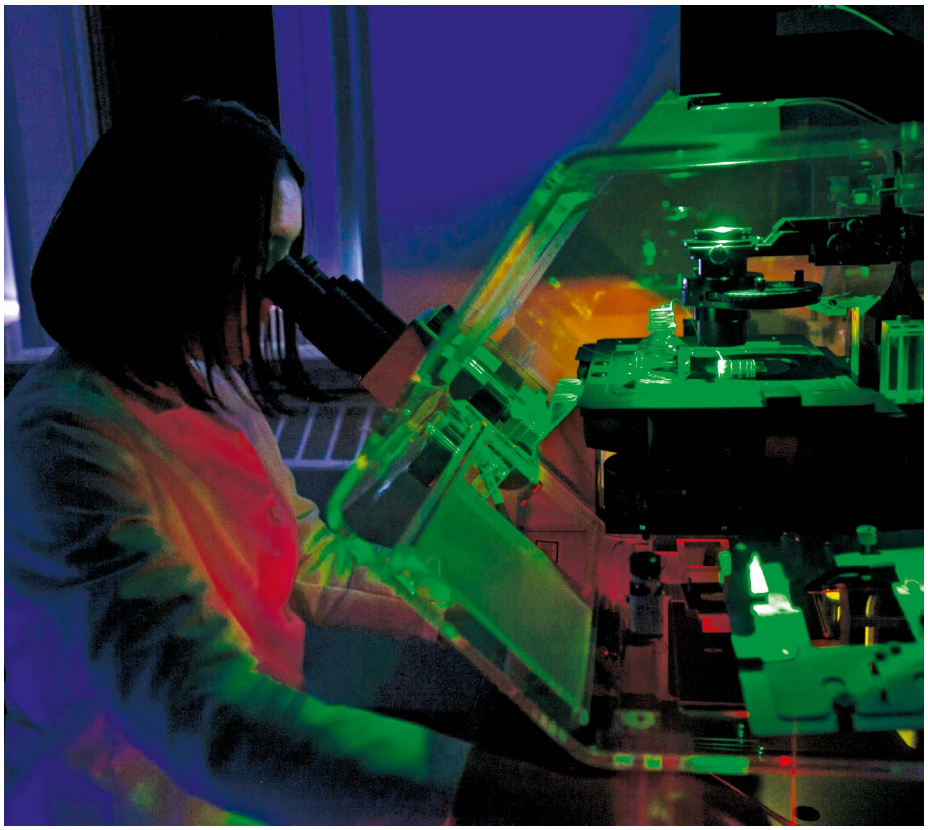

KELVIN MA/TUFTS UNIV.

Bree Aldridge is rethinking ways to search for drug candidates that overcome drug resistance.

Overcoming resistance

Facing a growing threat, researchers are finding ways to reinvigorate existing antimicrobial drugs and to create fresh ones. **By Neil Savage**

The infections show up everywhere. In India, at least 58,000 babies die each year as a result of 'superbugs' – microorganisms that are resistant to almost all known treatments. And the multidrug-resistant *Acinetobacter baumannii* has turned up in US military hospitals from Texas to Landstuhl, Germany. The bacterium can cause pneumonia, as well as infect wounds, the bloodstream and the urinary tract. Physicians presume it was introduced by wounded soldiers returning from the Middle East.

Much of the world's attention is focused on the virus that causes COVID-19, but resistance to antimicrobial drugs remains an urgent problem. The World Health Organization (WHO) has published a priority list of a dozen types of drug-resistant bacteria for which new antibiotics are needed. And the United Nations Interagency Coordination Group on Antimicrobial Resistance has noted that drug-resistant infections cause at least 700,000 deaths globally each year. If nothing changes, 10 million people a year could die from resistant infections by 2050.

The rise of resistance endangers life-saving procedures such as surgery, chemotherapy and organ transplants, all of which can expose people to deadly bacteria. Development of antimicrobial drugs has been flagging for decades, in part because there's little economic incentive for companies to take on the challenge. But now, researchers are working on ways to counter resistance, including reinvigorating existing drugs and looking for new candidates.

One of the bacteria on the WHO's priority list is *A. baumannii*. It is resistant even to a class of antibiotic known as carbapenems, which interfere with the bacterium's ability to form its cell wall and are usually reserved as the last line of defence. The US Centers for Disease Control and Prevention estimate that 8,500 people contracted the infection in US hospitals in 2017, and around 700 of them died.

A. baumannii is particularly worrisome because "we hardly have any antibiotics left to treat these infections", says Willem van Schaik, director of the Institute of Microbiology and Infection at the University of Birmingham, UK. Some strains are even resistant to colistin, a drug of last resort that is rarely used because it can cause kidney and neurological problems.

Top targets

The other top-priority organisms are *Pseudomonas aeruginosa* and various Enterobacteriaceae species – including *Escherichia coli*, which is often in the news for causing outbreaks of food poisoning. The WHO says that these microbes pose a particular risk to people in hospitals and nursing homes, especially those with invasive medical devices such as catheters or ventilators. "There are people right now in hospitals with bacterial infections that can no longer be treated with antibiotics," van Schaik says. "The numbers are still very low. And we need to keep it low."

Organisms listed as high or medium priority include those that cause the sexually transmitted disease gonorrhoea; *Shigella*, which causes a form of dysentery; and *Salmonella*, which causes food poisoning. Many of the priority microbes are Gram-negative bacteria. These organisms are particularly difficult to defeat because they have an extra, outer membrane that acts like a sieve, letting through only small molecules of exactly the right shape and charge. This keeps many drugs out, and if one does get through, it then faces another defence. Between the outer and inner membranes, the bacteria have 'multidrug efflux pumps' – molecular machines that recognize and capture foreign substances such as antibiotics, and squirt them back outside the membranes before they can do any damage[1].

Laura Piddock, a microbiologist at the University of Birmingham, UK, is searching for molecules that gum up this machinery, thereby restoring the potency of some antibiotics. In regard to these molecules, "they're taking up space within the pump that otherwise would be taken by drugs," she says. "So if they're there first, then the drugs aren't transported." As a result, the drugs should be able to stay in the bacterium long enough to have an antibiotic effect.

But as with all drug development, identifying compounds is only the first step. "So many of them have floundered at preclinical

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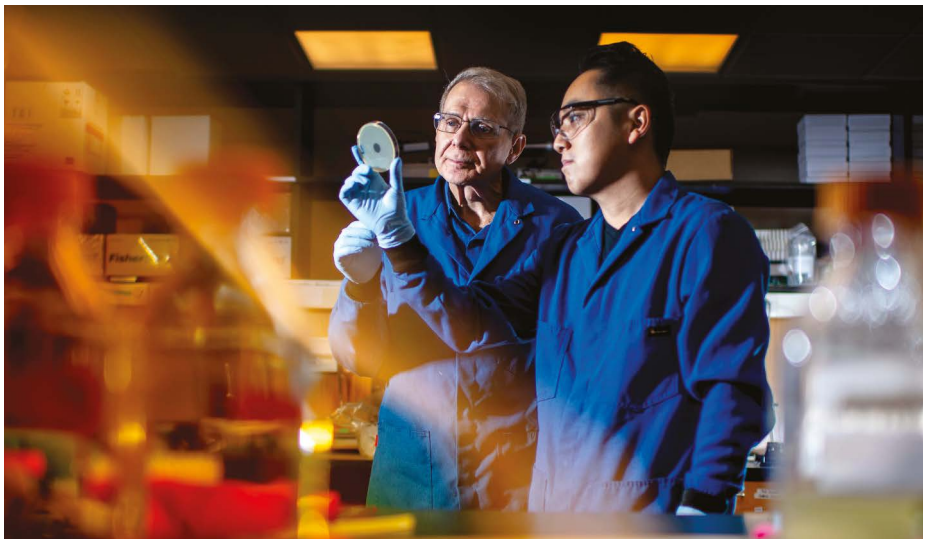

MATTHEW MODOONO/NORTHEASTERN UNIV.

Kim Lewis (left) is getting microbes to start the search for new antibiotics.

research because they're toxic or you can't formulate them to be drugs," Piddock says. "They're fantastic in the test tube, but you couldn't possibly give them to a person."

To overcome this stumbling block, Piddock is turning to brute force. Her laboratory has developed a test to screen known compounds for an inhibitory effect on the pumps. Whenever a drug starts to interfere with the pumps, the bacterium turns on a gene called *ramA*, which activates other genes that make more pumps. Piddock's screening system takes advantage of this by fusing the instructions for building a green fluorescent protein into the gene sequence of *ramA*. So when a substance activates *ramA*, the fluorescent protein is produced and the researchers can see the glow using high-throughput screening machinery. The team has now screened nearly 50,000 compounds, identifying 43 efflux-pump inhibitors, 11 of which have been shown to increase the efficacy of antibiotics in lab tests[2].

Searching in the wild

Kim Lewis, a biologist at the Antimicrobial Discovery Center at Northeastern University in Boston, Massachusetts, is taking a different approach, and getting the microbes to do the initial searching for him.

Microbes make their own antibiotics to fend off competitors, and they can share the compounds with each other through the underlying genetic code. (They can also spread genes for antibiotic resistance, contributing to the problem.) So Lewis reasoned that the antibiotics he's looking for might already be there. "If a bacterial group has been around for a couple of hundred million years, the chance that it will come across a gene that codes for a compound that can protect it against other bacteria is high," he says.

Lewis turned to *Photorhabdus*, which lives symbiotically with tiny parasitic worms called nematodes. Both *Photorhabdus* and nematodes use insect larvae as a food source, and it turns out that *Photorhabdus* secretes a compound that kills off competing bacteria. This

> **"They're fantastic in the test tube, but you couldn't possibly give them to a person."**

substance would have to be non-toxic to the nematode and be able to move through its tissues efficiently, so Lewis reasoned that it might behave similarly in humans. Lewis and his team named the antibiotic darobactin[3].

Darobactin doesn't try to penetrate the outer membrane of Gram-negative bacteria. Instead, it targets an essential protein on the outer shell. Tests showed that darobactin successfully cured infections in mice with *E. coli* and *Klebsiella pneumoniae*, which can cause pneumonia and urinary-tract problems. Although *E. coli* developed resistance to darobactin, it changed so much in the process that it could no longer infect cells and became harmless.

Lewis is also trying to tap into another potential source: the 99% of bacterial species that won't grow in a Petri dish. He and his colleagues have devised a way to trick the bacteria into thinking they are in their natural environment, thereby allowing them to grow in culture. That approach led the team to teixobactin[4] — an antibiotic that works on methicillin-resistant *Staphylococcus aureus*, commonly known as MRSA. Because teixobactin targets the precursors to lipid molecules that make up cell walls, it should be hard for bacteria to come up with a protective mutation, Lewis says.

The shape of things

There's a long road from discovering a potential drug to getting it approved for human use. Bree Aldridge, a biomedical engineer at Tufts University in Boston, Massachusetts, thinks the process could be speeded up by using a computerized imaging system that looks at how bacterial cells are deformed by a drug.

Such changes can give hints as to what part of a cell's biology the drug is acting on. "We think we know what a drug does, but then if you look at how it actually destroys the cells, we can sometimes see that it's a little bit different," Aldridge says. "This sort of method allows us to rapidly determine whether a drug is acting like known drugs or whether a drug is doing something that's novel." If it's novel, it might be a class that bacteria are not yet resistant to.

Her team tested its rapid-profiling system, dubbed Morphological Evaluation and Understanding of Stress, or MorphEUS, on existing tuberculosis drugs[5]. It found that 6% of the drugs used pathways that hadn't previously been identified, so could lead to innovative treatments. The system should be applicable to any pathogen that responds to drugs in subtle and complex ways.

Despite some promising leads, more candidates need to be discovered or created, and then make it through the development process. And then they need to be made available to those who need them, particularly in low-income countries. There are funding programmes to promote the development of new antimicrobials, such as the Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator, a global non-profit organization with US$500 million to support research, and the Antimicrobial Resistance Action Fund, launched in July by an alliance of pharmaceutical companies.

Such programmes make Piddock cautiously optimistic that drugs will be found before microbes overwhelm existing ones. "If I'd have spoken with you ten years ago, I'd be very, very gloomy and pessimistic," Piddock says. "Things have got much better."

Neil Savage is a freelance writer in Lowell, Massachusetts.

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