Antibiotics That Resist Resistance

A year ago drug-resistant bacteria were on the upsurge, with few new antibiotics in sight. Now companies are reporting a whole new crop. What went right—and will it continue?

In the war against bacterial disease, microbes recently have been on the offensive. Once on the verge of defeat, thanks to medicine's arsenal of roughly 160 antibiotic weapons, bacteria began a counterattack many years ago. Organisms that cause tuberculosis, pneumonia, and many other diseases evolved potent mechanisms of drug resistance. Scientists didn't notice the strength of this insurgency until relatively recently. And from the weapons contractors in this battle—the drug companies that design new antibiotics—there has been an unnerving silence.

The quiet has now been broken, and the early bulletins are encouraging. Researchers have recently reported work on nearly a dozen new antibiotics that show promise in controlling drug-resistant organisms. At a meeting of the Interscience Conference on Anti-Microbial Agents and Chemotherapy (ICAAC) in San Francisco in September,* for example, scientists from Upjohn unveiled 25 studies on a new class of antibiotics, compounds that may interfere with microbes' ability to synthesize proteins in a novel way. Other companies and academic researchers are retooling existing antibiotics by slightly altering their chemical structures so bacterial defenses can't recognize them, or by finding ways to sabotage and weaken those defenses.

NEW DRUGS FOR NEW BUGS

Compound	Company	Target
Oxazolidinones	Upjohn	Protein synthesis
Glycylcyclines	Wyeth-Ayerst	Protein synthesis
Streptogramins	Rhône-Poulenc Rorer	Protein synthesis
Boxazomycin	Parke-Davis	Protein synthesis
Ketolideas	Roussel-Uclaf	Protein synthesis
Inter.-based inhibitors	Cubist Pharm.	Protein synthesis
LY 333328	Eli Lilly	Cell wall formation
New *beta*-lactams	Bristol-Myers Squibb	Cell wall formation
2-Pyridone	Abbott Laboratories	DNA replication
New fluoroquinolones	Pfizer	DNA replication

In the works. Some new antibiotic compounds currently under development at pharmaceutical companies.

Most are still in the early stages of development, although one new compound, developed by the French pharmaceutical giant Rhône-Poulenc Rorer, is in late-stage clinical trials in the United States and Europe. But the reports from the lab benches are sparking new optimism among infectious-disease researchers. "At least now there's hope" that new antibiotics will be developed, says Prabhvathi Fernandes, who heads the drug screening research program at Bristol-Myers Squibb in Princeton, New Jersey. "Things have come quite a ways from where they were 2 to 3 years ago when there was very little" in the pipeline. Companies had walked away from antibiotic research in the 1980s, seeing little market for new drugs when the old ones were working so well. Says Alexander Tomasz, who heads the laboratory of microbiology at Rockefeller University in New York City: "The pharmaceutical industry is waking up."

It will, however, be a long time before the newly awakened industry brings these new compounds to the clinic. All of them must survive the battery of tests in humans to determine whether they are safe and effective, so "you're talking about drugs that are not going to make it out until the next century," if at all, cautions Stuart Levy, who heads the Center for Adaptation Genetics and Drug Resistance at the Tufts University School of Medicine in Boston, Massachusetts. Any successes, moreover, will themselves face resistance in time. To limit this problem, many are calling for companies to target future antibiotics at a narrower population of microbes—a move that could cut companies' profit margins and might reduce their incentives to develop new drugs.

Crumbling defenses

What all agree on is that in some quarters, the current problem is near crisis proportions. In recent years, strains of multidrug-resistant tuberculosis, for example, have spread around the world, killing thousands. In the United States alone, according to a report released last month by the now-defunct congressional Office of Technology Assessment (OTA), 19,000 hospital patients die each year due to hospital-acquired bacterial infections. Until recently these infections—typically caused by microbes such as *Staphylococcus epidermidis* and *Enterococcus faecium*—were treatable with antibiotics. That's no longer the case. Antibiotics, in fact, have been the victims of their own success: Heavily used, the drugs became an evolutionary force, selecting for and enhancing the survival of bacterial strains that could resist them.

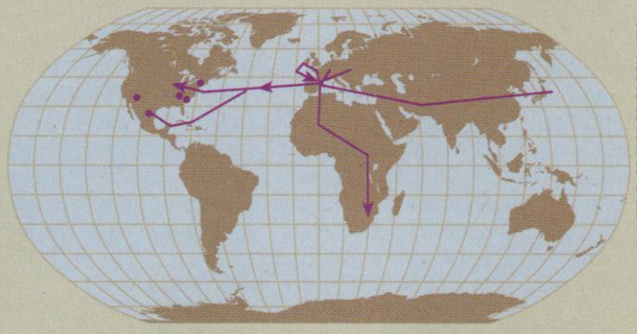
International health officials worry that multidrug resistance will spread from hospital-acquired pathogens to virulent organisms that cause disease in healthy people. Of particular worry is *Staphylococcus aureus*, which causes a variety of ailments from abscesses to surgical wound infections, says Barry Eisenstein, vice president of Eli Lilly Research Labs, in Indianapolis, Indiana. Currently, one common strain of the bug—known as methicillin-resistant *S. aureus*, or MRSA—is resistant to all current antibiotics except vancomycin, a drug traditionally used only as a last resort because it can cause serious side effects. In 1989, a strain of *E. faecium* acquired vancomycin resistance. Since then, vancomycin-resistant enterococci (VRE) have spread around the world. "It's just a matter of time" before this resistance spreads to other organisms such as *S. aureus*, says Eisenstein. If and when that occurs, adds Upjohn microbiologist Charles Ford, "that would be a major public health problem."

With the threat of resistant microbes growing, "people have been wondering where we've been," says Ford. The answer is that most drug companies shifted their research dollars to potentially more lucrative areas, such as drugs to treat chronic conditions such as heart disease, says George Miller, an infectious-disease expert at the Shering-Plough Research Institute in Kenilworth, New Jersey. "There was the perception that [bacterial disease] wasn't a problem any more," says Michael Cynamon, chief of the infectious diseases branch of the Veterans Administration Medical Center in Syracuse, New York. For profit-minded pharmaceutical giants, that meant a tiny market for

* 35th Interscience Conference on Anti-Microbial Agents and Chemotherapy, San Francisco, California, 17–20 September, 1995.

Tracking the Fingerprints of Drug Resistance

Antibiotic resistance doesn't just make pathogens difficult to treat. It also makes them harder to track. Traditionally, epidemiologists following the paths of disease-causing microbes have identified their suspects by features of bacterial polysaccharide coats, susceptibility to different antibiotics, or other schemes. But these tracking techniques "are losing their relevance," says Alexander Tomasz, a microbiologist at Rockefeller University in New York City. With the increase in drug resistance, a variety of resistant microbes can now wear the same coat or be resistant to the same drugs, making it harder and harder to keep tabs on individual strains.



AM. REVS. OF MICROBIOLOGY, 1996

Worldwide web. Researchers used DNA fingerprinting techniques to trace this drug-resistant pneumococcus strain from its starting point in Spain to various cities across the globe.

Epidemiologists, therefore, are increasingly turning to more precise molecular typing techniques, such as DNA fingerprinting, to distinguish resistant strains. Earlier this year, for example, the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta began using molecular methods in a nine-city effort to track drug-resistant *Streptococcus pneumoniae*, a major cause of pneumonia, meningitis, and middle-ear infections. The CDC set up a similar program in six U.S. cities in 1992 and 1993 to track multidrug-resistant tuberculosis. And in May, a privately run alliance of hospitals in 11 countries throughout Southern and Eastern Europe and South America, called the Centro de Epidemiologia Molecular Network for Epidemiologic Tracking, or CEM/NET, began molecular tracking of a variety of resistant pathogens including vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* (MRSA), and penicillin-resistant pneumococcus. A similar initiative, known as the Bacterial Antibiotic Resistance Group, was set up last year to track the same infections as well as tuberculosis in New York City metropolitan area hospitals.

DNA typing tools are, of course, not new. Indeed, some DNA-based methods, such as comparing plasmids (small rings of DNA outside the chromosomes), have been used by epidemiologists to track infections since the 1970s. But since plasmid DNA is transferred easily and often between different strains, that technique too has its limitations.

More recent techniques use restriction enzymes to cut apart entire bacterial chromosomes into strain-specific fragment patterns. Another method uses specific radiolabeled DNA probes, in a technique known as Southern hybridization, to test for the presence of a particular drug-resistance gene in a bacterial strain. "Such tools give epidemiologists unprecedented resolving power for identifying reservoirs and transition routes of genes and pathogens," says Tomasz. That's helped researchers track a number of drug-resistant clones as they travel vast distances. In one recent example, reported in the September issue of the *Journal of Clinical Microbiology*, researchers at Rockefeller and the Universidade Federal do Rio de Janeiro used DNA typing to show that a single MRSA clone caused hospital-acquired infections in several Brazilian cities as far as 5000 kilometers apart.

Such tracking methods also "help us learn about the mechanism of resistance," says CDC epidemiologist Robert Breiman. Resistance grows, he explains, either as one resistant organism spreads from one location to the next—as in the Brazilian MRSA—or as different strains and even species of microbes share the genes responsible for drug resistance, as a series of studies of vancomycin resistance recently demonstrated.

That knowledge also helps public health officials combat the spread. If resistance spreads "horizontally" as a microbe increases its range, Breiman says it's important to focus prevention efforts on minimizing person-to-person spread in hospitals and day-care centers. If, however, resistance genes are jumping between organisms, that suggests that overly aggressive antibiotic treatment is encouraging nonresistant bugs to acquire new genes (see main text). In such cases, "the focus needs to be on controlling antimicrobial use," says Breiman. The hoped-for result: fewer infections to track.

–R.F.S.

new anti-bacterial drugs.

But now that the market for new antibiotics is picking up again, "most [large pharmaceutical] companies are back into it," says Fernandes. They re-entered quietly, however, saying little about their new research efforts until they had developed promising compounds and patented their work.

Restocking the arsenal

Among the most auspicious of the new contenders, say researchers, are Upjohn's new compounds, known as oxazolidinones. Their promise is tied not only to the drugs' ability to stop the growth of drug-resistant organisms, but also to their chemical distinctions from current antibiotics. "I think [the compounds] are very exciting in the sense that they are a new [chemical] structure," says Levy. The hope is that because bacteria haven't yet come across this type of antibiotic, drug resistance won't come ready-made.

The chemicals were discovered in the mid-1980s at E. I. du Pont de Nemours & Co., but abandoned in 1989 when the company decided to get out of the anti-infectives research business. However, early results on oxazolidinones' anti-microbial activity caught the eye of a chemist at Upjohn, Steven Brickner. But after some preliminary studies, the Upjohn researchers noticed that a lead DuPont compound was toxic to animals, causing severe weight loss and death in rats.

Brickner and his colleagues set out to see if they could synthesize variants of the drugs that retained their antibiotic activity without the side effects. And at the San Francisco meeting the researchers described two that seem to fit the bill. In test-tube and animal studies, both of these compounds, known as U-100766 and U-100592, have proven very effective against MRSA and VRE; indeed, the drugs work on all gram-positive bacteria—related organisms that share a common cell-wall architecture. Company researchers also presented initial toxicology studies of the drug in humans, which showed no adverse effects at the delivered doses. Levy calls these results "promising."

Just how the compounds work remains something of a mystery, however. "We know the drugs inhibit protein synthesis at a very early stage," says Brickner. They share that

characteristic with a host of other antibiotics, such as macrolides and tetracycline, which gum up the protein production machinery by binding to ribosomes, the cell's protein-assembly factories, and halting protein synthesis in midstream. But, by tracking radiolabeled protein components in bacterial cells given oxazolidinones, DuPont and Upjohn researchers found the drugs inhibit protein synthesis even earlier; just where has remained elusive. "There are many players in that machinery. It's very tough to find out exactly what's going on."

One possibility raised originally by the DuPont researchers is that the drugs inhibit the complex interaction between messenger RNA, transfer RNA, and a piece of the ribosome known as the 30S ribosomal subunit, three key components involved in initiating protein synthesis. "But this is highly conjectured," says Brickner.

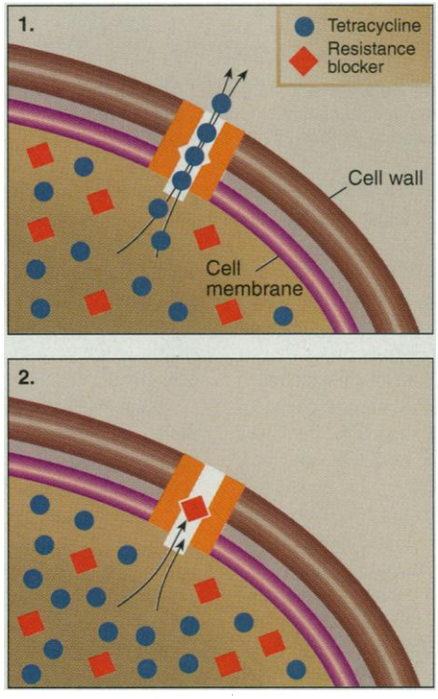
Revitalizing old weapons

While Upjohn chemists attempt to figure out how their new compounds work, other researchers are tinkering with the innards of old ones, including compounds that have been losing ground to the rising tide of drug resistance. One effort that has excited many researchers, for instance, is work at Eli Lilly on a new version of a vancomycin that kills VRE and is 100 times more potent than vancomycin itself against vancomycin-sensitive strains.

Originally derived from a micro-organism found in soil samples from the jungles of Borneo, vancomycin interferes with a bacterium's ability to synthesize peptidoglycan, a chainlike molecule essential for construction of the cell wall. When the wall falls, so does the microbe. The drug binds to a pair of amino acids on the end of certain peptidoglycan building blocks, preventing them from being added to the growing chain. It proved to be a powerful weapon—until 1987, when strains of *Enterococcus* began turning up with new components at the end of these building blocks. The new structure blunted vancomycin's ability to bind to its target.

Lilly researchers soon began screening a variety of compounds to see if they could find something capable of piercing the bacterium's new defenses. A close relative of vancomycin showed some promise, and when the researchers tinkered with the molecule's complex of sugars, adding an additional ring-bearing side chain, they were able to significantly increase the compound's ability to find and bind to its target. At ICAAC, Lilly researchers reported that in test tube and animal studies their new compound—a glycopeptide known as LY333328—kills a variety of gram-positive bacteria including vancomycin-resistant *Enterococcus* and MRSA. "It looks really good against the most feared organisms," says Tomasz, who saw the Lilly presentations at the meeting.

Another class of drugs in the shop for a facelift are Rhône-Poulenc compounds, known as streptogramins. Originally derived from a fungus, streptogramins, which inhibit protein synthesis, have been on the market as an oral antibiotic in France since the early 1960s. More recently, company researchers have made chemical modifications to allow it to be used in an injectable form. The new version, known as RP 59500, is currently in stage 3 clinical trials in Europe and the United States and has shown strong activity against gram-positive bacteria including VRE, MRSA, and penicillin-resistant pneumococcus.



SOURCE: S. LEVY

Pump clog. (1) Some bacteria can resist tetracycline because of "efflux" pumps that shoot the antibiotics from the cell. (2) Adding "resistance-blockers" designed to bind tightly to the pumps allows tetracycline to build up to lethal levels.

Retooling an old compound isn't the only strategy for defeating resistant microbes, however; another is to use reinforcements to push a drug past bacterial defenses. That's the tactic being employed by Levy and his colleagues at Tufts to revitalize an old standby, the tetracyclines. "In the past tetracyclines were very effective broad-spectrum antibiotics," says Levy. "But now resistance is so common you almost can't use them anymore." By understanding how bacteria evade the compounds, "we can attack the resistance mechanism head-on," says Levy.

Resistant organisms' first line of defense is to try to keep tetracyclines away from their targets, the bacterial ribosomes. They accomplish this by turning on one or more of a dozen different "efflux" pumps, which carry the drug out of the cell as fast as it comes in. "We went after the efflux pumps," says Levy. In essence, they clogged them up.

Efflux pumps consist of cell membrane proteins with a "pocket" that binds tetracyclines with just the right degree of force. The pump grabs an invading molecule, pushes it out of the cell, and then dislodges it, freeing the pump to repeat the process. So Tufts University chemist Mark Nelson synthesized tetracycline analogs that bind more tightly to the pump pocket; the pump has a much harder time shaking them free, which allows a lethal concentration of tetracycline to build up in the cell.

At ICAAC, Levy showed that when given in conjunction with tetracycline, the new compounds—which the Tufts group calls tetracycline resistance blocking agents—were able to kill resistant *E. coli* and several species of resistant *Staphylococcus* and *Enterococcus*. What's more, the blocking agents plugged up four different efflux pumps, each from one of the four main pump classes. "So we feel pretty optimistic the compounds will stop the other pumps as well," says Levy. Brickner agrees. "It's a new concept that you can get in and block these," he says. "I think it will probably prove to be more broadly applicable" as a way to restore other pump-prone antibiotics such as quinolones or macrolides, he says.

In addition to feeling optimistic about their compounds, the Tufts researchers are also feeling lucky. Some tetracycline-resistant organisms don't use pumps, but instead form a protein shield that forms around the ribosomes. Much to the Tufts researchers surprise, blocking agents killed these organisms as well. "We don't yet know why these work," says Levy. One possibility he offers is that the efflux and ribosomal protection proteins may have developed a similar three-dimensional binding site to initially grab tetracyclines. And the new drugs may clog up this site on both proteins.

Keeping the pipeline open

There are at least six more compounds under development, from companies like Wyeth-Ayerst and Abbott Laboratories. But if Levy's timetable is correct, it could take 5 to 15 years for these drugs to wind their way through the clinical trials and FDA reviews needed to make it to market. And there's no guarantee any of them will make it that far.

Such dilemmas aren't a drug company's only concern. In an effort to combat spreading resistance among nontarget bacteria, public health officials are calling on drugmakers to develop narrower spectrum antibiotics that target just one organism instead of whole classes. For drug companies that means a smaller market—and less of an incentive to make the drug. A compound effective only against MRSA, for example, would currently have a market of only about $60 million a year, well below the $100 mil-

lion benchmark used by industry to decide whether to invest in research in a particular area, according to the OTA report.

Hoping to boost those incentives, the OTA report outlined several possible strategies to encourage drug companies to focus their research. Among the proposals: federal funding of cooperative research between universities and drug firms, and extending the life of patents to make antibiotic research more profitable by extending the profit-making lifetime of the drugs.

Just encouraging the development of narrowly targeted antibiotics, however, won't necessarily encourage their use. "If we're going to have narrowly targeted drugs, we're going to have to see a lot of advances in diagnostics so exactly the right drug can be used in the right situation," says Lilly microbiologist Thalia Nicas. Without it, adds Fernandes, "doctors must essentially treat blind." And in such cases they typically choose broad-spectrum antibiotics. Like combating resistant microbes, concludes Fernandes, "slowing the development of resistance is a very complicated thing."

–Robert F. Service

OCEANOGRAPHY

Sea-Floor Data Flow From Postwar Era

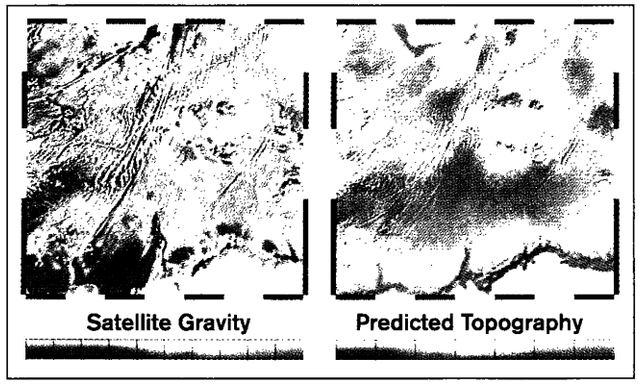
The Berlin Wall crumbled 6 years ago, and the Soviet Union dissolved in 1991. But it took the U.S. Navy until this summer to decide that the Cold War was history and scientists should have full access to a treasure-trove of satellite data on the world's oceans. The Navy's decision to declassify top-secret topographical maps of the sea floor, used by U.S. submarines to navigate the depths, represents the biggest victory to date for a government task force created in 1992 to look at the potential scientific utility of classified data—and more releases are on the way. The Navy also got a nudge from a civilian European satellite that has been spewing out similar, publicly available data for the past 4 years.

"This is a day of celebration, a data feast," says David Sandwell, a marine geophysicist at the Scripps Institution of Oceanography in California and one of the leaders in the lengthy campaign to declassify data collected by the Navy during the Cold War. But last week, as Sandwell and other scientists from the National Oceanic and Atmospheric Administration (NOAA) unveiled the detailed maps of the ocean floor at a press briefing, they reminded the audience that their real work was just beginning. "I'd really prefer to be back in my office feasting" than talking to the media, Sandwell quipped.

The feast comes after years of eating crumbs from a banquet of information that has been off-limits to oceanographers. From 1985 to 1990, for example, a classified Navy Geosat mission, using radar altimeters, beamed back detailed information on the presence of deep-ocean trenches and mountains by charting the bumps and depressions they make on the ocean surface. A 1500-meter underwater mountain, for example, produces a bump at the surface about 1.5 meters high. The method provides much greater coverage of the ocean floor than the oceanographer's standard tool, acoustic pulses sent out by ships. (The National Aeronautics and Space Administration had tried a similar approach in 1978 with its Seasat spacecraft, but it failed after only 3 months.)

The Navy kept a tight rein on the data because they revealed information on seafloor terrain and its effect on subsurface currents that helped submariners playing hide-and-seek with their Soviet counterparts. Although Sandwell and a group of researchers from NOAA did get a peek at some Geosat data in 1985, it wasn't until 1990 that the Navy agreed to release information from the Antarctic region—where few military submarines stray. The civilian researchers kept up their campaign, and in 1992 all data below 30 degrees south were declassified.

That same year, then-Senator Al Gore (D–TN) asked the Central Intelligence Agency to establish the Environmental Task Force to look at the potential scientific value of some classified data. This spring, the task force recommended that the Navy release all Geosat data, and on 19 July the Navy did so. Within a week, NOAA scientists had the tapes. Those data produced the maps presented last week.



Well-grounded. Geosat's gravity readings of southwest Indian Ocean ridge *(left)* will improve models of sea-floor topography.

And more data will be on the way. A report completed in June by a team of ocean scientists led by Otis Brown of the University of Miami in Coral Gables, Florida, urged the Navy to promptly declassify nine additional sets of data ranging from measures of ice thickness in polar waters to levels of salinity around the world. The data were gathered by a flotilla of Navy ships, aircraft, and satellites over the past 30 years, and the report concludes that "it is highly unlikely such an effort will ever be repeated."

The task force and the report weren't the only factors in the Navy's decision on Geosat, says NOAA marine geophysicist Walter Smith. The success of the European Space Agency's European Remote Sensing–1 (ERS-1) satellite, launched in 1991, provided data that closely matched the Geosat findings and made classification less relevant. The combined Geosat and ERS-1 data sets will "further the study of the ocean basins in the same way that the Hubble [Space] Telescope has promoted the study of the cosmos," says Marcia McNutt, an earth sciences professor at the Massachusetts Institute of Technology.

The ramifications of detailed maps of the ocean floor extend beyond oceanography. "The release of these new satellite gravity data will almost certainly change our thinking about the active geological processes in the world's deep ocean basins," says Jian Lin, an associate professor in the geology and geophysics department at Woods Hole Oceanographic Institution in Massachusetts. Tectonic plate theory will benefit from the increased level of detail of old fracture zones, says Sandwell, and more precise data will also improve sea circulation models used to predict global climate change.

For oil companies, data on sedimentation gathered by the Geosat and ERS-1 programs can provide clues to fossil fuel deposits. And more precise measurements of ocean depth could help the fishing industry by pinpointing shallow areas that harbor greater amounts of plant and animal life.

The fact that the Geosat data set is now flowing freely on the Internet (**http://www.ngdc.noaa.gov/mgg/announcements/**) offers another stark example of how much the world has changed since the United States and the Soviet Union were locked in underwater cat-and-mouse games. Although those military maneuvers may be a thing of the past, they are being replaced with competition of another kind. The new race, says Smith, is among scientists eager to digest the declassified data and publish their results.

–Andrew Lawler