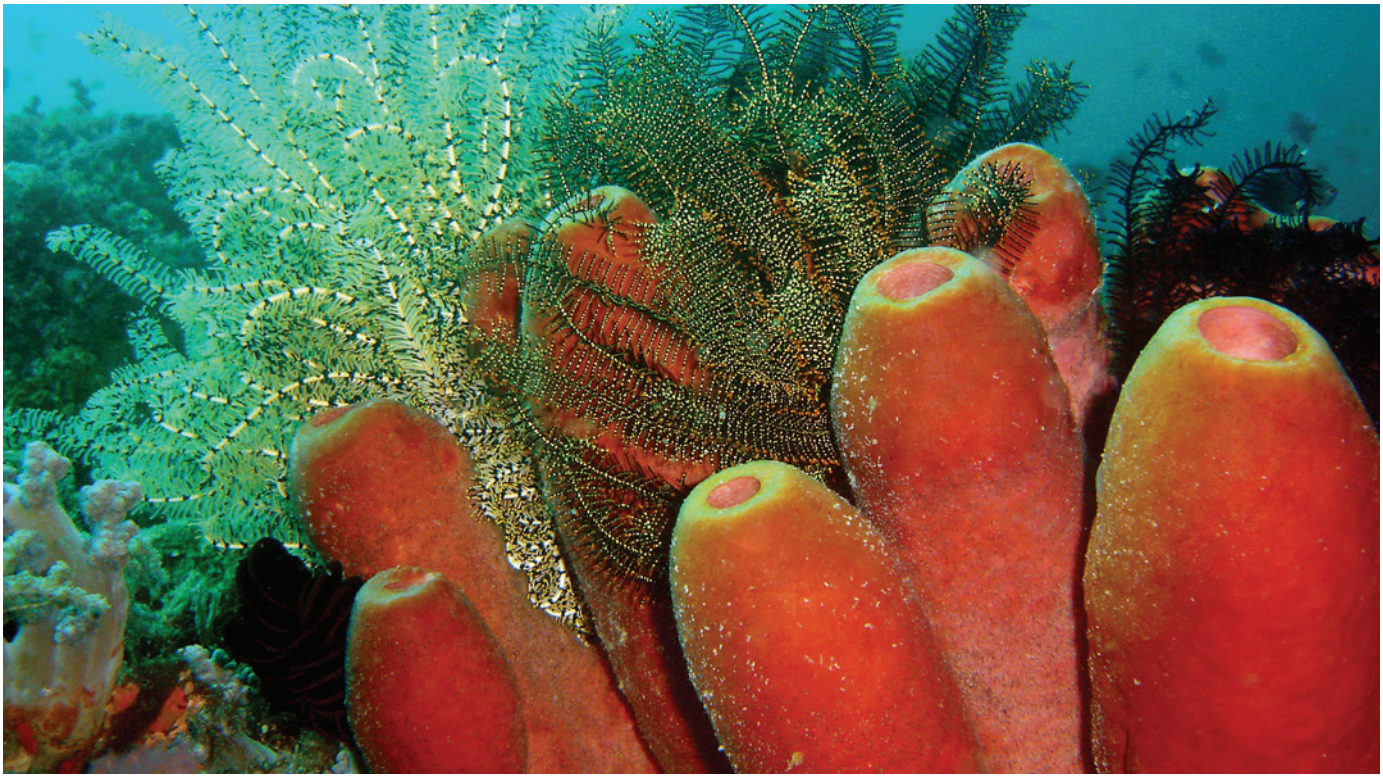

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Tomorrow's antibiotics might arise from unexpected sources, including this sponge, *Theonella swinhoei.*

DRUG DISCOVERY

Leaving no stone unturned

New antibiotic treatments could be found by combining novel and existing drugs, in drug-free nanoparticles, or at the bottom of the sea.

BY KATHARINE GAMMON

Under a microscope, the rod-shaped cells of the bacteria *Escherichia coli* look the same as ever. But there's a difference in the way they have been treated. Rather than giving them a growth medium containing the usual bonanza of nutrients, these bacteria face the nutrient-limited conditions they experience in nature. This simple change might provide a way to develop the next generation of antibiotics.

Unlike pharmaceutical scientists, who are governed by the odds of success, academic scientists have the freedom to carry out basic research in the hope it will lead to novel antibiotic agents. "What people struggle with in this area is how to do things in a fresh, new way," says Eric Brown, a biochemist at McMasters University in Hamilton, Ontario, in Canada. "There's not been a lot of success in recent years using modern drug discovery techniques." Brown realized that in order to beat *E. coli*, he had to think like *E. coli*, so he grows the bacteria in an environment that more closely mimics the inside of the human body during an infection — where paradoxically they are limited in vitamins and amino acids. It's work like this that has seen academic researchers rejuvenate the antibiotic pipeline.

Brown says he started to look for antibiotic targets in nutrient-limited conditions simply because no one else was looking there. First, he and his colleagues sifted through a library of 30,000 synthetic molecules, looking for any that can block the ability of *E. coli* to synthesize its own essential nutrients. About 300 molecules fit the bill. Then the researchers tested those molecules in nutrient-limited conditions, and 71 still worked. Ultimately, Brown and his colleagues identified three compounds[1] that might perform as antibiotics by blocking the bacteria's ability to make its own nutrients in an environment — like the human intestine — that lacks enough of them.

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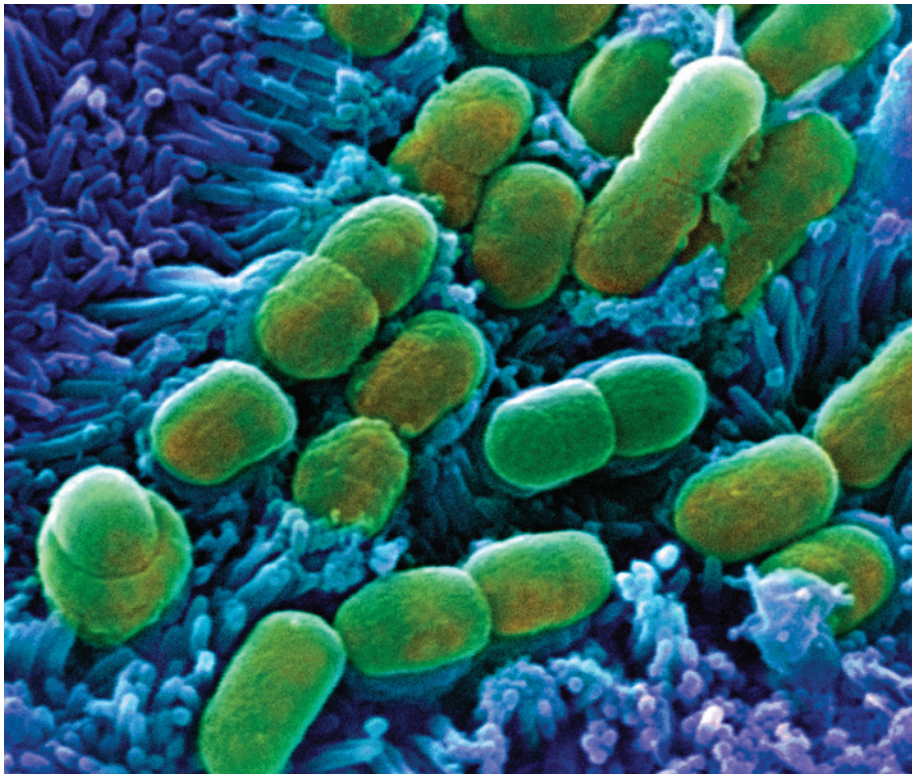
Such techniques might lead to fresh antibiotics — maybe many of them. Better still, the tool box includes a wide variety of strategies that could tackle the problem of antibiotics that are losing their power.

REVIVING FADED DRUGS

Instead of creating entirely new drugs, scientists might be able to reinvigorate antibiotics that have ceased to be effective by combining them with new agents, creating revamped compounds to refresh the drug pipeline. Robert Hancock, a microbiologist at the University of British Columbia in Vancouver, Canada, says that new approaches can breathe life into existing antibiotics. For instance, he created antimicrobial peptides called innate defence regulator peptides, which stimulate normal immune cells to sweep in and consume invading pathogens, and also suppress inflammation[2]. These peptides seem to be effective against a wide range of infectious bacteria, and are unlikely to lead to resistance because they have no direct effect on microbes.

Hancock says that although the peptides work as a stand-alone therapy, they are more likely to be used as an adjuvant to improve the



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Starved *Escherichia coli* bacteria (green) challenged with synthetic molecules could lead to new antibiotics.

effectiveness of existing antibiotics. To explore the creation of treatments based on these peptides, Hancock started a company, now called Soligenix, based in Princeton, New Jersey. One of these agents is now in phase 2 clinical trials.

Another method for resurrecting fallen antibiotics is to alter their structure. Researchers at St Jude Children's Research Hospital in Memphis, Tennessee, changed the chemical structure of an existing antibiotic, spectinomycin, to create a class of antibiotics called spectinamides that are effective against new targets. The original antibiotic was used primarily to treat gonorrhoea infections, whereas the new class of drugs is effective against other infections including tuberculosis (TB) — an illness with a pernicious drug resistance problem (about 4% of new TB cases are resistant to multiple drugs, and about 20% are in people already treated for TB). In trials in mice with both active and chronic TB infections, one particular spectinamide — an analogue known as 1599 — was as good as or better than current TB drugs at reducing levels of the bacteria in the lungs. In addition, 1599 caused no serious side effects[3].

To create the spectinamides, the researchers designed complex three-dimensional models to examine how spectinomycin binds to the TB ribosome, where proteins are synthesized. By disrupting the ribosome, the drug was able to stop the bacteria from synthesizing proteins that it needs to survive. Because spectinamides bind to a different part of the ribosome to other drugs, they can be used in conjunction with existing therapies.

Richard Lee, a chemical biologist at St Jude's and the lead scientist on the 1599 project, says that using a chemistry-first approach led to some interesting developments. The new class of drugs can stop efflux, which some bacteria use as a defence against antibiotics, pumping the medication out of their cells — overcoming efflux has been a goal for some time. "Because they overcome efflux, they may be more appropriate for treating chronic infections where bacteria grow rapidly," says Lee.

KILLING THE PERSISTERS

Instead of breathing fresh life into older drugs, some researchers are looking for ways to trick bacteria into killing themselves. Kim Lewis at Northeastern University in Boston, Massachusetts, for example, focuses on a class of cells called persisters inside methicillin-resistant *Staphylococcus aureus* (MRSA). Persisters account for only about 1% of bacterial cells but are deadly — they are often dormant but wake up after an antibiotic has run its course and wreak havoc. After years of work, Lewis and his colleagues created a peptide — acyldepsipeptide (ADEP) — that activates the dormant persister cells and triggers them to self-destruct by degrading proteins and forcing the cells to digest themselves. Resistance and persisters go hand in hand, but the researchers found that when the peptide was combined with traditional antibiotics, resistance was kept to a minimum. Lewis and his colleagues tested ADEP in the laboratory and in a mouse model of chronic MRSA infection, and found that the mice were free from infection within 24 hours and showed no side effects[4]. The researchers are now working on clinical trials.

"Antibiotic tolerance can be as big a problem as antibiotic resistance."

Lewis says that antibiotic tolerance can be as big a problem as antibiotic resistance. For infections such as cystic fibrosis, for which there is no cure, and infections in people with hip implants or artificial heart valves, antibiotics suppress the infection but do not kill all the bacteria. "The infection keeps coming back because the dormant persister cells are not killed by antibiotics," says Lewis. "The cells resuscitate and cause a relapsing infection." The US Food and Drug Administration has traditionally asked drug companies to test compounds only against rapidly growing cells, which is one reason why there are not many treatments for long-term infections.

A similar technique might reveal a faster way to treat TB, which typically requires several courses of antibiotics over a period of 6–9 months. Lewis says that potential new drugs aimed at triggering persister cells to commit suicide could treat chronic TB infections in 24–48 hours.

SOAKING UP INFORMATION

Sometimes researchers go to the ends of the Earth to find potential antibiotics. In the case of Micheal Wilson, a microbiologist at the Swiss Federal Institute of Technology in Zurich, Switzerland, the search took him to the bottom of the ocean. The ocean floor could yield many as-yet undiscovered natural products. "We know how fruitful our relationship has been with terrestrial microbes, so we believe the oceans provide a vast underexplored resource for new natural products," he says.

Wilson studies microorganisms that live on sea sponges, which he calls "the guts of the ocean" because they are teeming with microbiota. Because sponges can't move, they have evolved a diverse range of adaptive relationships with bacteria. Wilson and his colleagues found that thousands of different bacteria live on the surface of one particularly productive sponge, *Theonella swinhoei*, but just a single species of bacteria from the genus *Entotheonella* produces nearly all the sponge's biologically active compounds[5].

The work is still at an early phase, but Wilson says that he and his colleagues are using a 'metagenomic' approach to identify genes, associate them with known compounds, and develop biochemical systems to produce potential drugs. In addition, other sponges could be studied to find bacteria that produce compounds that might be turned into medicines.

CLEANING UP TOXINS

Other researchers are thinking small. At the University of California, San Diego, nanoengineer Liangfang Zhang has been developing

nanosponges that fight bacterial infections. The technique focuses on attacking pore-forming toxins, which are proteins produced by bacteria such as *S. aureus*. These toxins bore holes in cell membranes, essentially causing leaks that disturb a cell's normal function.

"For bacterial infections, it's not always necessary to use antibiotics to kill the bacteria," says Zhang. Instead, a treatment could target the bacterial toxins, which are the source of all the negative consequences of an infection. If there is an effective way of removing the toxin, the bacteria are disarmed and the immune system can finish the job of killing the bacteria.

Each nanosponge is about 100 nanometres across and is made up of a biologically compatible polymer core wrapped in segments of natural red-blood-cell membrane. The nanosponges — disguised as red blood cells — create a decoy that collects the toxins so they can't harm the host's cells. The sponges have a half-life of 40 hours in tests on mice, and are eventually safely metabolized in the liver, along with the toxins. There is no antibiotic, so there is no opportunity for antibiotic-resistant cells to grow. Unlike other anti-toxin treatments, the nanosponges can work against a variety of different toxins, from MRSA to bee stings and snake venom — anything that creates pore-forming toxins.

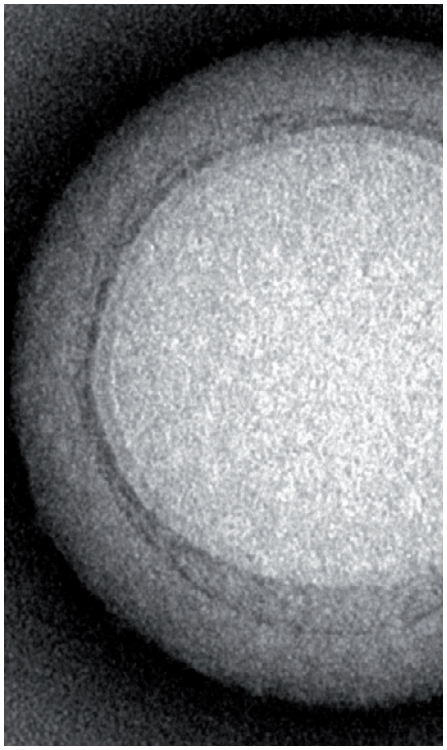
When the nanosponges were tested against alpha-haemolysin toxin from MRSA, pre-inoculation with nanosponges enabled 89% of mice to survive what would normally be lethal doses of toxin. Even administering nanosponges after the lethal dose led to 44% survival[6]. Further tests will combine the sponges with antibiotics with a view to making them even more effective.

Zhang is also working on a parallel nanosponge technique for vaccines that could prevent pore-forming toxins from taking hold[7]. He points out that there are more than 80 families of pore-forming toxin that this technology could disarm.

A SNIPER NOT A SHOTGUN

Instead of blasting someone with broad-spectrum antibiotics that can wipe out beneficial bacteria as well as disease-causing pathogens, it may be better to use a targeted approach. The biotech company AvidBiotics in South San Francisco, California, has created engineered versions of R-type bacteriocins, which are proteins used by *Pseudomonas aeruginosa* bacteria to kill other strains of bacteria by piercing their cell envelopes. AvidBiotics researchers say they chose this approach because the proteins are naturally species specific and easy to manipulate to new targets. The result is a protein-based drug called Avidocin.

So far, AvidBiotics has generated antibacterial proteins specific for a variety of bacterial pathogens, including *E. coli* O157:H7 (a strain that can cause fatal food poisoning), uropathogenic *E. coli* and *Clostridium difficile*. David Martin, AvidBiotics' chief executive, says the company has developed a large portfolio of proteins for



This nanosponge is made up of a nanoparticle wrapped in red-blood-cell membranes.

ZHANG RESEARCH LAB, UC SAN DIEGO

Gram-negative bacteria and a smaller one for Gram-positive bacteria. Clinical trials in humans are about a year-and-a-half away.

Martin points out that targeted attacks have many benefits. The protein against *C. difficile*, for example, is very narrow in focus and doesn't kill even related species. "The intent is that you can use it prophylactically, to prevent the disease rather than just treat the disease," says Martin. "These agents are so narrowly targeted that they don't disrupt the gut microbiota." They are designed to have a specific binding point and one dose is enough to kill the bacteria — a big advantage for avoiding potential side effects.

In hospitals and nursing homes, 3–10% of patients carry *C. difficile*, and Martin says they would benefit from treatment with Avidocin. The current use of broad-spectrum antibiotics can put them at risk of developing *C. difficile* colitis, which has a 20% mortality rate in people over 55 years of age. "Our strategy is to start with known bacterial pathogens, particularly in the gastrointestinal tract, and then eventually we want to be able to manage the gut microbiota using a sniper approach rather than a shotgun. Just kill the bad bugs," says Martin. "Don't kill beneficial bugs."

"No matter how successful the search for new antibiotics turns out to be, it might be best to use them in combination."

AvidBiotics is also collaborating with DuPont Nutrition and Health to create spray-on R-type bacteriocins that could fight pathogens such as *E. coli*, *Listeria* and *Salmonella* in packaged foods. Martin says his company is also creating narrowly targeted antibiotic alternatives to the drugs used in raising animals for food. In addition, the company has outlined a rapid-response platform with the potential to create targeted agents for use against emerging bacterial pathogens within days or weeks of acquiring the pathogen's genome sequence[8].

BETTER TOGETHER

No matter how successful the search for new antibiotics turns out to be, it might be best to use them in combination. Cancer, HIV and TB are all treated by combination approaches, and there's no reason why bacterial infections should be any different. Terry Roemer, who studies infectious diseases at Merck Research Laboratories in Pennsylvania, says scientists have long found that certain agents enhance each other's efficacy, for example using β-lactamase inhibitors to overcome bacterial resistance to β-lactam antibiotics. "The problem is, we're not really thinking rationally about which two antibiotics to pair that are synergistic together."

Roemer thinks that targets could be screened in a smarter, more rational way to create drug combinations that would decrease the likelihood of resistance. "For us, the mantra is: resistance is inevitable," says Roemer. "The best we can do is to try to reduce it as much as possible." Finding the best synergistic combinations of drugs would make it possible to do that.

As Roemer and colleagues have shown, MRSA infections were susceptible to β-lactam antibiotics when they treated the infection with two synergistic agents by containing resistance to a specific mutant that could be easily killed[9]. "So you can think of this as a situation where the potentiating agent restores the efficacy of the β-lactam, but resistance to that agent can drive two mutant forms of the pathogen that have a restored susceptibility," says Roemer.

In a world where effective antibiotics are becoming increasingly scarce, these approaches are set to benefit us all, from people receiving surgery to those with food-borne illness. And the advances are gaining speed. "Things have stagnated for a decade or more, but now it's a really exciting time," says Brown. "Pharma has been emboldened to take risks, but they still rely on crazy academics to do the wild things." ■

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