Mini-Reviews and Perspectives

Probiotics, Enteric and Diarrheal Diseases, and Global Health

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Enteric and diarrheal diseases are a major worldwide cause of death among children under the age of 5. In this age group, diarrhea occurs 2.5 billion times annually[1] and causes 15% of childhood deaths.[2] Diarrheal diseases claim 59 million disability-adjusted life years, nearly all from children in low- and middle-income countries.[3] Despite this enormous burden, these numbers fail to capture the full impact of enteric and diarrheal diseases. Early and frequent exposure to intestinal pathogens begins a cycle (Figure 1A) that affects digestion, nutrient absorption, growth, and immunity.[4] Repeated infections, with either overt diarrhea or subclinical enteropathy, produce acute and chronic undernutrition,[5] which leads to more frequent and severe infections.[6] Undernutrition contributes to 53% of childhood deaths[7] and is the leading risk factor for poor health outcomes in childhood[8]; survivors are at risk for developmental deficits in growth, fitness, and cognition that persist into adulthood with devastating consequences.[4] These consequences have a multiplicative effect on calculations of disability-adjusted life years from diarrheal disease.[9]

Fortunately, there are strategies to break this cycle, although each approach has limitations. Sustainable access to potable water and improved sanitation reduces pathogen exposure; a $70 billion annual investment would only begin to reduce the 2.5 billion people without these necessities by 2015.[10] Antimicrobial agents are effective against specific pathogens but are expensive and can exacerbate toxin-mediated diseases, disrupt the human microbiome, and induce antibiotic-associated diarrhea as well as drug resistance. Immunization with enteric vaccines can reduce the burden of severe diarrhea, but vaccines must be kept in the cold, only protect against specific pathogens, and are less effective in regions of high mortality. For example, the efficacy of the live, attenuated rotavirus vaccine against severe disease is only 48.3% in southeast Asia[11] and 39.3% in sub-Saharan Africa.[12] Zinc reduces the propensity to develop recurrent diarrhea and oral rehydration solution attenuates overt symptoms of diarrhea and dehydration.[13] However, these approaches do not adequately address broader growth and developmental processes that could yield long-term benefits.

Likewise, trials of therapeutics to reduce diarrhea severity, unplanned intravenous fluid administration, or duration of hospitalization fail to address the longer term, initially subclinical consequences of recurrent infections. Complementary outcome measures, including measures of growth and biomarkers of acute intestinal inflammation, barrier disruption, and impaired immunity, would provide greater insight into underlying pathology and therapeutic efficacy. Use of these measures could reduce acute, overt, as well as chronic, often unrecognized, intestinal diseases (Figure 1B).

No single intervention is sufficient to eliminate the global burden of enteric and diarrheal diseases. Vaccines, for example, can protect against limited infectious agents, but immunization can be overwhelmed by heavily contaminated water. Multiple interventions could work synergistically, such as the combination of improved water and sanitation, vaccines, micro- and macronutrient provision, and selectively targeted antimicrobial therapy (eg, single-dose albendazole for intestinal helminths). Do current global health strategies use the best available interventions?

One underexplored approach—probiotics—could combine favorable safety profiles with improved nutrition and microbiome function. Probiotics are live micro-organisms that confer a health benefit on the host,[14] and have been used to treat multiple gastrointestinal (GI) diseases.[15] Microbes are inexpensive to grow, and have the potential for rapid global scale-up. Is there compelling evidence to recommend developing probiotics-based strategies to complement current approaches against enteric and diarrheal diseases for children in developing countries? If so, what steps must be taken before these therapies are ready for clinical impact in the global health arena?
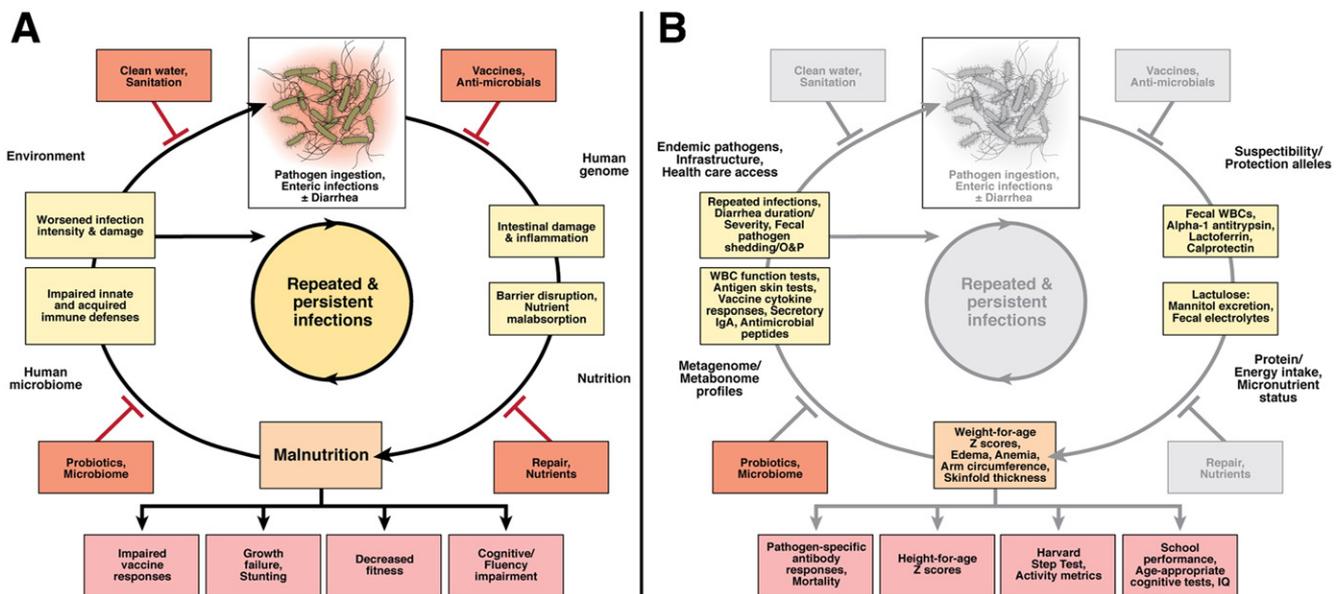
Figure 1. The vicious cycle of diarrhea and undernutrition in susceptible children. (*A*) The devastating synergy between enteric infections and undernutrition is influenced by the environment, the human genome, host nutrition, and the human microbiome. Various interventions (*red boxes*) may inhibit progression to the next step in the cycle, minimizing both acute and chronic morbidities. (*B*) Employing a spectrum of disease outcome measures would lend greater insight into the pathology underlying enteric and diarrheal diseases, while providing a more complete understanding of interventions targeting basic steps of enteric and diarrheal disease pathogenesis. Adapted with permission from Wiley: *Nutrition Reviews*,[4] copyright 2008. Available from: http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1753-4887.

Clinical Evidence

In 1907, Russian Nobel laureate Elie Metchnikoff suggested that ingestion of microbes could benefit human health.[16] As we learned about multidrug-resistant pathogens and the role of the human microbiome in health and disease, numerous trials showed the safety of probiotics[17] and their beneficial outcomes in patients with various illnesses.[18] A systematic review that included 12 randomized, controlled trials (RCTs) in the Cochrane database (the majority from affluent countries) concluded that probiotics reduced the mean duration of acute diarrhea in children by 29.2 hours in a fixed-effects model and by 30.48 hours in a random-effects model.[19] Two meta-analyses that evaluated similar studies found statistically significant but modest reductions of diarrhea duration.[20,21] Although combination analyses of trials with microbes of different genera, species, strains, and doses provide limited information about specific therapeutic interventions, it is clear that many probiotics reduce the duration of acute diarrhea.

There are studies of probiotics for enteric and diarrheal diseases targeting children in developing regions (Supplementary Table 1). The majority of RCTs studied acute gastroenteritis and reported modest reductions in diarrhea duration. However, the effects of probiotics were statistically equivalent to those of placebo in 25% of trials. Some of the negative results might be attributable to small sample size or administration of insufficient

doses[22-24]; each trial must be viewed in the context of the specific disease and probiotic strain analyzed. Two RCTs evaluated probiotics for children with persistent diarrhea and reported dramatic reductions in diarrhea duration—4.8 and 3.9 days in Argentina[25] and India,[26] respectively. Two trials evaluated probiotics for diarrhea prevention; children in Peru had 13% fewer diarrheal episodes after 15 months of *Lactobacillus rhamnosus*,[27] whereas diarrhea frequency was reduced by 14% among children in India who received daily doses of *Lactobacillus casei* for 12 weeks, with a 12-week follow-up period.[28]

Few studies have examined markers of acute or chronic immunity or underlying intestinal function. Administration of *Bifidobacterium bifidum* and *Streptococcus thermophilus* increased numbers of CD4+ T cells in HIV-infected Brazilian children.[29] A similar effect was observed after administration of *L rhamnosus* to HIV-infected adults in Tanzania.[30] Tropical enteropathy was studied in Malawian children using urinary carbohydrate excretion; *L rhamnosus* failed to improve ratios of lactulose:mannitol excreted,[31] despite evidence that probiotics ameliorated GI permeability defects in children with atopic dermatitis in Germany.[32] Probiotics improved growth among healthy children in Thailand[33] and Estonia,[34] but not among HIV-exposed infants in South Africa.[35] In Malawi, probiotics failed to improve nutrition status in severely malnourished children who received inpatient nutritional rehabilitation. Despite the negative primary out-

come, there was a trend toward decreased mortality among children treated with probiotics on an outpatient basis.[36] Probiotics also reduced the duration of rotavirus shedding in India.[37] Strategies to reduce fecal shedding of pathogens are important for the billions of people who live without adequate sanitation.

Probiotics could also have a role in immunization programs. *L rhamnosus* increased the virus-specific antibody response in children with acute rotaviral gastroenteritis,[38] so immunostimulatory probiotics might help children's immune systems to increase the memory responses to vaccines. Based on initial data from studies in industrialized regions, *Bifidobacterium longum* and *L rhamnosus*, administered during the first 6 months of life, increased vaccine-specific antibody production after vaccination against hepatitis B.[39] Infants that were given *Lactobacillus paracasei* from 4 to 13 months of age had increased titers of antibodies to *Haemophilus influenzae* type B capsular polysaccharide, diphtheria toxin, and tetanus toxoid.[40] Concentrations of antibodies against *H influenzae* type B increased among infants when women were given daily doses of probiotics during the final month of pregnancy; therapy continued for infants during their first 6 months of life.[41] Taking probiotics during pregnancy and lactation seems to be safe[42] and may yield postnatal benefits. Intriguingly, maternal consumption of *L rhamnosus* or *Bifidobacterium lactis* increased the amount of immunoglobulin A detected in breast milk.[43] Increased immunoglobulin A levels in breast milk might

protect infants from enteric pathogens and serve as a biomarker for studies of probiosis in lactating women.

It is a challenge to assimilate and analyze all the clinical evidence of the effects of probiotics. Study quality varies, randomization and blinding methods are rarely reported, and appropriate placebos are not always used. Exclusion criteria are numerous, limiting the generalization of findings to children who are very ill. Probiotic strain designations, bacterial growth phase, and variations in administration (in fermented dairy products, infant formula, solid food, oral rehydration solution, water, juice, capsules) are often unreported. It is important that studies report these parameters so that findings can be reproduced—proteins and metabolites synthesized by live microorganisms are strain specific and vary with growth conditions. Many probiotics have shown beneficial effects, but improving our knowledge of the mechanisms that mediate these effects would facilitate identification of more potent probiotics for specific applications.

Probiotic Mechanisms

There are 3 general classes of probiotic antipathogenic mechanisms: direct antagonism, immunomodulation, and exclusion (Figure 2).

Direct Antagonism

Many probiotics secrete small molecules or bioactive peptides that have antimicrobial activities. *Lactobacil-*

Figure 2. Three general mechanisms of probiosis for enteric infections. In direct antagonism, probiotics kill or inhibit the pathogen to limit infection, or they down-regulate the expression of virulence factors, such as adhesins or toxins, required for pathogenesis. Probiotics can also interact with the immune system (immunomodulation) to enhance the functionality of innate and/or adaptive immunity, or to limit the ability of the pathogen to initiate or facilitate an immune response. Through "exclusion," probiotics can alter the microenvironment to prevent pathogens from gaining access to appropriate receptors, to limit pathogen attachment, entry, or translocation, or to improve barrier function. A beneficial microbe may use a combination of these mechanisms and may employ different mechanisms against different pathogens. B, B cell; DC, dendritic cell; IEC, intestinal epithelial cell; M, M cell; MAC, macrophage; T, T cell; TJ, tight junction.

lus salivarius UCC118 protects mice against infection with *Listeria monocytogenes*; UCC118 produces a broad-spectrum bacteriocin (antimicrobial peptide) that kills *Listeria* in the lumen of the GI tract, preventing translocation and systemic spread of infection.[44] Mice were not protected when they were fed a derivative of UCC118 that no longer produced the bacteriocin or when they were infected with an engineered strain of *Listeria* that was resistant to the bacteriocin. *Listeria* pathogens were eliminated within 30 minutes after oral administration of UCC118, indicating a direct effect of the probiotic on the pathogen. UCC118 also protects mice against *Salmonella* infection, but the bacteriocin is not involved. Thus, a single probiotic can protect mammals against different pathogens via multiple mechanisms.

Several in vitro studies have reported down-regulation of virulence factors in pathogens exposed to probiotics or their cell-free supernatants.[45-47] *Lactobacillus acidophilus* LA-5 suppresses transcription of *Escherichia coli* O157:H7 genes involved in adherence; this corresponds with reduced colonization in mice.[48] Probiotics can also interfere with toxin production or directly antagonize enterotoxins. *Saccharomyces cerevisiae* var. boulardii (*S boulardii*), which reduces *Clostridium difficile*-associated diarrhea,[49] secretes a 54-kDa serine protease that hydrolyzes toxin A (a *C difficile* virulence factor) and its receptor, which is present in the intestinal brush border.[50,51]

Immunomodulation

Probiotics elicit a variety of responses from immune cells in vitro and in vivo, through mostly unknown mechanisms.[52] The responses of specific immune cells to particular microbes result from complex interactions between surface-bound and secreted ligands (eg, pathogen-associated molecular patterns) and host Toll-like receptors (TLRs).[53] Reductionist approaches to studying these interactions, such as analyses of knockout or transgenic mice, have provided limited information about probiotic immunomodulation. In one successful example, the presence of D-alanines in teichoic acids in the cell wall of *Lactobacillus plantarum* elicited production of proinflammatory cytokines by peripheral blood mononuclear cells.[54] Co-culture of dendritic cells with polysaccharide A derived from *Bacteroides fragilis* induced naïve T cells to generate an interleukin-10–producing regulatory T-cell population.[55] Purified polysaccharide A prevents intestinal inflammation in multiple mouse models.[56]

The immunomodulatory effects of probiotics can be species-[57] and strain-specific,[58,59] and involve multiple mammalian signaling pathways that affect immune cell phenotypes. Some probiotic *Lactobacillus* strains increase production of tumor necrosis factor through activation of the transcription factors nuclear factor-κB and STAT,[60] whereas others suppress tumor necrosis factor

production by inactivating nuclear factor-κB[61,62] or mitogen-activated protein kinase and c-Jun signaling.[58] Differential immune regulation might prime the immune system to limit infections, inflammation, and pathogen-mediated damage. Probiotic signaling molecules that regulate immunity have not been identified.

Exclusion

Exclusion is used as a "catch-all" term for probiotic mechanisms that make the GI environment less hospitable for pathogens. These mechanisms include altering the resident microbiota, decreasing luminal pH, improving epithelial barrier function, interfering with pathogen binding by down-regulating specific host receptors, and stimulating production of defense-associated factors, including mucins and defensins. Multiple probiotics have been implicated in each of these functions, but clear links between individual bacterial compounds and specific responses have been difficult to establish.[63]

Rats that consumed the probiotic mixture VSL#3 increased their luminal mucin content by 60% through an unidentified, heat-resistant, secreted, soluble compound.[64] Some bacterial products, including short-chain fatty acids produced by fermentation, can stimulate epithelial cell differentiation and improve barrier function[65,66]—this protects against pathogens that cause disease through loss of tight junction integrity, increased paracellular transport, fluid loss, and invasion of the submucosa.[67,68] Indole, an aromatic compound secreted by commensal *E coli* and detected in human feces, increases expression of genes whose products regulate production of mucins and organization of the cytoskeleton, tight junctions, and adherens junctions. Indole increases transepithelial resistance in enterocyte cultures.[69] The quorum-sensing molecule CSF, a 3-kDa heat-stabile, pepsin-sensitive pentapeptide from the probiotic *Bacillus subtilis*, activates heat shock protein 27 (Hsp27) after CSF is internalized by the enterocyte oligopeptide transporter OCTN2; Hsp27 activation protects epithelial cells from oxidant-induced stress.[70] The in vivo roles of these molecules in preventing infections have not been established.

Probiotics can also stimulate defensins, cationic antimicrobial peptides produced by cells of the intestinal epithelium.[71] The probiotic *E coli* Nissle 1917 increases synthesis of human β-defensin 2 by activating nuclear factor-κB and AP-1[72] via secretion of flagellin.[73] Increased β-defensin 2 levels were detected in stool samples from healthy volunteers 9 weeks after administration of nonpathogenic *E coli*.[74] Resistance to host-derived antimicrobials may be another important probiotic property.[75] Finally, some probiotics promote class switching to increase immunoglobulin A production, by inducing en-

terocytes to secrete B-cell stimulatory factors such as APRIL. Lipopolysaccharide- and flagellin-stimulated secretion of APRIL occurs in human enterocytes via TLR4 and TLR5 signaling.[76]

In addition to antimicrobial mechanisms, probiotics benefit host physiology, nutrition, and the ability to counteract pathogenesis. In mouse models, weight gain and adiposity are influenced by the intestinal microbiome.[77-79] Metabolism could be regulated by individual microbes; for example, *S boulardii* increases activities of brush border enzymes including sucrase, maltase, trehalase, lactase, aminopeptidase, and alkaline phosphatase.[80] Intestinal bacteria also synthesize niacin, pantothenic acid, biotin, folic acid, and vitamins K, C, and B_{12}.[81] These functions of probiotics have not been correlated with pathogen resistance.

Probiotics may also interact with the enteric nervous system to attenuate secretory diarrhea.[82] In mice, *Lactobacillus* inhibited postinfective intestinal hypercontractility through an unidentified, heat-labile fermentation product.[83] In rats, lactobacilli reduced hypercontractility by blocking calcium-dependent potassium channels.[84,85] Lactobacilli can also blunt visceral pain responses by increasing expression of enterocyte opioid and cannabinoid receptors[86] or by inhibiting sodium channels.[87] Further studies are needed to identify microbe molecular signatures associated with specific responses against pathogens.

Using Probiotics Worldwide

Step 1: Identify Molecular Mechanisms of Probiosis and New Therapeutics

The first step to realizing the full potential of probiotics is to define the specific microbial genes, small molecules, and host–microbe interactions that mediate their beneficial functions. Basic scientists must identify, isolate, and characterize bacterial fermentation products, immunomodulatory factors, antimicrobial agents, and cell-wall components that produce discrete physiologic effects through specific host interactions. These types of studies will improve our understanding of probiotic function and allow microbial libraries to be screened to identify new probiotics.

The Human Microbiome Project, MetaHit, and the International Human Microbiome Consortium published a partial catalog of microbial reference genomes to help identify new probiotic species[88]; studies to associate changes in microbial populations[89] or microbial gene content[90] with states of health and disease are underway. For example, the anti-inflammatory effects of *Faecalibacterium prausnitzii* were identified after reductions in this bacterium were associated with recurrence of ileal Crohn's disease.[91] However, Human Microbiome Project and International Human Microbiome Consortium studies have been limited to subjects in affluent, developed countries. Diarrhea disrupts the microbiota,[92] and environmental and lifestyle variations yield microbiomes that are specific to geographic regions, cultures, or ethnic groups.[93,94] The composition and function of GI microbiomes of undernourished children in disease-endemic regions must be studied separately from the microbiomes of people in developed regions.

Step 2: Develop New Biomarkers for Acute and Chronic Intestinal Disease

To more accurately assess intestinal pathology and therapeutic efficacy, new host and microbial biomarkers must be validated. Fecal samples are easily obtained, but their microbial composition primarily reflects that of the large bowel, a self-regulating community that can resist introduction of probiotics by virtue of niche exclusion.[95] Lactobacilli, administered daily, comprise only 0.001% of the fecal microbiota and quickly disappear once they are no longer ingested—they have only a minor presence in the large bowel biome.[96,97] Many enteric pathogens infect the small bowel, overgrowth of which is a significant feature of tropical enteropathy.[98] Thus, probiotics are likely to mediate their greatest effects against enteric and diarrheal diseases in the small bowel, where they comprise a substantial proportion of the biomass and functionally alter the proximal GI tract.[99] Ideal biomarkers would reflect the health status of these sites.

Metabolomics is a systematic, quantitative analysis of changes in the complete set of low-molecular-weight metabolites produced by cells in response to environmental or cellular changes.[100] Bacterial products are absorbed from the bowel lumen into lymph and blood circulations; body fluids, therefore, contain many bacterial and host metabolites that could serve as biomarkers of relationships between food, bacteria, and host cells and indicate health or disease.[101] Infection of mice with the nematode *Schistosoma mansoni* can be diagnosed based on alterations to the urinary metabolome, which reflect disruption of the bowel ecosystem by the intestinal parasite.[102] Urine, saliva, or bowel fluid, which are abundant and easily collected, could be sources of biomarkers for small bowel function.

Analysis of metabolomic profiles lags behind advances in detection methods, and new pattern recognition systems must be developed. Comprehensive reference sets of identified metabolites must be assembled to improve yields from metabolomic comparisons.[103] Linking metabolomics to probiotics research could lead to new ways to identify biomarkers and have practical applications in developing countries. Initial studies should compare metabolomes between healthy children and those with defined enteric infections, controlling for ethnicity, age, gender, socioeconomic status, and nutritional state. Biomarkers are likely to be identified that are associated with overt and subclinical intestinal disease.

Step 3: Optimize Therapeutic Regimens for Specific Populations

High-impact interventions must be developed for target populations—namely, children <2 years of age in disease-endemic areas. Core and variable components of their intestinal microbiomes should be catalogued. Children should be characterized with respect to overall health status, disease susceptibility, nutritional (macro and micro) status, and common enteric pathogens. Dietary evaluations must consider microbiomes and glycomes of breast milk and seek to identify natural substrates for probiotics that optimize their metabolic activities. This detailed picture of the microbiome and its interactions will guide selection of specific probiotic-based therapies for the pathogens relevant to each population.

Prospective RCTs should aim to reduce short-term pathologies associated with acute diarrhea, prevent long-term morbidities from recurrent or persistent infections, and increase vaccine efficacy. Specific strain and dose recommendations should be made, with the long-term goals of improving survival, growth, and development during childhood. With trials in multiple geographic locations and ethnic groups, patterns will emerge to guide selection of specific microbial-based therapies for specific regions of the world.

This plan has risks; although probiotics are assumed to be safe, undernourished children with immune and GI permeability defects could be more prone to bacterial translocation and sepsis. Safety monitoring will be critical. Immunostimulatory probiotics might not affect children whose immune systems have been highly stimulated by contaminated environments; in this case, other mechanistic bases of probiosis must be pursued. Finally, global application of probiotic therapies requires development of technologies to make freeze drying or other preparative methods of preserving probiotic viability feasible under challenging conditions. Strategies must also be developed to deliver probiotics through local distribution networks, and the products must be acceptable to diverse cultures.

Will Probiotics Be Ready for Worldwide Use in the Near Future?

Beyond the acute effects of severe diarrhea and dehydration, repeated and persistent infections yield devastating long-term consequences. For children in less-developed settings, many probiotics are effective for acute gastroenteritis, persistent diarrhea, and diarrhea prevention; their potential roles in growth, immunity, and vaccine efficacy must be further evaluated. Mechanisms that mediate their beneficial effects are being elucidated. Characterization of specific molecular interactions between probiotics and the host or microbiome will enable selection of more potent therapeutic microbes. Biomarkers of intestinal pathology must be developed to determine therapeutic efficacy of existing and new probiotics. Ultimately, clinical studies that test specific therapies in well-defined populations must be performed to determine efficacy against the overt and hidden consequences of enteric infections.

Basic scientists, clinical researchers, and industrial leaders have the opportunity to work together against one of the most pressing health problems facing the world today. If we accept the challenge, probiotic-based therapies might be incorporated into global health strategies, to reduce the burden of enteric and diarrheal diseases borne by millions of children worldwide.

Supplementary Material

Note: The first 5 references associated with this article are available below in print. The remaining references accompanying this article are available online only with the electronic version of the article. To access the remaining references, as well as additional supplementary data, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi:10.1053/j.gastro.2010.11.010.

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Supplementary Table 1. Summary of Prospective, Randomized Controlled Trials Investigating the Effects of Probiotics in Children With Enteric and Diarrheal Diseases in Less-Developed Regions

Location	Inclusion criteria	Exclusion criteria	n	Intervention	Outcomes	Reference
Acute diarrhea						
Pakistan, pediatric hospital	Children 1–24 m with >3 watery stools in previous 24 h for <14 days, moderate/severe dehydration	Severe malnutrition (<50% NCHS standard), suspected septicemia	40	L $rhamnosus$ GG, 10^{10}–10^{11} cfu freeze dried powder or placebo in 10 mL ORS every 12 h for 2 days	No change in stool frequency, stool output, or weight gain; reduced vomiting frequency at 2 days (4.0 to 2.0 per day); in watery diarrhea subset, reduced stool frequency (6.6 to 4.4 per day) and percent with diarrhea (75.0% to 31.3%) at 2 days	Raza et al., 1995[104]
Thailand, pediatric hospital	Children 1–24 mo with >3 watery stools in previous 24 h for <14 days	Exclusive breast feeding, septicemia	39	L $rhamnosus$ GG, 10^{10}–10^{11} cfu freeze dried powder or placebo in 10 mL ORS every 12 h for 2 days	No change in diarrhea duration or stool frequency; in watery diarrhea subset, reduced diarrhea duration (3.3 to 1.9 days) and stool frequency at 2 days (5.2 to 3.5 per day)	Pant et al., 1996[105]
Russia, pediatric hospital	Children 1–36 mo with ≥1 watery stool in previous 24 h for <5 days	None reported	123	L $rhamnosus$ GG, 5×10^9 cfu dried powder or placebo in 5 mL water and mixed with ORS or other drink/food, twice daily for 5 days	Reduced diarrhea duration (3.8 to 2.7 days); no difference in weight gain or hospital stay	Shornikova et al., 1997[106]
India, 2 pediatric hospitals	Males 4–48 mo with ≥5 liquid stools in previous 24 h for ≤96 h, malnutrition (≤80% NCHS standard)	Severe non-GI illnesses, gross blood in stools, exclusive breast feeding	102	S $thermophilus$ + L $bulgaricus$, 120 mL/kg/24 h yogurt made from 90 g standard starter in formula milk, or nonfermented milk, in 7 divided feedings daily, for 72 h or until recovery, whichever was later	No change in diarrhea duration or stool output; decreased percent weight gain (0.5 to –1.6%)	Bhatnagar et al., 1998[107]
Thailand, pediatric hospital	Children 3–24 mo with acute watery diarrhea ≤5 days, mild/moderate dehydration	Mucus bloody stools or major systemic illness	73	L $acidophilus$ LB, 2×10^{10} lyophilized heat-killed bacteria with 160 mg lyophilized fermented culture medium or placebo daily, mixed in 5 mL water, 5 doses over 48 h	Decreased diarrhea duration (57.0 to 43.4 h); increased percent with formed stools (2.8% to 18.9%); in subset not receiving antibiotics before admission, decreased diarrhea duration (74.0 to 42.9 h); in subset with rotavirus, decreased percent with diarrhea (56.3% to 15.8%)	Simakachorn et al., 2000[108]
Brazil, pediatric hospital	Males 1–24 mo with ≥3 watery/loose stools/24 h during ≥1 24-h period in previous 72 h, signs of moderate dehydration by WHO criteria	Severe malnutrition (≤65% NCHS standard), systemic infections requiring antibiotics, bloody diarrhea	124	L $rhamnosus$ GG, 10^{10} cfu capsule with 320 mg inulin or inulin alone in ORS, daily until cessation of diarrhea or up to 7 days	No change in diarrhea duration or stool output	Costa-Ribeiro et al, 2003[109]
Peru, pediatric hospital	Males 3–24 mo with ≥3 watery stools per day for <48 h, clinical signs of dehydration	Bloody stools, hypovolemic shock, coexisting acute systemic illness (ie, meningitis, sepsis, pneumonia) or chronic disease (ie, pulmonary tuberculosis), current antibiotic or antidiarrheal medication use, exclusive breastfeeding, malnutrition (<60% NCHS standard)	179	L $rhamnosus$ GG, 10^9 cfu/mL milk formula or milk alone, 150 mL/kg per day up to 1 L per day, every 4 h until cessation of diarrhea or up to 5 days	No change in diarrhea duration or hospital stay; increased total stool output (195.0 to 247.8 mL/kg)	Salazar-Lindo et al., 2004[110]
Turkey, pediatric hospital	Children 3–7 y with liquid, mucus, or bloody stools ≥2× as frequently as usual for previous 24 h–7 days	Chronic disease, malnutrition, recent history of antibiotics, antidiarrheals, or other drugs that influence intestinal motility	200	S $boulardii$, 250 mg granulated or placebo in water or juice daily for 5 days	Decreased diarrhea duration (5.5 to 4.7 days) and hospital stay (3.9 to 2.9 days)	Kurugol et al., 2005[111]
Bangladesh, pediatric hospital	Males 4–24 mo with ≥4 liquid stools/24 h for <48 h	Severe malnutrition (≤65% NCHS standard), systemic infection requiring antimicrobial therapy, bloody diarrhea, $Vibrio$ $cholerae$ by dark-field microscopy, antibiotics in previous 2 wks	230	L $paracasei$ ST11, 5×10^9 cfu lyophilized or placebo in milk formula twice daily for 5 days	No change in diarrhea duration, intravenous fluid administration, or stool output; for non-rotavirus diarrhea subset, reduced total stool output (381 to 225 g/kg) and number of stools (42.5 to 27.9)	Sarker et al., 2005[112]

Supplementary Table 1. Continued

Location	Inclusion criteria	Exclusion criteria	n	Intervention	Outcomes	Reference
India, pediatric hospital	Children 6 mo–12 y with acute watery diarrhea	Systemic infection, encephalopathy, convulsions, previous use of any pharmaceutical probiotic	98	L $acidophilus$, 1.5×10^{10} bacteria tyndalized in puffed rice powder or rice powder only, daily for 3 days	No change in diarrhea duration, frequency of stools, intravenous fluids needed, hospital stay, or weight gain; increased treatment failures (0%–8.3%)	Khanna et al., 2005[113]
Poland, pediatric hospital and outpatient department	Children 2 mo–6 y with ≥3 looser than normal stools per day for >1 but <5 days	Organic gut disease, underlying chronic disease, immunosuppressive condition or treatment, exclusive breast feeding	87	L $rhamnosus$ 573L/1, 573L/2, and 573L/3, 1.2×10^{10} cfu freeze dried or placebo in 2 mL of 10% glucose, twice daily for 5 days	No change in diarrhea duration or intravenous fluid duration; for rotavirus diarrhea subset, reduced diarrhea duration (115 to 77.5 h) and intravenous fluid duration (37.7 to 14.9 h)	Szymanski et al., 2006[114]
Indonesia, pediatric hospital	Males 3–12 mo with acute watery diarrhea >3 episodes for >24 h but <7 days	Malnutrition (<−2 SD below NCHS median), blood or mucus in stool, allergy to cow's milk, exclusive breast feeding, severe dehydration, fever >39°C, severe systemic infections, other disease requiring additional treatment	58	L $rhamnosus$ LMG P-22799, 5×10^8 cfu + 0.15 g inulin + 0.2 g soy polysaccharides + 0.4 mg zinc + 0.6 mg iron/100 mL formula, or formula only, up to 140 mL/kg per day for up to 7 days	Reduced diarrhea duration (2.45 to 1.63 days); no change in hospital stay, weight gain, or stool weight	Agustina et al., 2007[115]
Peru, 4 pediatric hospitals	Males 3 mo–4 y with ≥3 watery stools in previous 24 h for <72 h	Dehydration requiring hospitalization, bloody stools, chronic GI disease (eg, cystic fibrosis, celiac disease), chronic immunologic condition that could potentially cause diarrhea (eg, AIDS), lactose or fructose intolerance, hemodynamic abnormalities, neurologic disturbance, rectal body temperature >39.0°C, previous treatment with antibiotics or a drug interfering with intestinal motility	80	$Lactobacillus$ LB, 10^{10} killed bacteria with 160 mg neutralized supernatant spent culture medium or placebo in ORS twice daily for 5 days	No change in diarrhea duration or weight gain; in subgroup with diarrhea >24 h at presentation, reduced diarrhea duration (30.4 to 8.2 h)	Salazar-Lindo et al., 2007[116]
Argentina, outpatient pediatric department	Children 3 mo–2 y with ≥3 liquid/loose stools in previous 24 h for <7 days	Chronic intestinal disease, short bowel syndrome, ≥grade 2 malnutrition, severe disease (ie, dehydration requiring hospitalization), known immunodeficiency, use of probiotics, steroids, systemic antifungals, macrolides, or drugs that alter intestinal motility in previous 7 days	88	S $boulardii$, 250 mg capsule or placebo, once (<1 y of age) or twice (>1 y of age) daily, in liquid or solid food, for 6 days	Reduced diarrhea duration (6.2 to 4.7 days)	Villarruel et al., 2007[117]
India, pediatric hospital	Children mean age 1.2 y with ≥3 watery stools per day, stool microscopy with WBCs <10 per high power field and absence of RBCs and mucus flakes and bacteria, negative hanging drop preparation, negative bacterial stool culture	Blood or mucus in stool, systemic illness other than diarrhea, development of systemic complications of diarrhea during hospital stay	646	L $rhamnosus$ GG, 6×10^7 bacteria powder in 100 mL ORS or ORS alone, twice daily for 7 days or until diarrhea ceased, whichever was later	No change in diarrhea duration, diarrhea frequency, vomiting duration, vomiting frequency, or hospital stay	Basu et al., 2007[22]
Myanmar, pediatric hospital	Children 3 mo–10 y with acute watery diarrhea <7 days	Fever >38.5°C, severe dehydration, macroscopic blood in stool, use of antifungal drugs, severe malnutrition (<70% NCHS standard)	100	S $boulardii$, 250 mg in ORS or ORS alone twice daily for 5 days	Reduced diarrhea duration (4.7 to 3.1 days); increased percent with <3 stools per day at 2 days (30% to 54%) and 3 days (56% to 78%); increased percent with solid stools at 3 days (24% to 76%) and 4 days (60 to 98%) and 5 days (80% to 100%)	Htwe et al., 2008[118]

Supplementary Table 1. Continued

Location	Inclusion criteria	Exclusion criteria	n	Intervention	Outcomes	Reference
China, pediatric hospital	Children 6–36 mo with severe acute diarrhea for <48 h	Moderate/severe malnutrition (<75% NCHS standard), breast feeding, need for antibiotic therapy, allergy to cow's milk, GI or other chronic pathologies	224	B $lactis$ BB12, 10^8 cfu/g, + S $thermophilus$ TH4, 5×10^7 cfu/g lyophilized in milk-based lactose-free formula or BB12, 10^9 cfu/g, + TH4, 5×10^8 cfu/g, in formula or formula alone until 24 h after diarrhea had ended	No change in diarrhea duration, stools per day, or liquid stools per day; low-dose formula increased weight gain (27.5 to 38.3 g/kg)	Mao et al., 2008[119]
India, pediatric hospital	Children 3 mo–3 y with acute rotaviral diarrhea ≤3 days	Infectious diarrhea other than rotaviral, serum sodium >155 or <130 mmol/L, history of malabsorption syndromes, current respiratory or systemic infection	80	S $faecalis$ T-110, 3×10^7 bacteria, C $butyricum$ TO-A, 2×10^6 bacteria, $B.$ $mesentericus$ TO-A, 10^6 bacteria, and L $sporogenes$, 5×10^7 bacteria, in 20 mL water 3 times daily until recovered or up to 14 days	Reduced diarrhea duration (5.45 to 4.35 days) and percent with rotavirus shedding at hospital discharge (24.3% to 5.1%)	Narayanappa et al., 2008[37]
India, pediatric hospital	Children 6 mo–2 y with acute rotaviral diarrhea for <72 h	Systemic infection, chronic underlying disease, malnutrition (<60% NCHS standard), vomiting, need for antibiotics	224	VSL#3 (L $acidophilus$, L $paracasei$, L $bulgaricus$, L $plantarum$, B $breve$, B $infantis$, B $longum$, S $thermophilus$), 9×10^{10} bacteria or placebo in breast milk, formula milk, ORS or water, 2–4 sachets per day according to body weight, for 4 days	Reduced failure rate (38.7% to 7.1%) and mean stool frequency; improved stool consistency	Dubey et al., 2008[120]
India, pediatric hospital	Children mean age 1.6 y with ≥3 watery stools per day, stool microscopy with WBCs <10 per high power field and absence of RBCs and mucus flakes and bacteria, negative hanging drop preparation, negative bacterial stool culture	Blood or mucus in stool, systemic illness other than diarrhea, development of systemic complications of diarrhea during hospital stay	559	L $rhamnosus$ GG 10^{10} or 10^{12} cfu bacteria powder in 100 mL ORS or ORS alone, twice daily for 7 days or until diarrhea ceased, whichever was later	Reduced diarrhea duration (7.23 to 5.02 or 5.12 days), intravenous therapy duration (5.44 to 3.43 or 3.23 days) and hospital stay (9.75 to 6.21 or 6.24 days); no change in vomiting duration; no differences between the 2 doses	Basu et al., 2009[23]
Aboriginal Australia, pediatric hospital	Children 4 mo–2 y with ≥3 loose stools in previous 24 h for <7 days	Inability to tolerate ORS, supplemental oxygen requirement, chronic cardiac, renal, or respiratory disease, previous GI surgery, sucrose intolerance, suspected or known immunodeficiency, received probiotic supplementation before enrollment	70	L $rhamnosus$ GG, $\geq 5 \times 10^9$ cfu capsule with cellulose microcrystalline powder or powder only reconstituted in 5 mL sterile NaCl given via NG tube, 3 times daily for 3 days	No change in functional absorptive capacity of small intestine ($^{13}CO_2$ recovery after sucrose breath test), diarrhea duration, severity, or weight gain; reduced diarrhea frequency at 2 days (4.7 to 3.3 per day)	Ritchie et al., 2010[121]
Bolivia, pediatric hospital	Children 1–23 mo with ≥3 bowel movements more than the normal number, latex test positive for rotavirus within 24 h before or within 6 h after hospitalization	Malnutrition (≤−3 SD below NCHS median), dehydration >10%, severe electrolytic imbalance (K^+ <3.5 or Na^+ >145 mEq/L), bacterial and/or parasitic pathogens in stool, other infections (sepsis, pneumonia, urinary infection), immune deficiency, administration of antibiotics or anti diarrheal drugs or probiotics during the 7 days before admission	64	S $boulardii$, 4×10^{10} cells, or S $boulardii$ + L $acidophilus$ + L $rhamnosus$ + $B.$ $longum$ 1.25×10^9 total cells, lyophilized, or placebo, in 20 mL water, twice daily for 5 days	S $boulardii$ reduced diarrhea duration (84.5 to 58 h) and median duration of fever (67 to 18 h); 4-probiotic mix reduced median duration of vomiting (42.5 to 0 h)	Grandy et al., 2010[122]

Supplementary Table 1. Continued

Location	Inclusion criteria	Exclusion criteria	n	Intervention	Outcomes	Reference
Persistent diarrhea						
Argentina, pediatric outpatient clinical center	Children 6–24 mo with >3 loose stools per day for ≥14 days	Breast feeding, allergy to cow's milk, treatment with antimicrobials or antidiarrheals in previous 7 days, concurrent systemic illness, malnutrition (<60% NCHS standard), severe dehydration (>10% body weight), inability to take oral food	93	L $casei$ and L $acidophilus$ CERELA, 10^{10}–10^{12} cfu/g or S $boulardii$, 10^{10} cfu/g lyophilized in cow's milk or cow's milk only, 175 g twice daily for 5 days	Reduced diarrhea duration (8.5 to 3.7 or 3.8 days) and treatment failures (90% to 10% or 17%); no difference between probiotic treatments	Gaon et al., 2003[25]
India, pediatric hospital	Children mean age 4.2 y with persistent diarrhea ≥14 days, stool pH <5.5, and stool reducing substances >1%	Systemic illness other than diarrhea at admission, development of any systemic complication of diarrhea during hospital stay	235	L $rhamnosus$ GG, 6×10^7 cells powder in 100 mL ORS or ORS only, twice daily until diarrhea stopped or 7 days, whichever was last	Reduced diarrhea duration (9.2 to 5.3 days) and hospital stay (15.5 to 7.3 days); no change in vomiting duration	Basu et al., 2007[26]
Diarrhea prevention and/or growth						
Peru, peri-urban town	Children 6–24 mo with weight-for-age in the lower quartile for this community	Second or third-degree malnutrition (<−2 SD below NCHS median)	160	L $rhamnosus$ GG, 3.7×10^{10} cells or placebo capsule once daily, 6 days per week, in 1 oz liquid cherry gelatin, for 15 mo	Reduced episodes of diarrhea (6.02 to 5.21 episodes per child per year); no change in diarrhea duration	Oberhelman et al., 1999[27]
Thailand, orphanage	Children 6–36 mo	Chronic diarrhea	148	B $bifidum$ Bb12, 3×10^7 cfu/g, or Bb12 + S $thermophilus$ 3×10^7 cfu/g, lyophilized in infant formula or formula only, 400–600 mL per day in 3–6 servings, for 6 mo	Increased mean weight-for-age Z-scores and mean height-for-age Z-scores	Nopchinda et al., 2002[33]
Malawi, farming village	All village children 36–60 mo	Severe acute malnutrition, severe chronic illness	164	L $rhamnosus$ GG, 5×10^{10} cells or placebo capsules sprinkled onto maize porridge twice daily for 30 days	No change in excretion of mannitol, lactulose, or sucrose, diarrhea frequency, or weight gain	Galpin et al., 2005[31]
Estonia, 4 child health care centers	Healthy term infants 0–2 mo, taking formula for at least half of their feedings	None reported	120	L $rhamnosus$ GG, 10^7 cfu/g dry powder in infant formula or formula only for first 6 mo of life	Increased length-for-age (+0.07 to +0.44) and weight-for-age (+0.00 to +0.44) standard deviation score improvements	Vendt et al., 2006[34]
Brazil, outpatient pediatric HIV care center	HIV-infected children, 2–12 y, receiving regular antiretroviral therapy for at least 3 mo and no change in regimen	Chronic disease, diabetes, serious acute infection, allergy to proteins of bovine milk, hospitalization, ART failure	77	B $bifidum$ + S $thermophilus$, 2.5×10^{10} cfu or placebo powder, 14 g in 100 mL milk daily for 2 mo	Increased CD4 cell count (−42 to +118 cells/mm³); no change in liquid stool consistency	Trois et al., 2008[29]
South Africa, 3 pediatric hospitals	HIV-exposed or uninfected, exclusively formula-fed infants 0–7 days	Major congenital abnormalities and/or illness in the neonatal period, admission to an ICU or expected prolonged hospitalization, plans to introduce alternative formula feeds, unable to return for follow-up	132	B $lactis$ CNCM I-3446 in chemically acidified whey-adapted starter formula or formula alone ad libitum until age 6 mo	No change in weight-for-age, length-for-age, head circumference-for-age, or weight-for-length	Velaphi et al., 2008[35]
Malawi, pediatric nutrition rehabilitation unit	Children 5–168 mo admitted for nutritional rehabilitation	None	795	P $pentosaceus$ 16:1 LMG P-20608, L $mesenteroides$ 23-77:1 LMG P-20607, L $paracasei$ ssp $paracasei$ F-19 LMG P-17806, and L $plantarum$ 2362 LMG P-20606, 10^{11} cfu total, +2.5 g each prebiotic oat bran, inulin, pectin, and resistant starch, in RUTF or RUTF only, inpatient and outpatient basis	No change in nutritional cure rate; trend toward decreased mortality	Kerac et al., 2009[36]

Supplementary Table 1. Continued

Location	Inclusion criteria	Exclusion criteria	n	Intervention	Outcomes	Reference
India, peri-urban resettlement colony	Children 1–3 y	Severe malnutrition, milk allergy	624	*B lactis* HN019, 1.9×10^7 cfu per day with 2.4 g prebiotic oligosaccharides sachets in milk powder or milk powder only over 3 daily servings for 1 y	Increased velocity of weight gain (2.00 to 2.13 kg/y); reduced risk of anemia and iron deficiency by 45%	Sazawal et al., 2010[123]
India, urban slum community	All children 1–5 y	None reported	3758	*L casei* Shirota, 6.5×10^9 in 65 mL nutrient drink, or nutrient drink only, daily for 12 weeks	Reduced occurrence of acute diarrhea (1.03 to 0.88 cases/child per year) over 24-wk study period; no change in weight gain, height, or mid-upper arm circumference.	Sur et al., 2010[28]

ART, antiretroviral therapy; cfu, colony-forming units; NCHS, National Center for Health Statistics; NG, nasogastric; ORS, oral rehydration solution; RBCs, red blood cells; RUTF, ready-to-use therapeutic food; SD, standard deviation; WBCs, white blood cells; WHO, World Health Organization.