



BACTERIA *and the* BLANK SLATE

Humans are home to countless bacteria. These microorganisms are usually thought of as a menace, and often for good reason. But they are also vital to human health. Physician-researcher Juliette Madan is working toward a better understanding of how bacteria can both harm and help her youngest patients.

BY MATTHEW C. WIENCKE

LIKE MANY PEOPLE, JULIETTE MADAN IS FASCINATED BY BABIES. But unlike most other people, she is also passionate about babies' bacteria. "Babies are exciting to study when it comes to their bacteria, because they really are a blank slate," she says. "They are essentially sterile at birth."

Madan, an assistant professor of pediatrics at Geisel, combines these interests in her work as both a neonatologist and a scientist. She spends much of her time caring for infants in the intensive care nursery at the Children's Hospital at Dartmouth (CHaD). When she isn't working with patients, she can often be found in a lab in the Borwell Research Building, just a short walk through the hallways of DHMC from CHaD. There, Madan studies some of the conditions that cause newborns to end up in the intensive care nursery, such as cystic fibrosis, a genetic disorder that afflicts about 30,000 Americans. Her research specialty is something few others have examined: the bacteria that make their home in infants and young children.

Madan is interested in using bacteria both as an indicator of the progression of a disease—which could help physicians figure out which treatments are most likely to work—and as part of an actual treatment. Bacteria can cause a number of health problems, including the build-up of biofilms in the lungs of people with cystic fibrosis, but Madan thinks that some bacteria might also be part of the solution to treating cystic fibrosis and other diseases.

In recent years, scientists have started to learn much more about the relationship between humans and bacteria. Each of us is home to about 100 trillion bacteria, including thousands of different species, so studying them is an enormous challenge. Researchers are now taking a close look at these bacteria, which along with the other microorganisms that make their home in or on humans are called the human microbiome. These "bugs" (as scientists like to refer to microorganisms) are essential for our day-to-day life. They form walls to fight off other bacteria that cause disease, they help digest food, and they synthesize vitamins, among other vital tasks. But

until recently, no one knew how they vary from person to person.

"The thing that is still hard for me to wrap my head around is that there are 10 times more bacterial cells on our body than our body has [human] cells," says Bruce Stanton, a Geisel professor of immunology and microbiology and director of Dartmouth's Lung Biology Center. "One to three percent of our body-weight is bacteria."

Stanton chuckles. "Kind of creepy when you think about it. I'm starting to itch now."

In an effort to learn more about these bacteria, more than 200 scientists at more than 70 institutions collaborated on a five-year study of the microorganisms that inhabit humans. Results from this survey, called the Human Microbiome Project, were published in 2012 and showed the incredible variety of the bacteria found in 242 healthy people. Among the many interesting findings was that each person studied was home to many potentially harmful bacteria that, surprisingly, were not causing any harm.

Researchers at Dartmouth are well underway on their own microbiome project. They are classifying all the microorganisms found in the lungs of people with cystic fibrosis, with the hope that learning more about the diversity of bacteria, viruses, and fungi will lead to better treatments for the disease.

BACTERIA AND BABIES

When Madan came to Dartmouth in 2008, she had recently earned a master's degree in clinical research on nutrition and infection in high-risk infants. She was eager to study probiotics as a therapy for premature infants to decrease life-threatening health risks. Then she heard a lecture at Dartmouth by Mitchell Sogin, an environmental microbiologist at the Marine Biological Laboratory in Woods Hole, Mass., whose research helped lay the foundation for the Human Microbiome Project. Sogin discussed his studies of microorganisms in ocean aquifers and applications of this work to the microbiome of mice.

Madan had long been interested in the intestinal microbiome of preterm babies. She asked



Jon Gilbert Fox

Neonatologist Juliette Madan checks in on an infant who was born at 24 weeks gestation. As one aspect of her research, Madan is studying the relationship between bacteria and the health of babies born prematurely.

"One to three percent of our bodyweight is bacteria."



Madan talks with lab technician Molly Housman, who is in the process of storing a bacteria sample. Using such samples, Madan is trying to understand changes in the types of bacteria found in developing infants.

In one study, Madan examined microbial diversity in seven infants with cystic fibrosis, collecting intestinal and respiratory samples over their first 21 months of life. To identify the bacteria, the researchers used a technique called pyrosequencing to compare the DNA sequence of a gene (called 16S rRNA) that is present in all of the bacteria but that varies slightly from species to species.

Madan expected that over the time she followed the infants, microbial diversity would increase more rapidly in the intestine than in the respiratory tract. But she found just the opposite. In infants fed breast milk, diverse clusters of bacteria were forming more rapidly in the respiratory tract. The introduction of solid food also appeared to be associated with an increase in diversity in both the respiratory tract and the gut. Madan says this suggests that breast milk may be beneficial for combating colonization by pathogens in infants with cystic fibrosis by fostering the growth of diverse communities of bacteria.

Another finding was that many of the bacteria species first appeared in the gut and then were later found in the respiratory tract. One reason, Madan explains, is that babies are very prone to reflux. So it's possible that aspiration helps bacteria move from the intestine to the respiratory tract. More importantly, the beneficial or benign bacteria in the gut may "train" the immune system as to which bacteria do not pose a threat and which are pathogenic and need to be fought off. That hypothesis needs to be tested further, but Madan and other researchers believe that there is a connection between the gut microbiome and the immune system.

As part of this research effort, Madan has been working with Laura Filkins, a graduate student in the lab of George O'Toole. Filkins has been taking a close look at the intestinal and respiratory samples from Madan's infant patients. She has found that as the babies grew older, there was an increase in three types of bacteria: *Pseudomonas aeruginosa*, which is a dominant pathogen in

the lungs of people with cystic fibrosis; *Staphylococcus aureus*, a common cause of infection; and *Klebsiella*, which can cause pneumonia and bloodstream infections.

Filkins then took epithelial cells from the lungs of patients with cystic fibrosis and added various combinations of these bacteria to find out what would happen. She found that *Pseudomonas* kills off both *S. aureus* and *Klebsiella*, which would explain how *Pseudomonas* establishes itself as the dominant bacteria. Filkins plans next to figure out how *Pseudomonas* does this, which may give clues as to what drugs or probiotics may stop it and ultimately lead to clinical trials on new treatments for cystic fibrosis.

SEEING MORE CLEARLY

This sort of analysis takes a great deal of intellectual and technological firepower. In the study of seven infants, for example, the researchers had to sort through an enormous collection of data. The DNA sequence of the gene 16S rRNA is known for many bacteria species, so the team compared the sequences they found to already known sequences to establish the identity of each bacteria cell in every sample and to spot changes in the infants' microbiomes. Madan collaborates with experts in bioinformatics at Dartmouth, taking advantage of their experience to better understand the huge data sets produced by such studies.

The tools developed by Jason Moore and others at the Institute for Quantitative Biomedical Sciences make it possible to find and analyze relationships in huge data sets, relationships that might be virtually impossible to find using a spreadsheet or traditional charts and graphs.

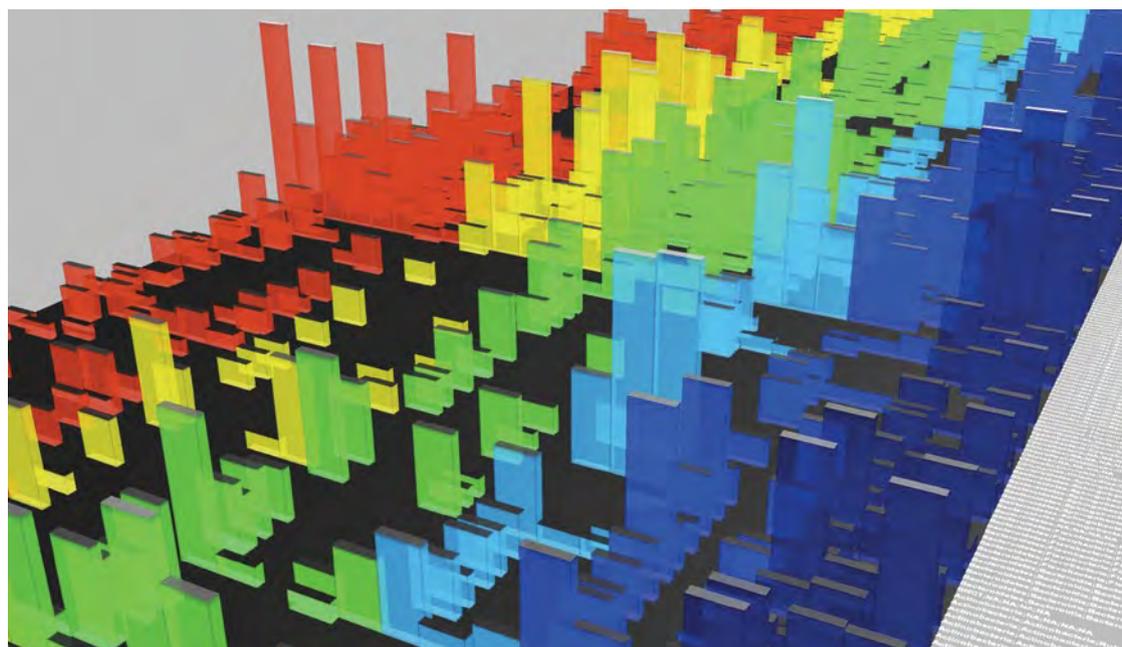
One program allows researchers to view their data in a type of heat map, but with the data points represented in three-dimensional forms, such as buildings, ribbons, or fish. Using a touch pad, the researchers can swim through the data, examining it from various perspectives. The animations and sounds make the experience something like playing a video

Sogin if he could help her analyze her data and found that he was excited to work with a physician on the clinical implications of this research. Together, they studied the DNA sequences of the microbes taken from preterm infants in a study Madan had conducted.

As Dartmouth is recognized nationally for its research and treatment of cystic fibrosis, it turned out to be a great place for Madan to channel her interests. So Madan set out to study how infants with cystic fibrosis acquire both beneficial bacteria and pathogens. "There's this very long window of time in early infancy where there's really no evidence of respiratory infection whatsoever," she says. She is one of the few researchers in the U.S. to focus on the microbiome of infants.

Madan collaborates with many others across Dartmouth, including Stanton; Jason Moore, an expert in bioinformatics and director of Dartmouth's Institute for Quantitative Biomedical Sciences; Devin Koestler, a bioinformaticist in Moore's lab; Geisel microbiologist George O'Toole; and DHMC pulmonologists Alix Ashare, Alex Gifford, and Margaret Guill.

A 3D heat map shows microbiome data from six premature infants. Each color represents data from a different infant, and each row provides data on a different species of bacteria. The height of the bars represents the abundance of each species at a specific point in time.



game, and, indeed, Moore used video-game technology to develop this tool.

Moore's goal is to make it possible to conduct statistical and computational analysis directly from a visualization. For example, if Madan spotted an interesting pattern, such as a cluster of certain bacteria, she would be able to find out immediately whether the cluster occurred by chance or was statistically meaningful.

Moore calls such tools "idea engines" and says they will allow Madan and others working with her—biologists, clinicians, and bioinformatics experts such as himself—to get together, examine the data, and answer questions they otherwise may never have even thought to ask.

Research on the microbiome of infants has applications for conditions other than cystic fibrosis as well. As one part of a new grant awarded to Geisel researchers to form a Center for Biomedical Research Excellence, Madan

overuse of antibiotics is a serious problem for infants' microbiome. In a study published in 2012, Madan and her team studied intestinal samples from six premature babies over a period of eight to twelve weeks. They found that prolonged antibiotic use led to a decrease in microbial diversity in the gut and an increase in *Staphylococcus*, one of the dominant pathogens for sepsis.

cystic fibrosis. Probiotics are live microorganisms that can potentially provide various health benefits—in effect, bacteria that act like a drug. Madan plans to work with her mentor, Patricia Hibberd, the chief of global health at Massachusetts General Hospital, to conduct the first probiotics trial on children with cystic fibrosis in the U.S. CHaD would be one of the primary sites for the trial.

In the U.S., routine practice does not include using probiotics in premature infants to treat or prevent disease. But according to some international studies, probiotics can be beneficial for premature infants. Madan and others are working to identify patterns in the microbiome of both healthy and ill infants to determine when using probiotics—or different antibiotic regimens—might be helpful.

Madan appears to be making her mark in the world of the developing microbiome, especially in the area of infants with cystic fibrosis. "Juliette has been a great member of our cystic fibrosis group," says O'Toole. "We really haven't worked with patients as young as Juliette has worked with. It's a real testament to her that she's developed this whole project from scratch. We're all excited to see where it's going to go."

Madan is, too. And not just to solve a scientific problem. As she plunges deeper into the complex infant microbiome, she hopes her work in the lab will give her better options to help the infants she treats in the intensive care nursery.

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Using a touch pad, the researchers can swim through the data, examining it from various perspectives.

will study the developing gut microbiome in 250 healthy infants over their first three years of life. She will focus on the relationship between the infants' microbiome and exposures (including antibiotic use, vitamin D levels, and the use of breast milk versus formula) and outcomes of infection and allergy.

The effects of antibiotics are a key part of this study. Madan and other researchers think the

Studies also suggest that antibiotics given to infants may contribute to childhood obesity. In one study, researchers found that children given antibiotics before they were six months old were 50% more likely to be overweight at age 10. Madan plans to study this possible connection.

Madan is also passionate about probiotics and how they can help infants and young children with