As the summer of 2016 wound down in Colorado, public health officials in two counties issued alerts: people drinking raw milk from a local dairy were getting sick from bacteria called *Campylobacter jejuni*. By the end of the outbreak, about a dozen people had fallen ill with diarrhea, fever, and vomiting. One person was hospitalized. Several were sick for more than 10 days, despite the fact that *Campylobacter* infections typically resolve quickly. Months later, federal public health officials revealed why the infection might have persisted for so long: bacteria they isolated had developed resistance to the antibiotics normally used to treat them.

It’s a story that has become increasingly common. Infections that were once easily treatable now require extraordinary doses of one or more antibiotics. Meanwhile, intravenous antibiotics with nicknames like “last resort” come off the shelf more and more often. These stories portend a bleak future, one in which small wounds could lead to death, a common occurrence of a bygone era, say scientists who spoke to C&EN about the antibiotic crisis.

“The real worry is what our world is going to be like for our kids” if antibiotics stop working, says Floyd Romesberg, who researches natural antibiotics at Scripps Research in California. “We risk living in a postantibiotic era.”

Finding new medicines to kill pathogenic bacteria is getting harder and harder, Romesberg says. Some teams are urgently trying to tweak tried-and-true antibiotics to squeeze a few more usable treatments out of them. Some researchers are looking for new compounds that can be used as templates to develop medicines. And still others are plumbing the unexplored crevices of bacterial physiology, hoping to unearth targets that could lead to whole new classes of antibiotics.

Future generations of bacteria-fighting drugs will be more targeted, likely more expensive, and, researchers hope, more quickly approved and deployed. But antibiotic hunters are worried. Bacteria are smart. They will keep developing resistance to anything scientists throw at them, and eventually, they will win, Romesberg and many of his colleagues say. “We’re buying time,” Romesberg says. “You just have to keep running as fast as you can to stay in place.”

### Bacteria’s tricks

Antibiotic development seems to drive in two lanes of traffic: one relies on tweaking the molecular scaffolds of tried-and-true medicines, like the β-lactam ring found in penicillin; the other involves the search for entirely new compounds to disrupt both known and novel targets.

In either case, says Lynn Silver, an antibiotic expert who consults for industry, antibiotic hunters face three big challenges: getting their compound into bacteria, especially Gram-negative species, which have tough-to-penetrate outer membranes; staving off resistance; and preventing toxic side effects in the person taking it.

Pathogens have a laundry list of ways to neutralize antibiotics. Some multidrug-resistant bacteria produce a high level of efflux pumps as a means to regurgitate antibiotics that find their way inside cells. Some develop enzymes that modify the drug, reducing its efficacy. And others find ways to produce redundancy. For example, if an antibiotic attacks a protein in a bacterium’s cell wall, that bacterium may simply replace the target protein with a different one that is immune to the antibiotic.
but preserves the target’s function.

These challenges are why the well has been fairly dry of truly new antibiotics for decades, says Eric Gordon, CEO of Arixa Pharmaceuticals, and why the pharmaceutical industry has virtually walked away from the search.

“The entire pharmaceutical industry, coming out of World War II, was an antibiotic industry,” Gordon says. Scientists have been building on the molecular scaffolds of the antibiotics from that era for the past 50 years and have reached the point of diminishing returns. “You might be able to squeeze one or two compounds out of these classic scaffolds, but they just don’t have much more to give.”

Gordon’s company is working on an oral version of a β-lactamase inhibitor, a compound administered along with antibiotics to try to overcome resistance. Bacteria produce enzymes called lactamasess that disable antibiotics containing the classic β-lactam ring scaffold. When given with β-lactam antibiotics, lactamase inhibitors can overcome resistance mechanisms, at least for a little while. Over the years, pathogens have evolved thousands of enzymes—to date, scientists know of about 2,700 of them—that neutralize β-lactams.

Arix’s drug is an oral version of an already US Food and Drug Administration–approved β-lactamase inhibitor called avibactam, which is given intravenously in conjunction with an antibiotic. The pill would allow consumers to take the medication outside the hospital.

Reformulating an existing drug means the company has fewer regulatory hurdles to jump through, making it a more reasonable pursuit, financially, than building a novel antibiotic, Gordon says.

Efforts like Arix’s are going to work for a while, Gordon says, but “it still doesn’t answer the question about what is going to be the source of the antibiotics of the future,” he says.

With so many ways to neutralize a β-lactam antibiotic, which works by preventing bacteria from building their cell walls, the field is running out of ways to use this structure to build antibiotics. And with few novel targets he can point to that researchers have successfully built antibiotics against, Gordon struggles to be optimistic.

“Most people think that with a big cash infusion into the area, maybe catalyzed by the government or something like that, antibiotics that we have came from nature,” he says. “It’s proven to be extraordinarily difficult for a chemist to come up with new scaffolds.”

Romesberg’s lab investigates natural compounds to take advantage of their ready-made scaffolds. One class of mol-


### Searching for new scaffolds

Eventually, tweaking known antibiotics will stop yielding new drugs. This is why several scientists, including Romesberg, have turned to natural products as a source of new building material. Like penicillin, “virtually all of the good

### Antibiotic resistance mechanisms

Pathogens have evolved several mechanisms to neutralize antibiotics: They can use inactivating enzymes such as β-lactamasess to destroy antibiotics containing β-lactam rings. They can increase the production of efflux pumps to spit antibiotics back out of the cell. They can alter the composition of their cell wall to decrease antibiotic uptake. They can alter the genetic targets of some antibiotics, and they can replace enzymes targeted by antibiotics with alternative enzymes that carry out the same function.
species, Romesberg argues. Picking up compounds at the narrow part of their cycle isn’t wasted time, he insists. When his team discovered arylomycin, which inhibits the activity of a signal peptidase that allows for bacteria to secrete proteins and lodge them in their membranes, the group found that despite a conserved target sequence, it was a narrow-spectrum compound.

The researchers tested it against several bacterial species, found one that was susceptible, and watched what happened during an experiment enabling that species to develop resistance in the lab. The species, *Staphylococcus epidermidis*, was changing its signal peptidase in a specific way, via a serine-to-proline substitution. A number of other bacteria that are resistant to arylomycins use the same trick to evade the antibiotics. From this finding, the team concluded that although arylomycins are currently narrow-spectrum, they were once powerful, broad-spectrum killers (*Antimicrob. Agents Chemother.* 2012, DOI: 10.1128/AAC.00785-12).

So to turn arylomycins into a broad-spectrum antibiotic once again, Romesberg’s team and others have focused on tweaking the natural scaffold to overcome the destabilizing effect of that proline.

RQx Pharmaceuticals, a biotech founded by Romesberg, partnered with Genentech in 2013 to take on that challenge. In September, Genentech scientists published early data on an arylomycin-based compound that can kill pathogens associated with some hospital-acquired infections. It works by covalently binding a lysine in the signal peptidase. This strong link, in theory, will thwart the effects of a proline being swapped in for a serine somewhere in the peptidase structure (*Nature* 2018, DOI: 10.1038/s41586-018-0483-6).

**Novel targets and novel products**

In the 1990s, with the advent of gene sequencing, pathogen scientists were sure that sequencing bacterial genomes would yield a treasure trove of proteins that bacteria couldn’t live without. Aiming at these targets with new drugs, either from known compounds or from libraries of untested compounds, seemed like it would energize the field.

It wasn’t the easy victory some had anticipated. Many of the genes described were already known and had already been through compound screens, antibiotic industry consultant Lynn Silver says. And Arixa’s Gordon adds that bacteria seemed to be able to compensate for antibiotic activity against those targets.

But some researchers are undeterred and say that they just need more time. “Exploring novel targets gives you an opportunity to surprise the bacteria,” says Concepción González-Bello, an organic chemist at the University of Santiago de Compostela.

González-Bello studies some of bacteria’s essential genes, including two in the metabolic shikimate pathway that are critical for survival. She believes that designing antibiotics for new targets will lead to medicines that could elude resistance strategies longer than medicines being developed for known targets.

While researchers continue to plug away at sussing out novel targets, González-Bello says some of the most intriguing work that could deliver sooner focuses less on building new antibiotics and more on what can be administered alongside them to make them work better.

The classic additive is the β-lactamase inhibitor, but newer research is looking at immune-boosting compounds called adjuvants. In the presence of pathogens, the human immune system goes to work immediately, recognizing chemical patterns on the surface of bacteria. The immune cell receptors that do this work are now targets for natural and synthetic compounds that can be paired with antibiotics to stoke the immune response.

Other promising ways to attack pathogens through the immune system include antimicrobial peptides, which are released by the human immune system in response to infection. Like antibiotic compounds, these peptides interfere with the cell membrane of bacteria, or nucleic acid and protein formation, both of which are required for survival. Medicinal antimicrobial peptides, which include vancomycin and daptomycin, are among last-resort treatments.

But a number of bacterial species are resistant even to last-resort treatments, so some members of industry are bucking the search for broad-spectrum antibiotics and focusing instead on these highly resistant species, which are the narrowest of targets. Genentech, for example, has a drug-antibody conjugate in Phase I trials, in partnership with Seattle Genetics and Symphogen. The antibody is engineered to attach to the cell wall of MRSA—methicillin-resistant *Staphylococcus aureus*—a common health-care pathogen that is resistant to all known β-lactam antibiotics, as well as some last-resort antibiotics like vancomycin.

On top of its resistance to antibiotics, MRSA hides inside circulating cells, allowing it to move from the site of initial infection to elsewhere in the body. The Genentech antibiotic is a spin on rifamycin that is inactive until it gets inside mammalian or human cells. The conjugate seems to work by bonding MRSA before it invades cells. Once a cell takes up the pathogen, the conjugate is cleaved, and the antibiotic is activated.

Some narrow-spectrum antibiotics are already showing progress in clinical use. In mid-November, a small biotech called Entasis Therapeutics released tantalizing clinical trial findings of zoliflodacin, a narrow-spectrum antibiotic that inhibits DNA synthesis in people with gonorrhea infection. It’s a needed advance: the sexually transmitted infection in some people has persisted, even after multiple rounds of antibiotics, including those of last resort.

While most people will still be able to use broad-spectrum antibiotics, Entasis Therapeutics CEO Manos Perros says that for a small group of people, “bacterial antibiotics are going to become like orphan-disease drugs, tailored to the resistance mechanism.”

That narrowness will come at a price. Although the FDA and Congress have created programs to fast-track antibiotics through approvals, and nonprofit groups, like Carb-X, provide financial support to promising programs, companies hint that developing drugs for specific uses will be expensive.

“You can do the math. If your market size is 5 million patients versus 50,000,” then you’ll have to adjust the price for the lower volume you will sell, Perros says. But he stresses that any high cost would be for a onetime treatment. People will get well, he says, and become productive again.

But like other scientists, Perros temper optimism with the reality that any approved antibiotics are short-term gains. “Every battle is going to be a losing battle,” he says. “We have to change how we do things. We have to change how we fight that war if we want to prevail. I can’t imagine that society would let things go back to the times when we could die from a minor flesh wound.”

—Manos Perros, CEO, Entasis Therapeutics

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