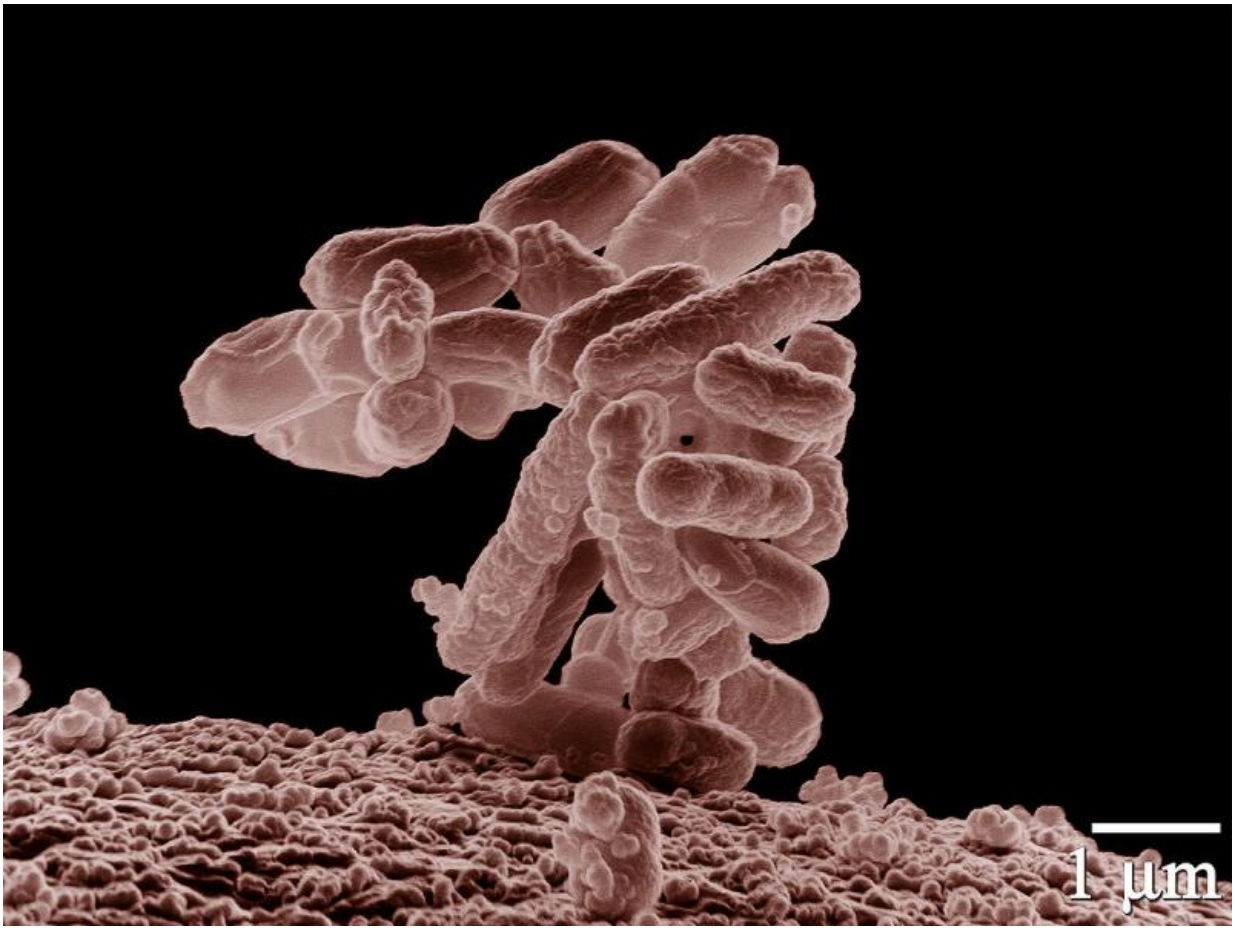
Structures reveal basis of recurring urinary tract infections

July 29 2015



A low-temperature electron micrograph of a cluster of *E. coli* bacteria, magnified 10,000 times. Each individual bacterium is oblong shaped. Credit: US Dept. of Agriculture, Agricultural Research Service

While the best antibiotics can wipe out most of the bacteria that cause notoriously difficult urinary tract infections, a few "sleeper cells" often remain. These "persisters," as they are called, survive by going dormant, essentially sleeping through the attack that kills off their more active brethren.

A new study has shown that a protein called HipA acts as a kind of molecular Sandman, putting bacterial cells to sleep so they can live another day. The Duke researchers behind the finding say understanding HipA may give them a way to combat drug-tolerant infections.

Their research, published July 29 in *Nature*, found that particularly potent, mutant versions of HipA cause multidrug tolerance in urinary tract infections. It explains how these mutations boost the protein's slumberous powers to help more bacterial cells avoid being obliterated by antibiotics.

"This discovery presents us with a new method for combating multidrug tolerance," said Richard G. Brennan, Ph.D., professor and chair of biochemistry at Duke University School of Medicine. "If we can find a way to block this protein, we may be able to awaken these problematic cells or keep them from falling asleep in the first place, so that we can eliminate them for good."

Multidrug tolerance occurs when a disease-causing microorganism manages to survive or tolerate an onslaught of antibiotics or other antimicrobials. It is not to be confused with the related phenomenon multidrug resistance, where pathogens alter their genetic makeup to become resistant to specific drugs. In multidrug tolerance, microbes instead change their behavior, temporarily shutting down cellular functions that are the typical targets of drugs so they are not seen as a threat.

Because only about one in a million bacterial cells employs this tactic, it is particularly difficult to decipher how these so-called "persisters" are able to emerge. More than three decades ago, researchers studying the common bacteria *E. coli* found that a protein called HipA was responsible for driving cells into dormancy. Studies showed that a mutated version of HipA, called HipA7, could generate 1000 times as many persisters.

Despite these advances, it still wasn't clear whether the HipA protein played a role in human disease. To investigate this possibility, the Duke researchers and their collaborators at Northeastern University sequenced the hipA gene of multiple *E. coli* samples from patients with urinary tract infections. They found that nearly two dozen of the samples harbored the hipA7 "high persister" mutations, which they then showed were responsible for causing recurrent infections in patients.

Oddly enough, the mutations were found to reside far from the part of the protein responsible for flipping the switch make a cell dormant. HipA acts as a kind of signaling protein, ordering other proteins to do the dirty work of driving dormancy. It has to be rather selective about sending out these signals or else all the bacteria will become catatonic. Therefore, HipA spends most of its time inactive, locked tightly in a complex with DNA and its partner protein HipB.

To see if they could explain the impact of the hipA7 mutations, the Duke team used x-ray crystallography to produce an atomic-level three-dimensional structure of the larger complex. When HipA is active in signaling, it appears as a single molecule or monomer. But they found that when it is bound in a complex with HipB and DNA to be quiet, it pairs up, or dimerizes, with another copy of itself. These dimers lock the complex into place, while also blocking HipA's active site. Because the hipA7 mutations are located where the two copies of the protein come together, they essentially keep the dimers from forming properly.

"It suddenly all made sense," said Maria A. Schumacher, Ph.D., lead study author and professor of biochemistry at the Duke University School of Medicine. "The protein is normally kept inactivated in this tight complex, but when it is set free, then and only then will it be activated. These mutations make it easier for HipA to be released so it can wreak havoc and promote persistence."

Now that the researchers understand how this structure enables cells to persist and outlast antibiotics, they can begin to explore new therapies that target this specific mechanism of multidrug tolerance. Brennan and Schumacher are currently searching for molecules that can keep HipA inactive so that it can no longer switch bacterial cells into sleep mode.

More information: "HipAB-promoter structures reveal the basis of heritable multidrug tolerance," Maria A. Schumacher, Pooja Balani, Jungki Min, Naga babu Chinnam, Sonja Hansen, Marin Vuli?, Kim Lewis, and Richard G. Brennan. *Nature*, July 29, 2015. DOI: 10.1038/nature14662

Provided by Duke University