

Gut check reveals vast multicultural community of bugs in bowels

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Mention the phrase "diverse ecosystem," and it conjures images of tropical rainforests and endangered coral reefs. It also describes the human colon.

A new study from the Stanford University School of Medicine reveals in greater detail than ever before the full extent of the bacterial community inhabiting the human bowel - 10 times more diverse than previous research had suggested. The technology that yielded this result offers the potential for much more accurate assessments of people's complex internal ecosystems, as well as more-sophisticated monitoring of the degree to which they are affected by, for example, antibiotics. The study will be published online Nov. 18 in the journal *Public Library of Science - Biology*.

The prospect of swallowing an antibiotic takes on a new meaning when you consider that for each human cell in your own body, there are roughly 10 single-celled microbes, most of which live in our digestive tracts. In sheer numbers, the mammalian colon harbors one of the densest microbial communities found on Earth. Estimates of the number of distinct kinds of bacteria in the human colon, based on the older technique of detecting lower-gut-dwelling organisms by growing them in petri dishes, have ranged upwards of 500. But these estimates have been hampered by huge numerical disparities between more abundant vs. rarer bacterial varieties, rendering the latter essentially undetectable (not to mention that only some varieties of organisms thrive in culture while others perish).

David Relman, MD, professor of medicine and of microbiology and immunology, and his associates used a technique known as pyrosequencing, developed outside of Stanford, to get a more complete count of the different varieties of bacteria colonizing the human colon, including myriad strains that exist in small numbers. Pyrosequencing has been used before to assess the richness of bacterial ecosystems in marine environments and soil, Relman said. "But this was one of the first times it has been employed to look inward at the ecosystems within our own bodies," he said.

It turns out that the colon is even more multicultural than had been imagined. In the new study, the investigators found at least 5,600 separate species or strains by looking at variations in DNA sequences that code for a molecule universal among all living cells. Pyrosequencing generates extremely large numbers of small DNA "tags" copied from the genes of organisms being examined.

"The new gene-sequencing technology lets us check far more 'bacterial ID cards' than the older methods did," said Les Dethlefsen, PhD, a postdoctoral researcher in the Relman laboratory who is the first author of the study. This, in turn, makes possible more-precise assessments of the effect of a disturbance in the lower-gut ecosystem - say, through the administration of broad-spectrum antibiotics - Dethlefsen said.

By and large, intestinal microbes just mind their own business, subsisting on complex carbohydrates impervious to our own digestive enzymes. But they also perform numerous critical functions of benefit to us, from fine-tuning our immune systems to producing nutrients such as vitamin K. Importantly, by occupying intestinal real estate and devouring its useless-to-us, tasty-to-them contents, friendly gut organisms prevent pathogens from taking over, much as a house filled with legal residents deters squatters.

The researchers tested the effects of a commonly used antibiotic, known for its mild digestive-tract side-effect profile, and were able to show that it had a measurable and pronounced effect on gut microbes. Fortunately, an approximately normal balance appears to be restored within a matter of weeks.

The researchers compared bacterial populations in stool samples provided by three healthy volunteers at various intervals over an eight-month time course that included a five-day oral regimen of the broad-spectrum antibiotic ciprofloxacin, or Cipro. Based on results from the powerful pyrosequencing methodology, ciprofloxacin treatment immediately caused a significant change in the abundance of about 30 percent of the distinct strains or species normally present, even though subjects themselves reported no digestive disturbances whatsoever. Most of this effect was due to a drop in abundance of various bacterial strains, including the complete disappearance of rare bacterial variants whose numbers were so low to begin with that their loss wouldn't have been noticed with older survey methodologies.

On a comforting note, the volunteers' gut-bacteria populations and diversity were more or less restored to normal within a month after the ciprofloxacin regimen was discontinued. "The bacterial ecosystems were knocked for a loop, but they snapped back quickly," said Dethlefsen.

"We clinicians would like to think antibiotics used on an otherwise healthy person would have only mild side effects," said Relman. He and Dethlefsen think the lack of noticeable side effects was due to bugs that were relatively unaffected by ciprofloxacin, or whose numbers were actually allowed to grow because of the reduction in their competition, picking up the slack left by their vanquished compatriots.

Nonetheless, Dethlefsen said, there could easily be subtle clinical effects that are not immediately apparent. For example, some of the low-

frequency bacterial varieties that are wiped out by even a relatively user-friendly drug such as ciprofloxacin may have important physiological functions whose loss manifests only later.

Now Dethlefsen, Relman and their colleagues are armed with a method that allows them to ferret out even minuscule subgroups of microbes with unprecedented precision. They are forging ahead to find out more about what short- and long-term effects various perturbations, such as dietary changes or other antibiotics, inflict on different individuals' lower-gut microbial populations.

Source: Stanford University Medical Center

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