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Research Article

Effect of Probiotics in the Treatment of Acute Watery Diarrhoea in Children Admitted to a Tertiary Care Hospital in Bangladesh: A Non-Randomized Prospective Clinical Trial

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Abstract

Objective: The study aimed to assess the efficacy of probiotics in the context of acute watery Diarrhoea and their effects on serum immunoglobulin in children.

Methods: Prospective clinical trial in children aged one month to 12 years hospitalized with acute watery Diarrhoea in Uttara Adhunik Medical College, and allocated to receive probiotics, antibiotics, or probiotics + antibiotics for 30 days in accordance with the standard treatment protocol of Diarrhoea. Clinical outcome measurements included duration of Diarrhoea and treatment adverse events. Stool culture and blood immunoglobulin were analyzed on days 0 and 30.

Results: 166 enrolled children were divided into three groups: Group A (probiotics), Group B (antibiotics) and Group C (probiotics + antibiotics) with 98 participants returning for a follow-up visit on day 30. All groups were comparable in their baseline characteristics. Causative organisms of Diarrhoea among final participants (N=98) were *Rotavirus* (69.4%), *E. Coli* (67.4%), multiple organisms (2 or more) (45.9%), *Campylobacter* (34.7%), *Vibrio cholerae* (20.4%), *Salmonella* (10.2%), *Shigella* (9.2%), and *Klebsiella* (1.0%). Fastest recovery occurred in Group A (3.03 ± 0.76 days; Group C: 3.80 ± 1.10 days; Group B: 4.11 ± 1.48 days; p=0.001). At follow-up, administration of probiotics was associated with presence of commensal *Lactobacillus* and *Bifidobacterium* in stool.

Conclusion: Inclusion of probiotics for treatment of acute watery Diarrhoea in children is effective, safe and results in shorter duration of Diarrhoea and faster discharge from hospital. Probiotics may provide future alternative prevention and treatment strategies in childhood Diarrhoeal diseases in Bangladesh.

Trial registration: CTRI/2020/04/024633, Date: 15/04/2020

Keywords: Lactobacillus • Bifidobacterium • Acute watery diarrhoea • Probiotics • Clinical trials

Introduction

Diarrhoeal disease is responsible for a substantial health-related burden on human society and remains the second leading cause of death in children below 5 years of age. In addition, it is also a major cause of considerable morbidity in children of all ages throughout the globe [1]. Diarrhoea consists in the symptomatic presentation of acute gastroenteritis, commonly caused by infectious agents, such as viruses (Rotavirus, Norovirus), bacterial pathogens (*Escherichia coli*, toxigenic *Clostridium difficile*, *Campylobacter jejuni* and *Vibrio cholerae*) and parasites. However, the most common cause of gastroenteritis in children, especially in young children aged 3-24 months, is rotavirus infection. Around 1.4 million of the 9 million child deaths reported in 2008 were due to acute Diarrhoea, with 49% of the deaths occurring in five countries, namely India, Nigeria, the Democratic Republic of the Congo, Pakistan and China. In 2010, in countries with Low and Middle Socioeconomic Status (LMIC), the incidence of acute Diarrhoea was estimated around 2.9 episodes per child annually, mostly affecting infants aged 6-11 months [2]. In Europe, the incidence of Diarrhoea in children up to 3 years of age ranges from 0.5 to 1.9 episodes per child per year. Furthermore, infectious agents, such as enteropathogenic *E. coli* (EPEC), may cause protracted Diarrhoea in children, increasing the risk of long-term morbidities. Wardlaw et al. (2010) have shown that early onset of Diarrhoeal episodes predisposes children to lasting disabilities, stunted growth, and impaired cognition and school performance [3].

Notwithstanding obvious improvements in the case management of Diarrhoea, including early administration of oral rehydration solutions, continued feeding, oral zinc, and antibiotics, Diarrhoea remains responsible for 1.5 million deaths annually, or 1% of deaths in children under 5 years [4][5]. Use of antibiotics is still highly prevalent even if inappropriate for the most part and responsible for the bulk antibiotic consumption in humans, and consequently for their contribution to the emergence of antibiotic resistance [6]. However, when antibiotic therapy is deemed necessary, it is useful to have an easily available, cost-effective, and safe method to prevent potential side effects associated with such treatment.

In recent years, the use of probiotics has gained increased popularity, even if the concept of using probiotics for prevention and treatment of some human illnesses has been around for more than a century [7]. The WHO defines probiotics as a "live microorganisms which, when administered in adequate amounts, confer a health benefit to the host" [5]. Such definition is echoed by both the International Life Science Institute (ILSI) and the European Food and Feed Cultures Association (EFFCA) [8,9]. In general, probiotics are beneficial bacteria that colonize and replicate in the human intestinal tract providing positive benefits to the host. Several clinical trials support the efficacy of certain probiotics in the prevention and treatment of various Diarrhoeal illnesses, with microorganisms such as *Lactobacillus, Streptococcus* and *Bifidobacterium* being frequently used [10]. The rationale for probiotic use in acute Diarrhoeal diseases is predicated on the assumption that they act against enteric pathogens via activation of immune

signaling pathways, produce factors against enteric pathogens, and operate by inducing the host to secrete anti-pathogenic factors [11]. To date, numerous studies assessing the efficacy and safety of various probiotic species and strains in preventing and treating childhood infectious Diarrhoea are available. The ESPGHAN/ESPID evidence-based guidelines for the management of acute gastroenteritis in children in Europe summarized data from several meta-analyses, and reported a significant effect and moderate clinical benefit of selected probiotic strains in the treatment of acute watery Diarrhoea (primarily rotavirus), mainly in infants and young children [12]. In a trial conducted by Guandalini, rotavirus-positive patients were treated with oral rehydrating solution, and *Lactobacillus GG* (LGG) administration significantly improved recovery [13]. A body of evidence suggests that probiotics are safe when used in healthy children, and effective in reducing the duration of acute infectious Diarrhoea [14].

In Bangladesh, no studies have been conducted so far using probiotics in the treatment of Diarrhoea in children, even though Diarrhoea is a significant health problem in Bangladesh. This trial was therefore designed to evaluate the role of probiotics in children with acute-onset Diarrhoea of all causes.

Materials and methods

Participants

This study was performed as a parallel-group, interventional non-randomized study with enrollment of pediatric patients according to eligibility criteria and being allocated to receive probiotic therapy (Group A), conventional antibiotic treatment (Group B), and probiotic + antibiotic therapy (Group C) during the period of April, 2020 to July, 2020 at the Pediatric Department of Uttara Adhunik Medical College & Hospital in Bangladesh. The investigators were not blinded to group allocation. The study protocol received approval from the Institutional Ethical Review Committee (Ref: UAMC/ERC/Recommend - 62/2018), and clinical trial registration was conducted (CTRI/2020/04/024633, date: 15/04/2020). Only patients whose parents or legal guardians were willing and able to give written consent were enrolled in the study, and consents were collected before administering any treatment and collecting data. Inclusion criteria were: children aged from 1 month to 12 years, both sexes, who were diagnosed with dehydrating acute watery Diarrhoea of less than 14 days' duration, patients with clinical signs and symptoms of dehydration as illustrated by the presence of thirst or eagerness to drink, sunken eyes, dry mouth and tongue, and loss of skin elasticity, children who retained their ability to take oral medications. Moderate Diarrhoea means having more than a few but not more than 10 Diarrhoea stools in a day. Mild Diarrhoea means having a few Diarrhoea stools in a day. Exclusion criteria were patients with a history of an episode of Diarrhoea in the month preceding onset of the present illness to exclude recurrent and persistent Diarrhoea, severe dehydration, severe malnutrition, Diarrhoea associated with another systemic illness (e.g., septicemia, pneumonia, urinary tract infection, otitis media), known hypersensitivity reaction to probiotics, and any child who received antibiotic therapy within 1 month prior to inclusion in study.

Protocol

Subjects fulfilling the inclusion criteria were divided into three groups, namely A, B, C, on the basis of their anticipated therapy. Patients who received only probiotics were designated as Group A. The dose regimen of giving probiotic was 2 capsules twice daily for 48 hours, then 1 capsule twice daily for 8 days and then 1 capsule daily (below 3 years of age) and 2 capsules daily (3 years to 12 years) till day 30. Each capsule contained *Lactobacillus acidophilus* (2 billion), *Lactobacillus bulgaricus* (1 billion), *Bifidobacterium bifidum* (1 billion) and *Fructo-oligosacharides* (as prebiotic) (100mg) (manufactured by Renata Pharmaceutical Ltd, Dhaka, Bangladesh). Attribution to Group A was predicated on the findings of stool culture and examination, whereby if a virus was detected and no pathogenic bacteria emerged then participants were assigned. Patients who were assigned to receive antimicrobial agents according to the standard treatment protocol in place in the hospital were included in Group B. Patients in Group C were those who received both antibiotics and probiotic therapy. All groups also received zinc supplements in standard dosages and hydration therapy with ORS. After initial correction of dehydration, children continued to receive ORS as maintenance therapy by matching stool volume and any other fluid losses until Diarrhoea ceased. Breast-fed children were allowed to continue breast feeding. Formula or animal milk or normal diet was also permitted. Parents had the option to withdraw their children from the study at all times. The final study cohort required continued enrollment through 30 days and returning for follow up. Data collected from participants were kept under strict anonymity.

Assessment during baseline and follow up

After enrollment, a complete clinical history was taken from parents or legal guardians and a thorough physical examination was conducted and findings were recorded. Socio-demographic information was collected from the parents using a semi-structured questionnaire. Blood for assessment of serum electrolytes (day 0) and for immunoglobulins (IgM, IgG, IgA,) (days 0, 30) were drawn from each subject and send to Clinical Biochemistry Laboratory of Uttara Adhunik Medical College for analyses. IgM, IgG, IgA concentrations in serum were measured by using ELISA N Antiserum to Human IgM, IgG, IgA kits according to the manufacturer's instructions (Dade Behring Marburg GmbH, Marburg, Germany).

Stool samples were obtained on day 0 before starting treatment and transported to the Microbiology Department of Jahangirnagar University and Uttara Adhunik Medical College for primary isolation and identification of bacterial enteric pathogens and on day 30 for the detection of *Lactobacillus and Bifidobacterium* in fecal samples. To culture and identify *V. Colerae, E. Coli, Salmonella, Shigella, Klebsiella*, and *Campylobacter*, the following media were used: Thiosulphate Citrate Bile Salts-Sucrose Agar (TCBS), Eosine Methylene Blue (EMB) agar, *Salmonella Shigella* (SS) agar, MacConkey agar, Campylobacter base agar with Campylobacter specific commercial supplements (Hi media, India). MRS agar was followed by gram staining for both *Lactobacilli* and *Bifidobacterium*. The Xpect™ *Rotavirus* test kit was used for the qualitative detection of *Rotavirus* antigens in human fecal specimens.

Rehydration

The degree of dehydration assessed clinically, was corrected and then fluid balance maintained using Oral Rehydration Solution (ORS) following WHO's recommended formulation and guidelines [19]. Briefly each child was given approximately 100 ml/kg of ORS during the first four hours. The ORS was administered in frequent sips using a spoon or by nasogastric tube. if vomit or stool output rates were high. After this period, ongoing fluid and electrolytes losses were replaced with the same solution on a volume-to-volume basis until Diarrhoea ceased.

Outcome measures

Clinical outcome measures:

- Rate of treatment failure: The proportion of children in every group who experienced recurrence or continued presence of signs and symptoms of Diarrhoea, worsening electrolyte abnormalities, or no weight gain from enrollment.
- Duration of Diarrhoea: Time from enrollment to passage of the last liquid stool.
- Occurrence of adverse events.

Laboratory outcomes:

Restoration of *Lactobacillus* and *Bifidobacterium* dominant gut flora.

Statistical analysis

All data initially were collected in pre-coded forms. Some inferential statistics (e.g., chi-square tests, fisher's exact tests, Kruskal Wallis test, etc.) were conducted to compare across treatment groups. Paired sample t-tests were also executed to investigate the effect of treatment on serum immunoglobulin during enrollment and follow up. Values were expressed as both frequencies (%) and mean (± SD). A two-tailed *p*-value <0.05 was considered to achieve statistical significance.

Results

A total of 166 participants were enrolled and allocated into the three groups as follows: Group A: n=64, Group B: n=41, and Group C: n=61. However, 68 patients had to be excluded from the study because of either parental unwillingness to participate during the study or because they did not return for follow-up. The final cohort includes therefore 98 children, with 30 patients in Group A, 38 patients in Group B, and the remaining 30 patients in Group C (Figure 1), and their characteristics are shown in (Table 1) and indicate that no significant differences were present on enrollment across the 3 groups. The clinical and serum electrolyte characteristics of the cohort are shown in (Table 2).

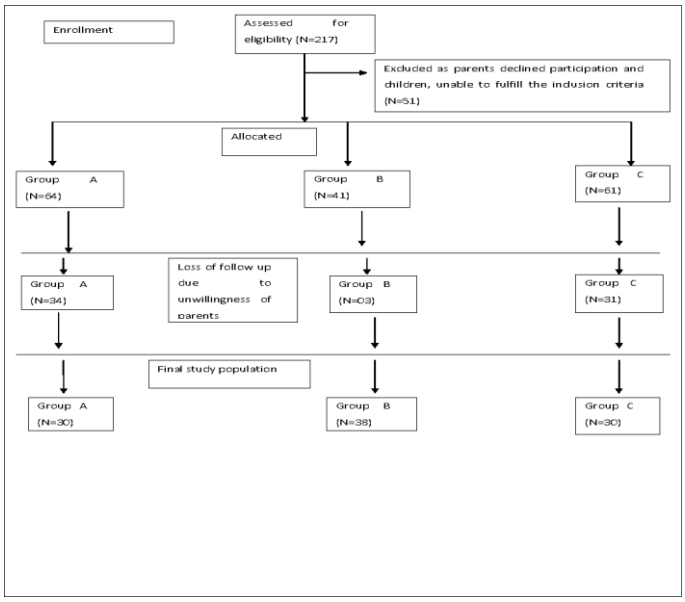

Figure 1. Flow chart of the study.

Table 1. Socio-demographic characteristics of the cohort based on treatment group allocation (n=98).

Variables	**Group A (Probiotics) (n=30) n (%)**	**Group B (Antibiotics) (n=38) n (%)**	**Group C (Probiotic + Antibiotics) (n=30) n (%)**
Sex			
Male	18 (60%)	25(65.8 %)	15(50%)
Female	12 (30.0)	13 (32.5)	15 (37.5)
Residence			
Urban	12 (40%)	23 (60.5%)	16 (53.3%)
Rural	18 (60%)	15 (39.5%)	14 (46.7%)
Economic status			
Poor	07 (23.3%)	06 (15.8%)	03 (10%)
Middle class	23 (76.7%)	32 (84.2%)	27 (90%)
	Mean ± SD (Median)	Mean ± SD (Median)	Mean ± SD (Median)
Age (months)	24.50 ± 34.70 -13	32.60 ± 35.64 -18.5	20.11 ± 16.41 -15
Mean duration of diarrhea prior to enrollment	3.50 ± 2.79 -2.5	3.39 ± 2.79 -2	2.37 ± 1.33 -2
(Note: SD = Standard deviation)			

Table 2. Clinical parameters and serum electrolytes of the cohort upon enrollment.

Clinical parameters	**Group A (Probiotics) (n=30)**	**Group B (Antibiotics) (n=38)**	**Group C (Probiotics + Antibiotics) (n=30)**
Loose watery stool	30 (100%)	38(100%)	30(100%)
Fever	8 (26.7%)*	34 (89.5%)	22 (73.3%)
Abdominal pain	1 (3.3%)	0	0
Nausea/vomiting	24 (80%)	28 (73.7%)	24 (80%)
Blood in stools	2 (6.7%)	5 (13.2%)	2 (6.7%)
Others (Convulsion, anorexia, feeding difficulty)	2 (6.66%)	3 (7.9%)	0
Laboratory features	Mean ± SD	Mean ± SD	Mean ± SD
Na^+(mmol/L)	139.43 ± 3.34	137.89 ± 3.80	139.67 ± 3.82
K^+(mmol/L)	4.18 ± 0.53	3.95 ± 0.49	4.29 ± 0.59
Cl^-(mmol/L)	104.4 ± 3.79	101.71 ± 3.74	103.57 ± 3.85
Co_2(mmol/L)	26.67 ± 1.27	27.26 ± 1.86	27.43 ± 1.68
(Note: SD = standard Deviation; * - p<0.05 vs. the other 2 groups.)			

Stool culture findings on admission are shown in (Table 3) for all 3 treatment groups. The distribution of enteric pathogens was similar across groups (*p*-not significant).

Table 3. Stool microbiological findings during admission to the study.

Organisms	**Group A (Probiotics) (n=30) n (%)**	**Group B (Antibiotics) (n=38) n (%)**	**Group C (Probiotics + Antibiotics) (n=30) n (%)**
E. Coli	24 (80%)	25 (65.8%)	17 (56.7%)
Campylobacter	11 (36.7%)	10 (26.3%)	13 (43.3%)
Vibrio	7 (23.3 3%)	7(18.4%)	6 (20.0%)
Salmonella	3(10.0%)	5 (13.2%)	2 (6.7%)
Shigella	1 (3.3%)	5 (13.2%)	3(10.0%)
Klebsiella	0	0	1 (3.3)
Rotavirus	21(70.0 %)	29 (76.3)	18 (60.0%)
No growth	1 (3.3%)	1 (2.6)	1 (3.3%)

Cessation of Diarrhoea occurred significantly fastest among Group A children (3.03 ± 0.76 days), followed by patients who received antibiotics + probiotics (Group C: 3.80±1.10 days) and slowest to recover were those in Group B (4.11 ± 1.48 days) (p<0.001); (Table 4).

Table 4. Duration of diarrhea after starting medications of all enrolled participants (N=98)

Groups	**(Mean ± SD)**	**Kruskal Wallis Test (*p*-value)**
Probiotic group (N=30)	3.03 **±** 0.76 days	0.001
Antibiotic group (N=38)	4.11 **±** 1.48 days	
Antibiotic + Probiotic group (N=30)	3.80 **±** 1.10 days	

After 30 days of treatment, stool analyses revealed significant differences among groups for the presence of *Lactobacillus* and *Bifidobacterium* (*p*<0.001); (Table 5). The changes in serum immunoglobulin levels for each of the treatment groups are shown in (Table 6).

Table 5. Stool culture findings during follow-up visit on day 30 for the cohort based on treatment group.

Treatment Groups	**Lactobacillus + Bifidobacterium**	**Campylobacter**	**No growth**
Group A (Probiotics) (n=30)	30 (100%)	0	0
Group B (Antibiotics) (n=38)	9 (23.7%)*	1 (2.6%)	28 (73.7%)

Group C (Antibiotics + probiotics) (n=30)	29 (96.7%)	0	1 (3.3%)
(Note: *- p<0.001 vs. the other groups)			

Table 6. Effect of treatment on serum immunoglobulin.

Groups	Immunoglobulins	Time frame of measurement			
		During enrollment (Day 0) (Mean ± SD)	During follow up (Day 30) (Mean ± SD)	Individual Change (Post-Pre) (Mean ± SD)	*p*-value
Group A (Probiotic) (N=30)	IgM (mg/dl)	86.93 ± 32.2	112.38 ± 25.32	25.45 ± 27.87	<0.001
	IgG (mg/dl)	695.4 ± 167.83	841.61 ± 164.79	146.21 ± 93.99	<0.001
	IgA (mg/dl)	132.55 ± 66.79	158.72 ± 57.80	26.17 ± 32.88	<0.001
Group B (Antibiotic) (N=38)	IgM (mg/dl)	81.71 ± 40.16	106.92 ± 23.48	25.21 ± 35.12	0.106
	IgG (mg/dl)	584.42 ± 167.9	808.72 ± 195.21	224.3 ± 172.88	0.014
	IgA (mg/dl)	166.79 ± 87.79	216.78 ± 69.17	50 ± 103.75	0.249
Group C (Antibiotic + Probiotic) (N=30)	IgM (mg/dl)	72.34 ± 28.94	102.08 ± 33.83	29.74 ± 40.55	<0.001
	IgG (mg/dl)	639.52 ± 124.66	787.14 ± 172.91	147.62 ± 135.32	<0.001
	IgA (mg/dl)	121.88 ± 63.27	172.19 ± 70.95	50.31 ± 43.5	<0.001

Discussion

In the present study, incorporation of probiotics in the treatment of acute watery Diarrhoea in children emerged as effective, safe and was associated with a shorter duration of Diarrhoea, leading to a faster discharge from the hospital. These findings support the institution of probiotic approaches as a routine component of the management of acute watery Diarrhoea in Bangladesh, while closely monitoring opportunities to further improve on such intervention while attempting to minimize the use of antibiotics in this context.

Before we discuss the potential implications of our study, some specific comments on the ancillary findings are worthy of mention. All groups were comparable in their baseline characteristics. Common clinical features of Diarrhoea were loose watery stool, nausea/vomiting, fever, blood in stool. Laboratory findings were overall comparable to those of Salazar-Lindo et al. in 2004 in Peru, except for bicarbonate levels which were higher in the present study, suggesting less severe dehydration status [15]. The most prevalent enteropathogens detected at the time of enrollment differed considerably from those of the trials conducted in India and Finland. In the Finish trial, Rotavirus accounted for more than 80% of Diarrhoeal cases [16], while in the study from India, *Rotavirus* (34.55%), *E. coli* (19.95%), no growth of pathogens (23.7%), *Vibrio cholera* (6.95%), and *Shigella* (2%) were reported. Thus, the greater diversity of etiologic agents and frequent co-pathogen associations as identified in the presemay reflect more accurately the findings occurring in LMIC.

In the current study, no treatment failures occurred, similar to the trial in Finland, in which all infants recovered within 5 days, and no treatment failure was reported [17]. We used *L. acidophilus, L. bulgaricus, and Bifidobacterium bifidum* as probiotic preparation in this trial, while Dubey et al. (2008) used strains of *L. Casei, L. bulgaricus, L. plantarum, S. thermophiles* for the treatment of *Rotavirus* associated Diarrhoea in children [18]. Narayanappa et al. (2008) showed that *Bifilac* (a combination of several probiotics) was safe and effective in patients with acute viral Diarrhoea [19]. Two different studies that were conducted with the aim to evaluate the efficacy of *L. rhamnosus* GG strain in acute watery diarrhoea in children showed inconsistent effects [20]. Furthermore, no beneficial effects of *Lactobacillus acidophilus* were observed in children suffering from acute Diarrhoea [21]. Probable explanations for the inconsistency of the findings across the multiple studies may include: (i) the fact that probiotic preparations and doses were not standardized in the Indian context; (ii) data generated in Western countries cannot be extrapolated to Indian or LMIC settings; (iii) the poor nutritional status of Indian or LMIC children may alter the responses to the probiotic interventions; (iv) different food habits may also affect the response to therapy; (v) the presence of a wide variety of both helpful and harmful intestinal microflora that may interfere with the efficacy of the treatment. Accordingly, the Indian trial showed that about 12% of patients had unresolved Diarrhoea and an additional 20% were classified as treatment failure mostly due to severe Diarrhoea. Findings of two more studies conducted also in India found similar results. In a randomized controlled clinical trial of *L. sporogenes* as probiotic in clinical practice on acute watery Diarrhoea in children, no treatment failures or adverse effects and complications were reported. However, the rate of treatment failure reported in a study conducted in Peru where *L. Casei* strain GG was used in the treatment of infants with acute watery Diarrhoea was 21.1% with LGG vs 18.0% with placebo.

We should also remark that the duration of Diarrhoea was significantly different among the three groups, indicative of a major beneficial effect of the probiotic intervention, particularly when in isolation and without the concurrent treatment with antibiotics. The duration of Diarrhoea in our study was comparable to that reported in two previous trials. Overall conclusions of a meta-analysis of 63 studies of probiotics involving more than 8,000 participants, mostly children, suggests that probiotics shorten the duration of Diarrhoea by ~24 hours with no evidence of adverse effect [22].

A few studies have demonstrated the presence of significant associations of probiotic species with altered gut microbiota composition. In our trial, stool analysis of participants at the 1-month follow-up revealed that *Bifidobacterium* and *Lactobacillus* were detectable and predominant among the majority patients treated with probiotics, while they were much less likely to be detected in those not treated with probiotics. If we assume that the presence such as probiotic strains is indicative of intestinal health and also signifies potential prevention of future Diarrhoeal episodes, then administration of probiotics during the acute Diarrhoeal episode may have long-term benefits that will need to be quantified in future studies. In experimental settings in rodents, a recent metagenomic analysis of 8-week-old Swiss mice fed a high-fat diet showed that treatment with a probiotic mixture of *Lactobacillus* and *Bifidobacterium* (*L. rhamnosus, L. acidophilus*, and *Bifidobacterium bifidum*) significantly altered the composition of the gut microbiota [23].

Similar work on obese mice revealed that several *Lactobacillus spp*., *Bifidobacterium* spp., and other coliform bacteria increased in the gut microbiota in mice with a high-fat diet treated with various *Lactobacillus* probiotic *strains (L. acidophilus IMV B-7279, L. casei IMV B7280, B. animalis VKL, and B. animalis* VKB) [24]. Studies have demonstrated that *Bifidobacterium* and *Lactobacillus* can inhibit harmful bacteria, improves gastrointestinal barrier function and *Bifidobacterium* alters the function of dendritic cells to regulate the intestinal immune homeostasis to harmless antigens and bacteria or initiate protective measures against pathogens [25-28]. Such basic studies have been somewhat corroborated by clinical trials as well. Indeed, a clinical study demonstrated that patients who received *L. plantarum* DSM 9843 showed the presence of *L. plantarum* in rectal samples of patients, along with reduced amounts of enterococci in fecal specimens [29].

In another study, analyses of the fecal microbiota of patients treated with a probiotic mixture of *L. acidophilus, L. plantarum, L. rhamnosus, Bifidobacterium breve, B. lactis, B. longum* and *Streptococcus thermophilus*. and analyses of the fecal microbiota of these patients revealed that the similarity of the microbial composition was more similar in probiotics-treated patients than that of the placebo group [30]. Another study analyzed the fecal microbiota of 6-month-old infants treated with daily supplements of *L. rhamnosus* (LGG), and showed an abundance of LGG and an increased index of evenness in the fecal microbiota of these infants, suggesting ecological stability [31].

Many probiotic bacteria have been tested for their immunomodulatory properties, especially *Lactobacillus sp.* and *Bifidobacterium sp* [32-35]. In our study, all children during follow-up on day 30 showed an increase of serum IgM, IgG, IgA antibodies with highest increase in serum concentration of IgG in patients treated with only probiotics. These findings may suggest that the elimination of pathogenic organisms and reestablishment of normal gut flora induce improvements in immune status. Indeed, a double blind, randomized controlled trial in healthy adults reported that oral administration of *Bifidobacterium lactis* Bl-04 and *Lactobacillus Acidophilus* La-14 changed the serum immunoglobulin concentrations compared with controls [36]. Shin et al. in a study in pigs also reported that administration of *L. plantarum* strain JDFM LP11 led to increased serum IgG was increased [37]. Oral introduction of *Bifidobacterium bifidum* was shown to enhance antibody response to ovalbumin and *Bifidobacterium breve* was shown to stimulate IgA response to cholera toxin in mice [38,39]. An increased humoral immune response, including an increase in *rotavirus* specific antibody-secreting cells in the IgA class, was detected in children with acute rotavirus Diarrhoea who received *L. rhamnosus* GG during the acute phase of Diarrhoea [40]. The mean serum rotavirus IgA antibody concentration at the convalescent stage was also higher in those individuals receiving *L. rhamnosus* GG [41]. In another trial, oral administration of *L. acidophilus*

LBKV3 strain as probiotic showed enhancement of IgG immunoglobulin levels, and regulation of gut microflora [42].

Limitations

To date, insufficient data justify the routine use of probiotics in Diarrhoea in Bangladesh. Although the sample size was small, this was the first clinical trial in Bangladesh, and provides initial support to expand these observations. However, we should also indicate that the present study has the following limitations: (a) It was conducted involving only a small population.; (b) It was conducted only in one tertiary care teaching hospital in Dhaka city; (c) It does not represent the whole pediatric population of Bangladesh; (d) it did not conduct an in-depth analysis of gut microbiome using metagenomic approaches. A continuation of this study involving a large number of patients (both children and adult patients) involving all age groups from all areas in Bangladesh (rural and urban areas) and addressing the current limitations will be required to provide more accurate and definitive recommendations.

Conclusion

In this study, administration of probiotics showed promise in becoming either an alternative or a complementary treatment option for acute watery Diarrhoea in the pediatric population. It was also shown that probiotics also helped to improve the immunity of children. As probiotics are already in use in many fermented products or in sue as over the counter supplements in many countries, there are no *a priori* major safety concerns. Randomized controlled trials that incorporate probiotics in the treatment of Diarrhoeal diseases may potentially lead to improved utilization of the traditional and virtually universally applied antimicrobial chemotherapy in Bangladesh. Probiotics may also provide effective prevention of Diarrhoea, as illustrated by the increased serum levels of immunoglobulins.

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