

IDENTIFICATION AND ISOLATION OF CAMPYLOBACTER JEJUNI

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Abstract

Campylobacter is widely spread in the nature, present in the intestine of numerous species of mammals as would be the she-goats, sheep, dogs, cats, rodents and birds and in the contaminated water with fecal matter. *C. doylei* was isolated only from the level of the human gastric mucous, *C. jejuni* from the intestine of the birds, of the pigs and bovines, and together with *C. coli* also from the residual water coming from slaughter houses. The source of infection in human beings is represented most frequently by the infected animals, including pets, dogs, cats, the infections with *Campylobacter* being in fact zoonotic. The most frequent epidemic outbreaks were quoted following the consumption of contaminated water or of the poultry with insufficient thermal preparation. The epidemics are more frequent in the warm months, but they can appear in any other period of the year.

Key words: campylobacter, isolated, epidemic, infection

INTRODUCTION

In 1886, Escherich observed organisms that resemble with campylobacter in the samples of stool of the children with diarrhea. In 1913 McFaydean and Stockman have identified campylobacter, called connate *Vibrio*, in the fetal tissues of the aborted sheep. In 1957 King described the isolation of connate *Vibrio* from the blood samples of the children with diarrhea and in 1972 the clinical microbiologists from Belgium have isolated for the first time campylobacter from the stool samples of the patients with diarrhea.

The bacilli are adapted to the colonization of the digestive tube and of the reproducing apparatus, presenting a particular motility that allows the bacteria to travel the covering epithelium by "corkscrewing" movements. According to some authors, *Campylobacter* is transforming, in unfavorable conditions, in intractable coccoid form, "dormient", by which they survive for a long time in the environment and return to the cultivable, virulent form, when they reach in a favorable host.

The infection appears generally in the summer and is the result of the ingestion of food not prepared accordingly. The severe cases of enteritis

with *Campylobacter jejuni* were due to the unpasteurized milk and some defects in the drainage system. The meat not prepared enough thermally, especially the chicken one was also associated to the infection.

Not all the infections with *Campylobacter* become symptomatic. Among the factors involved in the appearance of the manifested disease we can mention: the size of the bacterial inoculum that reaches in the small intestine, the virulence of the infected strain, the specific immunity of the host towards the ingested pathogen.

The clinical manifestations in the enteritis determined by *Campylobacter jejuni* are not distinguished of those caused by other etiologic agents of the acute diarrhea disease, as *Salmonella* and *Shigella*. After an average period of incubation of 3-4 days, the infection is located in jejunum, ileum and then in the bowel and rectum. The mucous appears ulcerated, swollen, bloody, with crypt abscess and infiltrated with PMN and eosinophils in lamina propria.

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.

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.

RESULTS AND DISCUSSIONS

The coproculture needs samples as fresh as possible (fast transport, in low temperatures), in exchange the determining of the antigen from the fecal matters can be performed also in the conditions when the coproculture is no longer possible.

In the department of bacteriology, from the recipients with the native sample were introduced in the transport medium, then it was performed the inoculation with the bacteriological loop with the diameter of 3 mm on the culture medium for the isolation and differentiation of the *Campylobacter*, such as agar mCCD, agar Skirrow, agar Kar. The incubation of the inoculated media is made in a temperature of 41,5°C in conditions of microaerobiosis.

The growth of *Campylobacter* on culture media for isolation and differentiation is examined daily: over 24, 48 and finally 72 hours with the recording of the results obtained in the respective registration forms.

On the culture medium agar mCCD the typical colonies of *Campylobacter* are of grey color, dense-or with metallic gloss, flat and humid with tendency of expansion. There can appear other forms of colonies:

C.jejuni forms small colonies, up to 1 mm in diameter, flat, semitransparent, of grey shade with smooth surface and regular margins, and *C.coli* – large colonies, approximately 2-4 mm in diameter, convex, juicy, of grey-yellow color, with smooth surface and regular margins.

On solid culture media with blood *Campylobacter* produces two types of colonies, among which the first has an irregular form with diameter of 3-8 mm. The colonies of this type are colorless or light grey, transparent, homogenous, having the aspect of water drops, non-hemolytic.

Occasionally, the time of long term cultivation, the surface becomes mat silvery, with a non thick consistency. The colonies of S type have a round form, 1-2 mm in diameter, regular margins, glossy smooth surface, gibbous, transparent, homogenous, most frequently colorless, after the prolonged cultivation are capable to form yellow pigment, don't cause hemolysis, possess a non-thick consistency.



Fig.1. *Campylobacter jejuni*, agar – blood medium. Colonies of S type.

Some species being able to grow also in conditions of anaerobiosis. They are oxidase-positive and the catalysis, production of H₂SO₄, the sensitivity to the nalidixic and cephalothin acid is used as tests of differentiation between the different species. In cultures older than 48 hours, on solid and liquid media, or after repeated passages, you can observe round forms of 0,5µm or larger, called “coccoid bodies”. These forms represent a “degenerative” phase of the bacteria under the influence of the medium conditions. *C. fetus* grows to 25°C, and *C. jejuni* to 42°C.

The major antigen of the type is lipopolysaccharide, situated on the level of the external membrane. The serologic heterogeneity of the strains of *C. jejuni* is determined by the presence of over 90 polyzaccharidic somatic antigens O and 50 of capsular antigens and flagellation. An important role in pathogenesis is that of the infective dose and the immune status of the patient. The patients contaminated with a large number of micro bodies, presenting gastric hypoacidity are more exposed and those that present hypogammaglobulinemia make prolonged and severe forms of disease.

The study “*Campylobacter jejuni*: molecular biology and pathogenesis”, affirms the fact that *Campylobacter jejuni* is a major cause of the bacterial diseases of food origin and its prevalence rivals or even passes that of the infections of food origin with *Salmonella* in the developed world.

Another study, “Epidemiologic and clinical characteristics of the infections with “*Campylobacter jejuni*”, affirms the fact that the greater incidence of the infections with *C. jejuni* and their tendency to invade the tissues and to induce the inflammation are compatible with a role in causing the Guillain-Barré syndrome.

CONCLUSIONS

1. In the strains of *C.jejuni* it was detected the activity of some endotoxins and enterotoxins, whose precise role wasn't established yet. The enterotoxin is thermo-stable and is similar with the one secreted by *E. coli*.
2. They are microaerophilic microbodies, some species being able to grow also in conditions of anaerobiosis.
3. They are oxidase-positive, and the catalysis, the production of H₂SO₄, the sensitivity to the nalidixic and cephalothin acid is used as tests of differentiation between the different species.
4. In cultures older than 48 hours, on solid and liquid media, or after repeated passages, it can be observed round forms of 0,5µm or larger, called “coccoid bodies”. These forms represent a “degenerative” phase of the bacteria under the influence of the medium conditions.

5. *C. fetus* grows to 25°C, and *C. jejuni* to 42°C.
6. The infection appears generally in the summer and is the result of the ingestion of unfit prepared food.
7. The severe cases of enteritis with *Campylobacter jejuni* were due to the unpasteurized milk and some defects in the drainage system. The meat not prepared enough thermally, especially the chicken one was also associated to the infection.
8. The etiology of the infections can be specified only by the diagnosis of laboratory.
9. The increased level of anti-*Campylobacter* antibodies indicate, usually, a recent infection in progress, even if the cultures of fecal matters can be negative.
10. The best proof for a recent infection is the significant modification of the level of antibodies in two pair-samples collected in a period of 2-3 weeks.

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