Blocking the spread of antibiotic resistance in bacteria

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It's as simple as A, T, G, C. Northwestern University scientists have exploited the Watson-Crick base pairing of DNA to provide a defensive tool that could be used to fight the spread of antibiotic resistance in bacteria -- one of the world's most pressing public health problems.

The resistant nasty pathogens cause thousands of deaths each year in the United States. Particularly virulent is methicillin-resistant *Staphylococcus aureus* (MRSA), which often cause hospital- and community-acquired infections. The Centers for Disease Control and Prevention calls antibiotic resistance one of its top concerns.

The Northwestern researchers have discovered that a special DNA sequence found in certain bacteria, called a CRISPR locus, can impede the spread of antibiotic resistance in pathogenic *staphylococci*. It blocks the DNA molecules (plasmids) that move from one cell to another, spreading antibiotic resistance genes. With the plasmids disabled, which the researchers believe is a result of the DNA itself being destroyed, the resistance cannot spread.

The blocking mechanism takes advantage of the fact that a small sequence of this CRISPR locus matches staphylococcal conjugative plasmids, including those that confer antibiotic resistance in MRSA strains.

The findings will be published in the Dec. 19 issue of the journal *Science*.

"If this mechanism could be manipulated in a clinical setting, it would provide a means to limit the spread of antibiotic resistance genes and virulence factors in staph and other bacterial pathogens," said Erik Sontheimer, associate professor of biochemistry, molecular biology and cell biology at the Weinberg College of Arts and Sciences. Sontheimer and postdoctoral fellow Luciano Marraffini carried out the study. Both are authors of the paper.

Generally, antibiotic resistance is spread through a process called horizontal gene transfer, the simple passing of genes from one individual to another. Bacteria are very adept at this, thus the interest among scientists in identifying biological pathways that limit horizontal gene transfer, particularly the process called conjugation, which is most commonly associated with the spread of antibiotic resistance.

Sontheimer and Marraffini studied the CRISPR locus in a clinically isolated strain of *Staphylococcus epidermidis*, bacteria that cause infections in patients whose immune systems are compromised or who have indwelling catheters.

The two found that the CRISPR locus can block the transfer of plasmids from one *S. epidermidis* strain to another or between *S. epidermidis* and S. aureus strains. The researchers' experiments show that the CRISPR locus limits the ability of the *S. epidermidis* strain to act as a plasmid recipient, essentially denying entry to the genes carrying the resistance.

They also found that "CRISPR interference," as this phenomenon is known, involves the targeting of the incoming plasmid or virus DNA directly. The CRISPR locus gives rise to RNA molecules (chemical cousins of DNA) that apparently recognize the incoming plasmid or virus DNA by the classic base pairing defined by Watson and Crick. This recognition then appears to lead to DNA destruction by unknown mechanisms.

Virtually any DNA molecule could be targeted with CRISPR interference. This blocking mechanism can, in principle, be "programmed" by incorporating into the CRISPR locus any desired A, T, G, C sequence that would match a target. It could potentially be used to fight antibiotic resistance in other pathogenic bacteria, including those that cause anthrax, tuberculosis, cholera and plague.

The programmable nature of CRISPR interference makes it analogous to RNA interference (RNAi), which has received much attention for its ability to block the functions of specific genes in human cells. Unlike RNAi, however, CRISPR interference operates naturally in bacteria.

The *Science* paper is titled "CRISPR Interference Limits Horizontal Gene Transfer in Staphylococci by Targeting DNA."

Source: Northwestern University