Solving the antibiotics crisis

Bacteriophages infect bacteria and kill them. They were used to aid in curing severe infections as long as a century ago. Now phage therapy is enjoying a renaissance – in the battle against multiresistant bacteria.

By Christine Broll

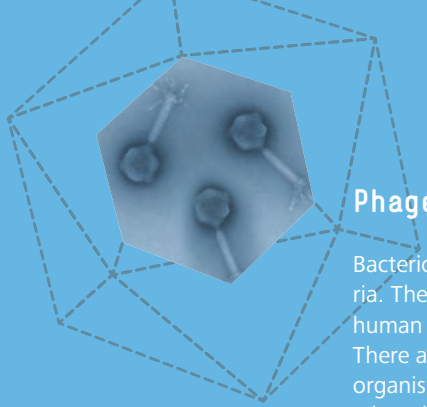
Antibiotic-resistant pathogens claim around 2400 lives in Germany each year. Fraunhofer ITEM is investigating antidotes. © *Fraunhofer ITEM*

Phage therapy has captivated Holger Ziehr ever since he saw a BBC documentary on the subject more than twenty years ago. Since then, he has been working to harness phages in the battle against antibiotic-resistant pathogens, but for a long time, few were willing to join him in this effort. Research proposals were derided and rejected. Companies declined when asked to manufacture investigational drugs for clinical studies. Only as more and more bacteria became resistant to antibiotics and pharmaceutical companies gave up searching for new antibacterial agents did his perseverance pay off.

Today, Prof. Holger Ziehr is an extremely sought-after expert on phage therapy. A division director at the Fraunhofer Institute for Toxicology and Experimental Medicine ITEM in Braunschweig, he is developing platform technology for the production of phages for use in pharmaceuticals. In parallel, he is working with regulatory authorities to establish a model process by which phages can be approved as a therapeutic agent in Germany. He sees phages as an important new component of treatment: "We do not want to replace antibiotics. Phages should be used where antibiotics come up against their limits."

Phage therapy was discovered before antibiotics were. It was in 1917, when French-Canadian microbiologist Félix d'Hérelle was working with shigellosis pathogens he had isolated in infected soldiers. While cultivating lawn cultures in lab dishes, he kept discovering small holes that spread over time. Further experiments showed that these holes were home to tiny microbes that ate the bacteria. He called these as yet unknown microbes bacteriophages – literally, bacteria eaters. ▶

In Germany, **30,000** to **35,000** people are infected with multi-resistant pathogens each year.



© Electron microscope image of a coliphage, Leibniz Institute DSMZ

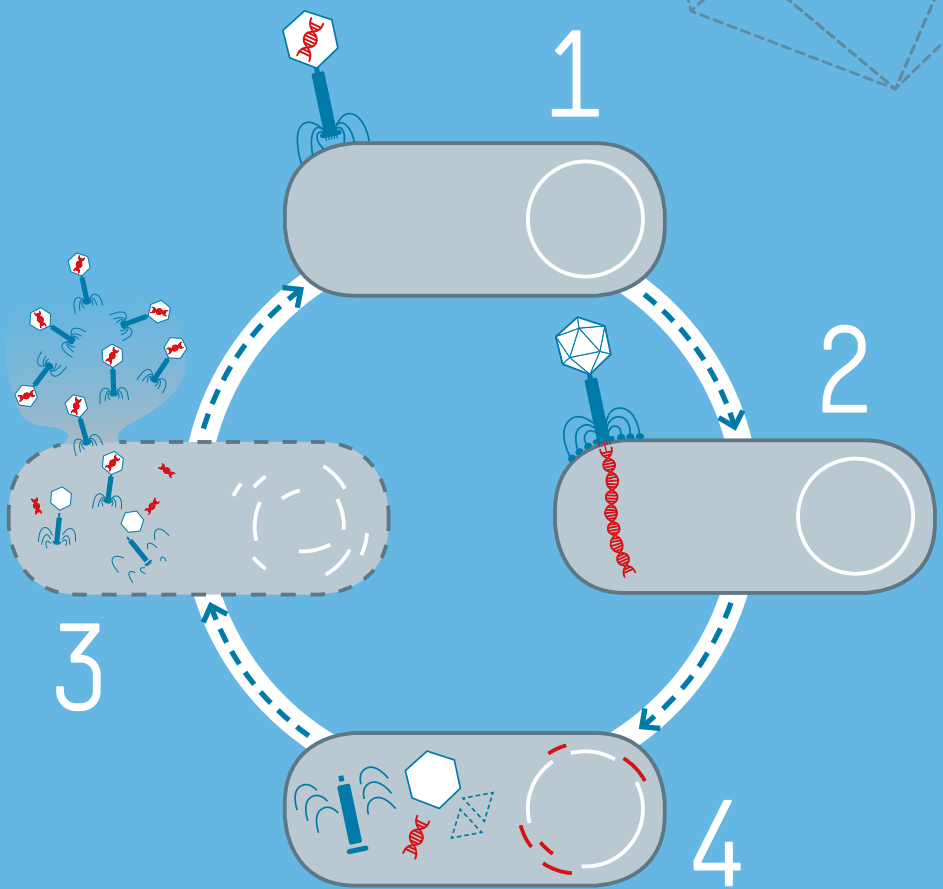
Phages

Bacteriophages are viruses that infect only bacteria. They are not capable of replicating in animal or human cells, so they pose no danger to humans. There are more phages on Earth than all other organisms combined. Phages are found everywhere bacteria live, so also in the human intestinal flora and on the skin. From a global standpoint, they contribute significantly to regulating the incidence of bacteria.

The head of a phage is shaped like an icosahedron, a sort of die with 20 faces and 12 vertices. It contains the genetic material and is perched on a long tail. The phage uses the tail fibers, which are shaped like spiders' legs, to attach itself to its host cell. Bacteriophages cannot self-replicate – they use bacterial cells for this. Every phage species is specific to a certain bacterial strain.

Phages recognize suitable hosts based on special receptors on the host's surface. In order for a phage to infect a bacterium, the bacterial receptors and the phage tail fibers must fit together like a lock and key.

After the phage attaches to the bacterium (1), it injects the genetic material from its head into the cell (2). The bacterium then begins to produce phage components (3). The phages are subsequently assembled and released (4). Some 50 to 100 new phages can be created from one bacterium.



Graphic © *Vierthaler und Braun*

▶ Félix d'Hérelle successfully treated the first shigellosis patients with a phage solution as early as 1919. In 1936, together with his friend Georgi Eliava, he founded the Eliava Institute of Bacteriophages, Microbiology and Virology in Tbilisi, Georgia, which seeded the spread of phage therapy in the Soviet Union. In World War II, Red Army soldiers were successfully treated with phages. Soviet researchers later continuously refined those methods, but after the Soviet Union collapsed, the old knowledge about phage therapy was forgotten.

Help for cystic fibrosis patients

Exactly one hundred years after bacteriophages were discovered, Holger Ziehr, together with two partners, launched Phage4Cure. Funded by the German Federal Ministry of Education and Research, this project is aimed at developing a phage-based drug for cystic fibrosis patients. People with this genetic disorder have a viscous mucus in their lungs that is an ideal breeding ground for bacteria. One such bacterium is Pseudomonas aeruginosa, which is now resistant to most antibiotics.

The phages used in the project stem from the DSMZ-German Collection of Microorganisms and Cell Cultures in Braunschweig. This collection also includes many phages that are specific to antibiotic-resistant bacteria. They were isolated from such sources as wastewater from clinics and municipal wastewater treatment plants.

Using more than 130 clinical samples from patients, the Fraunhofer ITEM researchers test which phages are most effective against the Pseudomonas bacteria. "There is no broad-spectrum phage that covers all Pseudomonas strains," explains Holger Ziehr. "So we created a cocktail of three different phages." ▶

Phage therapy was a success as far back as 1919 – then it was forgotten again.

The phages can be filled into vials in a sterile filling unit directly on the Fraunhofer ITEM premises and then shipped. © *Fraunhofer ITEM*

▶ The phages are manufactured on the second floor of the institute. This is the realm of Dr. Sarah Wienecke, who is responsible for phage production. When she starts a new batch, she places a flexible disposable plastic container on a heated shaking table, fills it with ten liters of clear nutrient solution and adds Pseudomonas bacteria from a safe production strain. The gentle rocking and pleasant warmth cause the bacteria to grow. When the nutrient solution has reached a certain turbidity due to the bacterial growth, Sarah Wienecke puts the phages in.

After just a few hours, the turbidity disappears and the nutrient solution becomes clear again. The phages have done an excellent job. They infected the bacteria, then replicated inside them and caused them to burst. Now the entire nutrient solution is full of phages. The biotechnologist then filters out the coarse components and uses other standard biotechnology methods to wash the phages. To produce the phages as investigational drugs, there are already clean rooms at Fraunhofer ITEM equipped with appropriate facilities that allow for production in compliance with stringent regulatory requirements.

Clinical studies begin in 2021

At Fraunhofer ITEM's headquarters in Hannover, Dr. Sabine Wronski then uses the phages to develop an aerosol that cystic fibrosis patients can inhale. Additional preclinical testing is underway at Berlin's Charité hospital, where the phages are tested on living lung tissue samples taken from portions of human lungs, for example following a tumor operation. Berlin is also where the first clinical studies are set to take place in 2021, at the Charité Research Organisation – initially on healthy study participants, then on patients.

The Phage4Cure team is also charting new regulatory territory, working closely with the German Federal Institute for Drugs and Medical Devices (BfArM) in the approval process. After all, there are as yet no specific rules for therapeutics that, like phages, can self-replicate. The process is guided by the requirements for biologic drugs, such as therapeutic antibodies. The quality parameters developed in this project can then serve as a template for approving additional phage therapeutics for clinical trials.

Phage therapy as a covered benefit?

The PhagoFlow project, which involves treating wounds infected with antibiotic-resistant germs, demonstrates just how important phage therapy has become. The project is funded by the Federal Joint Committee (G-BA), the body that makes legally binding decisions as to which benefits anyone insured under Germany's statutory health insurance scheme is entitled to. "Prof. Josef Hecken, Chairman of the Federal Joint Committee, personally advocated for PhagoFlow," says Holger Ziehr. The project is expected to provide indications as to whether and how phage therapy could be included in the service catalog of the statutory health insurance organizations.

> "Phage therapy is on its way to patients in Germany – at last!"
>
> Prof. Holger Ziehr

Many patients with gunshot wounds that have not healed despite treatment with antibiotics are treated at the German Armed Forces hospital (BwK) in Berlin. For PhagoFlow, Fraunhofer ITEM is collaborating with the military doctors. Once again, the German Collection of Microorganisms and Cell Cultures is responsible for choosing suitable phages. Fraunhofer ITEM produces the phages on a large scale and provides them to the BwK pharmacy. The pharmacists then select the appropriate phages for each patient based on a microbiological analysis of their wound secretions. Treatment entails simply putting the phage cocktail into the non-healing wound – just as was done for the Red Army soldiers more than 70 years ago, except that the quality standards for the cocktail are different today.

The initial successes in these publicly funded projects have awakened industry interest, too. Fraunhofer ITEM has already signed and sealed the first major contract with a pharmaceutical company. Holger Ziehr is very pleased with the resulting momentum: "Phage therapy is on its way to patients in Germany – at last." ■