Andrew Read, left, has studied how Robert Woods and other doctors decide how and when to use antibiotics.

RESISTANCE FIGHTERS

Evolutionary biologists are challenging old dogmas about the way antibiotics should be used

By **Kai Kupferschmidt**

PHOTO: BRAD ZIMMER

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One day in the spring of 2014, Robert Woods, a physician at the University of Michigan Health System in Ann Arbor, stopped by Andrew Read's office. "I've got a patient with an infection that can't be cleared and we have only two drugs left," he told Read. "How should we use them?"

Read, an evolutionary biologist, says his first reaction was: "What do you mean, 'we?'" Read rarely sees patients; based at Pennsylvania State University, University Park, he uses mice and math to study how microbes evolve resistance against therapeutics. He was spending 6 months at the hospital to better understand doctors' decisions about how to use drugs.

Woods's patient was a 56-year-old woman suffering from heart failure. A mechanical pump inside her body helped her blood circulate; it was connected to an external battery by a cable passing through her skin that kept spawning infections. First came methicillin-resistant *Staphylococcus aureus* (MRSA), a notorious hospital dweller, followed by *Enterobacter cloacae*, a species that often infects patients in long-term intensive care. The medical team tried several antibiotics in vain. High doses of ciprofloxacin finally got rid of MRSA, but *Enterobacter* developed resistance to it. They switched to meropenem, with the same result. Next, they tried cefepime—but by then it was too late. As Read and Woods would later write in a case study, "the patient died, in effect, from overwhelming evolution."

Drug resistance has become a huge problem in medicine, which scientists are battling on many fronts, from developing new antibiotics to improving diagnostics that help doctors decide which drug to use. But Read and a handful of other scientists are focused on an issue that gets surprisingly little attention: the evolutionary dynamics that lead to resistance in the first place.

Thinking about resistance in terms of evolution has led these scientists to ideas that fly in the face of conventional medical wisdom. Read's research suggests, for instance, that hitting infections with overwhelming antibiotic firepower, a standard strategy to prevent resistance from evolving, may be counterproductive. Applying a lower dose and letting the immune system do the rest might save countless lives in the long run, he says. Other researchers suggest that combining multiple antimicrobials, another method to avoid resistance, may sometimes backfire as well.

These insights come primarily from lab experiments and computer models; tests in humans are tricky because they might involve giving some patients a suboptimal dose of a life-saving drug. And many researchers and clinicians are skeptical, to say the least. But there's not much evidence for current practices either, says Hinrich Schulenburg, an evolutionary biologist at the University of Kiel in Germany who's also studying fresh strategies to avoid resistance. "We are developing new ideas," Schulenburg says. "I think that is important because we are breaking with dogmas … for which there is little empirical support."

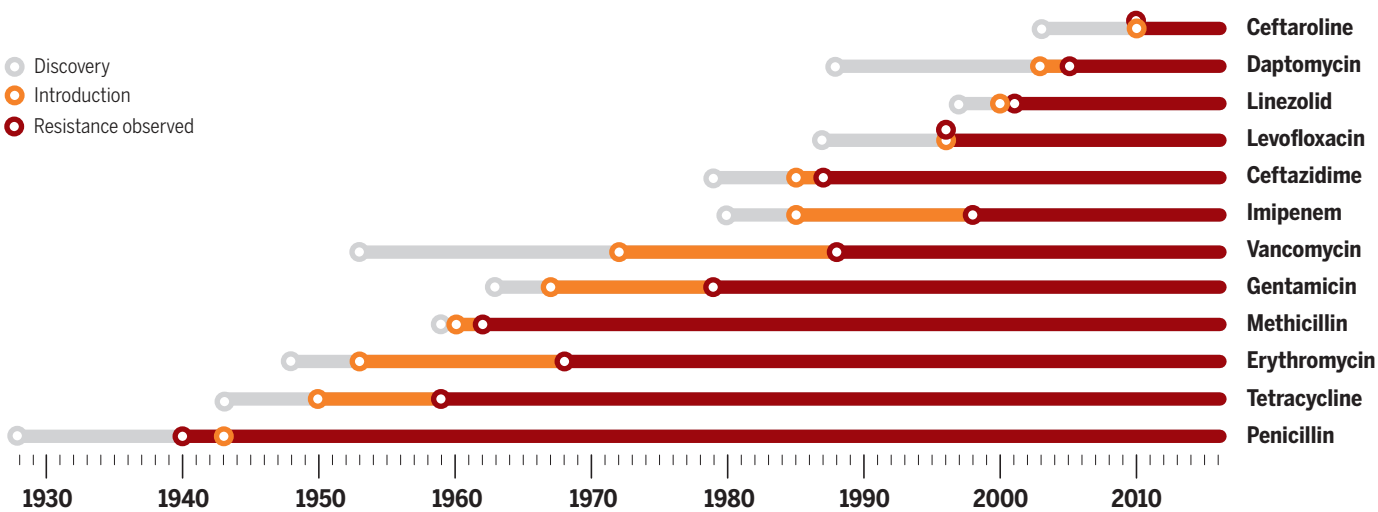
JUST A FEW YEARS after his discovery of penicillin in 1928, Alexander Fleming realized that many microbial species were able to develop resistance to the new wonder drug. Since then, bacteria have evolved resistance to every newly discovered antibiotic—sometimes even before the drugs came on the market (see graphic, below).

Drug resistance is taking an ever-larger toll. More than 2 million people become infected with resistant bacteria every year in the United States alone, and at least 23,000 of them die, according to the Centers for Disease Control and Prevention (CDC). (Some would have died even if antibiotics worked, but resistant infections generally lead to disease that is longer, more serious, and more often fatal.) By 2050, the number of deaths worldwide could be 10 million a year, according to a review commissioned by the U.K. government that will soon be published. Scientists have raised the specter of a return to a "preantibiotic era," when a simple thorn prick from a rose could kill you and even minor surgery carried major risks.

There's universal agreement that to avoid this, antibiotics shouldn't be used willy-nilly—for instance, against viral infections. (A CDC report published on 3 May found that one in three antibiotic prescriptions in the United States is inappropriate.) The debate is about what to do when antibiotics are actually useful and needed. Fleming believed he knew the answer: Treat at high doses. In his acceptance speech for the 1945 Nobel Prize, he warned of the dangers of using sublethal levels: "Mr. X. has a sore throat. He buys some penicillin and gives himself not enough to kill the *Streptococci*, but enough to educate them to resist penicillin. He then infects his wife. Mrs. X

The rise of resistance

Bacteria have developed resistance to every antibiotic discovered so far, sometimes even before the drug reached the market. The appearance of resistance does not mean that a drug has become completely useless.



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gets pneumonia and is treated with penicillin. As the *Streptococci* are now resistant to penicillin, the treatment fails. Mrs. X dies."

That logic still applies, researchers say. High doses kill many bacteria quickly; the fewer that are left to evolve, the less likely one of them is to develop resistance. As some say: "Dead bugs don't mutate." Yet a 2014 review authored by 23 scientists, including Read, noted that "there is surprisingly limited empirical evidence" to support this strategy. "The ignorance is frightening; the ignorance of the ignorance even more so," Read says.

He says the story is more complicated than Fleming realized. Many antibiotics are natural compounds that arose during millions of years of intermicrobial warfare. Antibiotic resistance is a product of natural evolution, too. In 1940, for instance, before penicillin was widely used, scientists found that some bacteria already had an enzyme making them resistant to it. Genes encoding resistance are literally everywhere, even in 30,000-year-old frozen sediments in the Yukon territory in Canada. Combined with the resistant microbes that have emerged more recently in hospitals and the wider community, this means that in many infections, a few resistant bacteria may be present from the start, Read says. The key issue is not keeping resistance from developing—it's stopping its spread.

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Hinrich Schulenburg, University of Kiel

This is why high doses of antibiotics may backfire, Read argues. Resistance usually comes with a "fitness cost" that limits growth: A bacterium may have to expend extra energy to pump out an antibiotic, for instance. High doses of antibiotics will kill susceptible bacteria rapidly, leaving resistant ones without any competition—a phenomenon known as competitive release—and giving them the upper hand.

With lower doses, in contrast, resistant bacteria would have to compete with susceptible bacteria, and would remain a minority. An antibiotic given this way simply holds the bacteria in check: The immune system—which seems able to kill resistant and susceptible bacteria equally well—then mops up the infection.

Read has tested this idea in mice that he infected with *Plasmodium chabaudi* (not a bacterium but a malaria parasite). At the start, one in every million or billion parasites was resistant to pyrimethamine, a malaria drug. When the mice were given an aggressive pyrimethamine treatment—one that mimics the recommended regimens for humans—the resistant parasites quickly became more common. "Once resistance is present in a patient, currently recommended regimens actually maximize its spread," Read says. But with a lower dose and a shorter course, the resistant parasites remained a tiny minority, and the mice didn't suffer more severe disease, Read reported in 2013. Several other studies have shown similar results.

Read acknowledges that high doses may prevent resistance when it isn't present from the start, but emerges during the course of an infection—the scenario Fleming described. But even then, dosing high isn't always the best solution, he has shown in a model developed with mathematician Troy Day of Queen's University in Kingston, Canada.

The model (see graphic, p. 761) has two extremes: When no antibiotics are given, bacteria can replicate freely, and any resistant bacteria that arise are quickly overwhelmed by susceptible ones. At very high doses, any resistant mutants would quickly take over—but because there is much less replication, such resistance is less likely to arise. This means that the likelihood of resistance emerging is equally low at very low and very high doses; it's elevated in between.

The model suggests that antibiotics should be developed and prescribed in a new way, Read says. Drug manufacturers should identify the highest doses patients can still tolerate and the lowest doses at which the medicine is still effective, and doctors should prescribe at one of the two ends of the spectrum. (In some cases, the high dose will turn out to be better, in others the low dose, Read argues.)

Inspired by these ideas, researchers at St. George's Hospital in London recently set up a trial in which children with pneumonia receive either a high or a low dose of amoxicillin, for 3 days or for 7 days. They will compare how often children in the different groups have to undergo another treatment and will take nasal swabs before, during, and after treatment to look for resistant bacteria.

MANY SCIENTISTS are unconvinced. Read's ideas may work in the lab, but "his recommendations are potentially dangerous," says Nicholas White, a malaria researcher at Mahidol University in Bangkok. For one, some people break down drugs very fast; if they receive a lower dose, they may not benefit from treatment at all, "and they may die," White says. "Read's argument is fundamentally fallacious because it assumes there is a safe way to undertreat infections. There isn't."

Bruce Levin, a biologist at Emory University in Atlanta, says there are two basic flaws in Read's argument against high doses. Resistance isn't an all-or-nothing phenomenon: Many resistant bacteria can survive lower antibiotic doses but are still susceptible to high doses, contrary to Read's assumption. And the fitness cost of resistance is often not that high, which means competitive release is not a very strong force, says Levin, who published a mathematical model supporting conventional views.

The dispute gets heated sometimes. Levin prefaced a recent talk with a slide that said: "Controversy is great for careers, whether warranted or not, but especially when not." But Read relishes the role of a rebel against what he calls "the curious orthodoxy of aggressive chemotherapy." "Many clinicians don't like my ideas, partly because they have been raised on this 100-year-old idea," he says. "And it's a natural instinct to use everything in your power to get rid of the infection as fast as possible."

Harvard University epidemiologist Marc Lipsitch adds that Read's ideas might saddle doctors with a dilemma. Low-dose treatments could benefit society as a whole but not the individual patient. (Indeed, the best way to prevent resistance is never to use antibiotics at all—obviously not a desirable strategy.) Especially with serious infections, "my guess is that few would be willing to risk the lower dose," Lipsitch says.

But Lipsitch thinks simple conservatism is also at work. "I do think there is a lot of conventional wisdom that prevents novel approaches," he says. "A big contribution of what Andrew has done is to shake up that conventional wisdom."

EVOLUTIONARY THINKING is challenging other widely held assumptions as well. Combining two, three, or even four drugs is the norm in treating HIV and tuberculosis, because it's thought to be much harder for a pathogen to develop resistance to all of them at once. Scientists hope combination therapy could thwart resistance in other

PHOTO: CHRISTIAN URBAN/UNIVERSITY OF KIEL

types of infections as well—but research by Schulenburg suggests it could backfire.

In one study, he challenged *Escherichia coli* cells with doxycycline, erythromycin, or both antibiotics together. One day into the experiment, the combination therapy performed better than either antibiotic alone. On day 2, the double whammy still successfully suppressed susceptible cells, but resistant cells had begun to grow. Because the combination therapy was so successful at suppressing susceptible cells, it gave a big competitive advantage to the resistant ones, ultimately producing a higher bacterial burden than either drug alone.

Along with casting doubt on combination therapies, Schulenburg says, the study shows that it's important to give experiments enough time for evolutionary dynamics to play out—many antibiotic studies last only 24 hours. Schulenburg thinks alternating different antibiotics may hold more promise. In lab experiments, he keeps finding that this strategy makes it harder for bacteria to adapt. "I think this is really something that should be tried in the clinic," he says.

Schulenburg is also studying which combinations are best. Doctors, fond of blasting bugs as hard as they can, usually prefer so-called synergistic combinations, in which the effect of two drugs together is greater than the sum of their individual effects. But Schulenburg's studies suggest such combos aren't always best at preventing resistance.

Antibiotic regimens can not only be changed, but also shortened, Read and others argue. Standard treatment courses are often a week or 10 days even though many infections clear within a few days. "People felt that if they treated for a few more days, there was no downside to it," says Ramanan Laxminarayan, who directs the Center for Disease Dynamics, Economics & Policy in Washington, D.C., and New Delhi. But longer treatments are more likely to favor resistant strains. What's more, recent discoveries about the human microbiome have shown that an antibiotic treatment can ravage the microbial ecosystem in the human gut, killing beneficial microbes and potentially giving harmful ones such as *Clostridium difficile* an evolutionary advantage. Gut microbes could also develop resistance and pass those genes on to pathogens later.

For tuberculosis it's critical that patients finish their full course, Laxminarayan says, because hidden pathogen reservoirs in the body take months to smoke out. But in 2012, a Cochrane review concluded that for children with streptococcal throat infections, 3 or 6 days of treatment are just as effective as 10 days. A 2009 review of clinical trials of acute bacterial sinusitis found that long-term treatment is no more effective than short-term. For many other infections, including meningitis and pneumonia, shorter courses still need to be investigated. It's a slow, incremental process; researchers usually err on the side of caution, testing durations well above the theoretical minimum.

Read, Lipsitch, and Schulenburg all say that if they needed antibiotics, they would stop taking the drugs as soon as they had recovered. "But if we are to challenge clinicians, we need much more and better data," Schulenburg says.

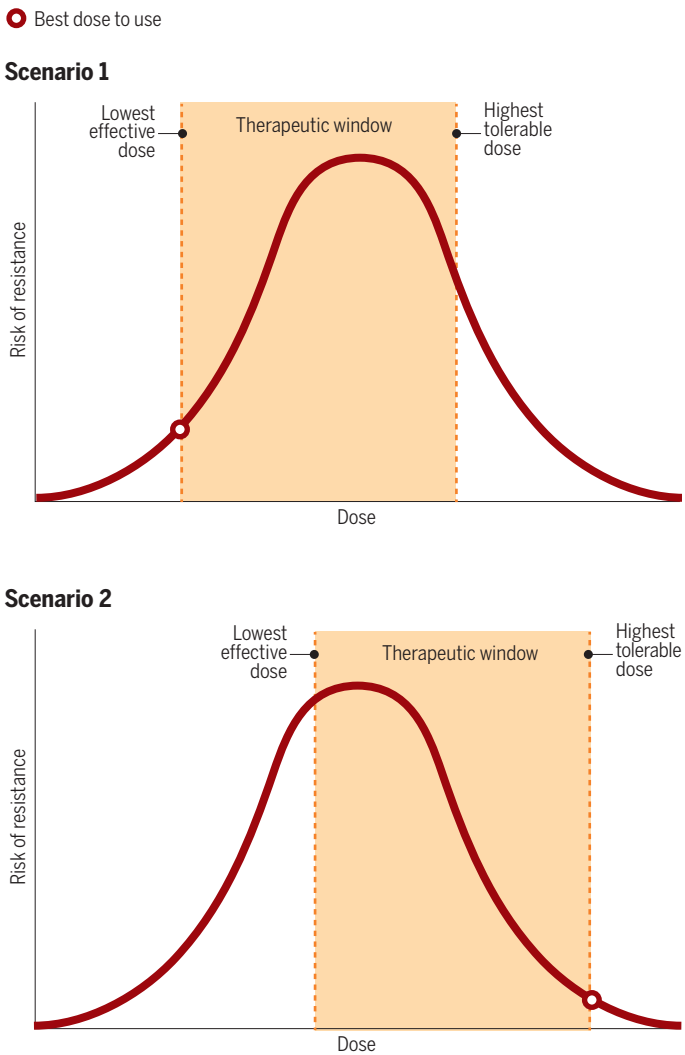
EVEN THOUGH clinical trials of lower doses or shorter courses of antibiotics could pose ethical challenges, Read says he can envision ways to test these new ideas in humans. Some people at increased risk of an infection—for instance with HIV—take drugs prophylactically; researchers could design studies in which different doses of the drugs are given and watch whether subjects become infected with susceptible or resistant strains, Read says. Lipsitch adds that researchers should find out whether infections include both antibiotic-susceptible and resistant strains from the start, as Read argues.

Perhaps the most important thing evolutionary biology can bring to the table is a change in perspective, says Roy Kishony, who studies antibiotic resistance at the Technion-Israel Institute of Technology in Haifa. Antimicrobial drugs have never wiped a pathogen from the planet, he notes, and probably never will. That means that doctors and researchers need to think about the drugs more as "selecting agents," Kishony says. "What you are doing is not really inhibiting, it's selecting for who is going to be there tomorrow or next year."

One way or another, Read adds, clinical medicine and evolutionary biology should start talking to each other. For him the biggest revelation at the Ann Arbor hospital was the "huge gulf" between the two. Not being able to help that patient was "hugely frustrating," he says. "When she died, [Woods] said it was a failure of our science. He was right." ■

High or low doses—what's better?

In Andrew Read's model, the risk of resistance emerging is lowest at very low and very high doses of a drug, and elevated in between (red curve). Which dose is best to use depends on the "therapeutic window" (orange), which ranges from the lowest effective dose to the highest dose a patient can tolerate. For some drugs, the risk of resistance emerging is minimal at the lower end of this window (Scenario 1), whereas for others it's at the higher end (Scenario 2).



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Resistance fighters

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