

Efficacy study of Indigenous Probiotics for Treatment of Acute Diarrhea in Infants: A Double- Blind, Placebo-Controlled Study

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ABSTRACT--- *Double- blind, Placebo-controlled clinical trial was conducted to study the efficacy of three indigenous probiotics in the treatment of acute diarrhea in infants. Patients were categorized by ages (birth to 6 months and 6 to 12 months) and observed daily for number of stools per day, vomiting episodes, loss of weight, and recovery days from diarrhea. In first category, the majority of patients were normalized in 16±2.7 days after intaking Lactobacillus-formula, while took 11±1.9 days in the case of Bifido-formula. Minimum number of days were calculated for the recovery of patients in the Mixed-formula (04±1.2 days). While the controlled group recovered in 21±4 days. In second category, a significantly late recovery response was observed in the controlled group (28±1.3 days) while Mixed formula again proved to be the best formula with only 08±2.2 recovery days in major cases with significant value of 3.4±1.4 mean number of stool per day, 0.9±0.3 mean vomiting episodes per infant, and 0.5±1.1 mean loss in weight per infant. The results revealed that these indigenously isolated probiotics, especially in the mixed multistrain cultures are effective in treatment of acute diarrhea in infants.*

Keywords--- indigenously isolated probiotics, acute diarrhea, multistrain probiotics, Double- blind, Placebo-controlled clinical trial, recovery time

1. INTRODUCTION

The World Health Organization (WHO) defines diarrhea as three or more loose or watery stools per day[1]. The oral rehydration solutions are generally used for treatment of acute diarrhea. However, these solutions are neither helpful in reducing the duration nor the severity of diarrhea [2]. Therefore, the use of probiotics is increasing in treatment of diarrhea since it is assumed that these lactic acid bacteria act against intestinal pathogens[3]. These probiotics are live microorganisms which can colonize in the gastrointestinal tract[4]. However, the mechanism of work is unknown; may include synthesis of antimicrobial substances[5], nutritional competition[6], adherence competition with pathogens[7], modifications in toxin receptors[8] and stimulation of immune responses[9]. *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, bifidobacterium, *Enterococcus faecium*, the yeast *Saccharomyces boulardii*, and combination of these strains are well known for the human gastrointestinal health[10, 11].

The mixed responses were found in the efficacy studies of probiotics in the treatment of diarrhea. Only two meta-analyses suggest a reduction in diarrhea by 60% [12]. *S. boulardii* was found effective in adult patients while *Lactobacillus GG* in children[13]. It is also investigated that the effectiveness of all probiotics is not same as a combination of *Lactobacillus acidophilus* and *L. bulgaricus* was found ineffective in preventing diarrhea in children during a double-blind placebo-controlled trial [14]. Moreover, another clinical trial failed to show superiority of *Lactobacillus GG* over placebo in 302 hospitalized patients in preventing diarrhea [15]. However, the clinical efficacy of probiotics for treating diarrhea in infants, especially in the form of multistrain preparations, has not been fully studied. Unfortunately, same is the case in Pakistan, no effort is made on the efficacy study of the indigenous probiotics in the treatment of diarrhea. Neither the data is available on the clinical trials of any commercial probiotic products nor on the effectiveness studies of indigenous probiotics.

This research was, therefore, designed to assess the effectiveness of indigenously isolated multistrain probiotics in the treatment of diarrhea in infants (up to 12 months of age). The three multistrain probiotic preparations were used in this study. The first preparation (Lb formula) was the consortium of five lactobacillus cultures (*L. acidophilus*, *L. lactis*, *L. plantum*, *L. reuteri*, *L. gasseri*), second (Bf formula) was the consortium of five Bifidobacterium cultures (*B. bifidum*, *B. infantis*, *B. longum*, *B. dentum*, and *B. brevis*), and third (M formula) the consortium of both *Lactobacillus* and *Bifidobacterium*. The aim of this double-blind placebo-controlled trial was to assess the potential of these preparations to attenuate the diarrhea in infants and evaluate the number of stools per day, vomiting episodes, loss of weight during the study, and days taken to recover completely from diarrhea. Number of episodes of regurgitation or vomiting were recorded on a weekly basis after daily observation of patients in the seven days from the day of enrollment for four weeks.

2. METHODS AND METHODS

2.1 Participants

A double-blind Placebo-controlled clinical study was conducted on the consecutive under 12 months aged 96 patients from four Children Care Hospitals of Lahore. Children were excluded if, at enrollment, they had a history of gastrointestinal disease or suspected malabsorption disorder. They were also excluded if they were receiving antibiotics, or soy based formula.

2.2 Study Design

Diarrhea was defined as the passage of unusually loose stool more than three times in 24 hours. The consent form was obtained from both parents. The patients were randomly divided into two main categories depending on their ages and further four groups were formed in each category on the basis of types of supplement including three supplement groups, one for lactobacillus- whey based supplement (Lb formula), second bifidus- whey based supplement (Fd formula), third multistrain-whey based supplement (mixed formula) and fourth was the control group. The supplement groups were fed with probiotic-whey based supplement for four weeks while the controlled group was on traditional treatment of antibiotics with no probiotic supplement. The supplement groups consisted of 14 patients in Lb formula group, 12 patients in Fd formula group, 11 patients in a mixed formula group while a control group of 11 patients in first under 6 months age category of infants while the second category of patients were containing 08 patients in Lb formula group, 12 patients in Fd formula group, 14 patients in mixed formula group while control group of 14 patients. The probiotic whey based supplements were guaranteed of having lived probiotic bacteria at 10^{12} CFU per gram of powder in each type of formula. The level of bacteria was confirmed before and after the study.

2.3 Follow up

If the patients discharged during four weeks of study, the clinical procedure was not disturbed and inquired on a daily basis. The patients were evaluated daily for number of stools per day, vomiting episodes, loss of weight during the study, and days taken to recover completely from diarrhea, while the formula intake was kept constant on the daily basis calculation of 1/4 teaspoon in single doses and twice a day was the recommended dose. Number of episodes of regurgitation or vomiting were recorded on a weekly basis after daily observation of patients in the seven days from the day of enrollment.

2.4 Statistical analysis

One-way ANOVA using SPSS 17 version was performed on data obtained. Significance was declared at $P \leq 0.05$.

3. RESULTS

The total of ninety six infants was studied with 1:1 sex ratio; divided into two categories on the basis of age (≤ 6 months and > 6 month to 12 months). Each category contained 48 infants (24 of each sex). Furthermore, each category was randomly divided into equal numbers based on the type of formula intake. At the time of enrollment, no significant difference was found in respect of age, weight, height, and family history of allergy or gastrointestinal disease in the respective category of patients. The features and findings of the 96 patients at the time of enrollment and during the follow-up is given in Table 1.

Table 1: Features of patients at enrollment and during the follow-up

	≤ 6 months of age				> 6 month to 12 months of age			
	Lb formula	Bd formula	M formula	Control	Lb formula	Bd formula	M formula	Control
Number of infants	14	12	11	11	8	12	14	14
Age (months)	3.1±2.2	3.9±2.1	3.3±2.5	3.5±2.4	6.4±2.1	5.1±2.2	7.1±2.2	8.4±2.2
Weight (Kg)	4.9±2.0	5.7±1.9	5.1±2.1	6.2±0.8	7.9±2.8	7.2±1.2	8.7±0.4	9.4±1.0
Height (cm)	59.2±2.9	64.1±2.0	59.7±3.7	61.9±5.4	7.1±2.9	6.9±3.9	8.4±2.1	8.9±2.0
Breastfeeding	Y	Y	Y	Y	N	N	N	N
Solid food	N	N	N	N	Y	Y	Y	Y
Intake days	21±3	18±2	8±1	32±4	16±2	21±4	11±3	37±6
Follow up days	32	32	32	32	32	32	32	32
Mean no. of stool per day	5.1±2.2 ^b	4.6±1.5 ^b	3.6±1.6 ^a	6.1±1.5 ^c	4.6±1.9 ^{ab}	5.9±0.9 ^b	3.4±1.4 ^a	7.3±1.2 ^b
Mean vomiting episodes per infant	2.5±2.0 ^b	2.0±0.9 ^b	0.5±1.3 ^a	3.5±0.6 ^c	3.5±0.8 ^b	2.4±1.2 ^b	0.9±0.3 ^a	2.1±0.5 ^b
Mean loss in weight per infant	1.8±1.2 ^c	1.1±0.9 ^b	0.3±0.4 ^a	2.2±0.1 ^c	0.7±0.6 ^a	1.1±0.3 ^a	0.5±1.1 ^c	1.9±0.4 ^b
Recovering from diarrhea (total mean days)	16±2.7 ^{bc}	11±1.9 ^b	04±1.2 ^a	21±4 ^c	12±3.3 ^{ab}	16±1.8 ^b	08±2.2 ^a	28±1.3 ^c

Lb formula= *Lactobacillus* formula, Bd formula=*Bifidobacterium* formula, M formula= Mixed formula containing 1:1 *Lactobacillus* and *Bifidobacterium*, Control= no probiotic formula given to infants of this group, Y= breastfeeding infants, N= Infants not on breastfeeding

In the present clinical trials, the admitted patients of under 6 months of age category were receiving the breastfeeding at the time of enrolment and not the solid food but later during the period of feeding trails, mothers were not allowed to feed their children till the normality in number and consistency of patient's stool. The significant data was observed and found that the after 16±2.7 days of Lb formula majority of patients were normalized with mean number of stool of 5.1±2.2 per day, mean vomiting episodes of 2.5±2.0 per infant and Mean loss in weight of 1.8±1.2 per infant while it took 11±1.9 days in normalization of stool number and consistency in the case of Bd formula with 4.6±1.5, 2.0±0.9, and 1.1±0.9 mean no. of stool per day, mean vomiting episodes per infant and mean loss in weight per infant, respectively. Minimum number of days were calculated for the recovery of patients to their normal life in the M formula (04±1.2) which contained the mixture of both *lactobacillus* as well as *bifidobacterium* in ratio of 1:1, while the controlled group of patients recovered in 21±4 days, which have significantly different response than the formula groups and the latest response of patients against diarrhea in this study in respect to category of under 6 months of age.

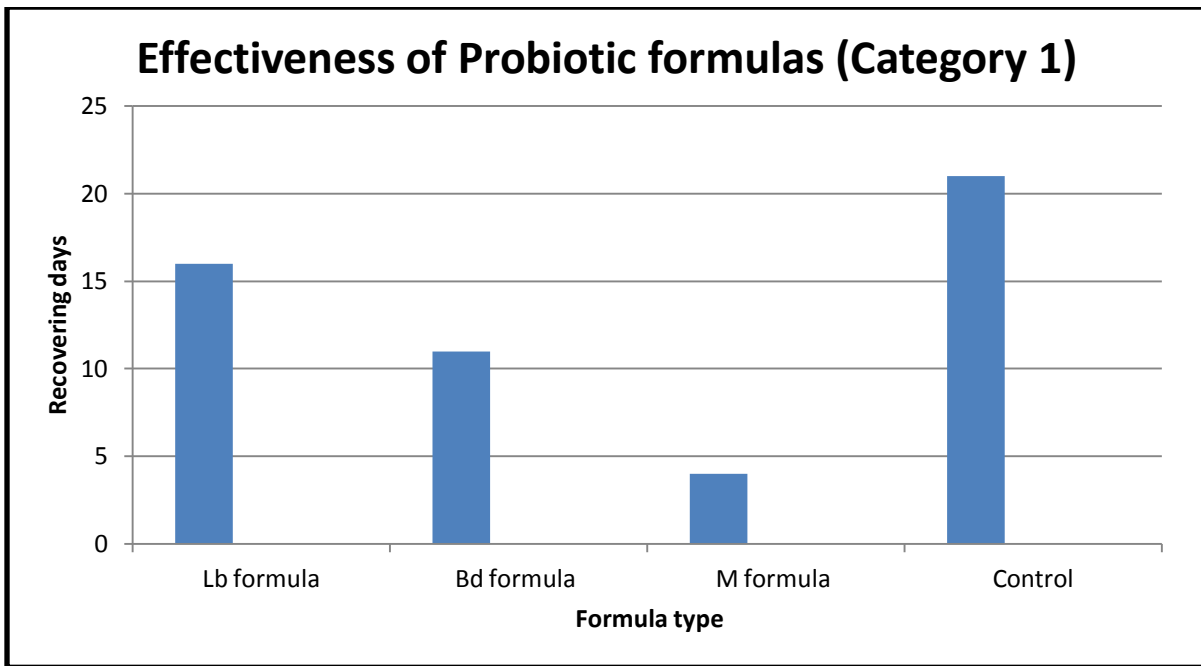


Figure 1: Effectiveness of Probiotic formulas against the recovery days (Category 1)

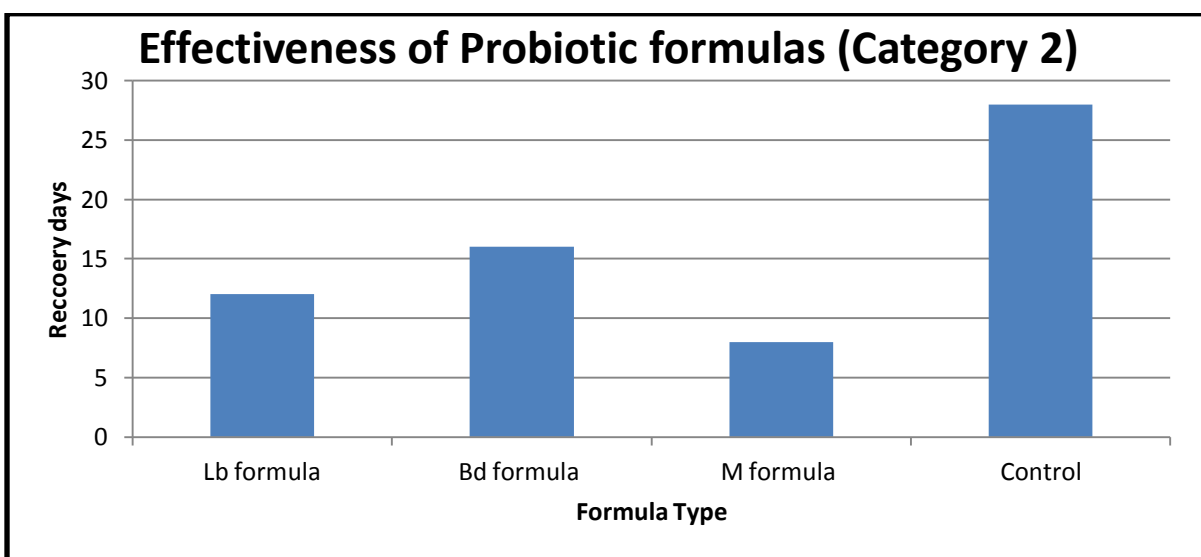


Figure 2: Effectiveness of Probiotic formulas against the recovery days (Category 2)

The second category of patients age between 6 month to 12 months showed a significantly late recovery response in the controlled (28 ± 1.3 days) while Mixed formula again proved to be the best formula for the infants of this age group also with only 08 ± 2.2 recovery days in major cases with significant value of 3.4 ± 1.4 mean number of stool per day, 0.9 ± 0.3 mean vomiting episodes per infant, and 0.5 ± 1.1 mean loss in weight per infant. The *lactobacillus* -whey based (Lb) formula took 12 ± 3.3 days for normalization of stool number and consistency while *bifidobacterium* -whey based (Bd) formula took 16 ± 1.8 days in normalization of patients.

4. DISCUSSION

The findings of first category, in the case of bifidobacterium, agrees with the previous study on the efficacy of bifidobacterium based acidified supplement by Chouraqui et al. which had provided more protective effects against acute enteritis [16]. At the time of enrolment, only the patients on solid food were selected with no breastfeeding at all to test

the prepared supplement in the proper way. There was no difference in the monitoring time and volume of formula intake by each patient. The potential mechanism by which these probiotic bacteria might exert a protective effect against the acute gastroenteritis are numerous [17, 18]. A large variety of probiotic bacteria survive through the gastrointestinal tract and proliferate in the intestine of healthy adult as well as in the children [19, 20]. The use of a probiotic-whey based formula with low casein and phosphate, but high lactose and protein contents and synergic effect of living lactobacillus and bifidobacterium, known as probiotic bacteria, have a significant protective impact on infantile acute gastroenteritis. The probiotic-whey based formula is economically beneficial, as it is inexpensive as well as reduces the severity of diarrhea. There is a possibility that the preventive effects of the formula studied are because of high nutritional composition of whey or may be the influence of coating of bacterial strains with prebiotics, under the process of microencapsulation, as various studies highlight synergistic effects of prebiotic and probiotics in the human intestine especially in infants [21-24]. Another reason behind the potential finding of the supplement may be the effects of bovine-colostrum immunoglobulin G. The mammary glands of mother are responsible for transfer of immunoglobulin (IgG) to young through a highly selective mechanism. These IgG are transported and absorbed across the wall of the small intestine of infant, ultimately reaching into the bloodstream to perform vitally important immunological protection and defence against infections in infants [25, 26]. For this reason, an additional dose of IgG is provided in the formulas that have been extracted from colostrum milk. As the Bovine colostrum is generally used as a raw material for immune milk preparations because of their immunological properties to treat or prevent infections of the gastrointestinal tract [27, 28].

The present clinical trial shows that the potential benefit of indigenously isolated multistrain probiotic supplement in the treatment of acute diarrhea. The future studies will aim to identify the mechanism of these potential beneficial effect of indigenous probiotics.

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