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Role of Probiotics as Therapeutics against Gastrointestinal Disorders

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Gastrointestinal disorders are the major causes of morbidity and mortality worldwide. Diarrhea can be caused by several mechanisms including malabsorption, increased secretion of fluid, electrolytes and nutrients, motility disturbance, various types of diarrheal infections caused by microbes, antibiotic-associated diarrhea or due to stress. Such disturbances are becoming increasingly difficult to treat due to the increasing dissemination of antibiotic resistance among microorganisms and the emergence of the so-called 'superbugs'. Taking into consideration these problems, the need for novel therapeutics is essential. Although probiotics are being used over a century, they have only been extensively researched in recent years. Their use in the treatment and prevention of gastrointestinal disorders has yielded many successful results, some of which are outlined in this review. Probiotics are live microbial food ingredients that alter the enteric microflora and have a beneficial effect on health. Probiotics maintain the composition of micro-flora in the gut, enhance the immune system of the body and hence, prevent infections. Several health-related effects associated with the intake of probiotics are discussed here. Several clinical studies have been discussed which provide evidences for the efficacy of probiotics against these infections. Recently, a number of experiments have also been conducted mostly on intestinal cells to test the antiviral potential of probiotics against different viruses.

Key words: Probiotics, Gastrointestinal disorders, Antibiotic-associated diarrhea, Immune system; cell lines.

Diarrheal diseases continue to be the second most common cause of death in young ones of not only human beings but other animals also, mostly younger than 5 years of age[1, 2]. Acute watery infectious diarrhea and dysentery (diarrhea with blood) are leading causes of childhood deaths worldwide particularly in developing countries. Diarrhea can be caused by several categories of mechanisms including malabsorption as well as increased secretion of fluid, electrolytes and nutrients and motility disturbance. Growth of invasive microorganisms in the intestinal cells may reduce the absorption rate either by stimulating secretion or inhibiting absorption. According to the mechanism involved, there are several types of diarrhea prevalent in the world. Diarrheal infections, which are caused by certain microbes such as viruses (*Rotavirus*, *Calciviruses*, *Reoviruses*, *Norovirus*), bacteria (*E. coli*, *Salmonella* species, *Clostridium difficile*, *Shigella* etc.) are known as Microbe Associated Diarrhea which is accompanied by exudation of proteins and blood. Infections can also occur due to excess intake of antimicrobial substances which cause

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side effects resulting in diarrhea such as antibiotic associated diarrhea. Diarrhea due to growth of pathogenic bacteria is the most common side effect of using antibiotics like rabeprazole, clarithromycin, tinidazole, etc. Excess use of antibiotics can cause changes in composition of intestinal microflora which further results in imbalance in bowel syndrome and due to which person suffers from diarrheal infections.

Bacterial and viral pathogens seem to be the most common cause of gastroenteritis in people of all ages. Among them rotaviruses are the major etiological agents of severe gastroenteritis in infants and young children of a wide variety of mammalian and avian species throughout the world[2,3,4]. It has become the significant cause of the morbidity and mortality all over the world[5]. Probiotics can be used as an effective measure to treat diarrheal infections. In the host, the first barrier against food-borne pathogens, including viruses is represented by intestinal epithelium, followed by the responses of mucosal immune system. The gut barrier behaves as a physical blockade to the pathogen entry, if it is not disturbed[6,7]. Concomitantly, recent studies, both experimental and clinical, demonstrate a dependency on healthy host-microbe interaction i.e. interaction of probiotics with host to cope up with the pathogens' challenges. Probiotic bacteria promote the host defense and modulate immune system by being a part of gut micro-flora[8,9]. Probiotics maintain the composition of micro-flora in the gut and hence, prevent infections. In the present review, several clinical studies have been discussed which provide evidences for the efficacy of probiotics against these infections.

Probiotics in use

Probiotics are the living microorganisms that are similar to beneficial microorganism found in the human gut. They are also called the "friendly bacteria or good bacteria" or GRAS (Generally Recognized As Safe). One widely used definition developed by WHO and FAO of United Nations is that probiotics are "live microorganisms which when administered in adequate amount confer health benefit on the host." Another way to re-establish the microbial equilibrium in body and prevent diseases is introduction of beneficial bacteria (probiotics) to gastrointestinal tract[10]. Before characterization of bacteria as probiotics, certain measure should be considered as, it should be nonpathogenic, non-toxigenic, should retain viability during storage and use, should have the capacity to survive and metabolize in the gut. The probiotics bacteria most commonly studied include member of genera *Lactobacillus* and *Bifidobacterium*, *Saccharomyces boulardii*, *Escherichia coli* and *Enterococcus* strains. Currently used probiotics are listed in Table 1[11-14]. The colonization factors in the gut are the key factors which are responsible for the survival of probiotic organisms. They also depend upon the organelles which enable them to resist the antibacterial mechanisms that operate in the gut. In addition to the antibacterial mechanisms, they need to avoid the effects of peristalsis, which tend to flush out bacteria with food[13].

Effects of probiotics on immune system

Probiotics are nonpathogenic microorganisms that, when ingested, exert a positive influence on the health or physiology of the host. They can influence intestinal physiology either directly or indirectly through modulation of the endogenous ecosystem or immune system[15]. Several probiotics which claim to stimulate immune system by several mechanisms which are responsible for the beneficial effects on intestinal micro flora are listed in Fig. 1 [16]. According to the mechanism involved, there are several types of diarrhea prevalent in the world as summarized in Fig. 2 [16]**.** Diarrhea means the increased liquidity with decreased consistency of stools, usually associated with increased frequency and increased fecal weight. Osmotic diarrhea occurs due to insufficient absorption of osmotically active substances in the gut. Secretory diarrhea occurred in most cases of viral and bacterial enteritis which are accompanied by increased secretion or decreased absorption of ions into the gut. Microbe associated diarrhea are accompanied by exudation of proteins and blood.

Probiotics as therapeutics against Antibiotic-Associated Diarrhea

Diarrhea due to growth of pathogenic bacteria is the most common side effect of using antibiotics. Probiotic might be the effective measure for this infection by releasing inhibitory substances or by other mechanisms as listed in Fig. 1[16]. So far several studies have shown that probiotic bacteria *Lactobacillus* GG can prevent

antibiotic associated diarrhea. A randomized double blind placebo-controlled trial was done in which 135 patients aged over 50 were prescribed antibiotics. The participants were divided in two groups: A and B. Group A received probiotic treatment and B received placebo-controlled treatment. Only 7 patients were found as diarrheal patients from group A whereas from group B, 19 patients were found to be suffering from infection[14]. Another randomized study was done in which 119 hospitalized children received either *Lactobacillus* GG or placebo treatment. It was observed that 5 % of *Lactobacillus* GG group and 16% of placebo group children within 2 weeks of antimicrobial therapy have faced diarrheal incidence[17]. A placebo controlled trial has been reported in which 188 patients were observed. LGG, 1 x 10(10) - 2 x 10(10) colony forming units (cfu) per day, or comparable placebo was administered in a double-blind randomized trial to children receiving oral antibiotic therapy in an outpatient setting. Twenty-five placebo-treated but only 7 LGG-treated patients had diarrhea. *Lactobacillus* GG overall significantly reduced stool frequency and increased stool consistency during antibiotic therapy by the tenth day compared with the placebo group. *Lactobacillus* GG reduces the incidence of antibiotic-associated diarrhea in children treated with oral antibiotics for common childhood infections[18]. Another multicentre placebo-trial was reported with 123 patients. The use of *Enterococcus* SF68 shows significant reduction in antibiotic associated diarrhea as only 8.7% patients reported diarrhea as compared to 27.2% of placebo[19].

In an another trial, twenty-four healthy subjects received 150 mg clindamycin four times daily for 7 days and 250 ml yogurt twice daily for 14 days. A combination of three probiotics *L. acidophillus, L. paracasei* F19 and *Bifidobacterium lactis* were administered to the patients. Fecal samples were collected before, during and after administration of clindamycin. There was an increase in number of enterococci after treatment in both the placebo group ($P \leq 0.05$) and the active group, whereas other Gram-positive microorganisms decreased. Probiotics act as an effective measure in infants and children with severe bacterial infections and receiving broad spectrum antibiotics[20]. The results of another double blind placebo control study showed better results in group receiving probiotics since fewer diarrheal episodes (37.5%) were observed in this group than the control group (80%)[21].

Bacterium *H. pylori* is an etiological factor in the gastritis type B and peptic ulcers. Sixty healthy asymptomatic subjects screened positive for *H. pylori* infection were randomized to 1 week rabeprazole (20 mgb.d.), clarithromycin (500 mg b.d.), tinidazole (500 b.d.) and the probiotic *Lactobacillus* GG for 14 days or to the same regimen with a placebo preparation. Probiotic *Lactobacillus* reduced the diarrheal and nausea infections in 60 patients who were found to be positive for *H. pylori* and were randomized to 1 week with antibiotic[22]. Probiotic *Bacillus clausii* has also been observed as measure for treating diarrheal infection associated with antibiotic. A randomized study has been done in 120 patients receiving antimicrobial therapy for *H. pylori*. Four week therapy showed that the incidence of diarrhea has been reduced significantly as compared to placebo group[23]. In another study a total of 85 *H. pylori* positive, asymptomatic patients were randomized in four groups to receive probiotic or placebo both during and for 7 days after a 1-week triple therapy scheme (rabeprazole 20 mg b.id., clarithromycin 500 mg b.i.d., and tinidazole 500 mg b.i.d.). Group I (n = 21) received *Lactobacillus* GG; group II (n= 22), *Saccharomyces boulardii*; group III (n = 21), a combination of *Lactobacillus* spp. and *Biphidobacteria*; and group IV (n = 21), placebo. In all probiotic-supplemented groups, there was a significantly lower incidence of diarrhea and taste disturbance during the eradication week with respect to the placebo group. No differences in the incidence of side effects between the probiotic groups were observed. The *H. pylori* eradication rate was almost identical between the probiotic and placebo groups[24]. An eight day long randomized, double blind placebo trial was conducted in 89 patients suffering from antibiotic associated diarrhea and only seven of 44 patients in *Lactobacillus* group were found to be infected with diarrhea as compared to the 16 of 45 patients of placebo[25]. In a double blind controlled study, 740 patients undergoing cataract surgeries were administered the antibiotic ampicillin and cloxacillin with or without protected *Lactobacilli*. The incidence of diarrhea in patients receiving plain

antibiotic was 13.3% compared to 0.0% in patients receiving antibiotic with protected *Lactobacilli*[26]. Effectiveness of probiotics was studied in children (aged 3 months to 14 years) with common infections. Children were enrolled in a double-blind, randomized, placebo-controlled trial in which they received standard antibiotic treatment plus 2 x 10 (10) colony forming units of a probiotic *Lactobacillus rhamnosus* (strains E/N, Oxy and Pen) (n = 120) or a placebo (n = 120), administered orally twice daily throughout antibiotic treatment. Diarrheal incidences were found in only 9 (7.5%) patients in probiotic group and 20 (17%) patients in placebo group. Three (2.5%) children in the probiotic group developed AAD (diarrhea caused by *Clostridium difficile* or otherwise unexplained diarrhea) compared to nine (7.5%) in the placebo group. No adverse events were observed[27].

Saccharomyces boulardii another probiotic also underwent randomized double-blind placebo controlled clinical trial in the treatment of antibiotic associated diarrhea. A total of 269 children (aged 6 months to 14 years) with otitis media and/or respiratory tract infections were enrolled, in which they received standard antibiotic treatment plus 250mg of *S. boulardii* (experimental group, n = 132) or a placebo (control group, n = 137) orally twice daily for the duration of antibiotic treatment. 3.4% and 17.3% patients were found infected with diarrheal episodes in probiotics and placebo groups, respectively[28].

Probiotics as therapeutics against Microbe-Associated diarrhea

Most common microbes that infect the GI tract and become the prime cause of diarrhea are viruses (*Norovirus, Rotavirus, Adenovirus, Calcivirus*, etc.); bacteria (*Salmonella enteritidis, Escherichia coli, Shigella species, Campylobacter species, Vibrio* species, *Staphylococcus aureus, Clostridium difficile, Yersinia*, etc.) and protozoa (*Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum*). A number of studies have been conducted in treating the diarrheal infections with the help of probiotics, some of which have been discussed below.

Generalized Diarrhea

Oral bacteriotherapy promotes recovery from acute childhood diarrhea, but only few strains have been shown to have therapeutic potentials. A prospective, randomized, double-blind, placebo controlled study was conducted in which 304 children infected with acute diarrhea were enrolled. Patients were randomized to receive either placebo or Biothree (a mixture of *Bacillus mesentericus*, *Enterococcus faecalis* and *Clostridium butyricum*) in 3 divided dosages by oral administration for 7 days. The mean duration of diarrhea after start of therapy was 60.1 hours in the probiotics group versus 86.3 hours in the placebo group (P = 0.003). Hospital stay was shorter in the probiotics group than in the placebo group (P = 0.009). IL-10 was found to increase in the serum and supernatants of cell culture in the probiotics group, and tumor necrosis factor-α value were down-regulated. Interferon-γ and IL-12 were found to be mildly elevated in the probiotics group, compared with the placebo group[29]. The effect of two newly identified probiotic *Lactobacillus* strains was examined in acute childhood diarrhea. Sixty-nine children were randomized during hospitalization for acute diarrhea to receive a mixture of *Lactobacillus rhamnosus* 19070-2 and *Lactobacillus reuteri* DSM 12246, 10(10) colony-forming units of each strain or placebo twice daily for 5 days. In patients receiving probiotics, the diarrheal phase was reduced by 20%. In patients with diarrhea for <60 h before start of treatment (early intervention), a clear effect of the probiotics was demonstrated. After early intervention, the length of hospitalization was reduced by 48% (3.5 vs. 1.7 days, P = 0.03). At the end of the intervention, rotavirus antigen was found in 12% of patients from the treatment group vs. 46% from the control group (P = 0.02). The two probiotics, *L. rhamnosus* 19070-2 and *L. reuteri* DSM 12246, ameliorated acute diarrhea in hospitalized children and reduced the period of rotavirus excretion. The beneficial effects were most prominent in children treated early in the diarrheal phase[30].

In most cases, acute diarrhea becomes self-limiting during the first few days after onset. For young children, however, health risks may develop when the disease lasts longer than 3 days. A trial was conducted in which a total of 113 children (aged 2-47 months) with acute diarrhea (> three watery or loose stools in 24 h) were randomized to either a group receiving the probiotic *E. coli* strain Nissle 1917 (EcN) suspension (n = 55) or a group receiving the placebo suspension (n = 58) in a confirmative, double-blind clinical trial. The causes

of the diarrhea were viral rather than bacterial, but they were mainly unspecific infections. The number of patients showing a response was clearly higher ($p < 0.0001$) in the EcN group (52/55; 94.5%) than in the placebo group (39/58; 67.2%). EcN was found to be safe and well-tolerated, and it showed a significant superiority compared to the placebo in the treatment of acute diarrhea in infants and toddlers[31].

***Clostridium difficile* associated diarrhea**

Clostridium difficile is the leading cause of nosocomially acquired intestinal infection, affecting virtually all cases of pseudo-membranous colitis and most of the cases of antibiotic-associated diarrhea[32]. *Clostridium difficile* is a gram-positive, anaerobic bacillus that colonizes human large intestine, and produces at least two exotoxins: toxin A, which is primarily an enterotoxin; and toxin B, a cytotoxin. Colonization by this organism and subsequent infection occur in response to disruption of the stability of the indigenous micro-flora. The altered colonization resistance frequently occurs following antibiotic therapy in hospitalized patients[33]. The pathogenesis of *Clostridium difficile* associated diarrhea (CDAD) involves a triad of factors i.e. exposure to antibiotics, exposure to *C. difficile*, which typically occurs in hospitals and hostfactors[34]. Initial approach for the treatment of CDAD involves the use of antimicrobial therapy but the infection reoccurs in patients and can be fatal. So it needs an improved approach for the treatment. Treatment of the CDAD disease with combination of antimicrobial agents and probiotic culture reduced the infection significantly[33,35]. It has been reported that *Lactobacillus plantarum* 299v (Lp299v) has been found to reduce recurrence of CDAD as a controlled trail was performed on twenty-two ICU patients which were given a fermented oatmeal gruel containing Lp299v, and 22 patients received an equivalent product without the probiotic bacteria. Colonization with *C. difficile* was detected in 19% (4/21) of controls but in none of the Lp299v- treated patients[36]. A double blind placebo controlled trial was conducted on 138 patients (69 probiotics and 69 placebo group). The probiotics product comprised of 2×10^{10}cfu *Lactobacillus acidophilus* and *Bifidobacterium bifidum* capsule; the placebo. The incidence of samples positive for *C. difficile*- associated toxins was 2.9% in the probiotics group compared with 7.25% in the placebo-control group. When samples from all patients were tested (rather than just those developing diarrhea), 46% of probiotic patients were toxin-positive compared with 78% of the placebo group[33]. A trial was reported in which yeast *Acharromyces boulardii* was used in combination with antibiotics (vancomycin hydrochloride or metronidazole) for the treatment of CDAD. Thirty out of 67 patients who were receiving the placebo were found to fail in the treatment and only 15 out of 57 in probiotic–antibiotic combined group failed.

Table 1. List of probiotics currently being used

Bacteria
Lactobacillus: acidophilus, sporogenes, plantarum, rhamnosum, delbrueck, reuteri, fermentum, lactus
Bifidobacterium: bifidum, infantis, longum, thermophilum, animalis
Streptococcus: lactis, cremoris, alivarius, intermedius
Leuconostocsp.
*Pediococcus*sp.
*Propionibacterium*sp.
Bacillus sp.
Enterococcus: faecium
Yeast and Mould
Aspergillus: cerevisiae, niger, oryzue
Candida: pintolopesii,
Sacharomycesboulardii.

The efficacy of *S. boulardii* was found to be 41.3% for the treatment and prevention of CDAD recurrence. The combination of standard antibiotics and *S. boulardii* was shown to be an effective and safe therapy for these patients with recurrent CDAD; no benefit of *S. boulardii* was demonstrated for those with an initial episode of CDAD[37].

Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) is a functional disorder of colon, accompanied by abdominal pain, bloating and problems with bowel movements. The IBS follows an acute, presumably infectious diarrheal illness in approximately 15% of patients. There may be a persistent, mild inflammatory state with changes in mucosal function or structure. Colonic bacteria normally metabolize nutrients with the formation of gas and short chain fatty acids. The latter may induce propulsive contractions and accelerate colonic transit or they may enhance fluid and sodium absorption in the colon. Probiotics provide an alternative treatment for IBS. The mechanisms influenced by probiotics that are of potential relevance to the development of IBS include immune function, the intra luminal milieu and motility[38]. Animal model and human studies have evaluated the immunologic modulation with specific probiotic bacteria. The potential antiinflammatory effect of *Lactobacillus reuteri* in an experimental rodent study demonstrated an inhibition of tumor necrosis factor-α (TNF- α)

Probiotic bacteria stimulate immune system by:

- → Lower intestinal pH
- → Producing bactericidal substance
- → Agglutinating pathogenic microorganism
- → Competing for adherence site and substrate
- → Releasing gut protective metabolites
- → Binding and metabolizing toxic metabolites

Fig. 1. Mechanisms responsible for beneficial effects of probiotics on intestinal micro flora

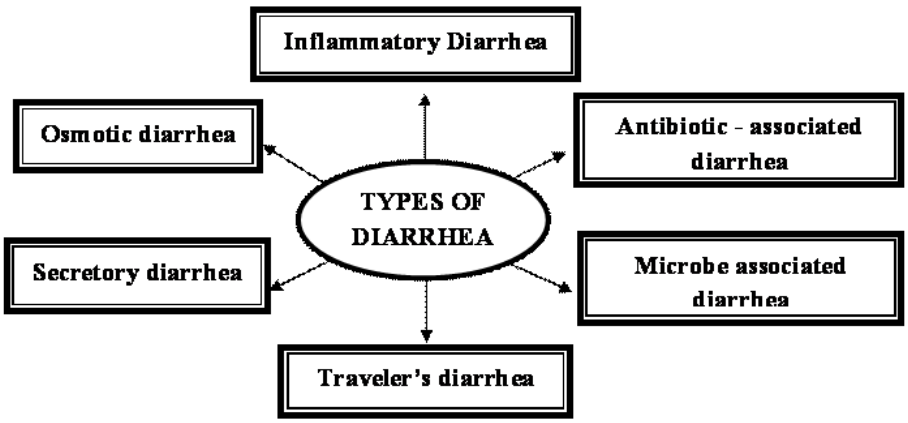

Fig. 2. Types of diarrhea

induced production of IL-8. *L. reuteri* is effective in inhibiting colitis in interleukin-10 (IL-10)-deficient mice. The conclusion was that *L. reuteri* has potent direct anti-inflammatory activity on human epithelial cells, which is likely to be related to the activity of ingested probiotics. *L. reuteri* also upregulates an unusual anti-inflammatory molecule, NGF, and inhibits NF-kappaB translocation to the nucleus[39]. In a clinical trial, 10 patients suffering from irritable bowel syndrome or functional diarrhea were administered the probiotic preparation VSL#3 (CD Pharma India). In 10 day long treatment, preliminary results indicated that administration of VSL#3 improved the clinical picture and changed the composition and biochemistry of fecal micro-biota. A significant increase in *lactobacilli, bifidobacteria* and *Streptococcus thermophilus* was observed as a consequence of probiotic treatment, while *Enterococci, Coliforms, Bacteroides* and *Clostridium perfringens* did not change significantly[40]. The intraluminal milieu, colonic bacteria normally metabolize nutrient substrates reaching the colon with the formation of gas and production of short chain fatty acids. *Lactobacillus* and *Bifidobacterium* subspecies are able to deconjugate and absorb bile acids. This may reduce the amount of intracolonic bile acids and could therefore have a beneficial effect on diarrhea by decreasing colonic fluid secretion or motility. Probiotics have been shown to have a beneficial effect in acute infectious diarrhea and inflammatory bowel disease and thus could presumably be of potential benefit in post infectious IBS. Another rationale for using probiotics in IBS is their potential to influence fermentation processes and diminish gas production by changing the colonic flora. Even though evidence from controlled clinical trials supporting a beneficial role of probiotics in the treatment of IBS is still limited, improvement of different IBS symptoms and normalization of inflammatory cytokine levels have been demonstrated[41].

Rotavirus associated diarrhea

Rotavirus is the main cause of acute diarrheal infections, which is the leading cause of death and disease among children[4]. There is ample evidence that probiotics reduce the duration of diarrheal episodes. A double blind placebo controlled trial consisting of 287 patients was organized. All the patients i.e. children from age 3 months to 6 years, were divided into two groups. Group A received placebo treatment and group B received probiotics *Lactobacillus* GG. In rotavirus positives cases, diarrhea lasted 76.6+ 41.6 hours in group A versus 56.2+ 16.9 hours in group B. Diarrheal infections lasted longer than 7 days in 10.7% of group A and 2.5% of group B[42]. Children between 6 and 36 months of age admitted for rotavirus-associated diarrhea were randomized into three groups to receive either 10(10) (n=21) or 10(7) cfu of *L. reuteri* (n=20) or a matching placebo (n=25) once a day for up to 5 days. This trial got significant results in watery diarrhea cases, 80% patients persisted with diarrhea infection in placebo group where as 70% and 48% were found infected on second day in *L. reuteri* small dosage and large dosage groups, respectively. The mean duration of watery diarrhea was 2.5 in placebo, 1.9 in *L. reuteri* small dosage group and 1.5 in large dosage group. *L. reuteri* effectively colonized the gastrointestinal tract after administration and significantly shortened the duration of watery diarrhea associated with rotavirus[43]. A double blind randomized controlled trial was conducted for evaluation of efficacy and tolerability of VSL#3 (CD Pharma India) in the treatment of acute rotavirus diarrhea in children. Use of probiotic mixture VSL#3 in acute rotavirus diarrhea resulted in earlier recovery and reduced frequency of ORS administration reflecting decreased stool volume losses during diarrhea. However, the overall recovery rate was higher in placebo group[44]. The effectiveness of nitazoxanide and probiotics was reported in comparison with a control group which was used in randomized, single blind study. Seventy-five children aged from 28 days to 24 months, with rotavirus diarrhea, were randomly assigned to receive either oral nitazoxanide (15mg/kg/day) twice a day for three days, a combination of oral probiotics, 1g twice a day for five days, or only oral or systemic rehydration solutions. The median duration of diarrhea was significantly reduced ($p = 0.009$) in children who received nitazoxanide (54 h) and probiotics (48 h)compared to the control group (79 h). Treatment with nitazoxanide and probiotics is effective in the management of children with acute rotavirus diarrhea[45]. A prospective clinical study was carried

out at Cathay General Hospital to determine the effect of *Lactobacillus acidophilus* and *Bifidobacterium infantis* on the course of acute diarrhea in hospitalized children. Altogether 100 children between 6 and 60 months of age were randomly allocated into 2groups. Study group (n=50) received mixture of both probiotics called InfloranBerna and controlled group (n=50) received parenteral rehydration. The frequency of diarrhea for study group improved on the first and second day of hospitalization with statistical difference ($p < 0.01$). The duration of diarrhea during hospitalization in study group also decreased (3.1 vs. 3.6 days, $p < 0.01$). Oral bacterial therapy is an effective adjuvant therapy in rotavirus positive and negative children with diarrhea and can safely be administered during an episode of acute diarrhea[46]. A trial of oral administration of live *Lactobacillus casei* strain GG on 100 children suffering from diarrhea was conducted. The children were divided into two groups, 48 received oral rehydration therapy and 52 received oral rehydration therapy along with oral bacterial therapy. Diarrheal duration was reduced by approximately 50% ($p <0.01$) in children receiving oral bacterial therapy compared with control subjects. It was concluded that oral administration of *Lactobacillus* GG is effective in rotavirus-positive and rotavirus-negative ambulatory children with diarrhea. Furthermore, it reduces the duration of rotavirus excretion[47].

Recent trends in cell culture based studies of probiotics on diarrheal infections

Besides case studies on experimental animals and children, antiviral potential of probiotics is being been elucidated in cell culture also. Recently, a number of experiments have been conducted mostly on intestinal cells, in which significant reduction in ill-effects due to rotaviruses after using probiotics have been found. Human intestinal and macrophage cell lines are being used as models to test the antiviral potential of probiotics against rotaviruses. Certain assays like cytopathic reduction assays, determination of reactive oxidation species and adhesion competence of probiotics etc. have been employed against pathogens to justify the antiviral potential of probiotics. It has been shown in many intervention studies that probiotic bacteria can have a beneficial effect on rotavirus and HIV-induced diarrhea. Inspite of this fact, antiviral effects of probiotic bacteria have not been systematically studied yet. It has been reported that non-tumorigenic porcine intestinal epithelial cells (IPEC-J2) and alveolar macrophages (3D4/2) were treated in different experimental designs with probiotic and other lactic bacteria and their metabolic products[48]. Vesicular stomatitis virus (VSV) was used in the study as a model virus. Cell survival and viral inhibition were determined by antiviral assay and confirmed by immunofluorescence. Pre-incubation of cell monolayers with probiotic bacteria reduced viral infectivity up to 60%. All the probiotic bacteria used prevented VSV binding to the cell monolayers by direct binding of VSV to their surface. Probiotic and other lactic bacteria prevented viral infection also by establishment of the antiviral state in pre-treated cell monolayers by secreting antiviral substances during their growth. It was observed that the infectivity of virus was diminished by 68% when bacterial supernatants were tested. It was shown for the first time that probiotic and other lactic bacteria exhibit an antiviral activity in acell culture model. Possible mechanisms of antiviral activity include, hindering the adsorption and cellinternalization of the VSV due to the direct trapping of virus by the bacteria, "cross-talk" with the cells in establishing the antiviral protection and production of metabolites with a direct antiviral effect[48]. Antagonistic activity of *Lactobacillus acidophilus* LB against intracellular *Salmonella enterica* Serovar *Typhimurium* infecting human enterocyte-Like Caco-2/TC-7 cells has been studied. LB (LB-SCS) strain decreases the number as well as inhibits the growth and development of apical serovar *Typhimurium*-induced F-actin rearrangements in infected cells. LB-SCS treatment efficiently decreases transcellular passage of *S. enterica* serovar *typhimurium*[49]. A study was aimed to examine the antiviral activity of lactic acid bacteria (LAB) using animal and human intestinal and macrophage cell line models of non-tumor origin. LAB strains selected on the basis of previous *invitro* trials were co-incubated with cell line monolayers, which were subsequently challenged with rotavirus and transmissible gastroenteritis virus (TGEV). In order to elucidate the possible mechanism responsible for the antiviral activity, the induction of reactive oxygen species (ROS) release as well as the

attachment ability of LAB on the cell lines was investigated. Highest protection effects were recorded with the known probiotics *Lactobacillus rhamnosus* GG and *Lactobacillus casei* Shirota against both rotavirus and TGEV, while notable antiviral activity was also attributed to *Enterococcus faecium* PCK38, *Lactobacillus fermentum* ACA-DC179, *Lactobacillus pentosus* PCA227 and *Lactobacillus plantarum* PCA236 and PCS22, depending on the cell line and virus combination used. A variable increase (of up to 50%) on the release of $NO^{\cdot}$ and H_2O_2 (ROS) was obtained when LAB strains were co-incubatedwith the cell lines, but the results were found to be LAB strain and cell line specific, apart from a small number of strains which were able to induce strong ROS release in more than one cell line. In contrast, the ability of the examined LAB strains to attach to the cell line monolayers was LAB strain but not cell line specific. Highest attachment ability was observed with *L. plantarum* ACA-DC 146, *L. paracasei* subsp. *tolerans* ACA-DC 4037 and *E. faecium* PCD71. Clear indications on the nature of the antiviral effect were evident only in the case of the *L. casei* Shirota against TGEV and with *L. plantarum* PCA236 against both rotavirus and TGEV. In the rest of the cases, each interaction was LAB-cell line–virus specific, barring general conclusions. However, it is probable that more than one mechanism is involved in the antiviral effect[50].

CONCLUSION

Probiotics are living organisms that are similar to the beneficial micro-organisms found in the human gut. There area variety of microorganisms present which exhibits characteristics of probiotics. Fermentation products of probiotics include organic acids, oxidation-reduction potential, bacteriocins and antibiotic substances which are responsible forthe antibacterial or antiviral potential. Probiotics are gaining importance because of the innumerable benefits, e.g. treating lactose intolerance, hyper-cholesterol problem and managing cardiac problems likeatherosclerosis and arteriosclerosis[51]. Probiotics play very important role in the management of lactose malabsorption and acute diarrhea, particularly acute infant diarrhea with viral etiology. Lactic acid bacteria are known to release various enzymes and vitamins into the intestinal lumen, which provide synergistic effects on digestion, alleviating symptoms of intestinal malabsorption, lactose intolerance and calcium deficiency. Lactic acid produced by the LABs lowers the pH of intestinal content and drives the proton motif forces into action due to which leakage occurs and pathogenic cell dies. Probiotics may also have a prophylactic effect in terms of decreasing the incidence of illness when taken regularly, the effect of which appears to be more in high risk problems. With the current focus on disease prevention and the quest for optimal health at all ages, the probiotics market potential isenormous. The use of food supplements as medication opens the discussion to create a category of "medical food" [52]. Most of the agents currently being studied and in use appear to be safe, with no apparent adverse effects noted in the thousands of subjects as reported. Future studies involving the comparisons between agents and doses, cost-benefit analyses, and efforts to determine the exact mechanisms by which these agents yield their effects need to be conducted. Further investigations to elucidate the underlying mode of action and to develop a cell line model as a system for selection of probiotic strains suited for particular application is the need of the hour.

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