In late post-surgical colon 'leaks,' finger points to microbes

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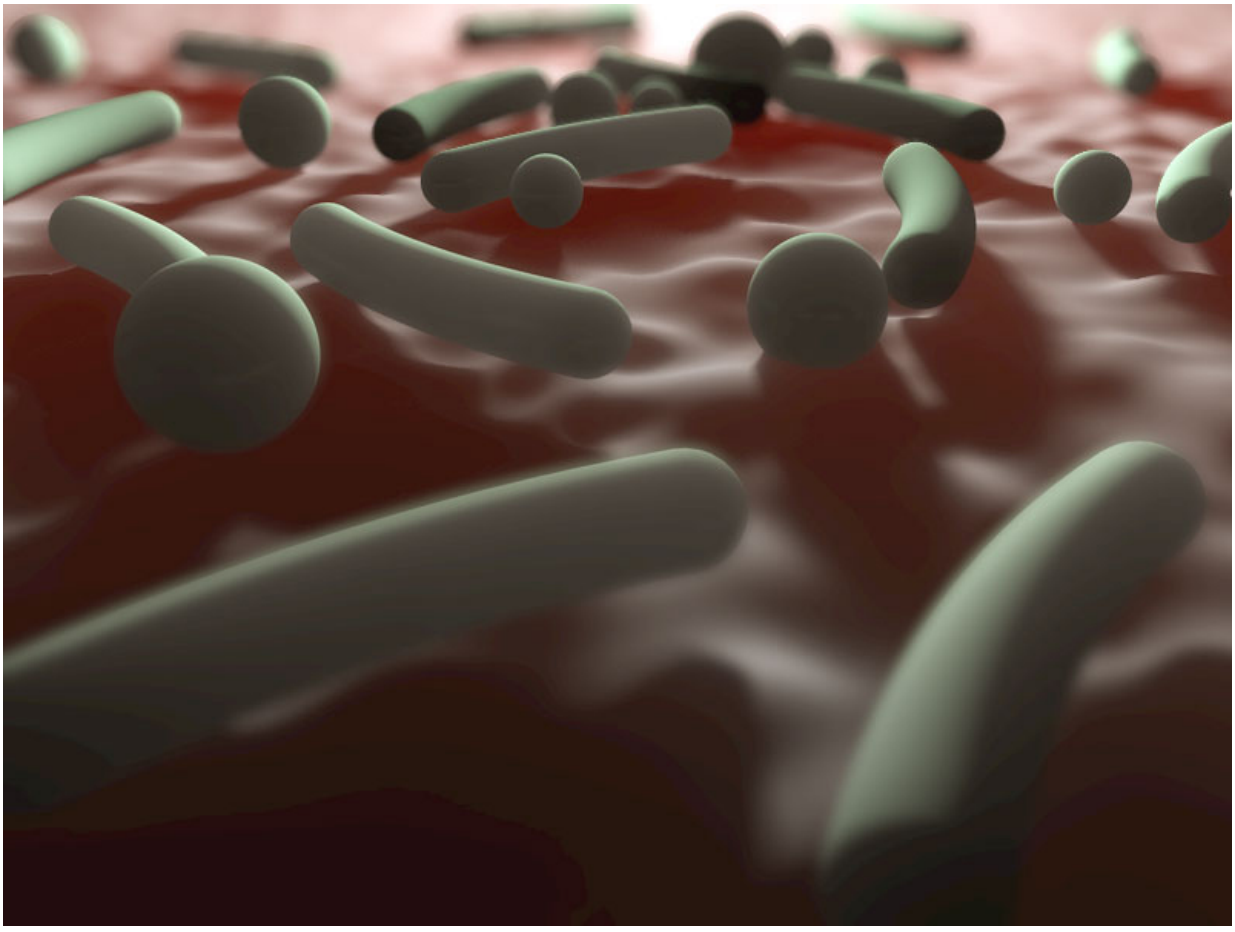

Enterococcus faecalis are commensal bacteria of the human gut microbiome. Benjamin Shogan and colleagues find that E. faecalis can destroy tissue and prevent intestinal leak from healing. Credit: V. Altounian / Science Translational Medicine

Post-surgical leaks that develop after a segment of the colon has been removed and stitched back together often are caused not by negligence or technical error but by bacteria in the bowel that elude antibiotics, according to new evidence about this devastating complication of gastrointestinal surgery.

Such leaks, which can develop days or weeks after the procedure, allow the bowel's contents to spill into the abdomen and can cause pain, fever, sepsis and even death.

In patients undergoing high risk surgery such as in the rectum, leak rates can approach 30 percent. This can compel surgeons to perform a temporary ileostomy or colostomy so that the stool stream empties directly from the intestine into an external bag. This approach diverts stool away from the newly formed intestinal connection, giving it a chance to heal, but it can be distressing to patients and requires a second operation to restore intestinal continuity.

Findings of the study provide insight into why post-surgical leaks occur and could lead to more effective ways to reduce them.

"For more than 60 years, surgeons have suspected that intestinal microbes played a causative role in leakage after intestinal surgery," said the study's senior author, John Alverdy, MD, professor of surgery at the University of Chicago, "but we were never certain how much they contributed, which bacteria were involved or how to prevent it. Identification of one of the primary microbial culprits points us toward better ways to reduce the risk."

In the May 6, 2015, issue of *Science Translational Medicine*, the researchers trace the bulk of the damage to one strain of the intestinal microflora: *Enterrococcus faecalis*. The concentration and prevalence of this species increases dramatically in the intestine during the first weeks

of recovery following intestinal surgery

"This microbe has all the tools to complicate healing," Alverdy said.

E. faecalis degrades intestinal connective tissues such as collagen I, which plays a central role in wound repair as it helps to form scar tissue. It activates the enzyme matrix metalloprotease 9 (MMP9), which degrades collagen IV, another connective tissue involved in wound healing. Standard antibiotics used in colon surgery often do not eliminate this microbe.

By dissolving the scar tissue needed to seal the intestine during healing, *E. faecalis* creates small holes in the intestine at the anastomosis, the place where part of the bowel has been removed and the two flanking segments reconnected.

The scientists searched for the cause of these leaks by performing colon resections on healthy rats, removing 1 centimeter of the lower colon, then reconnecting the two adjoining segments. Then they used genetics to track the bacteria found at the surgical site as they evolved over time.

Of all the bacteria at these sites, *E. faecalis* stood out as the dominant microbe. It was far more common in rats that had developed post-surgical leaks than those without. By the time leaks occurred, the relative abundance of *E. faecalis* had increased 500-fold.

The leaks appeared to be caused by the breakdown of collagen in the healing intestinal wall, so the researchers looked for bacteria that might make enzymes capable of collagen degradation. Again, *E. faecalis* had "the raw goods to be able to breakdown healthy scar," Alverdy said, making it the central focus of the study.

They found two different strains. One of them, labeled E1, produced

limited collagenase. E2 produced a great deal. This E2 strain began degrading collagen much sooner and was more virulent.

When the researchers exposed rats to each of the two strains via enemas soon after surgery, those that received E2 all developed leaks at the surgical site within six days. None of the rats receiving strains that produced negligible amounts of collagenase produced a leak.

The choice of antibiotics and how they were delivered also affect the risk of a leak. Rats that received three antibiotics—ciprofloxacin, metronidazole and neomycin—directly applied to the intestinal tissues via enema immediately after surgery and the next day had no *E. faecalis*, less MMP9 and no leaks. Rats that received antibiotics intravenously had *E. faecalis* remaining on the tissues and a high leak rate.

In the 1970s and '80s, most surgeons routinely administered oral antibiotics that targeted intestinal bacteria such as *E. faecalis*. These were replaced in the 1990s by a group of intravenous antibiotics, the cephalosporins, many of which do not eliminate *E. faecalis*.

"It is noteworthy that the most commonly used antibiotics in colon surgery today do not eliminate *E. faecalis* in the gut, but in fact allow it to proliferate and predominate," the study authors point out. "In fact, *E. faecalis* has been shown to 'bloom' in the intestine following a single parenteral dose of a cephalosporin."

Alverdy said identifying *E. faecalis* is only the beginning.

"There are many other intestinal bacteria that can produce collagenases and cause leaks," he said. "We need to identify those that disrupt the course of healing and eliminate them."

Alverdy and colleagues are planning a clinical trial that will test their

hypothesis in humans. In order to identify the bacteria that cluster at the surgical site and determine their role in causing leaks, the researchers plan to monitor patients via colonoscopy for up to three weeks after surgery, to observe daily how their anastomotic tissues heal.

More information: Collagen degradation and MMP9 activation by Enterococcus faecalis contribute to intestinal anastomotic leak, *Science Translational Medicine*, stm.sciencemag.org/lookup/doi/ … scitranslmed.3010658

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