

Studying Multidrug Resistant Pathogens

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The United States Center for Disease Control and Prevention (CDC) reports at least two million infections and 23,000 deaths are caused annually by multidrug resistant pathogens. [1] Within the U.S. military, there is serious concern about the use of broad-spectrum antibiotics for combat wounds, due to growing resistance. [2] Additionally, new antibiotic development has dwindled, meaning fewer novel therapies are available to replace drugs losing their efficacy. There is a clear need for alternative strategies to combat multidrug resistant pathogens.

One way of anticipating and limiting the spread of resistance is by reconnaissance: studying how resistance to a particular antibiotic will occur before it emerges. These insights are then applied to develop strategies to minimize and limit resistance, for instance, determining when a combination of therapies should be used rather than an individual antibiotic. Researchers at Rice University, in Dr. Yousif Shamoo's lab, successfully developed an approach to adapt and then characterize bacteria as they evolve to antibiotics resistance, which provides an understanding of how resistance will occur in patients. By applying this high throughput approach to clinically relevant antibiotics and pathogens, insights allow us to take a preemptive approach to combating antibiotic resistance.

One leading cause of hospital-associated infections is vancomycin-resistant enterococci (VRE). Infections caused by VRE can be difficult to treat because they frequently become resistant to more than one antibiotic. [3] One drug of 'last resort' for treating VRE is often tigecycline, a third-generation tetracycline derivative that functions by binding to the bacterial ribosome and halting the production of proteins. Tigecycline is not widely used and resistance remains rare. [4, 5] The Shamoo lab group applied their reconnaissance approach to VRE and identified the most important genetic and biochemical changes responsible for future tigecycline resistance. [6]



Kathryn Beabout, a graduate student in the Shamoo lab, working with a bioreactor used to study antibiotic resistance. (Photo courtesy of Dr. Yousif Shamoo)

To adapt cells to resistance, the researchers used a bioreactor system that keeps the bacteria growing at their fastest rate, while exposing the cells to increasing concentrations of antibiotic: essentially, fast evolution in a mini-reactor. Replicate experiments were run with pathogenic VRE lasting for 24 and 19 days. Resistant cells were isolated from the end of the experiments and genome sequencing was used to determine what mutations arose as the cells evolved resistance. To look at how adaptation to resistance occurred over the course of the experiments, genome sequencing was also used on samples of the population from each day of the experiments. This revealed the frequency of every mutation in the population of bacteria on each day of the experiment, which revealed what mutations are the most important and most common to becoming resistance.

The researchers found that the bacteria acquired mutations in a ribosomal protein called S10. By mutating and altering S10, it is likely that tigecycline cannot bind the ribosome as efficiently, which in turn makes the antibiotic less effective.

Additionally, researchers discovered that deletions upregulating the expression of a tetracycline resistance gene, known as *tetM*, gave the cells a high level of resistance to tigecycline. TetM binds to the ribosome and displaces tetracycline, therefore restoring the ability of bacteria to make proteins. Typically TetM does not work against tigecycline, but when an excessive amount of the protein is made it is able to have an effect.

Interestingly, the mutations that caused an increase in TetM production occurred on Tn916, a conjugative transposon, or a small segment of DNA that can move into different locations on the genome and transfer between different bacterial cells. These mutations not only made the bacteria resistant to tigecycline, but also caused Tn916 to be passed between cells at a highly elevated rate. Typically Tn916 moves very rarely, happening in only one in 120,000 bacterial cells. However, the resistance mutations caused Tn916 movement to increase to one in every 50 bacterial cells. As a consequence, the bacteria began to acquire more copies of Tn916 during the experiments and resistance spread

quickly through the population.

The high mobility of the mutant Tn916 means that tigecycline resistance could potentially spread rapidly, which has serious implications for hospital settings.

“This is really a double whammy,” Shamoo said. “Our finding that these bacteria become more antibiotic-resistant while at the same time spreading their resistance more efficiently was really surprising and worrying.” [7]

The Shamoo group hopes that this research can be applied to reduce the likelihood of resistance occurring in patients undergoing tigecycline therapy and to maintain the effectiveness of tigecycline.

The work was published in [Molecular](#)

[Biology and Evolution](#).

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