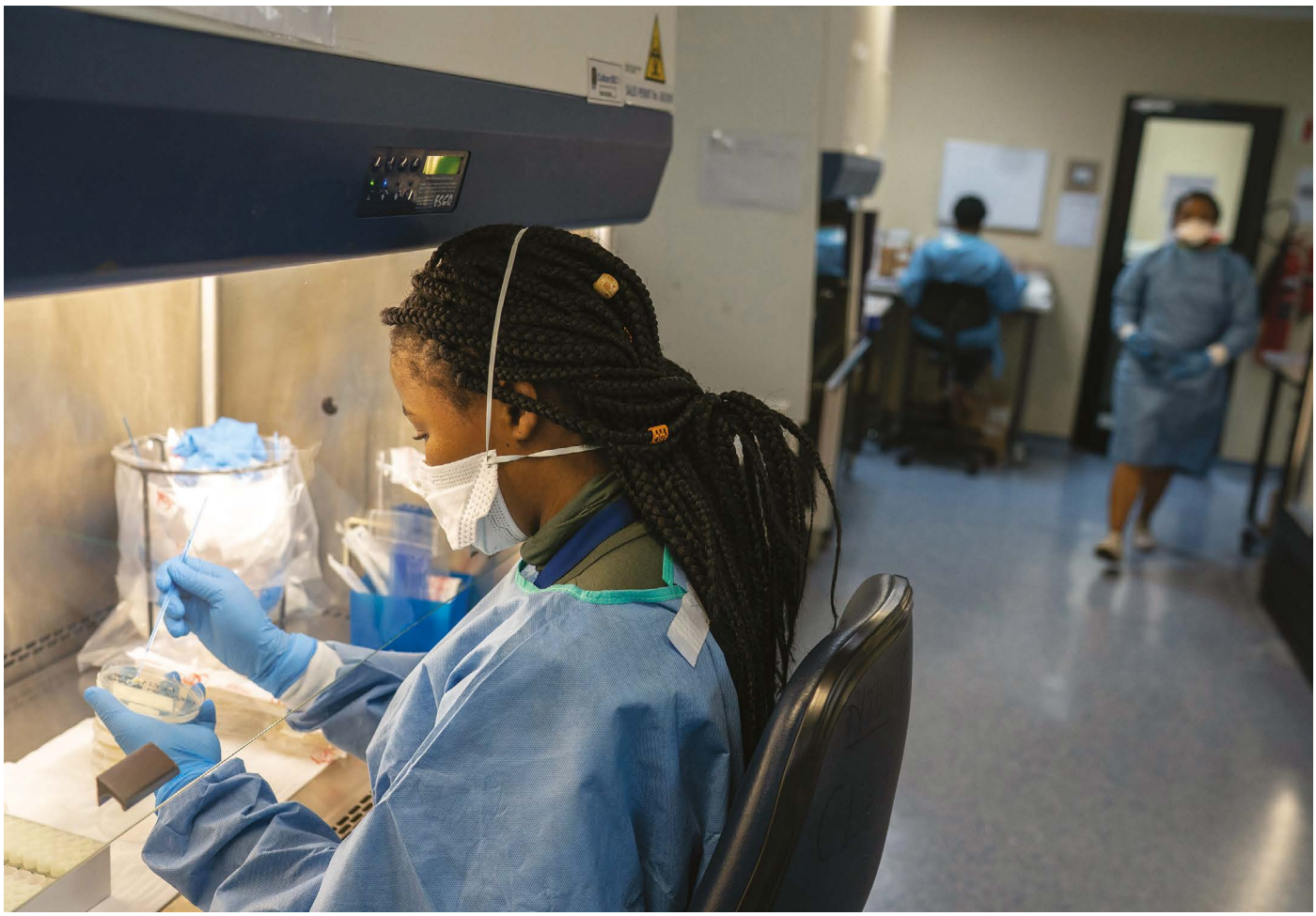


JOAO SILVA/NYT/REDUX/EYEVINE

Bacterial resistance to antibiotics is a major problem around the world. New solutions are hoping to change that.

TOOLS TO TACKLE ANTIBIOTIC RESISTANCE

Diagnostics that rely on bacterial movements, genomics and machine learning could help to address a global crisis. **By Jyoti Madhusoodanan**

Maha Farhat spent months in 2007 tending to patients at a hospital in Durban, South Africa. Many were infected with HIV. But the infection that preyed on the then-medical-resident's mind, and her patients', was caused not by a virus, but by a bacterium: *Mycobacterium tuberculosis*, the pathogen that causes tuberculosis. In particular, she was concerned about strains that are resistant to common antibiotics.

Although immunocompromised individuals are especially susceptible to tuberculosis, the infection isn't unique to people with HIV: *M. tuberculosis* claimed 1.4 million lives worldwide in 2019, 208,000 of whom had HIV. "Tuberculosis was briefly superseded by COVID-19, but it's still the top infectious-disease killer globally," says Farhat, now a physician and bioinformatician at Harvard University in Boston, Massachusetts. Drug-resistant forms of tuberculosis are a major contributor to the problem.

Drug-resistant pathogens of all types have precipitated an antibiotic-resistance crisis that threatens public health, agriculture, animal husbandry and more. But spotting these strains and identifying effective treatments is tricky. Labs equipped to handle especially infectious pathogens, such as *M. tuberculosis*, can be hard to come by in resource-limited countries, and instruments for testing for drug sensitivity can take days to return results. In many cases, physicians test for resistance only after one or more standard antibiotics fail. While waiting, patients might begin an unnecessary or ineffective course of antibiotics, or leave the clinic without treatment.

Farhat and other researchers are turning to tools such as atomic force microscopy, genomics and machine learning to create point-of-care diagnostic tests that they hope will provide results in minutes, minimizing the use of incorrect or unnecessary prescriptions. "An increase in rapidity is the most important advance needed," says clinical microbiologist Evgeny Idelevich at the University Medical Center Greifswald in Germany.

Gauging growth

The gold-standard method for assessing drug susceptibility of microbes, known as a disk diffusion test, dates to 1889. Researchers

culture bacteria on an agar plate, then place tiny paper disks loaded with drugs on the growing cells; zones around the disks become transparent if a drug kills bacteria or stalls their growth, indicating that the microbes are susceptible to the medication.

Companies have automated that same principle in antimicrobial sensitivity testing instruments, such as the BD Phoenix from BD Biosciences, headquartered in New Jersey, and the VITEK 2 from bioMérieux, based in Marcy-l'Étoile, France. These systems seed bacteria in liquid cultures with antibiotics and look for optical changes that indicate bacterial growth or death. The tests typically require somewhere between 4 and 8 hours, although results can take a day or more to arrive because clinicians must send samples to clinical microbiology labs[1].

But researchers are also exploiting assays that are more commonly associated with the physical sciences than with microbiology labs.

In 2018, for instance, Idelevich devised a miniaturized version of the liquid culture test that relies on MALDI–TOF, a mass-spectrometry technique that uses laser-induced ionization and a long 'flight tube', through which ions travel, to identify molecules on the basis of their mass and charge. Idelevich and his colleagues placed microdroplets of cultures of two pathogens — *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* — directly on a solid support matrix used for MALDI–TOF and incubated each droplet with a different drug. They then processed the sample with a system specifically designed for bacterial identification: the MALDI Biotyper from Bruker Daltonik. Intensities of characteristic spectral peaks indicated whether the cultures were susceptible or resistant to the antibiotic[2].

In 2013, Giovanni Longo, at the National Research Council of Italy in Rome, and his colleagues found that when they bound pathogenic *Escherichia coli* to miniature diving-board-like structures called cantilevers and exposed them to antibiotics, the cantilever bobbed up and down because of small movements of attached, living bacteria. The movements ceased if the microbes were susceptible to the antibiotics. The movement was visible under an atomic force microscope within minutes — long before microbes replicated — meaning the test can identify live bacteria far faster than is possible with an assay that looks for bacterial growth[3].

Rachel McKendry, a nanotechnology researcher at University College London, and her then-graduate-student Isabel Bennett wanted to take that approach into the clinic. But attaching the bacteria to cantilevers that were 200 micrometres long in a Petri dish was easier said than done. "Only a fraction of the cantilevers would have bacteria attached, and sometimes they'd be either clumpy or too few attached," Bennett says.

As she worked with Longo's team to fine-tune the process, Bennett detected large differences in reflected light, which suggested that similar bacterial movements could be spotted even when the microbes were not tethered to the cantilevers. So, the team switched tactics: they altered the set-up to track bacteria as they floated across the structures' surfaces. They made the cantilevers from a hard, reflective material, and developed software to analyse bacterial movement so that the readout was proportional to the number of bacteria in solution[4]. "This deceptively simple signal turned out to be really a nice way to detect resistance compared to current methods," McKendry says.

Although not yet commercialized, the system could be adapted and scaled up for clinical use, Bennett suggests. The reflective surfaces could be turned into inserts placed in routinely used microtitre plates, and the atomic force microscope replaced with a DVD player's optical reader to capture the signal. "It could potentially be a very easy, low-cost set-up," she says.

Physicist Kamil Ekinci, at Boston University in Massachusetts, is pursuing another proxy for bacterial antibiotic resistance: electrical current. His team placed a urine sample spiked with *K. pneumoniae*, a common cause of urinary-tract infections, directly into a single channel of a microfluidic device with an antibiotic, and tracked electrical conductance through the channel[5]. "If the bacteria grow and clog the channel, they create more electrical resistance," Ekinci says. "We're basically transducing the bacterial growth into an electrical signal."

"The more data we collect, the more we stand a chance of improving our ability to predict resistance."

The advantage, Ekinci adds, is that an electrical signal is easier to amplify and visualize than are microscopy images. "In principle, our technique can detect a single bacterial division," he says — although he adds that the method might not work for all bacteria, particularly slow-growing pathogens such as *M. tuberculosis*.

Measuring molecular markers

Tests based on bacterial growth are easy, cheap and non-specific: a single test works across a wide range of pathogens. But because test results depend on growth conditions and using the right concentration of antibiotics, "everything else is a disadvantage", says Susanne Häussler, who studies medical microbiology at Rigshospitalet in Copenhagen.

As an alternative, Häussler and others are turning to genomics for clues to drug resistance. This 'culture-independent testing' is the next big shift in the field, says epidemiologist Sophia Koo at Harvard University.

Relying on genes that are clearly linked to antibiotic-resistance mechanisms is an ideal route to a quicker diagnostic because it doesn't require lengthy periods of bacterial incubation, says infectious-diseases researcher Gary Schoolnik at Stanford University in California. But it's important to know which sequences in the bacterial genome are important for drug resistance, says Thomas Grys, a clinical microbiologist at Mayo Clinic in Phoenix, Arizona. "If you don't, you could easily miss a new mechanism or detect a fragment of a gene that's not actually conferring resistance."

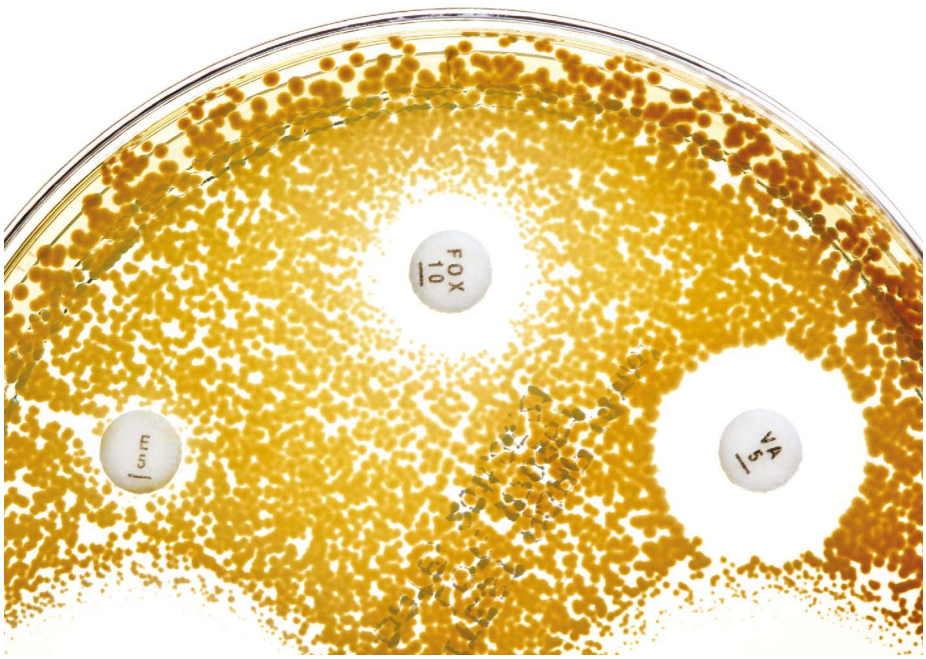
Schoolnik is also chief medical officer at Visby Medical, a California-based start-up that won US$19 million in 2020 as part of an Antimicrobial Resistance Diagnostic challenge sponsored by the US National Institutes of Health and the US Department of Health and Human Services' Biomedical Advanced Research and Development Authority.

The company's test is a single-use, point-of-care diagnostic, run using a simple handheld device, to spot drug resistance in sexually transmitted pathogens such as *Neisseria gonorrhoeae*. The assay focuses on mutations that confer resistance to ciprofloxacin, a commonly used oral antibiotic for gonococcal infections. Mutations in the gene encoding the enzyme gyrase A spell the difference between *Neisseria* strains that are resistant or susceptible to ciprofloxacin.

PCR-based tests to detect such variants are of limited use in clinics because of the need for instruments, reagents and technicians who are trained to perform reactions. Visby's diagnostic bypasses these constraints by reducing the assay to a simple colour change. Amplified fragments flow into a chamber on the device that contains capture probes for each variant of the gene. The binding results in a colour change that reflects whether a strain is sensitive or resistant to ciprofloxacin[6].

Others continue to explore whole-genome sequencing to capture the spectrum of variants that confer resistance. But developing low-cost, speedy tests based on such information remains a challenge. "It's not just about the presence of a resistance gene, but also its expression," says Nicole Wheeler, a data scientist at the University of Birmingham, UK, who studies machine-learning approaches to genomics. "The more transcriptomic and proteomic data we collect, the more we stand a chance of improving our ability to predict resistance," Wheeler says.

Techniques based solely on genome



DONCASTER AND BASSETLAW HOSPITALS/SPL

A disk diffusion test is a common way to uncover how effective an antibiotic is.

sequencing work well for some pathogens, such as *Salmonella enterica*, but mutations in multiple regulatory genes can alter gene-expression patterns (and thus resistance) in others, including *P. aeruginosa*. "In principle, all transcriptome data is in the genome," Häussler says. "But it's sometimes easier to look at the transcriptome instead of looking for all the possible mutations that alter gene expression."

In 2014, for instance, Chikara Furusawa, a bioengineer at RIKEN in Osaka, Japan, studied lab strains of *E. coli* adapting to growth in the presence of different antibiotics, and found that he could use changes in gene expression to predict resistance more accurately than he could with genomic DNA sequences themselves[7]. "The correlation between gene expression and resistance was significantly higher than that between resistance and genomic markers," Furusawa says.

Forecasting future resistance

In their work, Häussler and her colleagues honed in on a mix of genomic and transcriptomic markers as the best 'signature' to predict antibiotic resistance in *P. aeruginosa*[8].

But to improve their model, they turned to machine learning. Instead of simply identifying resistance-conferring mutations, they used their algorithms to identify a signature of DNA and RNA variations that predicted a strain's resistance to antibiotics[8]. The algorithm helps to identify only key traits — it won't be a part of an eventual diagnostic test, Häussler says. Still, such approaches can overcome the problem of capturing all 'bug–drug' combinations through the genome alone, Wheeler says.

Rather than simply informing clinicians of a pathogen's current resistance profile, these algorithms could also reveal which antibiotic-resistance mechanisms a strain might develop in response to treatments. Still, "deciding whether an algorithm should be trusted or not is challenging", Wheeler says. "They're black boxes. Even if you have all the code and all the data, you don't necessarily know what's driving the model to say sample A is resistant to azithromycin, for instance."

Another problem developers are working to overcome is overfitting, Wheeler says: an algorithm might "memorize a whole lot of unimportant features" in data, rather than learn to find true correlations. Because bacterial gene sequences can be very similar, machine-learning tools might oversimplify a problem and draw the wrong conclusions. Wheeler likens the problem to a flawed image search: an algorithm that is trained on many pictures of farm animals in fields might identify a photograph of an open space as a sheep. Bacteria frequently pass antibiotic-resistance genes around to each other on small circular chunks of DNA that aren't part of their genomes. "But because the rest of the genome is the same, the algorithm might say the strain is still sensitive," she says. "What we really want from these models is for them to learn the biology of resistance."

Given the constraints of studying and testing tuberculosis, Farhat adopted a machine-learning approach that uses whole genome sequences to make predictions. In April, she and her colleagues described a web-based tool called GenTB that can predict resistance to several tuberculosis drugs[9]. The tool's performance varies with the quality of input sequence data and the drug in question. Whereas one common mutation is responsible for up to 80% of resistance to the first-line drugs used for TB, several rare variants confer small boosts in resistance to second-line drugs, Farhat says. "Sometimes, you only see the resistance when several such mutations are present."

Work in progress

Whichever approach they use, researchers face the same fundamental challenge: to design a diagnostic that improves significantly on current devices. Current tests can already return results to clinicians in less than 24 hours for a dollar or two per test, Grys says. "The question is not whether a test is good," he says. "The question is: is it better than what we have right now? It's important to set a trajectory that helps us meet the goals."

Some tests in development are restricted in the kinds of sample they can process, or the bacteria or antibiotics they can test. Visby's diagnostic is currently limited to gonococcal infections, for instance, and Ekinci's microfluidic device requires urine samples and cannot handle infections caused by more than one species of bacterium. Others that require advanced microscopes or spectrometry, such as cantilevers, will need to be adapted before they can be used by non-specialists working in resource-poor clinics around the world. Because many of these approaches test one or two microbes against a handful of drugs, they've conquered only "the tip of the iceberg", says Alex van Belkum, director of microbiology research at bioMérieux. "There's still a big lag between these technologies and the automated antibiotic susceptibility testing systems currently in laboratories."

As in the COVID-19 pandemic — during which rapid tests have proved crucial to detecting and stopping the spread of the virus — low-cost, point-of-care diagnostics are essential in reducing the misuse of antibiotics, says McKendry. "Antimicrobial resistance is a very complex problem, and new tests are only one part of the solution."

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