Detection of drug resistance in *Mycobacterium tuberculosis*

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Editor

The report by Florea et al. (Broad-range PCR coupled with mass-spectrometry for the detection of *Mycobacterium tuberculosis* drug resistance, GERMS 2016) describes the use of a commercial PCR assay coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) to detect isoniazid (INH) and rifampin (RIF) resistance in 63 *M. tuberculosis* isolates obtained from Romanian patients with pulmonary tuberculosis (TB). They compared the results of the assay to those based on the absolute drug concentration method in Löwenstein-Jensen medium. The sensitivity and specificity of the PCR/ESI-MS assay was 100% and 95% for INH resistance, while the sensitivity and specificity was 92% and 97% for RIF resistance, respectively. The results were compared to a large-scale application of this technology used to detect drug resistance in 1340 *M. tuberculosis* isolates from different regions of the world reported by Massire et al. in 2010.¹ This study found a sensitivity of 89.3% and 96.3%, and specificity of 95.8% and 98.6% for INH and RIF, respectively, compared to the indirect proportion method in Middlebrook 7H10 medium.¹

Many tests based on multiplex nucleic acid amplification of genes with mutations associated with drug resistance in *M. tuberculosis* have been

reported over the last 20 years.²⁻⁹ The PCR/ESI-MS is yet another approach that is based on coupling multiplexed nucleic acid amplification to mass spectrometry. It was first reported in 2005 by Ecker et al., who described the use of an universal biosensor Ibis T5000 to analyze samples obtained from military recruits during respiratory disease outbreaks as part of a biodefense application.¹⁰ The process used high-performance electrospray ionization mass spectrometry and base composition analysis of PCR amplicons from a target DNA sequence (e.g., strain-specific alleles) to determine both the bacterial species and genotype of the strain from a mixed population of bacteria in clinical samples.¹⁰ Primers can be multiplexed to target multiple highly variable regions of bacterial genomes, and mass spectrometric analysis of the PCR-amplified products can then be performed to genotype bacterial strains. If the variable genes that are targeted involve mutations in genes associated with drug resistance, this approach can be used to identify resistance.

The major advantage of the PCR/ESI-MS assay is its ease in detecting resistance in *M. tuberculosis*, especially resistance to INH and RIF. If resistance to both INH and RIF (defined as multidrug resistance) can be detected easily, appropriate multidrug treatment regimen can be initiated in most situations.

However, there are several limitations that restrict its practical application. The need for a multiple set of primers to genotype bacterial pathogens or identify drug resistance associated with multiple mutations for a particular group of antibiotics (e.g. mutations in β -lactamase genes) can add to the complexity and cost of this technology. Although resistance to INH and RIF in most strains of *M. tuberculosis* is associated with

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mutations in restricted regions within a limited set of genes (*katG*, *inhA*, *ahpC* promoter gene for INH resistance, and *rpoB* for RIF resistance), if resistance to other drugs need to be identified (e.g., ethambutol [EMB] or fluoroquinolone [FQ]), additional primers would be required. Furthermore, *M. tuberculosis* gene mutations associated with resistance to other drugs (e.g., *embB* codon 306 with EMB resistance and *gyrA* with FQ resistance) are not as strongly correlated as those for INH and RIF resistance.

The positive predictive value (PPV) of all tests based on amplification of genes to detect drug resistance is affected by differences in the geographic source of the *M. tuberculosis* isolates tested. In this study by Florea et al., all but one of the 63 isolates belonged to the Euro-American lineage (PGG 2 and 3), a lineage in which mutations associated with INH and RIF resistance have been previously well characterized. A test for mutations conferring drug resistance on a small collection of isolates belonging to a related lineage from a geographically confined source would show high sensitivity. Another small study from the United States that applied the PCR/ESI-MS assay on 48 isolates reported a sensitivity of 100% for both INH and RIF.¹¹ A study in China that applied the PCR/ESI-MS assay to characterize *M. tuberculosis* isolates from Shanghai (low TB endemic region) and Chongqing (high TB endemic region) found that mutations conferring drug resistance to INH and RIF were more diversified in the region of low TB endemicity than in the region of high TB endemicity.¹² This diversity was revealed not by PCR/ESI-MS but by sequencing the target genes.¹²

The abovementioned study in China demonstrates that the PCR/ESI-MS assay does not have the resolution of the sequence-based information. This assay may not be able to

distinguish mutations that result in no mass change, such as C-to-G or G-to-C changes. However, such potential ambiguity can be prevented by designing the primer pairs to avoid amplification of such regions.

There are other issues that preclude the practical implementation of PCR/ESI-MS, including its cost, especially for its implementation in resource-limited high-burden settings, where TB diagnosis is most needed. With a rapid *M. tuberculosis* drug-susceptibility test such as GeneXpert⁸ (which is also costly) already endorsed by WHO, the advantage of PCR/ESI-MS is not obvious. However, its use in a research setting to screen drug-resistant *M. tuberculosis* strains for further analyses (e.g., sequencing drug-resistance genes, whole genome sequencing) is one practical application of this technology.

Conflicts of interest: None to declare.

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