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IDENTIFICATION OF *ENTEROPATHOGENIC E. COLI* AND THE ANTIGENIC STRUCTURE

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Abstract

Entereobacteriaceae is the bacteria that includes many of the most familiar pathogen agents or pathogen conditioned as would be Salmonella, Shigella, E. Coli. Following the genetic studies, they were placed in Proteobacteria having their own order, Enterobacteriales.

E. Coli, in the human intestine, represents dominant flora of the large intestine having an important role in the maintaining of its normal physiology and in the synthesis of some proteins from the groups B and K. Eliminated in the external environment with the excrements it contaminates the water, the soil, the food. Inside the species are distinguished numerous versions that express characteristics of pathogenicity, versions named pathovars, patotypes. E. Coli is pathogen by virulence and/or toxigenicity. The determinants of pathogenicity are various. Depending on the determinants of pathogenicity that the strains of E.Coli are having can cause different affections. The enterobacteriaceae of the Escherichia type are mainly saprophyte or conditioned pathogen. There are patotypes of E.Coli involved in the human pathology as the diarrhea patotypes, uropathogen and other types are. The intestinal infections or infectious enterocolites are determined by the five diarrhea patotypes, each having serologic subgroups and determining distinct clinical manifestations.

Key words: pathogenicity, toxigenicity, infections

INTRODUCTION

Enterobacteriaceae is a microorganism that can be found in the environment which evolved in order to compose the normal intestinal flora.

Enterobacteriaceae are non demanding germs, they grow on simple media. They are aerobe germs, facultative anaerobe that need for an optimum growth, temperatures of 22-37°C. On the non selective media will form colonies of grey color of the "S" type, "M" type. On the blood agar, some species determine β hemolysis.

It is a resistant bacteria in the environment. Destroyed by the usual antiseptics and disinfectants and by the colorants as malachite green, crystal violet.

It presents a special capacity of adapting to the modifications from the environment as would be the presence of some minerals, modifications of ph, temperature, osmolarity.

It can sense the presence or absence of some chemical substances or gases in the environment or of life and with the help of the cilia it can come closer or farther of them.

It can also become immobile and to produce adhesion fimbrae that would allow the adherence to the specific substrate.

Due to the temperature or osmolarity they modify the dimension of the pores by the modification of porins constituent of the external membrane. Thus it can increase the dimension of the pores to accumulate large molecules of nutrient or to eliminate inhibitor substances.

They poses a complex mechanism of regulation of the bacterial cell metabolism that allows it to assure an easy life. They synthetize only those enzymes necessary for the obtaining of some metabolites when they are present in the environment where it can use them.

It is a microorganism that develop the resistance towards the antibacterial substances by the elaboration of enzymes that hydrolyses the beta lactamases or by mutations that affect the porins thus becoming resistant to the aminosides.

The enterobacteriaceae present pils that assure the adherence to mucous, allowing the bacteria to colonize and multiply.

The mannose-sensitive pils are connected to the host cell in the same place as D-mannose.

They are important for the normal colonization of the gastric-intestinal tube. Other mannose-resistant pils are not connected to the host cell in the same place with the D-mannose.

There are pils that represent a major importance as factor of virulence, helping the microorganisms to determine affections outside their normal area of action.

The invasins are proteins that act locally destroying or invading the host cell, facilitating the growth and the spreading of the pathogen agent.

MATERIAL AND METHOD

For the accomplishing of the objectives proposed was used the retrospective study.

Collection and transport of samples

The collection has to be made as close to the beginning of the disease and before the beginning of any antimicrobial treatment.

• The collection from the stool made spontaneously – it is preferred and is indicated in all the forms of acute diarrhea when the emission of excrements is frequent.

• For bacterial and parasite examinations, the collection is made with the “spoon” of the coproculture tube, concerning the liquid parts and

especially, those mucous and/or sanguinolent, if they exist. The volume of the collection has to be of minimum 5 ml or 3-5 cm^3, if the stool is formed[3].

• For the isolations or virological exams is collected 5-10 cm^3 of excrements or minimum 5 ml, if the stool is not formed[3].

• The rectal collection – is recommended in:

– chronic shigellosis, where the curettage of the rectal mucous with the probe or the tampon offers greater chances to the isolation;

– the investigation of the carriers of Shigella and Salmonella, with the exception of the those with S. Typhi.

For this type of collection are used Nelaton probes (no.14-16) or adequate tampons, as the following: with the tampon, wet in saline isotone solution (not to be used lubricant gels), is penetrated the anal sphincter by slow rotation, introducing in the rectum approximately 15 cm. It will proceed identically also with the Nelaton probe, to which is adapted a syringe (10 ml) used for 1-2 aspirations. After the collection, the probes and tampons are introduced in sterile recipients that contain preservation medium, are labeled correspondently and are sent to the laboratory[3].

The transport of the samples and their processing is made in maximum 1h, if they were collected in recipient without medium of transport (with transport at the room temperature), or can be kept up to 24h at room temperature, if they were collected in recipients that contain Cary-Blair medium of transport which assures a good viability of the bacterial intestinal pathogens. An exception to these rules are the samples collected for the suspicion of infection with Shigella spp, very sensitive bacteria, which needs seeding on the culture media immediately after collection[3;4] .

For the viral etiology, the samples that are not processed immediately have to be kept at – 70°C[3] .

The isolation of the aerobe bacteria

• It is seeded the sample on two culture media, one weakly selective (Mac Conkey) and one moderately selective (Hektoen) and is incubated 24 h at 35-37ºC, following the cultures at 24 and 48 h for the appearance of characteristic colonies. For the Vibrio type, the recommended selective medium is BSA (bile salts agar), and for yeasts – the Sabouraud medium with Cloramfenicol.

• In order to increase the chances of isolation, the sample is sub cultivated on media of enriching that favors the multiplication of the pathogen (ex. selenite broth sodium acid for Salmonella spp., alkaline peptone water or broth with taurocholate and peptone at pH=8,0-9,0 for Vibrio where, after incubation can be made smears and cultures from the superior part of the medium). It is incubated 24 h at 35-37°C, then are made transmissions on the culture mediums.

• The colonies characteristic to each type will be transplanted in order to identify on the level of species and agglutination with specific serums.

RESULTS AND DISCUSSIONS.

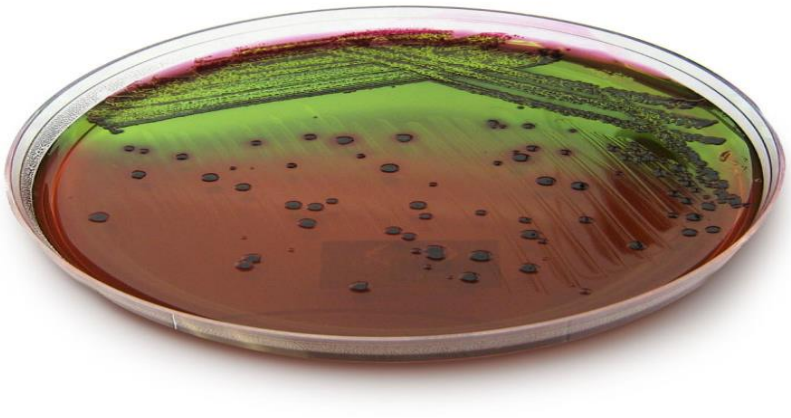

Fig.1. *E. Coli.*

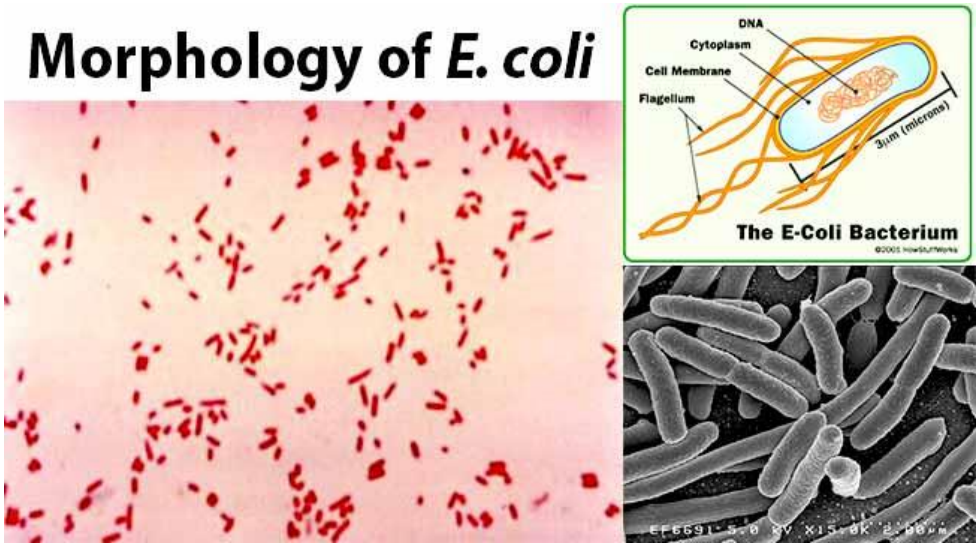

Fig.2. *E. Coli.*

E. Coli is a germ well adapted to its living environment. It is not demanding. It grows on simple mediums in which the glucose is the only organic constituent.

It is an aerobe germ, discretionary anaerobe that can have both fermentative and respiratory metabolism. On solid mediums it grows under the form of colonies of "S" type, and in the liquid medium it determines uniform disorder and adherent ring on the wall of the tube.

From the point of view of the metabolism and of biochemistry, *E. Coli* ferments the glucose and other carbohydrates with production of acid and gas. The majority of strains are oxidase-negative, are capable to reduce the nitrites in nitrates.

They don't produce urease, don't decompose the proteins with formation of H2S, don't use the citrate as unique source of carbon. They decompose the lactose with emission of acid, decompose the proteins with formation of indole, give the positive methyl red reaction.

Some strains, usually those involved in urinary infections, determine the beta hemolysis of blood agar.

The antigen "o" or somatic one, is the antigen with specificity of group that corresponds to the polyoside fixed on the lipopolysaccharides of the bacterial wall. It is thermo stable and acid-alcohol-resistant. By the reactions of agglutination there were identified 180 "o" antigenic blood groups that can present reactions of crossing with antigens of some strains of Klebsiella, Salmonella, Providencia, Vibrio, and others are common with the Shigella group.

The Antigen ''H" or the flagella antigen is present only in the ciliate strains, it is made of a specific protein named flagellin. It has a specificity of type, it is thermolabile and is inactive in alcohol. By reactions of agglutination there were distinguished 56 "H" serotypes.

The Antigen ''K" or the capsular antigen is of polysaccharide nature, has a specificity of type and was identified in the uropathogen strains of E.Coli and in strains involved in the case of newborn meningitis. The B antigen is an antigen of surface.

The strains of enteropathogenic *E.Coli* induce a watery diarrhea similar to that from the infections with enterotoxigenic *E.Coli*, affecting especially the children. enteropathogenic *E.Coli* doesn't produce enterotoxins TL or TS but only small quantities of Shiga-like toxin with enterotoxic and cytotoxic effect.

By the Bfp adesins is connected to the intestinal epithelia on the level of the colon determining the destruction of the microvilosities with inflammatory response, without sign of invasion. As a consequence is

installed the malabsorption, the watery diarrhea, persistent, accompanied by fever and vomit.

The enterotoxigenic strains affect all the categories of age though it represents the major etiology of the children's diarrhea and of the tourists in the countries with scarce sanitation, named also "diarrhea of tourists".

Nataro and Kaper, presented the fact that, from its description, many cases and outbreaks of diseases associated to the diarrhea were associated to the EAEC strains, but the clinical characteristics and the results of these infections are diverse.

In 2002, Elias and others in the studies on the EAEC strains showed its heterogeneity.

Jenkins and others, 2006b, Okeke and others, 2010, offered information on the heterogenic pathogenesis of CEEA in the human being, but also the importance of the host.

Recently, Okeke and others confirmed the heterogeneity of the EAEC strains by the molecular phylogenetic analysis, because 96 types of sequence (ST) were identified using the impression of the multilocus sequencing (MLST) and isolated EAEC identified by the HEp-2 tests, mainly from a site, although 40% of the EAEC strains belong or were locus versions of three STS.

In this review we will discuss about the relevance for the public health of the infection with EAEC and its complexity due to the heterogeneity of the strains.

CONCLUSIONS

The diagnosis of laboratory is bacteriologic and is based on the identification of the strains of Escherichia Coli in the pathologic products.

The identification based on the biochemical characteristics is completed with the serotyping for EPEC and EHEC strains.

Numerous andesine, toxins and proteins associated with virulence were described, and also many factors that contribute to the inflammation induced by EAEC.

None of these factors is found in all the isolated EAEC and no single factor was ever involved in the virulence of EAEC.

EAEC producing Shiga toxins is increasing its pathogen potential and interest to find real pathogen factors that can define this prototype.

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