Typhoid fever bacteria collect on gallstones to perpetuate disease

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A new study suggests that the bacteria that cause typhoid fever collect in tiny but persistent communities on gallstones, making the infection particularly hard to fight in so-called "carriers" - people who have the disease but show no symptoms.

Humans who harbor these bacterial communities in their gallbladders, even without symptoms, are able to infect others with active typhoid fever, especially in developing areas of the world with poor sanitation. The disease is transmitted through fecal-oral contact, such as through poor hand-washing by people who prepare food.

Typhoid fever is rare in the United States, but it affects an estimated 22 million people worldwide, causing symptoms that include a high fever, headache, weakness and fatigue, and abdominal pain. It leads to hundreds of thousands of deaths each year.

Scientists and physicians have known for decades that these bacteria, *Salmonella enterica serovar Typhi*, accumulate in the gallbladder. In fact, the most widely accepted treatment of chronic typhoid infection is removal of the gallbladder.

"We're trying to get to the heart of why this is. Why does Salmonella sit in a pool of highly concentrated detergent, which is what bile is, but not die?" said John Gunn, professor of molecular virology, immunology and medical genetics at Ohio State University and senior author of the study. "It's got to survive in some way, and a good way to survive is by forming

a biofilm."

Biofilms - in this case, the collection of bacteria on gallstones - typically do not respond well to antibiotics or the human immune response. But now that the biofilms themselves have been discovered in association with asymptomatic typhoid infection, they present a potential treatment alternative to expensive and invasive gallbladder removal, Gunn said.

Specifically, targeting a sugar polymer on the bacterial surface that promotes development of the biofilm might be a strategy to prevent biofilm formation in the first place, he said.

The research appears this week in the online early edition of the *Proceedings of the National Academy of Sciences*.

Gunn and colleagues observed this biofilm formation in mice infected with a strain of Salmonella bacteria similar to the strain that causes typhoid fever in humans. The scientists also detected these biofilms on gallstones in about 5 percent of humans in a Mexican hospital who had their gallbladders removed because of complications from gallstones. Typhoid fever is widespread in Mexico.

"The mouse data coupled with the human data suggest strongly that biofilms lay a foundation that allows for establishment and maintenance of chronic typhoid infection," said Gunn, also a vice director of Ohio State's Center for Microbial Interface Biology.

And the researchers suspect biofilms are at play in the gallbladder's association with typhoid fever because in most cases, the only way to treat a biofilm-related infection is to remove whatever the biofilm has attached to from the body. For example, infections that form on catheters, implanted joints or artificial heart valves typically result from biofilms, and the only way to clear the infection is to remove those

devices.

"Information in our lab and in the literature that gallstones were associated with how people became carriers of typhoid bacteria, that organisms were confined to one site, and that antibiotics are ineffective so one has to remove the gallbladder for successful therapy - it all fit with biofilm-related disease," Gunn said.

In the study, the researchers fed mice either normal food or a high-cholesterol diet for eight weeks, intending to induce gallstones in the animals on the fatty diet. The scientists then gave these mice a type of Salmonella bacteria designed to mimic a chronic human typhoid infection without causing actual illness in the mice. A control group of mice received no bacteria.

The number of bacteria harbored in the gallbladders of mice with gallstones increased over time, becoming abundant within 21 days, and was significantly higher than bacteria in mice that did not have any stones. No bacteria were detected in mice that weren't given the infection, even if they had gallstones.

In the infected mice, the Salmonella bacteria also could be seen in the gallbladder lining and in bile as well as on the surface of the gallstones. The gallstones were the focus of this study because Gunn's lab has determined in previous experiments that Salmonellae are attracted to cholesterol-coated surfaces.

There are two common types of gallstones - cholesterol stones and brown or black stones composed primarily of calcium bilirubinate, which can be found in bile. Gunn's test-tube research to date had suggested that *Salmonella Typhi* bacteria bind particularly well to cholesterol gallstones to form biofilms, and this current study supported that.

Three weeks after infection, biofilms covered about 50 percent of the surfaces of the gallstones removed from the infected mice.

"What we think is that having gallstones makes you more susceptible to becoming a carrier because it provides that environment for Salmonella to bind to the surface, form a biofilm and establish infection," Gunn said. "Whether that happens 100 percent of the time, nobody knows."

In a second component of the mouse study, the researchers tested fresh fecal pellets from infected mice to test the association between gallstone biofilms and transmission of a typhoid-like infection via feces, a phenomenon called "shedding." The mice with gallstones shed three times more bacteria than did infected mice without gallstones.

"The mice that had gallstones and were infected with bacteria had a much higher rate of shedding, meaning those bacteria were released, probably because they had more bacteria in the gallbladder itself," Gunn said.

The mouse data not only supported Gunn's hypothesis that gallstones present at least one surface on which Salmonella biofilms form and maintain the carrier state of typhoid fever. The researchers also realized they had developed a new mouse model for further study of asymptomatic typhoid carriage.

Gunn and colleagues also obtained data from humans at a hospital in Mexico whose gallbladders were removed as a treatment for gallstone complications. Though none of the patients had ever shown symptoms for typhoid fever, 5 percent of them ended up being carriers of Salmonella Typhi bacteria biofilms on their gallstones. In the single patient determined to be a typhoid carrier who didn't have biofilm on his gallstones, the stones were dark in color, suggesting they were likely composed of something other than cholesterol, Gunn said.

This ability of a single individual to harbor latent bacteria elsewhere in the gallbladder leads Gunn and colleagues to suspect that biofilms can form elsewhere in the gallbladder - perhaps in its lining or persisting within specific cells of the gallbladder wall. Gunn's lab is exploring those possibilities.

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