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Probiotics and prebiotics to combat enteric infections and HIV in the developing world

A consensus report

Marc Monachese,[1,2] Susanna Cunningham-Rundles,[3] Maria Alejandra Diaz,[4] Richard Guerrant,[5] Ruben Hummelen,[6] Rober Kemperman,[7] Marko Kerac,[8] Remco Kort,[9] Dan Merenstein,[10] Pinaki Panigrahi,[11] Balakrishnan Ramakrishna,[12] Nasia Safdar,[13] Andi Shane,[14] Livia Trois[15] and Gregor Reid[1,2,*]

[1]Canadian Research and Development Centre for Probiotics; Human Microbiology and Probiotics; Lawson Health Research Institute; [2]Departments of Microbiology, Immunology and Surgery; The University of Western Ontario; London, ON Canada; [3]Department of Pediatrics; Cornell University Weill Medical College; New York, NY USA; [4]Baylor College of Medicine; Texas Children's Hospital; Houston, TX USA; [5]Department of Medicine; University of Virginia School of Medicine; Charlottesville, VA USA; [6]Department of Public Health; Erasmus MC; University Medical Centre Rotterdam; Rotterdam, The Netherlands; [7]Unilever; Vlaardingen, The Netherlands; [8]UCL Centre for International Health & Development; London UK; [9]TNO Microbial Genetics Group; Zeist and VU University; Department of Molecular Cell Physiology; Amsterdam, The Netherlands; [10]Department of Family Medicine; Georgetown University Medical Center; Washington, DC USA; [11]UNMC College of Public Health Department of Epidemiology; Center for Global Health and Development Nebraska Medical Center Omaha; NE USA; [12]Christian Medical College; Vellore, India; [1] [3]University of Wisconsin School of Medicine and Public Health; Madison, WI USA; [14]Emory School of Medicine; Atlanta, GA USA; [15]Department of Nutrition; Unilasalle, Brazil

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Infectious disease in the developing world continues to represent one of the greatest challenges facing humanity. Every year over a million children suffer and die from the sequela of enteric infections, and in 2008 was estimated almost 2.7 million (UNAIDS 2009 update) adults and children became infected with human immunodeficiency virus (HIV). While oral rehydration therapy for diarrhea and antiretrovirals (ARV) for HIV are critical, there is a place for adjunctive therapies to improve quality of life. The importance of the human microbiota in retaining health is now recognized, as is the concept of replenishing beneficial microbes through probiotic treatments. Studies have shown that probiotics can reduce the duration of diarrhea, improve gut barrier function, help prevent bacterial vaginosis (BV) and enhance immunity even in HIV-infected subjects. However, many issues remain before the extent of probiotic benefits can be verified, and their application to the developing world realized. This consensus report outlines the potential probiotic, and to a lesser extent prebiotic, applications in resource disadvantaged settings, and recommends steps that could bring tangible relief to millions of people. The challenges to both efficacy and effectiveness studies in these settings include a lack of infrastructure and funding for scientists, students and research projects in developing countries; making available clinically proven probiotic and prebiotic products at affordable prices; and undertaking appropriately designed clinical trials. We present a roadmap on how efficacy studies may be conducted in a resource disadvantaged setting among persons with chronic diarrhea and HIV. These examples and the translation of efficacy into effectiveness are described.

*Correspondence to: Gregor Reid; Email: gregor@uwo.ca
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Introduction

Many citizens of developing countries face extreme challenges every day of their life. The term 'resource disadvantaged' is generally accepted to describe areas with a low gross domestic product and with a below-average human quality of life index. Many countries in Africa, Southeast Asia and the Middle East fit this profile.[1]

In addition to personal safety concerns, access to high quality food and clean water is a daily struggle.[2] This, and a lack of adequate daily nutrient intake, is particularly devastating for children. A 2008 World Health Organization (WHO) regional review showed that infectious diseases are responsible for the majority of deaths among children <5 years of age.[3] An estimated four billion cases of diarrhea are reported yearly leading to over two million deaths, mostly among children residing in areas without access to clean water, latrines, healthcare and adequate nutrition. Diarrhea is caused by a wide range of microbial pathogens leading to the passage of loose or liquid stools more frequently than normal.[4] Of these, rotavirus infections in children are one of the most common, although they generally pass without complications as long as oral rehydration therapy is administered. The introduction of a vaccine against rotaviruses along with oral rehydration salts, zinc, antibiotics for dysentery, vitamin A supplementation, sanitation, hygiene and breastfeeding has been stated to avert five million deaths.[5]

However, enterotoxigenic bacteria such as *Escherichia coli,* Shigella, Campylobacter and Salmonella, as well as *Vibrio cholera* can produce a number of virulence factors that induce a fatal outcome.[6,7]

Confounding the burden of disease on these resource disadvantaged areas is the global epidemic of HIV, the prevalence of which has increased in women through heterosexual contact. In some countries, the prevalence is as high as 35% amongst the

adult population aged 15–49.[8] This devastates the work force, including teachers and caregivers, and leads to orphans and a breakdown of family structures. Diarrhea is highly prevalent in HIV-infected subjects and compounds the inability to absorb nutrients, decreases energy to carry out daily activities and reduces the ability to tolerate anti-retroviral treatment (ART).[9,10]

While great strides have been made to provide water purification systems and inexpensive or free pharmaceutical agents to treat infections and stabilize immunity, millions of people particularly in rural areas and high density urban slums still suffer from conditions, which in the developed world are manageable. Of the armamentarium of agents used to manage diarrhea, probiotics defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'[11] are increasingly explored.

Workshop Objectives

In August 2010, a workshop was hosted by the International Scientific Association for Probiotics and Prebiotics (ISAPP) to discuss the rationale for global application of probiotics. Two conditions identified as potentially receptive to probiotic intervention include diarrheal diseases and complications associated with HIV infection. A previous closed workshop on a similar topic was held in the UK in 2010 by the Bill and Melinda Gates Foundation, but the present meeting is the first to openly discuss findings from a review of the literature by researchers with experience working in resource disadvantaged regions and with knowledge of probiotics. Although no representative from an African nation was able to attend, several scientists with experience with this region did participate, thus each continent had representation.

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Three objectives were established:

(1) Apply the scientific rationale for using probiotics and/or prebiotics to combat diarrheal diseases and assist with the adverse events and treatment regimens associated with HIV to improve outcomes.

(2) Design evaluations to determine the optimal probiotic and prebiotic for specific conditions and populations.

(3) Discuss the development of strategies for local production, distribution, and uptake of probiotics and prebiotics.

Scientific Rationale for Probiotic Use

The rationale for use of probiotics in children and adults in resource disadvantaged regions comes from essentially three factors. First, the role the microbiota plays in the maintenance of the epithelial barrier function of the gut. Studies have shown that bacteria can upregulate tight junction proteins and increase resistance of the cell layer.[12,13] An intact epithelium is required for efficient nutrient uptake in a healthy host.[14] Secondly, microbes play a role in modulating innate and acquired immunity in a manner that protects the host from infection. Certain strains of *Lactobacillus casei* have been shown to stimulate immunoglobulin IgA production, increasing basal levels of this circulating antibody. In addition numerous strains have been shown to increase cytokine production, the type upregulated depends on the strain but include; inflammatory [tumor necrosis factor alpha (TNFα), gamma interferon (IFNγ); and interleukin-12 (IL-12)] and regulatory (IL-4, IL-10) cytokines.[15,16] Gill et al. reported an increase in IFNγ and IL-4 production from 100 units/ml to 200 units/ml or higher following addition of strains of *Lactobacillus rhamnosus*, *L. acidophilus* and *Bifidobacterium lactis* versus controls in mice. Thirdly, beneficial microbes, from which probiotic strains are selected, often express factors such as bacteriocins, hydrogen peroxide and other antimicrobials that kill pathogens or inhibit their growth.[18-20] They may also release signaling molecules that downregulate toxin production or release by pathogens,[21-23] as well as interfere with binding of pathogens to receptor sites on the gut mucosa.[24]

Probiotics for Diarrhea

There have been many studies on the use of probiotics to help treat diarrhea, but it has generally been accepted that the main role is to prevent this condition.[11] A meta-analysis has shown that at least two strains, *L. rhamnosus* GG and *Saccharomyces cerevisiae* subsp. *boulardii* Lyo, are effective in reducing the duration of diarrhea when supported by oral rehydration.[25] In addition, there is some evidence that probiotics can reduce episodes of diarrhea, severity of symptoms, vomiting and fever.[26] Two Cochrane reports have recently been published on the topic. In one, the aim was to assess the effects of probiotics in proven or presumed acute infectious diarrhea.[27] Although the report claimed that 63 studies met the inclusion criteria with a total of 8,014 participants, their inclusion criteria of what constitutes a probiotic (by definition[11]) was flawed and even reported a study with dead lactobacilli. Nevertheless, the analysis concluded that probiotics provide tangible benefits in shortening the duration and reducing stool frequency in acute infectious diarrhea, when used with oral dehydration. Given that some probiotic interventions come in liquid form, it would be interesting to perform a meta-analysis of the efficacy of these products in cases where oral rehydration was not available or used. In the second recent meta-analysis, four trials with 464 participants were assessed to evaluate probiotics for treating persistent diarrhea (lasting more than 14 days) in children.[28] The authors concluded that evidence for probiotics to ameliorate this condition was limited. Clearly, the root cause of the diarrheal persistence needs to be addressed before expecting probiotics or any other intervention to be successful. In a study of 571 children aged 3–36 months visiting a family paediatrician for acute diarrhea, the type of probiotic agent used had profound effects, with *L. rhamnosus* GG being the most effective in reducing duration of diarrhea.[29]

The administration of probiotics to children facing chronic diarrhea is of great importance for more than just gut health. While diarrhea may be life threatening if severe and untreated, infants who suffer from frequent episodes or chronic diarrhea tend to have stunted growth (low height for-age, a marker of chronic malnutrition), abnormally low body mass indices and impairment of cognitive function with detrimental lifelong consequences.[30-32]

Probiotics for HIV

Probiotics appear to support maintenance of a strong gut epithelia layer[33] and stimulation of innate immunity[34] which act as the first layer of defense against translocation of viral particles and bacterial pathogens.[35] There is preliminary evidence that *L. rhamnosus* GR-1 in yogurt and a combination of *Bifidobacterium bifidum* with *Streptococcus thermophilus* can confer some immunostimulatory activity in adults and children.[36-38] In addition, the nutrients in yogurt and the ability of probiotics to improve gut barrier function likely also play a role in enhancing nutritional and immune competence.[39]

The Role for Prebiotics in Amelioration of Diarrhea

Defined as 'the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host',[40] prebiotics have been used in resource disadvantaged settings for diarrhea treatment. Intriguingly, a recent study showed that a hypotonic oral rehydration solution (ORS) containing zinc and prebiotics (0.35 g/L xylooligosaccharides) was more effective at resolving diarrhea at 72 hours than regular oral rehydration therapy in young children.[41] Such prebiotic products could potentially be made available in countries where no cold chain storage and transportation exists to maintain the viability of probiotic foods and supplements. Another prebiotic, galacto-oligosaccharide mixture (5.5 g) was shown to be better than maltodextran at reducing the severity and/or incidence of travellers' diarrhea (TD) in healthy subjects who stayed in a country of low or high risk for TD for a minimum of 14 days and a maximum of 60 days.[42] High-risk destinations included Asia, The Middle East, Africa, Mexico, Central and South America, while low-risk areas included Turkey, South Africa and The Caribbean Islands.

The combining of probiotics with prebiotics into a 'synbiotic' product has been promoted as enhancing the effects of single agents. However, results have been mixed. In a randomized, double-masked, controlled trial, healthy newborns >35 weeks of gestational age and >1,800 g birth weight were randomized between 1 and 3 days after birth to receive an oral synbiotic preparation (*Lactobacillus plantarum* and fructooligosaccharides) or a dextrose saline placebo for 7 days.[43] The main outcomes were persistence of the lactobacilli and reduction in Gram-negative species in the infant gut. The application of a synbiotic (Synbiotic 2000 Forte) freeze-dried food (containing *Pediococcus pentosaceus* 16:1 LMG P-20608, *Leuconostoc mesenteroides* 23–77:1 LMG P-20607, *Lactobacillus paracasei* ssp *paracasei* F-19 LMG P-17806 and *Lactobacillus plantarum* 2362 LMG P-20606 and oat bran [rich in β-glucans], inulin, pectin and resistant starch) to 399 Malawian infants did not result in detectable improvements in nutritional status or secondary outcomes such as diarrhea reduction.[44] Prebiotic and synbiotic products need to be further studied if their primary goal is to impact the gut health of children in resource disadvantaged areas.

Diarrheal Disease-Proposed Study Design for Future Study

If probiotic-based therapies are to be incorporated into global health strategies to reduce the burden of enteric and diarrheal diseases, target populations must be carefully identified[45] with a high likelihood of responders.[46] Such studies follow a randomized, double masked, placebo-controlled protocol with primary endpoints of: reduction of diarrhea episodes, decline in hospital visits, mortality and a general improvement in public health, with follow-up for at least one year. In addition, secondary endpoints should include the impact on stunting, weight and cognitive defects. As high population density urban slums are often sites of frequent/severe diarrhea outbreaks in resource disadvantaged countries, these would provide ideal populations to study the direct effects of probiotics or prebiotics/synbiotics on treating diarrhea and improving symptoms. A clinical trial conducted in Kolkata, India showed that probiotic *Lactobacillus casei* strain Shirota could reduce episodes of acute diarrhea during a 24 week follow.[47] However, that study did not take into account weight, height and other biomarkers which can be valuable sources of data explaining what is occurring in the gut microbiota as a result of intervention. It may be possible broadening the outcomes to analyze and applying different organisms in a different dose may change the study outcome.

An additional point of extreme importance is that the study materials (yogurt, sachet) should be either in a form that is affordable to the population being tested, or after the trial it will be made available for an extended period of time to the community. One method to establish this is to create a local economy for production and distribution of the product as has been achieved in several African countries using probiotic yogurt.[38,48] This is critical, otherwise the local community see the benefits of an effective intervention which they played a part in, only for the product to disappear from their neighborhood. This is exploitative, demoralizing and creates an outcomes gap.

In order to turn the introduction of probiotics in resource poor countries into a sustainable and large-scale operation, it is essential to provide long-term access to probiotic products by creating a local infrastructure for their production and distribution. This provision of affordable products can be based upon commercial Bottom of Pyramid (BoP) principles.[49] Accordingly, increase of health and wealth can be achieved by creating the appropriate distribution channels, assuring affordability of the product, and/or increasing buying power and dedicated consumer education. Initiatives aimed at the distribution of license-free probiotic starter cultures could enable local communities with existing yogurt production facilities to upgrade their product portfolio with probiotics at a minimal cost increase, such as with a Dutch initiative in Uganda.[50] This approach not only introduces affordable health products in the region, it also increases income for farmers, producers and local distributors. One of the low-cost but effective ways to inform people about the potential benefits of the probiotic products include local radio stations or the M-Health concept which could potentially reach people via mobile electronic devices.[51] Complementary partnerships with health care

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organizations, insurance companies, cattle trade, schools, hospitals and large employers also help. Product development should not only focus on dairy, but also consider traditional fermentation of home grown foods,[52] as well as dried preparations.

In the study being proposed here, the probiotic would be provided in the form of a sachet which can be added to the meals participants regularly consume. Sachets are already used in some resource disadvantaged countries and products such as micronutrient-rich Moringa are used widely by HIV/AIDS patients to fortify their diet. The possibility of adding a probiotic strain(s) to an existing sachet should be explored. Several European probiotic producers have offered to provide their strains free-of-charge in sachet form, for the envisaged study, but long term implications will need to be considered. If such companies could also assist with transferring the sachet production capabilities to a local site near where the trial is being performed, this would encourage long term sustainability if the product is effective. Using a sachet avoids complications of asking participants to consume a food product which they may not like. However, a probiotic yogurt or other nutrient rich food could also supply nutrients such as vitamin A, zinc and glutamine which have been shown to improve gut conditions.[53-55] In addition, administering probiotics early allows for intervention when the child is most sensitive to the environment and is growing both cognitively and physically.[56,57] The rationale for use of a placebo powder without the probiotic, in addition to blinding and assessing efficacy is that this follows standard care of children in these impoverished communities; that is, they do not receive interventional therapy.

Participants would be divided into control or experimental groups randomly; neither the participant nor investigators would know the designation of the participant, to avoid bias. The study proposed herein would involve a longitudinal follow-up of children for up to two years minimum, in order to note any long term differences such as height and weight gain in addition to changes in frequency/intensity of diarrheal episodes and effects on the gut microbiota. It also allows for determination of improvements in quality of life. Recruitment would commence in children at age six months and after baseline data was collected, the probiotic intervention would begin at month seven and continue until the children are 12–18 months of age.[56,58] The children would be followed until age 2, with measurements taken monthly. It is accepted that the microbiota of a breast-fed child differs from formula fed children.[56] As breast feeding correlates with fewer episodes of diarrhea, when breast feeding ceases around 7 months of age, the provision of a probiotic supplement at this time point could help protect the weaning child at a critical time of growth.

While the focus is on reducing diarrhea, simply counting this as the only outcome marker would limit the power and impact of the study. For example, improving the quality of life of patients, reducing mortality, reducing stunting, improving weight gain, showing a long term impact on cognitive function, and improving the overall effect on the public health burden are all integral to success. Public health workers need to be aware of the benefit of probiotics and this is where improvement in education could be crucial.

Several other biomarkers should be studied, such as serum lipopolysaccharide (LPS) or anti-LPS core antibody levels to measure inflammation, lactulose and mannitol to asses gut barrier and nutrient absorption,[59,60] nutritional assessments to get a clear understanding of each child's specific situation, height, weight, head circumference and IQ scores.[61] Long-term reductions in cognitive function as assessed by the relative language- and culture-independent TONI-III test have correlated with more frequent episodes of diarrhea.[57] Taking baseline measurements of cognitive function and comparing after study completion may reveal the immediate impact, if any, that probiotics and nutrition can have on improving this factor. However, long-term assessments that include cognitive testing (such as TONI-III or Raven scores) at >4–6 years of age may be necessary.

It is well known that children suffering from chronic or recurrent diarrhea are often stunted and thinner. Increases in weight with probiotic treatment can be viewed as a positive change implying improvements in gut health and nutrient uptake. A microbiota study of fecal samples will help determine what organisms are dominant in healthy vs. diarrhea subjects. Such studies have been performed in favellas in Brazil using culture-based techniques,[62] so it is feasible. However, without on-site facilities and expertise with culturing methods, at the very least samples should be collected at time of study for later processing by sequencing methods such as Illumina[63,64] to profile the depth of microbes present and their relative abundance.

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HIV-Study Design

The global threat of HIV/AIDS has been well documented in recent history. Although prevalence of the virus is still exceedingly high, in many African countries reported rates are decreasing. This positive trend is likely due to the increased investment of resources in these countries and improving education on the disease and its prevention. Many of the current treatments for HIV focus on antiviral drugs, and the discovery of effective vaccines. While these endeavours are noble, they do not answer the question of how can we make an immediate improvement in the quality of life and outcomes for people already infected by HIV.

As stated above, the gut barrier is a natural defense mechanism preventing translocation of pathogens into the bloodstream. This barrier becomes damaged in HIV infected subjects, leading to inflammation and the increased ability of pathogens to cause bacteremia via translocation.[65] Studies suggest that probiotics can aid in repair of the damaged epithelial barrier and help prevent pathogen translocation.[66] HIV infects and destroys T cells, including Th 17 $CD4^+$ T cells that mediate host defenses against bacterial translocation in the gut.[67] By lowering the T cell levels in the blood and preventing a proper immune response upon infection, there is an increased likelihood of future susceptibility to infections. A clinical trial in Brazil analyzing the immune stimulatory abilities of a mixture of *Bifidobacterium bifidum* and *Streptococcus thermophilus* found that $CD4^+$ T cell counts could be increased from a mean of 580 cells mm^{-3} in the control group to 673 cells mm^{-3} in the probiotic group.[37] This study in HIV infected children age 2–12 years, demonstrated an

immune stimulatory effect induced by probiotics. When given in conjunction with antiretroviral drugs, probiotic supplementation improved $CD4^+$ T cell counts and reduced viral load, delaying clinical onset of AIDS.

The proper functioning of a child's immune system to combat infections relies on their nutritional status. Nutrients regulate the priming of immune responses postnatal through effects on signal transduction pathways and immune cell development.[68] Differences have been found in the ability of neonates to respond to pathogens depending on their nutritional status and intake of micronutrients including vitamins A and E, calcium, iron and zinc.[39] A probiotic supplement delivered in a medium, such as yogurt, which can be enriched with micronutrients would provide additional fortification to the child's immune system. Probiotics delivered to children at a young age can fortify the gut microbiota. This is especially important for children not being breast-fed by HIV-infected mothers due to the potential of HIV transmission via human milk.[69] One study has shown that the number of lactic acid bacteria present in the intestinal tract of children infected with HIV is far less than in healthy children, with *Lactobacillus plantarum* and Bifidobacterium spp not found in the infected children.[70] This implies that replenishment of these species might provide benefits.

A study design similar to the successful Brazilian study cited earlier[37] would verify whether nutrition supplemented with probiotics can improve the quality of life of HIV-infected children. Enrolment would consist of children aged 2–10 with $CD4^+$ T cell counts between 200–500 cells/μL, without co-infection and receiving highly active anti-retroviral treatment (HAART). Note, this will need to be assessed and controlled as treatment regimens vary by country and patient profile. Targeting children allows for signs of early intervention to be noticed, such as reduction of diarrhea and secondary infections caused from a weakened immune state. Participants would be randomized into two groups; with the control group receiving unsupplemented yogurt and the experimental group receiving yogurt with probiotic supplementation. In this instance, no prebiotics would be added. Baseline measurements would be taken daily for one month to determine fluctuation in T cell levels and estimates of viral load. Testing would then commence with the groups receiving probiotic supplemented yogurt or unsupplemented yogurt daily for two months. Measurements would ideally be taken every day (or twice weekly depending upon compliance and practicality) once treatment began. Following the two month intervention period, treatment with probiotic yogurt and daily measurements would stop but, participants would be followed and have measurements taken weekly for the next year, to determine longitudinal changes.

Counts of $CD4^+$ T cells and T-regulatory (T-reg) cells (including Th17 cells) would be examined to aid in determination of immune stimulatory effects of the intervention. As this was used in the Brazilian study,[37] comparisons can be made with the new findings. In HIV-resistant woman there are higher amounts of T-reg cells compared to non-resistant woman.[71] T-reg cells are believed to suppress $CD4^+$ and $CD8^+$ T cell activation. By suppressing activity and transcription, virus infected cells become unsuitable for viral replication. Loss of Th17 cells and reduction of precursor CD161 CD4 cells, which may limit Th17 reconstitution in untreated HIV infection has now been shown to be associated with a gradual decline in T-regs, increased immune activation and disease progression.[72] There has been some connection between probiotics and increased T-reg cell activity,[73] but not yet related to HIV. By including T-reg cell functional data in the proposed study, the plasma viral levels would be measured to determine if changes in cell responses correlate with lowering viral load.

The gut microbiota will also be analyzed using stool samples collected from study participants both in control and experimental groups, as for the diarrhea study above. Analysis will be done using a high throughput sequencing platform such as Illumina which has been used to sequence the vaginal microbiota down to species level.[63] The use of Illumina or a similar high throughput system is attractive, as DNA samples can be isolated on site but when stored properly at -80 degrees Celsius or on liquid nitrogen, they can last for years. This allows the investigators to return to their own institutions and have the data analyzed if such sequencing facilities are not present on site. In addition, with the advancement of the technology, the prices for sequencing are becoming very economical. This makes it feasible to perform sequencing of large sample sizes and improve statistical power. Particular interest will be placed in how species abundance changes over the course of infection and if probiotic use has an effect on these, inflammation, diarrhea, weight/body mass index BMI and energy.[74] The ability of probiotics to modify weight and energy is of interest, as the primary goal of such a study is to improve quality of life. The Medical Outcomes Study HIV Health Survey (MOS-HIV) is a very widely used quality of life measure. It includes 35 items that address the domains of: role function, pain, physical functioning, cognitive functioning, overall health perception, mental health and vitality.[75] The weighted sub-scores in these domains are then combined to produce two summary scores measuring physical health and mental health. By using such a scoring system, it is possible to determine quality of life and notice differences in experimental vs. control group.

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Important Additional Issues

Clinical designs such as those proposed allow opportunities to test probiotic effects. This is required in order to justify setting up a community kitchen with the goal of providing probiotic benefits daily for members of the community. But, the long term establishment of an operation requires acknowledging feasibility issues and how the project will be managed embracing local people and their customs. In approaching the proposed studies, careful consideration must also be given to strain selection. Unfortunately this decision must be based upon limited understanding of how probiotics might work in people challenged with malnutrition and infection. Still, strains can be selected with respect to safety, some in vitro documentation that supports the proposed mechanisms of action, and their ability to produce a food with satisfactory taste and texture for the users. These

selection criteria are crucial, along with careful identification of subjects more likely to response.[46]

Another issue comes from understanding how clinical trials are performed in resource limited settings. Factors to consider include cultural, political, language and ethical factors in recruitment of subjects.[76,77] Establishment of studies may require special partnerships either with government support or local research institutions in order to succeed. Local researchers and community leaders are critical to the development, conduct and sustainability of implementation of ethical and relevant outcomes. Three particularly important issues must be considered.

(1) **Funding.** Currently clinical trials related to probiotics are not receiving the attention of many governments and funding agencies. Despite best efforts of dedicated and respected scientists and data proving a positive effect of probiotics against diarrhea, HIV and other infections, the generalized misinformation on probiotic use and effects has posed a challenge to secure funding of these important clinical trials. In order to counter this, progress must be made on understanding how probiotics work. Focus should also be put on understanding how the strains of interest grow, their metabolic pathways, and how they react in vitro with other strains. While these are important considerations, the complexity of the probiotic field suggests that clinical trials conducted in collaboration with evaluating mechanisms of probiotic response may be the most efficient means of developing the basic science and clinical applications of the field. Sequencing of the genome of the strain(s) to be used can provide valuable data and permit a transcriptomic study to assess which genes might be upregulated in vivo. Such studies are expensive and not yet part of trials in developed countries, so it is unrealistic in the settings discussed above. Nevertheless, they remain a worthy goal.

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Awareness of the scientific (and non-scientific) community, including grant review panel members, needs to be raised so there is an understanding of the importance and beneficial impact probiotic use can have on health and quality of life. Product safety must always be an issue, even though past studies have shown minimal risk of probiotic yogurts. For locally-produced trial material, safety standards must be established and products monitored regularly and thoroughly, in particular microbial culturing should be performed to rule out contamination by pathogens. Storage of products at point-of-distribution to point-of-ingestion also requires safety monitoring for tampering. In addition, the safety of study subjects must be considered, either for them traveling to and from the study site, or for issues related to their participation, such as being ostracized in their community.

(2) **Selection of and access to strains.** Currently the majority of strains are owned by for-profit companies such as Chr Hansen, Danisco, Lallemand, Danone, General Mills, Nestle and others. These companies have the right to control who has access to the strains and what studies can be performed with them or their by-products. Advancement of scientific knowledge gained from projects aimed at improving the lives of millions of people living in countries where such strains and products are not available would ultimately benefit business partners. While ownership and proper management of such strains are important in terms of business ventures, partnerships need to be established with strain owners to allow access to the organisms for what amounts to humanitarian studies. These partnerships would constitute win-win scenarios in which industries controlling the strains would have their interests understood and protected in the study, while allowing researchers access to the organisms. More importantly, with the studies' success, measures could be put in place to allow long term use of the strains in these settings. One option would be to allow not-for-profit strain usage, such as granting the local community the ability to set up community kitchens,[48] and leaving the option with the intellectual property holder to use their strain in the future in these countries if they decide to set up commercial ventures. Freedom to publish results must also be considered in these partnership negotiations.

In terms of the strain(s) itself, the basis for selection is far from easy. In both diarrhea treatment and use for improving gut health in HIV subjects, a case could be made for a strain that increases epithelial barrier function and upregulates antimicrobial immunity,[33,34] as tested in vitro. The in vitro ability to adhere to epithelial cells or mucus, or to inhibit pathogen growth may or may not be essential. Probiotic strains can confer beneficial properties as planktonic cells, and they can adhere by a range of specific and non-specific mechanisms that may not be assessed in the assays commonly used. Also, a large range of microbial pathogens can cause diarrhea, so it can be difficult to prove a correlation between pathogen growth inhibition in vitro and in vivo outcome. The exception to the latter point is *Lactobacillus salivarius* UCC118 which in animal studies has been shown to have a bacteriocin essential for preventing infection by *Listeria monocytogenes*.[78] Cell line studies could also be used to identify strains that inhibit viral invasion and replication.[79,80] The concept of using a multi-strain product is worthy of consideration, but requires not only mechanistic justification (one strain that upregulates barrier function and another that stimulates innate immunity), but also step-wise evidence that the addition of strains improves the effectiveness of a single-strain product.

(3) **Setting up a long lasting operation.** The freedom-to-operate issue is critical to resolve because in order to improve the lives of those living in the developing world, any positive findings related to probiotic studies need to translate into long term benefits for the communities in which the studies were performed. Questions must be asked before the study begins, such as what will be the best way to deliver the probiotic, (i.e., a sachet, a yogurt or in milk?). What financial considerations must be resolved to allow some future control by the local community over the strains, supplies, safety, quality assurance, fixed costs and maintenance of a viable production set-up. In Mwanza Tanzania, a community kitchen is operated by the local Kivulini woman's group with support from the National Institute for Medical Research, and other partners along with The University of Western Ontario.[48] This kitchen produces a probiotic yogurt which is given to all-comers, including people infected with HIV. Mimicking such a set up and adapting it to different locations would allow for an initial set-up which can be modified to suit the cultural and specific needs of the population. Members of these communities are impoverished and cannot afford to pay high prices for probiotic products. Thus, funds must be secured

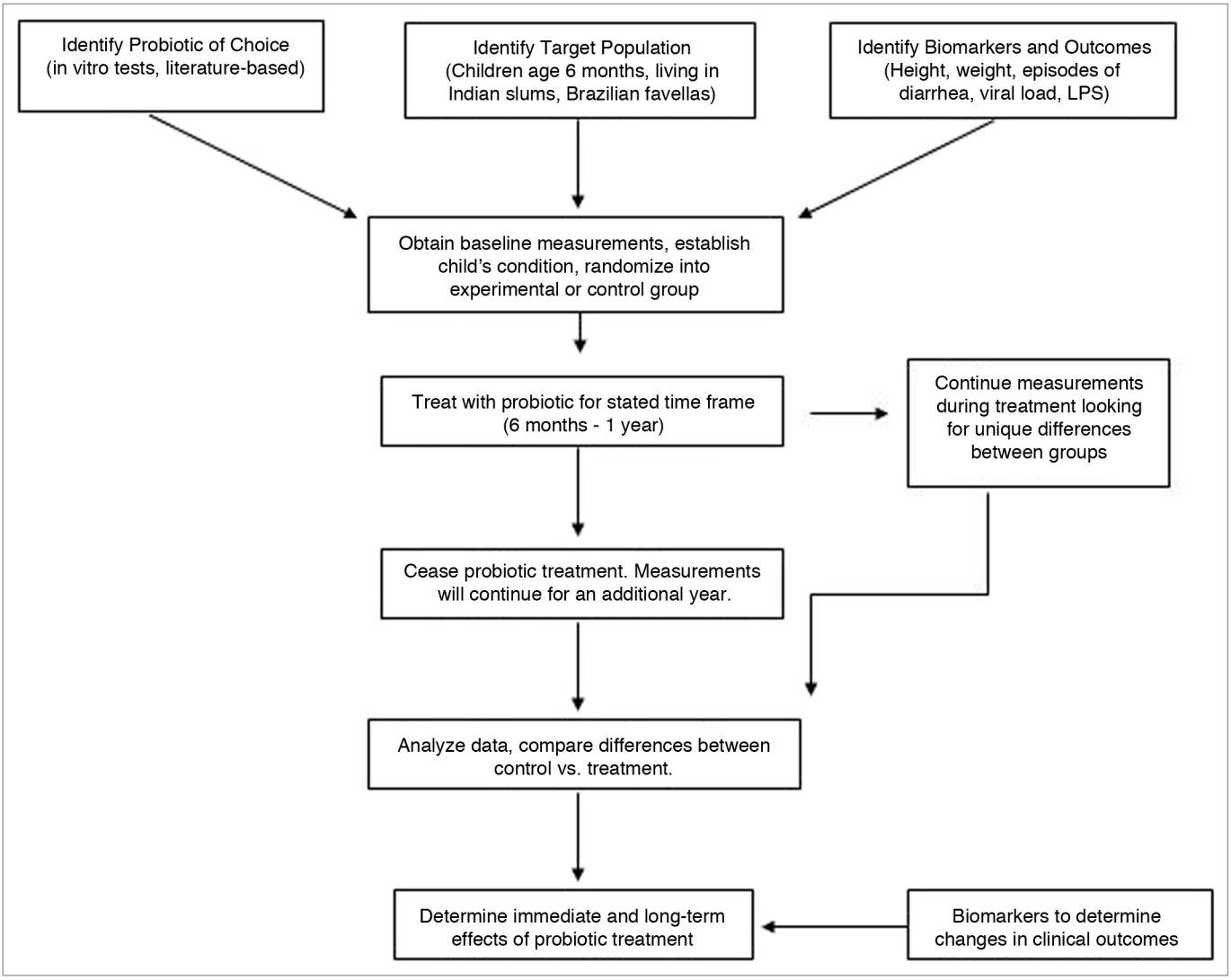

Figure 1. Road map for the design of the clinical study.

initially from developed countries, and used to supplement local in-kind support, while conjointly lobbying for local funds (such as through the Tanzanian Commission for AIDS, TACAIDS) which for example could pay for HIV/AIDS patients to receive the yogurt for free.

Maintaining product quality is crucial when setting up such an operation. Ensuring reproducibility of the product, inclusion of the probiotic in all batches, and having the ability to problem solve (such as if taste and texture change) cannot be understated. Contamination needs to be avoided at every level of production and distribution, or quality will not be retained and possible adverse health effects may occur. Stocks of strains could be pre-made, kept frozen at a nearby institute or hospital, then prepared and delivered to the kitchen as needed. However, this requires buy-in from the institute/hospital site. Alternatively, stocks could be kept at the kitchen and prepared as needed, in which case personnel training, access to equipment including freezers, refrigerators and incubators is needed and external, independent monitoring arranged. The latter scenario also relies upon the goodwill of the strain owner to provide stock cultures. Ideally, if dried powdered strains in pre-set vials could be provided, it would allow for easy preparation of the end-product.

A road map for the design of clinical studies which attempts to summarize key steps and outcomes is presented in **Figure 1**.

Recommendations

In conclusion, it was felt four key points must be addressed in order to improve probiotic clinical trials in resource disadvantaged settings.

(1) **Identify the right population.** Researchers must understand the population, not only its health challenges, but just as important its cultural, social, demographic and ethical nuances. For example, factors such as the daily diet, whether children are being breast-fed, whether the same child receives the study product each day, whether health records for the subjects are reliable, and what factors ensure compliance are much greater challenges

in many instances in the developing world where education levels are often very low. Baseline assessment of the microbiota will be important to understand whether or not subjects respond in the same manner to probiotic treatment as a North American or European citizen who had been shown to respond to similar treatment. Knowing how to maintain compliance if the study subjects (say children) may be left alone for many hours of the day is crucial. This may require working with a local clinic or community facility where children can come to receive treatment daily. It may also require recruiting an initial large sample size if a large percentage may be expected to drop out or be non-compliant. The disease of interest must also be validated in all participants. Control subjects must be equally prone to have the disease so that comparisons can be made on the extent of the effect for probiotic treatment.

(2) Selecting study strains. With the emphasis being on treating diarrhea and improving health/gut function and quality of life of those suffering from HIV, the selection of strains with an immunomodulatory function and an ability to improve gut barrier function need to be considered. Combination strains must be shown to complement each others' activity and survive to appropriate levels in the yogurt that is the delivery vehicle. In vitro tests may be helpful, if not already performed on the strains, but translating the results to the clinical setting must occur with caution. Decisions must be made about whether to recover the strain from the stool or how best to establish if the strain is functioning in some or all subjects. Such research questions will require a separate budget. Advancements in basic knowledge attained from the trials will benefit others in the future.

(3) Identifying biomarkers. How do we measure the effect we are looking for in an individual subject? For analyzing diarrhea would one only look at reduction of episodes as an effect of probiotic activity? Identification of biomarkers is crucial in order to track clinical outcomes. A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of normal and disease processes or pharmacological response.[81] A condition such as diarrhea has been shown to affect the body in many ways including being detrimental to growth, adversely impacting long term cognitive function, gut health and levels of energy. The ability to measure, particularly long term, biomarkers of relevance to the test community is critical. Quality of life factors in developing countries are not 'biomarkers' per se, nor necessarily the same as in developed countries. But, the ability to have enough energy to work each day may certainly be as important as T-reg cell status or LPS levels in the blood indicating improved gut barrier function. Determining whether the subject has diarrhea lasting 7 days or longer ("prolonged diarrhea") may provide a helpful, simple marker of the impact of the treatment on overall diarrhea burden and growth shortfalls. Metabolic by-products recoverable from feces might also provide markers of the host's response to the intervention,[82] although these tests are expensive, only performed at a few sites and not available in resource disadvantaged countries. It is known that lactobacilli, bifidobacteria and prebiotics can alter the metabolic print-out,[83] so if a preferred readout associated with health could be identified, the administration of different candidate probiotics or prebiotics could be assessed in one or two volunteers, to see if they achieve resolution of diarrhea and restoration of health based upon the metabolome.

(4) Partnerships with industry, communities and government. Partnerships are vital for successful implementation of clinical studies in resource-poor communities. These must include local community leaders to engage subjects and help subjects understand the ethical, practical and compliance issues of the study and to educate the community about expectations. An ideal team may consist of government officials who can provide access to funds and oversee health and security issues; scientists and clinicians who can promote the study amongst the population, help recruit patients and coordinate sample collection and analysis; local business people such as farmers to provide milk in a reliable and consistent manner; donors to provide payments for subject travel; and people to distribute the product. Cultural or religious leaders may also be required to ensure the project is aligned with community values.

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Conclusion

A lack of information and clinical trial data have made it difficult to secure funding for these studies, to date. Public awareness of the scientific (and non-scientific) community needs to be raised so there is an understanding of the importance and beneficial impact probiotic use can have on public health and quality of life in the developing world. We would like to conclude with an invitation to industry, government and scientific institutions to join us and participate in these efforts to alleviate disease and improve the quality of life of those in need.

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References

1. United Nations Development Programme. Human Development Index-2010; http://hdr.undp.org/en/statistics
2. WHO. Global Water Supply and Sanitation Assessment (2000) WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation, Geneva and New York 2001; http://www.who.int/docstore/water_sanitation_health/Globassessment/GlobalTOC.htm
3. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, et al. Child Health Epidemiology Reference Group of WHO and UNICEF*. Global, regional and national causes of child mortality in 2008: a systematic analysis. Lancet 2010; 375:1969-87.
4. Ashbolt NJ. Microbial contamination of drinking water and disease outcomes in developing regions. Toxicology 2004; 198:229-38.
5. Walker CL, Friberg IK, Binkin N, Young M, Walker N, et al. Scaling up diarrhea prevention and treatment interventions: a lives saved tool analysis. PLoS Med 2011; 8:1000428.
6. Gibson GR, McCartney AL, Rastall RA. Prebiotics and resistance to gastrointestinal infections. Br J Nutr 2005; 93:31-4.
7. Sears CL. Molecular physiology and pathophysiology of tight junctions V. Assault of the tight junction by enteric pathogens. Am J Physiol Gastrointest Liver Physiol 2000; 279:1129-34.
8. Godwill OA, Kufa E, Ntabangana S, Alisalad A, Calleja JG. HIV/AIDS epidemiological surveillance report for the WHO African region: 2007 update; http://www.who.int/hiv/pub/surveillance/epi_afro2007/en/index.html
9. Sheehan LA, Macallan DC. Determinants of energy intake and energy expenditure in HIV and AIDS. Nutrition 2000; 16:101-6.
10. Arpadi MS. Growth failure in HIV-infected children, Consultation on nutrition and HIV/AIDS in Africa: evidence, lessons and recommendations for action. WHO, Department of Nutrition for Health and Development 2005; http://www.who.int/nutrition/topics/Paper%20Number%204%20-%20Growth%20failure.pdf

11. FAO/WHO. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report 2001; http://www.fao.org/ag/agn/Probio/probio.htm
12. Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut 2003; 52:988-97.
13. Thompson-Chagoyán OC, Maldonado J, Gil A. Aetiology of inflammatory bowel disease (IBD): Role of intestinal microbiota and gut-associated lymphoid tissue immune response. Clin Nutr 2005; 24:339-52.
14. Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. Science 2001; 292:1115-8.
15. Kaila M, Isolauri E, Soppi E, Virlanen E, Laine S, Arvilommi H. Enhancement of the circulating antibody secreting cell response in human diarrhoea by a human Lactobacillus strain. Pediatr 1992; 32:141-4.
16. Perdigon G, Fuller R, Raya R. Lactic acid bacteria and their effect on the immune system. Curr Issues Intest Microbiol 2001; 2:27-42.
17. Gill HS, Rutherford KJ, Prasad J, Gopal PK. Enhancement of natural and acquired immunity by *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN107) and *Bifidobacterium lactis* (HN019). Br J Nutr 2000; 83:167-76.
18. Sinead CC, Yin L, Christian RU, Paul OW, Colin H, Cormac GMG. Bacteriocin production as a mechanism for the anti-infective activity of *Lactobacillus salivarius* UCC118. Proc Natl Acad Sci USA 2007; 104:7617-21.
19. Silva M, Jacobus VN, Deneke C, Gorbach LS. Antimicrobial substance from a human *Lactobacillus* strain. Antimicrob Agents Chemother 1987; 31:1231-3.
20. Aiba Y, Suzuki N, Kabir AMA, Takagi A, Koda Y. Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. Am J Gastroenterol 1998; 93:2097-101.
21. Carey CM, Kostrzynska M, Ojha S, Thompson S. The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic *Escherichia coli* O157:H7. J Microbiol Methods 2008; 73:125-32.
22. Asahara T, Shimizu K, Nomoto K, Hamabata T, Ozawa A, Takeda Y. Probiotic bifidobacteria protect mice from lethal infection with shiga toxin-producing *Escherichia coli* O157:H7. Infect Immun 2004; 72:2240-7.
23. Li J, Wang W, Xu SX, Magarvey NA, McCormick JK. *Lactobacillus reuteri*-produced cyclic dipeptides quench agr-mediated expression of toxic shock syndrome toxin-1 in staphylococci. Proc Natl Acad Sci USA 2011; 108:3360-5.
24. Bernet FM, Brassart D, Neeser RJ, Servin LA. *Lactobacillus acidophilus* LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut 1994; 35:483-9.
25. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. Am J Gastroenterol 2006; 101:812-22.
26. Grandy G, Medina M, Soria R, Teran C, Araya M. Probiotics in the treatment of acute rotavirus diarrhoea. A randomized, double-blind, controlled trial using two different probiotic preparations in Bolivian children. BMC Infect Dis 2010; 10: 253.
27. Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. Cochrane Database Syst Rev 2010; 11:3048.
28. Bernaola Aponte G, Bada Mancilla CA, Carreazo Pariasca NY, Rojas Galarza RA. Probiotics for treating persistent diarrhoea in children. Cochrane Database of Syst Rev 2010; 11:7401.
29. Canani RB, Cirillo P, Terrin G, Cesarano L, Spagnuolo MI, De Vincenzo A, et al. Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. BMJ 2007; 335:335-40.
30. Guerrant RL, Oriá RB, Moore SR, Oriá MO, Lima AMA. Malnutrition as an enteric infectious disease with long-term effects on child development. Nutr Rev 2008; 9:487-505.
31. Moore RS, Lima LN, Soares MA, Pinkerton FRJL, Guerrant LR, Lima AMA. Effects of prolonged episodes of acute diarrhea on childhood growth and risk of persistent diarrhea in northeast Brazil: A community birth cohort study. Gastroenterol 2010; 139: 156.
32. Guerrant DI, Moore SR, Lima AMA, Patrick PD, Schorling JB, Guerrant RL. Association of early childhood diarrhoea and Cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in northeast Brazil. Am J Trop Med Hyg 1999; 61:707-13.
33. Generoso SV, Viana M, Santos R, Martins FS, Machado JA, Arantes RM, et al. *Saccharomyces cerevisiae* strain UFMG 905 protects against bacterial translocation, preserves gut barrier integrity and stimulates the immune system in a murine intestinal obstruction model. Arch Microbiol 2010; 192:477-84.
34. Pagnini C, Saeed R, Bamias G, Arseneau KO, Pizarro TT, Cominelli F. Probiotics promote gut health through stimulation of epithelial innate immunity. Proc Natl Acad Sci USA 2010; 107:454-9.
35. Hummelen R, Vos AP, van't Land B, van Norren K, Reid G. Altered host-microbe interaction in HIV: A target for intervention with pro- and prebiotics. Int Rev Immunol 2010; 29:485-513.
36. Anukam KC, Osazuwa OE, Osadolor BH, Bruce A, Reid G. Yogurt containing probiotic *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 helps resolve moderate diarrhea and increases CD4 count in HIV/AIDS patients. J Clin Gastroenterol 2008; 42:239-43.
37. Trois L, Cardoso ME, Miura E. Use of probiotics in HIV-infected children: A randomized double-blind controlled study. J Trop Pediatr 2008; 54:19-24.
38. Irvine LS, Hummelen R, Hekmat S, Looman C, Changalucha J, Habbema DF, et al. Probiotic yogurt consumption is associated with an increase of CD4 count among people living with HIV/AIDS. J Clin Gastroenterol 2010; 44:201-5.
39. Cunningham-Rundles S, Lin H, Ho-Lin D, Dnistrian A, Cassileth BR, Perlman JM. Role of nutrients in the development of neonatal immune response. Nutr Rev 2009; 67:152-63.
40. Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, et al. Prebiotic effects: metabolic and health benefits. Br J Nutr 201; 104:1-63.
41. Passariello A, Terrin G, De Marco G, Cecere G, Ruotolo S, Marino A, et al. Efficacy of a new hypotonic oral rehydration solution containing zinc and prebiotics in the treatment of childhood acute diarrhea: A randomized controlled trial. J Pediatr 2011; 158:288-292.
42. Drakoularakou A, Tzortzis G, Rastall RA, Gibson GR. A double-blind, placebo-controlled, randomized human study assessing the capacity of a novel galacto-oligosaccharide mixture in reducing travellers' diarrhoea. Eur J Clin Nutr 2010; 64:146-52.
43. Panigrahi P, Parida S, Pradhan L, Mohapatra SS, Misra PR, Johnson JA, et al. Long-term colonization of a *Lactobacillus plantarum* synbiotic preparation in the neonatal gut. J Pediatr Gastroenterol Nutr 2008; 47:45-53.
44. Kerac M, Bunn J, Seal A, Thindwa M, Tomkins A, Sadler K, et al. Probiotics and prebiotics for severe acute malnutrition (PRONUT study): a double-blind efficacy randomised controlled trial in Malawi. Lancet 2009; 374:136-44.
45. Preidis GA, Hill C, Guerrant RL, Ramakrishna BS, Tannock GW, Versalovic J. Probiotics, enteric and diarrheal diseases and global health. Gastroenterology 2011;140:8-14.
46. Reid G, Gaudier E, Guarner F, Huffnagle BG, Macklaim J, Murcia Munoz A, et al. Responders and non-responders to probiotic interventions: how can we improve the odds? Gut Microbes 2010; 1:200-4.
47. Sur D, Manna B, Niyogi KS, Ramamurthy T, Palit A, Nomoto K, et al. Role of probiotic in preventing acute diarrhoea in children: a community-based, randomized, double-blind placebo-controlled field trial in an urban slum. Epidemiol Infect 2010; 1-8; DOI: 10.1017/S0950268810001780.
48. Reid G. The potential role for probiotic yogurt for people living with HIV/AIDS. Gut Microbes 2010; 1:1-4.
49. Prahalad CK. In: Harris B, Ed. Fortune at the Bottom of the Pyramid. Strategy and Business issue 26. Uppersaddle River, NJ: Wharton School Publishing 2002.
50. Kort R, Sybesma W. Yoba for Life Foundation. http://www.yoba4life.com
51. Free C, Phillips G, Felix L, Galli L, Patel V, Edwards P. The effectiveness of M-health technologies for improving health and health services: a systematic review protocol. BMC Res Notes 2010; 3:250.
52. Odunfa SA. African fermented foods: from art to science. World J Microbiol Biotechnol 1988; 4:259-73.
53. Sazawal S, Dhingra U, Hiremath G, Sarkar A, Dhingra P, Dutta A, et al. Effects of *Bifidobacterium lactis* HN019 and prebiotic oligosaccharide added to milk on iron status, anemia and growth among children 1 to 4 years old. J Pediatr Gastroenterol Nutr 2010; 51:341-6.
54. Chen P, Soares MA, Lima AA, Gamble VM, Schorling JB, Conway M, et al. Association of vitamin A and zinc status with altered intestinal permeability: analyses of cohort data from northeastern Brazil. J Health Popul Nutr 2003; 21:309-15.
55. Williams EA, Elia M, Lunn PG. A double-blind, placebo-controlled, glutamine-supplementation trial in growth-faltering Gambian infants. Am J Clin Nutr 2007; 86:421-7.
56. Bhutta ZA, Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, et al. What works? Interventions for maternal and child under nutrition and survival. Lancet 2008; 371:417-40.
57. Niehaus MD, Moore SR, Patrick PD, Derr LL, Lorntz B, Lima AA, et al. Early childhood diarrhoea is associated with diminished cognitive function 4 to 7 years later in children in a northeast Brazilian shantytown. Am J Trop Med Hyg 2002; 66:590-3.
58. Victora CG, de Onis M, Hallal PC, Blossner M, Shrimpton R. Worldwide timing of growth failure: revisiting implications for interventions. Pediatrics 2010; 125:473-80.
59. Brignardello J, Morales P, Diaz E, Romero J, Brunser O, Gotteland M. Pilot study: alterations of intestinal microbiota in obese humans are not associated with colonic inflammation or disturbances of barrier function. Aliment Pharmacol Ther 2010; 32:1307-14.
60. Kelly P, Shawa T, Mwanamakondo S, Soko R, Smith G, Barclay GR, et al. Gastric and intestinal barrier impairment in tropical enteropathy and HIV: limited impact of micronutrient supplementation during a randomised controlled trial. BMC Gastroenterol 2010; 10:72.
61. Gladstone M, Lancaster GA, Umar E, Nyirenda M, Kayira E, van den Broek NR, et al. The Malawi Developmental Assessment Tool (MDAT): the creation, validation and reliability of a tool to assess child development in rural African settings. PLoS Med 2010; 7:1000273.
62. Mello RM, Morais MB, Tahan S, Melli LC, Rodrigues MS, Mello CS, et al. Lactobacilli and bifidobacteria in the feces of schoolchildren of two different socioeconomic groups: children from a favela and children from a private school. J Pediatr (Rio J) 2009; 85:307-14.
63. Hummelen R, Fernandes AD, Macklaim JM, Dickson RJ, Changalucha J, Gloor GB, et al. Deep sequencing of the vaginal microbiota of women with HIV. PLoS One 2010; 5:1-8.
64. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010; 464:59-65.

65. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006; 12:1365-71.
66. Ewaschuk JB, Diaz H, Meddings L, Diederichs B, Dmytrash A, Backer J, et al. Secreted bioactive factors from *Bifidobacterium infantis* enhance epithelial cell barrier function. Am J Physiol Gastrointest Liver Physiol 2008; 295:1025-34.
67. Ancuta P, Monteiro P, Sekaly RP. Th17 lineage commitment and HIV-1 pathogenesis. Curr Opin HIV AIDS 2010; 5:158-65.
68. Yu Y, Sitaraman S, Gewirtz AT. Intestinal epithelial cell regulation of mucosal inflammation. Immunol Res 2004; 29:55-68.
69. Orne-Gliemann J, Mukotekwa T, Miller A, Perez F, Glenshaw M, Nesara P, et al. Community-based assessment of infant feeding practices within a programme for prevention of mother-to-child HIV transmission in rural Zimbabwe. Public Health Nutr 2006; 9:563-9.
70. Dicks LMT, Fraser T, Doeschate K, Van Reenen CA. Lactic acid bacteria population in children diagnosed with human immunodeficiency virus. J Paediatr Child Health 2009; 45:567-72.
71. Card CM, McLaren PJ, Wachihi C, Kimani J, Plummer AF, Fowke RK. Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of $CD4^+$ $CD25^+$ $FOXP3^+$ regulatory T cells. J Infect Dis 2009; 199:1318-22.
72. Prendergast A, Prado GJ, Kang HY, Chen F, Riddell AL, Luzzi G, et al. HIV-1 infection is characterized by profound depletion of $CD161^+$ Th17 cells and gradual decline in regulatory T cells. AIDS 2010; 24:491-502.
73. Baroja ML, Kirjavainen PV, Hekmat S, Reid G. Anti-inflammatory effects of probiotic-yogurt in inflammatory bowel disease patients. Clin Experimental Immunol 2007; 149:470-9.
74. Steenhout PG, Rochat F, Hager C. The Effect of *Bifidobacterium lactis* on the growth of infants: A pooled analysis of randomized controlled studies. Ann Nutr Metab 2009; 55:334-40.
75. Stewart AL, Hays RD, Ware JE Jr. The MOS short-form general health survey: reliability and validity in a patient population. Med Care 1988; 26:724-35.
76. Farmer P, Campos NG. Rethinking medical ethics: a view from below. Dev World Bioeth 2004; 4:17-41.
77. Brody BA. Ethical issues in clinical trials in developing countries. Stat Med 2002; 2:2853-8.
78. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. Proc Natl Acad Sci USA 2007; 104:7617-21.
79. Botic T, Klingberg TD, Weingartl H, Cencic A. A novel eukaryotic cell culture model to study antiviral activity of potential probiotic bacteria. Int J Food Microbiol 2007; 115:227-34.
80. Conti C, Malacrino C, Mastromarino P. Inhibition of herpes simplex virus type 2 by vaginal lactobacilli. J Physiol Pharmacol 2009; 6:19-26.
81. Atkinson AJ Jr, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, et al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001; 69:89-95.
82. Martin FP, Sprenger N, Montoliu I, Rezzi S, Kochhar S, Nicholson JK. Dietary modulation of gut functional ecology studied by fecal metabonomics. J Proteome Res 2010; 9:5284-95.
83. Vitali B, Ndagijimana M, Cruciani F, Carnevali P, Candela M, Guerzoni ME et al. Impact of a synbiotic food on the gut microbial ecology and metabolic profiles. BMC Microbiol 2010; 10:4.

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