Chapter-1: Introduction

Gastrointestinal Tracts (GI) contain different normal microbiota which plays a significant role in the maturation and function of the immune system in the gut. Microbial colonization begins at birth that is mostly affected by the children. The microbiota is continuously interacting with the environment, including other bacteria, the gut epithelium, and the mucosal and endocrine systems. The different microbiota in the Gastrointestinal Tracts (GI) between healthy and diseased individuals was identified during the late 19^{th} century. These type of beneficial microorganisms which found in the GI tract were named probiotics. Probiotic therapy is based on the concept of maintaining a healthy, balanced microbiota.

Probiotic means "for life", comes from the Greek. In 1907, the Nobel laureate Metchnikoff is credited with the health benefits of probiotics suggesting that the consumption of living lactic acid bacteria in fermented foods may promote health and longevity by favorably modulating the gastrointestinal microflora (Paraschiv *et al*.,2011). Consumption of Probiotic has health benefit effects in a host of disorders including diarrhoea, irritable bowel syndrome, inflammatory bowel disease, food allergies, lactose intolerance, urogenital infections, and atopic eczema (Metchnikoff ,1907). The concept of probiotics which are characterized as microorganisms that promote growth of other microorganisms (Lilly *et al*., 1965). Nowadays, the universal meaning of the term "probiotic" was established by the World Health Organization and the Food and Agriculture Organization of the United States. These two organizations defined as probiotics are live microorganisms which when administered in adequate amounts, have a beneficial effect on health of the host organism.

A number of studies have found probiotic consumption to be useful in the treatment of many types of diarrhea, including acute diarrhea, travelers' diarrhea, and diarrheal diseases in young children.*Lactobacillus* and *Bifidobacterium* families probiotic bacteria are most commonly used in market. These probiotics may be sold as single components, marketed in combination products with other probiotic ingredients, or added to enriched fermented foods such as yogurts. The "dosage" of bacteria probiotics is mainly measured in CFUs, or colony-forming units. Probiotics are essential 5 to 10 billion CFUs for a child and 10 to 20 billion CFUs for an adult dosages daily. Probiotic yogurt products and fermented milks contain varying amounts of bacteria, depending on the brand and ounces consumed. The most

commonly studied probiotic species in these studies have been found to be *Lactobacillus casei* sp. (Strain GG), *Lactobacillus* casei, *Bifidobacterium bifidum*, *Streptococcus thermophilus* (Isolauri *et al*., 1991).

Probiotics are primarily used for gastrointestinal (GI) conditions such as diarrhea caused by infections, inflammation, or extended use of antibiotics. They increase the amount of healthy microorganisms in the gut, thereby controlling the amount of harmful organisms that cause the diarrhea.

About three times per day most healthy people open their bowels and their normal stools are usually solid. Dirrhoea is defined as more than three times loose and watery stools in a day when stools are passed. The common cause of an attack of acute diarrhoea is an intestinal infection and also food poisoning is a common form of acute diarrhoea, most commonly caused by the bacteria Salmonella and Campylobacter, these infections are passed on through contaminated food, such as poultry and eggs, or water; and sometimes by a person such as food handler who has the infection, but has no symptoms of that infection that are easily affected to infants and children. In mechanisms of acute diarrhoea,small intestine absorb nutrients,fluids and salts of food and large intestine absorb remaining extra fluid,that's way healthy people's stools are solid. But Diarrhoea occurs when these processes are impaired when the lining of the gut is damaged by bacteria or viruses and when there is excessive secretion of fluid into the bowel. The aim of treatment is to reduce the period that a person is infectious. Treatment options available are oral rehydration solution, antibiotics, and gut motility suppressing agents such as loperamide, codeine, and probiotics. This review considers the use of probiotics and oral rehydration solution (Allen *et al*., 2003).

Acute diarrhoea management consists of the replacement of lost fluid with oral rehydration solution. This solution, however, reduces neither the severity nor the duration of diarrhoea. The search for such agents started over 20 years ago (Alam, 2003). Several trials with probiotic preparations have been conducted in different settings and with different end points. Few of these studies, however, meet the criteria of properly controlled trials (Allen, 2004).

Probiotic supplements are generally considered safe for most people and rarely cause side effects. Little is known about the long-term effects of regular use of these supplements, however, and they may be less safe for use in people with poor immune

systems. The most common side effects reported are temporary bloating, gas, and mild stomach upset. Side effects are rare, primarily because probiotic microorganisms already are found naturally in the GI tracts of healthy people. Probiotic supplements basically restore the body's balance between good and harmful bacteria and yeasts (Allen *et al*., 2003).

In recent studies of children with diarrhoea, probiotics were the most commonly prescribed treatment. With the increasing availability and widespread use of probiotics, it is important to identify products which are most effective. We evaluated the efficacy of three probiotic products for the treatment of acute diarrhoea in children.

Study Objective

General Objective

To assess the effectiveness of probiotics among under five years of children in the treatment of acute diarrhoea

Specific Objectives

1. To assess the effectiveness of Oral rehydration solution (ORS) among studied children for reducing/shorter the duration (hour) of acute diarrhoea
2. To assess the effectiveness of Probio probiotic among studied children
3. To assess the effectiveness of Enterogermina probiotic among studied children
4. To assess the effectiveness of TS6 probiotic among studied children
5. To assess the comparison the effectiveness of three types of probiotics with ORS and ORS only among the studied children who suffer from acute diarrhoea
6. To assess the comparison of the effectiveness of these three types of probiotics among the studied children who suffer from acute diarrhoea

Chapter-2: Review of Literature

2. 1 Probiotics

Probiotics which defined as "Live micro-organisms which, when administered in adequate amounts, confer a health benefit to the host" (FAO/WHO, 2001). Probiotics are live microorganisms administered in adequate amounts which has beneficial physiological effects on the host (Reid *et al*., 2003). The term probiotic is currently used to name ingested microorganisms associated with benefits for humans. Probiotics should have some basic characteristics such as they must be able to survive in the gastrointestinal tract where they must be resistant to gastric juices and bile and they should pretend benefits to the host through growth and activity in the human body. In gastrointedtinal tract, the intestinal microbiota plays a significant role in the maturation and function of the immune system in the gut. Microbial colonization begins at birth which is affected to the infant's diet, and by the age of two years gradually alters to assimilate an adult but children easily affected by these others bacteria. The mature intestinal microbiota provides a physical and immunological barrier against microbial and other harmful exposures from the environment (Salminen *et al.*, 2006). Other bacteria living in environment,is being continuously interacted by these microbiota which resists the colonisation of pathogens,participates in the elimination of foreign antigens, and regulates the immune responses. The healthy host-microbe interaction is disturbed, leading to increased intestinal permeability, inflammatory immune responses, and infection in disease condition. Probiotics are used to maintain a healthy, balanced microbiota.

Probiotics positively affect the gut microflora and decrease the microbial toxic activity. Probiotics must be non-pathogenic and non-toxic. Probiotic is a nonpathogenic organism, such as yeast or bacteria, in foods that can exert a positive influence on the host's health (Brown and Vallere ,2004) . These live microorganisms in food or supplements improve microbial balance of the intestinal tract. Probiotics have health benefit effects which should protect against pathogenic micro-organisms by means of multiple mechanisms (FAO/WHO, 2001). They associated with a range of health benefits including protection against diarrheal diseases, stimulation of the immune system,nosocomial and respiratory tract infections, lowering of cholesterol,

attenuation of overt immuno inflammatory disorders and anticancer effects (Gill and Prasad,2008; Britton and Versalovic ,2008). Probiotics are also used to assess biochemical and clinical effects of an oral probiotic dietary supplement in chronic kidney disease (CKD) patients (stages 3 and 4) ,a 6-month pilot scale trial in Canada (Ranganathan *et al.*,2009) .

Health effects are strain-specific that is important to identify the genus and species of the probiotic strains. The probiotic species most widely used in food products are mainly *Lactobacillus* ssp.(Gram-positive, non-spore-forming and non-flagelated rods or cocobacilli,aerotolerant, fastidious,acid-tolerant, and strictly fermentative) and *Bifidobacterium* ssp.(also Gram-positive). The consumption of 1x10^6 – 1x10^10 viable cells of probiotic per day is usually required for beneficial effects to be seen (Bernardeau *et al.*, 2006), although higher doses of 10^9 –10^10 are needed for faecal recovery (Saxelin *et al.*, 1993). *Lactobacillus* bacterial strains are typically used for non-pathogenic infections. *Lactobacillus* strains are normally found in the small intestine. Of more than 100 *Lactobacillus* bacterial species are commonly used such as: *L. acidophilus*, *L. casei*, *L. rhamnosus*, and *L. fermentum*. *Bifidobactrium* bacterial strains are also used for non-pathogenic infections and some isolated cases of human infection. They are anaerobic and normally found in the large intestine. There are some common *Bifidobactrium* bacterial strains such as: *B. bifidum*, *B. adolescentis*, *B. animalis*, and *B. therophilum*. Spores of a number of *Bacillus* bacterial strains are used as probiotics Some examples of the *Bacillus* species are: *Bacillus cereus, B. subtilis,* and *B. coagulans*. These strains of bacteria that are naturally occurring in our intestinal tract. Different probiotic strains have been used to treat and/or prevent certain diseases (Hamilton, 2004; Isolauri, 2001).

The concept of using probiotic has advanced rapidly over the last several years. There are most commonly used species of probiotics given in Table 1. A bacterium or product containing bacteria is not considered a probiotic unless the bacteria are viable at the time of use in sufficient quantity to confer a physiologic health benefit. The intentional modification of the intestinal microbiota with probiotics leads to select clinical beneficial effects, such as reduction of diarrhea (Peate, 2010).

Table 1: The most commonly used species of Probiotics (Adam *et al*., 2012).

Lactobacillus Sp.	*Bifidobacterium Sp.*	*Enterococcus Sp.*	*Streptococcus Sp.*	*Bacillus Sp.*	*Pediococcus Sp.*
Lactobacillus acidophilus, Lactobacillus delbrueckii, Lactobacillus casei, Lactobacillus cellobiosus, Lactobacillus fermentum, Lactobacillus curvatus, Lactobacillus lactis, Lactobacillus plantarum, Lactobacillus reuteri	*Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium thermophilum, Bifidobacterium animalis, Bifidobacterium longum*	*Enterococcus faecium, Enterococcus faecalis*	*Streptococcus salivarius, Streptococcus diacetylactis, Streptococcus cremoris, Streptococcus intermedius*	*Bacillus cereus vartoyoi, Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans, Bacillus polyferminticus, Bacillus laterosporus, Bacillus pumilus, Bacillus polymyxa, Bacillus cla*	*Pediococcus cerevisiae, Pediococcus acidilactici*

2.2 Diarrhoea

Diarrhoea is defined by the World Health Organization (WHO) as 3 or more loose or watery stools in a 24-hour period.Infectious diarrhoea is an episode of diarrhoea that is caused by an infectious agent. Many infectious agents cause diarrhoea.Rotavirus is the most common cause of severe diarrhoea and diarrhoea mortality in children worldwide (Cunliffe,1998). Important bacterial pathogens are: enterotoxigenic *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, and *Vibrio cholera*. The main parasitic causes of diarrhoea are *Cryptosporidium* and *Giardia*. An aetiological study of young children attending hospitals in China, India, Mexico, Myanmar, and Pakistan showed that rotavirus, enterotoxigenic *E. coli* and *Shigella* spp. were the most commonly isolated pathogens (Huilan, 1991).

Acute diarrhoea is one kind of diarrhoea which have non-specific response of the intestine to several different factors and conditions. There are some conditions such as drugs, functional bowel disorders or inflamatory bowel diseases may cause of diarrhoea but the most common cause of acute diarrhoea is infection. Acute diarrhoeal infectious remains a major cause of morbidity and mortality of the worldwide. Infectious diarrhoea is one of the major problem of morbidity in industrialized countries and has a major financial impact on health care systems but increased mortality and the burden is much heavier in developing countries. So, the prevention of diarrhoeal illnesses would have considerable public-health implications (Cheng *et al.*, 2005).

In prospective cohort studies, during a 6-12-month follow-up of children aged 6 months to 7 years attending day-care centres, 46-51% of the subjects developed acute gastroenteritis and under two years of age among children,these incidence is hightest and age-dependent (Rosenfeldt *et al*., 2005). The incidence of diarrhoea is occurs to be higher in boys than in girls and day-care attendance is a major risk factor for diarrhoea in children (Staat *et al*., 1991). Diarrhoeal episodes in day-care centres is 49% in 1-year-olds and 37% in two-year-olds (Louhiala *et al*., 1997). The peak value of diarrhoea occurs during the winter, between January and April (Rosenfeldt *et al*., 2005).

Most cases of diarrhoea are the results of an infection and this can be occurred by some specific organisms and can be identified in patients. Rotavirus is the most frequent cause of acute diarrhoea, resulting in annual winter epidemics of high attack rates,and affecting young children (Waters *et al.*, 2000; Rosenfeldt *et al*., 2005). Rotavirus are the causative organism which are found in children admitted to hospital with symptoms of acute diarrhoea (36%), than in the diarrhoeal episodes of children attending child-care centres (15%) (Waters *et al*.,2000). Rotavirus is transmitted by the faecal-oral route with small infectious doses.The virus enters the epithelial cells where it elaborates toxin, causing diarrhoea during the incubation period of 18-36 hours. It destroys the epithelium, leading to extensive damage and the shedding of huge masses of virus in the stools (Glass *et al*., 2006). Other viruses are found as causative agents of diarrhoea among toddlers and children (19% of outbreaks), astrovirus (7%), adenovirus (4%), torovirus (3%) and noroviruses (2%), (Waters *et al*., 2000; Rosenfeldt *et al*., 2005). Bacterial pathogens are detected in only 6%, and parasites in 2% of the episodes in day-care centres and these measures for 24-40% of the diarrhoeal episodes (Waters *et al*., 2000; Rosenfeldt *et al*., 2005). There are some bacteria such as *Campylobacter jejuni* and *Clostridium difficile* have been found in children (Rosenfeldt *et al*., 2005).

The etiology of diarrhoea may sometimes be non-infectious, but epidemic diarrhoeal illnesses are common that caused by a variety of bacterial and viral agents (Garibaldi, 1999). Norovirus is one kinds of virus which is highly infectious because of the small infectious dose. These virus, transmission occurs by the faecal-oral route, via aerosol sprays and through the ingestion of contaminated food and water. Norovirus which is very stable and resistant to many disinfectants, and may shed for prolonged periods of up to one month (Estes *et al.*, 2006). Some bacteria such as *Salmonella* and toxigenic *Escherichia coli* are the major bacterial agents that cause diarrhoea and *Clostridium difficile* which may be carried asymptomatically or may cause diarrhoea. Infectious nosocomial diarrhoea is mainly caused by *C. difficile* and the prevalence of these colonisation in long-term-care facilities in the absence of an outbreak has ranged between 4-20%, while up to 30% of residents have been found to harbour *C. difficile* during outbreaks (Simor *et al.*, 2002).

Diarrhoea which is defined as an alteration in the normal bowel movement characterised by an increase in the water content, volume or frequency of stools.

Acute diarrhoea is defined as three or more soft or liquid stools per day lasting 14 days or less (Guerrant *et al.*, 2001). These diarrhoea persist more than 14 days, and chronic when it lasts over 30 days. There are some symptom Infectious diarrhoea such as nausea, vomiting and abdominal pain (Guerrant *et al*., 2001).Other symptoms, such as headache and diarrhoea is generally a self-limiting illness lasting 5-7 days. The duration of diarrhoea among day-care children has been 33 hours which is median value, ranging between 5-169 hours (Rosenfeldt *et al*., 2005).

Acute diarrhoea is frequent among travelers in whom enterotoxigenic *E. coli* is particularly common (Black, 1986). In practice, most episodes of acute diarrhoea that are assumed to be caused by an infectious agent are treated without the causative agent being identified. Major causes of acute infectious diarrhoea will differ according to local factors such as availability of clean water and sanitation. In contrast with acute infectious diarrhoea, infection is likely to be only one of several factors that contribute to the pathogenesis of persistent diarrhoea (Walker, 1993).

2.3 Diarrhoeal Treatment

In developing countries, diarrhea is a major cause of mortality in children. The management of diarrhoea is related to these relief such as the reduction of the duration of the illness and the correction of fluid loss and electrolyte imbalance (Cheng *et al*., 2005; Gadewar and Fasano, 2005). Oral rehydration therapy is cheap, simple, and effective and remains the mainstay of management of infant diarrhea.The development of an oral rehydration solution that has provided a simple approach for maintaining hydration in children suffering from acute watery diarrhoea. In recent meta-analysis studies, 17 case-control and intervention trials, proper hand washing resulted in a 47% reduction in diarrhoea risk (Curtis and Cairncross., 2003). Vaccine development against several pathogens including *Salmonella typhi*, *Cholera*, *Campylobacter*, *Shigella*, and rotavirus has also been under extensive study during the past decades (Gadewar and Fasano, 2005). Two live rotavirus vaccines (containing an attenuated human monostrain or a combination of five bovine-human reassortant strains) are currently licensed in 35 countries in Europe and America (Glass *et al*., 2006). Dirrhoea reduction drugs are widely used by adults to treat the condition but are contra-indicated in young children.

2.4 Probiotics in the prevention of Acute Diarrhoea:

In most of the studies, diarrhoeal disease (acute diarrhoea) have prevented by health benefit probiotics. On the basis of probiotics criteria, they are used to treat and prevent diarrhoeal disease that they modify the colonic microbiota and act against enteric pathogens. Some of the probiotic preparations of Lactobacillus and Streptococcus species showed inconsistent results in RCTs among children, used singly or in combination, for the treatment of diarrhoea (Basu *et al.*2007, 2009; Dubey *et al*. 2008). *Lactobacillus sporogenes (Bacillus coagulans*) is one of the most commonly available and marketed probiotic preparations in many European and Asian countries, including India (Sanders *et al*. 2003). Aged 6–24 months who had diarrhoea with some dehydration, double-blind, randomised, placebo-controlled, hospital-based clinical trial with children by treating L. sporogenes (B. coagulans) which products are managed of acute dehydrating diarrhoea (Dutta *et al*.,2011).

In several studies among children by using different *Lactobacillus* species (such as *L. rhamnosus* and *reuteri*) have shown to reduced duration of acute diarrhoea mainly caused by rotavirus, reduced excretion of rotavirus and reduced length of hospitalisation (Rosenfeldt *et al.*, 2002a;Szymanski *et al.*, 2006). In clinical trial conducted with 287 hospitalised children suffering from acute diarrhoea, *Lactobacillus* GG administered with an oral rehydration solution reduced the duration of diarrhoea from 71 to 58 hours, and in rotavirus-positive patients even more markedly, from 77 to 56 hours (Guandalini *et al.*, 2000). They reduce frequency of diarrhoea, the number of watery stools and the risk of a prolonged course of disease. The effect was evident among rotavirus-positive children and among those with diarrhoea of unknown etiology, but not among those with invasive diarrhoea caused by *Salmonella, Shigella, Campylobacter, Yersinia* or *Entamoeba* (Guandalini *et al*., 2000). It thus seems that the effect of probiotics in treating acute diarrhoea is well established. The beneficial effects of probiotics in the treatment of acute diarrhoea have also been shown in systematic reviews and meta-analyses carried out among hospitalised children (Szajewska and Mrukowicz, 2001; Huang *et al*., 2002) (Table 2). The risk of prolonged diarrhoea has been reduced by 57% by the probiotic therapy (Szajewska and Mrukowicz, 2001). In another syndrome,Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder ,is characterized by

abdominal pain or discomfort, diarrhea, constipation, abdominal bloating and flatulence, which are associated with changes in the frequency and form of stool and may markedly lower the quality of life (King CK *et al.*,2003;Tuohy KM *et al.*,2003). Bacillus coagulans MTCC 5856 has been marketed as a dietary ingredient at a dose of 2 × 10^9 cfu/day along with standard care of treatment was found to be safe and effective in diarrhea predominant IBS patients for 90 days of supplementation and management of diarrhea predominant IBS patients (Majeed M et al.,2016).

Table 2 .Some reviews on the effects of probiotics for treating or preventing acute diarrhoeal illness

Design	Studies included	Inclusion criteria	Subjects	Probiotics used	Main findings: probiotic vs. placebo	Reference
Syste-matic review	RDBPC studies:	Acute diarrhoea defined as > 3 loose or watery stools /d	Children or infants n=731 / 773	*L. rhamnosus GG* *L. reuteri* *L. acidophilus* *S. thermophilus* *Saccharomyces boulardii*	**RR = 0.43** for diarrhoea lasting > 3 days. Only LGG showed a consistent effect. **Duration** of diarrhoea ↓ **-18.2 h** for all **-24.8 h** for LGG and *L. reuteri.*	(Szajewska and Mrukowicz, 2001)
Meta-analysis	RPC treatment studies	Acute diarrhoea, duration of diarrhoea reported	Healthy children < 5 years n=1917	Coadministration of *L. rhamnosus GG* *L. reuteri* *L. acidophilus* *L. bulgaricus* *S. thermophilus* *Sacch.boulardii* *Enterococcus* *B. subtilis* with rehydration therapy	**Duration** of diarrhoea ↓ **- 0.8 day** **-1.2 days** with LGG **- 0.6 day** with other lactobacilli	(Huang *et al*.,2002)
Meta-analysis	RPC prevention trials	Risk of diarrhoea available: travellers' / AAD / acute infectious / nosocomial diarrhoea	Children and adults n=4886	*L. rhamnosus GG* *L. reuteri* *L. casei* *L. acidophilus* *L. bulgaricus* *S. thermophilus* *Sacch. boulardii* *Enterococcus* *B. longum* *B. bifidum* *B. lactis* and combinations	**RR** = **0.65** **RR** = **0.48** for AAD **RR = 0.92** for travellers' diarrhoea **RR = 0.43** for children **RR = 0.74** for adults **RR = 0.83 (n.s.)** for single strains **RR = 0.48** for combinations	(Sazawal *et al*., 2006)

RDBPC = Randomised, double-blind, placebo-controlled,
RPC = Randomised, placebo-controlled,
AAD = antibiotic-associated diarrhoea,
RR=relative risk

The preventive effect of probiotics occurring of community-acquired diarrhoea among children have been examined in some studies that apart from hospital-acquired diarrhoea (Oberhelman *et al.*, 1999; Pedone *et al.*, 1999; 2000; Chouraqui *et al.*, 2004; Thibault *et al.*,2004; Weizman *et al*., 2005) (Table 3). In these studies, they examined the effect of an infant formula containing bifidobacteria or lactobacilli on the incidence of diarrhoea in infants. These *Bifidobacterium or Lactobacillus has reduced* infant's diarrhoeal disease and duration of diarrhoea. The incidence of diarrhoea in infants and small children have been reduced but not in men by *Lactobacillus* GG (Oberhelman *et al*., 1999) and *L.casei* (Pedone *et al*., 1999; 2000) that used as probiotic(Pereg *et al.*, 2005). Specific probiotics that effect on health in reducing the duration of acute diarrhoea. And these specific strain probiotics also prevented duration of diarrhoea and decreased frequency of diarrhoea among children.

Table 3. Probiotics in preventing diarrhoea in healthy children.

Study design and settings	Subjects	Probiotics used and length of the intervention	Main findings: probiotic vs. placebo	Reference
RDBPC Peri-urban Peruvian town	204 undernourished infants and children age: 6-24 mo	*L. rhamnosus* GG or placebo in gelatine capsules for 15 mo	**Diarrhoea episodes** ↓ 5.2 vs. 6.0 **Incidence of diarrhoea** ↓ in nonbreastfed 4.7 vs. 5.9 **Duration of diarrhoea** ◄►	(Oberhelman *et al*., 1999)
RPC Day-care centres	287 infants and children age: mean 19 mo	*L. casei* DN-114001 or placebo in fermented milk for 6 mo	**Incidence of diarrhoea** ◄► **Duration of diarrhoea** ↓ 4.3 vs. 8.0 days	(Pedone *et al*., 1999)
RDBPC Multicentre	928 infants and children age: 6-24 mo	*L. casei* DN-114001 or placebo in fermented milk for 4 mo	**Incidence of diarrhoea** ↓ 16% vs. 22%	(Pedone *et al*., 2000)
RDBPC Multicentre residential care settings	90 infants age < 8 mo	*B. lactis* Bb-12 or placebo in infant formula for 4.5 mo	**Incidence of diarrhoea** ◄► 28% vs. 39% (ns.) **Duration of diarrhoea** ↓ 1.2 vs. 2.3 days	(Chouraqui *et al*., 2004)
RDBPC Pediatric centres	971 infants age: 4-6 mo	*B. breve* + *S. thermophilus* or placebo in infant formula for 5 mo	**Incidence or duration of diarrhoea, hospital admissions** ◄► **Medical consultations** 46% vs. 57%↓ **Need for dehydration** 2.5% vs. 6.1%↓ **ORS presriptions** 42% vs. 52%↓	(Thibault *et al*., 2004)
RDBPC Day-care centres	201 infants age: 4-10 mo	*B. lactis* Bb-12 *or* *L. reuteri* or placebo in infant formula for 3 mo	**Diarrhoeal episodes :** ↓ 0.02 for *B. lactis* group 0.13 for *L. reuteri* group 0.31 for controls **Duration of episodes** : ↓ 0.15 vs. 0.37 vs. 0.59	(Weizman *et al*., 2005)

RDBPC = Randomised, double-blind, placebo-controlled,
RPC = Randomised, placebo-controlled,

Chapter-3: Methods

An experimental study was designed to assess the effectiveness of probiotics in children with acute diarrhoea received written instructions to purchase a specific brand of probiotic. Data collection was conducted between 1st April to 31st May 2016.During this period, all the admitted childrens of hospital, aged 6 to 60 months were enrolled of the study . From all children, one hundred and sixty childrens were purposively studied for the research purposes. The study was performed in collaboration with family members and with assigned doctors. It was also collected from doctor's prescriptions at the Chittagong Maa-Shishu General Hospital in Bangladesh care for children 6 to 60 months.

These childrens were diagnosed with acute watery diarrhea of more than 3 times for more than 24 hours and less than 7 days with moderate dehydration by World Health Organization (WHO) criteria.All children who were three or more outputs of loose or liquid stools a day were included in the study. Childrens with visible blood in the stool, history of allergy to cow's milk, severe dehydration,fever of more than 39°C and severe systemic infections(e.g. pneumonia,sepsis) and other disease requiring additional treatment were excluded from the study.

All children were given oral rehydration solution for three to six hours. Children were also received to oral rehydration solution only; probiotics with ORS such as Probio (*Lactobacillus acidophilus,L bulgaricus,Bifidobacterium bifidum);* Enterogermina (*Bacillus clausii having poly-antibiotic resistant*);TS6(*L acidophilus,L casei,B bifidum,L rhamnosus GG,B longum,B infantis,L lactis,L paracasei*) which are given below(Table 4). One hundred and sixty children were divided into four groups: Group 1, Group 2, Group 3 and Group 4 respectively. Group 1(control group) received Oral rehydration solution; Group (2 to 4) received Probiotics with ORS.

3.1 Use of Probiotics

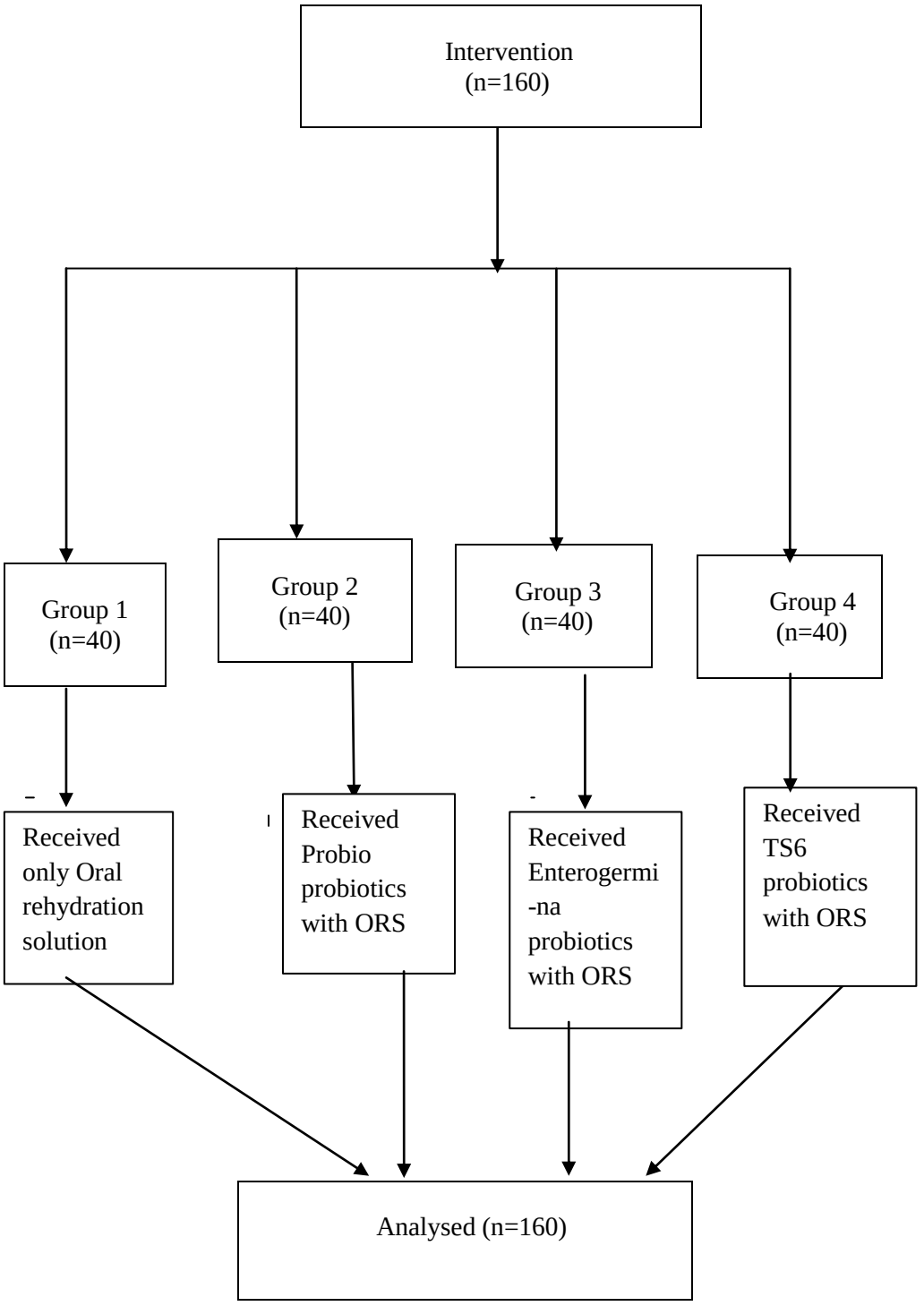

Figure 1: Flow diagram of the uses of probiotics for the treatment of children suffering from acute diarrhoea

Table 4: The characteristics of the probiotics:

Groups	Micro-organisms	Each product(capsule/liquid)contains	Dose/ Day	Brand
2	*Lactobacillus acidophilus,L bulgaricus,Bifidobacterium bifidum*	4 billion microorganism with 100mg fructo-oligosaccharides	One/two capsule with two spoons water	Probio
3	*Bacillus clausii having poly-antibiotic resistant*	2 milliards/5ml-2 billion/5 ml	one	Enterogermina
4	*L acidophilus,L casei,B bifidum,L rhamnosus GG,B longum,B infantis,L lactis,L paracasei*	350 mg/tablet	One to Two capsules daily	TS6

Probiotics were prescribed for seven days and administered orally according to the manufacturers' instructions. All the probiotics used in this study were available only in pharmacies and had a similar brand image. The group of children who received only oral rehydration served as controls.

The total duration of diarrhoea and daily stool outputs were recorded,during hospitalization. Duration of diarrhoea was the time in hours from the first to the last abnormal (loose or liquid) stools preceding a normal stool output. Stool consistency was evaluated through a score system (Guarino *et al*., 1997) and faeces were graded as 1 (normal), 2 (loose), 3 (semi liquid), and 4 (liquid).

3.2 Data Analysis

Duration of diarrhoea (hours) or recovery from diarrhoea (hour) of one hundred and sixty studied children were entered into the SPSS for analysis.

3.3 Statistical Analysis

Statistical analysis was performed by using SPSS for Windows versions 16.0 (SPSS Inc, Chicago, IL). Duration of diarrhea (hours) or recovery from diarrhoea (hours) outcomes were presented as mean difference between the probiotic treatment and controls (ORS) with 95% confidence intervals. Two-tailed P values < 0.001 were considered significant.

Chapter-4: Results

The anthropometric measurements (age and weight) of one hundred and sixty studied children were shown in Table 5.

Table 5: Anthropometric measurements of the studied under 5 years of children

	Group1 (ORs)	Group2 (probio)	Group3 (Enterogermina)	Group4 (TS6)
Age (months) Mean (range)	27.80 (6-60)	21.30 (6-54)	15.68 (6-30)	22.43 (6-54)
Weight (kg) Mean (range)	9.57 (5-16)	8.33 (4-12.50)	7.93 (4.50-9.50)	8.61 (4.90-13.20)

Table 5 depicts that anthropometric measurements, the mean of age(months) of study group 1, group 2, group 3 and group 4 were 27.80,21.30,15.68 and 22.43 respectively and the mean of weight (kg) of study group 1, group 2, group 3 and group 4 were 9.57,8.33,7.93 and 8.61 respectively.

Table 6: Association between ORS (group 1) and Probio probiotic (group 2) for reducing duration of diarrhoea (hour)

Treatment	Mean (Std.Deviation) (hour)	Median (Rang) (hour)	P value	Mean difference (95%CI)
Group 1	117.5 (18.02418)	114.00 (96-168)	P<0.001	(33.37-49.43) 41.400
Group 2	76.1 (18.07277)	72.00 (48-120)		

Table 6 shows that duration of diarrhoea (hour) or recovery from diarrhoea (hour) was significantly lower (P <0.001) in children receiving Probio probiotic (group 2) with ORS than in patients receiving oral rehydration solution only (group 1).

Table 7: Association between ORS (group 1) and Enterogermina probiotic (group 3) for reducing duration of diarrhoea (hour)

Treatment	Mean (Std.Deviation) (hour)	Median (Rang) (hour)	P value	Mean difference (95%CI)
Group 1	117.5 (18.02418)	114.00 (96-168)	P<0.001	(24.01-43.94) 33.98
Group 3	83.6 (26.03444)	84 (48-144)		

Table 7 shows that duration of diarrhoea (hour) or recovery from diarrhoea (hour) was significantly lower (P <0.001) in children receiving Enterogermina probiotic (group 3) with ORS than in patients receiving oral rehydration solution only (group 1).

Table 8: Association between ORS (group 1) and TS6 probiotic (group 4) for reducing duration of diarrhoea(hours)

Treatment	Mean (Std.Deviation) (hour)	Median (Rang) (hour)	P value	Mean difference (95%CI)
Group 1	117.5 (18.02418)	114.00 (96-168)	P<0.001	(32.81-50.59) 41.70
Group 4	75.8 (21.75020)	75 (48-144)		

Table 8 depicts that duration of diarrhoea (hour) or recovery from diarrhoea (hour) was significantly lower (P <0.001) in children receiving TS6 probiotic (group 4) with ORS than in patients receiving oral rehydration only (group 1).

Table 9: Duration of diarrhoea (hour)or recovery from diarrhoea by using three probiotics in study groups of children

Group	Treatment	Number of Children	Age (years)	Mean	Median (Range) (hour)	Std.Deviation
2	Probio probiotic	40	6 to 60 months	76.1	72 (48-120)	18.07277
3	Enteroger-mina probiotic	40	6 to 60 months	83.5	84(48-144)	26.03444
4	TS6 probiotic	40	6 to 60 months	75.8	75(48-144)	21.75020

Table 9 shows that Enterogermina probiotic had relatively lower effect in the recovery from diarrhoea (83.5 hours) than Probio (76.1 hours) and TS6 probiotics (75.8 hours) (mean).

Table 10: Daily stool outputs of U-5 years children from the first day (24 hours) of probiotic application

Group	Treatment	Hour						
		24	48	72	75	84	96	114
1	Oral rehydration solution	>15	10-12	8	6	5-6	4-5	3
2	Probio probiotic	>20	8-10	2	-	-	-	-
3	Enterogermina probiotic	>20	10	5-6	4	2-3	-	-
4	TS6 probiotic	>20	8-10	4-5	3	-	-	-

Table 10 shows that daily stool output was shorten in group 2, group 3 and group 4 than group 1 starting the day after the first probiotic application.

Chapter-5: Discussion

Diarrhoea in most of the developing countries including Bangladesh is usually self-limiting and does not require active treatment except replacement of fluids and electrolytes for prevention or correction of dehydration (Bhatnagar *et al.* 2006). Many probiotics are prescribed by Doctors and some probiotics have proven efficacy in children in developing countries but not all.

Acute infectious diarrhoea which is still a major cause of childhood morbidity . It is also a source of worry to families of affected children and represents a heavy economic burden for families and for society (Zimmerman *et al*., 2001). Most of the children are spontaneously affected by diarrhoea during April and May in Bangladesh. In the previous study, the peak value of diarrhoea occurs during the winter, between January and April (Rosenfeldt *et al*., 2005). Family members worried about this problem and most of the family mambers purchase drugs but they do not know about probiotics. These drugs that affect intestinal motility, ion transport and adsorptive moieties, and living bacteria have been used to reduce the duration of diarrhoea (Zimmerman *et al.*, 2001). Probiotics have gained in credibility for the treatment of diarrhoeal diseases. Probiotics have properties which are considered to be food additives rather than drugs. Therefore, only safety features probiotics and not proof of efficacy are required for marketing (Young *et al*, 1998). We did not conduct a qualitative and quantitative study of the microbial content of the probiotics in this trial because we wanted to carry out a field trial of the clinical effectiveness of commercially available probiotics that had been prescribed by the doctors. But in recent study, commercially available three probiotics have clinical effectiveness for treating diarrhoeal disease.

We conducted this clinical trial to evaluate the clinical efficacy of three probiotics (Probio, Enterogermina and TS6) , which are most commonly marketed probiotics in Bangladesh and are prescribed frequently by doctors for treatment of diarrhoea in children. These probiotics has been marketed in many Countries, because it is cheap to produce, easy to prepare, robust to production process and has a long shelf life over wide range of temperatures; it is quite stable in formulation of powder, granules, dry syrup, tablets, capsule, resistant to high moisture and oxygen, and compatible with pharmaceutical ingredients such as vitamins, minerals, amino acids (Vecchi and

Drago, 2006) easily received by parents because it is easy to prepare and easily they feed their child.In hospital,parents were easily prepared probiotics with water or ORS before feeding their child because of existing these properties.

This clinical trial showed that probiotics had a beneficial effect in reducing diarrhoea of 6 months to 60 months of children that was close in previous studies who found beneficial effects on diarrhoea in children, were 4 months to 2 years old (Chouraqui *et al*., 2004; Thibault *et al*., 2004; Weizman *et al*., 2005), and this trail was statistically significant in the study. Three probiotics early reduced the duration of diarrhoea than ORS only. A recent Cochrane meta-analysis of 23 randomised controlled trials found mild therapeutic benefit from probiotics that was generally reproducible regardless of organism for treating infectious diarrhea (Allen *et al*., 2004). Table 11 depicts that different trails of various Probiotics for the treatment of diarrhea among children.In previous study, Probiotics had early reduced duration of diarrhoea (days) of children than control group above the three trails.

Table 11: Different trails of various probiotics for the treatment of diarrhoea among children

Number of Children	Children (Age in months)	Probiotic	Duration (days)	Probiotic (days)	Controls (days)	Reference
100	1-36 mon	*L rhamnosus GG*	5	3.3±2.9	5.9±2.8	Guarino.,1997
73	3-24	*L acidophilus*	3	1.8±1.1	2.4±1.5	Simakachorn., 2000
100	6-60	*Lactobacillus acidophilus,Bifidobacterium infantis*	4	3.1±0.7	3.6±0.8	Lee., 2001

Probio probiotic which contains bacterial strains such as *Lactobacillus acidophilus,L bulgaricus,Bifidobacterium bifidum;* Enterogermina probiotic which contains bacterial strains such as *Bacillus clausii having poly-antibiotic resistant*; and TS6 probiotic which contains bacterial strains such as *L acidophilus,L casei,B bifidum,L rhamnosus GG,B longum,B infantis,L lactis,L paracasei*. These probiotics were associated with shorter duration of diarrhoea and two probiotics contained *Lactobacillus* strains among three probiotics. In previous study, *Lactobacillus* GG was associated with a shorter duration of diarrhoea, which was expected because proof of efficacy of this strain has been obtained in children in hospitals and outpatients in both industrialised and developing countries (Szajewska et al., 2006). The probiotic effects are also known to be strain and species dependent. Mixing two or more probiotic strains/species has been marginally more effective than using single strains in preventing diarrhoea (Sazawal *et al*., 2006). Probio probiotics and TS6 probiotics were mix of three strains and eight stains respectively whereas Enterogermina probiotic was *Bacillus clausii* stain which were relatively lower than mixture of stains probiotic. Bacillus species, except Bacillus cereus and Bacillus anthracis, are generally regarded as non pathogenic but it was felt that the safety of B. coagulans as probiotics should be evaluated by experts (Vecchi and Drago, 2006). *Bacillus clausii* which existing in Enterogermia probiotics had beneficial effect in children.

In recent study, uses of probiotics reduced the duration of diarrhea which was 117.5 hours in group 1 comparing with 76.1 hours in group 2 , 83.6 hours in group 3 and 75.8 hours in group 4 (mean value) with ORS and three probiotics had positive ($P<0.001$) effect on children for reducing of acute diarrhoea that was close to a clinical trial, which were conducted with 287 hospitalised children suffering from acute diarrhoea, *Lactobacillus* GG administered with an oral rehydration solution reduced the duration of diarrhoea from 71 to 58 hours, and in rotavirus-positive patients even more markedly, from 77 to 56 hours (Guandalini *et al.*, 2000). Among the three probiotics, Probio and TS6 probiotics worked the best than Enterogermina probiotics of studied children .

Children (6 to 60 monthes) need semi-solid foods,breast milk(bottle milk) that contain important source of nutrients. Over 6 months of age children should begin to eat from the family pot, taking those foods that are most nutritious (WHO., 1988). Here most of the studied children were taken breast milk or bottle milk depending on age and with small amounts of nutritious and digestible food such as coconut water, Hotchpotch (Khichuri) were suggested to correct the dehydration. Fluids were given to the child but fruit juice and sugary drinks which were avoided increase diarrhea. Table 12 depicts that diet management can be used as a treatment for diarrhoea of children. Breast feeding,coconut water, Hotchpotch (Khichuri) were suggested from 6 to 24 months of children and coconut water, Hotchpotch (Khichuri) from 24 to 60 months of children were provided. And Table 13 shows that among one hundred and sixty childrens were taken daily food pattern of children by family.

Table 12: Daily dietary pattern suggested from hospital in studied children

Age(months)	Hospital suggested diet/food
6 -24	Breast feeding, Coconut water, Hotchpotch(Khichuri)
24-60	Coconut water, Hotchpotch(Khichuri)

Table 13: Daily food intake pattern of studied children in family

Age(months)		children taken daily food pattern
6 -24	Morning	Breast feeding/Bottle feeding, Ruti/ Hotchpotch(Khichuri) Coconut water
	Afternoon	Breast feeding/Bottle feeding, Rice, Vegetables, Fish/Meat, Or Hotchpotch(Khichuri) Coconut water
	Evening	Breast feeding/Bottle feeding, cake Juice
	Night	Breast feeding/Bottle feeding, Rice, Vegetables, Fish/Meat
24-60	Morning	Ruti Banana Juice Coconut water
	Afternoon	Rice/Biriani, Vegetables, Fish/Meat, Juice Some fruits
	Evening	Cake Juice
	Night	Rice Vegetables, Fish/Meat, Juice Some fruits

Chapter-6: Conclusions

The current study concluded that the acute diarrhoeal disease of children who received probiotics such as Probio, Enterogermina and TS6 with ORS had a shorter duration of diarrhoea (hour) or recovery from diarrhoea than frequency of diarrhoea with ORS only. These three probiotics with ORS receiving children demonstrated significant efficacy ($p < 0.001$) towards the treatment of acute diarrhoeal disease when compared to ORS receiving children. Among the three probiotics, Enterogermina probiotic had relatively lower effect than Probio and TS6 probiotics on studied children. Probiotics had a beneficial effect in reducing diarrhoea of 6 months to 60 months children and these probiotics are included in the everyday diet for reducing the acute diarrhoea during their hospital stay or the follow up period, they can be considered a suitable, easy and safe method for balancing the body's natural defences against infections.

Chapter-7: Recommendations and Future perspectives

Probiotics may play a beneficial effect in several medical conditions including acute diarrhea. The following recommendations have been found out from this study –

1. The evidence supports the use of probiotics in the prevention and treatment of infectious or acute diarrhea. However, further research is needed. Research is also needed to guide the use of particular probiotic in the elderly patients.

2. Time constrains and small sample size were another limitations of this current research. If the sample size was large, we would divided the group into sub-groups according to their physiological conditions.

3. The studied childrens did not follow up after discharging from hospital.

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