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**EFFICIENCY OF PROBIOTICS ASSOCIATION IN  
THE ETIOPATHOGENIC TREATMENT OF ACUTE  
ENTEROCOLITIS IN INFANTS AND TODDLERS**

**PHD THESIS  
ABSTRACT**

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## Introduction

Infectious diarrhea is a disease in which dysfunction of intestinal barrier plays an important role. It is defined as diarrhea caused by a bacterial, viral or parasitic gastro-intestinal tract, the emission of at least 3 watery stools in large amount in one day. Acute diarrhea character is defined by the evolution of this disease within 2 weeks of onset.

Acute diarrhea can be defined as a temporary malabsorption of water, electrolytes and food principles (oligosaccharides and fat) leading to accelerated evacuation of intestinal contents, which leads to weight loss.

The episodes of diarrhea are a major health problem in children worldwide; diarrheal disease incidence remained unchanged in the last decade, estimated at 3.2 episodes per child per year. In developing countries diarrheal diseases are associated with an increased risk of mortality and thus is a major concern.

The use of probiotics to modulate intestinal bacterial flora has been proposed for many years and there are various studies in the literature that support it for the prevention and treatment of various gastrointestinal disorders: diarrhea viral or bacterial etiology (including *Clostridium difficile*) and associated antibiotic treatment, gastroenteritis caused by *Helicobacter pylori*, maltase deficiency, lactose intolerance, chronic inflammatory bowel disease, irritable colon.

Some effects of probiotics on normal or pathological functions of the body are well documented and their use, either alone or in combination with other therapies, may be considered evidence of their effectiveness.

Alteration of intestinal microbial flora following dietary changes may affect the mucosal immune system. Bacteria from the digestive tract forms a protective barrier that prevents colonization by pathogens. This barrier can be affected by various pathological circumstances or injudicious antibiotic treatments and consider that it can be obtained by ingestion of live bacteria, even if they temporarily colonize the intestine (which requires their regular consumption). In addition to the barrier effect, many metabolites of the lactic bacteria can inhibit the growth of pathogens.

The results of animals and humans studies support the concept that lactic acid bacteria have immunomodulatory effects, although there are few studies in humans. Until now, overall, the findings of studies on the immunological effects of lactic bacteria, support the hypothesis that

consumption of lactic acid bacteria can modulate the immune response, which in turn increase resistance to immune-mediated diseases.

Managing acidic dairy and lactic bacteria leads to cure moderate gastroenteritis or diminish specific symptoms, including to infants and toddlers. The effects vary considerably, depending on the species and the product used, as well as pathogenic bacteria. The best results were obtained with *L. acidophilus* NCFB 1748 Johnson rhamnosus GG, casei Shirota, *Bifidobacterium lactis* and with *Enterobacter faecium*. Pathogens on which were most favorable effects were pathogenic *E. coli*, *Salmonella*, *Shigella*, *Staphylococcus aureus*, Rotavirus and many others.

Research has shown that probiotics help reduce:

- Symptoms of bloating and diarrhea in cases of lactose intolerance;
- Diarrhea as a side effect of antibiotics;
- Exacerbations of inflammatory bowel disease that causes diarrhea and abdominal pain;
- Inflammation of the ileum in patients who have undergone surgical removal of a portion of the colon.

In most cases, the probiotics are used for the prophylaxis of diarrhea caused by antibiotic. Antibiotics kill the bacteria that causes the disease, but also kill the beneficial bacteria of the digestive tract. A decrease of beneficial bacteria may cause diarrhea. Administration of probiotic supplements (as capsules, powder or liquid) may help replace the lost beneficial bacteria and thus can prevent diarrhea. The decrease of beneficial bacteria may lead to the development of other infections, such as vaginal candidiasis and urinary tract infections.

By far the best clinical results have been obtained using probiotics in acute diarrhea in children. A number of studies have documented the therapeutic use of probiotics as supplements onset. Most studies have included various species of *Lactobacillus*, but the most used was *L rhamnosus* (GG). The efficacy of *L rhamnosus* (GG) has been shown, in particular, when administered as a supplement to the rotavirus diarrhea. The most important effect reported was the reduction in the duration of illness. It was taken into account the reduction in the incidence of acute diarrheal disease. Several studies with different levels of importance, have documented a reduction in the incidence or severity of acute diarrhea using bifidobacteria, mainly *B lactis* and lactobacilli, mainly *L rhamnosus* (GG).

Keywords: probiotics, lactobacilli, bifidobacteria, acute enterocolitis, interleukine, immunoglobulins.

## **Study's aim**

The study aims to highlight the influence of completing treatment of acute enterocolitis in infants and small children with probiotics, following symptoms change during disease progression and changes in laboratory parameters values.

The purpose of this research is to prove the beneficial influence of the combination of probiotics in the treatment of acute enterocolitis, aiming at the development of clinical and laboratory parameters.

To achieve its purpose we will pursue the following specific objectives:

- Establishment of study groups and achieving a database, identifying the main parameters of interest: gender, age, personal history of physiological and pathological conditions, family history, medical history, presumptive clinical diagnosis.

- Identification of clinical and laboratory parameters: the number and frequency of daily stools, their appearance, coprocitograma, stool culture, blood counts (CBC), PCR and values of serum IgA, IL10, TNF $\alpha$ .

We will make correlations and associations between the studied parameters and the results of investigations in determining the statistically significant using various statistical programs. Finally we will develop conclusions regarding the association of probiotics in the etiopathogenic treatment of acute diarrhea.

## **Objectives**

Through this study we evaluate the positive influence of probiotics association in etiopathogenic treatment of acute enterocolitis on length of hospital stay and on the evolution of symptoms. Also we will follow the risk factors for acute enterocolitis in infants and children aged 1-3 years.

## Material and method

The positive diagnosis was based on personal clinical history and the laboratory data. We insisted on the circumstances that caused diarrhea, the clinical manifestations that allow us assessing disease severity and etiology. We collected data about new foods introduced into the diet, infections, administration of drugs (oral antibiotics, laxatives), recent travel (endemic factors and seasonal variations), excessive intake of liquids, etc. Clinical examination helped identify the etiology, sometimes reflected by the complications: dehydration, fever, bloody stools, abdominal pain, vomiting. Laboratory testing included CBC, ESR, blood immunoassays and examination of the stool.

Were included in the study three groups of children:

- The first group, whose study was prospective, consists of all cases of infants and young children hospitalized with the diagnosis of acute enterocolitis to Filantropia Hospital Craiova, from January 2011 to December 2012, and received symptomatic and etiopathogenic treatment, 131 cases (control group).
- The second group, whose study was also a prospective one, consists of all cases of infants and young children hospitalized with the diagnosis of acute enterocolitis to Filantropia Hospital Craiova, between January 2011 and December 2012 and who received etiopathogenic and symptomatic treatment and probiotics, 222 patients (study group).
- The third lot, subject to a prospective study, consists of infants and young children hospitalized at the Hospital for Infectious Diseases "Victor Babes" Craiova, January 2012 - April 2012, with the diagnosis of acute enterocolitis, who received etiopathogenic and symptomatic treatment and probiotics, and to which we follow the evolution of immunological markers, IL10, TNF, IgA, during hospitalization.

We excluded cases of admission which have not presented diarrhea and those who were discharged after 24-28 hours, unable to follow their evolution over a significant period.

To emphasize the benefic role of the association of probiotics, we realized a comparative study of the cases listed in the first two groups and a descriptive study of the evolution of values of immunological markers for the third lot.

## Results

Realizing the analysis of the study group in terms of the association of probiotics in the etiopathogenic treatment of acute enterocolitis, we found that in 222 cases (62.89%) the treatment was supplemented with probiotic and only a third, 131 cases (37.1 %) did not receive probiotic.

Factors related to infancy, such as, prematurity and low birth weight increases the risk of acute enterocolitis by increased susceptibility. In the study group there was a slightly higher incidence of prematurity (24 cases; 6.8%) and birth weight below 2800g (53 cases, 15.01%).

Knowing the protective qualities of breast milk against diarrhea, we quantified the incidence of breast feeding for the study group. It was found that a large number of 232 children (65.72%) received only breast milk for the first 4 months of life, and a percentage halved, 105 cases (29.75%) received formula milk from the first day of life. So, it can be considered that the absence of natural food was a risk factor for the occurrence of acute enterocolitis., the fact stated and published by the literature.

We divided the two subgroups by the length of hospital stay in category of duration and we compared the distributions by Chi square test results. We observed that, in the group treated with probiotic had a higher weight patients admitted for a period of less than 5 days and more than 7 days, and a smaller share those hospitalized during 5-7 days, but not significant compared to the group treated without probiotic - Chi square = 0.183  $p > 0.05$ .

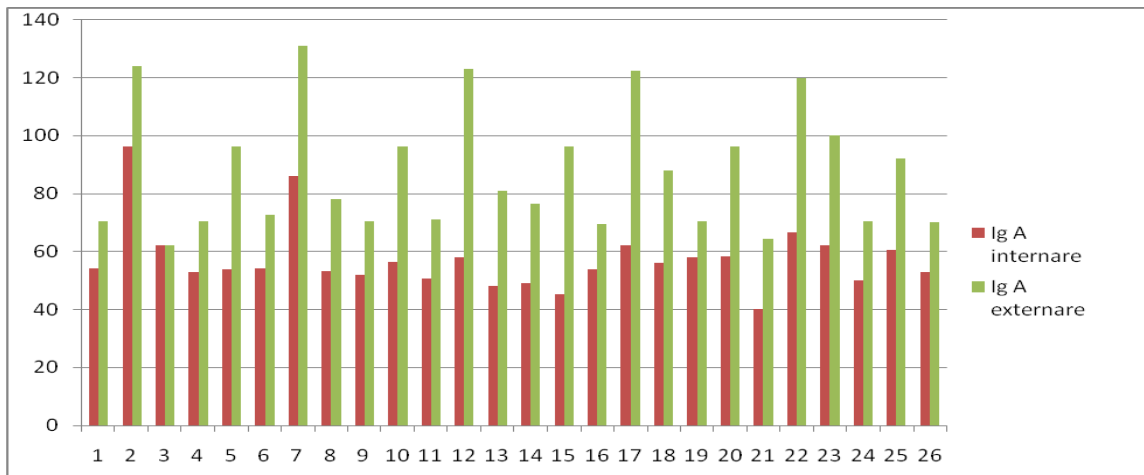
### Evolution of IgA values

Probiotics participate favorable to host immunity by stimulating the synthesis of immunoglobulins, especially by stimulating the synthesis of IgA.

As shown in Figure 3, in all the studied cases, the value of IgA increased after using the combination therapy etiopathogenic treatment-probiotics.

According to WHO, the normal value for serum IgA is 90-450 mg / dl.

The results are consistent with those mentioned in the literature, which supports the hypothesis that the probiotics stimulate the host immunity by acting on cells involved in natural immunity and specific immunity.



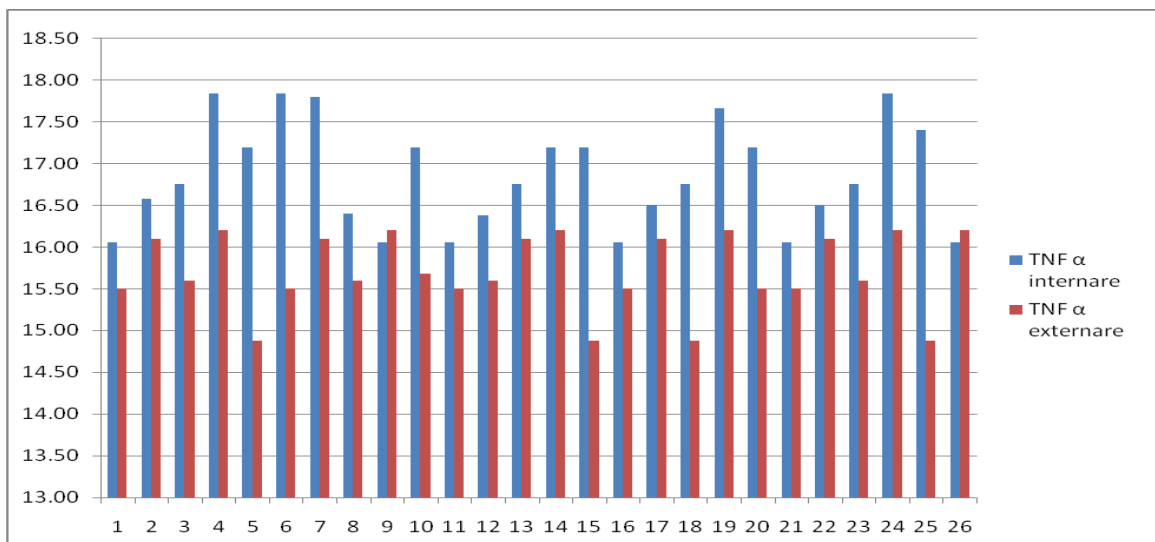
**Figure 54.** Evolution of IgA values on admission and discharge

### Evolution of TNF $\alpha$ values

The probiotics of the genus *Lactobacillus* inhibit the secretion of tumor necrosis factor TNF $\alpha$ , a murine pro-inflammatory cytokine produced by macrophages. Probiotics have been shown to be recognized by the portion of the antigen-presenting cells of Peyer's patches and can inhibit the production of cytokines such as TNF $\alpha$ .

Our results confirm the decreases of TNF $\alpha$  during hospitalization days, by adjuvant treatment with probiotics.

Note that TNF $\alpha$  is not detectable in the serum of apparently healthy donors.



**Figure 56.** Evolution of TNF $\alpha$  values on admission and discharge

## Conclusions

- The term of "probiotic" was first used in 1965 by Lilly and Stillwell, then Parker in 1974 define them as "organisms or substances which contribute to the intestinal microbial balance." A group of experts commissioned by FAO (Food and Agriculture Organization) and WHO defined probiotics as "live microorganisms" which when administered in adequate amounts confer a health benefit on the host.
- Worldwide, acute diarrhea continues to be one of the most important causes of morbidity and mortality among children between 0-5 years. Many of the episodes of acute diarrhea in children remain unknown, each child having in the first year of life, on average, 5-7 episodes of diarrhea.
- Environmental influence of provenance, socio-economic and sanitary conditions differ from those in underdeveloped countries, the cases we studied come from urban environment, from with good and very good socio-economic status.
- Analysis of prematurity and low birth weight involvement as risk factors, showed no statistically significant difference, indicating that in this study the two parameters do not affect the solitary disease.
- It is noted that a high percentage of children in the study group were fed artificially or mixed. Knowing that breast milk contains anti-infective factors, lack of natural food in the first 4 months of life seems to play an important role in the emergence and evolution of acute enterocolitis episode.
- There is an increased incidence of viral etiology enterocolitis, especially with Rotavirus and the bacteria with highest weight are Proteus and Klebsiella.
- Acute dehydration signs were set at about half the cases, observation correlates with the literature, that the presence of dehydration is a risk factor for acute enterocolitis episode extension.
- Performing analysis study group in terms of the combination of probiotics in the etiopathogenic treatment of acute enterocolitis, we found that two thirds of patients received therapy with probiotics
- The selected probiotics chosen for the therapy are part of the Lactobacillus, Bifidobacterium and Streptococcus. Their election was left to the treating physician according to the endowment of the clinic, to the moment of admission and to the followed



treatment at home, because many of the patients with probiotic regimens had previously introduced it.

- Assessing the impact of probiotics on reducing the number of stools during hospitalization, compared with control group who did not received probiotics, we found that the difference between the two groups was observed in reducing the number of stools on the short period of 1-3 days of hospitalization, also specified in other studies.
- In the case of our study group, we found that the effect of the probiotic association was observed on the average hospitalization period of 5-7 days. Perhaps, as stated by other studies, duration less than 5 days is too small to assess the efficacy of probiotics in the treatment and the cases who needed a hospitalization stay longer then 7 days are too complex to assess in this regard.
- In all studied cases, the amount of IgA increased after using probiotics combination with etiopathogenic treatment. The results are consistent with those in other trials, which supports the hypothesis that probiotics stimulate host immunity by acting on cells involved in natural immunity and specific immunity
- Although all the patients received a second blood sample collected after clinical and paraclinical improvement, meaning after 4-5 days of hospitalization, only 23.8% of patients noted increased IL 10.
- In terms of influence on the evolution of  $TNF\alpha$ , the results of our study confirm decreases of  $TNF\alpha$  during hospitalization days by adjuvant treatment with probiotics.
- Relative to the safety of treatment with probiotics, we found no adverse effect following administration of probiotic products, and this is supported by a number of studies.
- After the study we conducted with probiotics in acute diarrhea in children, we inclined to propose the introduction of probiotics in the group of biological medicinal products considering them more than food supplements.

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