PROBIOTICS IN CLINICAL PRACTICE

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Abstract

In recent years, probiotics supplementation has been advised in the prevention and treatment of a number of clinical conditions, but their efficacy still remains a debatable question due to paucity of information. However, the concept of probiotic therapy is not new as examples of probiotic therapy can be dated back to 76 BC.

Probiotic supplementation has shown significant positive result in certain conditions like infectious diarrhoea, antibiotic associated diarrhoea, necrotizing enterocolitis, atopic dermatitis, irritable bowel syndrome, inflammatory bowel disease and few other conditions.

In this review article, an effort has been made to throw some light on the efficacy, judicial usage and justification of probiotic therapy in various clinical conditions.

Keywords for the Paper: *Probiotic, prebiotic, microbiota, symbiotic, antibiotic, diarrhoea, caesarean section, proton pump inhibitors, necrotising enterocolitis, irritable bowel syndrome, inflammatory bowel disease, obesity, bacterial vaginosis, Lactobacillus, Bifidobacterium, dysbiosis, faecal transplantation.*

Introduction

The concept of probiotic therapy is not very new. Several instances in history reveal the usage of probiotics time and again. The first known reference of probiotic was in 76 BC, when the Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis[1]. In a Persian version of the Old Testament (Genesis 18:8) it is stated that "Abraham owed his longevity to the consumption of sour milk"[2]. The traditional methods followed by our great grandparents also depict that fermented milk products were used to manage diarrhoea and some other gastrointestinal upsets.

In 19th century when the pioneering works of Louis Pasteur, Robert Koch, Joseph Lister and others highlighted the harmful effects of pathogenic bacteria, the positive effects of nonpathogenic bacteria residing in our system were greatly neglected.

Introduction

The scientific rationale for the use of live microbes in the prevention and treatment of infections came to the lime-light at the beginning of the 20th century when Elie Metchnikoff in 1907 hypothesized that replacing or diminishing the number of 'putrefactive' bacteria in the gut with lactic acid bacteria could normalize bowel health and prolong life[3].

Lily and Stillwell first coined the term 'Probiotics'[4]. Etymologically the term is a composite of the Latin preposition *pro* (for) and the Greek adjective *biwitikos* (biotic), the latter deriving from the noun *bios* (life).

In 2001, the Food and Agricultural Organization and the World Health Organization adopted a definition of the term 'probiotic' as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'[5].

Symbiotic action of prebiotic and probiotic

The efficacy of probiotics is enhanced by prebiotics. Prebiotics are non-digestible fibres that pass undigested through the upper part of gastrointestinal tract and stimulate the growth and/or activity of advantageous bacteria that colonize the large bowel by acting as substrate for them. Breast milk contains a variety of non-digestible oligosaccharides which function as prebiotics preferentially stimulating proliferation of Bifidobacteria and Lactobacilli which causes initial colonization in newborns[6]. Foods such as tomatoes, onions, garlic, chicory, asparagus, berries, bananas, legumes, flax seeds are natural source of prebiotics in the adults. Prebiotics and probiotics are a symbiotic combination that may convert dysbiosis (i.e. microbial imbalance on or inside the body) to a symbiosis by balancing potential pathogens with health promoting bacteria.

Mechanism of action

The average human being harbours about 10 times more bacterial cells than their own cell numbers[7]. These microbes colonize the skin, respiratory tract, urogenital tract and gastrointestinal tract. Among all these sites, the gastrointestinal tract is the most densely populated area.

Probiotics have multiple and diverse influences on the host. Different organisms can influence the intestinal luminal environment, epithelial and mucosal barrier function, and the mucosal immune system. They exert their effects on numerous cell types involved in the innate and adaptive immune responses, such as epithelial cells, dendritic cells, monocytes/macrophages, B cells, T cells, including T cells with regulatory properties, and NK cells[8].

Probiotics can antagonize pathogenic bacteria by reducing luminal pH, inhibiting bacterial adherence and translocation, or producing antibacterial substances and defensins. Productions of antimicrobial compounds, termed bacteriocins, by probiotics are also likely to contribute to their beneficial activity. Several bacteriocins produced by different species from the genus ***Lactobacillus*** have been described[9].

Mechanism of action	Intestinal barrier function is maintained by several interrelated systems including mucus secretion, chloride and water secretion, and binding together of epithelial cells at their apical junctions by tight junction proteins. Enhancement of barrier function by probiotics has been observed both in vitro models and in vivo. The probiotics mixture VSL#3 normalized barrier integrity as assessed by short circuit currents, transepithelial potential differences, and mannitol fluxes in excised tissue from mice[10]. The signaling pathways that allow epithelial cells to distinguish commensal or probiotics from pathogenic organisms appear to be different. Pathogenic bacteria induce proinflammatory responses in intestinal epithelial cells by activating the transcription factor NF-κB. In contrast, nonpathogenic species can attenuate proinflammatory responses by blocking the degradation of the counter-regulatory factor IκB[11].
Proposed therapeutic actions	Till date clinical trials have not been able to provide us with conclusive indications for use of probiotics. However some positive outcome with probiotic therapy have been documented in few conditions like infectious diarrhoea, antibiotic associated diarrhoea, inflammatory bowel disease, irritable bowel syndrome, necrotizing enterocolitis, bacterial vaginosis, recurrent urinary tract infection, obesity, certain atopic conditions, immunocompromised conditions, glossitis, H. pylori infection, dental caries[12], rheumatoid arthritis[13], radiation induced diarrhoea, constipation etc. Several documents have stated the potential benefits of probiotics in modifying the intestinal flora in certain high risk groups like premature infants[14], infants born by caesarean section[15,16,17], traveler's diarrhoea[18] and geriatric age group[19,20]. A comparative analysis based on the clinical study of Floch et al and Natural Standard helps us to understand the efficacy of probiotic therapy on certain specific conditions[21,22].

Proposed therapeutic actions

Table shows the comparative results of the clinical study on probiotic supplementation by Floch et.al and Natural Standard. (Ratings: A= Strong, B= Good, C= Fair).

Name of the condition	Floch et al (2006)	Natural Standard (2006)
Ulcerative colitis	C	B
Crohn's disease	C	-
H. pylori infection	C	A
Necrotising enterocolitis	-	C
Bacterial vaginosis	C	C
Urinary tract infection	-	C
Infectious diarrhoea	A	B
Antibiotic-associated diarrhea	A	C
Diarrhoea prevention	B	B
Irritable bowel syndrome	C	B
Atopic dermatitis/allergy	?B	B/C

Probiotic supplementation in pregnancy and at birth

A seminal study from Finland shows *Lactobacillus rhamnosus* when given to pregnant women having a family history of allergy during the latter stages of gestation, resulted in infants with 50% lower incidence of atopic dermatitis than control infants. The protective effect was apparent till 7 years of age[23,24]. Thus probiotic supplementation in late pregnancy and early infancy shows positive result with atopic sensitivity although effect on asthma or wheezing is still not clear.

Phases of bacterial colonization is most likely to occur during vaginal delivery when the baby passes through the birth canal consuming its first bolus of maternal vaginal and colonic microbiota which enters the baby's gastrointestinal tract. Phases of initial gut colonization are as follows[25]:-

- 'Germ free' intrauterine environment
- Phase 1: Acquire maternal vaginal/colonic flora (full term vaginal delivery).
- Phase 2: Introduction of oral feedings (breast milk/formula food)
- Phase 3: Weaning period
- Phase 4: Acquire complete adult colonization (12-18 months; more than 1000 species).

Children born by caesarean section and receiving injudicious antibiotics are deprived of the Phase-1 of initial gut colonization and have aberrant mucosal immune function[26]. If antibiotic treatment is used during Phase 4, the timing and nature of colonization is disrupted and prolonged[27]. A fully colonized intestine can function as an ancillary organ in the body. It consists of 1-2 kg of body weight in human adult and has a 10 fold greater number of cells than the cells of the human body as well as a 100-fold greater number of genes than the human genome. Moreover, the metabolic activity of colonizing bacteria is greater than the liver, the most active body organ[28].

Probiotic supplementation in infants

Studies have proved that breastfed infants have relative resistance to infectious gastroenteritis and probiotic supplementation to their diet provide protection against infectious diarrhea (e.g. Rotavirus) and antibiotic associated diarrhoea[29],[30].

Probiotic supplementation has shown positive results in many causes of infant mortality including preterm babies[31], premature babies and babies born with birth weight less than 1500g[32]. However the effect in babies weighing less than 1000g need further study[33].

Necrotizing enterocolitis is one devastating intestinal disorder that a preterm infant may face within a neonatal intensive care unit. Necrotizing enterocolitis is characterized by abdominal distension, bilious emesis, bloody stools, lethargy, apnoea, and bradycardia [34]. The disease progresses through an inflammatory cascade with septic shock and intestinal necrosis. Necrotizing enterocolitis has been reported to occur in 10 to 25% of preterm infants (<1,500 g in weight) admitted to the neonatal intensive care unit, and it may involve approximately one third to one half of all very low birth weight infants [35].

The gut microbiome of infants developing necrotizing enterocolitis (NEC) is characterised by increased abundance of gamma Proteobacteria[36]. Bacterial colonization or infection of the intestine by pathogens such as Clostridium, Escherichia, Klebsiella, Salmonella, Shigella, Campylobacter, Pseudomonas, Streptococcus, Enterococcus, Staphylococcus aureus, and coagulase-negative staphylococci increases the risk of necrotizing enterocolitis. If nonpathogens, such as lactobacilli and bifidobacteria, colonize the intestine, or if breast milk rather than formula is used, the incidence of necrotizing enterocolitis has been reported to fall[37].

Increased incidence of necrotizing enterocolitis has been associated with administration of Histamine-2 receptor antagonist as acid blockers to preterm infants[38],[39]. The histamine signalling pathway may provide an opportunity for targeted implementation of probiotics based on known mechanisms. Probiotics capable of converting the dietary amino acid L-histidine to histamine have been reported[40], and histamine may suppress inflammation by promoting H2R signalling in the intestinal mucosa.

Probiotic supplementation in infectious diarrhoea and antibiotic associated diarrhea

It is estimated that every 15 second a child dies from diarrhoeal disease somewhere in the world. In a study in 204 undernourished, 6- to 24-month-old children in Peru, once-daily intake of Lactobacillus rhamnosus GG 6 days a week for 15 months led to significantly fewer episodes of diarrhoea (5.21 versus 6.02 episodes of diarrhea per child per year in the placebo group; P = 0.028)[41]. However, this type of study is difficult to verify because there is little control over the organisms to which the children are exposed and the compliance in taking the treatment. At the least, probiotics provide a safe and potentially beneficial remedy, especially when delivered in milk, which provides the child with nutrition and a means to overcome adverse effects of fluid loss. Current WHO recommendations state that clinical management of acute diarrhoea should include replacement of fluid and electrolytes losses along with nutritional support[42]. As such, oral rehydration salts are widely used in diarrheal disease management.

The strongest evidence of a beneficial effect of probiotics has been established with L. rhamnosus GG and B. lactis BB-12 for prevention and L. reuteri SD2222 for treatment [43],[44],[45],[46],[47] of acute diarrhoea mainly caused by rotaviruses in children.

Several potential mechanisms have been proposed for how lactobacilli reduce the duration of rotavirus diarrhoea. The first is competitive blockage of receptor sites[48], in which lactobacilli bind to receptors, thereby preventing adhesion and invasion of the virus. The second potential mechanism may be that the immune response is enhanced by lactobacilli, leading to the observed clinical effect[49]. This is supported by the protective effect which local immunoglobulin A (IgA) antibodies appear to confer against rotavirus[50]. A third mechanism could involve a signal from lactobacilli to the host that downregulates the secretory and motility defenses designed to remove perceived noxious substances. Glycosylated intestinal mucins inhibit rotaviruses[51] , and MUC2 and MUC3 mRNA expression is increased in response to lactobacillus signaling, protecting cells against pathogenic bacterial adhesion[52]. A final theory is that lactobacilli produce substances that inactivate the viral particles. This has been shown in vitro[53] , with supernatants from Lactobacillus rhamnosus GR-1 and L. fermentum RC-14 inactivating 109 particles of the double-stranded DNA adenovirus and the negative-stranded RNA vesicular stomatitis virus within 10 min.

It is well known that prolonged and indiscriminate use of antibiotic therapy leads to eradication of bacteria as a whole including both harmful and beneficial bacteria. It is important to replenish the gut microbiota at the earliest to prevent future digestive, metabolic and immune dysfunction. Antibiotic associated diarrhoea usually occurs due to change in carbohydrate metabolism with decreased short chain fatty acid absorption resulting in osmotic diarrhoea. Several studies conducted in children have demonstrated that probiotics are effective at suppressing antibiotic-associated diarrhoea[54],[55] and probiotics promote restoration of microbial diversity as a mechanism for amelioration of the disease phenotype[56].

Probiotic Supplementation In H.pylori infection:

Probiotic Supplementation In H.pylori infection:

Helicobacter pylori is a gram-negative bacterial pathogen responsible for type B gastritis and peptic ulcers and may be a risk factor for gastric cancer. There are some in vitro and animal data to indicate that lactic acid bacteria can inhibit the pathogen's growth and decrease the urease enzyme activity necessary for it to survive in the acidic environment of the stomach[57],[58],[59],[60]. In humans, there is also evidence that probiotic strains can suppress infection and lower the risk of recurrences[61],[62],[63]. Studies show that on administration of combination of *Lactobacillus* and *Bifidobacterium sp* (VSL#3), bacteriocins are produced, which inactivates *H. pylori* or dislodge the bacteria from its site[64].

Probiotic supplementation in indiscriminate use of Proton Pump Inhibitors:

Probiotic supplementation in indiscriminate use of Proton Pump Inhibitors:

A mutualistic relationship exists between the normal gut flora and human body. Loss of the normal gastric acidity or profound gastric suppression has been associated with dysbiosis and abnormal colonization of opportunistic bacteria in the normally sterile upper gastrointestinal tract[65]. Prolonged use of proton pump inhibitors lead to rebound hypersecretion of acid and increased risk of enteric infections like food borne enteric infections, increased risk of *Clostridium difficile* infections and spontaneous bacterial peritonitis[66]. Injudicious and prolonged use of proton pump inhibitors result in disturbed gut flora and may lead to vitamin B12 deficiency thereby leading to megaloblastic anaemia[67]. The effectiveness of probiotics to restore normal gut flora in such cases needs to be studied in detail[68,69].

Probiotic supplementation in Irritable Bowel Syndrome:

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Irritable bowel syndrome is a disorder of unknown pathophysiology characterized by symptoms like cramping, abdominal pain, bloating, constipation, diarrhoea in the absence of any organic cause and diagnosed on the basis of Rome III criteria[70]. Researchers suspect that the main cause of the condition is imbalance in the enteric flora. There is a strong basis for the therapeutic use of probiotic organisms (especially, *B infantis* 35624 and *B animalis* DN-173010 based on clinical trials) as a component of multidisciplinary approach for treating symptoms associated with irritable bowel syndrome. In a study carried out in India on 200 patients *Lactobacillus plantarum* 299v also showed positive results[71].

Probiotic supplementation in Inflammatory Bowel Disease:

Inflammatory bowel diseases such as pouchitis and Crohn's disease may be caused or aggravated by alterations in the gut microbiota[72]. Preliminary evidence suggests that a combination of strains[73] rather than a single organism[74] may alleviate symptoms of disease. In the study with the VSL#3 product containing very high doses of four strains of lactobacilli, three strains of bifidobacteria, and one strain of Streptococcus salivarius subsp. thermophilus (5 × 1011 per g of viable lyophilized bacteria), 40 patients in clinical and endoscopic remission were randomized to receive VSL#3, 6 g/day, or placebo for 9 months[73]. Three patients (15%) in the VSL#3 group had relapses within the 9-month follow-up period, compared with 20 (100%) in the placebo group ($P < 0.001$). It was not surprising that the faecal content of lactobacilli, bifidobacteria, and S. thermophilus increased significantly from baseline levels in the VSL#3-treated group ($P < 0.01$), given the high numbers of probiotics administered.

Randomised control trial on patients of ulcerative colitis concluded that short term synbiotic treatment of active ulcerative colitis resulted in improvement of the full clinical appearance of chronic inflammation[75, 76.]

Probiotic supplementation in vaginosis:

The vaginal microbiota is often in a state of flux, as shown by Nugent score analysis, culture, and molecular tracking[77,78,79,80]. The Nugent score[81] is determined by microscopic analysis of vaginal cells collected from the vagina. When the field of view is dominated by lactobacillus morphotypes, the score is low (0 to 3). When it is dominated solely by gram-negative rods (indicative of anaerobes like Gardnerella vaginalis or uropathogens like Escherichia coli) or gram-positive cocci like group B streptococci or enterococci, the score is high (8 to 10). Intermediate values indicate the presence of pathogens and lactobacilli in a sort of transition state. The factors that contribute to the transition from asymptomatic to symptomatic infection or a return to one that is healthy remain to be determined.

The concept of restoring the Lactobacillus content of the vaginal microbiota as a barrier to prevent infection was first conceived by Canadian urologist Andrew Bruce in the early 1970s. Extensive research since then has shown that certain Lactobacillus strains are able to colonize the vagina following vaginal suppository use[82] and reduce the risk of urinary tract infection, yeast vaginitis[83], and bacterial vaginosis[84].

Probiotic supplementation in obesity:

Probiotic supplementation in obesity:

Clinical trials carried out on mice revealed that weight gain and insulin resistance could be suppressed with VSL#3, a probiotic mixture mainly containing L. bacilli and Bifidobacterium sp.[85]. The mixture acts by releasing hormones that reduce food cravings and promote glucose tolerance thereby preventing obesity and its associated diseases. The efficacy of probiotics in the treatment of obesity is inconclusive and needs further research.

Probiotic supplementation in other conditions

Studies have been carried out on the efficiency of probiotics on various other conditions like cholesterol control and hypertension control. In cholesterol control, few strains have been found to increase serum HDL level and improve the LDL:HDL ratio[86]. In hypertension control[87], milk fermented with Lactobacilli has been recommended by some researchers. In both cases, substantial data is still not available to show the effects of probiotics.

The ability of lactobacilli and bifidobacteria to modify the gut microbiota and reduce the risk of cancer is in part due to their ability to decrease β-glucuronidase and carcinogen levels[88]. Cancer recurrences at other sites, such as the urinary bladder, also appear to be reduced by intestinal instillation of probiotics, including L. casei Shirota (the strain present in Yakult, a Japanese milk-based drink taken by an estimated 26 million people every day)[89]. In vitro studies with L. rhamnosus GG and bifidobacteria and an in vivo study with L. rhamnosus GG and LC-705 and a Propionibacterium sp. showed a decrease in availability of carcinogenic aflatoxin in the lumen[90,91]. Increased activity of glutathione transferase (induced by Bifidobacterium longum and lactulose and resistant starch), colonic NADPH-cytochrome P450 reductase, and enhanced removal of O6-methylguanine from colonic mucosa may also play a role in disease prevention[92,93].

The human skin contains several dominant bacterial genera across different sites, including Corynebacterium, Eubacterium, Propionibacterium, Staphylococcus and Streptococcus[94], and one dominant fungal genus Malassezia[95]. Focused studies on specific body compartments have highlighted key features of colonization in healthy individuals. Relative differences in composition and function of bacterial communities on the human skin may explain different patterns of atopic diseases involving the skin and airways. In cases of atopic dermatitis, staphylococci including S. aureus and S. epidermidis populations appear to 'bloom' and contribute to disease flares and relapse at specific skin sites[96]. Perhaps, the topical application of probiotics or skin bacterial communities that suppress pathogen 'blooms' on specific body surfaces may help prevent or mitigate these atopic disease flares in the future. Unfortunately for patients, the identification of probiotic strains that bestow beneficial effects on the human skin has not been defined, and such applications in dermatology await further investigations. Past limited successes with oral probiotics and amelioration of atopic skin disease features in children[97,98] have generated optimism for the potential roles of oral or topical probiotics in the treatment of atopy. However, this enthusiasm has been tempered by the realization that many gaps exist in our knowledge of the skin microbiome, probiotics and pediatric allergic diseases, and no single probiotic strain can be recommended at this time[99].

Prescribing probiotics

Extensive research is needed to standardize the prescribing dosage of probiotics. Till date *Lactobacillus rhamnosus* has been the best studied bacteria. The combination products are still not well studied, but they might work as well. Typical dosages vary based on the product, but common dosages range from 5 to 10 billion colony-forming units per day for children and from 10 to 20 billion colony-forming units per day for adults[100]. The common side effects noted so far are bloating and nausea.

One promising approach for relieving dysbiosis-associated diseases is called faecal transplantation. It is the re-establishment of normal microbiota via transplantation of a healthy donor's stool into a symptomatic host[101]. One possible future venue for faecal transplantation is the use of a patient's own stored healthy stool to restore their intestinal microbiota following antibiotic treatment or disease onset. Due to its inexpensive nature, faecal transplantation might be particularly favourable in populations where expensive treatments are not easily accessible.

Conclusion

The research on probiotics has advanced considerably in the last three decades, spurred by global progress in understanding of the role of the human microbiota in health and disease. Well controlled intervention trials, systematic reviews and meta-analyses provide convincing evidence of the benefits of probiotics, including ones with valuable public health implications.

As we proceed into the era of metagenomics medicine, patients may be tested for their own microbial compositional and functional features so that probiotics may be customized and tailored to the disease state and the individual patient. The fusion of the microbiome with microbe-based therapies in medicine will advance the causes of holistic and personalized medicine.

Stepping into the times of therapeutic probiotic supplementation we should always keep in mind that probiotic supplementation is not the solution for 'unhealthy' practice by healthcare providers. Thus injudicious use of proton pump inhibitors and antibiotics should be condemned and vaginal delivery should be encouraged.

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