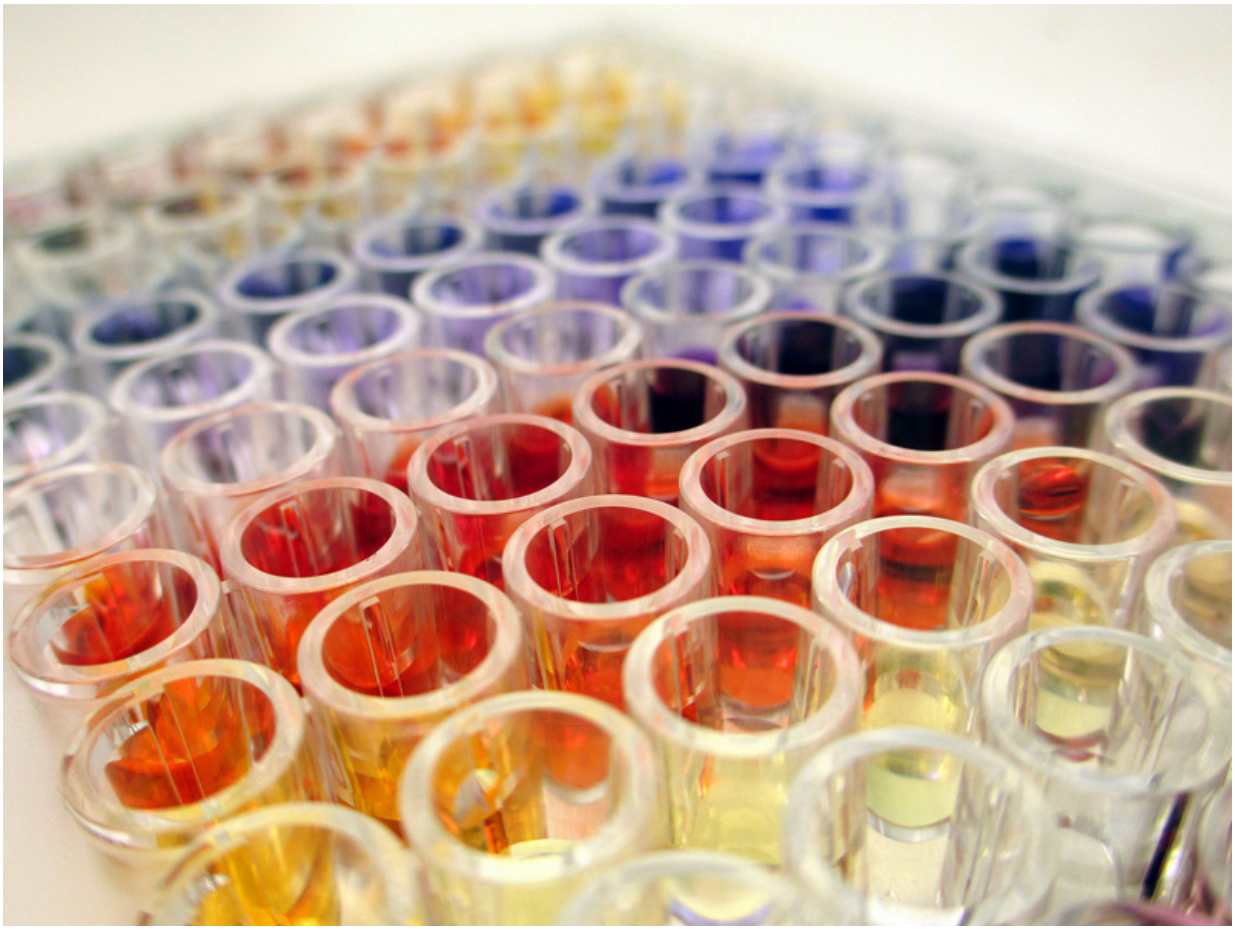
Two-for-one bacterial virulence factor revealed

January 15 2016, by Diana Lutz



Rainbow of colors is created by different concentrations of siderophores, molecules secreted by bacteria to steal iron from a host during an infection. Credit: Shangwen Luo/ University of Illinois at Chicago

We've all seen the headlines. "Man found to be shedding virulent strain of polio"; "Virulent flu strain in Europe hits the economy"; "Most virulent strain of E. coli ever seen contains DNA sequences from plague bacteria."

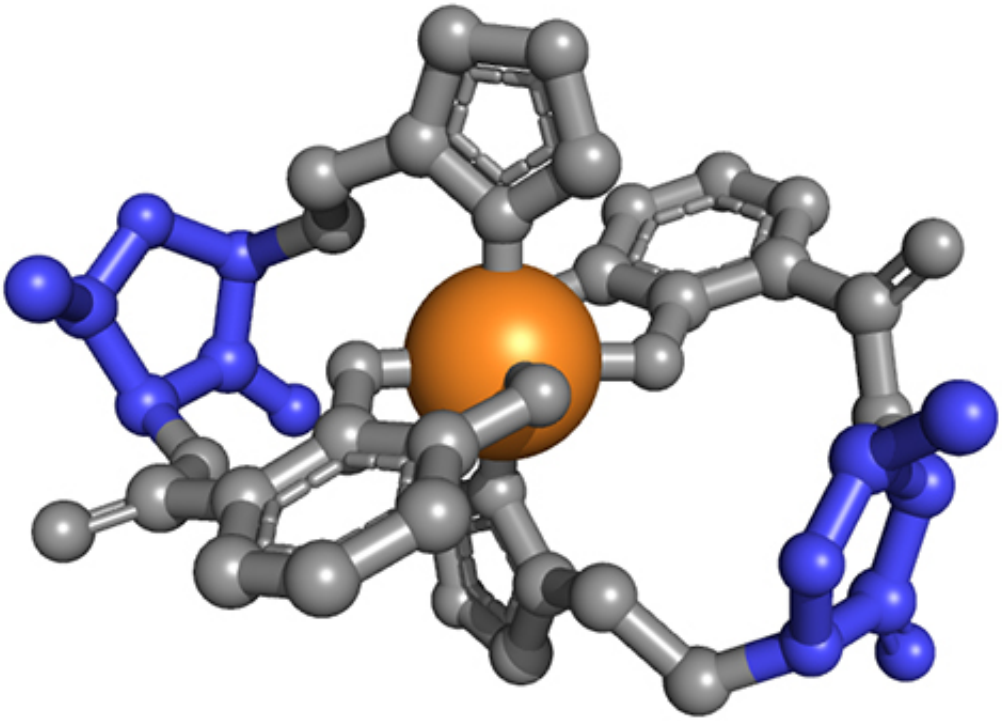
To most of us "virulent" means "aggressive," or just plain "bad," but to a microbiologist it has a more specific meaning. Virulent strains of bacteria are ones that produce "virulence factors," small molecules and proteins that convert a benign bacterium into a pathogen.

They make the difference between *E. coli* that are helpful members of our gut microbiome and the *E. coli* O157:H7 responsible for the Jack-in-the-Box outbreak.

Virulence factors allow bacteria to evade the human immune system, to infect tissues and cells and to establish a foothold within the body. Without them, bacteria would be rapidly cleared by the immune system and unable to establish an infection.

Tim Wencewicz, PhD, assistant professor of chemistry in Arts & Sciences at Washington University in St. Louis, thinks we should be looking for agents that block virulence factors rather than continuing to search for ones to kill bacteria outright. In his vision, antivirulence antibiotics would replace failing bactericidal ones.

"Do we have to find molecules that kill bacteria to fight bacterial infections?," he asks. "Is that really what we have to do?"



The siderophore actinobactin secreted by the bacterium *A. baumannii* clutching an iron atom (orange) it has stolen from the host for the bacterium's benefit. Credit: Tim Wencewicz

Traditional antibiotics carry with them the seeds of their own destruction, he said. The megadoses of broad spectrum antibiotics often given to patients in clinical medicine apply tremendous selective pressure to bacterial communities, creating rich opportunities for resistant strains by eliminating all susceptible ones.

"Antivirulence antibiotics would apply much less selective pressure," Wencewicz said. "If you treat bacteria in a test tube with an antivirulence antibiotic, the bacteria will grow as if there is no antibiotic there. But if you treat bacteria in the human body, bacterial growth will be suppressed. The antivirulence antibiotic behaves like a traditional bacteriostatic antibiotic, suppressing pathogen's growth until the immune

system has time to recognize and clear it.

"We could give anti-virulence antibiotics to people with healthy immune systems, who would be able to clear infections with this assistance," he said, "and traditional antibiotics combined with antivirulence therapies to people with compromised immune systems, who really need them."

In the online issue of the February issue of the ACS journal *Infectious Diseases*, Wencewicz describes one possible drug target: an iron-seeking molecule secreted by the bacterium *Acinetobacter baumannii*. Now that the complex biochemistry of this virulence factor is better understood, he plans to start looking for agents that block its synthesis or activity.

Know your enemy

The bacterium Wencewicz studied illustrates how quickly and spectacularly traditional antibiotics can fail.

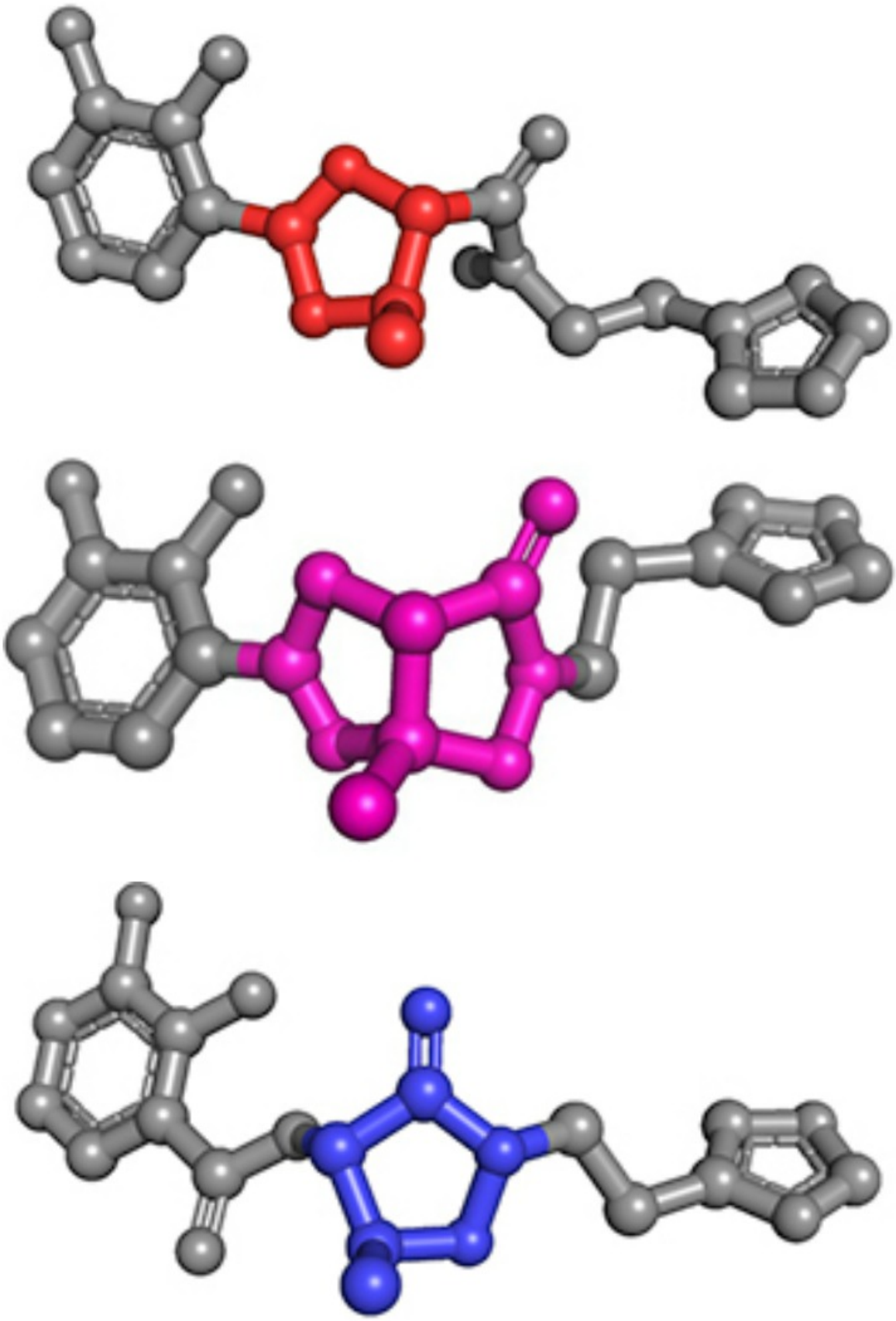
A. baumannii, sometimes called "Iraqibacter," emerged as a battlefield pathogen during the wars in the Middle East. "People would be injured, go to the hospital with an open wound and get an Actinetobacter infection," Wencewicz said. "Doctors tried to treat these infections with every drug at their disposal and they found that they were resistant to almost every FDA approved antibiotic. Nothing worked against these infections."

The resistant bacteria soon spread globally and leaked out of hospitals into communities.

A. baumannii is a bad pathogen not because of the number of infections it causes— *Staphylococcus aureus* causes many more—but because its hallmark is multi-drug resistance.

A Gram-negative bacterium, it has a double cell wall and so is intrinsically resistant to most classes of antibiotics. "Just the fact that it is Gram-negative tosses off the table many antibiotics that would work against Staph, which is Gram-positive," Wencewicz said.

"So you start with a smaller set of antibiotics, but *A. baumannii* are often resistant to those as well because they swap resistance genes," Wencewicz said. A strain that caused an outbreak in China in 2008 carried a "massive" plasmid, or mobile DNA element, that included 45 resistance genes, he said.



As the pH of its surroundings rises, the siderophore pre-actinobactin (top) rearranges itself structurally to form the molecule actinobactin (bottom) that functions better at the new pH. Two copies of the molecules join to grasp an iron atom. Credit: Tim Wencewicz

If a patient has resistant *A. baumannii*, the standard of care is to drop back to polymixins, compounds developed in the 1950s that were abandoned in the 1950s because they are so toxic to kidneys, he said. Now, strains resistant to polymixins are being found in hospitals.

"Given the speed with which pathogens resistant to bactericidal antibiotics took over when we selected for them with broad spectrum antibiotics, we should design the next generation of drugs with bacterial evolution in mind," he said.

Starving them out

One class of virulence factors common to many pathogens is siderophores, small molecules whose job is to seek out iron in the environment, wrap around it, and bring it back to the bacterial cell.

"When you get an infection, your body's first response is to starve out the invader. You hide all your nutrients: you flush your amino acids into your kidneys, and drain all nutrient supplies in the blood," Wencewicz said.

This is a particularly effective strategy in the case of iron, because iron is in short supply to begin with. The concentration of iron in the blood can be 10-24 molar (one yoctomole or about one atom of iron per 1.6

liters of blood). Living things need about 10-6 molar concentrations of iron (micromoles) to survive, Wencewicz said.

"Bacteria have to fight this huge concentration gradient in order to grab enough iron to proliferate," he said.

A. baumannii makes three siderophores which work in concert to create a gradient of iron chelation that feeds the metal back to the bacterial cell. But in this research he focused on acetintobactin, a siderophore found in every single clinical isolate of *A. baumannii*.

A fish pathogen provides a clue

The structure of acinetobactin was published in 1994, and, by 1997, ,scientists had discovered that it was created by the rearrangement (isomerization) of a precursor molecule called pre-acinetobactin.

Wencewicz knew that the fish pathogen Vibrio anguillarum makes a similar compound, called pre-anguibactin, but pre-anguibactin is locked in the "pre" form and does not isomerize. So in this case, it seemed, the "pre" form is a functional siderophore.

He wondered which of the two forms of acinetobactin was the real siderophore: the pre-acinetobactin, the acinetobactin—or both.

He also knew from the literature that *A. baumannii* could prosper over a wide range of pHs but most sites of infection are acidic. In fact, *A. baumannii* can induce lactic acidosis by lowering the pH of its surroundings by converting glucose to lactic acid and secreting the acid.

So as a first step his lab measured pre-acinobactin's rate of isomerization over a pH range from 5.5 to 8.0 (roughly from the pH of Pepto Bismol to the pH of baking soda).

"We discovered that pre-acinetobacin is stable at a slightly acidic pH of 5, but at the more basic pH of 8, it rapidly isomerizes to acnetobactin," Wencewicz said.

Why is this siderophore pH sensitive? "Suppose *A. baumannii* has established itself in a slightly acidic open wound," Wencewicz said, "but depletes the resources there. To gain access to more nutrients it enters the bloodstream, but the pH of blood is 7.4 not 5. When the pH of the bacterium's environment changes, the pre-acinetobactin converts to acinetobactin, which performs better at the new pH."

In short, *A. baumannii* has evolved a two-for-one siderophore whose conversion from one form to another is triggered by a change in pH.

This strategy pays off, Wencewicz said, because siderophores are metabolically expensive to make. If the bacteria can make one convertible molecule, they don't have to build and maintain two separate pathways for two siderophores.

"We think this may be a general strategy," he said, "because there are other classes of siderophores that also isomerize.

"Now that we know how this siderophore works, we can properly frame techniques to block it," he said. "For example, we might attach something bulky to the siderophore so that when it docks on the bacertium's receptor, it plugs it, preventing the siderophore from ferrying the iron inside."

Wencewicz does not underestimate the enormous distance between a potential drug target and a safe and effective clinical drug.

But given the rapid evolution of bacteria, he feels we shouldn't be turning over every stone looking for new bactericidal compounds that

would be just as brittle as those that have already failed. Instead, we should look for antibiotics that, if not evolution-free, at least apply much less selective pressure on bacterial communities to evolve resistance.

More information: Justin A. Shapiro et al. Acinetobactin Isomerization Enables Adaptive Iron Acquisition in through pH-Triggered Siderophore Swapping , *ACS Infectious Diseases* (2015). DOI: 10.1021/acsinfecdis.5b00145

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