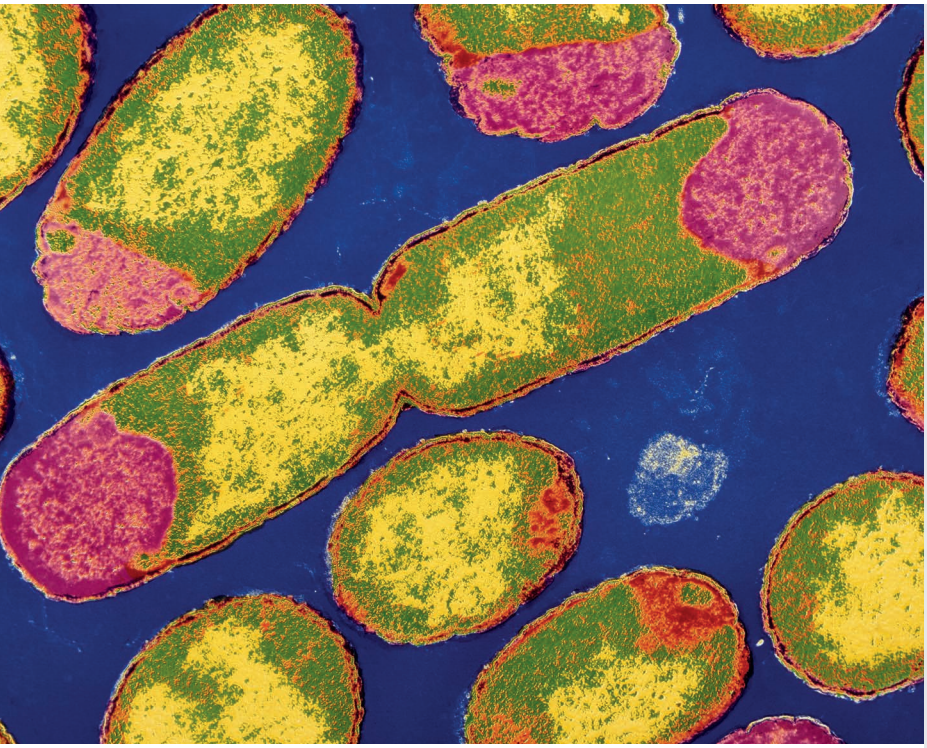


A. B. DOWSETT/SPL

The bacterium *Escherichia coli* is a common source of infection in the gut.

ONE STEP AHEAD

Old drugs and new tricks are helping to restock the antibacterial armoury.

BY NATASHA GILBERT

The world is running out of effective antibiotics. Without action, bacterial infections that can now be shrugged off with a simple course of treatment could again become common causes of death. Antibiotic resistance in bacteria is blunting the effectiveness of drugs on which people have relied for almost a century, and too few new drugs have moved from the laboratory into clinical trials to add to the armamentarium. According to the World Health Organization, 51 antibiotics are in trials in people, but only 17 of those are considered to be innovative — with the remainder closely related to existing drugs. Fewer than 10 of the 51 are likely to make it through the minefield of drug development to market within five years.

More-responsible use of existing antibiotics will go some way to averting disaster. But the root of the problem is the historic neglect of research and development, says Suzanne Hill, director of the Department of Essential Medicines and Health Products at the World Health Organization. "Antibiotics are no longer of market value for pharmaceutical companies to research and develop," she says. Instead, companies prefer to make more profitable investments in drugs that patients take over a long period of time. There is also a lack of basic research into the complex biology of bacteria, which has stalled the discovery of innovative drugs, she adds.

This call to arms is spurring researchers to search for new antibiotics. Here, *Nature* profiles some promising drug candidates and discoveries. Some comprise fresh twists on existing drugs or known targets long thought to have been overexploited. Others can better withstand attempts by bacteria to develop resistance, or are radical approaches — including a way to turn the bacterium's immune system against itself. ■

RENEWED STRENGTH

The emergence of microbial resistance doesn't have to spell 'game over' for an antibiotic. Entasis Therapeutics, a pharmaceutical firm in Waltham, Massachusetts, is working to revitalize the antibiotic cefpodoxime. This drug used to be a common line of attack against multidrug-resistant members of the Enterobacteriaceae family of microbes, which can cause serious infections in areas of the body such as the urinary and gastrointestinal tracts.

Cefpodoxime belongs to a group of broad-spectrum antibiotics known as β-lactams — named after the β-lactam ring in their chemical structures. Bacteria have evolved resistance to such drugs by producing enzymes called β-lactamases that break open the ring, destroying the drugs' antibiotic properties. It's a big problem — there are at least 3,000 types of β-lactamase, says Ruben Tommasi, chief scientific officer at Entasis.

Now, Entasis is turning the tables on resistant bacteria. The company is developing a compound called ETX1317 that binds to and inhibits β-lactamase, enabling β-lactam antibiotics to work uninhibited. Because ETX1317 has to be administered intravenously, it is best suited to use in hospitals to treat serious multidrug-resistant infections.

The company has also produced a version that can be taken orally, known as ETX0282. The World Health Organization has urgently called for new oral formulations of antibiotic, which will be of considerable benefit in the outpatient setting — for example, when treating people with urinary-tract infections that are complicated by resistant bacteria such as *Acinetobacter baumannii*. "This is a big deal. Doctors needed a go-to drug for urinary-tract infections," says Robert Bonomo, who studies antibiotic resistance at Case Western Reserve University in Cleveland, Ohio.

Both Entasis compounds are effective against several multidrug-resistant Gram-negative bacteria, including *Klebsiella pneumoniae* and *Escherichia coli*, grown in culture, as well as their infections in mice. The company is conducting safety studies, and hopes to begin phase I clinical trials next year, says Tommasi.

Gram-negative bacteria are particularly difficult to beat because they have two cell membranes that drugs must traverse to be effective. By contrast, Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*, have just one membrane, which makes them easier to penetrate. The World Health Organization has warned that "there is a serious lack of treatment options" for Gram-negative bacterial infections. Bonomo tips his hat to Entasis for taking on the challenge. "These are hard pathogens to treat," he says.

The company is also looking to develop antibiotics to take the place of β-lactams, including a class that — like β-lactams — directly targets the penicillin-binding proteins that help to build bacterial cell walls. "All the β-lactam class of antibiotics are suffering some kind of emergence of resistance," says Tommasi, but Entasis's replacements are unaffected by the β-lactamases responsible. He is unable to reveal more about the new drugs, but the project has already attracted investors' attention — winning up to US$10.1 million from CARB-X, an international public–private partnership that funds preclinical antibiotic development. ■

EVADING RESISTANCE

As quickly as researchers discover antibiotics, bacteria will evolve workarounds. But Kim Lewis, a microbiologist at Northeastern University in Boston, Massachusetts, is bullish that his candidate compound will stand up to the threat of resistance.

Many antibiotics bind to proteins inside bacteria. But pumps nestled in the bacterial cell wall can eject unwanted molecules from inside the cell. Lewis's antibiotic, teixobactin, fights microbes in a different way. It attaches to the outer surface of bacteria to avoid the ejection mechanism. Specifically, teixobactin binds to the molecular building blocks of two biopolymers — peptidoglycan and teichoic acid — that make up the bacterial cell wall. The compound acts to inhibit cell-wall synthesis.

As well as binding to the outside of the cell, teixobactin has other advantages, says Lewis. The building blocks it targets are not directly encoded by DNA; rather, they are the product of a series of reactions catalysed by enzymes. This makes it less probable that bacteria will develop resistance because the extent of the changes required could not be achieved through a simple mutation alone. And as teixobactin binds to important regions of the building blocks, he adds, it is more probable that any mutation that did confer resistance would also adversely affect cell function, leading to a defective cell wall and the death of the bacterium.

Teixobactin is produced by *Eleftheria terrae*, a soil-dwelling species of bacterium that Lewis and his colleagues discovered using a nifty tool they developed. Because many microbes are difficult to cultivate on agar plates, the team invented the iChip — a thumb-sized device that holds hundreds of wells filled with a mixture of soil and agar that is diluted so that each well holds only one bacterium. The device is then planted in soil and the microbes grow successfully. "The bacteria are tricked into perceiving that they are growing in their natural environment," says Lewis. Teixobactin is one of 30 potentially useful compounds that have been derived from the more than 10,000 microbes cultured using the iChip. Lewis is confident that further exploration of the soil microbiome using methods such as the iChip will yield important antibiotics. Researchers have identified only 1% of all microbes that exist, he estimates.

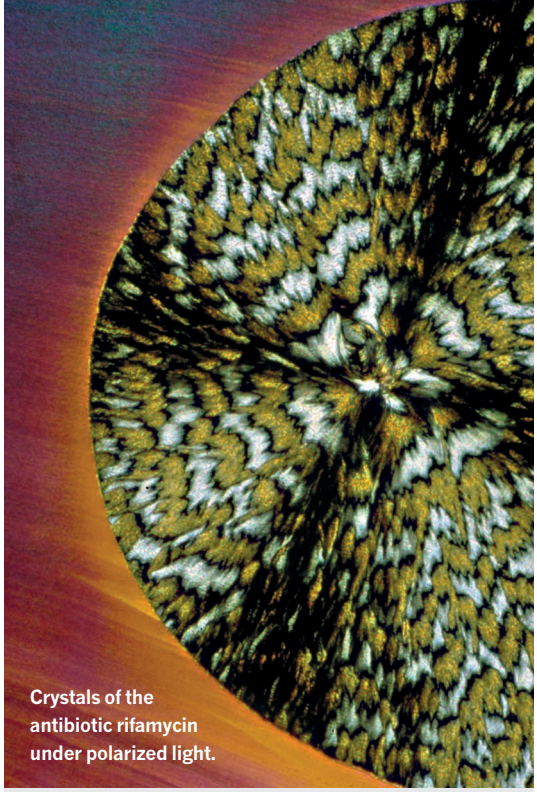
Tests in mice show that teixobactin is effective against the drug-resistant bacterium methicillin-resistant *Staphylococcus aureus*, as well as *Streptococcus pneumoniae*, which can cause pneumonia and meningitis. In culture, the compound defeated other disease-causing bacteria, including *Mycobacterium tuberculosis* and *Clostridium difficile*. Teixobactin will now undergo the testing required by the US Food and Drug Administration for researchers to get permission to start trials of the drug in people.

To examine how teixobactin holds up against the development of resistance, Lewis and his colleagues exposed *S. aureus* and *M. tuberculosis* to low doses of the compound. Such doses fail to kill all bacteria, and those that escape have the potential to evolve resistance. But no resistant microbes were found. "This indicates that teixobactin evolved to be largely protected against the development of resistance," says Lewis.

The resistance-hardy compounds have buoyed researchers such as Timothy Lu, a synthetic biologist at the Massachusetts Institute of Technology in Cambridge, who is engineering antibiotics using the CRISPR–Cas9 gene-editing system. At a time when doctors are increasingly seeing antibiotic resistance, "it's very exciting to find a molecule like this", he says. ■

The antibiotic teixobactin (yellow) disrupts cell-wall formation in Gram-positive bacteria, leading to their rupture during cell division.

CLAUS LUNAU/SPL



Crystals of the antibiotic rifamycin under polarized light.

DENNIS KUNKEL MICROSCOPY/SPL

FRESH ATTACK ON A RESISTANT TARGET

Rifamycins are a group of front-line antibiotics that are used to treat infections such as tuberculosis and those that lead to pneumonia. These drugs help to kill bacteria by stopping the microbes from making RNA, a molecule that is essential for the production of protein, by inhibiting an enzyme called bacterial RNA polymerase. However, bacteria have evolved resistance to rifamycins by making a simple change to the amino-acid sequence of the RNA polymerase, which prevents such drugs from binding but does not impede the enzyme's ability to build RNA.

Scientists have discovered several other molecules that target bacterial RNA polymerase yet are different enough to evade the defences of bacteria. One such researcher, Richard Ebright, is close to being able to turn two of these molecules into potent antibiotics.

A molecular biologist at Rutgers University in Piscataway, New Jersey, Ebright has spent about two decades studying the structure of RNA polymerase in bacteria. He has been searching for uncharted binding sites on the enzyme, and then surveying bacteria that live in soil to see if any produce compounds that latch on to the sites. Although Ebright is bringing innovative techniques to the search, in many ways he is using an old-fashioned approach. "Our best source of new molecules has been microbial-extract screening," he says. "Some people have said it is tapped out, but it's not remotely tapped out. One simply needs to know how to look."

Ebright has discovered six areas of interest on bacterial RNA polymerase. "The sites don't overlap with the current drug-binding sites," he says, meaning that any molecules that bind to them should be effective even in microbes that have already developed resistance to rifamycins. What's

more, these binding sites are common to the RNA polymerases of all bacteria, which makes Ebright's compounds good candidates for broad-spectrum antibiotics.

One of the sites is a hinge-like region that enables the RNA polymerase to open up, letting in DNA for translation into RNA. Ebright has found a compound called myxopyronin that stops the hinge from opening. Produced by the bacterium *Myxococcus fulvus*, myxopyronin has been used successfully to treat infections in mice. His team has since worked to improve the compound's potency and pharmacological properties, and myxopyronin is ready to enter clinical trials, says Ebright.

Another site is found in part of the enzyme that produces RNA from nucleotide building blocks. Ebright has found that a molecule called pseudouridimycin can take the place of a nucleotide, preventing the RNA polymerase from working. Pseudouridimycin has been shown to clear infection with *Streptococcus pyogenes* in mice. Ebright's team is now tweaking its chemical structure to increase the molecule's potency and stability.

Ebright hopes that, because these binding sites are in crucial areas of the RNA polymerase, this will prevent — or at least delay — the evolution of resistance to the new antibiotics. It will be harder for bacteria to alter such sites without affecting the activity of the enzyme. But he warns that, eventually, "bacteria will always find a way".

Ebright's discoveries have made other researchers sit up and take notice. "The new chemistry is really exciting," says Gerry Wright, who studies antibiotic resistance at McMaster University in Ontario, Canada. "It's a brand new chemical scaffold that hits the RNA enzyme in places that other drugs do not." But the compounds have a long way to go before they prove themselves, Wright adds. "The difference between finding a new molecule and finding a new drug is huge." ■

PUSHING THE SELF-DESTRUCT BUTTON

Many bacteria defend themselves against invading viruses through an immune system called CRISPR — more widely recognized in the past few years for its application in genome editing. After a bacterium has been exposed to a virus, also known as a bacteriophage, its CRISPR system generates a short RNA sequence that is complementary to a specific part of the phage's genetic code. When the bacterium is infected again, the RNA can then guide enzymes to cut the phage's DNA — often destroying the virus.

Locus Biosciences, a biotechnology company in Research Triangle Park, North Carolina, aims to subvert this CRISPR system. "We're able to harness and activate the bacterium's natural immune system to kill itself," says Paul Garofolo, the company's co-founder and chief executive.

Researchers at Locus are arming phages by loading them with DNA that matches sequences found in the bacterial genome. The viruses can then infect bacteria and insert their genetic material into the nucleus. When the viral DNA is transcribed, the resulting RNA guides the CRISPR system's cutting enzyme to several targets in the bacterial genome. However, unlike CRISPR-mediated genome editing, which uses the enzyme Cas9 to make clean cuts to both strands of DNA, the Locus system uses Cas3. "Cas3 doesn't just cut DNA, it degrades it at the same time, so it can't be repaired," says Dave Ousterout, co-founder and chief technology officer at Locus.

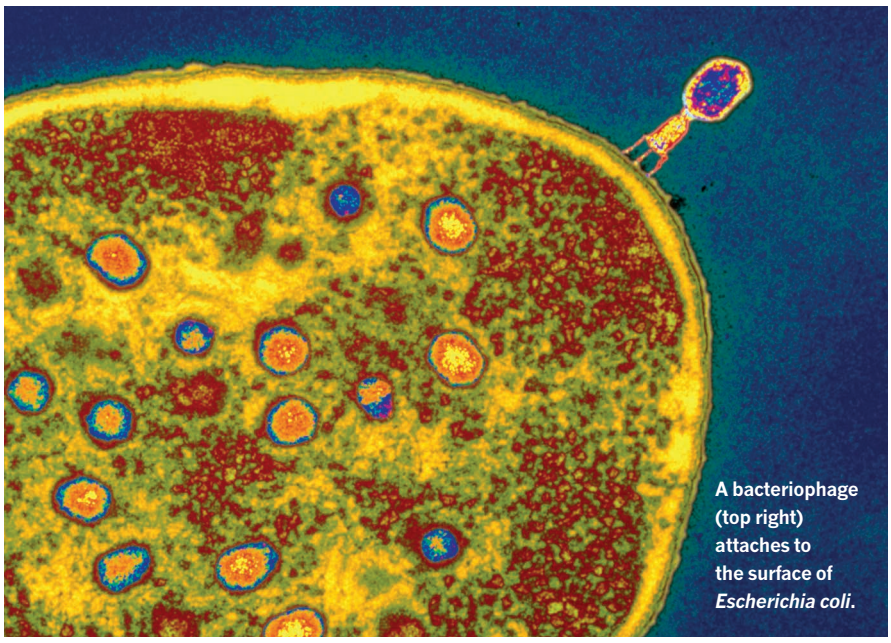
Timothy Lu, a synthetic biologist at the Massachusetts Institute of Technology in Cambridge, has developed a method of targeting bacteria that is based on the CRISPR–Cas9 system. He says that both the Cas3 and Cas9 enzymes are "useful ways to trigger intracellular DNA cutting", and that "both of these strategies may be employed to kill".

Locus is mainly focused on tackling intestinal pathogenic agents such as *Clostridium difficile* and *Escherichia coli*. Antibiotic-resistant *C. difficile* poses one of the most urgent threats to human health, and *E. coli* can cause life-threatening infections of the blood and urinary tract. In laboratory tests, the CRISPR–Cas3 antibacterial tool cleared *C. difficile* infections in mice, says Garofolo. The company hopes to start phase I trials in 2019, but must first obtain approval from the US Food and Drug Administration. Garofolo adds that the treatment would probably be administered to people who do not respond to existing first-line drugs such as vancomycin.

Countries such as Russia and Poland have long used phages to treat bacterial infections in people. The advantage of this treatment is that it targets only specific bacteria, rather than wiping out a swathe of beneficial bacteria along the way. But phage therapy hasn't taken off more widely, in part because bacteria can easily develop resistance to the phages.

Garofolo hopes to see higher efficacy with the CRISPR–Cas 3 system than with conventional phage therapy because the viruses used by Locus have been boosted with bacterial DNA. Furthermore, the team hopes to limit the risk of bacteria developing resistance by using multiple phages to attack the bacterial genome at several sites — ensuring that the bacteria cannot survive. And restricting the use of the CRISPR–Cas3 treatment to people with the most severe infections will also help to limit the opportunities for resistance to develop. "We anticipate that some sort of antibiotic stewardship upfront will help our product maintain efficacy," says Ousterout.

The phage therapy developed by Locus has broad potential, says Garofolo. The company's goal is to use the technology to treat long-term conditions such as irritable bowel syndrome or colorectal cancer. "If the technology breaks through," he says, "it should be able to address any number of additional bacterial targets." ■



EYE OF SCIENCE/SPL

A bacteriophage (top right) attaches to the surface of *Escherichia coli*.

Natasha Gilbert *is a freelance science writer based in Washington, DC.*