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Original Research Article

Comparative study of *Bacillus clausii* and multistrain probiotics in the management of acute diarrhoea in children

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ABSTRACT

Background: Diarrhoea is the second most common cause of under-five mortality especially in developing world. Many studies have been conducted so far using different probiotic strains with variable outcome. So, the aim of the present study was to compare the clinical efficacy of *Bacillus clausii* and multi strain probiotic formulation as adjunct treatment of acute diarrhoea.

Methods: This prospective single blind randomized controlled clinical trial included 300 infants and children between 6 months to 6 years of age admitted in a tertiary care hospital Sylhet, Bangladesh with acute watery diarrhoea having varied dehydration status ranging from no to severe dehydration excluding shocked state. Cases were randomly assigned to three groups which were group I (n=100) comprised of children who were treated with standard treatment (according to WHO guideline) only as control group, group II (n=100) who received standard treatment plus *Bacillus clausii* and group III (n=100) who received standard treatment plus multi strain probiotic formulation (*Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Streptococcus thermophilus*). Primary outcome variables were duration, frequency of diarrhoea and consistency of stool. Secondary outcome variable was duration of hospital stay.

Results: Mean duration of diarrhoea was significantly shorter (p=0.001) in group III (2.62 days) compared to group I (3.26 days) and group II (3.22 days). Frequency of diarrhoea was significantly lower on day 3 of probiotic administration in group III ($p < 0.05$) and on day 5 of treatment in group II ($p < 0.05$). Stool consistency significantly improved on day 3 in group III ($p < 0.05$) while it was on day 4 in group II. The duration of diarrhoea, hospital stay, stool consistency and frequency of stool on day 3 were not statistically significant ($p > 0.05$) in group II in comparison to group I and group III.

Conclusions: Multistrain probiotic formulation is effective in reducing the duration, frequency of diarrhoea and duration of hospital stay.

Keywords: Bifidobacterium breve, Bifidobacrerium infantis, Bacillus Clausii, Diarrhoea, Lactobacilli casei, Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus bulgaricus, Probiotic, Streptococcus thermophilus

INTRODUCTION

Acute diarrhoea is still a major health problem worldwide and a frequent cause of death especially in developing countries.[1] This is usually treated according to WHO guideline using oral rehydration solution, intravenous fluid as indicated, and zinc supplementation.[2] This treatment doesn't halt the progression of the disease, but

to minimize the complications which are the causes of death in diarrhoea. The concept of using probiotic as an adjuvant therapy in existing diarrhoeal treatment has been introduced decades ago and till now studies are being taken in both developed and developing countries to evaluate its beneficial effect. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host.[3] The rationale for using probiotics in acute infectious diarrhoea is based on the assumption that they act against intestinal pathogens and possible mechanisms include the synthesis of antimicrobial substances, competitive inhibition of adhesion of pathogens, modification of toxin and non-toxin receptors and stimulation of nonspecific and specific immune responses to pathogens.[3,4] Scientific evidence points to the fact that the ability of a probiotic bacterium to confer a health effect largely depends on the particular strain being used.[4] While some probiotic strains have shown benefit others have demonstrated no visible difference. Most of the studies conducted so far evaluating multistrain probiotics, especially *Lactobacillus* species came up with favorable outcome. Studies conducted using *Bacillus clausii* probiotic were very few and most of them did not recommend its use.[5-11] As probiotics are extensively used by Bangladeshi paediatricians and general physicians in paediatric diarrhoea, randomized controlled clinical trials are necessary before prescribing the most beneficial probiotic strain. The two probiotic strains used in this study are commercially available in Bangladeshi market as single strain *Bacillus clausii* (Enterogermina) and multistrain formulation (Protexin). So, the aim of the present study was to determine the comparative clinical efficacy of a single strain *Bacillus clausii* with multistrain probiotic formulation (*Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium infantis, Streptococcus thermophilus*) as adjunct treatment of acute diarrhoea.

METHODS

This prospective single blind randomized controlled trial was carried out in a tertiary care hospital in Sylhet, Bangladesh over a period of 1 year from March 2017 to February 2018. Previously healthy 6 months to 6 years old infants and children diagnosed as acute watery diarrhoea with no to severe dehydration excluding shocked state clinically on the basis of history and physical examination were included in the study. Children with dysentery, chronic diarrhoea, other acute systemic illness, severe malnutrition and/or immunosuppressive state, use of probiotic or antibiotic in previous three weeks were excluded from the study. Informed consent was obtained from parents/guardians of individual participant included in the study. Ethical clearance was taken from the institution's ethical clearance committee before the study. Author analyzed total 300 patients fulfilling the inclusion criteria. Cases were randomly assigned to three groups which were group I (n=100) comprised of children who were treated with standard treatment (according to WHO guideline) only as control group, group II (n=100) who received standard treatment plus *Bacillus clausii* and group III (n=100) who received standard treatment plus multi strain probiotic formulation (*Lactobacilli, Bifidobacteria and Streptococcus thermophilus*). Standard treatment was used according to WHO guideline, the use of oral rehydration solution, intravenous fluid as indicated, and zinc supplementation. Group II received 2 billion spores of *Bacillus clausii* contained in a small bottle 12 hourly for 5 days. Group III was given multistrain probiotics formulation (*Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium infantis, Streptococcus thermophilus*) in single oral sachet (1×109 CFU) once daily for 5 days. Data were entered into prepared proforma, which included the information regarding baseline characteristics of patients (age, sex, nutritional status, and dehydration status), duration of symptoms of study groups before admission (duration and frequency of diarrhoea), primary outcome variables (duration of diarrhoea in days, frequency of diarrhoea per day and consistency of stool) and secondary outcome variable (duration of hospital stay in days). Consistency of stool was evaluated through a scoring system such as: 1= Normal, 2= Loose, 3= Semi liquid and 4= Liquid.

Patients were followed up daily. Clinical responses were evaluated in terms of improvement in symptoms and dehydration status. Data were processed and analyzed by using SPSS statistical software version 17 employing appropriate statistical tests. Any probability value of less than 0.05 was considered statistically significant.

RESULTS

Mean age of patient's was 15.81 (±10.5) months in group I, 16.62 (±8.5) months in group II and 16.36 (±8.6) months in group III showing no statistical significance. Most of the patients in study groups were male (M:F=1.5:1 in group I versus 2.1:1 in group II and 2:1 in group III). More than half of the patients in three groups had no malnutrition. Grade I and grade II malnutrition was comparable in all groups. Regarding dehydration status, most of the patients in all groups suffered from some dehydration (Table 1).

Before admission, duration of diarrhoea in all three groups was comparable showing no significant difference ($p > 0.05$). Frequency of diarrhoea per day was 7.17 (±2.4), 7.24 (±2.4), and 7.23 (±2.7) in group I, group II and group III respectively. This was also not statistically significant ($p > 0.05$) (Table 2).

After intervention, duration of diarrhoea was significantly reduced in group III when compared to group I and group II ($p=0.001$). There was significant reduction in the duration of hospital stay in group III in comparison to group I ($p=0.001$) and group II ($p=0.002$). Frequency of

diarrhoea decreased significantly at day 3 of treatment in group III (p <0.05) showing statistical difference. Group II showed statistical difference with control group on day 5 (p=0.046). Stool consistency improved significantly on day 3 of treatment in group III compared to group I (P=0.001) and group II (P=0.012). Group II also showed significant difference on day 4 of treatment (p=0.032) (Table 3 and 4).

Table 1: Baseline characteristics of study groups.

Parameter		Group I (n=100)	Group II (n=100)	Group III (n=100)	p value
Mean age (Months±SD)		15.81 (±10.5)	16.62 (±8.5)	16.36 (±8.6)	0.540
Sex	Male no. (%)	64 (64%)	60 (60%)	67 (67%)	
	Female no. (%)	36 (36%)	40 (40%)	33 (33%)	
Nutritional status	No malnutrition	56 (56%)	52 (52%)	52 (52%)	
	Grade 1 malnutrition	34 (34%)	30 (30%)	36 (36%)	
	Grade 2 malnutrition	10 (10%)	18 (18%)	12 (12%)	
Dehydration status	No dehydration	15 (15%)	11 (11%)	11 (11%)	
	Some dehydration	75 (75%)	78 (78%)	80 (80%)	
	Severe dehydration	10 (10%)	11 (11%)	09 (09%)	

Mean age of patients in control and study groups showed no statistical difference. Most of the patients in all groups were male. Grade I and grade II malnutrition was comparable in all groups. Most of the patients in all groups suffered from some dehydration.

Table 2: Comparison of duration of symptoms of study groups before admission.

Symptoms (Mean±SD)	(Mean±SD) (n=100)	Group II (n=100)	Group III (n=100)	Significance		
				Group I vs Group II	Group I vs Group III	Group II vs Group III
Duration of diarrhoea (days)	3.58 (±1.7)	3.85 (±1.5)	3.92 (±1.4)	0.693	0.281	0.842
Frequency of diarrhoea (per day)	7.17 (±2.4)	7.24 (±2.4)	7.23 (±2.7)	0.842	0.855	0.977

Before admission, duration and frequency of diarrhoea in all three groups were comparable showing no significant difference.

Table 3: Comparison of outcome variables of study groups.

Outcome variables (Mean±SD)	Group I (n=100)	Group II (n=100)	Group III (n=100)	Significance		
				Group I vs Group II	Group I vs Group III	Group II vs Group III
Duration of diarrhoea (days)	3.26 (±1.1)	3.22 (±1.3)	2.62 (±1.2)	0.809	0.001*	0.001*
Day 1	6.81 (±1.9)	6.73 (±2.1)	6.80 (±2.2)	0.794	0.975	0.810
Day 2	4.94 (±1.4)	4.90 (±1.6)	4.88 (±1.7)	0.847	0.772	0.933
Day 3	3.04 (±0.9)	3.03 (±0.9)	2.60 (±1.0)	0.941	0.011*	0.018*
Day 4	2.13 (±0.8)	2.00 (±0.7)	1.80 (±0.6)	0.724	0.005*	0.002*
Day 5	1.08 (±0.5)	0.93 (±0.5)	0.91 (±0.6)	0.046*	0.034*	0.812
Duration of hospital stay (days)	3.84 (±1.0)	3.22 (±1.3)	2.62 (±1.2)	0.809	0.001*	0.001*

Duration of diarrhoea and duration of hospital stay were significantly reduced in group III when compared to group I & group II. Frequency of diarrhoea decreased significantly at day 3 of treatment in group III, while Group II showed statistical difference with control group on day 5.

Table 4: Comparison of outcome variables of study groups.

Stool consistency	Group I (n=100)	Group II (n=100)	Group III (n=100)	Significance		
				Group I vs Group II	Group I vs Group III	Group II vs Group III
Day 1	4 (3-4)	4 (3-4)	4 (3-4)	0.682	0.677	1.000
Day 2	3 (3-4)	3 (3-4)	3 (2-4)	0.657	0.408	0.566
Day 3	3 (2-3)	3 (2-3)	2 (2-3)	0.077	0.001*	0.012*
Day 4	1 (1-2)	1 (1-2)	1 (1-1)	0.032*	0.001*	0.004*
Day 5	1 (1-2)	1 (1-2)	1 (1-1)	0.103	0.014*	0.158

The patients in control group (I) and study groups (II &III) had grade 3-4 consistency of stool day1 &2, while it improved significantly in group III on day 3 and in group II on day 4.

DISCUSSION

The present study showed that the group which received multistrain probiotic formulation had a significantly lesser duration of diarrhea compared to the control group and the *Bacillus clausii* group. The duration of diarrhea was lesser by 0.64 days in children who were treated with multistrain probiotic formulation compared to control group and by 0.60 days compared to *Bacillus clausii* group. Frequency of diarrhoea decreased significantly on day 3 in multistrain probiotic group in comparison to control and *Bacillus clausii* group, the later showed significant reduction of frequency on day 5 of treatment. Consistency of stool improved significantly on day 3 in multistrain probiotic group while it was on day 4 in *Bacillus clausii* group. There was significant difference in the duration of hospital stay in multistrain group. It was 1.22 days lesser compared to the control group and 0.60 days lesser compared to the *Bacillus clausii* group. *Bacillus clausii* did not have significant outcome comparing different variables in this study.

This result was consistent with the study conducted by Canani RB et al, evaluating five probiotic preparations in children with acute diarrhoea proved that only two preparations are *L. rhamnosus* (LGG) and the mix of (*L. bulgaricus, L. acidophilus, S. thermophilus and B. bifidum*) had a significant effect on reducing the frequency and duration of diarrhoea after the first day of administration. *Bacillus clausii* did not show any significant effect.[6] A systematic review and meta-analysis conducted by McFarland LV et al, included 228 trials and significant efficacy evidence was found for 7 (70%) of probiotic strains among four preventive indications and 11 (65%) probiotic strains among five treatment indications. Significant efficacy was demonstrated by the mixture of *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosu, Lactobacillus reuteri and Saccharomyces boulardii*.[7]

A review by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) was published in 2014 on the use of probiotic in diarrhoea reporting that *Lactobacillus rhamnosus GG* and *S. Boulardii* are very potent, while *L. reutrri* and *L. acidophilus* have a lower recommendation. *Bacillus clausii* and other probiotics cannot be recommended.[8] Szajewska H et al, conducted meta-analysis revealed that probiotic (*L. rhamnosus GG, L.reuteri and S. boulardii*) compared with placebo significantly reduce the risk of diarrhoea.[9] Rosenfeldt V et al, showed that *Lactobacillus rhamnosus* and *Lactobacillus reuteri* improved acute diarrhea in hospitalized children and reduced the duration of rotavirus expulsion.[5] A Cochrane review suggested that probiotics mainly combination of *Lactobacillus* species and *S. boulardii* may appear to be a useful adjunct to rehydration therapy when managing both adults and children.[10] Applegate JA et al, evaluated eight RCTs which studied different combination of probiotics and individual probiotic showed reduction in duration and frequency of diarrhoea with *Lactobacillus rhamnosus GG* with other combination but not with *Bacillus clausii*.[11]

A study conducted in Thailand concluded that *Lactobacillus acidophilus* and *Bifidobacterium infantis* might be an effective treatment in acute diarrhoea in infants which supported the finding.[12] Another recent study conducted by Bhat S et al, which showed that *Bacillus clausii* did not significantly affect duration and frequency of diarrhoea in comparison to control and *Saccharomyces boulardii* group.[13]

In this study, *Bacillus clausii* did not show any significant improvement in term of duration, frequency of diarrhoea and duration of hospital stay, but improved stool consistency one day later than that of multistrains group. Maugo BM conducting a study in under 5 children in Kenya concluded that there was a significant decrease in the frequency of stool on Day 3 and 4 of treatment but no significant difference in reduction of duration of diarrhoea and duration of hospital stay with *Bacillus clausii*.[14]

However, a recent study on *Bacillus clausii* done by Ianiro G et al, in Italy showed promising result. It concluded that *Bacillus clausii* might represent an effective therapeutic option in acute childhood diarrhoea.[15] Another study conducted by Jayanthi N et al, supported the use of *Bacillus clausii* in pediatric diarrhoea.[16] Lahiri et al, conducted a study on *Bacillus clausii* in pediatric acute diarrhoea and it showed reduction of diarrhoeal duration, and hospital stay but it was regarded as poor quality by meta-analysis done by Ianiro et al.[15,17]

However, another most recent study conducted by Freedman SB et al, in Canada concluded that *Lactobacillus rhamnosus* and *L. helveticus* did not have a role in pediatric diarrhoea.[18] A study done in USA using *Lactobacillus rhamnosus* did not show better outcome than placebo.[9] but the authors concluded that other *Lactobacilli* strains, either single or multiple might be beneficial in pediatric diarrhoea.

CONCLUSION

It is well supported from the present study as well as from literature review that multistrain probiotic formulation is effective as an adjunctive treatment in acute diarrhoea. Single strain probiotic, *Bacillus clausii* cannot be recommended based on this study result and from most of the western studies. Multicentre randomized controlled trials need to be undertaken using *Bacillus clausii* probiotic to actually evaluate its role in diarrhoea.

The limitation of the study was that author did not identify the offending organism by stool examination.

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