Microbe Management

Bacteria and other microscopic organisms inhabit our bodies in huge numbers. Most do not harm us, and some help us.

By A. J. Smuskiewicz

The alarming red splotches on Kyle's leg were driving him crazy with itching. Kyle knew that his blood *glucose* (sugar) level had been unusually high lately, and he wondered if the skin problem was somehow related to his diabetes. A visit to the doctor and a few tests revealed that Kyle had contracted a fungal infection. "These fungal spores," said the doctor, "are always present on our skin. But they gain a foothold when the normal mix of microscopic organisms on the skin gets out of whack. I think that happened because your skin chemistry was disturbed by high glucose levels."

Kyle's experience illustrates a fundamental reality of human health and disease—that the proper functioning of our bodies is strongly influenced by the *microbes* (microscopic organisms) that inhabit us. To emphasize this point, molecular biologist Bruce Birren of the Broad Institute at Massachusetts Institute of Technology (MIT) has said, "We're not individuals, we're colonies of creatures." In fact, recent research demonstrates that microbial cells outnumber human cells within our bodies by an amazing ratio of 10 to 1.

Bacteria may be the best-known microbe, but other microscopic organisms, including yeasts, protists, archaea, and viruses, also inhabit the human body. Scientists refer to all the microbes that naturally live on and in the human body as the human microbiome. (A biome is a community of organisms living in a particular environment; a microbiome is such a community of microscopic organisms.)

Contrary to popular notions, not all the bacteria and other microbes are harmful to us. In fact, we could not live without these microorganisms. They help us digest food, *synthesize* (produce) vitamins, fight off disease-causing bacteria, and perform other tasks.

Identifying and understanding the microbial species that make up the human microbiome has been the goal of the Human Microbiome Project (HMP). This massive, five-year undertaking has involved hundreds of researchers affiliated with some 80 research institutions—including MIT's Birren. In June 2012, HMP researchers published 14 scientific articles that provided the most detailed information ever on the human microbiome.

Scientists have long known that we have many kinds of microbes living on our bodies. A small percentage of these organisms were known to be *pathogenic* (disease-causing); others were known to be beneficial. But researchers thought that the vast majority of the microbial species on and in the human body did not cause any harm or provide any particular benefits. Thanks to the HMP project and other recent research, scientists have discovered that the number of microbiome species that significantly affect their human hosts—mainly in helpful ways—is much larger than previously thought.

The HMP team began its work with funding from the National Institutes of Health, an agency of the United States Department of Health and Human Services. The objectives of the project were to fully describe the microbial communities found at multiple sites on the healthy human body and to investigate how changes in the microbiome may affect health. In addition to the Broad Institute of MIT, research institutions participating in the HMP included the Baylor College of Medicine in Houston; Washington University School of Medicine in St. Louis; and the J. Craig Venter Institute in Rockville, Maryland, among others. The latter institute was founded by J. Craig Venter, the geneticist who in the 1990's spearheaded the private effort to *sequence* (determine the order of) the genetic subunits that make up the human *genome* (entire set of genetic information). The HMP is comparable to that genome project, considered a landmark in the history of science.

During the course of the HMP project, researchers collected more than 11,000 microbial samples on three occasions from 242 healthy volunteers (129 men, 113 women) between the ages of 18 and 40. Because scientists knew that different species of microbes live on different parts of the body, the researchers sampled 15 body sites in men and 18 sites in women. Parts of the body from which samples were obtained included the nostrils, the mouth and throat, the skin, the colon (by means of stool samples), and the vagina in women. The HMP scientists believe that the samples represent 81 to 99 percent of the genera of the human microbiome. A *genus* (singular of

genera) is a group of closely related species.

To identify the individual species of microbes in these samples, the investigators could not use standard laboratory techniques for *culturing* (growing) microbes. Many microbes of the human body are so specialized for living in their particular habitats of the body that they cannot be grown in culture—that is, on Petri dishes. Instead, the researchers used advanced analytical techniques of genetics to study the microbiome. These techniques primarily involved sequencing the microbial DNA in laboratory machines.

DNA, the chainlike molecule that determines heredity in living things, consists, in part, of varied patterns of four compounds, called bases. The way in which the patterns of bases are combined in the DNA chain is known as the DNA sequence. It is this pattern that *codes for* (contains the instructions for constructing) specific proteins in a living organism. Using various tools and technologies, geneticists sequence the DNA to determine the unique order of patterns in a species.

One such technology is 16S ribosomal RNA (16S rRNA) sequencing. Ribosomes are cellular structures that use RNA, a chainlike molecule somewhat similar to DNA, to produce proteins. 16S rRNA genes are segments of DNA that code for the synthesis of certain parts of ribosomes in bacteria and other microbes. Each species of microbe has its own unique variation of these genes, with slightly different subunits. However, humans do not have any 16S rRNA genes. As a result, the HMP scientists were able to separate out and identify individual microbe species and determine relative proportions of these species—without the possibility of contamination by human DNA.

The HMP researchers also used a sophisticated technology called shotgun metagenomic sequencing, which enabled them to sequence long DNA segments and, indeed, entire genomes of the microbes. Armed with 16S rRNA sequencing and shotgun metagenomic sequencing, the researchers were able to gain an understanding of the overall functional capabilities of microbial communities from specific body areas.

To gain additional insights into the microbiome, the scientists compared their sequencing data with data from "reference genomes"—previously decoded genomes of hundreds of microorganisms. These comparisons helped the researchers more easily narrow down the species that corresponded to their genetic data.

The laboratory analyses by the HMP team reveal that human bodies can collectively host more than 10,000 species of microorganisms. However, the combination of species varies from person to person, with each individual having as many as 1,000 different species. If these microorganisms could be removed from the body and placed on a scale, they would weigh from about 1 to 2.7 kilograms (2 to 6 pounds). The HMP researchers further estimated that the microbes in the human microbiome contain a total of 8 million different genes, massively outnumbering the roughly 22,000 genes in human cells by a ratio of 360 to 1.

One major surprise of the HMP research was the wide variation in microbe species from one body site to another. For example, most of the microbe species found in the nose or mouth belong to different groups than the species found in the gastrointestinal tract. The researchers also found that species vary widely from person to person.

The most common individual microbial species in the mouth and throat belong to the genus *Streptococcus,* such as *Streptococcus mitis* (abbreviated *S. mitis),* which dominates the inner cheek. These bacteria produce chemical substances that help the immune system fight off harmful bacteria, but they can also cause heart inflammation if they enter the bloodstream and reach the heart. Common species of bacteria in the mucus of the nostrils and respiratory tract also secrete proteins that boost the human immune system.

Among the most common species on the skin is *Propionibacterium acnes,* a usually harmless bacterium that produces *enzymes* (molecules that help carry out chemical reactions) that break down excess skin oil. However, this microbe can also cause acne. Another bacterium that helps regulate the skin's oil content, *Pityrosporum ovale,* also may play a role in the development of certain skin disorders. The moist, oily skin surface normally maintained by such bacteria serves as an effective barrier to infection. Yet another skin microbe, *Staphylococcus epidermidis,* helps to protect against bacterial infections but can cause serious infections if moved inside the body. For example, such bacteria might enter the body on the surface of a catheter (a tube to remove urine or other body fluid).

Within the gastrointestinal tract, the most common bacteria in many people belong to the genus *Bacteroides.* Such bacteria include *B. fragilis,* which produces a molecule that stimulates the activity of certain cells of the immune system. However, this species of bacteria can cause serious infections if it invades internal organs other than the intestines. *Streptococcus thermophilus* —used in the production of yogurt—is among the gut microbes that aid in digestion of *lactose* (milk sugar). *Lactobacillus casei* is a gut microbe suspected of lowering one's risk of developing such autoimmune diseases as asthma and allergies.

The HMP researchers detected various *strains* (subtypes) of *Escherichia coli* in the intestines of most individuals. Many of these bacteria produce vitamin K2, important for bone and cardiovascular health. *E. coli* and certain other microbes produce enzymes that break down toxins (poisons), rendering them harmless or less harmful to the human body. However, some strains of *E. coli* can cause severe diarrhea and even death. *Helicobacter pylori* is a common gastrointestinal bacterium that plays a role in regulating appetite, though it can also trigger the formation of stomach ulcers.

In the vagina, the dominant microbial species belong to the genus *Lactobacillus.* Some species of these bacteria synthesize compounds that make the vagina acidic, creating an inhospitable environment for other, potentially harmful, microbes. This environment may help protect newborns from infections during birth. *L. johnsonii,* which normally inhabits the gut and produces enzymes that digest milk, moves into the vagina before delivery. The baby becomes coated with these bacteria during birth. The newborn baby soon swallows some of the bacteria, which take up residence in its gastrointestinal system.

As a baby passes through the vagina during birth, it picks up many other beneficial organisms that will become part of its own microbiome. As the baby grows, its microbiome develops together with its immune system. Because of this codevelopment, the immune system does not attack the microbes as foreign invaders.

Some body sites have many more microbial species than other sites. Evidence obtained from feces indicates that the lower gastrointestinal tract can be home to more than 4,000 species. In fact, about half the mass of a typical stool consists of microbes, the HMP researchers discovered. Bacteria-rich stains on the teeth known as plaque can contain about 1,300 microbial species. The nostril openings host about 900 species; and the inner lining of the cheeks, about 800 species. In total, more than half the microbial DNA found by the researchers came from the gastrointestinal tract or mouth; about 20 percent came from the skin; and lesser amounts came from the airways, *urogenital tract* (the passage that leads from the bladder to the exterior opening for discharge of urine), and other body sites.

Although there is wide variation among individual human microbiomes, people living in the same community tend to have more similar species than people living widely apart. For example, bacterial species in saliva are similar among people who live near each other but vary greatly among people living in different areas. The greatest microbial diversity among individuals was found in the gut and mouth and on the skin. In the gut, some individuals have almost all *Bacteroides* bacteria, but other individuals have virtually no bacteria of this genus. Some individuals have a gut microbiome that produces more vitamin B7; in other people, gut microbes produce more vitamin B1. Despite these variations, there is a small "core microbiome" of species that are shared by most people at each body site.

The HMP investigators were not sure why microbes vary among people. They speculated that one's microbial makeup might be related to diet, environmental factors, genetic factors, body chemistry, lifestyle, age, and other factors. Further research is needed, the researchers note, to explore the reasons for this human variability.

Despite differences in microbe species among individuals, the overall collection of microbes performs similar tasks in all people. For example, people may have different species of microbes on their tongues, but the microbiome on each person's tongue uses similar chemical reactions to break down such energy sources as sugars and other carbohydrates.

Investigators do not know why harmful bacteria in the microbiome remain harmless until some trigger causes them to turn pathogenic. For example, HMP researchers detected the bacterial species *Staphylococcus aureus* in the noses of 30 percent of the study participants, all of whom were healthy. Under certain conditions, *S. aureus* can cause skin infections, food poisoning, and an especially dangerous condition called toxic shock syndrome. One strain of *S. aureus* has become resistant to many antibiotics and is known as methicillin-resistant *S. aureus* (MRSA). MRSA infections are difficult to treat and can be life-threatening. Nevertheless, MRSA can be present on people's skin without causing illness.

One factor that may activate the pathogenic activities of bacteria in the microbiome, according to the researchers, is the use of antibiotics. These drugs have the potential to alter the microbiome, killing abundant helpful or neutral strains and allowing rare harmful strains to take their place. The HMP researchers speculated that illness or pregnancy could also upset a normal, balanced microbiome. Whatever the cause of disruption, researchers assert that a healthy, diverse microbiome is crucial to preventing infection and fending off disease.

The great achievement of the HMP, analysts observe, is providing scientists and medical researchers with information on the normal makeup of the healthy human microbiome—and, thus, a baseline for studies of how changes may lead to disease. Researchers believe that the microbiome not only keeps people healthy, but also plays a role in determining why individuals react differently to drugs and why some people are more susceptible to certain infections than other people. Among the many conditions suspected of being associated with an altered microbiome are asthma, atherosclerosis, dermatitis, diabetes, esophageal cancer, inflammatory bowel diseases, meningitis, obesity, pediatric abdominal pain, pediatric fever, psoriasis, and *vaginosis* (a bacterial infection of the vagina). Scientists involved in the HMP advocate further research to better understand the factors that tip the microbial balance toward disease.

A study reported by researchers with the University of California at San Francisco (UCSF) in June 2012 illustrates the role that the microbiome plays in health—and in the increased risk of disease when the microbiome is disrupted. The researchers sampled microbial communities in the noses of 10 healthy individuals and 10 individuals previously diagnosed as having chronic rhinosinusitis (CRS)—commonly known as sinus infection. The scientists found that the people with CRS had fewer total nasal microbes, as well as fewer species of microbes. Moreover, the researchers detected in these subjects' noses an overabundance of a particular bacterial species in the genus *Corynebacterium.*

The UCSF researchers next designed a study with mouse subjects to test their hypothesis that the *Corynebacterium* strains were causing sinusitis in the absence of a healthy, diverse nasal microbiome. They treated some of the mice with antibiotics for seven days in order to reduce their microbiome population. The researchers then exposed both treated and untreated mice to *C. tuberculostearicum.* Mice that had been treated with antibiotics displayed symptoms of sinusitis, but the untreated mice did not. This result seemed to confirm the researchers' hypothesis.

The HMP researchers and other scientists expect that analyses of the human microbiome will lead to new tools for diagnosing and treating many diseases. Such treatments are likely to involve the manipulation of certain microbiomes to help keep people healthy and to defend against disease. Microbiome-oriented therapies could even reduce our dependency on antibiotics and, thus, slow the development of antibiotic-resistant strains of bacteria. But the importance of understanding the microorganisms that are responsible for so much biological activity in

humans goes beyond practical, clinical applications. Knowing that we have more microbial DNA than human DNA in our bodies may change how we think about what it means to be human.

Help from "Good" Microbes

The explosion of knowledge about the human microbiome has raised expectations of new treatments for a variety of diseases. But in fact, medical science and practice already provide several important therapies for managing the human microbiome.

One therapy is the use of probiotics. Probiotics are preparations that contain bacteria, yeast, or other microbes known to benefit the human body. For example, the "good" bacterium Lactobacillus acidophilus, which is in yogurt, acidophilus milk, and some other foods, promotes bowel regularity and other aspects of intestinal health. In addition to food products, probiotics come in such forms as specialty teas, dietary supplements, and skin lotions.

According to the Harvard Medical School (HMS), clinical studies have shown that probiotic therapy can help treat gastrointestinal disorders (such as severe diarrhea), as well as vaginal and urinary infections in women. Studies also suggest that the use of probiotics may delay the development of allergies in children. However, HMS scientists advise consumers to consult with their primary care physician about using such products and to pay attention to the microbial species listed on product labels. Probiotic supplements are not regulated in the United States by the Food and Drug Administration, so the saying caveat emptor (buyer beware) applies.

A relatively new medical therapy called fecal transplant shows great promise as a treatment for certain diseases of the colon. In this procedure, an extract of feces containing health-promoting microbes from a healthy donor is given via a suppository or colonoscopic procedure to a patient suffering from an intestinal disorder. Fecal transplants, many medical researchers suspect, may eventually have broader application as a treatment for such diseases as colon cancer and allergies.