

# STUDY OF THE EFFECT OF LACTOBACILLUS ACID FAST AS A PROPHYLAXIS FOR ENTER-COLITIS FOLLOWING SURGERY FOR HIRSCHSPRUNG'S DISEASE IN CHILDREN

By

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## ABSTRACT

**Background:** Hirschsprung's associated enterocolitis (HAEC) after surgery is a common problem. The exact pathogenesis of HAEC still remains unclear. Considering the complex interrelation between the epithelium, the immune system, and the micro biome of the intestine, disturbances of the intestinal microbial composition may predispose a patient to develop HAEC. Surgery reduces, but does not eliminate the risk of enterocolitis, as up to 40% of the patients continue to suffer from HAEC despite correct surgical treatment.

**Objective:** To evaluate use of the lactobacillus acid fast (probiotic) as a prophylactic treatment for enterocolitis following surgery for Hirschsprung's disease (HD) .

**Patients and Methods:** This numerical randomized control study was done on fifty patients. Their ages ranged from 0 day up to 10 years. All of them had undergone surgical treatment for Hirschsprung's disease in Al-Azhar University Hospitals, Al Sheikh Zayed specialized Hospital and Al haram Hospital, during the period from January 2017 to December 2019. They were classified into 2 equal groups; Group A received lactobacillus acid fast (probiotic) as a prophylactic treatment for two years post-operative compared to group B did not.

**Results:** Treatment with probiotics did not only significantly diminish the incidence, but also decreased the severity of HAEC.

**Conclusion:** The use of probiotics as a prophylactic treatment following surgery for Hirschsprung's disease did not only significantly diminish the incidence, but also decreased the severity of Hirschsprung-Associated Enter-colitis (HAEC).

**Keywords:** Hirschsprung-Associated Enter-colitis (HAEC).

## INTRODUCTION

Hirschsprung's disease (HD) is a serious birth defect where nerve cells are completely missing from the end of the bowel. The absence of ganglion cells causes the muscles in the bowels to lose their ability to move stool through the

intestine (peristalsis). This leads to stool backing up in the intestines. Affected individuals can develop constipation and partial or total obstruction of the bowels. Pain and discomfort can result. If not treated, a potentially serious bacterial infection may develop (*McLaughlin and Puri, 2015*).

Newborn with failure to pass the meconium within a short time after birth for 24-48 hours is suggestive of HD. Infants with HD will very often have abdominal swelling (distention), abdominal pain, and vomiting. Affected infants have constipation and often exhibit poor weight gain, and slow growth.

HD can sometimes lead to a condition called enterocolitis, which is inflammation of the small intestines and colon. This is often referred to as Hirschsprung-associated enterocolitis. Hirschsprung-associated enterocolitis is the most frequent complication of HD occurring in about 40% of individuals with HD and can be mild to severe in nature. Hirschsprung-associated enterocolitis often presents with fever, explosive diarrhea, abdominal swelling, lethargy, and vomiting. Some individuals with either severe or untreated Hirschsprung-associated enterocolitis may develop sepsis, which is a widespread bacterial infection of the bloodstream and is potentially life-threatening. Severe or untreated enterocolitis can also lead to toxic megacolon, another life-threatening complication. It cannot be over emphasized that an individual with HD who develops these symptoms should urgently seek medical attention (*Burkhardt et al., 2014*).

Most reports from developing countries indicate that majority of children with Hirschsprung's disease present late to a tertiary health facility for management. This late presentation, among other factors, may be responsible for the less than optimal outcome for this disease in some developing countries (*Poenaru et al., 2010*).

Diagnosis of HD can be suspected based on a physical examination, a complete patient and family history, identification of characteristic symptoms and a variety of specialized tests. Most people (85-90%) are diagnosed in early infancy. The first symptom is usually failure to pass the first bowel movement (meconium). The preferred diagnostic test for HD is a suction biopsy of the rectum. A biopsy involves surgically cutting out a small sample of affected tissue and studying it under a microscope. Absence of ganglion cells confirms the diagnosis. Additional tests that can be used include an abdominal x-ray, which can reveal the presence of an intestinal blockage, an anorectal manometry, which involves using balloons and pressure sensors to assess the health and function of the rectum, and a contrast or barium enema (*Ralls et al., 2012*).

In almost all cases, treatment of HD requires surgery to remove the part of the colon and/or rectum that lacks the normal nerve development, and to join the two healthy ends together. There are three standard surgical procedures designed to correct this disorder. Each procedure removes the affected part and attaches the healthy part of the bowel to the rectum completing what is known as a "pull-through" procedure. Currently most procedures are performed in a single stage. But if the child is very ill or the bowel is much dilated, it is more common to create a safer approach multistage strategy. The first stage is to create a temporary ostomy to let the child grow, let bowel inflammation resolve, or let the bowel shrink back down to normal size before the second stage "pull through" operation is carried out at which time the

stoma may be closed (*Demehri et al., 2013*).

Enter-colitis is a potentially dangerous problem for children with Hirschsprung disease. Symptoms may worsen over the course of hours and include "explosive", foul smelling or bloody diarrhea, abdominal distension, lethargy and fever. If a rectal exam is performed there may also be an "explosive" release of gas or stool. In some cases of enter-colitis, bacteria can enter the blood stream from the bowel to cause sepsis, a problem that can be fatal. Children with Hirschsprung disease may have episodes of enter-colitis at any time, even after surgical treatment (see below). Typical symptoms include "explosive diarrhea" that may be foul smelling or bloody (*St. Louis, 2016*).

In many cases, Metronidazole (Flagyl) and vancomycin are most commonly used antibiotics. Surgery reduces, but did not eliminate the risk of enter-colitis. About 35 percent of children have at least one episode of enter-colitis after surgery and some children have recurrent or prolonged episodes of enter-colitis. This was usually treated with (Metronidazole) and with rectal irrigation. Occasionally, additional surgery is needed in children with frequent enter-colitis unresponsive to these approaches (*St. Louis, 2016*).

Probiotics have been used to prevent colitis in a number of pediatric conditions and may be beneficial in the prevention of Hirschsprung-Associated Enter-colitis (HAEC). *Lactobacillus* sp. And *Bifidobacteria* sp. in children with HAEC. Proponents suggest that replacement of these commensal strains may restore bacterial equilibria and thereby play a

preventive role against HAEC (*Wang et al., 2012*).

**The aim of this work was to** evaluate the use of the lactobacillus acid fast as a prophylactic treatment for enter-colitis following surgery for Hirschsprung's disease.

## PATIENTS AND METHODS

This randomized prospective study was approved by the Ethics Board of Al-Azhar University. All patients were collected from the Outpatient Clinic and Pediatric Surgery Department at Al-Azhar Hospitals, Al Sheikh Zayed specialized Hospital and Al Haram Hospital over a period from January 2017 to December 2019, with appropriate consent from patients' parents to participate in this study. Their ages ranged from 0 day up to 10 years. They had undergone surgery for Hirschsprung's disease. Those subjects were categorized into 2 equal groups. Group A received lactobacillus acid fast (probiotic) as a prophylactic treatment for two years post-operative compared to group B did not receive the drug and considered as a control group.

All data, including sex, age, medication, and post-operative complications were collected. Clinical follow-up data were obtained by direct communication with the patients. All patients answered a questionnaire concerning changes in number of motions per day or per week, as well as abdominal distension and postoperative enterocolitis in the form of loose watery motions associated with offensive explosive diarrhea, vomiting, fever, lethargy, shock and overall satisfaction with the post-operative results.

**Inclusion criteria:** Age from 0 day up to 10 years, both sexes, children with Hirschsprung's disease undergoing surgical intervention.

**Exclusion criteria:** Patients unfit for surgery for medical problems, patients with other post-operative complications (leak, stenosis), other reasons of enterocolitis, infection or food intolerance, other causes of chronic constipation and Patients with associated major gastrointestinal anomalies.

All patients were be subjected to complete history taking with special stress on attacks of enter-colitis (abdominal distention; foul-smelling, watery diarrhea; lethargy; and poor feeding). General, local examination investigations; full laboratory (CBC, CRP, KFT, LFT), stool analysis and stool culture.

**Follow up the follow up was as follow:** once per week in the first month, then monthly till 6 months, then every 3 months until 2-years post-operatively.

**Mean outcome measurement:** Enterocolitis clinical picture, investigation (stool analysis, stool culture and others) and images. Type of organisms in group A with treatment, and group B without treatment. Group A with different ages, less than 2 years were 7 Children. From 2-5 years were 15 Children and from 5-10 years were 3 Children, 16 male patients and 9 female patients. They have received treatment with probiotics as a prophylactic treatment for enter-colitis following surgery for 2 years in details (Lactobacillus acid fast, was the most

common probiotic used in our study as sachet or tablet in regime of one sachet bid or one tablet bid) They received one sachet of Lactobacillus Acid Fast (Lactobacillus sp. And Bifidobacteria sp) in the morning and one sachet of Lactobacillus Acid Fast (Lactobacillus sp. And Bifidobacteria sp) in the evening. Lacteal fort (lactobacillus delbruekii and fermentum 10 billion). Group B with different ages, less than 2 years were 10 Children. From 2-5 years were 10 Children and from 5-10 years were 5 Children, 15 male and 10 female as comparative group post-operative were receiving other treatment than probiotics for the duration of study (two years).

During the duration of our study every, patient had at least one monthly visit, during this visit History was taken with special stress on attacks of enter-colitis (abdominal distention, foul-smelling, watery diarrhea, vomiting, fever, lethargy, poor feeding and shock), clinical follow-up, full investigations and stool samples were taken for microbial assessment and Plain abdominal X-ray films erect and supine were done.

#### **Statistical analysis:**

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. Results were expressed as mean  $\pm$  standard deviation or number (%). Comparisons between the median values of different parameters in the two groups were performed. P Value  $< 0.05$  was considered Significant.

## RESULTS

In this study, Group A was twenty-five patients with different ages, 16 males and 9 females, and Group B was twenty-five patients with different ages, 15 males and

10 females. Demographic representation of comparison (Age, sex, Age at operative (years) and 1st. visit post-operative between two groups (**Table 1**).

**Table (1): Demographic representation of comparison. (Age, sex, Age at operative (years) and 1<sup>st</sup> visit post-operative)**

Parameters		Groups		P-value
		Group A No. = 25	Group B No. = 25	
Age	Median (IQR)	3 (2 - 4)	2.9 (1.9 - 4.1)	0.778
	Range	1.1 - 9	1.1 - 7.2	
Gender	Female	9 (36.0%)	10 (40.0%)	0.771
	Male	16 (64.0%)	15 (60.0%)	
Age at operative (years)	Median (IQR)	2.4 (1.2 - 3.11)	2 (1.7 - 3.6)	0.521
	Range	0.5 - 8.5	1.11 - 5.9	
1st. visit P.O (Months)	Median (IQR)	1.6 (1 - 4)	5 (2 - 8)	0.019
	Range	0.5 - 36	1 - 37.2	

The use of probiotics Lactobacillus Acid Fast (10 billion cfu/dose) either sachet, capsules, or tablet bid post-operative reduced the episodes of enterocolitis. As in group A, just 4 Children have suffered from episodes entero -colitis about 16 %. 3 males and one female, 4 Children had episodes of abdominal distention ± constipation and pain, 3 Children had episodes loose watery motions, 2 Children had episodes of vomiting, one Child had episodes of fever, one Child had episodes of lethargy and one Child had presented with severe HAEC and admitted to the intensive care unit and improved with aggressive fluid resuscitation and broad-spectrum antibiotics (**Table 2**).

But in Group B, as patients did not receive Probiotics as prophylactic treatment there were 11 Children suffered from episodes entero-colitis about 44%. 7 males and 4 females, 11 Children had episodes of abdominal distention ± constipation and pain, 10 Children had episodes loose watery motions associated with offensive explosive diarrhea, 5

Children had episodes of vomiting, 6 Children had episodes of fever, 4 Children had episodes of lethargy and 3 Children had presented with severe HAEC and sepsis they had admitted to the intensive care unit one of them refractory to medical treatment so diversion with enterostomy had been done. 3 Children from those 11 Children has been identified with recurrent HAEC during the observation period (**Table 2**).

The results of 15 Stool samples which were taken for microbial assessment in Group B during diarrhea episodes for 11 Children, 5 samples were Clostridium difficile and anaerobes (Bacteroidaceae), 4 samples were gram negative bacilli and yeast (mainly C. albicans) and pus cells, gram negative bacilli (Enter pathogenic E. coli) in 3 samples, gram negative bacilli (salmonella spp) in 2 samples and one sample was pseudomonas aearugimos but in Group A the results for 9 stool samples which were taken during diarrhea episodes for 4 Children, were gram negative bacilli (salmonella spp) in 3 samples, gram negative bacilli (Enter

pathogenic *E. coli*) in 2 samples, 3 samples were gram negative bacilli and yeast (*Candida* spp) and pus cells (60-65) / HPF and one sample was *Clostridium Difficile* and anaerobes (*Bacteroidaceae*) (Table 2).

The radiograph findings in Group B, 3 films were included the “cutoff” sign in the rectosigmoid colon with absent distal air with one film free intra-abdominal air from intestinal perforation, 7 films were

with dilated loops of bowel, air fluid levels, 2 films with dilated loops of bowel and 2 films with pneumatosis intestinalis. But in Group A, we observed that 3 films of plain radiograph included dilated loops of bowel with two or more air-fluid levels, one film finding an intestinal cut-off sign in the left lower quadrant and no evidence of speculation, pneumatosis, and no free intra-abdominal air from intestinal perforation (Table 2).

**Table (2): Demographic representation comparison of presenting symptoms of enters colitis, complications, stool samples, investigations and radiological examination**

Parameters	Groups	Group A		Group B		P-value
		No.	%	No.	%	
<b>History Clinical date</b>						
Abdomen Distention pain	Negative	21	84.0%	14	56.0%	0.031
	Positive	4	16.0%	11	44.0%	
Diarrhea	Negative	22	88.0%	15	60.0%	0.024
	Positive	3	12.0%	10	40.0%	
Vomiting	Negative	23	92.0%	20	80.0%	0.221
	Positive	2	8.0%	5	20.0%	
Fever	Negative	24	96.0%	19	76.0%	0.042
	Positive	1	4.0%	6	24.0%	
Lethargy	Negative	24	96.0%	21	84.0%	0.157
	Positive	1	4.0%	4	16.0%	
Shook	Negative	24	96.0%	22	88.0%	0.297
	Positive	1	4.0%	3	12.0%	
<b>Investigations</b>						
Stool analyses, cu	Negative	20	80.0%	16	64.0%	0.208
	Positive	5	20.0%	9	36.0%	
Others WBC CRP	Negative	21	84.0%	19	76.0%	0.480
	Positive	4	16.0%	6	24.0%	
<b>Imaging X-rays</b>						
Negative		22	88.0%	16	66.7%	0.074
Positive		3	12.0%	8	33.3%	

## DISCUSSION

*Austin (2012)* reported that the post-operative enterocolitis associated with Hirschsprung's disease can occur in the immediate post-operative period or later, even more than a year after corrective surgery and the exact pathogenesis of HAEC still remains unclear. Considering

the complex interrelation between the epithelium, the immune system, and the microbiome of the intestine. Disturbances of the intestinal microbial composition may predispose a patient to develop HAEC independent of correct surgical treatment as despite correction endorectal

pull-through for HD, up to 40% of the patients continue to suffer from HAEC

In this study, it was noted that in Group B there were 11 children suffering from episodes entero-colitis about 44%., 3 children from them had presented with severe HAEC and sepsis they had admitted to the intensive care unit one of them refractory to medical treatment so diversion with enter-ostomy had been done and three children from those eleven children has been identified with recurrent HAEC during the observation period.

*Temple et al. (2012)* stated that although pull-through operations relieve the obstructive symptoms of HD, there is a persistent risk of the development of enterocolitis, occurring in up to 42 % of patients. *Austin (2012)* mentioned that up to 40% of the patients continue to suffer from Hirschsprung's associated enterocolitis as a clinical condition with diarrhea, abdominal discomfort, fever, and eventually subsequent septic shock despite of correct endorectal pull-through for HD.

In Group A, we observed that the use of probiotics post-operative reduced the episodes of enterocolitis, as just 4 Children have suffered from episodes entero -colitis about 16 %., and only one Child from them had presented with severe HAEC and admitted to the intensive care unit and improved with aggressive fluid resuscitation and broad-spectrum antibiotics.

*Wang et al., (2012)* reported that Probiotics have been used to prevent colitis in a number of pediatric conditions and may be beneficial in the prevention of HAEC. Use of Lactobacillus, Bifidobacterium, Saccharomyces, and Streptococcus sp. has been reported in

children. These organisms have been evaluated for treatment of infectious diarrhea, antibiotic associated diarrhea, atopic dermatitis, necrotizing enterocolitis, Helicobacter pylori infection, Crohn's disease, and ulcerative colitis. They reported that use of Lactobacillus, Bifidobacterium, Saccharomyces, and Streptococcus sp in children may be beneficial in prevention of HAEC. These organisms have been evaluated for treatment of infectious diarrhea, antibiotic associated diarrhea, atopic dermatitis, necrotizing enterocolitis, Helicobacter pylori infection, Crohn's disease, and ulcerative colitis Shen et al. reported markedly decreased Lactobacillus sp. and Bifidobacteria sp. in children with HAEC.

According to this study, 15 Stool samples which were taken for microbial assessment in Group B during diarrhea episodes. 5 samples were Clostridium difficile and anaerobes ,4 samples were gram negative bacilli , yeast and pus cells, gram negative bacilli (Enter pathogenic E. coli) in 3 samples, gram negative bacilli (salmonella spp) in 2 samples and one sample was pseudomonas aearugimos but in Group A .Nine stool samples during diarrhea episodes in 4 Children, were gram negative bacilli (salmonella spp) in 3 samples, gram negative bacilli (Enter pathogenic E. coli) in 2 samples, 3 samples were gram negative bacilli and yeast (candida spp) and pus cells (60-65) / HPF and one sample was Clostridium Difficile and anaerobes.

*Georg Singer et al. (2018)* Published report described the intestinal microbiome of a patient suffering from Hirschsprung's disease during episodes with and without

enterocolitis and during treatment with probiotics. The fecal microbiome differed significantly between healthy and HAEC episodes. HAEC episodes were associated with significant decreases of Actinobacteria and significant increases of Bacteroidetes and Proteobacteria. One of the most striking changes was seen for the genus *Bifidobacterium* which was reduced from 13% to 5% during HAEC episodes.

The patient was started on continuous treatment with probiotics for three months. In detail, he received one sachet of OMNi-BiOTiC® in the morning and one sachet of OMNi-BiOTiC® in the evening. In the period of probiotic treatment, stool samples were taken for microbiome analysis during diarrhea episodes and during healthy episodes. Probiotic treatment led to a significantly increased alpha diversity irrespective of healthy or HAEC episodes, the most striking findings were that, during HAEC episodes under probiotic treatment, the significant increase of Bacteroidetes and the decrease of Actinobacteria were not encountered on the genus level; *Bifidobacterium* and *Streptococcus* were significantly increased during probiotic treatment. So, these results ascertain that Probiotic treatment significantly changed the fecal microbiome it increases of *Bifidobacterium* and *Streptococcus* as well as decreases of Rikenellaceae, *Pseudo butyrivi brio*, *Blautia*, and *Lachno spiraceae*.

Also, in this study we observed that the radiograph findings in Group B. 3 films were included the “cutoff” sign in the rectosigmoid colon with absent distal air with one film free intra-abdominal air from intestinal perforation, 7 films were

with dilated loops of bowel, air fluid levels, 2 films with dilated loops of bowel and 2 films with pneumatosis intestinalis. But in Group A. we observed that 3 films of plain radiograph included dilated loops of bowel with two or more air-fluid levels, one film finding an intestinal cut-off sign in the left lower quadrant. And no evidence of speculation, pneumatosis, and no free intra-abdominal air from intestinal perforation.

According to *World Gastroenterology Organization (WGO-2011)*, Probiotics affect intestinal bacteria by increasing the numbers of beneficial anaerobic bacteria and decreasing the population of potentially pathogenic microorganisms, probiotics affect the intestinal ecosystem by stimulating mucosal immune mechanisms and by stimulating non-immune mechanisms through antagonism and competition with potential pathogens. These phenomena are thought to mediate most beneficial effects, including reduction of the incidence and severity of diarrhea.

Finally, it has been shown that treatment with probiotics not only significantly diminishes the incidence but also decreases the severity of HAEC.

## CONCLUSION

The use of probiotics as a prophylactic treatment following surgery for Hirschsprung’s disease not only diminished the incidence, but also decreased the severity of Hirschsprung-Associated Enter-colitis (HAEC).



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## دراسة إستخدام باسيلات الأسيدي فاست كعلاج وقائي في حالات إتهاب الأمعاء ما بعد جراحه القولون المتضخم في الأطفال

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**خلفية البحث:** إتهاب القولون المرتبط بهيرشسبرونغ في الأطفال بعد الجراحة مشكلة شائعة و قد يتم إستخدام توسيع المستقيم أو ري المستقيم كطرق للتغلب علي هذه المشكلة. ويؤدي هذا إلى إطلاق المحتويات المعوية تحت الضغط ويقلل ذلك من خطر تسمم الدم. وفي كثير من الحالات، تُستخدم المضادات الحيوية أيضاً لعلاج إتهاب القولون المعوي، مثل ميترونيدازول (فلاجيل) وفانكوميسين. وفي بعض الأحيان، قد نحتاج إلى تدخل جراحي إضافي للأطفال الذين يعانون من الإلتهابات المتكررة للأمعاء والقولون الذي لا يستجيب لهذه الأساليب.

**الهدف من البحث:** تقييم إستخدام حمض الباسيلات الأسيدي فاست (البروبيوتيك) كعلاج وقائي لإلتهاب القولون بعد الجراحة لمرضى هيرشسبرونغ.

**المرضى وطريقة البحث:** تم تسجيل خمسين مريضاً في هذه الدراسة تراوحت أعمارهم بين ٠ يوم ١٠ سنوات و خضع جميعهم للعلاج الجراحي لمرض هيرشسبرونغ. تم إستخدام

حمض الباسيلات الأسيد فاست كعلاج وقائي لـ ٢٥ مريض منهم بعد الجراحة مباشرة ولمدة عامين مجموعة (أ) و ٢٥ مريض الآخرون مجموعة (ب) لم يتم استخدامه.

**نتائج البحث:** لا يقلل العلاج باستخدام البروبيوتيك من الإصابة بالتهاب القولون فحسب، بل يقلل أيضاً من شدة ومضاعفات التهاب القولون المرتبط بهيرشسبرونغ.

**الاستنتاج:** استخدام البروبيوتيك كعلاج وقائي بعد الجراحة لمرض هيرشسبرونغ يقلل من الإصابة بالتهاب القولون بشكل ملحوظ بل ويقلل أيضاً من شدة وخطورة هذا الإلتهاب.

**الكلمات الدالة:** التهاب القولون المرتبط بهيرشسبرونغ .