Swan song for antibiotics? Can phage therapy and gene editing fill the gap?

It's time to face the music: the golden age of antibiotics is over.

Humans have been in an endless war against bacteria — indeed it can kill us — and for decades antibiotics have been our only real weapon against them. They revolutionized medicine in the 20th century, and have together with vaccination led to the near eradication of many diseases in the developed world.

But despite their amazing success, and they've had a miracle like run, drug resistant superbugs like MRSA, CRE and VRE are slowly winning. Every year more and more people die from drug resistant infections as we inch closer back to the pre-penicillin days. Their effectiveness and easy access led to overuse, especially in live-stock raising, prompting bacteria to develop resistance. This led the World Health Organization last year to classify antimicrobial resistance as a "serious threat [that] is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country"

There is a lot of blame to be shared for this reversal: doctors prescribing antimicrobials for the common cold, patients demanding the drugs for the flu, governments de-incentivizing the development of new antibiotics and pharmaceutical companies deeming unprofitable.

Regardless of who might be blamed, this current situation was bound to happen; our haphazard behavior simply hastened the arrival of this day. Whenever the goal is to kill a living organism resistance is inevitable. It's how nature works: natural selection. In this case an antibiotic kills off all susceptible bacteria, but a hardy few resistant to the drug because of random mutations survive, and eventually thrive. On the verge of wiping out a problem, it roars back even worse than how it started.

This is how all life responds to stressors. We adapt. It shows up in the plant world as well. Look at the firestorm debate over herbicide resistant weeds in agriculture. Whether a farmer uses organic approved herbicides, conventionally bred ones (such as conventionally bred sunflower crops) or crops genetically engineered to resist glyphosate, mutant weeds survive and adapt and supersedes develop. It's the law of nature. Yes, we can change up herbicides or create new ones, but the process then begins again. That's true about antibiotics as well. So even if we create a new class of antibiotics, resistance will eventually follow, no matter how responsible we are with using it.

In this light, it's clear that terms like "superbugs" and "superweeds" are misnomers because these organisms aren't special in any way, they are simply doing exactly what nature intends them to do.

What compounds the challenge for antibiotics is that bacteria appear to be able to evolve resistance faster than any other type of organism. Conjugation (bacterial sex) allows bacteria to share resistance genes with not only members of their species, but across species and even across bacterial type (i.e. gram positive to gram negative). This is called horizontal gene transfer and allows advantageous traits to be spread among members of the current generation, instead of in future generations. Furthermore, some evidence suggests that bacteria can also sense when their back is against the wall and mutate their

genome at faster rates in a last ditch effort for one of their descendants to mutate enough to develop resistance to the antibiotic.

But Darwin aside, it's in our best interest to move beyond the era of antibiotics. Using antibiotics to treat an infection is like using a sledge hammer to pound in a nail. Sure the nail will get pounded in but there's also going to be a large hole in your wall. Even the most narrow spectrum of antibiotics drastically affect our normal flora in ways that can have far reaching consequences on our physiology.

Phage therapy

What we need in our war against microbes are biocidal agents that are effective but also have more species specificity. One strategy in the works involves turning to the natural enemy of the bacteria, the virus for help. In other words *the enemy of my enemy is my friend.*

Viruses don't just infect humans, in fact every species on the planet has numerous species of virus that can infect them. When a virus infects a bacteria, scientists have a special name for them: bacteriophage, or just phage. In bacteria, as they do in humans, viruses work by gaining access to the interior of a cell and manipulating the host's cell machinery (i.e. enzymes, ribosomes etc) into making more copies of the virus. At a certain point, the number of copies of a virus induces the cell to burst releasing sometimes thousands of new copies of the virus, which can then go on to infect another cell and the process repeats.

Corrupting this process to fit human needs in the fight against antibiotics has actually been going on since the early days of the cold war. While the U.S. was investing in antibiotics, the Soviets were developing what is now called phage therapy. In fact in places like the country of Georgia, Russia and Poland phage therapy is still widely used today to treat infections.

One of the major benefits of phage therapy over traditional antibiotics is the specificity a phage has for its host. For the most part, a phage that infects an *E. coli* will not be able to infect another bacteria type, like a commensal *Staphylococcus*. There is even some evidence that phages are strain specific meaning we can develop phages that can differentially infect pathogenic strains of *E. coli* (i.e. *E. coli* O157:H7) while leaving the healthy commensal *E.coli* strains alone.

Another advantage of phage therapy is that it is self-repleting. A single phage infecting a cell will produce thousands of new copies of itself that can go on to kill more bacteria. However, some point to the mechanism in which viruses kill cell, by bursting it open, presents a drawback to the treatment as it will release bacterial toxins which can lead to sepsis, a potentially deadly condition in which the immune system overreacts to dieing bacteria.

But researchers are already working up a solution to this problem. At MIT, they have developed "phagemids": engineered phages that carry plasmids instead of a whole genome. Plasmids are shortpiece of circular DNA that carry a couple of genes which often code for virulence factors and/or forantibiotic resistance factors. When bacteria undergo conjugation this is generally what they share to passaround resistance genes. In a phagemid, the plasmid is encoded with genes that kill the bacteria withoutthe cell being lysed. No lysis means those internal toxins aren't released into the body. These scientistshave already had a high degree of success treating mice for peritonitis.

CRISPR-Cas9

Gene editing using CRISPR-Cas9 (clustered repeating interspaced short palindromic repeats) could be used to fighting bacteria. If bacteria successfully fights off infection from a phage, they save a short portion of the viruses genome in the genome. The bacteria expresses these sequences as short RNA sequences that are complementary to parts of the phage's genome. If the bacteria becomes reinfected with the same phage, the RNA sequence binds complementary to that portion of the phages genome. Inside the cell, these RNA sequences are associated with a DNA cutting protein called Cas9, which cleave the phage genome, rendering it ineffective.

The trick with CRISPR-Cas9 is that RNA sequence can be made to be complementary to anything including bacterial resistance genes. For example, a group at Tel Aviv University has created a CRISPR-Cas9 system to target the Beta lactamase gene. This gene produces an enzyme that inactivates a wide variety of antibiotics including penicillins, cephamycins, and carbapenems. Other groups have seen excellent success targeting the genes that turn *Staphylococcus aureus* into MRSA.

The CRISPR sequence and the Cas9 protein will be delivered to bacteria via an engineered phage and the technique is successful at targeting both chromosomal genes and plasmid based ones. Currently, much of the research on this technique has been to re-sensitize bacteria to common antibiotics, but it is not out of the realm of possibility that CRISPR-Cas9 could be engineered to target essential bacterial genes, thus making the technique bactericidal.

The use of phages, phagemids and CRISPR-Cas9 present to us a more sophisticated and targeted way to treat infections from bacteria. However, in no way should this be thought of as a magic bullet; that's the kind of thinking that got us into trouble with antibiotics. Resistance to these techniques will happen. It is inevitable that these organisms will adapt.

Resistance to CRISPR-Cas9 in phages has already been documented in some species, so it is possible bacteria could evolve resistance to them too. Bacteria will also develop CRISPR-Cas9 systems to create a memory of the engineered phages we will use in phage therapy. But these are not reasons to abandon this technology. These should serve as a blueprint for what we should be prepared for when the day comes that resistance to our initial phage therapy options is observed. In essence, we now have the enemy's battle plans for how they will react to our newest defense. We need to use that information to have our counter attacks waiting in the wings.

One way though to maximize the time until resistance is observed is to develop multiple phages that are

distinct genetically (i.e. create multiple phage strains with distinct targets) and then use a cocktail of phages in the treatment given to patients. The odds of a bacteria developing resistance to one phage are good but the odds of a bacteria developing simultaneous resistance to multiple phage strains during the course of one infection are infinitesimally small. And if this were to happen, then even I'd be ok with using the term "superbug".

Nicholas Staropoli is the associate director of GLP and director of the Epigenetics Literacy Project. He has an M.A. in biology from DePaul University and a B.S. in biomedical sciences from Marist College. Follow him on twitter @NickfrmBoston.