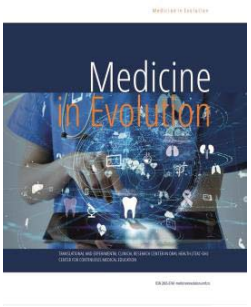
Methods of isolation and identification of Enterobacteriaceae



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Abstract

Aim and objectives: The purpose of this study on the isolation and identification of Enterobacteriaceae is aimed at bacteriological examination, following the basic examination of the microbiological diagnosis of pathological samples, being represented by the cultivation of feces on specific culture media.

Methods: We conducted a prospective and retrospective study on eighty-six isolated clinical trials, including Enterobacteriaceae, to identify and highlight genotypic and/or phenotypic characteristics between strains isolated from single infections and those isolated from recurrent cases based on microbiological diagnosis registered in the bacteriological register of the medical analysis laboratory, SC Diaser, Oradea.

Results: Antibodies detected by the Western blot method are guided against the 3 species of Yersinia: enterocolitica, pseudotuberculosis and pestis. For the detection of specific anti-Yersinia antibodies, the antigen-loaded band is incubated together with the patient's diluted serum. The weakly selective media, MacConkey, Eosin-Methylene Blue, allow the growth of all Enterobacteriaceae lactase positive and negative, even other groups of gram-positive bacilli.

Conclusions: Poorly selective media, MacConkey, Eosin methylene blue, allow the growth of all Enterobacteriaceae lactase positive and negative, even other groups of gram-positive bacilli.

Keywords: Enterobacteriaceae, gram negative, anaerobe, pathogen agents

INTRODUCTION

The habitat for Enterobacteriaceae is the intestinal flora and it is one of the most frequent human pathogen agents [1,2]. Also they are the source of infections achieved in the community and in the hospitals. They have the tendency to spread easily among the people by the transport of food, contaminated water and to achieve genetic material by horizontal transfer of genes, mediated in the greatest part by plasmids and transposons.

For the fast differentiation, although the test of catalase was used for many years, of types of gram-positive microorganisms, it was told very little about its utilization for Enterobacteriaceae [3]. It was found the fact that, there is a great variety of methods for the accomplishing of the test of catalase that there is no universally accepted concentration for the hydrogen peroxide and that there were no gradations mentioned for the strength and speed of the reaction.

It was observed that Serratia, Proteus and Providencia are strong reactors of catalase [4-6]. In exchange, Salmonella and rarely isolated Escherichia, Enterobacter şi Klebsiella were moderated catalase reactors. In great part, the strains of Escherichia and Shigella have nonreactive, while the majority of Enterobacter strains had the tendency to be weakly reactive. The Klebsiella strains were divided equally between non-reactive and weakly reactive [7-9].

A special role is also the association of more characteristic activities and namely, the fermentation of sugar, the split up of amino acids or other compounds, respectively the production of hydrogen sulphide together with the adequate indicators.

Another group if that of selective components that inhibits the associated flora allowing the development of enteric pathogens. There were proposed and are used selective agents or groups of agents of inhibitory capacity and of enteric bacteria, that have to be isolated [10].

In the last years there was a category of different selective mediums that was extended very much using antibiotics and sulphonamides as pressing factors. The advantages to dose with precision the quantities in relation to the associated flora and the more reduced price are taken into consideration in the promoting of these mediums.

Aim and objectives

The purpose of this study regarding the isolation and identification of the Enterobacteriaceae has as target the bacteriological examination, following the basic examination of the microbiological diagnosis of the pathological samples, being represented by the cultivation of the fecal matters on specific mediums of culture.

This presupposes the isolation of the bacterial etiologic agent on adequate mediums and its identification based on the morphological characteristics, of culture, exoenzymatic and antigenic, of biochemical reactions, respectively.

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

MATERIAL AND METHODS

We accomplished a prospective and retrospective study, on eighty-six clinical isolated samples including Enterobacteriaceae to identify and underline the genotypic and/or phenotypic characteristics between the isolated strains from cases of unique infection 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.

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, as the following: with the tampon, moisten in isotone saline solution (not to be used lubricant gels), is penetrated the anal sphincter by slow rotation, being introduced 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 the prelevation, the probes and tampons are introduced in sterile recipients that contain preservation medium, are labeled correspondently and are sent to the laboratory.

RESULTS

The antibodies detected by the Western blot method are guided against the 3 species of Yersinia: enterocolitica, pseudotuberculosis and pestis. The test uses secretory antigens coming from Yersinia serologically relevant, that are separated based on the molecular weight by the electrophoresis in gel of polyacrylamide in the presence of sodium dodecyl sulphate and transferred afterwards electrophoretic on a membrane of nitrocellulose. The free connection situses from the membrane are saturated with a solution of proteins, after which the matrix is washed and cut in strips. For the detection of the specific antibodies anti-Yersinia the strip loaded with antigens is incubated together with the diluted serum of the patient. If in the serum are present specific antibodies, they are connected to the corresponding antigens from the strip.

The mediums of enriching frequently used for the isolation of the aerobe enteritis pathogens from the groups Salmonella, Yersinia, Vibrio are presented in Table 1.

The medium of culture, broth, for Salmonella with sodium selenite acid in many variants, has the specificity the fact that the selenite with cysteine gave the best results for the isolation of the serotypes met equally in human (Figure 1).

Its capacity can be enriched and the period of incubation shortened 12-18 hours by incubation 40-41°C. When the possibilities don't allow by a single medium of enriching, the medium with selenite is preferred. It is inhibitor for other enterobacteria (especially lactose-positive), but Proteus and Shigella are developed relatively frequently.

Rappaport-Vasiliadis broth, also highlighted as having a good capacity of enriching, is recommended and mentioned with superior results for the enriching of all the other serotypes, with the exception of Salmonella serotype Typhi.

The medium of culture with tetrathionate broth was used for the isolation of Salmonella serotype Typhi. It is used in more restraint quantity due to the laborious preparation and the difficulties of sale as industrial product "ready to be used" or in dry form.

Table 1. The medium of enriching for the isolation of the aerobe enteritis pathogens

Medium	Inhibitors for associated flora	Temperature of incubation	Duration of incubation	Duration of incubation	Observations
			Salmonella	Yersinia	
Alkaline peptone water	Ph 9,0-9,2	35°C/37°C	—	—	—
Broth for gram-negative bacilli	Sodium deoxycholate	35°C/37°C 22°C	18-24 hours	18-24 hours	Allows also the multiplication of other gram-negative bacilli
Selenite broth sodium acid (Leifson)	Sodium selenite	35/40°C	18-24 hours	—	Shigella increases inconstantly, has multiple variants
Tetra-thionate broth	Biliary salts Brilliant green Iodine	35°C/37°C	18-24 hours	—	—
Rappaport broth	Malachite green	37°C	18-24 hours	—	—
Tampon phosphate solution	—	3-5°C	—	2-4 weeks	—

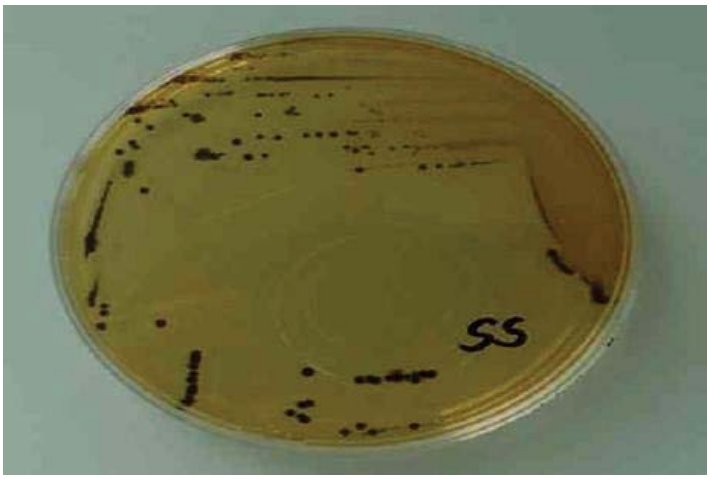

Figure 1. Salmonella colonies "cat eyes". Medium of culture SS

For Yersinia a current procedure of enriching is the keeping of the sample tampon in phosphate tampon solution 2-3 weeks at 4-5°C after which is seeded by selective media. Because the bacteria from the gender Yersinia are developed preferentially at 22-29°C, the simple incubation at this temperature accomplishes the enriching of the broth for gram-negative bacilli.

The inoculation of the media of enriching was performed by the suspension of fecal matters that is seeded with pipet: 7-10 drops for each tube with enriching medium (the maximum proportion 1/10). The tampons from sample were transferred directly on enriching media.

The incubation is performed at 35-37°C maximum 24 hours. The selenite broth is incubated at 40-41°C, 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 the seeding of the sample and the suspension of fecal matters is accomplished directly on selective media for the obtaining of characteristic isolated colonies, in order to identify them.

The selective media are always solid media (agar) that contain mainly three groups of constituents. Thus, there is a group of nutritive constituents that favor the bacterial growth (meat extract, peptone, yeast extract, factors of growth).

DISCUSSIONS

The coprocitogram usually indicates the presence of the erythrocytes and leucocytes is large number [11]. The test has a significance but there is a number of polymorphonuclear (PMN) greater than 10/hpf (microscopic field with large power – objective 40x). The most frequently involved are the germs from the group Shigella, Salmonella, Campylobacter, Escherichia coli enteroinvasive and enterohemorrhagic.

In regard to the pre-identification of the isolated samples, is accomplished by the 3rd phase of the bacteriologic diagnosis that will be approached [12,13]. The classification in genders and even in species is useful but the practical diagnosis and the further study, in the specialized laboratories and reference centers, of the isolated strains. This study starts from primary elements that characterize the isolate and namely, morphological aspects, aspects of cultivability and minimum data regarding the exoenzimatic behavior [14,15].

In a study on frozen beef pate, performed in USA, APC (aerobic plate count) was smaller than 3,0 〖log〗_10 UFC/g, and coliform and Escherichia coli biotype I was under 1,0 〖log〗_10 UFC/g. These researchers observed a lack of correlation between the small number of E.coli biotype I and E.coli O157:H7. A Canadian study showed that the number of coliform bacilli and E.coli recovered from the tables and from the transporting belt, in a unit of processing the meat was similar to the one recovered from the pieces of meat sectioned from the side margins, which underlined the importance of the devices as source of this microorganism for the parts of cut meat.

The efficiency of the medium of culture for Salmonella-Shigella is underlined in the study regarding the "Superiority of MacConkey Agar compared to Salmonella-Shigella Agar for the isolation of Shigella dysenteriae Type 1".

The efficiency of MacConkey Agar in isolating different types of Shigella was compared with that of Salmonella-Shigella (SS) during an extended outbreak of disease of 18 months caused by Shigella. Totally there were 1580 isolated samples of Shigella from 12307 samples of rectal tampons and fecal matters from patients with diarrhea and their contacts by direct plating on MacConkey agars and SS. Shigella dysenteriae type I and Shigella flexneri were 55% and, respectively, 33% of all the isolated samples, with a smaller number of Shigella boydii and Shigella sonnei. MacConkey Agar was superior to the SS agar in detecting S. dysenteriae type I; 83% of the isolated samples were detectable on MacConkey Agar compared to 40% on SS agar. In exchange 84% of the isolated samples S. flexneri were detectable on agar SS, compared to 51% only on MacConkey Agar. This discovering confirm the fact that, for the culture of fecal test tubes about which is considered that S.S. dysenteriae type I, one of the media used, should be non-inhibiting.

CONCLUSIONS

The weakly selective media, MacConkey (MC), Eosin methylene blue (EMB), allow the growth of all the Enterobacteriaceae lactase-positive and negative, even of other groups of gram-positive bacilli. The moderate selective media have a more selective capacity than the gram-negative bacilli. The media moderate selective have a higher selective capacity inhibiting considerably the lactase-positive Enterobacteriaceae.

The coprocitogram is a usual technique that establishes a presumptive diagnosis in the infectious diarrhea, as a consequence of the microscopic examination of the fecal matters, indicating usually the presence of erythrocytes and leucocytes in large number. The test has a significance if there is a larger number of polymorphonuclear (PMN) than 10/hpf.

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