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

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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 sequelae 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.

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 cholerae 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
Probiotics for HIV

Probiotics appear to support maintenance of a strong gut epithelia layer and stimulation of innate immunity which act as the first layer of defense against translocation of viral particles and bacterial pathogens. 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. 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.

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/species in the gut microbiota that confer(s) health benefits to the host', 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 xyooligosaccharides) was more effective at resolving diarrhea at 72 hours than regular oral rehydration therapy in young children. 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 maltodextrin 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. 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. 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. 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 with a high likelihood of responders. 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 Shirata could reduce episodes of acute diarrhea during a 24 week follow. 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, sachets) 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. This is critical, otherwise the local community may 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, dehumanizing 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. 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. 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. Complementary partnerships with health care
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. 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. 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. 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. This implies that replenishment of these species might provide benefits.

A study design similar to the successful Brazilian study cited earlier 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 probiotics 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, 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. 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 Tregs, increased immune activation and disease progression. There has been some connection between probiotics and increased T-reg cell activity, 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. 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. 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 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. 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.

**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
identify probiotic of choice (in vitro tests, literature-based)

identify target population (children age 6 months, living in indian slums, brazilian favelas)

identify biomarkers and outcomes (height, weight, episodes of diarrhea, viral load, LPS)

obtain baseline measurements, establish child's condition, randomize into experimental or control group

Treat with probiotic for stated time frame (6 months - 1 year)

continue measurements during treatment looking for unique differences between groups

cease probiotic treatment. Measurements will continue for an additional year.

analyze data, compare differences between control vs. treatment.

Determine immediate and long-term effects of probiotic treatment

biomarkers to determine changes in clinical outcomes

**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 preset 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