The Efficacy of Bacterial Multi-strain/Mono-strain and Yeast Probiotics Versus Placebo on The Duration of Hospital Stay Among Children Admitted For Acute Diarrhea*

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Background:

Acute gastroenteritis is one of the leading causes of childhood morbidity and mortality in developing countries worldwide. The recommended treatment by the World Health Organization (WHO) is the use of oral rehydration solution, intravenous fluid if indicated, and zinc supplementation. Several studies advocate the use of probiotics as adjunct treatment for acute gastroenteritis.

Objective:

To determine the efficacy of multistrain and monostrain bacterial probiotics, and yeast probiotics as compared to placebo in relation to the duration of hospital stay among children admitted for acute diarrhea

Methods:

This is a prospective cohort study of 129 pediatric inpatients from April- May 2012 at the Department of Pediatrics, Region 1 Medical Center. It is a randomized, double blind placebo controlled clinical trial of patients aged 6 to 60 months admitted for acute gastroenteritis. They were randomly assigned into 4 groups to receive supplements of 1. multistrain bacterial probiotics 2. monostrain bacterial probiotics 3. yeast probiotics 4. placebo for probiotics. All patients received IV hydration and zinc supplementation. Duration of hospital stay of the different groups was the primary outcome measure.

Results:

A total of 129 patients were eligible for the study. Groups given the Multi-strain and mono-strain bacterial probiotics revealed duration of hospital stays with an average of 2.18 days having no significant difference with placebo group (p-value of 0.054). Patients given yeast probiotics had a shorter duration of hospital stay, with an average of 2.09 days as compared to the placebo group with average hospital stay of 2.58 days, which was statistically significant (with p-value of 0.026). There were no adverse events observed during the course of treatment of the different treatment groups.

Conclusion:

The study showed that yeast probiotics significantly reduced the length of hospital stay of patients with diarrhea as compared to bacterial probiotics.

Key words: probiotics, acute diarrhea

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Introduction

Diarrheal diseases are a major cause of morbidity and mortality in infants in the developing world. Department of Health (DOH) annual report showed that among infants, diarrhea ranked second in morbidity and second in mortality.[1] In this institution, acute gastroenteritis is the second most common cause of admission (21.31% of the total admission of year 2011).[2]

World Health Organization (WHO) defines diarrhea as passage of unusually loose stools, usually at least three times in a 24 hour period. However, a change in stool consistency versus previous stool consistency is more indicative of diarrhea than stool number, particularly in the first months of life.[3] Diarrheal disease is widespread all over the world, not only threatens human health but also greatly affects society and economy. Along with acute respiratory infections, it is the leading cause of death for children less than five years of age.[4] Essential elements in the management of acute gastroenteritis as recommended by the World Health Organization (WHO) includes the guidelines on the use of reduced osmolality oral rehydration solution formulation, intravenous fluid if indicated, and zinc supplementation, which have been shown to reduce duration and severity of diarrheal episodes.

Several studies advocate the use of probiotics as adjunct treatment for acute gastroenteritis. To date, several types of probiotics were developed and individually studied on their effects on different illnesses, including acute gastroenteritis.

Probiotics are nonpathogenic live microorganisms that provide beneficial effects on the health of the host.[5] In recent years, the use of probiotics have entered mainstream medical practice, as a decrease in the severity and duration of infectious gastroenteritis has been noted with use of some strains.

The use of probiotic microorganisms for the prevention or therapy of gastrointestinal disorders is an obvious measure and perhaps the most usual application of probiotics because most health effects attributed to them are related directly or indirectly (i.e., mediated by the immune system) to the gastrointestinal tract. The mechanisms and the efficacy of a probiotic effect often depend on interactions with the specific microflora of the host or immunocompetent cells of the intestinal mucosa. The gut (or its associated lymphoid system, GALT) is the largest immunologically competent organ in the body, and maturation and optimal development of the immune system after birth depend on the development and composition of the indigenous microflora and vice versa. Protection by probiotic bacteria and yeasts with immunostimulatory properties or the alleviation of symptoms and shortening of acute infections are perhaps the best-documented probiotic effects, and these have been demonstrated many times in the past in clinical studies fulfilling scientific requirements.[6]

This study was designed to determine the effects of multistrain and monostrain bacterial

probiotics, and yeast probiotics on the duration of hospital stay of patients admitted for acute gastroenteritis.

General Objective:

To evaluate the effect of probiotics using multistrain and monostrain bacteria and yeast on the clinical course of diarrhea in patients aged 6 months to 60 months admitted at Region I Medical Center, Dagupan City, April-May 2012.

Specific Objectives:

1. To determine the duration of hospital stay of patients with acute diarrhea who received:

a. multistrain bacterial probiotics supplementation
b. monostrain bacterial probiotics supplementation
c. yeast probiotics supplementation
d. placebo probiotic

2. To determine the significant differences among those given multistrain and monostrain bacterial and yeast probiotics compared to placebo on the duration of hospital stay of patients
3. To observe adverse events related to the use of mutistrain/monostrain bacterial probiotics and yeast probiotics

Methodology

Study Population

Children aged 6-60 months of either sex who were sufficiently ill to be admitted at the Region 1 Medical Center at Dagupan City, Pangasinan with a clinical diagnosis of acute gastroenteritis from April to May 2012 were considered in the study. Diagnosis of acute gastroenteritis were based on the presence of episodes of loose bowel movement with the presence of signs of dehydration, which may include irritability, sunken fontanelle, sunken eyeballs, poor skin turgor and inability to feed. After informed consent was obtained from the parents of eligible patients, they were entered into the study. The following were the inclusion criteria:

1. Patients aged 6 months to 60 months old;
2. Admitted for Acute Gastroenteritis with moderate to severe signs of dehydration;
3. Diarrhea of less than two weeks duration; and
4. Gastroenteritis was not due to other disease processes.

The following were the exclusion criteria:

1. intractable vomiting;
2. any intake of antibiotics before or during the illness;
3. diagnosed case of Crohn's disease, short gut syndrome, and ulcerative colitis;
4. presence of severe respiratory infection, meningitis, congenital heart disease, or another gross congenital malformation and malnutrition; and
5. bloody diarrhea.

All patients admitted as cases of acute gastroenteritis were randomized to receive either probiotics or placebo using random numbers. The randomization codes remained sealed until the blinded analysis had been carried out. For comparisons, patients were randomly assigned into groups to receive supplements of 1. multistrain bacterial probiotics 2. monostrain bacterial probiotics 3. yeast probiotics 4. placebo for probiotics.

All patients in the study received zinc supplementation at a dose 20 mg once daily for patients 6 months or more. Multistrain and monostrain bacterial probiotics were given at a dose of 1 sachet once a day, given with or after food intake. Yeast probiotics was given 1 sachet twice a day. It can be added to food, water or milk according to the recommendations of the manufacturer. Multistrain probiotic used contains 1 billion CFU per sachet of probiotic cultures including Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, Lactobacilus acidophilus, Bifidobacterium infantis, Lactobacillus bulgaricus, and Bifidobacterium longum. Monostrain probiotic which contain 3 billion CFU of Lactobacillus casei.

Yeast Probiotic used contains Saccharomyces boulardii 250mg per sachet. Placebo for probiotics consisted of the flour base. Both the participants and those administering and evaluating the patients were unaware of the treatment allocation (double blinded). Compliance with study treatment was evaluated by direct observation by the researcher or a nurse cohort and indicated in their respective medication sheets.

Criteria for discharge of patients include:

1. good oral intake i.e. no vomiting, with restoration of appetite as compared to the premorbid state;
2. decreased frequency of purging; and
3. resolution of the signs and symptoms of dehydration.

Research Design

This is a prospective cohort research utilizing randomized, double blind placebo controlled clinical trial with intention to treat, evaluating the comparative efficacy of all the treatment arms including placebo on the duration of hospital stay of patients with acute diarrhea.

Statistical Treatment

Data were analyzed using descriptive statistics. To compare the length of hospital stay of the three different treatment groups to placebo, non-parametric test of individual Pearson's chi-square test was employed at $P < 0.05$ level of significance. (Actual p values were computed in relation to the placebo.))

RESULTS

One hundred twenty nine (129) patients were included in this study. There were thirty two patients in each of two groups, those who received multi-strain and mono-strain bacterial probiotics. There were 31 patients who received yeast probiotics, while 34 patients received the placebo an almost equal distribution among the 4 groups (Table 1)

Table 1. Distribution of the study population on the 4 treatment arms

Treatment Group	No. of cases	Percentage
1. Multi-strain bacterial Probiotc	32	24.8 %
2. Mono-Strain Bacterial Probiotic	32	24.8 %
3. Yeast Probiotic	31	24 %
4. Placebo	34	26.4 %
TOTAL	129	100 %

As to gender distribution, there were more females at 73 cases (56.6 %) compared to 56 males (43.5 %) admission of Acute Gastroenteritis cases at Region I Medical Center.

Age distribution of patients included in this study is reflected in Figure 1. The result shows that majority of patients were >1-3 years old with 69 (53.5%) followed by >3-5 years old which comprises 34 (26.4%) and 26 (20.2%), respectively.

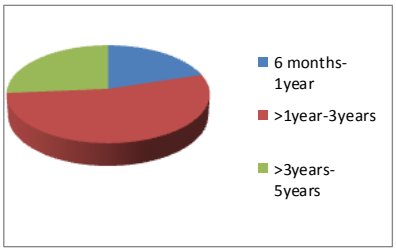

Figure 1. Distribution of Population According to Age.

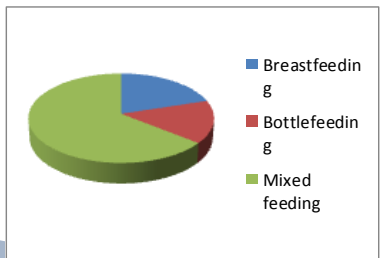

Figure 2. Distribution of population according to feeding.

Data in Figure 2 reveal that majority of cases (n = 83) admitted with acute gastroenteritis were on mixed feeding (breastmilk + formula milk) roughly at 64.3%, followed by breastfed children with 26 (20.2%) and bottlefed children with 20 (15.5%). This result showed that feeding habit (significantly) affects the occurrence of diarrhea in children.

The length of hospital stay of patients who received different treatment conditions (probiotic supplementation and placebo) can be seen in Tables 2A-C. The comparative difference in the duration of hospital stay between the probiotics groups and placebo imply the efficacy of probiotics supplementation. Tables 2A and 2B reveal the same results. They showed that the two bacterial probiotics groups and the placebo have majority of their patients staying in the hospital for 1 to 2 days with 77.2% of the cases. Fifteen (22.8%) of them had hospital stay for 3 or more days. The mean hospital stay of patients was 2.18 days in the multistrain and monostrain bacterial probiotics group and 2.58 days for the placebo.. There is no observed significant statistical difference between the multistrain bacterial probiotics group and the placebo group and the monostrain probiotic groups with the placebo in relation to the length of hospital stay of patients.

Table 2A. Length of Hospital Stay Between Cases with Multi-strain Probiotics Supplementation and on those on Placebo

Intervention	HOSPITAL STAY		Mean Hospital Stay	Pearson Chi-square
	1-2 days	3 days and more		3.700
Multistrain Bacterial Probiotics	28(87.5%)	4 (12.5%)	2.18 days	
Placebo	23(67.6%)	11(32.4 %)	2.58 days	
TOTAL	51 (77.2 %	15 (22.8 %)		
	Chi-Square p = 0.054			

Table 2 B. Length of Hospital Stay Between Cases with Monostrain Probiotics

Intervention	HOSPITAL STAY		Mean Hospital Stay	Pearson Chi-square
	1-2 days	3 days and more		
Yeast Probiotics	28(90.3%	4(12.5%)	2.18 days	3.700
Placebo	23(67.6%)	11(32.4%)	2.58 days	
TOTAL	51 (77.2 %)	15 (22.8 %)		
	Chi-Square p =0.054			

Supplementation and those on Placebo

Table 2C. Length of Hospital Stay Between Cases with Yeast Probiotics Supplementation and those on Placebo

Intervention	HOSPITAL STAY		Mean Hospital Stay	Pearson Chi-square
	1-2 days	3 days and more		
Yeast Probiotics	28(90.3%	3(9.7%)	2.09 days	4.934
Placebo	23(67.6%)	11(32.4%)	2.58 days	
TOTAL	51 (78.5 %)	14 (21.5 %)		
	Chi-Square p =0.026			

Table 2C revealed the efficacy of yeast probiotics supplementation in relation to length of hospital stay of patients. Majority of patients in both groups stayed in the hospital for 1 to 2 days with 51 (78.5%). Fourteen (21.5%) of them had hospital stay for 3 or more days. The mean average hospital stay of patients was 2.09 days in the yeast probiotics group and 2.58 days for the placebo group. Statistically significant difference is observed in the duration of hospital stay between yeast probiotics group and placebo group ($P < .05$ level). There were no adverse events observed during the course of treatment associated with probiotics.

Discussion

Treatment of diarrhea by administering living or dried bacteria to restore a disturbed intestinal microflora has a long tradition. Yogurt had originally been developed in Spain and introduced into the market as an inexpensive, easy to prepare, and easily available remedy against diarrhea in children. There were earlier reports on the successful use of *Enterococcus faecium/faecalis*, strains of *Escherichia coli,* or freshly isolated members of the patients' own intestinal microflora as a remedy as well. However, most were case reports and open studies rather than well-documented, randomized, double-blind, controlled clinical studies. In the last two decades, investigations in probiotic microorganisms by in vitro studies, animal experiments, and appropriate well-designed clinical studies have put this "bacteriotherapy" on a more rational basis.[6]

The mechanisms and the efficacy of a probiotic effect often depend on interactions with the specific microflora of the host or immunocompetent cells of the intestinal mucosa. The gut (or its associated lymphoid system, GALT) is the largest immunologically competent organ in the body, and maturation and optimal development of the immune system after birth depend on the development and composition of the indigenous microflora and vice versa.[6] Many strains of probiotic microorganisms have been shown to inhibit growth and metabolic activity as well as the adhesion to intestinal cells of enteropathogenic bacteria (*Salmonella, Shigella,* enterotoxigenic *E. coli, or Vibrio cholerae*) to modulate (temporarily) the intestinal microflora and to have immunostimulatory or -regulatory properties.[7]

Suggested mechanisms for the effects on the intestinal microflora are lowering the intestinal pH, the production of bactericidal substances such as organic acids, H_2O_2 and bacteriocines, agglutination of pathogenic microorganisms, adherence to the cellular surface of the mucosa, and competition for fermentable substrates or receptors, strengthening the barrier effect of the intestinal mucosa, release of gut-protective metabolites (arginine, glutamine, short-chain fatty acids, conjugated linoleic acids), binding and metabolism of toxic metabolites, immunologic mechanisms, or regulation of the intestinal motility and mucus production.[7] Ample laboratory evidence show that ingestion of probiotic microbes, especially lactic acid bacteria and bifidobacteria, alleviates of prevents disorders, such as rotavirus diarrhea, lactose intolerance and atopy.[8]

Functionality of a multistrain probiotic could be more effective and more consistent than that of a monostrain probiotic. Colonization of an ecosystem providing a niche for more than 400 species in combination with individually determined host-factors is anticipated to be more successful with multistrain (multispecies) probiotics than with monostrain preparations.[9] Famularo et al. (1999) have envisaged that probiotic preparations containing bacteria of only one strain have little chances of

successfully colonizing the GI-tract.[10] Furthermore, probiotics are expected to control multi-factorial diseases demanding a variety of probiotic properties, whereas such properties are strain-specific.[11] Dunne et al. (1999) have suggested that probiotics should consist of a combination of strains[12]. In 1992 a group of probiotic experts concluded that the optimal prophylactic culture is a mixed one: 'Different strains can be targeted toward different ailments and can be blended into one preparation' (Sanders, 1993).[13] Mixed cultures may contain bacteria that complement each other's health effect and thus have synergistic probiotic properties.

A study by Rosenfeldt et al. indicates the efficacy of a mixture of L. rhamnosus and L. reuteri used at high doses (10^{11} CFU 2x a day) in treatment of acute diarrhea in children. By comparison with a control, the probiotic mixture reduced hospital stay and viral shedding. Although the study did not compare the mixture to individual strains, the authors noted that the combination seems to be no more effective than a single-strain probiotic, Lactobacillus GG.[14]

This study supports the use of probiotics as adjunct treatment in the management of acute diarrhea, especially in shortening the duration of hospital stay of patients. Although statistically not significant at $P < 0.05$ level, the use of bacterial mono or multi-strain probiotics may still be beneficial as seen from the trend in the data presented. The ability to differentiate significant effects between the use of bacterial probiotics as contrasted to placebo could have been maximized if the test had an increased power to discriminate (1-β) by enrolling more of the study population through lengthening the study period or increasing the size of the test (α level).

Some studies used the yeast probiotics (Saccharomyces boulardii) as monostrain probiotics in comparing with the multistrain bacterial probiotics. Grandy et al. observed a reduction in rotavirus diarrhea in groups taking both single- and multi-strain products. The duration of fever was only reduced in the single strain group despite the multistrain product also containing that single strain (Sacc. Boulardii). This suggests that the strain-specific effect was diminished either by dose reduction of that particular strain, or by the presence of competitive probiotics. in the multi-strain product (Sacc. boulardii competes for nutrients or binding sites).[15]

S. boulardii is the only yeast commercialized for human use, and consequently the single preparation formally recognized as probiotic.[16] In this study, yeast probiotics significantly shorten the hospital stay of patients admitted for acute diarrhea. This supports the study done by Villaruel et al., concluding that S. boulardii, as an adjuvant to ORS, decreased the duration of diarrhoea, accelerated recovery and reduced the risk of prolonged diarrhea. [17]

Saccharomyces boulardii possesses many properties that make it a potential probiotic agent,

i.e. it survives transit through the GI tract, its temperature optimum is 37 °C, both *in vitro* and *in vivo,* it inhibits the growth of a number of microbial pathogens.

Conclusion

The study showed that multistrain and monostrain probiotics shorten the duration of hospital stay with an average of 2.18 days, while yeast probiotics supplementation had shortened duration of hospital stay at an average of 2.09 days, as compared to the placebo which was statistically significant. Statistical analysis showed no significant difference between the multistrain and monostrain probiotics and placebo group with regards to length of hospital stay though increasing the power of the test may offer significant results.

Recommendation

The following are the recommendations:

1. Use a larger population group to increase the power of the test

2. Studies should be made on the different dosing regimens particularly on bacterial probiotics

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