Overcoming Resistance
In the face of bacterial threats that can evade modern medicines, researchers are trying every trick in the book to develop new, effective antibiotics.

By The Scientist Staff | April 1, 2014

Although researchers and drug developers have been sounding warnings for years about bacteria out-evolving medicine’s arsenal of antibiotics, the crisis is coming to a head. In the United States alone, some 23,000 people are killed each year by infections caused by drug-resistant bacteria, according to the Centers for Disease Control and Prevention’s 2013 Threat Report. Many more patients die of other conditions complicated by infection with resistant pathogens. Such maladies cost the health-care system more than $20 billion annually, in part because patients suffering from drug-resistant infections require more than 8 million additional hospital days.

The statistics are sobering, and they’re made even more so by the fact that the US Food and Drug Administration (FDA) has only approved two new classes of antibiotics since 1998. In fact, only five new classes have hit the market in the last 45 years; the vast majority of today’s antibiotics were developed before 1968.

Overuse—and not just in people, but in animals, too—is a primary driver of the antibiotic-resistance epidemic. One of the most controversial antibiotic practices has been the “nontherapeutic” treatment of farm animals with low doses of the drugs to promote growth and prevent disease in crowded factory-farm conditions. (See “Antibiotics in Animals We Eat,” The Scientist, April 2012.) Up to 80 percent of the antibiotics used in the U.S. is fed to animals, and the Natural Resources Defense Council recently criticized the FDA for allowing livestock producers to include 30 different antibiotics in the animals’ feed and water, 18 of which the agency itself had rated as “high risk” for introducing antibiotic-resistant bacteria into the human food supply.

While debates rage over what is driving the recent onslaught of antibiotic-resistant pathogens and how to best stem the bacterial tide, many researchers are now focused on developing new treatment regimes to combat these deadly superbugs. One thing most of these scientists on the front lines agree on is that antibiotic resistance is not a single-solution problem. Here, The Scientist surveys four strategies being explored to overcome even the most resistant bacteria: tweaking old compounds into entirely new classes of antibiotics; combining modern antibiotics in a one-two punch against infection; supplementing existing antibiotics with adjuvants that can render resistant pathogens susceptible once more; and reviving the field’s roots by

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Blasts from the Past

The golden era of antibiotic discovery is well behind us. In the mid-20th century, numerous new classes of antibiotics came on the market, and scientists tinkered with these molecules to create ever more powerful versions of the drugs. Since then, however, the well has dried up. Even genomics has failed to rescue the stalled antibiotics field, says Anthony Coates, an antibiotic researcher at St. George’s, University of London, and the founder of HelperBy Therapeutics.

One approach researchers are undertaking to break the dry spell is to alter old drugs, including those that have been abandoned by Big Pharma, using new techniques. Richard Lee at St. Jude Children’s Research Hospital in Memphis, Tennessee, for example, is studying an old antibiotic called spectinomycin that was introduced in the 1960s to treat gonorrhea. While the drug worked against the sexually transmitted bacterium, large doses were required, and, eventually, drug makers developed more potent antibiotics. Although spectinomycin only has weak effects against most microbes, Lee saw potential in remodeling it to treat certain bacterial infections, thanks to its ability to bind to the bacterial ribosome and dog protein synthesis.

“What we could take advantage of, which wasn’t available 20 years ago, is the crystal structure of spectinomycin bound to the ribosome,” Lee says. With a tweak to adjust how the molecule binds to the ribosome, the modified drug was able to fight off tuberculosis in vitro and in mice (Nat Med, 20:152-58, 2014). The changes not only maintained the affinity of the drug for the ribosome, but allowed the antibiotic to avoid the efflux pump that normally ejects this drug from the tuberculosis bacterium. “It works better than we would have dreamed,” Lee says, although its potency against gonorrhea was not improved.

Jason Sello, a biochemist at Brown University, has also found that slight chemical tweaks can make a profound difference to antibacterial activity. His group has been tinkering with the structure of ring-shape compounds called acyldepsipeptides (ADEPs). Discovered in the 1980s, ADEPs were initially of interest to pharmaceutical companies because of their antibacterial activity, but were ultimately set aside in pursuit of other endeavors and never brought to market. One unfavorable aspect of ADEPs is that bacteria tended to become resistant to them quite quickly, rendering the drugs incapable of clearing an infection. But because ADEPs work by the drug working against the bacterial enzyme ClpP, which normally clears misfolded proteins—they’re extremely appealing for development as a drug to fight bacterial infections, says Sello.

He and his colleagues have focused on the rigidity of the cyclic structure of ADEPs and found that strengthening the hydrogen bonds of the ring can increase the antibacterial power of the compound, seemingly by allowing the drug easier entry into target cells (J Am Chem Soc, 136:1922-29, 2014). “Why it’s better at killing bacteria is not because it has a better mechanism of action, but we think it’s more cell-permeable,” says Sello. Preliminary studies in mouse models show that the modified ADEP is good at treating staph and Enterococcius infections, and so far, there’s no evidence that the drug is toxic.

At Oregon State University, microbiologist Bruce Geller has picked up on yet another discovery that was made decades ago. Phosphorodiamidate morpholino oligomers (PMOs) are short, synthetic versions of genetic material that were invented in the 1980s. Their molecular backbone makes them resistant to nucleases, so they can sneak past bacteria’s defenses against foreign DNA, and the sequence of each PMO is custom-designed to interfere with mRNA expression by a particular gene. Geller’s group bonded the PMOs to membrane-penetrating peptides to enhance entry into bacteria. The resultant peptide-conjugated PMOs (PMPMOs) are “the ultimate narrow-spectrum therapeutic, because they’re species- and gene-specific,” Geller says.

Geller has designed PMPMOs to target a variety of bacterial genes, including acpP, a gene required for lipid biosynthesis. “If you knock it out, it’s a lethal event,” he says. Sure enough, when treated with acpP-specific PMPMOs, mice infected with multidrug-resistant Acinetobacter baumannii survived for at least one week, while control mice died, most within a day (J Infectious Diseases, 208:1553-60, 2013).

Geller and the other researchers hope their work will one day bear fruit in the clinic, but for now, such new drugs emerging from old discoveries remain merely a preclinical glimmer of hope, with many years of work ahead before medicine gets a desperately needed novel class of antimicrobials. “The scientific difficulty [of developing a new antibiotic] is not to be underestimated,” says Coates.—Kerry Grens

It Takes Two
Given the difficulties in bringing an entirely new class of antimicrobials to market, some researchers are setting their sights on what they see as a more readily attainable goal: to combine existing drugs into more effective therapies. "Finding a brand-new chemical scaffold that has all the wonderful chemical properties of the [antibiotics] we have now is going to be extremely hard to do," says McMaster University chemical biologist Gerard Wright. "All future antibiotics should be developed as combinations."

To explore the potential of new combination therapies, microbiologist Kim Lewis, director of the Antimicrobial Discovery Center at Northeastern University in Boston, looked to one of the seemingly failed ADEP antibiotic compounds, ADEP4. Bayer Healthcare scientists discovered the drug in 2005 but later dropped it after in vitro experiments showed that bacteria rapidly developed resistance to it. But, like Sello, Lewis and his colleagues thought that it just needed a little help. So they combined ADEP4 with a conventional antibiotic, rifampicin, in the hopes that the treatment would be effective—and stay effective—against Staphylococcus aureus, which readily forms antibiotic-resistant biofilms harboring dormant cells known as persisters. (See "Bacterial Persisters," The Scientist, January 2014.)

The therapy worked better than anyone had dared to hope: while ADEP4 and rifampicin each reduced microbial populations in vitro and in mice, administered together they obliterated the bacteria (Nature, 503:365-70, 2013). "What we discovered unexpectedly was that with the combination of this ADEP compound with another antibiotic we got complete sterilization," Lewis says.

Lewis and his colleagues don’t yet know the precise mechanism of vulnerability that the ADEP4/rifampicin combination exploited, but it likely involved ADEP4’s activation of the ClpP protease. Triggering ClpP to degrade proteins nonspecifically in persister cells within biofilms may have caused the breakdown of hundreds of proteins, forcing the cells to self-digest, Lewis says. While some bacteria could have evolved to lack functional ClpP and therefore resist ADEP4’s strike, rifampicin, which inhibits RNA polymerase, likely stepped in and killed those cells. "We have to do some additional toxicity testing, but the goal is to move this into clinical studies," Lewis says.

Yannin Hu, a medical microbiologist at St. George’s, University of London, and director of research at Helpergy Therapeutics, also had recent success with a combination antibiotic therapy. Hu and Helpergy founder Coates used high-throughput screening to identify HT61, a small antibiotic compound that exhibited selective bactericidal activity against methicillin-susceptible S. aureus (MSSA) and methicillin-resistant S. aureus (MRSA) by depolarizing the bacterial cell membranes. "We thought, ‘Okay, if we combine our compound with existing antibiotics, let’s see what we can get,’” Hu recalls. The result in vitro and in mouse models: HT61 enhances the antimicrobial activities of traditional antibiotics, especially aminoglycosides such as neomycin, gentamicin, and chlorhexidine, against MSSA and MRSA (J Antimicrobial Chemother, 68:374-84, 2012).

Hu says that the combination therapy likely worked so well—far better than either antibiotic administered alone—because HT61 was essentially punching holes in the membranes of nondividing bacterial cells, allowing the aminoglycosides to flood in. Used as a topical agent in combination with the antibiotic mupirocin, HT61 has cleared Phase 1 and 2 trials for the treatment of latent MRSA infections, Hu says. She and Coates have also identified a plethora of other potential compounds that might serve to enhance the effects of existing antibiotics. "We have about 300 similar compounds that show very good activity against persistent organisms," Hu says.

Antibiotics can also be combined with existing, nonantibiotic drugs, as Wright is doing. In 2011, he and his colleagues screened more than 1,000 approved drugs for compounds that augmented the ability of the antibiotic minocycline to fight infection. They identified a suite of promising nonantibiotic drugs—for indications as diverse as Parkinson’s disease, irritable bowel syndrome, cancer, and diarrhea—which, in combination with minocycline, were able to fight infections of Pseudomonas aeruginosa, E. coli, and S. aureus in vitro and in mice (Nat Chem Biol, 7:348-50, 2011). "We’ve really missed a whole section of antimicrobial target space," says Wright, who adds that he feels strongly that combination therapies are the best way to tackle the antibiotic resistance threat. "The idea of a magic bullet is gone. We need a magic shotgun."

—Bob Grant

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Resensitizing Bacteria
Rather than combining antibiotics with new compounds found to have antibiotic activity, some researchers are looking to simply add adjuvant compounds. Although adjuvants themselves are unable to kill bacteria, when added to antibiotic regimens they render resistant microbes susceptible once again.

"We are developing agents to sensitize bacteria to the agents we already have," says microbiologist Anders Hakansson of the State University of New York at Buffalo, who in 2012 found that treatment with a protein-lipid complex from human milk could potentiate the effect of common antibiotics against drug-resistant *Streptococcus pneumoniae* (*PLOS ONE*, 7:e43514, 2012).

From a financial standpoint, antibiotic adjuvants make sense. Developing and validating a small-molecule sensitizer to be used in conjunction with an existing antibiotic should cost far less than developing and validating a completely new drug. The aim is "to extend the utility and lifetime of existing antibiotics," says biomedical engineer James Collins of Boston University. "There is still some activity, in some cases, of the antibiotic, it just doesn’t get to the lethality threshold. The adjuvant allows one to shift that threshold."

One way microbes are evolving resistance to first-line antibiotics is by blocking entry of the drug into the cell. Many gram-negative bacteria pose the additional challenge of producing β-lactamase enzymes that block antibiotics containing a β-lactam ring, such as penicillins, cephamycins, and some carbapenems, from inhibiting bacterial cell-wall biosynthesis. And even if an antibiotic is able to penetrate the bacterial cell wall and avoid degradation by β-lactamases in the cytoplasm, the drug must also fight against efflux pumps to stay inside the bacterium long enough to kill the cell.

It’s a tall order, says Laura Piddock, a professor of microbiology at the University of Birmingham, who leads the Antimicrobials Research Group there. "These molecules have to not only get through the outer [bacterial cell] membrane, they then have to get past all these enzymes, and then they’re almost certainly going to be pumped out," she says. "These three things together make a very, very tough challenge."

But new adjuvant sensitzers can target any one of these bacterial defenses—by damaging cell walls, inhibiting β-lactamase, or stopping efflux pumps—and a handful of biotech companies now have antibiotic adjuvants in their discovery and development pipelines. For example, the Boston-based firm Collins cofounded, EnBiotix, is working on potentiators such as silver compounds that sensitize persistent bacteria to existing antibiotics by increasing bacterial membrane permeability. Oklahoma City-based Synereca is working to validate inhibitors of the bacterial protein RecA, which plays a role in recombinational DNA repair. And Venus Remedies in Chandigarh, India, secured approval in a handful of countries last year to sell Elores, a β-lactamase inhibitor combined with the antibiotic adjuvant disodium edetate.

By and large, however, progress has been slow, limited in part by the toxicity of these small molecules. "People are starting to look [for antibiotic adjuvants]," says Hakansson, "but right now, there’s not really a critical mass of molecules that really work" without causing unacceptable side effects.

Nevertheless, he and others continue to search for new adjuvants that could render increasingly useless antibiotics effective once again. "Different drugs synergize with each other," says Hakansson, who envisions a future in which antibiotic-resistant bacterial infections are treated much like HIV, with a cocktail of drugs. Once identified and validated, adjuvant sensitzers could be as common to pharmacy shelves as the antibiotics themselves. —Tracy Vence

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**Discovery Zone**

Since Alexander Fleming’s serendipitous 1928 observation that a *Penicillium* fungus prevented growth of staphylococci bacteria, the search for new antibiotics has largely been focused on fungi and microbes living in the soil, in the hopes of discovering another natural product with the broad effectiveness and low toxicity of penicillin. But as more recent searches result in disappointment, some investigators are turning to new sources—plants, insects, and marine organisms—to find antibiotics that can kill our most common and persistent pathogens.
When chemist Simon Gibbons of the University College London School of Pharmacy went in search of plants harboring compounds with antimicrobial properties in 2008, he paid particular attention to those that have been used in traditional medicine—especially for wound healing. "If a plant is used as a wound-healing agent, it's quite likely that it contains chemicals that kill the bacteria in the wound," he says. Although best known for its psychoactive properties, cannabis, historically ingested in parts of Afghanistan and India to treat infection, fit the bill. Gibbons and his colleagues isolated five cannabinoids from Cannabis sativa and found that each one was effective against MRSA (Nat Prod, 71:1427-30, 2008). "It hasn't been confirmed in vivo, but certainly in the lab, we know that these things kill drug-resistant bacteria," he says.

Gibbons has also found chemicals with antibiotic properties in other familiar plant groups. For instance, plants in the Allium genus, which includes garlic and onions, produce sulfur-containing compounds that have activity against MRSA and Mycobacterium (Nat Prod, 72:360-65, 2009). And many of the hypericums—the family that includes St. John's wort—make chemicals called acylphloroglucinols that also effectively kill MRSA in vitro (Nat Prod, 75:336-43, 2012). "We've had leaks... and a series of compounds, which have been patented," says Gibbons, and those compounds are now being synthesized and modified to improve their activity.

Andreas Vilcinskas of Justus Liebig University Giessen in Germany is using another vast resource to identify novel antibacterial compounds: insects. "Insects are considered the most successful group of organisms in the world," says Vilcinskas, who suspects that one of the keys to their success is the ability to manage microbes. And it's likely, he adds, that different insects have different strategies for protecting themselves against pathogens. "I'm convinced that the biodiversity that you see at the species level is also reflected at the molecular level."

In 2012, he decided to home in on invasive insect species, which he hypothesizes have a particularly strong immune system to allow them to succeed in new environments. Harvesting hemolymph from harlequin ladybird beetles (Harmonia axyridis), which have successfully outcompeted native beetles the world over, Vilcinskas discovered more than 50 novel antimicrobial compounds. One compound, called harmonine, demonstrated activity against both Mycobacterium tuberculosis and MRSA (Biology Letters, 8:308-11, 2012), and Vilcinskas's group is now making chemical modifications to harmonine and other compounds to produce even more potent antibiotics.

Other researchers, such as William Fenical of the Scripps Institution of Oceanography in San Diego, California, have moved the quest for antibiotics away from terrestrial environments entirely. From offshore shallows to depths of nearly 6,000 meters (more than 19,000 feet), Fenical and his colleagues collect ocean-floor samples, then culture the microorganisms contained within and test the compounds they produce against antibiotic-resistant microbes such as MRSA. "Seventy percent of the Earth is the ocean," Fenical says. "We feel the ocean has enormous potential."

Last year, the group detailed its discovery of a unique antibiotic made by a species of Streptomyces bacteria isolated from marine sediments off the coast of Santa Barbara. They named the compound anthracmycin because of its high activity against the potential bioterrorism agent Bacillus anthracis, but the compound also demonstrated inhibition of MRSA in nutrient broth assays (Angew Chem Int Ed, 52:7822-24, 2013).

Moving from the lab to the clinic is not trivial, however, Fenical notes. Improving the compound's solubility and activity, lowering its toxicity, and scaling up its production can all present challenges. And in such early stages, the impact of these discoveries on the problem of antibiotic resistance remains to be seen. "To be completely honest, the jury's out," says McMaster's Wright, whose group has explored compounds made by microbes found in an isolated Mexican cave and in a Cuban mangrove forest. Nevertheless, given the diversity of natural products now being discovered, he adds, "it's certainly worthwhile exploring." —Abby Olena

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methicillin-resistant staphylococcus aureus, infectious disease, drug resistance, disease/medicine, antibiotics and antibiotic resistance

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