SHARON NATOLI

Urine testing can reveal the presence of microorganisms in the bladder.

MICROBIOME

A bag of surprises

Once thought to be sterile, the bladder contains microbes that could influence the development and treatment of cancer.

BY CLAIRE AINSWORTH

It was with some trepidation that Jeremy Burton started his new job in 2013 as professor of urology at the Lawson Health Research Institute in London, Canada. The terms of the chair endowment stipulated that he should research probiotics — live bacteria that can be used to promote health — in relation to urology. But Burton, who had recently returned to academia after a decade working in industry, was wondering what microbiology had to do with a healthy urinary system. At the time, bacteria were known mainly as a cause of urinary-tract infections, and microbes in the gut had been implicated in kidney stones. "Other than that, it was pretty nebulous whether bacteria had any other kind of role," he recalls.

Now, a mere four years later, everything has changed. The dogma that urine, and by extension the bladder, must be sterile to be healthy has been overturned, and microbes are being discovered throughout the urinary system. Researchers are investigating potential roles for them in healthy bladders and in a range of conditions, including urge incontinence — where people experience a sudden need to urinate — and in some cancers. Burton's team has found traces of bacteria in cancerous kidneys, for example. Although still at the early discovery stage, research into the bladder's microbes promises to transform understanding of the urinary tract. "It's really grown and exploded rapidly," says Burton.

CLOUDED JUDGEMENT

The idea that healthy urine is sterile dates back to the mid-nineteenth century, when early microbiologists such as Louis Pasteur showed that, in contrast to urine left open to the air, urine contained in sealed vials did not become clouded with growing microbes. Later methods that screened patients for urinary-tract infections by culturing bacteria on slabs of agar jelly reinforced this idea. But such methods detect only microbes that thrive under culture conditions, and miss those that do not. If unculturable microbes were present in the bladder, they would not be detected.

The same used to be true of other parts of the body, such as the gut. Suspicious that they were missing something, researchers in the mid-2000s applied 'culture-independent' methods, originally developed to find unculturable microbes in environmental samples, to body parts. Instead of trying to grow the bacteria, researchers sequenced all the DNA in a clinical sample, an approach known as metagenomics. The two main methods are 16S ribosomal RNA (rRNA) sequencing and whole-genome shotgun sequencing.

In the first method, researchers look for the 16S rRNA gene, which is unique to bacteria and varies enough between them to allow broad identification. This lets them detect bacteria that are present even in low numbers and determine their genera, although not usually their species. The method cannot detect non-bacterial microorganisms, such as viruses and fungi, that lack a 16S rRNA gene. By contrast, whole-genome shotgun sequencing can identify every type of microorganism at the species level. It can reveal all the microbial genes in a sample, as well as the metabolic pathways they encode, but it sometimes fails to detect microbes that are present in low numbers.

These techniques have been used on various body sites, including the gut, skin, vagina and mouth, as part of the Human Microbiome Project, which was launched in 2008. But the bladder and urinary tract were not included, partly because the 'sterile urine' dogma was so deeply entrenched.

A handful of researchers had questioned this dogma as far back as the 1970s, but it persisted until 2012, when researchers published results of 16S rRNA sequencing studies on urine samples and found evidence of microbes in people without urinary-tract infections. Researchers led by Linda Brubaker of Loyola University Chicago in Maywood, Illinois, found[1] a range of unculturable bacteria in women's urine. However, it was not clear what the bacteria were doing there, or even if they were alive. A few months later, Derrick Foutts of the J. Craig Venter Institute in Rockville, Maryland, and his colleagues reported[2] that they had found bacteria in urine from both men and women, and observed that the two sexes had different patterns of bacterial species.

Microbes can be found in a large proportion of clinical samples, so Burton is convinced that they are not merely passengers that have accidentally been transferred into the bladder from the vagina or anus, for example. "They are

definitely there, and they are definitely alive," he says. But what they are doing there, and whether they have any physiological role, remains mysterious — so much so that some researchers are wary of using the term 'microbiome' to describe them. "A microbiome reflects a community of bacteria that have a functional relationship to each other or to their host environment," says Rembert Pieper, who studies infectious diseases at the J. Craig Venter Institute. When it comes to microbes in the bladder, "that's very difficult to prove".

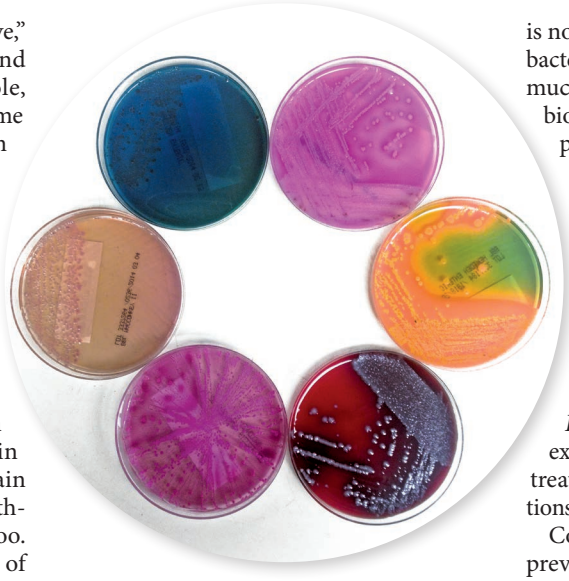
One difficulty when studying urinary microbes is that, although they occur in many people, they are generally found in low numbers. This makes it hard to obtain results using whole-genome shotgun methods, and it can skew 16S rRNA findings too. Compounding this rarity is the problem of contamination, because urine passing through the lower urethra can pick up microbes from the genital tract, for example. Samples would ideally be collected part-way through urination, allowing the first flow to wash away any microbial interlopers. Such 'mid-stream' samples are thought to be representative of the bladder, but they might still contain traces of contaminating bacteria. This contamination can be avoided by collecting samples using a catheter or a needle inserted through the abdomen, but such procedures are invasive.

Burton and his team have been developing methods that can detect low numbers of bacteria and other microorganisms, and minimize the effects of background microbial contamination. "We've spent a lot of time deriving methodologies to try to make sure that what we are looking at is a real result," he says.

COMMUNITY ACTION

Despite these problems, technologies such as metagenomics and metaproteomics — the study of all the proteins present in a sample — have the potential to offer fresh insights into the composition and function of the microbial communities in the bladder, says Pieper. They could also allow researchers to explore associations between bladder microbes and disease, and could potentially unravel causal effects. For example, Pieper and his colleagues recently presented the results of an unpublished metagenomic study of urine samples from people with suspected urinary infections. Their whole-genome shotgun results revealed infection-related bacteria and identified bacteria, viruses and fungi not known to colonize the urinary tract. Meanwhile, his team conducted a metaproteomic study[3] on the same samples that allowed them to explore the relationship between disease-causing bacteria and proteins arising from the patient's immune response, and also with proteins associated with damage to the bladder lining.

"They are definitely there, and they are definitely alive."



EVANN HILT

Enhanced quantitative urine culture can grow bacteria previously thought to be unculturable.

There is currently no hard evidence linking microbes with bladder cancer, but researchers are beginning to explore several plausible mechanisms. One is chronic inflammation. Transient inflammation is an important part of the body's immune defence against infection, but if that inflammation persists, it could potentially contribute to the development of cancer. Inflammatory bowel disease, for example, is thought to increase a person's risk of colon cancer several-fold. In the bladder, a parasitic-worm infestation called schistosomiasis is associated with cancer development if the infestation is chronic and remains untreated (see page S46).

The potential link with chronic inflammation raises the question of whether repeated urinary-tract infections might be involved in the development of bladder cancer. One of the largest epidemiological studies of bladder cancer conducted so far reported[4] in 2015 that repeated, regular bouts of cystitis were associated with increased risk, but whether the association was causative is unclear. Further studies will be needed to confirm any links, which remain "a little tenuous" at the moment, according to Burton.

As well as exploring whether microbes are involved in generating bladder cancer, researchers are investigating how they might affect its treatment. An established bladder-cancer therapy involves introducing the bacillus Calmette–Guérin (BCG) vaccine into the bladder (see page S36). Scientists think the bacteria in the vaccine stick to molecules in the extracellular matrix — the jelly-like substance that surrounds the body's cells — and stimulate an immune response that attacks the cancer cells.

Although BCG treatment works in many cases, up to 40% of people do not respond. It is not clear why, and it is possible that other bacteria in the bladder have a role. At different mucosal sites, such as the mouth, the microbiota reduce the inflammatory response to pathogens that would otherwise stimulate the immune system for their own advantage, says Burton. His team has shown that *Lactobacillus iners*, a bacterium found in healthy bladders, binds particularly strongly to fibronectin, the same extracellular-matrix molecule as that bound to by the BCG bacteria, thereby potentially affecting BCG's interaction with it. Could the activity of *L. iners* or other bacteria in the bladder explain the differences in response to BCG treatment? "These are the sort of basic questions we don't have the answer to," says Burton.

Could manipulating the microbiota help to prevent bladder cancer or stop it recurring? Bladder cancer has one of the highest rates of recurrence of all cancers, with between 50% and 70% of patients whose bladders are not removed experiencing a relapse within five years of treatment (see page S34). Several studies have suggested probiotics as a promising strategy.

In 2008, for example, a team in Japan reported[5] that people with bladder cancer who drank a probiotic containing *Lactobacillus casei* (sold commercially as Yakult), while also receiving chemotherapy treatments infused into the bladder, had recurrence rates that were 15% lower than those of subjects receiving chemotherapy alone. Critics of the study said that the pattern of patient dropout and lack of blinding may have undermined its conclusions, although the authors disagreed. Previous studies in animals, conducted by several research groups, also suggest that probiotics can have anticancer effects in the bladder. These studies suggest that probiotics deserve further investigation, says Burton.

Bladder microbiologists now have plenty of avenues to explore, and face several questions relating to cancer. Are bacteria exerting a protective effect by reducing inflammation? Do they mop up or inactivate compounds known to promote cancer development, including heavy metals such as cadmium? Are bacteria in other microbiomes, such as the gut, influencing bladder health with crosstalk between the two? If Burton was wondering what to study four years ago, he certainly isn't now. "I started off thinking, 'there's nothing to do here'," he says. But now his thoughts are "Oh my God, there's too much to do". ■

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CORRECTION

The Outlook article 'A bag of surprises' (*Nature* **551,** S40–S41; 2017) incorrectly identified fibronectin as a molecule produced by BCG bacteria. Fibronectin is produced by the human body and is a putative binding site for the BCG bacteria.