

MICROBIOME

Detecting diversity

Surveys of the microbes that live in the vagina have revealed unexpected variability. More research might reveal links between these microbes, infection and birth complications.

BY COURTNEY HUMPHRIES

Vaginal health requires beneficial microorganisms. These microbes, so the story goes, are bacteria from the genus *Lactobacillus*, which feed on carbohydrates in cells shed from the vaginal walls to produce lactic acid. The acid dampens the growth of yeast and other bacteria, and protects the vagina from infections. “The dogma is that a *Lactobacillus*-dominated microbiome is more healthy than a complex microbiome,” says Gregory Buck, principal investigator of the Vaginal Microbiome Consortium at Virginia Commonwealth University in Richmond.

But as studies over the past decade or so have characterized the vaginal microbiome in greater detail, they have complicated this simple view of what's healthy and what's not. Research is finding, for instance, that not every species of *Lactobacillus* confers good vaginal health. Instead, good health seems to be determined by complex interactions between the microbes and the vaginal cells. But the biggest puzzle is that there are clear racial and ethnic differences, with women of African descent being less likely to have a microbiome dominated by *Lactobacillus*.

Some researchers think these differences can help to explain why African-American women bear a disproportionate burden of certain health problems, such as HIV infection and premature delivery. The depletion of *Lactobacillus* is known as vaginal dysbiosis, and it increases the risk of both conditions, says Janneke van de Wijkert, an infectious-disease researcher at the University of Liverpool, UK. The most common type of vaginal dysbiosis is a disorder called bacterial vaginosis (BV), which can cause an unpleasant, fishy odour and a watery discharge, and this is more common in women of African ancestry. These and other epidemiological findings suggest that, beyond being embarrassing, BV and other forms of dysbiosis could compromise a woman's reproductive health.

The improved understanding of the vaginal microbiome is also causing scientists to ponder the consequences of symptomless BV. “If you just go and look at asymptomatic women, a fair number of them will have a vaginal microbiota that looks a lot like bacterial vaginosis,” says David Fredricks, a microbiologist at the Fred Hutchinson Cancer Research Center in Seattle, Washington. So the question is: are

these symptomless women healthy, or does their vaginal microbiota put them at greater risk of health problems?

Restoring a beneficial balance of microbes could be an important strategy for protecting women's reproductive health. But to achieve that, researchers will need to go beyond simply cataloguing which bacteria are present in the vagina, and gain a fuller understanding of the connections between vaginal bacterial communities and women's health.

THE ACID TEST

Studies of the vaginal microbiota are showing how specific bacterial communities, species and strains might influence reproductive health. One striking finding is that *Lactobacillus* species differ in their effects. “Some of these *Lactobacillus* seem to be more healthy than others,” says Buck. *Lactobacillus crispatus* has consistently been linked with good vaginal health, and women in which this species is dominant have a fivefold lower risk of developing BV than women predominantly colonized by other species. By contrast, *L. iners* is often found in women who have BV, and it seems to provide less protection against colonization by other organisms. Other *Lactobacillus* species — *L. gasseri*, *L. jensenii* and *L. vaginalis* — are dominant in fewer women, and their role in health is less clear.

Most women have a microbiota dominated by one or more *Lactobacillus* species, but a significant proportion have a more diverse array of bacterial species, with no one dominant organism. Many of these species are anaerobic microorganisms that have been associated with BV, including *Gardnerella vaginalis* bacteria. But whereas *G. vaginalis* has sometimes been proposed to be the cause of BV, recent studies have identified several culprits. “We think there's a good chance it's caused by combinations of bacteria,” says Buck.

What's more, he says, “there are racial and demographic differences in terms of what is actually healthy”. A 2011 study¹ analysed the bacteria in nearly 400 women without vaginal symptoms and found that *Lactobacillus* species were dominant in about 80% of Asian women and nearly 90% of white women, but in only about 60% of African-American and Hispanic women. In 2014, Buck's team reported² that African-American women had more-diverse bacterial communities and were more likely to be colonized by *L. iners* or BV-related bacteria,

whereas women of European ancestry were more likely to be colonized by *L. crispatus*. Van de Wijkert led a recent study³ on an ethnically mixed population of women in Amsterdam and found that *L. crispatus* was most prevalent in those of European descent, whereas women of sub-Saharan African descent were more likely to have a diverse microbiome containing *G. vaginalis*.

It is not known how and why these differences in microbiota arose. Cultural practices could have a role, such as douching and other forms of vaginal ‘cleansing’ that may encourage the growth of different communities of organisms. A woman's vaginal microbiome may be acquired from her mother early in life, or it may be influenced by genetic differences in her response to microbes. And sexual activity is a risk factor for BV, suggesting that it can be transmitted from person to person, although BV sometimes occurs in women who are not sexually active.

Jacques Ravel, a microbiologist at the University of Maryland School of Medicine in Baltimore, led the 2011 study¹ on ethnic differences. He says that making sense of the consequences of having different bacterial communities will require more than lists. “We need to be a lot more functional and look at the microbiota not just as the bacteria, but what the bacteria are doing,” he says, including their interactions with a woman's vaginal cells.

Chemical profiles of vaginal samples offer some clues. A study⁴ led by Fredricks analysed metabolites from 40 women with BV and 20 without to understand how the bacteria are changing the vaginal environment chemically. Fredricks says that the anaerobic

bacteria in the women with BV seem to be feeding on amino acids in the mucus, rather than on the carbohydrates favoured by *Lactobacillus*. This difference in food source could be driving some of the BV-related symptoms. Enzymes in the bacteria may be breaking down the protein in mucus to create a thinner, less-protective layer. And the degradation of amino acids creates amines, which give off the unpleasant odour. Biopsies of women with BV have also found that *G. vaginalis* and other bacteria associated with BV form biofilms on vaginal walls

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ZARA PICKEN

that resist breakdown, perhaps explaining why BV is so persistent in some women.

Links between the vaginal microbiome and health outcomes should become clearer as more prospective studies follow women over time. The Vaginal Microbiome Consortium, in partnership with the Global Alliance to Prevent Prematurity and Stillbirth, based at Seattle Children's Hospital, is focusing its current data-gathering efforts on preterm birth, which affects at least 10% of pregnancies. Buck says the team has collected samples from about 1,500 pregnant women at several prenatal clinical visits, as well as during and after birth. The researchers will use a combination of DNA sequencing and chemical analysis to identify factors that are potentially involved in premature deliveries, including bacteria associated with BV.

REBALANCING ACT

Studies are under way to improve the treatment of BV by exploiting the growing understanding of the relationship between the vaginal microbiota and health. Doctors already have protocols for diagnosing and treating BV, but the treatments leave much to be desired.

Hilary Reno, an infectious-disease physician at Washington University School of Medicine in St Louis, Missouri, sees many people with BV at her clinic for sexually transmitted diseases. They usually have the characteristic vaginal odour and discharge.

Reno tells them about the known behavioural risk factors, such as having a new sex partner. She warns them to avoid douching or using heavily scented bath products, saying that the vagina can take care of itself. And she prescribes a course of antibiotic pills (the most common treatment for BV is metronidazole or clindamycin). Recurrent infections can be managed with a metronidazole gel, but Reno says that this is a more expensive product and some women balk at applying it twice a week. Other antibiotics are being developed, including a product currently in clinical trials that would be a one-dose treatment for BV.

In the clinic, the most common way of diagnosing BV is to use the Amsel criteria, in which three of four conditions must be met: a vaginal pH above 4.5 (less acidic than normal); the presence of inflammatory cells, called clue cells, in the vaginal fluid; a milky, homogeneous vaginal discharge; and a 'whiff test', based on the detection of a fishy odour when potassium hydroxide is added to fluid samples. Researchers sometimes use a laboratory test called a Nugent score, in which a stain is added to vaginal fluid to make bacteria visible under a microscope, because lactobacilli are a different shape from most bacteria that cause BV. But doctors rarely take this extra step because the Amsel criteria are sufficient for a diagnosis.

Although treatment for BV is usually effective, at least in the short term, "30–70% of

women will recur in 3–6 months", says Craig Cohen, a gynaecologist and clinical researcher at the University of California, San Francisco. The lack of an effective long-term treatment has made it hard to confirm the hypothesis that eliminating BV reduces the risk of associated health problems.

Several studies have failed to show that metronidazole treatment during pregnancy reduces the rate of preterm delivery, for example. It is not clear whether failures such as these are because BV is not actually causing other health problems, or because the treatment for BV is not effective enough.

Cohen is now leading a clinical trial that will test whether an experimental probiotic treatment called Lactin-V, which delivers *L. crispatus* directly into the vagina, can establish a healthier microbiome and prevent BV. Reno, who is also involved in the study, says that her patients have been receptive to the idea. "Many of them have friends who have BV too," she says. "We've actually been able to recruit by word of mouth because of that."

Lactobacillus supplements are available as both oral and vaginal therapies, but so far, trials of these probiotics have shown only a modest benefit at best — and many have failed to colonize the vagina and prevent recurrent disease. Osel, the company based in Mountain View, California, that developed Lactin-V, is putting the product through clinical testing as a therapeutic drug, rather than as a



Craig Cohen (right) is testing Lactin-V, which delivers powdered *Lactobacillus crispatus* into the vagina.

supplement, which means it must demonstrate efficacy. Lactin-V is a powdered form of *L. crispatus* that is delivered in a tampon-like applicator. A small phase IIa trial showed that it was able to colonize some of the treated women several days after treatment. The planned phase IIb study will enrol more than 200 women and more thoroughly examine the drug's efficacy.

Some researchers question whether a single strain of bacterium can possibly restore healthy microbial communities, however. Laura Ensign, a biomolecular engineer at Johns Hopkins University in Baltimore, Maryland, wants to go further. She thinks it makes more sense to transplant entire microbiomes from healthy women to women with BV, rather like the faecal-microbiota transplants used to treat *Clostridium difficile* infections.

"It's such a complex situation and there are things about the vaginal environment that we don't really understand," Ensign says. She plans to first use vaginal secretions to transplant the full microbiota, as a proof of principle. Only later, she says, should scientists isolate the most important components to make a manufactured product.

Ensign is currently working on the protocols for screening donors and for collecting, freezing and delivering samples of vaginal mucus to women who have BV. But she does not yet know whether she will be able to proceed with small proof-of-principle studies or whether she must seek investigational new-drug status with the US Food and Drug Administration, which governs faecal-microbiota transplants.

DEFINING HEALTH

The growing body of research on the vaginal microbiome has sparked debate about how to define vaginal health. Under the current diagnostic criteria, 20–30% of healthy women of childbearing age are classified as having BV, but that figure is 50% for African-Americans. Under current guidelines, only women who have symptoms are given antibiotics; some researchers think that treating the many asymptomatic women who lack *Lactobacillus* would be an unnecessary overuse of antibiotics.

Even so, some researchers argue that BV is already overdiagnosed and overtreated. Steven Witkin, an obstetric-immunology researcher

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at Weill Cornell Medical College in New York City, is among them. He believes that host factors, such as inflammation or genetic variation, could also be important in defining what

is healthy. "You have to look at the woman, not just at the bacteria," he says. "A specific microbiome may be fine for one woman but a problem for another"

Claims of overdiagnosis might seem to conflict with current research suggesting that women whose vaginas do not contain abundant *Lactobacillus* are at higher risk of health problems, regardless of whether or not they experience symptoms of BV. Van de Wiggert and others compare *Lactobacillus* depletion to risk factors such as high blood pressure or elevated cholesterol levels. But so far, there

is not enough information about when the risk is high enough to intervene. "In the cardiovascular field, researchers have designed evidence-based risk scoring systems to decide who needs treatment," van de Wiggert says, including factors such as age, smoking, blood pressure and cholesterol levels. She thinks that microbial imbalances will need similar kinds of scoring systems to guide treatment decisions, based on both the microbes present and risk factors such as pregnancy and exposure to sexually transmitted infections.

The question of what is healthy has important consequences for women with vaginal dysbiosis. African-American women have a 50% higher risk of giving birth prematurely, and up to twice the risk of miscarriage, than white mothers, and they face higher rates of infant mortality and low birth weight. Such health disparities can have many causes, such as socio-economic factors, poor health care, lack of access to care, and the cumulative effects of institutional racism. However, says Elizabeth Corwin, who studies maternal health at Emory University School of Nursing, in Atlanta, Georgia, "the list of adverse birth outcomes is really so much higher in African-American women, even if you account for most risk factors you can think of". Researchers need to find out why.

The microbiome could be an answer. But it is not enough to compare black women with other groups. To truly understand the dynamics of health disparities, says Corwin, it is important to look within the group experiencing the disparity. To do this, Corwin is leading a prospective study with her colleague Anne Dunlop that has nearly finished enrolling 540 pregnant women, all African-American and from a wide range of socio-economic levels in the Atlanta area. The study will analyse blood and samples from the oral, gut and vaginal microbiomes, and will also survey women about their experience of chronic stress, racism, violence and abuse, as well as their health status and personal-care practices. "It doesn't just look at the microbiology, it looks at the women's lives," she says. The goal is to tease apart the various roots of risk that may be threatening African-American families. Early results indicate that about 25% of the women have BV and low levels of *Lactobacillus*.

Research on the vaginal microbiome is starting to give a clearer picture of how women vary. But it has also raised the question for Corwin, as it has for others, of what is normal in the population. ■

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1. Ravel, J. et al. *Proc. Natl Acad. Sci. USA* **108** (Suppl. 1), 4680–4687 (2011).
2. Fettweis, J. M. et al. *Microbiology* **160**, 2272–2282 (2014).
3. Borgdorff, H. et al. *PLoS ONE* **12**, e0181135 (2017).
4. Srinivasan, S. et al. *mBio* **6**, e00204-15 (2015).