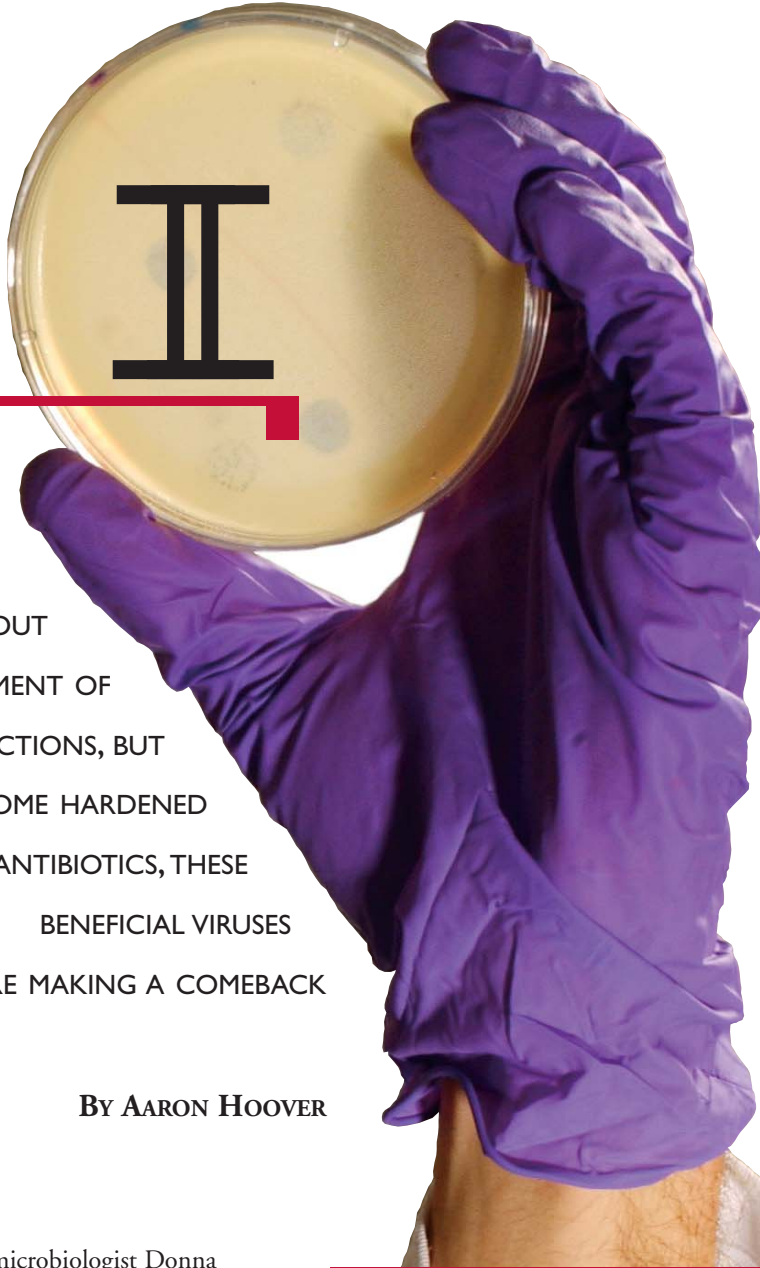


# PHAGE II



70 YEARS AGO, PHAGES LOST OUT TO ANTIBIOTICS AS THE TREATMENT OF CHOICE FOR INFECTIONS, BUT AS BACTERIA BECAME HARDENED AGAINST ANTIBIOTICS, THESE BENEFICIAL VIRUSES ARE MAKING A COMEBACK

BY AARON HOOVER

**A**fter decades of being met with blank stares or rolled eyes, veteran microbiologist Donna Duckworth had almost stopped talking about an alternative to antibiotics called phage therapy.

Phages are naturally occurring viruses that attack bacteria. Discovered more than a century ago, they were a common treatment for infection before the popularization of antibiotics.

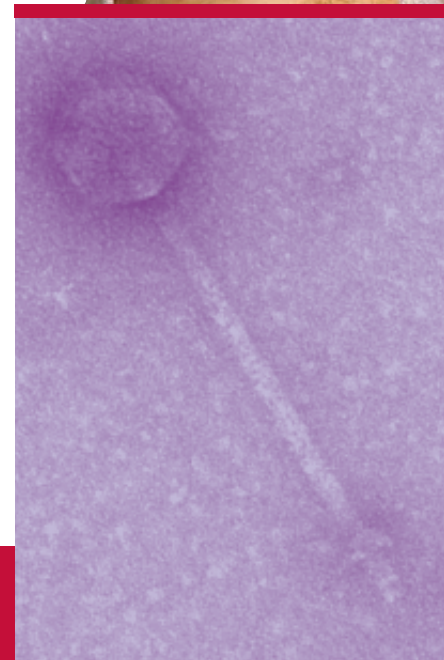
“For a long time, if you talked about phage therapy, people would look at you like you were crazy,” says Duckworth, a professor of molecular genetics and microbiology in the University of Florida College of Medicine.

But that has not deterred Duckworth and colleague Paul Gulig from experimenting with the little-known treatment on a deadly disease humans can get from eating raw oysters.

*Vibrio vulnificus*, a relative of the cholera bacterium that is difficult to treat with antibiotics, causes dozens of serious illnesses and a handful of deaths annually. Duckworth and Gulig, also a professor of molecular genetics and microbiology at UF, tested *Vibrio*-attacking phages in diseased mice. The results, reported last fall in the journal *Infection and Immunity*, were impressive.

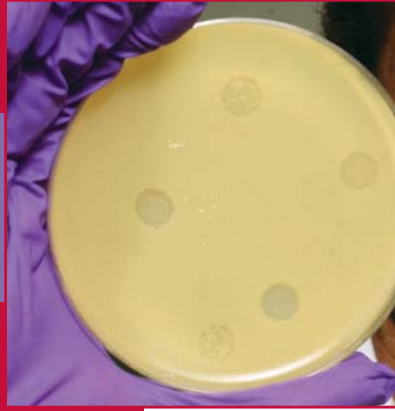
“It was very clear that for many of the mice the phage treatment could completely protect them,” Gulig says.

*An illustration of a Bacteriophage T4 virus attacking a bacterial cell. At left, a phage has attached to the bacterial cell wall. At right, the phage tail has contracted and the phage DNA (red rope-like structure) is shown entering the cell.*



Phage

THE UF RESEARCH HAS SINCE EVOLVED INTO A UNIQUE PROJECT — PART MEDICINE, PART AQUACULTURE — TO RID OYSTERS OF THE VIBRIO BACTERIA BEFORE THEY REACH CONSUMERS.



Photography by Ray Carson

The UF research has since evolved into a unique project — part medicine, part aquaculture — to rid oysters of the *Vibrio* bacteria before they reach consumers. The work is at the forefront of a small but growing body of research suggesting that phages may offer hope in dealing with bacteria that are naturally resistant to antibiotics — or have become resistant due to their widespread use. The latter is a growing public health problem, with doctors already facing antibiotic-resistant strains of bacteria behind maladies ranging from childhood ear infections and pneumonia to sinusitis, blood infections and meningitis.

“The reason why people are going back to this is because they are running out of effective antibiotics,” says Alexander Sulakvelidze, a University of Maryland microbiologist and chief scientist at a Maryland biotech start-up that hopes to become the first to market phages in the United States.

## Bacteria Eaters

The recent resurgence in interest in phages is only the latest chapter in the twisted tale of these tiny therapeutic viruses.

By some accounts, a British chemist named E.H. Rankin first detected the presence of phages in 1896. The story goes that Rankin found that water from the River Ganges stopped the spread of cholera bacteria. However, when the water was boiled, its powers subsided, suggesting that a living agent was killing off the cholera.

A Canadian microbiologist named Felix d’Herelle was among the first to identify the bacteria-killers as viruses. While dealing with an epidemic of dysentery among soldiers at a Paris hospital in 1917, he noticed that some soldiers recovered on their own. Curious, he found through experiments that the feces of the recovered soldiers contained a substance that zapped cultures of the dysentery bacteria. He called this substance a “bacteriophage” for its ability to “eat” bacteria,

according to Duckworth and Gulig.

d’Herelle’s publication of his findings touched off a decade-long flurry of research. In 1926, d’Herelle published a book that described treating more than a dozen diseases with phages — including typhoid fever, cholera and bubonic plague. In the 1930s, Eli Lilly was among the major pharmaceutical companies that successfully marketed phages. However, when some commercial phage treatments were reported to be ineffective and others were blamed for illnesses and deaths, the viruses began to fall out of favor, Gulig and Duckworth say.

The 1928 discovery of penicillin and subsequent rise of antibiotics in the West proved to be the death knell for phages, which quickly faded both from medical research labs and the public mind. However, due to the relatively high cost of antibiotics, research and clinical use of phages continued in the East. Today, phages continue to be sold at pharmacies in Russia, Poland and the former Soviet Republic of Georgia for intestinal and wound infections, Duckworth says.

Western researchers have long used phages for basic science, where they have been pivotal to most major advances in molecular biology this century, including the discovery of restriction enzymes. Researchers found out about these enzymes — which cut a DNA molecule at specific place and therefore are essential to recombinant DNA technology — while investigating why some phages infected some strains of *E. coli* bacteria but left others untouched. However, only a handful of researchers, mostly in Eastern Europe, have probed phages for clinical uses. Over the past decade or so, that has started to change as more and more bacteria have become resistant to even the most potent antibiotics.

The journal *Nature* recently reported that more than 40 percent of the bacteria that cause meningitis, pneumonia, bloodstream infections, sinusitis and childhood ear infections



*After growing phages in petri dishes, master's student Julio Martin introduces them into a tank of oysters. Wearing four gloves for protection, Martin shucks the oysters, then places them into an industrial-style blender called a stomacher that beats them into a brown mush. Martin then uses a pipette to place samples of the mush into agar growth medium on petri dishes.*

will be resistant to the widely used antibiotics penicillin and erythromycin by next year, up from just 9 percent in 1996. Other evidence of the pressing need for an antibiotic alternative abounds. For example, in recent months authorities in Los Angeles and several other major cities have reported outbreaks of a drug-resistant form of staph, which causes blood infections in patients in nursing homes and hospitals, according to news articles.

Phages and antibiotics kill bacteria in markedly different ways. Antibiotics kill indiscriminately, poisoning any bacteria they encounter. Phages are tuned only to a unique host, destroying its DNA and replacing it with their own, then replicating and venturing forth to infect more host bacteria.

“Phages are more like a laser-guided rocket, while antibiotics are more like an H-bomb,” Sulakvelidze says.

The difference is at the heart of the promises and pitfalls of phages for treating disease. On the one hand, their specificity means they don't kill off “good” bacteria such as the ones that naturally break down food in the intestines. It also means they have none of the side effects associated with antibiotics. On the other hand, in order for a phage to work, doctors have to find and administer just the right one. That means they also have to know the exact bacteria causing the patient's infection. While this was difficult in the early 20th century and contributed to phages' decline in popularity, modern technology has made identification easier.

## Vibrio Vulnerability

The *Vibrio vulnificus* bacteria occur naturally with microscopic algae in seawater. When oysters eat the algae, the bacteria become concentrated. Healthy people who eat these oysters or are otherwise exposed usually won't get sick, but people who suffer from previous liver damage or other conditions can become seriously ill or die.

Patients in advanced stages suffer from quarter-sized blood blisters on their arms and legs. Nationwide, at least 30 people come down with *Vibrio* annually. It's not a public health crisis, but even one sickness or death can cast a pall over the oyster industry regionally, shutting down harvesters and hurting

*Donna Duckworth*





Paul Gulig

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from Sea Grant, Gulig and Duckworth are working on the project in collaboration with Anita Wright, an assistant professor of food science and human nutrition in UF's College of Agricultural and Life Sciences. An early stage of the experiment involves intentionally exposing live oysters to Vibrio bacteria, then introducing phages and figuring out how many bacteria the virus kills. Preliminary results indicate that the phages kill about four-

seafood markets and restaurants. Earlier this year, California banned the sale of all raw oysters harvested from the Gulf of Mexico from April to October, when the bacteria are most prevalent.

“One death is too many for us,” a spokesman for the California Department of Health Services told the *New Orleans Times-Picayune* newspaper.

Antibiotics are effective in treating Vibrio only if it's caught early on. However, the disease is often missed because initial symptoms resemble the flu.

Seeking a better treatment, Gulig and Duckworth collected oysters and mud from the Gulf coast and local seafood markets and then isolated the naturally occurring phages in the oysters. They grew the phages in a laboratory and then injected solutions containing concentrated amounts into mice infected with *Vibrio*. The researchers found the phages cured the mice if administered during a short window of the disease's progression.

“We showed that, in typical infections of mice, we get 100 million bacteria per gram of tissue, and in these treated mice we could not detect any bacteria at all,” Gulig says.

As encouraging as the results were, any use of the phages to cure people suffering from Vibrio — or any other disease — is years, if not decades, away in the United States. Among other steps, the phages would have to go through clinical trials which, as with all new therapies, would be highly controlled and closely scrutinized by the U.S. Food and Drug Administration (FDA). Americans seeking cures for infections have journeyed to Eastern Europe for phage treatment, Gulig says.

So the project, funded by a \$64,000 grant from the U.S. Department of Commerce Sea Grant Program, is very much the first in a long series of steps.

But if follow-up UF research pans out, phages may reach the market much sooner as sterilizing agents that rid oysters or other foods of harmful bacteria before they ever reach consumers' dinner plates. Funded with a second \$144,000 grant

fifths of the bacteria in the oysters, dropping bacterial counts from 100,000 to 20,000 per gram. That's about the same as another experimental technique that uses ultraviolet light to zap the bacteria in water surrounding the oysters.

“We would like to knock it down to near zero, since we don't know the safe limits for Vibrio,” Gulig says, explaining that future experiments will probably use “cocktails” of different phages aimed at attacking many different forms of the Vibrio bacteria.

If the researchers can come up with a suitable cocktail, the next step would be for oyster harvesters, distributors or seafood markets to “treat” oysters with it — probably by submerging them briefly in vats of water containing the phage. There's no health threat to people because anyone who eats oysters already eats the natural phage.

Other researchers have adopted a similar approach but are even closer to a commercial product. Sulakvelidze is chief scientist at a start-up called Intralytix, which is developing phages for sterilizing food-processing plants. He said the Baltimore-based company is now conducting FDA-approved experimental tests of a phage that zaps *Listeria monocytogenes*, a deadly pathogen, in a poultry-processing plant. If the results are as good as lab tests, the company hopes to become the first to market a phage in the United States.

Says Duckworth, “I don't think phages will ever replace antibiotics, but they will provide one more tool.”

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