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Researchers Determine How "Hospital Staph" Resists Antibiotics

Structural studies of a key enzyme have revealed how dangerous strains of the bacterium, *Staphylococcus aureus*, become resistant to antibiotics.

Resistant strains of *Staphylococcus aureus*, which are also called "hospital staph" because of their prevalence in hospitals, constitute 34 percent of the clinical isolates in the United States, more than 60 percent in Japan, Singapore and Taiwan, and more than 50 percent in Italy and Portugal. And the emergence of strains of *Staphylococcus* that are resistant to vancomycin -- the antibiotic of last resort -- makes public health concerns about drug-resistant strains of the bacterium even more urgent.

In an article published online on October 21, 2002, in the journal *Nature Structural Biology*, Daniel Lim and Natalie Strynadka, who is a Howard Hughes Medical Institute international research scholar, reported structural studies of the enzyme known as penicillin-binding protein 2A (PBP2a). Lim and Strynadka are at the University of British Columbia.

"A computer-generated model of the penicillin-binding protein 2a from the bacterium, *Staphylococcus aureus* ."

Before the advent of drug-resistant strains of *Staphylococcus aureus*, staph infections were treated using beta-lactam antibiotics such as methicillin, which block the bacterial enzyme PBP. This enzyme -- called a transpeptidase -- normally catalyzes the cross-linking of structural molecules in the bacterial cell wall. Blocking PBP with methicillin weakens the cell wall, which ultimately bursts, killing the bacterium.

However, a methicillin-resistant strain of the bacteria has evolved that has acquired the gene for a new version of PBP -- PBP2a -- from another bacterium. The challenge, as well as the opportunity, said Strynadka, is to understand why PBP2a is resistant to beta-lactam antibiotics.

"What is very attractive from a therapeutic point of view is that PBP2a constitutes a single target, in terms of developing new antibiotics that can overcome this resistance," she said.

To understand the detailed structure of PBP2a, Lim produced a version of the enzyme that lacked a segment that anchored it to the cell membrane, but which retained the enzymes catalytic activity. Eliminating the anchoring segment rendered the protein soluble, so that the researchers could crystallize the protein for use in x-ray crystallography studies. In x-ray crystallography, researchers direct an x-ray beam through crystals of a protein to deduce its structure by analyzing the pattern of diffraction that is produced. Analysis by Lim and Strynadka revealed critical differences between the structures of PBP2a and other beta-lactam antibiotic sensitive PBPs.

"By comparing the native enzyme with previously known structures of transpeptidases, we came to understand that PBP2a had evolved distortions of the active site that prevent an effective reaction with the antibiotic," said Strynadka. An enzymes active site is the pocket within which the enzyme carries out its catalytic reaction. In the case of PBP2a, this catalytic reaction drives the essential cross-linking of cell-wall proteins in the bacterium.

"Although beta-lactam-sensitive bacteria still have a number of these normal transpeptidases, they also have PBP2a, which because of its distorted active site doesnt react easily with the antibiotic," said Strynadka. "Thus, PBP2a can produce sufficient cross-linking in the cell wall so that the bacterium survives."

The researchers studies showed that PBP2a is different from normal PBPs throughout its structure, and not just at the active site. This suggests that the distorted active site is an integral part of the enzyme, said Strynadka. The good news is that the PBP2a active site structure has unique features which can be used to design new types of antibiotics that block its resistance activity.

"The active site of PBP2a is quite extended and relatively hydrophobic," said Strynadka. "The structures we observe now allow for the rational design of specific PBP2a inhibitors that are tailored to better fit these features of the PBP2a active site allowing better affinity and inactivation of the enzyme."