Medical Xpress

# Scientists discover dangerous new method for bacterial toxin transfer

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Scientists have discovered a new way for bacteria to transfer toxic genes to unrelated bacterial species, a finding that raises the unsettling possibility that bacterial swapping of toxins and other disease-aiding factors may be more common than previously imagined.

In a laboratory experiment, the scientists from NYU School of Medicine discovered that Staphylococcus aureus, a notorious bacterium that causes toxic shock syndrome and many other types of infections and is the scourge of hospitals nationwide due to its growing antibiotic resistance, could co-opt viral parasites as secret pipelines for transferring toxin genes to vastly different bacterial species.

Microbes have been known to gain antibiotic resistance through the transfer of plasmids, extra-chromosomal pieces of DNA that can be shuttled between unrelated bacteria. Some Staph aureus strains, in particular, have become major public health concerns after gaining antibiotic resistance through plasmids transferred from other species.

The startling new finding, published in the Jan. 2, 2009, issue of *Science* by John Chen, Ph.D., and Richard Novick, M.D., suggests that Staph aureus also can take advantage of bacteriophages, viruses that infect bacteria, to pass genetic material on to completely unrelated bacteria. In the lab, the researchers showed that Staph could transfer genes for deadly toxic shock to Listeria monocytogenes, which is already known to cause a potentially deadly form of food poisoning. This is the first time that phages have been observed to serve as shuttle vehicles for bacterial

toxins between different species.

The experiments were part of a general exploration of bacteriophages. Until now, scientists did not believe that bacterial viruses could transfer genes between unrelated bacterial species. When Dr. Chen, a postdoctoral fellow in Dr. Novick's laboratory, suggested doing some transfer experiments with bacteriophages, Dr. Novick, said "go ahead, but it won't work." To his surprise, the bacterial viruses did the completely unexpected.

"We have found that a bacteriophage can transfer genetic elements, or DNA, between unrelated bacterial species in a way that was really not expected," Dr. Novick says. Although the study uncovered only transfer to Listeria, he said the relatively high efficiency suggests that other cases may well exist in nature, raising new questions about the ease with which unrelated microbes might gain some of the more notorious S. aureus toxins. Since bacteriophages are extremely common among bacteria—a drop of seawater contains millions of bacteriophages, for example—other combinations of disease-causing species could engage in phage-mediated toxin gene transfer.

"We happened to be looking at the nasty toxic shock toxin. As far as we know, this toxin is produced only by Staphylococcus aureus. No other species have been shown to produce it," Dr. Novick says. "If it suddenly appeared in Listeria, it could enable that species to cause toxic shock, which could have a major impact on human pathogenicity." In other words, Listeria could become an even more dangerous threat to human health.

The high frequency of genetic transfer suggested that other bits of DNA could be passed along, and the scientists showed in another experiment that staphylococcal plasmids containing antibiotic resistance genes could also be transferred to Listeria via bacteriophages.

Although Drs. Chen and Novick successfully transferred DNA from an S. aureus strain to L. monocytogenes via bacteriophages in the lab—using a strain in which the gene for toxic shock toxin had been inactivated to preclude the potential hazard of toxic-shock-causing Listeria—the researchers stress that they have not yet discovered any Listeria strain that contains the transferred genetic elements or is able to produce toxic shock toxin or other staphylococcal toxins in the wild. Both microbes, however, are known to afflict dairy cows, sheep and goats with a serious bacterial infection called mastitis, an inflammation of the udder, which costs the global dairy industry billions in lost revenue every year.

In treating mastitis, veterinarians have tried to get around the vexing issue of antibiotic resistance with a new strategy that uses bacteriophages to attack the infection-causing microbes. When they modeled that phage therapy in the lab, however, the NYU researchers observed a phage-mediated transfer of pathogenic S. aureus genes to L. monocytogenes in raw milk. The unwitting selection of a phage with similar properties for mastitis therapy could abet the transfer of new toxin genes, Dr. Novick warns. Given that possibility, he suggests that all phages being considered for mastitis treatments should be tested beforehand to ensure they couldn't be used as bacterial DNA conduits.

Most bacteriophages have a very narrow host range and are often limited to different strains, or variants, of the same bacterial species. "Phages normally have a pretty specific cell receptor that they must find to infect the cell," Dr. Novick explains. The ability of several Staphylococcus-specific phages to infect Listeria with high efficiency, he says, suggests that the two unrelated bacterial species share a similar receptor, possibly a protein protruding from the cell where the phage can dock.

Bacteriophages also generally kill their infected hosts by bursting open the cells, a process that forms obvious plaques, or clear circles, on a lawn

of bacteria in a Petri dish. If a phage does not form plaques on a specific bacterial strain, researchers usually conclude that the strain is not susceptible to phage infection. Surprisingly, the genetic transfer observed by Drs. Chen and Novick occurred even though the phages did not form telltale plaques on the L. monocytogenes cell cultures. "The transfer is happening by stealth, silently, without anyone noticing," Dr. Novick says.

The distinct chromosomal regions within Staph aureus that carry many of its toxins, the pathogenicity islands, are actually related to phages, allowing them to be easily activated and packaged into phage particles, says Dr. Novick. Most phages, however, require a very specific DNA sequence for attaching this packaged genetic material to the genome of other bacteria. "If you knock out that attachment site, the phage has a very difficult time finding another one," he says.

He and Dr. Chen found that the rules of the attachment process in both Staph aureus and L. monocytogenes have been relaxed to allow attachment at more DNA sites, thus removing a major barrier for integrating new genetic elements like the pathogenicity islands.

Drs. Chen and Novick have not thus far been able to demonstrate that other bacterial species whose outer cell walls are similar in structure to that of L. monocytogenes could take up the Staph aureus pathogenicity islands through phage infection. They hope to cast a wider net in follow-up studies to determine whether other species could be hosts for pathogenic Staph aureus genes. In addition, they plan to follow the fate of an S. aureus bacteriophage once it injects its own DNA into L. monocytogenes, and they would like to test whether the phage-mediated exchange of genetic information goes only one way or in both directions between the microbes.

Source: York University School of Medicine