSSIONS

From the suspension of fecal matters are performed directly dispersions on two selective media. The isolation with enriching consist of the procedure of enriching which was recommended and is used currently for a sum of enteric pathogens that are dispersed in a small number on the unit of volume of fecal matters. The pathogen process being developed, the excreted bacteria are dispersed in a fecaloid mass becoming abundant by the inhalation of intestinal hydro-electrolytic. As a consequence the reduced density of pathogen has determined the introduction of a process of enriching of the etiological agent in Sallmonelosis, Yersiniosis, cholera.

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

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

Fig.1. *Salmonella*: colonies "cat eyes".
Culture Medium SS

In regarding the culture medium, the broth for *Salmonella* with selenite acid of Sodium in many variants, the fact that the selenite with cysteine has specificity gave the best results in isolation of the serotypes met equally in human being.

For *Yersinia* a current procedure of enriching is the keeping of the tampon sampled in tampon solution Phosphate for 2-3 weeks at 4-5°C after which is seeded in selective media. Because the bacteria from the *Yersinia* type are developed preferentially at 22-29°C, the simple incubation at this

temperature accomplishes the enriching on the broth for gram-negative bacilli.

For *Vibrio* alkaline peptone water (pH 9,0-9,2) is the most efficient method of enriching. *V.cholerae* and *V.parahaemolyticus* grow promptly, so that after 6-12 hours of incubation at 35-37℃ it can be done the subpassing on selective media specific to the isolation of the vibrios.

The inoculation of the enriching media was accomplished by the suspension of fecal matters, which is seeded with the pipet: 7-10 drops for each tube with enriching medium (maximum proportion 1/10). The tampons from the sample were transferred directly on the enriching media. The incubation is performed at 35-37℃ maximum 24 hours. The selenite broth is incubated at 40-41℃, but in this case the passing on selective media is made at 12-18 hours.

The isolation without enriching or the direct isolation consists of seeding the sample, and the suspension of fecal matters is accomplished directly on selective media to obtain the characteristic isolated colonies for their identification.

The study "Food gastroenteritis caused by *Vibrio, Yersinia and Campylobacter"* affirms that the gastroenteritis determined by *V. parahaemolyticus* is transmitted exclusively by fish products. The involvement of other food is due to the cross contamination with fish products. Another particular characteristics of this syndrome is the natural habitat of the etiological agent: the sea. Beside the fact that it causes gastroenteritis, *V. parahaemolyticus* causes extraintestinal infection in human being.

The *Vibrio* type includes at least 28 species; *V. vulnificus, V. alginolyticuse V. cholera* are often associated with *V. parahaemolyticus* in the aquatic media and in fish products. *Parahaemolyticus* is common in the ocean waters and the coast waters. Its presence in the water is connected to their temperature, not being detectable as long as it remains under 19-20 ° C.

CONCLUSIONS

1. In regarding the culture medium, the broth for *Salmonella* with selenite acid of Sodium in many variants, the fact that the selenite with cysteine has specificity gave the best results in isolation of the serotypes met equally in human being.
2. The *Yersinia* type is developed preferentially at 22-29℃, the simple incubation at this temperature accomplishes the enriching on the broth for gram-negative bacilli.
3. For *Vibrio* alkaline peptone water (pH 9,0-9,2) is the most efficient method of enriching they grow promptly, so that after 6-12 hours of

incubation at 35-37°C it can be done the subpassing on selective media specific to the isolation of the vibrios.

4. The weak selective media, MC, EMB, allow the growth of all the lactase-positive and negative enterobateriaceae even of other groups of gram-negative bacilli as *Vibrio,* including *V.cholerare*, *Pseudomonas.*

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