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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 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.

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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

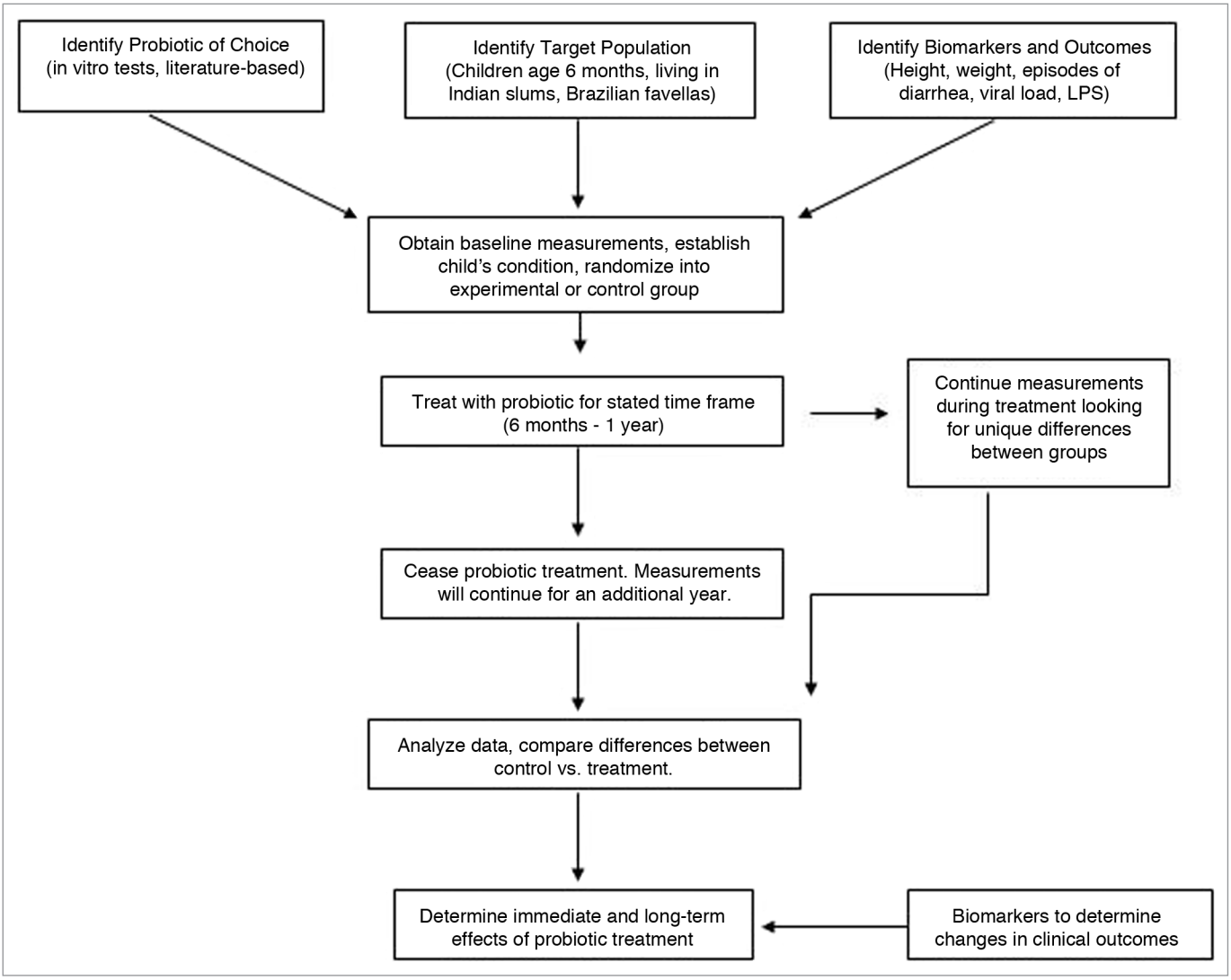

Figure 1. Road map for the design of the clinical study.

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.

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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