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New Ways to Squash Superbugs

Scientists are using new tools and tactics in the race to discover novel antibiotics

By Christopher T. Walsh and Michael A. Fischbach

"S"uperbug Strikes in City" sounds like a horror movie title, but instead it is a headline printed in the October 26, 2007, edition of the New York Post. Twelve days earlier a 12-year-old Brooklyn boy, Omar Rivera, died after a wound he received on the basketball court became infected with methicillin-resistant Staphylococcus aureus (MRSA), a bacterium that has become resistant to one of the most potent drug classes in the current antibiotic arsenal.

The prospect of healthy people contracting an untreatable bacterial infection may have seemed remote a decade ago, but it has now become a reality. In 2007 a research team led by Monina Kleven at the Centers for Disease Control and Prevention reported that MRSA causes 19,000 deaths every year in the U.S., which is more than HIV/AIDS causes. The number is especially alarming because almost 20 percent of people who contract MRSA die from it, and an increasing number of its victims are young, healthy people who were infected going about everyday activities. The problem was once limited to hospitals or nursing homes, where many people were already vulnerable because of impaired immunity. Even for those who survive, the price of MRSA is high: a patient who contracts it while hospitalized stays an average 10 days longer and costs an additional $30,000.

The total annual expenditure on treating MRSA infections in U.S. hospitals is an astounding $3 billion to $4 billion, and staph is just one of the pathogens that are becoming more and more difficult to subdue. Modern medicine is losing the war against disease-causing bacteria that were once considered vanquished, and new approaches to discovering and creating antibiotics are needed to turn the tide.

Recurring Resistance

The story of MRSA illustrates how quickly drug resistance can arise. Indeed, the natural mechanisms that cause resistance in staph and other bacteria make the problem almost inevitable, creating a constant need for new antibiotics.
Methicillin, a derivative of the better-known penicillin, was introduced in 1959 to treat infections caused by strains of bacterial species—such as *S. aureus* and *Streptococcus pneumoniae*—that had become resistant to penicillin. European hospitals, however, observed methicillin-resistant strains of *S. aureus* just two years later, and by the 1980s MRSA was becoming widespread in health care facilities throughout the world. By the mid-1990s a new class of MRSA infections had emerged: those that were contracted in the “community,” rather than a health care setting.

MRSA is challenging to treat, in part because it can spread quickly if it gains access to the bloodstream. But the most pernicious quality of MRSA is its ability to resist a major class of antibiotics known as beta-lactams (which includes cephalosporins and all variations of penicillin) by producing an enzyme that cleaves and destroys the drugs. Resistance to beta-lactams limits the physician’s arsenal of anti-MRSA weapons to a small set of antibiotics, each of which has serious side effects. And some strains of MRSA have already become resistant to the most effective of these, namely, vancomycin.

The advent of vancomycin resistance among bacteria already resistant to methicillin exemplifies a daunting problem for doctors and drug developers alike: from the moment an antibiotic is introduced in the clinic, its useful lifetime begins to tick down. The culprit is natural selection: the mere presence of an antibiotic creates an environment in which a bacterial strain that happens to be resistant will suddenly have a growth advantage over its competitors. Vancomycin was approved by the U.S. Food and Drug Administration in 1958, and once MRSA arose, it became the mainstay of therapy for MRSA infections. But in 2002 strains of MRSA that were also resistant to vancomycin began to emerge in hospitals. These strains, known as vancomycin-resistant *S. aureus* (VRSA), were progeny of MRSA that had acquired a set of five genes that travel together as a “cassette” and confer vancomycin resistance. The enzymes encoded by these genes allow VRSA to replace vancomycin’s target on the bacterial cell wall with a variant structure that vancomycin is no longer able to bind. As a result, vancomycin—a drug once known as the “antibiotic of last resort”—no longer inhibits the growth of VRSA.

Replacing an antibiotic’s target is just one of three major strategies bacteria use to evade death. As a second strategy, many antibiotic-
Typical antibiotics in doctors' arsenals today aim to kill bacteria by interfering with some aspect of their essential life functions. In turn, bacteria have several ways of destroying or evading the drugs.

BREAKING THE CYCLE
Current antibiotics attack cellular activities that a bacterium must carry out to survive, such as expanding its outer wall to grow, making proteins and unwinding its DNA for copying. Several drug mechanisms, along with the antibiotic classes that employ them (and examples of drugs in those classes), are highlighted below.

DNA and copied using the bacterial host's own replication machinery. To aid their spread, plasmids also bear genes that promote the survival of their host, including antibiotic-resistance genes. One plasmid isolated from bacteria in a sewage treatment plant was found to encode nine different antibiotic-resistance genes.

The process of horizontal gene transfer was also witnessed in action when MRSA, VRSA and a third bacterium, Enterococcus faecalis, were isolated from the same dialysis patient in a Michigan hospital in 2002. Genetic analysis of these strains showed that a plasmid containing the vancomycin-resistance gene cassette (along with resistance genes for three other antibiotics and one class of disinfectants) had been transferred from E. faecalis to MRSA, creating a novel strain of VRSA.

That one chronically ill patient became co-infected by two different bacterial pathogens that gave rise to a third is, sadly, not surprising. Because hospital intensive care units and nursing homes are often populated with immunocompromised patients undergoing intense antibiotic treatment, they are the best-known breeding grounds for new antibiotic-resistant bacteria. Nurses and doctors can unwittingly facilitate bacterial transfer by shutting from patient to patient to change intravenous lines and

resistance genes, such as the one that makes MRSA resistant to beta-lactams, encode an enzyme that destroys or chemically modifies the antibiotic, rendering it ineffective. Still other resistance genes carry instructions for a pump that gets mounted in the cell membrane and excretes antibiotic molecules that enter the bacterial cell, keeping its internal concentration of the antibiotic low enough to avoid death.

Where do these resistance genes originate? Some arise through random mutations in the bacterial cell's own genes, such as the variant gene that replaces the enzyme target of ciprofloxacin and other fluoroquinolone antibiotics with a resistant form of the same enzyme. Other resistance genes are picked up from nearby bacteria; for instance, the five-gene cassette of Enterococcus faecalis that confers vancomycin resistance originally came from a bacterium that produces the antibiotic. It needed the genes to protect itself from its own chemical weapon, but other bacteria probably acquired the same defense through the ongoing genetic swap meet that bacteria engage in, known as horizontal gene transfer.

Such transfers are often carried out by circular pieces of DNA called plasmids, which behave like stripped-down viruses: they transfer themselves from one bacterium to a new host cell, where they are recognized as a native piece of

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catheters, which is why programs that encourage hospital staff to sanitize their hands between each patient encounter inevitably lead to a reduction in the number of infections.

VRSA, which has not yet spread widely, is sensitive to very few antibiotics in clinical use and has a high death rate. Another class of emerging pathogens, the pan-drug-resistant gram-negative bacteria, has an even scarier profile of resistance. Gram-negative bacteria have, in effect, two cell membranes, and the additional outer membrane prevents many antibiotics from getting inside gram-negative cells. Gram-negative pathogens that are resistant to almost all clinically used antibiotics include strains of the food-poisoning pathogen Escherichia coli, its relative Klebsiella pneumoniae, and two opportunistic bugs—Pseudomonas aeruginosa and Acinetobacter baumannii—that can cause pneumonia, meningitis and bacteremia in immunocompromised hospital patients.

Clearly, health care providers must do everything possible to help prevent the spread of resistant bacteria—and, therefore, resistance genes—in clinical settings and in communities. But new antibiotics are also needed to complement those efforts and to combat already resistant bugs.

The period from the late 1930s to the early 1960s was a Golden Age for antibiotic discovery in which almost all the major classes of currently used antibiotics were introduced. Unfortunately, the four decades between the launch of the quinolones in 1962 and the approval of the oxazolidinones in 2000 represent an innovation gap in which no new antibiotic classes made it to the clinic. One reason is a slack of economic disincentives for pharmaceutical companies to invest in antibiotic research—in part because it is so challenging, with small profit margins when compared with so-called lifestyle drugs that must be taken long-term for a condition such as high blood pressure or arthritis. Another reason is that the current antibiotics were discovered by techniques that are now dated, and finding new ones will require novel discovery strategies.

Seek and Synthesize
Most antibiotics sold today are produced by bacteria and fungi or are chemically modified derivatives of these natural antibiotics. Microbes wield their antibiotics against one another as “chemical warfare” and perhaps also secrete them in lower concentrations as signaling molecules. Investigators have traditionally searched for such natural antibiotics by isolating microbes, often from soil samples, growing them in the laboratory, then extracting their secretions from the culture medium. By testing those chemicals
against disease-causing bacteria, drugmakers look for individual molecules that might have therapeutic potential. Pharmaceutical companies have tested millions of bacterial extracts in this way, yet only about 10 classes of natural antibiotics are on the market. Others have been discovered, but for various reasons—including weak antibacterial activity and unmanageable toxicity—none are widely prescribed.

These approaches worked well during the Golden Age of antibiotic discovery, but all the low-hanging fruit has now been picked. Despite the continued efforts of pharmaceutical companies over the past five decades, the rate of antibiotic discovery actually declined. One frustrating reason is rediscovery: because most antibiotic-producing microbes form spores that travel the globe—and the genes responsible for antibiotic production can be transferred horizontally, just like antibiotic-resistance genes—many different microbes make the same antibiotics. According to a recent estimate, for example, about one in 2,500 strains of the most commonly mined order of antibiotic-producing bacteria, the actinomycetes, makes tetracycline. Although the high rate of rediscovery has caused some research groups to conclude that the antibiotic “mother lode” has been mined out, genetic analyses of bacteria have recently cast doubt on this conclusion and instead suggest that new tactics are necessary.

Technological advances often lead to the rebirth of an old discipline, however, and antibiotic discovery seems to be on the verge of such a renaissance. Current strategies for developing new antibiotics fall into two categories: modifying existing ones and discovering entirely novel ones. Chemically modifying microbiologically produced antibiotics yields “semisynthetic” antibiotics in which the warhead is intact and the periphery has been altered. A recent example of this approach started with antibiotics in the tetracycline class, which inhibit the bacterial cell’s protein-making factory, the ribosome. Resistance to the tetracyclines is often caused by a pump in the bacterial cell membrane that ejects them before they can do their work—which has become a serious problem among pan-drug-resistant gram-negative bacteria.

Scientists at Wyeth synthesized a chemically modified tetracycline, called tigecycline, that can no longer be pumped out of target cells. Approved by the FDA in 2005, tigecycline is now used against a variety of tetracycline-resistant pathogens, although its use is restricted to health care settings because it requires intravenous administration. Ominously, resistance to tigecycl-
cline has already been observed among strains of A. baumannii; time will tell how quickly tigecycline resistance spreads.

Instead of chemically tweaking microbiologically produced antibiotics such as penicillin, vancomycin and erythromycin, scientists can also modify drugs by genetically altering the organisms that produce them. Most organisms synthesize natural antibiotics using enormous assembly lines of enzyme teams, or “modules,” each of which inserts a building block into the growing antibiotic molecule. By making genetic changes that alter particular enzyme modules, investigators can induce the organisms that serve as antibiotic factories to produce drugs that differ by a single building block at a specified position. Kosan, a biotechnology company recently purchased by Bristol-Myers Squibb, applied this form of programmed genetic engineering to generate dozens of derivatives of the antibiotic erythromycin that would otherwise have been difficult to make using standard synthetic chemistry.

Even though modifying existing antibiotics has been a fruitful strategy, discovering brand new antibiotic classes would be even more desirable because they are less likely to suffer from the rapid rise of resistance that plagues successive generations of existing antibiotics.

**Mining Genomes**

Research efforts in recent years have focused on identifying enzymes that bacteria require for survival, in the hope that molecules that inhibit these essential enzymes could be found in chemical libraries and turned into drugs. Before embarking on that search, however, the first step is to establish what effect losing the enzyme would have on the bacterium. Once researchers have deciphered a bacterium’s genome—its full sequence of DNA code—they can then disable genes encoding certain enzymes to see whether the bacterium can survive without them.

Although efforts to identify new enzyme targets this way have disappointingly not yet yielded new antibiotics, they may bear fruit in the coming years. One major challenge is the formidable barrier to entry presented by the bacterial cell wall: even when a small molecule that potentially inhibits an important bacterial enzyme is discovered, it is useless if it cannot reach its target inside the cell. Instead of seeking weak spots in pathogens, another way of discovering new antibiotics is to study antibiotic-producing microbes. Genomics can be useful here, too.

The first genome sequences of antibiotic-producing bacteria, in 2002, raised an intriguing mystery: these microbes, in the actinomycete class, had 23 to 39 gene sets that, according to their sequences, looked as if they were blueprints for enzyme modules that produced antibiotic-like molecules, but the bacteria did not appear to use most of those genes. When cultured in the laboratory, they synthesized just one or two of the molecules.

To see if such apparently dormant genes encode machinery for making novel antibiotics, we, along with several of our co-workers at Harvard Medical School and the Broad Institute, are sequencing the genomes of 20 additional actinomycete strains and applying sophisticated computer algorithms to pick out any genes that might contain instructions for antibiotic-producing
Scientists can modify drugs by genetically engineering the organisms that produce them.

**NOVEL WAYS TO BEAT BUGS**

Beyond improving existing antibiotics and seeking new molecules with antibiotic-like effects, researchers are also pursuing novel approaches to killing or disabling pathogenic bacteria. Many of these have the added advantage of avoiding mechanisms that usually lead to resistance.

**POKING HOLES**

Rather than attacking bacterial enzymes or life processes, pore-forming tubes kill by simply puncturing a bacterial cell's membrane. Small, naturally occurring proteins called defensins perform a similar function in vertebrates to defend against microbes. Several research groups are developing synthetic protein fragments called peptides that would self-assemble into tubes within bacterial membranes.

**NARROW TARGETING**

Bacteriophages (green) are viruses that infect a bacterium (blue), typically preferring only one host. Phages have long been studied for possible use against pathogenic bacteria, but they also exemplify the principle underlying new "narrow spectrum" drugs, which target only a single pathogen, leaving human cells and friendly bacteria unharmed.

**SUBDUED WITHOUT KILLING**

Sparing the pathogen itself but taking away its ability to cause illness is one way to treat disease without promoting antibiotic resistance. An example of this approach is genetically engineered E. coli (red) designed to mimic cells in the human gut. When the harmless E. coli are consumed, they soak up deadly Shiga toxin (blue) produced by another microbe.

enzyme modules. Studying the genome sequences around these modules should also help reveal the regulatory mechanisms that cells use to determine when an antibiotic gets made. With both sets of information, we can engineer the cells to switch on the desired genes so we can test the cryptic molecules for antibiotic activity.

Instead of trying to coax bacteria into making their antibiotics on demand, however, a research group at Saarland University in Germany has chosen to move antibiotic-producing genes from their recalcitrant producers to different bacteria that seem better suited for antibiotic manufacture. Rolf Müller and his colleagues work with myxobacteria, an order of bacteria that, like actinomycetes, are prolific producers of antibiotics. Because myxobacteria can be more difficult to culture in the laboratory than actinomycetes, far fewer efforts have been made to screen them for novel antibiotic production.

Müller circumvented this problem by splicing the genes involved in producing myxochromide, an antibiotic-like molecule, from the myxobacterium *Stigmatella aurantiaca* into *Pseudomonas putida*, a bacterial strain that is easier to grow. Indeed, *P. putida* is commonly used for commercial production of industrially useful enzymes. In meeting two key challenges—finding a genetically manipulable bacterial host that has the metabolic infrastructure required for antibiotic production and developing techniques to move large DNA fragments from one microbe to another—Müller's work opens the door to discovering and producing a treasure trove of new antibiotics from myxobacteria and suggests that a large-scale myxobacterial genome-sequencing effort would be well worth undertaking.

In addition to exploring underexploited soil microbes, researchers can turn their attention to as yet unexplored ecological settings that might be fruitful because organisms in exotic settings are more likely to make antibiotics that have not been discovered already. Roderich Süssmuth and his colleagues at the University of Tübingen in Germany made just such a discovery: a new antibiotic called abyssomicin from a actinomycete isolated from a sediment sample taken at a depth of 289 meters in the Sea of Japan. Another group studying marine bacteria—Bradley Moore, William Fenical and their colleagues at the Scripps Institution of Oceanography in La Jolla, Calif.—sequenced the genomes of two formerly unknown marine actinomycete strains. These genomes displayed a diverse array of genes for antibiotics and related molecules, providing further evidence for the potential of marine bacteria to yield new classes of antibiotics.

A different approach to mining new ecological settings for useful drugs is to study microbes that take part in mutualisms—interspecies interactions in which both parties benefit. For instance, the southern pine beetle is known to carry around a mutualistic fungus that digests the insides of pine trees the beetle invades. How the beetle protects its fungal mutualist from a second, antagonistic strain of fungus that competes with the first strain for food was a mystery, however, until Cameron Currie, Jon Clardy and their research groups at the University of Wis-
conscin-Madison and at Harvard Medical School recently showed that the beetle totes a second mutualistic microbe—an actinomycete—that produces a powerful and previously unknown antifungal agent. This molecule, called mycangimycin, kills the antagonistic fungus but not the mutualistic one.

Jörn Piel of the University of Bonn in Germany has shown that a different kind of beetle and a marine sponge both harbor bacterial symbionts that produce related antibiotic-like molecules. Also in Germany, Christian Hertweck of the Hans-Knöll Institute in Jena has discovered a fungus that carries its own bacterial symbiont that produces an antibiotic-like drug called rhizoxin. Indeed, podophyllotoxin and camptothecin, two widely used antineoplastic drugs long thought to be made by plants, are actually produced by fungi living inside the plants. Although symbiotic microbes have only begun to be explored, they are among the most promising sources of naturally occurring antibiotics, perhaps including compounds that define new antibiotic classes or have novel mechanisms of action. In addition, explorations of the role of symbiotic microbes within our own bodies are yielding new approaches to antibiotic treatment.

Preserving Allies
Humans, like insects and sponges, harbor a rich variety of bacterial symbionts that perform an array of useful functions, from helping us digest food to promoting the proper development of our immune systems. Unfortunately, all the antibiotics sold today are blunt instruments; they not only kill the pathogens that cause infections, they also kill the helpful bacterial mutualists that inhabit our gut. In some cases, this eradication of a patient’s gut microflora clears the way for a different harmful strain of bacteria, such as Clostridium difficile, to multiply and cause a new, “secondary” infection that can sometimes be more dangerous than the first.

Using friendly microbes or substances that foster the mutualists’ growth, so that they can outcompete pathogens, is one approach to preventing bacterial infections. Although such “probiotic” treatments can be helpful in avoiding the kind of widespread antibiotic use that promotes resistance, probiotics have never been demonstrated to be effective at treating an existing infection.

Nevertheless, growing recognition that our natural gut microflora can help stave off infection has led to a new strategy for antibacterial discovery: developing narrow-spectrum drugs designed to kill the pathogen causing the infection, while leaving the rest of our bacterial mutualists untouched. Neil Stokes and his colleagues at Prolysis, a company based in Oxford, England, for example, recently developed a new potential antibiotic that kills S. aureus and its relatives by preventing them from undergoing cell division, while leaving other bacteria intact. Victor Nizet and Andrew Wang, both at the University of California, San Diego, with Eric Oldfield of the University of Illinois, led a team that took this concept one step further. They discovered a drug that blocks synthesis of a pigment molecule that contributes to S. aureus virulence, thereby inhibiting S. aureus’s ability to make someone ill without actually killing the bacterium.

Experimental approaches to inhibiting bacterial virulence have the added benefit of possibly avoiding mechanisms that generate resistance. If a therapy does not kill a pathogen, then natural selection cannot favor the “survivors,” and resistant strains are less likely to evolve. Similarly, the narrow-spectrum approach relies on finding a target that is unique or essential to the pathogenic bacterium but does not occur in others. Thus, even if the target microbe eventually develops resistance to the drug, it is at least a form of resistance that is unlikely to spread and be useful to other pathogens.

Whether such therapies, on their own or as part of a combination of treatments, will prove practical in the real world remains to be seen. For one, these drugs would require rapid diagnostic tests that could pinpoint the pathogen responsible for a patient’s infection; such tests have been developed, but they are not yet in widespread use. Narrow-spectrum antibiotics, with their limited applications, might also be economically unattractive to drug companies.

The idea of one-size-fits-all antibiotics is no longer viable, however. During the 1960s and 1970s infectious disease was widely believed to be on the verge of being conquered. More recently, reports in the popular press have proclaimed that multidrug-resistant bacteria have brought about the “end of antibiotics.” We now know that neither is true: humans may never definitively win this race against time, but for the past century new therapies have kept us a step ahead of the pathogens. Every effort must be made to retain our lead.