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Concordance between Molecular and Phenotypic Testing of *Mycobacterium tuberculosis* Complex Isolates for Resistance to Rifampin and Isoniazid in the United States

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Multidrug-resistant (MDR) isolates of *Mycobacterium tuberculosis* complex (MTBC) are defined by resistance to at least rifampin (RMP) and isoniazid (INH). Rapid and accurate detection of multidrug resistance is essential for effective treatment and interruption of disease transmission of tuberculosis (TB). Overdiagnosis of MDR TB may result in treatment with second-line drugs that are more costly, less effective, and more poorly tolerated than first-line drugs. CDC offers rapid confirmation of MDR TB by the molecular detection of drug resistance (MDDR) for mutations associated with resistance to RMP and INH along with analysis for resistance to other first-line and second-line drugs. Simultaneously, CDC does growth-based phenotypic drug susceptibility testing (DST) by the indirect agar proportion method for a panel of first-line and second-line antituberculosis drugs. We reviewed discordance between molecular and phenotypic DST for INH and RMP for 285 isolates submitted as MTBC to CDC from September 2009 to February 2011. We compared CDC's results with those from the submitting public health laboratories (PHL). Concordances between molecular and phenotypic testing at CDC were 97.4% for RMP and 92.5% for INH resistance. Concordances between CDC's molecular testing and PHL DST results were 93.9% for RMP and 90.0% for INH. Overall concordance between CDC molecular and PHL DST results was 91.7% for RMP and INH collectively. Discordance was primarily attributable to the absence of known INH resistance mutations in isolates found to be INH resistant by DST and detection of mutations associated with low-level RMP resistance in isolates that were RMP susceptible by phenotypic DST. Both molecular and phenotypic test results should be considered for the diagnosis of MDR TB.

Two essential drugs for the first-line treatment of tuberculosis (TB) are rifampin (RMP) and isoniazid (INH). Isolates of *Mycobacterium tuberculosis* complex (MTBC) that are resistant to at least both these drugs are classified as multidrug resistant (MDR). Rapid and accurate detection of resistance to either RMP or INH is crucial for selection of treatment regimens and public health interventions. In 2012, the Centers for Disease Control and Prevention (CDC) reported 9,945 cases of TB in the United States (http://www.cdc.gov/tb/statistics/reports/2012/default.htm). For 7,188 of these cases, for which initial drug susceptibility to first-line antituberculosis drugs was reported, 660 (9.2%) were at least INH resistant, and 83 (1.2%) were MDR. The American Thoracic Society, CDC, and the Infectious Diseases Society of America have issued guidelines for treating INH-monoresistant TB (1). Early detection of RMP resistance, which correlates well with multidrug resistance, is critical for the initiation of effective second-line treatment regimens and interruption of disease transmission.

CDC offers the molecular detection of drug resistance (MDDR) for mutations associated with resistance to RMP at the RMP resistance-determining region (RRDR) of the *rpoB* locus and with resistance to INH at the *katG* and *inhA* loci (2, 3). Other loci that are examined are *embB* (EMB resistance), *pncA* (pyrazinamide resistance), *gyrA* (fluoroquinolone resistance), *rrs* (kanamycin, amikacin, and capreomycin resistance), *tlyA* (capreomycin resistance), and *eis* (promoter region mutations associated with kanamycin resistance) (2). MDDR is available by request in coordination with state public health laboratories (PHL) for *M. tuberculosis* isolates and sediments meeting submission criteria (http://www.cdc.gov/tb/topic/laboratory/MDDRsubmissionform.pdf). Although molecular testing can rapidly detect mutations associated with drug resistance, it should complement, not supersede, conventional phenotypic drug susceptibility testing (DST) (4). Therefore, all submissions to MDDR undergo growth-based DST for a full panel of first-line and second-line drugs (4). Submitters receive a preliminary report with molecular test results and a final report upon completion of DST, with both the molecular and DST results and interpretive comments.

In this study, we examined the concordances between molecular testing and DST for RMP and INH to determine the performance characteristics of CDC's MDDR service for rapid detection and confirmation of MDR TB. Through an electronic survey using a secure data collection instrument, we collected phenotypic DST results from state and local PHL for isolates submitted for testing at CDC. We examined the concordances between molecular and phenotypic DST performed at CDC and compared our results for the two methods to results from phenotypic DST performed by submitting laboratories.

MATERIALS AND METHODS

MTBC isolates. A flow chart presented in Fig. 1 represents the process for analyzing RMP and INH testing results for MTBC isolates submitted to

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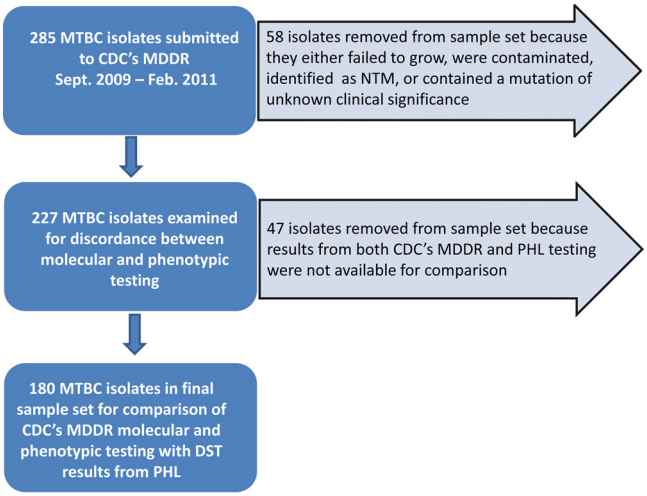

FIG 1 Flow chart representing the process for determining concordance of molecular and phenotypic results between testing conducted by CDC's MDDR service and DST from PHL for MTBC isolates.

CDC's MDDR service by PHL from September 2009 to February 2011. Phenotypic DST results were successfully collected from state and local PHL for 241 (84.6%) of the 285 MTBC isolates submitted during the study period.

Molecular testing and phenotypic DST. Phenotypic DST for RMP and INH was performed at CDC (Atlanta, GA) using the indirect agar proportion method according to the Clinical and Laboratory Standards Institute (CLSI)-approved standard (4). Test concentrations in supplemented Middlebrook 7H10 agar were 1 μg/ml for RIF and 0.2 and 1 μg/ml for INH. DNA sequencing for detection of mutations at loci associated with resistance to RMP (*rpoB*) and INH (*katG* and *inhA*) was performed as previously described (2).

Collection of phenotypic DST results from PHL. This data collection received expedited approval under an Office of Management and Budget (OMB) generic clearance package (OMB no. 0920-0879). A survey instrument was designed using Snap Surveys software (version Snap 10 Professional; Snap Surveys) to collect phenotypic DST results for RMP and INH securely online from PHL. Each isolate was assigned a CDC specimen identification number (CSID) linked to the specimen number used by the submitting laboratory. Each site was sent a list of CSIDs and corresponding specimen numbers enabling retrieval of their local DST results. No personally identifiable information was collected. Respondents submitted a separate survey for each CSID. CDC determined that this study was non-human subject research, that is, it did not require Institutional Review Board approval.

Data analysis. Phenotypic DST data from PHL were downloaded into a Snap Surveys database and exported to a PASW Statistics (version 18; IBMSPSS software) spreadsheet for further analysis. Concordance between phenotypic DST and molecular testing performed by CDC's MDDR service was determined through cross-tabulation of results and calculation of the percentage agreement. Similarly, concordance was calculated between testing conducted at CDC (both molecular testing and phenotypic DST) and phenotypic DST performed by PHL. MTBC isolates received by CDC that failed to grow or were contaminated, identified as nontuberculous mycobacteria (NTM), or contained mutations of unknown clinical significance were not included when calculating concordance.

RESULTS

Concordance between CDC's MDDR molecular testing and phenotypic DST. The cross-tabulation of results to determine concordance between molecular testing and phenotypic DST conducted at CDC is shown in Table 1. Where results were available for the 285 isolates submitted to CDC during the study period, the

TABLE 1 Concordance between CDC molecular results and phenotypic DST for MTBC isolates submitted for MDDR

		Phenotypic DST result (no. of isolates)							
Discordance	Mutation(s) detected	MDR[a]	RMP-R[b]	INH-R[c]	Susceptible	No growth	Contaminated	NTM[d]	Total no. of isolates
No	*rpoB* and either *katG* or *inhA*	68	0	0	0	0	0	0	68
	rpoB only	0	4	0	0	0	0	0	4
	katG or *inhA* only	0	0	26	0	0	0	0	26
	No mutation	0	0	0	106	0	0	0	106
	No amplification	0	0	0	0	0	0	2	2
Yes	*rpoB* and either *katG* or *inhA*	0	1	4	0				5
	rpoB only	11	0	0	0				11
	No mutation	0	2	5	0				7
Unknown	*rpoB* and either *katG* or *inhA*	0	0	0	0	9	4	0	13
	katG or *inhA* only	0	0	0	0	2	0	0	2
	No mutation	0	0	0	0	15	20	0	35
	No amplification	0	0	0	0	1	0	0	1
	UCS *rpoB* only[e]	0	0	0	1	1	0	0	2
	rpoB and UCS *katG*[f]	1	0	0	0	0	0	0	1
Total		80	7	35	107	28	26	2	285

[a] MDR, multidrug resistant, growth in the presence of rifampin and isoniazid.
[b] RMP-R, rifampin resistant, growth in the presence of rifampin and no growth on isoniazid.
[c] INH-R, isoniazid resistant, growth in the presence of isoniazid and no growth on rifampin.
[d] NTM, nontuberculous mycobacteria, no conventional or molecular results.
[e] Mutation of unknown clinical significance (UCS) within rifampin resistance-determining region (RRDR) of *rpoB*.
[f] Mutation within rifampin resistance-determining region (RRDR) of *rpoB* and mutation of unknown clinical significance (UCS) in *katG* loci.

TABLE 2 Concordance between CDC's MDDR service and PHL phenotypic DST results for MTBC isolates

Discordance	Mutation(s) detected by MDDR	Phenotypic DST result from PHL (no. of isolates)				Total no. of isolates
		MDR[a]	RMP-R	INH-R	Susceptible	
No	*rpoB* and either *katG* or *inhA*	56	0	0	0	56
	katG or *inhA* only	0	0	18	0	18
	rpoB only	0	3	0	0	3
	No mutation	0	0	0	77	77
Yes	*rpoB* and either *katG* or *inhA*	0	0	4	1	5
	katG or *inhA* only	2	0	0	0	2
	rpoB only	9	0	0	0	9
	No mutation	3	2	5	0	10
Total		70	5	27	78	180

[a] For an explanation of abbreviations, see Table 1.

mean turnaround time (range) for completion of molecular testing was 2.3 days (1 to 8 days) and for phenotypic DST was 41.4 days (14 to 117 days). Of the 285 isolates, 56 were not included in calculations of concordance because of the absence of phenotypic DST results due to failure to grow (28 isolates), contamination (26 isolates), and identification as NTM (2 isolates) (Fig. 1). Results for two isolates were of unknown discordance because they contained mutations of unknown clinical significance. One of these isolates contained a Pro439Leu mutation of unknown clinical significance in the RRDR of *rpoB* but was susceptible by phenotypic DST. The other isolate contained a mutation in the RRDR region of *rpoB* and a Trp351Arg mutation of unknown clinical significance in the *katG* region but was found to be MDR by phenotypic DST. Thus, results for a total of 58 (20.4%) isolates submitted to CDC were not included in calculations to determine concordance.

Concordance between molecular testing and phenotypic DST performed by CDC's MDDR service for determining RMP and INH resistance was 94.9%. There was 100% concordance between methods for isolates determined to be susceptible to RMP and INH by phenotypic DST. No mutations associated with either RMP or INH resistance were detected in 107 (47.1%) isolates that were susceptible to these drugs by phenotypic DST.

For RMP, there were six discordant results. Two isolates were RMP monoresistant by phenotypic DST but no mutations associated with resistance were detected in the RRDR of *rpoB*. Four isolates were contained mutations associated with both RMP and INH resistance but were INH monoresistant by phenotypic DST. Thus, concordance for molecular testing and phenotypic DST determination of RMP resistance was 97.4%.

For INH, concordance between molecular testing and phenotypic DST for the identification of INH resistance was 92.5%. There were 17 discordant results between molecular testing and phenotypic DST. By phenotypic DST, 11 isolates were determined to be MDR, but no mutations known to be associated with INH resistance were detected in either the *inhA* or *katG* loci sequenced. Five isolates were INH monoresistant by phenotypic DST, but no mutations for INH resistance were detected. One isolate had mutations associated with both RMP and INH resistances but was RMP monoresistant by phenotypic DST.

Only molecular test results were available for 56 of the 283 isolates of MTBC submitted to CDC's MDDR service. For 13 of these isolates, mutations associated with both RMP and INH resistance were detected indicating MDR. When both molecular test and phenotypic DST results were interpreted collectively, 34.5% (98) of the MTBC isolates submitted to CDC during the study period were determined to be MDR.

Concordance between molecular testing performed at CDC and phenotypic DST results from PHL. Results for RMP and INH from molecular testing and phenotypic DST performed at CDC were available for comparison with phenotypic results from PHL for 180 MTBC isolates (Table 2). Overall concordance was 91.7% between molecular testing performed by CDC's MDDR service and PHL phenotypic DST for determination of INH and RMP resistance.

For RMP resistance, molecular testing at CDC and phenotypic DST results from PHL were in concordance for 169 MTBC isolates (93.9%) with discordant results between the methods for 11 isolates (6.1%). Phenotypic DST results submitted by PHL indicated that five isolates were monoresistant to RMP. However, molecular testing at CDC indicated that two of these isolates did not possess mutations associated with RMP resistance. For five isolates found to be MDR by phenotypic testing, molecular testing at CDC did not detect a mutation in the RRDR of *rpoB*. Conversely, five additional isolates determined to be MDR by molecular testing at CDC were either INH monoresistant (four isolates) or susceptible to both RMP and INH (one isolate) by phenotypic testing.

For INH resistance, molecular testing performed by CDC's MDDR service was in agreement with PHL phenotypic DST for 162 of the 180 MTBC isolates (90.0%) where both sets of results were available for comparison. There were 18 isolates (10.0%) with discordant INH results. Nine isolates reported as MDR by PHL using phenotypic DST possessed only a mutation associated with RMP according to molecular testing at CDC. There were eight isolates for which no mutations associated with INH resistance were detected by molecular testing at CDC but which were determined to be either INH monoresistant (five isolates) or MDR (three isolates) by PHL phenotypic DST. One isolate susceptible to both INH and RMP according to PHL phenotypic DST results was determined to be MDR by molecular testing at CDC, as stated above.

Phenotypic DST results for RMP and INH resistance from PHL indicated that 70 isolates (38.9%) were MDR. Phenotypic DST results were concordant for 56 (80.0%) isolates, with results from CDC's MDDR service confirming they were MDR. Interpreted collectively, test results from CDC and PHL indicated 76 MTBC isolates (42.2%) were MDR.

TABLE 3 Concordance between CDC and PHL phenotypic DST results for MTBC isolates

Discordance	MDDR phenotypic DST result	Phenotypic DST result from PHL (no. of isolates)				Total no. of isolates
		MDR[a]	RMP-R	INH-R	Susceptible	
No	MDR	63	0	0	0	63
	RMP-R	0	3	0	0	3
	INH-R	0	0	21	0	21
	Susceptible	0	0	0	75	75
Yes	MDR	0	0	2	0	2
	RMP-R	1	0	0	1	2
	INH-R	3	1	0	2	6
	Susceptible	3	1	4	0	8
Total		70	5	27	78	180

[a] For an explanation of abbreviations, see Table 1.

Concordance between CDC and PHL phenotypic DST results. CDC and PHL phenotypic DST results for 180 isolates of MTBC were compared by cross-tabulation (Table 3). Results were in concordance for determination of both RMP and INH resistance for 162 MTBC isolates (90.0%). Concordance between CDC and PHL phenotypic DST for determination of RMP or INH resistance was 93.9%. There were 11 isolates with discordant phenotypic DST results for RMP or INH. Among six isolates determined to be MDR by PHL phenotypic DST, three were INH monoresistant and three were susceptible to both RMP and INH by CDC phenotypic DST. Two isolates determined to be MDR at CDC were INH monoresistant by PHL phenotypic DST. CDC found eight isolates susceptible to INH, while PHL found four of these to be resistant to INH and four to be MDR.

When examining both CDC and PHL phenotypic DST results for determination of MDR, 72 isolates of MTBC were determined to be MDR at either CDC or by PHL. Results were in agreement for 63 isolates which were determined to be MDR by both CDC and PHL, for an overall concordance of 87.5%.

Summary of discordant results between CDC's MDDR service and PHL phenotypic DST. A total of 30 MTBC isolates had at least one discordant result between testing conducted by CDC's MDDR service and phenotypic DST performed by PHL. Details of mutations associated with discordant results are displayed in Table 4. Of note, isolates with the *rpoB* mutation Asp516Tyr did not consistently test as RMP resistant, with four of these isolates testing as INH monoresistant by phenotypic DST at CDC and two reported as INH monoresistant by PHL.

DISCUSSION

Determination of primary drug resistance through molecular detection of mutations associated with RMP and INH resistance can provide results within days versus weeks required for phenotypic DST. Due to gaps in knowledge regarding mechanisms of resistance, however, these molecular assays do not yet provide sufficient sensitivity to replace phenotypic DST. In this study, most discordances between molecular testing and phenotypic DST were due to not detecting a mutation in either the *katG* or *inhA* locus for an isolate later determined to be INH resistant by phenotypic DST. Four discordant results were due to phenotypic DST failing to reveal RMP resistance while molecular testing detected an Asp516Tyr *rpoB* mutation known to be associated with low-level resistance (5, 6). We determined that the sensitivity of molecular testing for detection of multidrug resistance compared to phenotypic DST was 85.0%. Failure to detect MDR isolates through molecular testing was attributable primarily to the lower sensitivity (90.6%) for determining INH resistance through detection of mutations at either the *katG* or *inhA* locus (2).

Molecular testing can be used to obtain information regarding resistance when phenotypic DST is complicated by isolates that either fail to grow when subcultured or are contaminated. Thus, the availability of molecular results from services like CDC's

TABLE 4 Summary of discordant results between CDC's MDDR service and PHL phenotypic DST

No. of isolates	CDC molecular results			CDC phenotypic DST result	PHL phenotypic DST result
	rpoB mutation	*katG* mutation	*inhA* mutation		
5	Ser531Leu	None	None	MDR	MDR
3	Asp516Val	None	None	MDR	MDR
1	His526Tyr	None	None	MDR	MDR
1	ΔSer512Gln513Phe514[a]	Ser315Thr	None	INH-R	MDR
1	WT	Ser315Thr	None	INH-R	MDR
1	Thr480Asn	WT	C-15T	RMP-R	MDR
1	Asp516Tyr	Ser315Thr	C-15T	INH-R	INH-R
1	Asp516Tyr	Ser315Thr	WT	INH-R	MDR
1	Asp516Tyr	Ser315Thr	C-15T	INH-R	Susceptible
1	Asp516Tyr	Ser315Thr	None	INH-R	INH-R
1	Leu511Pro, Asn518Asp	Ser315Thr	None	MDR	INH-R
1	Ser531Leu	Ser315Thr	None	MDR	INH-R
3	None	None	None	Susceptible	MDR
4	None	None	None	Susceptible	INH-R
1	None	None	None	Susceptible	RMP-R
1	None	None	None	INH-R	RMP-R
1	None	None	None	INH-R	INH-R
1	None	None	None	INH-R	Susceptible
1	None	None	None	RMP-R	Susceptible

[a] In-frame deletion.

MDDR service can contribute to rapid initiation of effective treatment without delays caused by lengthy repeated attempts to complete phenotypic DST. A survey conducted by CDC of PHL who submitted isolates to CDC's MDDR service indicated that over 85% of respondents reported molecular results as soon as they were available to health care providers without waiting for completion of phenotypic DST (M. A. Yakrus, presented at the 2013 National TB Conference, Atlanta, GA, 11 to 13 June 2013). In theory, this should have sped the initiation of proper treatment, hastened sputum conversion, reduced days of isolation, and interrupted transmission of disease by shortening the infectious period, but these effects remain to be confirmed by operational studies.

Discordant results were obtained for some isolates containing an Asp516Tyr mutation in *rpoB* which has been previously reported as associated with low-level RMP resistance and discordant test results with phenotypic DST methods (5, 6). The widely used Bactec MGIT liquid culture system is more prone than solid media to miss low-level RMP resistance conferred by mutations at specific codons (7, 8). Our molecular and phenotypic results for 285 isolates revealed that six isolates possessed an Asp516Tyr mutation, of which four were found to be RMP susceptible by phenotypic DST (data not shown). Detection of *rpoB* mutations in isolates with phenotypic susceptibility to RMP at recommended test concentrations (MICs of 1 to 2 μg/ml using the 7H10 agar proportion method) have been significantly associated with treatment failure (9). Low-level resistance to RMP supports the suggestion to investigate if higher doses of RMP may be needed for patients infected with strains possessing this trait (6).

Molecular detection of MDR is hampered by the limited sensitivity for detection of INH resistance through identification of mutations in the *katG* or *inhA* regions (2). Where results were available for 283 MTBC isolates submitted to CDC's MDDR service during the study period, molecular testing initially identified 86 isolates as MDR while phenotypic DST determined that 80 isolates were MDR. However, collectively these methods identified 98 isolates as MDR. The advantages of a reference laboratory that can concurrently perform both molecular detection of drug resistance and phenotypic DST for a full panel of first-line and second-line drugs and quickly provide reliable results to health care providers are apparent. Emphasis needs to be placed on quickly identifying drug resistance at the local level and sending specimens for rapid confirmatory testing to a suitable reference laboratory such as CDC's MDDR service.

Molecular testing and phenotypic DST results must be considered collectively to reach a final interpretation of drug resistance. This same conclusion was reached in a recent study where rare *rpoB* mutations were associated with low-level phenotypic RMP resistance for isolates from treatment failure and relapse patients (10). We conclude that when RMP-resistance is indicated by either a molecular or a phenotypic technique, results need to be reported promptly to the health care provider pending further analysis (11). When discordance occurs between molecular testing and phenotypic DST, a final interpretation requires full consideration of the clinical picture and consultation with an expert (11). In this study, molecular testing and phenotypic DST by CDC's MDDR service revealed that 5.8% of the MTBC isolates tested were RMP monoresistant, relative to first-line and second-line drugs examined through this service. Our test setting contained an inherently high prevalence of MDR isolates (35%) because many of the isolates were submitted either for confirmation of suspected drug resistance or were from patients believed to be at high risk for MDR TB. In one cohort of retreatment cases with a prevalence of MDR TB over 17% and containing 6,308 cases with resistance to RMP, 534 cases (8.5%) of the RMP-resistant isolates were INH susceptible (12). Therefore, monoresistance to RMP is more common when MDR is suspected. For any test, even with high sensitivity and specificity, the positive predictive value is low for a rare condition (11). To confirm a positive result, genetic loci associated with RMP resistance (to include the RRDR of *rpoB*), as well as INH resistance (to include *inhA* and *katG*), should be sequenced to assess for MDR TB. If mutations associated with RMP resistance are confirmed, rapid molecular testing for other known mutations associated with drug resistance (to first-line and second-line drugs) is needed for health care providers to select an optimally effective treatment regimen as soon as possible, while awaiting phenotypic results (4, 11). A panel of experts meeting at CDC recommended that all molecular testing should prompt phenotypic DST (http://www.cdc.gov/tb/topic/laboratory/rapidmoleculartesting/MolDSTreport.pdf). With the increasing incorporation of molecular tests like the Xpert MTB/RIF assay into testing algorithms, it will be essential that health care providers consult with PHL concerning the above recommendations for confirming drug resistance and initiation of treatment.

To determine potential causes and associated outcomes of discordant results, CDC is conducting further evaluation. Such analyses will include information regarding how results from CDC's MDDR service and PHL are interpreted, how quickly they are used by health care providers, and the subsequent effects on patient outcomes.

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