Probiotics in Health and Disease in Children

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Abstract

Probiotics are living microorganisms which are being used in the management of multiple medical conditions associated with disturbance of normal microflora of human mucosa. The expected benefits and potential risks of probiotics use have been evaluated and guidelines have been suggested for their safe and effective use in hospitalized and ambulatory pediatric patients. If minimal benefit is expected then financial cost should also be taken into account. Research is going on to develop probiotics by isolating the beneficial component of the microbe, and thus decrease the potential risks of using live microorganisms.

Introduction

Any physician who prescribes antibiotics is familiar with antibiotic associated diarrhea. The incidence of Clostridium difficile associated diarrhea is increasing.[1] Change in gut microflora due to antibiotic therapy can contribute to post infectious diarrhea, not associated with *C.difficile*. Systemic antibiotics, whether given orally or parenterally will alter the normal microflora of the human gut by decreasing antibiotic susceptible bacteria in the microbiome. It is only in the last decade that we have understood the mechanism of the antibiotic associated diarrhea and how the commensal microbes maintain the integrity of the gut mucosa, decrease the paracellular permeability induced by pathogens and inhibit proinflammatory cytokines.[2]

Systemic antibiotics as well as local application, such as antibiotic eye drops, antibiotic cream applied to nares or nebulized antibiotics will alter the microflora of the oropharynx and respiratory tract. The vaginal microflora is similarly changed by systemic antibiotics.

In many instances, as antibiotics decrease the commensal bacteria, Candida which is part of normal flora, overgrows leading to oral thrush, vaginitis, esophagitis, intertriginous dermatitis and diaper rash.

These everyday clinical observations have encouraged many clinicians to use probiotics. Generally probiotics are living microorganisms but components of microbial cells that have a beneficial effect on human host are also called probiotics. Probiotics are often used with prebiotics; these are substances which enhance the proliferation of probiotic microorganisms. These include fructo and galacto oligosaccharides, inulin, germinated barley and psyllium. These substances pass unchanged through upper GI tract. Combined probiotics and prebiotics are called synbiotics.[2]

The United Nations Food and Agriculture Organization (FAO) and World Health organization (WHO) have defined probiotics as "live microorganisms, which when administered in an adequate amount confer a health benefit on the host."[3]

Objective

This review aims to evaluate present evidence which is often contradictory and many times incomparable due to use of different microbes, different species, and different subspecies and number of CFU used. The preparations available in Karachi, their components, experimental and clinical data supporting their use, possible drawbacks and usual price will also be considered, without supporting any particular brand.

Background

A standard Pediatric textbook, Nelson Textbook of Pediatrics, 18th edition, published in 2007, has a comprehensive chapter: Probiotics in Gastrointestinal Diseases by David Branski and Michael Wilschanski of Hebrew University Medical School, Israel. This chapter refers to the basic science of immunomodulation by probiotics as well as clinical studies in childhood diarrheal and atopic disorders. It addressed lactic acid producing bacilli, Lactobacillus and Bifidobacterium and the yeast Saccharomyces boulardii and reiterated that as probiotic therapy introduces living microbes in a person's body it should meet certain criteria for safety and efficacy (Table1,2,4). After discussing the clinical studies of probiotic use in acute infectious diarrhea, antibiotic associated diarrhea, Neonatal Necrotizing Enterocolitis (NEC), Lactase deficiency, Irritable Bowel Syndrome, Inflammatory Bowel disease, Celiac disease, food protein sensitivity and Helicobacter pylori infection; the chapter ended with exhortation to define the beneficial strains and optimal combinations of strains. It raised concerns regarding transfer of virulence and antibiotic resistance. as well as safety in immune deficient infants and possible induction of dental caries.[2]

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Table 1: Criteria for microorganisms to be used as Probiotics

1. It should be of human origin.
2. It should be nonpathogenic and safe.
3. It should resist digestion by gastric and pancreatic juices and bile.
4. It should adhere to enterocytes.
5. It should produce antimicrobial substances.
6. It should produce favorable immunomodulation.
7. It should be able to influence metabolic activities.

Table 2: Commonly used Probiotic Microorganisms for Prevention and Treatment of Disease

BACTERIA

Lactobacillus
L. acidophilus
L. bulgaricus
L. caseii
L. fermentum
L. paracasei
L. plantarum
L. rhamnosus (GG)

Bifidobacterium
B. bifidum
B. breve
B. infantis
B. lactis
B. longum

Streptococcus
S. boulardii
S. salivarius
S. thermophilus
Lactococcus lactic
Enterococcus faecium SF68 (nonpathogenic)
Escherichia coli Nissle 1917 (nonpathogenic strain)
Clostridium butyricum

Yeast
Saccharomyces boulardi

From Feign and Cherry's Textbook of Pediatric Infectious Diseases. 7th edition,2014.
Harrison GJ. Probiotics chapter 242, Page 3370.

The 20th edition of Nelson Textbood of Pediatrics published in 2016 discusses probiotics in the chapter: The Microbiome and Pediatric Health, and supports probiotic use in antibiotic associated diarrhea, reduction and recurrence of C. difficile infection and prevention of NEC. Probiotics were deemed to be of possible use in recurrent Urinary tract infections and respiratory infections. There do not seem to be clear guidelines or strong endorsement despite intervening nine years of clinical use.[4]

Clinical Use of Probiotics
In clinical use probiotics have been considered from three aspects[1]

1. Role in suppressing the pathogenic bacteria.
2. Role in improving intestinal barrier function.
3. Role in development and modulation of immune system.

Gastrointestinal Disorders
Gastroenteritis (GE) remains a leading cause of disease worldwide. The role of probiotics in suppressing intestinal pathogens and improving the barrier function of lining enterocytes (by decreasing paracellular permeability) has raised interest in use of probiotics in gastrointestinal disorders.

Management of GE includes fluid repletion, refeeding, zinc supplementation which has been shown to decrease stool output and duration of diarrhea.[5-7] Adsorbents (attapulgite and smectite) can potentially bind mucus, toxins and reduce water loss but only smectite was shown to reduce duration of diarrhea by one day.[8] Antibiotics and antiparasitic agents should be used when indicated.

Probiotics have been advised in the care of children with diarrhea. Although probiotics have been part of human diet in fermented food such as yogurt, kimchi etc. use of probiotics supplementation in large doses (10^{10} 10^{12} cfu) should be recognized as a deliberate attempt to alter the intestinal microflora and its potential for benefit and harm should be evaluated.

Clinically, these beneficial microorganisms are used in much larger doses than present in fermented food. Large doses, (billions of live microbes daily) are used so that at least some of the orally administered microbes may survive the gauntlet of gastric acid, proteolytic enzymes of stomach and intestine and bile. These surviving probiotic microorganisms then attach to enterocytes to produce their metabolic effects.

Modest therapeutic benefit has been noted in acute infectious diarrhea[9] including that caused by Rotavirus.[10] In undernourished children, use of ORS supplemented with Lactobacillus rhamnosus GG (10^{10} 10^{12} cfu) was associated with significant decrease in frequency and duration of diarrhea.[11]

Present information suggests that presumed infectious diarrhea be treated with probiotics preferably with Lactobacillus GG or Saccharomyces boulardii which have been most commonly studied. Probiotics also attenuate the muscle hypercontractility seen in postinfectious gut motility.[12]

Probiotics (*Lactobacillus and S. boulardii*) have shown benefit in prevention and treatment antibiotic associated diarrhea

Table 3: Preparations available in Karachi (alphabetically)

Brand (manufacture)	Supplied as	Contents	Price
Amybact (ICI Pakistan)	Sachet	Bifidobacterium BB12 90% CFU? Lactobacillus paracasei,L. casei 5% CFU? Streptococcus thermophilus, TH 4 5% CFU?	480 Rs/10
Biflor (Tabros)	Sachet	Saccharomyces boulardii 250 mg	400 Rs/10
Captain AD (PharmEvo)	Sachet Tablet	Bifidobacterium BB12 11 billion CFU/tablet	?
Enflor (Hilton)	Sachet capsule	Saccharomyces boulardii 250 mg	480 Rs/10
Enterogermina (Sanofi)	suspension	Spores of Bacillus clausii polyantibiotic resistant, 2 billion	800 Rs/20
Florabion (Promise Health Foods)	Sachet	Lactobacillus acidophilus 5 billion CFU Lactobacillus sporogenes 4 billion CFU Bifidobacterium lactis 5 billion CFU Fructo oligo saccharides 50 mg	285 Rs/5
FlorAid (Angelini)	Sachet and capsule	Saccharomyces boulardii 250 mg	
Gutcare (Searle)	Sachet and capsule	Clostridium butyricum and Bifidobacterium 420 mg	415 Rs/10
Montum (MAKSONS)	Sachet	Lactobacillus paracasei ssp paracasei and Lactobacillus acidophilus >3 billion CFU	595 Rs/10
Newflora (RG Pharmaceutica)	?	Enterococcus faecium SF68	?
Prepro (Matrix)	Sachet	Lactobacillus rhamnosus GG 5 billion CFU	400 Rs/10
Protectis (Ferozsons)	Drops and Tablets	Lactobacillus reuteri DSM 17938 1 billion CFU drops (need refrigeration)	1511.92Rs/5ml
Resiton (MAKSONS)	Sachet	Lactobacillus paracasei ssp paracasei> 1 billion CFU	595 Rs/10
Restore	Sachet	Saccharomyces boulardii 1 gram	?
Yugud	Sachet	Lactobacillus acidophilus 1 GG Bifidobacterium	?

(13).Review of 82 probiotic trials for antibiotic associated diarrhea showed 42% reduction in diarrhea. Sixty nine percent trials had used *Lactobacillus* alone or with other organisms. Fifteen trials (20%) used *S. boulardii* alone, in these the risk of antibiotic associated diarrhea was reduced 52%.[14]

Supplementation of Infant Milk Formula with Probiotics
Lactobacilli and Bifidobacterium which predominate in the stool of breast-fed infants were added to infant formula. In a meta-analysis of 11 trials of infant formula supplemented with probiotics, Lactobacilli and Bifidobacterium, it was seen that when supplemented infant formula was given at or before 28 days of life and continued for at least two weeks, the infants (n=1459) had stools which were similar in appearance and consistency to the stools passed by breast fed infants and their stool bacteria were similar to those of breastfed babies. Ten trials reported that supplemented formula was well tolerated but one reported significantly increased incidence of diarrhea, irritability and eczema in the infants receiving probiotic supplemented formula.[15] In a meta analysis of 35 trials no benefit was seen in probiotic, prebiotic or synbiotic addition to infant formula. It provided no improvement in growth or in

protection from diseases including "infantile colic" or excessive crying. No adverse effects were noted.[16] This review published in 2012 did not support routine supplementation of term infant formula with probiotics, prebiotics or synbiotics.

Thus probiotics are not recommended to treat or prevent infantile colic which generally resolves on dietary changes and parent counselling. However a meta-analysis concluded that Lactobacillus reuteri may be beneficial in reducing colic (excessive crying) in exclusively breastfed babies.[17]

Necrotizing Enterocolitis

The pathogenesis of necrotizing enterocolitis (NEC) which occurs in prematurely born neonates or small infants is multifactorial. Abnormal gut flora may play a role.

Normally in the first month of life there is an enormous shift from sterile milieu in utero to microbial colonization of gut. Normally *Lactobacilli* and Bifidobacterium predominate in infants. Antibiotic usage and the NICU environment hinder development of normal gut flora in these in infants; this may be one of the factors causing NEC.

Thus probiotics therapy seems logical for preventing NEC. A meta-analysis of trials using Lactobacillus, Bifidobacterium, *Streptococcus salivarius* and *Saccharomyces boulardii*, showed that Infants who received probiotics vs placebo were less likely to get severe NEC and had a lower mortality rate. Also there was no difference in the rate of nosocomial sepsis in the groups receiving probiotics vs placebo.[18-19] A follow up of one of the study groups showed no difference in neurodevelopment at 3 year age.[20]

Complications of Probiotics Use

The use of probiotics still remains a concern in the prematurely born vulnerable population, as systemic infections have occurred with probiotics in physically impaired hosts.Lactobacillus septicemia occurred in two children with short bowel syndrome who were receiving receiving Lactobacillus rhamnosus GG supplementation.[21]

Saccharomyces cerevisiae was recognized as an emerging infectious disease in adults when three case occurred in a two week period in an ICU. The only risk factor was treatment with probiotic containing S. boulardii (manufacturer, Bristol-Myer Squibb) for prevention of Clostridium difficile associated diarrhea. Patients had received the probiotic by nasogastric tube for mean 8.5 days before positive blood culture. Only two of four control hospitalized patients had received the probiotic. Surveillance cultures of controls were negative and S.cerevisiae with DNA identical to the blood culture isolate was cultured from the probiotic capsules.Literature review showed this fungemia to be associated with probiotic therapy in 50% patients, and with high mortality.[22]

S. cerevisiae fungemia has also been reported in an immunocompromised patient who was not treated with *S. boulardii* preparation[23] and there is a report of catheter related fungemia caused by *S. cerevisiae* in a newborn.[24] With so many patients and healthy caregivers taking probiotics as food supplement, it remains a possibility that these two cases were also related to accidental exposure to this otherwise uncommon pathogen.

The potential for contamination of probiotic preparations by other microbes with disastrous results is further illustrated by the following report: An infant developed a fatal case of intestinal mucormycosis with Rhizopus oryzae, after receiving a probiotic supplement on first four days of life. The product (Solgar ABC Dophilus powder) was supposed to contain Bifidobacterium lactis, Streptococcus thermophilus and Lactobacillus rhamnosus but the unopened samples were found to be contaminated with the fungus Rhizopus oryzae.[25]

The probiotic preparations can produce unexpected allergic results as their contents are not labelled fully. To study this risk skin tests were performed on children who were allergic to cow milk protein using three probiotic preparations. All showed an allergic response to these products (Reuterin (Lactobacillus reuteri, Bio Gaia), Fiorilac and Dicoflor). This led allergists to advise that children who are allergic to milk should not receive probiotic preparations which contain cow milk protein and that all these products should be labelled clearly, for the potential presence of cow milk protein.[26]

Children with autism often have gastrointestinal problems and diarrhea. Probiotics have been tried but no benefit nor was any harm documented.[27]

Role of Probiotics in Promoting Immunity

Although probiotics are generally promoted for use in GI disorders, they are of immense interest to allergists and pediatricians dealing with eczema and asthma.

Gut microbiota confer specific immune protective effects which are mediated through complex pathways within gut associated lymphoid tissue (GALT). This is mediated by local IgA synthesis and production of tolerogenic dendritic cells and regulatory T cells. These effects may have influence on the immune system of the whole body. The regulatory T cell population of GALT produces immunomodulatory cytokines, Interleukin 10 (IL 10) and transforming growth factor beta (TGF-beta).[28]

Atopic dermatitis, eczema, is a chronic inflammatory skin condition which generally starts in infancy.Topical corticosteroids and topical calcineurin inhibitors (tacrolimus and pimecrolimus) are the mainstay of management. Skin and nares of children with eczema are often colonized with Staphylococcus aureus In meta-analysis of trials using probiotics, the supplementation was found to decrease the incidence of eczema if it was given

to mothers during pregnancy and to babies in early infancy.[29] However a prior meta-analysis of 39 trials using probiotics on young infants had failed to show any reduction of eczema but it was found that babies receiving probiotics were more likely to spit up.[30]

The failure of probiotics in preventing eczema can be understood by following the process by which the functionally immature neonatal immune system develops a complex balance between host defences and immune tolerance and this can only occur in presence of gut flora.

Animal experiments with germ free mice show that immune function can develop if normal gut flora is restored but only in young animals. So if probiotic are given to develop a healthy gut flora and thus help develop a normal (non-allergic) response to antigenic challenge, they will need to be given very early in life.[31]

The possibility of promoting commensal bacteria and eliminating colonization of upper airway with pathogens has drawn attention in management of children who are prone to recurrent episodes of acute otitis media (AOM).

In theory administration of probiotics to a child after antibiotic treatment of AOM will re-colonizes the nasopharynx with bacteria that will hinder the growth of pathogenic bacteria and thus end nasopharyngeal colonization with the pathogens preventing recurrence.

However in two randomized trial, each of which included more than 200 children with recurrent otitis media, treatment with oral probiotics produced no change in outcome.[32,33]

Another placebo controlled trial which used the novel approach of intranasally administered spray of Alpha Streptococci kept more children AOM free for three months (42% vs 22%).[34]

Discussion

The benefit of probiotics use is outlined in Table 4. It shows that in otherwise healthy infants and children, probiotics (*Lactobacillus acidophilus*, Bifidobacterium and Saccharomyces) moderately decrease the severity and duration of acute infectious diarrhea. They are a better choice than antibiotics, smectite and nitazoxanide.

In undernourished children Lactobacillus reuterii GG decreased frequency and duration of diarrhea.
S. boulardii seems preferable in the prevention and treatment of antibiotic associated diarrhea and may be used in children receiving outpatient antibiotic therapy.

The beneficial effects may be related the type of organism being used and generalization may not be valid.

Probiotics are living organism. They cannot be sterilized and thus they carry the risk of being contaminated with pathogens and causing infections. Probiotics have the potential to cause systemic infection when mucosal or skin barriers are breached. Given the widespread use and the large numbers of living microbes in each dose we need to identify high risk groups of patients who may develop systemic disease, even if these sachets are opened in the their room, ward, ICU or NICU.

These high risk groups include all patients with central lines, all immune compromised patients; these include patients on chemotherapy with mucositis and/or neutropenia, organ transplant recepients, patients with AIDS and third degree

Table 4: Diseases in Infants and Children in Which Probiotics Have Been Used for Treatment and Prevention

Diseases	Prevention	Treatment
Gastrointestinal Diseases		
Diarrhea		
Acute infectious diarrhea (especially rotavirus)	++	++
Traveler's diarrhea	+	?
Antibiotic-associated diarrhea	++	+
Clostridium difficile	+	?
Infantile Colic	?	+
Inflammatory bowel disease		
Ulcerative colitis	?	+
Crohn disease	?	-
Helicobacter pylori gastritis	?	+
Hepatic encephalopathy	+	+
Irritable bowel syndrome	-	+
Constipation	-	-
Lactose intolerance	-	+
Pancreatitis	-	-
Allergic/Atopic Disease	+	+
Upper Respiratory Tract Infections	+	-
Neonatal Disease		
Necrotizing enterocolitis	++	-
Neonatal Sepsis	++	-
Cancer	?	?

\+ indicates limited evidence or conflicting evidence for benefit
++ indicates clear evidence for benefit.
\- Indicates no clear evidence for benefit or possible deleterious effect.
? Indicates no studies conducted to date

From Feign and Cherry's Textbook of Pediatric Infectious Diseases. 7th edition,2014.
Harrison GJ. Probiotics chapter 242, Page 3371.

malnourished young children; who frequently present with presumed infectious diarrhea.

If probiotics are used in the inpatient setting, rules should be made to open the sachets or capsules in a room away from the high risk patients and the site of intravenous fluid handling and preparation. The person mixing them in fluid or milk for in-patient oral or NG administration should wear disposable gloves and apron to not track these germs to the high risk patients.

Probiotics are stimulators of immune system; theoretically probiotics might cause excessive immune stimulation leading to autoimmune type disorders.

The risk of Gene transfer from probiotic bacteria to gut bacteria should be regarded as a risk especially when antibiotic resistant strains are used. Use of antibiotic resistant bacterial strains as probiotic, would not only prevent treatment of a rare probiotic infection but may even allow transfer of genetic material conferring resistance to other gut flora.

Strains used for probiotic therapy should be chosen from commensal human flora and bacterial strains should not carry intrinsic resistance to antibiotics. One of the preparations being given to small infants (Enterogermina drops) is in flagrant disregard of this safeguard.

These problems will be ultimately solved when biologically active isolated products of microbial agents become available and are used in probiotic therapy.

Conclusions

Therapeutic use of some probiotics, Saccharomyces boulardii and Lactobacillus species have been efficacious in decreasing the duration of infectious diarrhea and risk of antibiotic associated diarrhea. Probiotics are protective against development of NEC in prematurely born infants. There is no benefit of probiotic supplementation of normal infants.

The importance of normal gut flora in development of immunity and normal enterocyte function emphasizes the need to limit unnecessary antibiotic use in respiratory viral infections and diarrhea; Rather than correcting the subsequent deleterious effects on microflora.

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