NGS Used to Monitor Gut Microbiota Replacement after *Clostridium difficile* Infection

Michael Sadowsky, PhD, investigates how fecal microbiota transplantation restores healthy balance by sequencing gut microbiota with HiSeq® and MiSeq® systems.

Introduction

If it could save your life, you would do it. While it might sound repulsive, fecal microbiota transplantation (FMT) can bring someone suffering from certain bacterial gut infections back from the brink of death in a matter of days. It took a unique partnership between a gastroenterologist and a microbial ecologist to analyze how this process works.

Michael Sadowsky, PhD, is a microbial ecologist. He is a professor in the Department of Soil, Water, and Climate and Director of the Biotechnology Institute at the University of Minnesota (UMN) where he teaches microbial ecology and researches applications of microbial ecology in water and soil. He never considered working in human systems until he was approached by his colleague, Dr. Alexander Khoruts, a gastroenterologist. "That's the versatility of metagenomics or amplicon-based sequencing," said Dr. Sadowsky. "You can use it to study any microbial environment."

They began their collaboration in 2009, studying a new approach for people with *Clostridium difficile* (*C. difficile*) diarrheal infection (CDI). Dr. Sadowsky chose Illumina HiSeq and MiSeq systems to determine the taxonomic identities of microorganisms following gut microbiota restoration after FMT. "Illumina next-generation sequencing (NGS) platforms are ideal for studying microorganisms that cannot be successfully grown in culture," said Dr. Sadowsky.

iCommunity caught up with Dr. Sadowsky to hear about the progress he's made in monitoring bacteria that keep *C. difficile* in check.

Michael Sadowsky, PhD, is a professor in the Department of Soil, Water, and Climate, and Director of the Biotechnology Institute at the University of Minnesota.

Q: What is CDI?

Michael Sadowsky (MS): CDI is a debilitating disease that affects hundreds of thousands of people and causes 14,000 U.S. deaths each year[1]. The disease is caused primarily by the use of antibiotics. Antibiotics might be given for a bladder infection or a cut on the hand, which ends up causing dysbiosis or a dysfunctioning intestinal tract. As the antibiotic halts infection, it also kills off important members in the gut microbial community, allowing *C. difficile* to gain a foothold in the intestinal tract. When antibiotics are administered to eliminate the *C. difficile* infection, the microflora change again. After antibiotics are withdrawn, *C. difficile* spores start to germinate and take over the intestinal tract, producing a toxin and causing further changes in the microflora.

Q: What is the mechanism that makes *C. difficile* so invasive?

MS: *Bacteroidetes* and *Firmicutes* represent the largest numbers of microorganisms and appear to be of primary importance in restoring normal gut function. For example, Bacteroides is present in feces at up to 10^{12} organisms per gram. Yet, it's more than just the reintroduction of key microorganisms that restores gut functioning. Microorganisms also produce various compounds that they use to signal among themselves and the host. One important compound group is bile acids, which are produced by the liver, stored in the gall bladder, and released into the intestinal tract while eating. Bile acids are converted to bile salts by the host. Bacteria convert these bile acids (also called primary bile acids) into secondary bile acids. Interestingly, primary bile acids act as germinants of *C. difficile* spores. If the bacteria in your gut are absent or not functioning, they cannot convert primary bile acids to secondary bile acids, leading to germination of the toxic *C. difficile* spores and, ultimately, CDI.

Q: How is CDI treated?

MS: Treatment for dysbiosis is through a process called fecal microbiota transplantation (FMT). It involves applying purified fecal microbiota preparations to the individual from a healthy donor. The intestinal tract is cleaned out using various

solutions and these microorganisms are applied to restore a healthy microbial balance in the gut.

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Q: What is the source of the fecal microbiota?
MS: It can come from a relative or our qualified donor pool. We try to extract all the microorganisms from the feces that we can. We call this full spectrum microbiota. The idea is to capture the genetic diversity because that is what leads to a functioning gut ecosystem. We have a rigorously controlled system where we produce feces under GMP conditions and carry out our own FMTs. The material is purified microbiota that's stored in glycerol, frozen to -80°C. It's thawed and rehydrated prior to being applied via colonoscopy.

Q: How do you follow the microbiota changes after FMT?
MS: One way is to take biopsies of the intestinal tract and extract microorganisms from those biopsy samples, but it is not very pleasant for the individual. Although the intestinal tract has been studied for hundreds of years, most microorganisms present have not been cultured.

We found that NGS performed with DNA extracted from fecal samples provides a good assessment of the microbiota in the intestinal tract. Our research uses Illumina sequencing to analyze engraftment of fecal microbiota into the new host. We take samples of the donor and the individual, while they have CDI and after FMT using NGS to follow daily, weekly, monthly, and even yearly changes in the microbiota in the intestinal tract. After a successful transplant, the individual's gut microbiota resembles that of the donor's, and acquires subtle differences thereafter.

Q: How has Illumina technology impacted this project?
MS: Since we can't culture the majority of intestinal microorganisms, we're left with only sequencing technology to determine which microorganisms are present in the intestinal tract before, during, and after FMT. We have three HiSeq and two MiSeq systems. Which one we use depends upon the length of the queue, how many samples we can pool together at a specific time, and how fast we need the data back. We typically run 48 samples on MiSeq and between 200–300 samples on HiSeq.

The multiplexing ability of the HiSeq and MiSeq systems enable us to sequence multiple samples in a very cost effective and rapid manner. We can simultaneously sequence large numbers of samples using bar-coded sequence primers and obtain data on a many individuals in a relatively short period. We couldn't do this without NGS. It's the perfect technology to follow which microbes are present in the gut, and at what period of time.

Q: What is the recipe for a healthy balance of gut microbiota?
MS: It's very complex. There are 450–1,000 species of microorganisms present in the adult gastrointestinal tract, so there isn't a high degree of diversity. We know what those microorganisms are taxonomically and what the relative proportions are. Within two days after FMT these microorganisms have taken over the intestinal tract, resembling that of the donor.

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Q: What are the next steps for this program?
MS: Microbiota encapsulated in a pill form is in development here and among various groups around the U.S. Our current frozen preparation has been successfully stored for more than a year, so we know it is stable long term. We've also developed a microbial therapeutics program at UMN where we're starting a clinical trial to look at the effect of microbiota on obesity and metabolic syndromes.

References

1. www.cdc.gov/hai/organisms/cdiff/cdiff_infect.html (December 14, 2014).

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