Drug-susceptibility testing for TB: poised to take a turn?

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January 2019—In a large international study, whole genome sequencing with next-generation sequencing technology has proved its ability to accurately assess susceptibility of *Mycobacterium tuberculosis* isolates to four first-line drugs. The data are convincing enough that the new technique has replaced phenotypic drug-susceptibility testing in some public health laboratories in the United States and Europe.

"What is remarkable is the lack of progress [in this area] in the last 100 years. It is only recently that we have made any advance at all," says Timothy M. Walker, DPhil, of the Department of Microbiology, John Radcliffe Hospital, Oxford, United Kingdom. "Until the late 1990s we were still dependent on phenotypic testing [to determine resistance in *M. tuberculosis* isolates]. Now things appear to be moving."

Dr. Walker led the study in 16 countries across six continents that analyzed 10,209 clinical specimens. It demonstrated the power of whole genome sequencing to correctly predict resistance and susceptibility of *M. tuberculosis* isolates (*N Engl J Med.* 2018;379[15]:1403–1415). "There is still a lot of work to be done," he tells CAP TODAY, before this method can become standard in the clinical microbiology laboratory.

Dr. Walker

In the United States, whole genome sequencing to determine susceptibility of *M. tuberculosis* isolates is being performed at Wadsworth Center, the laboratory of the New York State Department of Health, in Albany. "We developed and validated a test based on whole genome sequencing that provides comprehensive resistance detection for this organism," says Kimberlee A. Musser, PhD, Wadsworth's chief of bacterial diseases. It was brought online in February 2016. "We performed that test side by side with culture-based susceptibility testing for more than two and a half years," Dr. Musser says. Agreement was excellent, so whole genome sequencing was implemented as Wadsworth's first-line clinical test.

Dr. Musser and Dr. Walker believe that direct detection of *M. tuberculosis* resistance in clinical samples by whole genome sequencing is feasible. Their laboratories are among several working now to achieve it.

Dr. Walker, who is an academic clinical lecturer in infectious diseases and microbiology, says there are two important conclusions from the international study, conducted by Comprehensive Resistance Prediction for Tuberculosis: an International Consortium (CRyPTIC) and the 100,000 Genomes Project. First, "We are now at the point where our understanding of the molecular causes of resistance to first-line [antituberculosis] drugs is at a sufficiently high level that we can replace routine resistance testing by phenotypic methods with whole genome sequencing." WGS can provide a result within 10 days rather than weeks to months, he notes.

Second, Dr. Walker says, "We argue that for the first time we can use a molecular method to predict susceptibility rather than just resistance. This is a slightly trickier concept to get our heads around. It is not complicated, but it is not the way people typically think about it."

Cepheid's GeneXpert MTB/RIF assay, for detection of rifampin resistance, lacks sufficient sensitivity, Dr. Walker says. As a result, "In the absence of a positive result we haven't been able reliably to predict susceptibility." In

contrast, with a negative resistance result with whole genome sequencing, “we can now confidently say you can give this drug and avoid these others.”

In the study published Oct. 11, 2018 in the *New England Journal of Medicine*, there was a strong correlation between WGS-based predictions of susceptibility to four first-line tuberculosis drugs—isoniazid, rifampin, pyrazinamide, and ethambutol—and phenotypic susceptibility as determined by culture-based testing. Sequencing correctly predicted resistance, with sensitivities ranging from 91 percent to 98 percent. As a result, whole genome sequencing correctly predicted susceptibility to the four drugs, with specificities ranging from 93 percent (ethambutol) to 99 percent (isoniazid).

“[W]hole-genome sequencing can now characterize profiles of susceptibility to first-line antituberculosis drugs with a degree of accuracy sufficient for clinical use,” Dr. Walker and coauthors write, adding that the importance is twofold. “First, it shows that the genomic approach could be used to guide the choice of which drugs to prescribe and not just which drugs to avoid, in a way similar to phenotyping. Second, the data can be used to support plans to reduce the workload associated with culture and susceptibility analysis in places where routine whole-genome sequencing is performed.”

Dr. Musser’s laboratory started to work on a whole genome sequencing resistance test about five years ago. “We piloted a whole genome sequencing test to detect in a comprehensive way mutations that were known to cause resistance in *M. tuberculosis* strains,” she says. “In New York we have a mechanism to validate clinical tests of any kind.” Following this protocol produced reassuring results. “We found that whole genome sequencing predicted susceptibility and resistance with high sensitivity and specificity. Each year we refined our bioinformatic pipeline”—developed by a Wadsworth bioinformatician—“to a point where we felt we could detect all known indicators of resistance in *M. tuberculosis* as well as identify other mutations that never cause resistance.”

They studied resistance and susceptibility to the same four first-line drugs that the international consortium studied. “The value we think is most important is the susceptibility predictive value,” says Dr. Musser, whose laboratory wasn’t part of the large study. With the whole genome sequencing test, these values were rifampin, 100 percent; isoniazid, 99 percent; ethambutol, 99 percent; and pyrazinamide, 98 percent. Overall they saw a 99 percent predictive value for susceptibility to the four first-line drugs based on high-confidence mutations. Specificities were also between 99 percent and 100 percent (Shea J, et al. *J Clin Microbiol.* 2017;55[6]:1871–1882).

Dr. Musser

Dr. Musser and her colleagues added a third group of mutations that are currently unknowns. They were from archival strains that are sometimes resistant and sometimes susceptible. “Usually they have low-level resistance,” she says. “In our newest update to our pipeline, we added in those unknown mutations. At the same time, on Oct. 1, 2018, we went live using whole genome sequencing as our first line of testing. For any strains that test susceptible, we don’t do any additional testing. We report those out as susceptible with high confidence.”

For strains that test resistant or unknown, the Wadsworth laboratory continues to perform culture-based susceptibility testing. “Since strains in New York and the U.S. generally have a low level of resistance, we reduced the amount of culture-based susceptibility testing by about 70 percent,” Dr. Musser says. “Now we can focus more on drug resistance.”

There is work to be done to make the new technology accessible in resource-poor countries, Dr. Walker says, adding that Cepheid’s GeneXpert has the advantage of literally being a black box. “You put in a sample and, with

minimal preparation, it gives you a result. To get DNA from clinical samples requires technical expertise. The new method won't be rolled out around the world until someone completely automates it. In the meantime," he says, "I suspect it will be restricted to labs with trained staff." That's what happened even with GeneXpert, he adds. "It was envisioned as a bedside test, but that didn't happen."

In Dr. Musser's laboratory, all whole genome sequencing data are used to answer other questions. Epidemiological tracking is one application. To which member of the tuberculosis complex is a particular isolate related? Dr. Musser and colleagues relate each new organism to past strains and send a report including the strain or strains most closely related (by assessing the entire 4.4 million nucleotide genome) to the tuberculosis control epidemiologist. "It helps with those investigations," Dr. Musser says. Testing with whole genome sequencing is faster than culture-based susceptibility testing and thus allows tuberculosis controllers and physicians to use the lab's information more quickly, which is especially helpful for multidrug-resistant TB.

Thus, one whole genome sequencing test provides rapid, accurate, comprehensive drug prediction and replaces tests used for other purposes. WGS is an additional cost but one that is partially offset by reduced costs for conventional testing, she says, and ultimately the more rapid results save health care dollars. Dr. Musser's conclusion: WGS susceptibility testing "pays for itself." In New York there are about 800 cases per year, of which about 20 percent are drug resistant. "For states with fewer cases and a lower percentage of drug-resistant cases, it may not make sense to implement sequencing," she says.

Cost for a whole genome sequencing assay is about $200 start to finish. "If we moved to a larger sequencing instrument, that might bring the cost down to $100," Dr. Musser says. They are now using an Illumina MiSeq. "It fits our volume very well. We run about 15 specimens per week. We are using a NextSeq for some foodborne bacterial testing," which she says can do 80 specimens per run. "We are thinking about also going to that for bacteria on which we do whole genome sequencing."
Like Wadsworth, Public Health England decided to stop phenotyping isolates predicted to be susceptible to all first-line drugs, and "similar decisions have been made in the Netherlands," according to the published international study.

Can the WGS assay be performed on DNA extracted directly from sputum, rather than wait for bacterial growth in culture? Says Dr. Walker: "We did all of our testing on culture isolates. The goal for many people in this field is to replicate those results without culture. The obvious thing is to sequence directly from clinical samples; that way you could get same-day results, which would transform the way we manage cases. We and others are working on isolating DNA from sputum." They are making progress, he says: "I don't think we are far from being able to isolate DNA from clinical samples." Though considerable work is yet to be done, "it's where we have to go," he adds.

Dr. Musser's group is exploring methods to enrich for *M. tuberculosis* DNA and to do target-based sample amplification. Their goal is to amplify only drug resistance prediction genes. "This is something we think about and work on every day," she says.

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