Inner Workings: Malnutrition, gutted

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In 2010, Rashidul Haque spent a week at Washington University in St. Louis getting up to speed on a seemingly unpleasant task: the art and practice of collecting and storing human excrement. By learning the necessary steps for sample preservation and DNA extraction, Haque, who has spent the past 25 years developing new ways of diagnosing pathogens from clinical stool samples as head of the parasitology laboratory at the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), would become a crucial partner in a finely tuned international, collaborative project.

Haque is part of a diverse team spearheaded by Washington University microbiome researcher Jeff Gordon, who has taken on the mammoth task of determining how gut microbes contribute to malnutrition in the developing world. In Malawi, Gordon has shown that children with a protein deficiency disorder, called kwashiorkor, have a different type of gut community than their unaffected twin siblings (1). In Bangladesh, Gordon has identified a set of bacterial species associated with both healthy growth in children (2) and recovery from cholera in adults (3). Gordon has other ongoing research projects in India, Peru, South Africa, and Brazil.

But he's not merely on a microbial cataloguing adventure. "Our goal isn't to sample the world," Gordon says. "The goal is to critically test the hypothesis that the microbiota are an important contributor to disease pathogenesis and a potential target for therapy and prevention."

In Mirpur, a densely populated urban slum in the Bangladeshi capital of Dhaka, Gordon, Haque, and the other scientific investigators call on a group of 16 field research assistants. The idea is to conduct genetic analysis of fecal samples to determine how the microbiome differs between healthy versus malnourished children. However, before analysis can begin, a careful collection process must be in place.

Time is of the essence. Mothers must save the stool from their children. Field research assistants then have to gather these samples within 30 minutes and bring them on ice to the ICDDR,B, where they are processed and preserved in −80 °C freezers for long-term storage. The protocol recently changed to use precharged liquid nitrogen shippers, rather than ice packs, to transport samples back to the laboratory for long-term cryopreservation. ICDDR,B scientists extract DNA from the samples that is shipped to Gordon's laboratory for analysis.

The ICDDR,B and its forerunner (the Pakistan-SEATO Cholera Research Laboratory, which dates back to before Bangladeshi independence) have been working in the community for over half a century, long enough that they "know their populations and have spent many years building their trust," says Michael Barratt, executive director of Washington University's new Center for Gut Microbiome and Nutrition Research. Even so, there are ethical and cultural caveats inherent in collecting microbiological samples from people in the Dhaka slums.

For example, the researchers must explain to poor and often uneducated participants why they want to study the invisible world that's inside their children's intestines. "It's important to do this in a way that's not just about getting the sample," Gordon says. By carefully explicating the consent documents, Tahmeed Ahmed, director of nutrition and food security at the ICDDR,B, says his team helps the study participants understand how these efforts may provide insights into the origins of undernutrition. The mothers are primarily concerned with the health and well-being of their children, he notes, so most are willing to help advance the research if it could one day help their families or neighbors. "When you explain this to the caregivers and the mothers in very simple Bangla, the local language, I think they understand," Ahmed says. Gordon is acutely aware of the cultural sensitivities at play. "We feel very strongly that these microbes represent family, community, and national assets," he says.

Building a multidisciplinary collaboration has been key. For example, anthropologists Amber Benezra and Joe DeStefano, a husband-wife duo who were each visiting scholars in Gordon's laboratory in the early 2010s,

As part of a microbiome study, a field research assistant from the ICDDR,B asks for consent from a mother in Mirpur, a slum in Dhaka, Bangladesh. Image courtesy of William Petri Jr. (University of Virginia, Charlottesville, VA).

surveyed participating households in Mirpur to learn about their food customs, personal hygiene practices, and childcare routines (2). According to Benezra, a sociocultural anthropologist now at New York University, knowing how study subjects live, bathe, and eat can help in the interpretation and application of the microbiome data, especially as the researchers start to study probiotic therapeutics that could improve the kids' microbial balance. "The more information that the scientists have, the fuller picture they can see of these lives and these guts and these microbiota," she says.

The picture suggests that malnourished children have a less-diverse gut community than their healthy peers. Nutritional interventions can improve the gut composition, but they only offer only a temporary reprieve (2). Much of Gordon's research also involves transplanting human microbes into germ-free mice for further analysis. By transplanting a community indicative of a healthy gut in Bangladeshi children, the researchers revealed a bacterial pathway that helps the gut ward off infections (3).

Building on these initial results, Gordon and his collaborators in Bangladesh are probing the gut microbiota's role in cognitive defects, and in the failure of oral vaccines to protect against polio and rotavirus. A newly launched trial will explore whether a therapeutic food made entirely of rice, lentils, and other local ingredients leads to more sustained microbiome improvements than the internationally used, peanut-based supplements.

But all of this required buy-in from the local communities, the result of decades of close contact between the ICDDR,B and the people of Mirpur. "When I walk through this community with the field research assistants, it's kind of like being with the Godfather in Little Italy," says William Petri Jr., an infectious disease researcher at the University of Virginia School of Medicine in Charlottesville, who has partnered with Haque to study intestinal diseases in Bangladeshi children for the last 22 years. "You bask in the admiration that the people have for the team."

1 Smith MI, et al. (2013) Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science* 339(6119):548–554.
2 Subramanian S, et al. (2014) Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature* 510(7505):417–421.
3 Hsiao A, et al. (2014) Members of the human gut microbiota involved in recovery from *Vibrio cholerae* infection. *Nature* 515(7527):423–426.