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Researchers Identify New Mechanism Behind Spread of Antibiotic Resistance

Howard Hughes Medical Institute (HHMI) researchers have discovered that the commonly prescribed antibiotic, ciprofloxacin, is among those that may inadvertently trigger the spread of antibiotic resistance genes between different species of bacteria. The new studies show how indiscriminate use of antibiotics can promote the evolution of new antibiotic-resistant organisms.

The new details about how antibiotic resistance spreads among cholera bacteria emerged during studies by HHMI investigator Matthew Waldor, John Beaber and Bianca Hochhut at Tufts University School of Medicine. The researchers emphasize that their findings about how bacteria transmit antibiotic resistance further underscore the hazards of widespread chronic use of antibiotics in humans and farm animals.

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— **Matthew K. Waldor**

"This new understanding of the mechanism of transfer is very worrisome, for it shows how indiscriminant antibiotic use can promote the dissemination of antibiotic resistance," Waldor said.

The researchers published their findings December 21, 2003, in an advance online publication in the journal *Nature*.

The group's experiments were designed to probe how bacteria transfer certain collections of genes through contact with one another. These mobile gene regions, or integrative conjugative elements, are part of the normal mechanism by which bacteria exchange genes with one another. Genes in these mobile regions can excise themselves from one bacterial chromosome and integrate into the chromosome of a new host bacterium.

Waldor and his colleagues focused on the collection of antibiotic-resistance genes called SXT, which beginning in 1993 appeared in almost all strains of cholera found in Asia. The presence of SXT confers resistance to four different antibiotics, and has forced clinicians in that part of the world to largely abandon previously effective treatments for cholera.

When the researchers sequenced the SXT region in earlier work, they found that it included a gene called *setR*, which encodes the SetR repressor protein. The protein is similar to one found in viruses that infect bacteria, where it normally maintains the viral genes in an inactive state in the host bacterium. However, when damage to the bacterial chromosome activates the “SOS response,” the viral repressor switches off, triggering the virus to replicate itself and spread from the damaged bacterium.

Reasoning that the same repressor process might control SXT, the researchers set out to explore the function of the SetR protein. In their initial studies, they found that SetR repressed two gene regions within SXT, called *setC* and *setD*, which are activators of the excision and transfer of the SXT collection of genes. They also showed that SetR is itself inactivated by a protein called RecA, which is turned on during the SOS response. Waldor and his colleagues found that when the RecA protein is activated, it inactivates SetR by snipping it apart, in a process called proteolysis.

The researchers pinpointed the target of SetR repression, a promoter region upstream from *setC* and *setD* on the bacterial chromosome. They found that SetR ordinarily inhibits the excision and transfer of SXT. When the SOS response leads to degradation of SetR, *setC* and *setD* become active, allowing transfer of the antibiotic resistance genes to other bacteria.

The SOS response can be activated by environmental stimuli such as ultraviolet radiation, and also by certain classes of antibiotics. Importantly, the researchers found that the widely used antibiotic, ciprofloxacin, was among those that induced the SOS response and enhanced transfer of SXT.

“This is very different from the process by which antibiotic use selects for resistant organisms that are already in the population,” said Waldor. “We have shown how antibiotic use can promote the evolution of new antibiotic-resistant organisms that have obtained their resistance genes from other species.”

According to Waldor, while their studies concentrated on SXT in cholera, similar integrative conjugative elements exist in other pathogenic bacteria. “However, at present we're not sure how widespread this type of repressor-regulated element is, so we can't say for sure how important this mechanism is for the spread of antibiotic resistance,” he said.

Further studies will aim at understanding in greater molecular detail how the SetR repressor regulates the expression of the *setC* and *setD* genes, said Waldor. The researchers will also seek to understand how other stimuli govern transfer of SXT from one bacterium to another.