PUBLIC HEALTH

New Test Catches Drug-Resistant TB in the Spotlight

When an antibiotic treatment for tuberculosis (TB) was found in the 1940s, the disease was transformed in the developed world from a lethal plague to a vanquished and vanishing illness. But now TB is on the rise again. Thriving among the homeless and AIDS patients, it reached a total of 28,000 cases nationally last year. And some strains of the bug have an ominous new weapon: drug resistance. In New York City, where the problem is worst, one-fourth of all new TB cases are resistant to one or more of the 11 drugs in the anti-TB arsenal. But a new screening test reported in this issue of *Science* (page 819) may soon allow doctors to spot resistant cases quickly, avoid treating them with drugs that don't work, and keep them from infecting others.

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>
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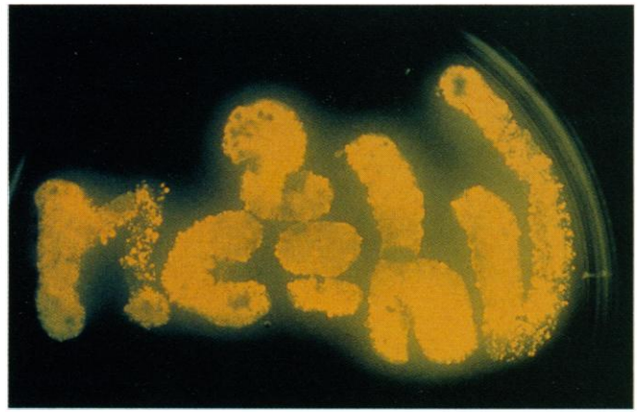
Because TB is a slow-growing organism, it typically takes 2 to 3 months to culture enough bacteria from a patient's sputum and test the patient for drug sensitivity. During that time doctors usually treat the patient with anti-TB drugs even though they don't know if the drugs are working. That can lead to a population boom among the resistant bacteria. "If you start with three drugs and the [TB] is already resistant to two of those, not only is the treatment not likely to succeed, but you are also likely to select out additional drug resistance," says Jack Crawford, chief of the mycobacteriology laboratory at the National Centers for Disease Control and Prevention.

Those on the front lines of the TB war are hoping the new assay, which takes only a few days to perform, will literally spotlight the resistant cases. The assay, developed in the laboratories of microbiologists William Jacobs and Barry Bloom of the Albert Einstein College of Medicine and Graham Hatfull of the University of Pittsburgh, uses a light-producing reaction catalyzed by the firefly enzyme luciferase to distinguish resistant and nonresistant TB bacilli rapidly. The team engineered bacterial viruses called phages to insert the luciferase gene into the genome of the TB bacillus, *Mycobacterium tuberculosis*. The bacteria then produce the luciferase enzyme coded by the gene. When supplied with a substance called luciferin, the luciferase in the bacteria produces light.

This light show also requires adenosine triphosphate (ATP) as an energy source, says Bloom, and "a sick or dead bug is not going to have as much ATP as a live bug." So a bacillus weakened by an anti-TB drug will not shine as brightly as a resistant organism.

The group tested both drug-sensitive and drug-resistant TB organisms that had been treated with the three main tuberculosis drugs: isoniazid, rifampin and streptomycin. After the drug treatment, the bugs were mixed with the engineered phage and then given luciferin. "If the sample was drug-sensitive," says Jacobs, "the lights were out. And if it was resistant, the lights would go on."

"This is a very important breakthrough," says Lee Reichman, president of the American Lung Association and director of the New Jersey Medical School National Tuberculosis Center. It is good news, he says, for the individual patient, and for the public health. "When it takes 13 weeks to make a decision as to whether someone has resistant organisms, you run the risk of having some people not respond [because they are being treated with the wrong drugs] and give resistant TB to others," Reichman says. "If [a patient] is treated appropriately, he very rapidly becomes noninfectious."



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A test with glowing results. *Mycobacteria* streaked across an agar plate glow with luciferase-generated light, equating Einstein's mass-energy conversion formula with the energy of a photon.

The new test, however, is not yet quite as simple as watching lights go on. An instrument called a luminometer is required to detect the light, and sputum samples would need to be cultured for several days at least to provide the 10,000 bacteria required for the test. But improvements are already in the works. Hatfull's group has engineered a new phage with DNA that makes more luciferase when it is inserted into *Mycobacterium bacilli*. Their early results suggest that fewer than 100 bacteria infected with the new phage may make enough of the enzyme to produce detectable light. That, says Jacobs, would make it feasible to take a sputum sample containing 1000 bacteria and, without any culturing at all, simply "split it to 10 tubes, throw in the antibiotics, and then a few hours later tell them what to treat the patient with."

It could take another year for Hatfull's group to perfect this feat, however. The present 2-to-3 day version is enough of an improvement over currently available tests that the team is planning to set up a first generation trial on actual clinical samples at the Montefiore Hospital in New York. "We should know a lot about its practicality in a year," says Bloom. Several companies are interested in commercializing the test, and if it proves practical, a version might then become available to U.S. hospitals.

Even before the test starts lighting up patients' bacteria, however, it will be useful in the search for new TB drugs. Its speed in highlighting the effectiveness of drugs has several drug companies interested. "The real value is the time saved," says Richard Baltz, a research adviser at Eli Lilly and Co. Many drugs break down over time, making it difficult to use them in long tests, he adds, but the luciferase assay "lets you test drugs and combinations of drugs that would be unstable for 4 to 6 weeks on a Petri dish." Both Lilly and Bristol-Myers Squibb are negotiating to use the test in TB drug discovery efforts.

Bloom hopes eventually to produce a more low-tech version for use in developing countries. TB now infects 8 million people worldwide, and kills 3 million each year. As TB drugs become more widely available, drug resistance needs to be better monitored, says Basil Vareldzis of the World Health Organization's TB program, and what is needed is a simple, inexpensive test. Bloom envisions a "highly sensitive assay in a little plastic box," in which film could be used to detect the glowing bacteria. But that, too, is probably more than a few years off. Nevertheless, the luciferase test looks like it might soon be brightening the lives of embattled TB workers right here at home.

–Marcia Barinaga