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DNA Sequencing for Confirmation of Rifampin Resistance Detected by Cepheid Xpert MTB/RIF Assay

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DNA sequencing of *rpoB* and culture-based drug susceptibility results were evaluated for samples referred for confirmation of rifampin resistance detected by the Cepheid Xpert MTB/RIF assay. Silent mutations and mutations associated with low-level resistance were found in the study population. These data support CDC recommendations to confirm Xpert rifampin resistance results.

Rapid diagnosis and effective treatment are two of the most important strategies in a tuberculosis (TB) control program to prevent ongoing transmission of disease and to improve patient outcomes (1). To ensure an effective treatment regimen, drug susceptibility testing (DST) must be performed. Culture-based methods can take 4 to 12 weeks and are considered the "gold standard" for DST of *Mycobacterium tuberculosis* complex (MTC). To provide more rapid DST results, the Centers for Disease Control and Prevention (CDC) implemented a clinical laboratory service, Molecular Detection of Drug Resistance (MDDR), for U.S. Public Health TB programs in September 2009. MDDR uses PCR to amplify targeted genetic loci and then DNA sequencing to detect mutations associated with resistance to four first-line drugs, rifampin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB), and 4 second-line drugs, ofloxacin (OFX), amikacin (AMK), capreomycin (CAP), and kanamycin (KAN) (*2*). Culture-based DST by the agar proportion method and MGIT for PZA, is performed concurrently on all samples received for MDDR. Culture-based DST is necessary to complement molecular results because the clinical relevance of some mutations is unknown, and not all mechanisms of resistance are understood (3). However, culture-based DST for RIF is imperfect, and DNA sequencing may yield more information in some situations (4–6). More than 95% of RIF-resistant (RIF^r) strains contain a mutation in the RIF resistance-determining region (RRDR) of *rpoB*. Previous studies revealed mutations in the RRDR (e.g., 511Pro, 516Tyr, 526Asn, 526Leu, and 533Pro) that are "disputed" or associated with highly discordant results among culture-based DST methods because they yield low-level RIF^r that may not be detected by some methods. TB cases that are caused by strains exhibiting these mutations may not respond well to a rifampin-based treatment regimen (4–6). Some strains may harbor silent mutations in the RRDR that do not result in an amino acid change and do not confer resistance (*7*).

In July 2013, the Cepheid Xpert MTB/RIF assay (Xpert) received FDA market authorization for the primary identification of MTC and the detection of RIF^r by molecular analysis. This assay can be performed on raw or concentrated sputum sediments using the fully automated GeneXpert Instrument and provides rapid results weeks earlier than culture-based methods (8). However, some experts have recommended that RIF^r detected by Xpert be confirmed by DNA sequencing due to the potential low positive predictive value for detection of RIF^r, attributable to the low prevalence of drug resistance among U.S. TB cases and because the output of Xpert does not provide the specific *rpoB* mutation detected (i.e., the probes detect the presence of wild-type sequence) (9). Knowing the specific mutation detected is necessary because silent mutations can lead to false resistance by Xpert and "disputed" mutations may lead to discordant culture-based DST results. In this report, we describe *rpoB* DNA sequencing results of the RRDR and correlate them with the RIF DST results for clinical isolates and specimens referred to the CDC for confirmation of RIF^r detected by Xpert at laboratories in the United States.

Molecular and culture-based DST results were retrospectively analyzed for 84 isolates and specimens containing MTC from 80 patients, received between February 2011 and June 2014 for confirmation of RIF^r detected by Xpert. Excluded from the analysis were four duplicate patient samples, two samples that could not be DNA sequenced, and 14 samples without a DST result, due to contamination, no growth in the DST media, or with no MTC growth detected. Forty-two (66%) of the remaining 64 sample results analyzed were RIF^r by agar proportion DST (Table 1). Of these, 39 (93%) had mutations commonly associated with RIF^r and the remaining 3 had mutations associated with low-level RIF^r. Twenty-two (34%) samples were susceptible to RIF by agar proportion DST. Of these, 12 (55%) possessed silent mutations that lead to false RIF^r by Xpert, and 6 had mutations associated with low-level resistance (Table 1).

In this evaluation, 12 of 64 samples (19%) that were tested for confirmation of Xpert-detected RIF^r had silent mutations not associated with phenotypic resistance and 9 (14%) had "disputed" mutations associated with low-level resistance. The prevalence of these types of mutations is unknown and warrants a larger survey. However, our data are consistent with recent reports (6, 9) and support the recommendation to confirm Xpert RIF^r results with

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TABLE 1 Frequency of *rpoB* mutations identified in study samples

RRDR result	No. (%) of samples with DST result:		
	RIF^r	RIF^s	Total
Ser531Leu	26	0	26 (41)
His526Tyr	3	0	3 (5)
His526Asp	2	1	3 (5)
Ser531Trp	2	0	2 (3)
Gln513Leu	1	0	1 (2)
Asp516Val	1	0	1 (2)
His526Arg	1	0	1 (2)
Phe514PhePhe	1	0	1 (2)
His526Arg/Cys/Tyr[a]	1	0	1 (2)
Leu511Pro[b]	0	2	2 (3)
Asp516Tyr[b]	0	2	2 (3)
His526Ser[b]	0	1	1 (2)
Leu533Pro[b]	0	1	1 (2)
His526Leu[b]	1	0	1 (2)
Leu511Pro and Asp516Ala[b]	1	0	1 (2)
Ser512Arg and His526Asn[b]	1	0	1 (2)
Asp516Glu and Ser522Leu	1	0	1 (2)
Asp516Gly and Ser522Leu	0	1	1 (2)
Phe514Phe[c]	0	11	11 (17)
Leu521Leu[c]	0	1	1 (2)
No mutation	0	2	2 (2)
Total	42 (66)	22 (34)	64
Mutations associated with RIF^r	39	1	40 (63)
Mutations associated with low-level RIF^r	3	6	9 (14)
Silent mutations	0	12	12 (19)

[a] Mixed peaks were observed (CAC > YRC).
[b] Mutation associated with low-level RIF^r (i.e., disputed mutation).
[c] Silent mutation.

both molecular methods that allow for the specific DNA sequence and culture-based DST. DNA sequencing results are useful for interpretation of results from Xpert and for resolving potential discordant results between the Xpert and culture-based methods. False-positive results by Xpert and false-negative results by culture-based DST could contribute to less effective treatment regimens and delay the diagnosis of multidrug-resistant (MDR) TB. The CDC recommends the use of minimum reporting language for results from Xpert. If a mutation is detected, the reporting language should include the following statement (10): "A mutation in *rpoB* gene has been detected, indicating possible rifampin resistance. Confirmatory testing should follow." As more laboratories in both the public and private sectors expand their use of molecular diagnostics, such as the GeneXpert platform, the need for understanding molecular results and their limitations is paramount. Consistent, precise reporting language contributes to a balanced interpretation of results. In addition, all laboratory results should be placed in the context of clinical indicators to ensure optimal patient care.

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