Centers for Disease Control and Prevention
Model Performance Evaluation Program

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Report of Results
November 2014
Performance Evaluation Survey

National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
Division of Tuberculosis Elimination

CS255757

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for November 2014 Survey

Purpose	The purpose of this report is to present the results of the U.S. Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for *Mycobacterium tuberculosis* complex drug susceptibility testing survey sent to participants in November 2014.
Report Content	The material in this report was developed and prepared by Cortney Stafford, MPH, MT (ASCP), Health Scientist, Laboratory Capacity Team, NCHHSTP, DTBE, LB Acknowledged contributors: Beverly Metchock NCHHSTP, DTBE, LB; Lois Diem NCHHSTP, DTBE, LB; Mitchell Yakrus NCHHSTP, DTBE, LB; Angela Starks NCHHSTP, DTBE, LB
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Table of Contents

***Mycobacterium tuberculosis* Complex Drug Susceptibility Testing MPEP Report for November 2014 Survey**

Introduction: Overview of MPEP Final Report

The Model Performance Evaluation Program (MPEP) is an educational self-assessment tool in which five isolates of *M. tuberculosis* complex (MTBC) are sent to participating laboratories biannually for staff to monitor their ability to determine drug resistance among the isolates. It is not a formal, graded proficiency testing program. This report includes results for a subset of laboratories performing drug susceptibility tests (DST) for MTBC in the United States. MPEP is a voluntary program, and this report reflects data received from participating laboratory personnel. This aggregate report is prepared in a format that will allow laboratory personnel to compare their DST results with those obtained by other participants using the same methods and drugs, for each isolate. We encourage circulation of this report to personnel who are either involved with DST or reporting and interpreting results for MTBC isolates.

CDC is neither recommending nor endorsing testing practices reported by participants. For approved standards, participants should refer to consensus documents published by the Clinical and Laboratory Standards Institute (CLSI), "Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard," M24-A2 [1].

Expected Susceptibility Testing Results

The tables below provide the anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in November 2014. Although CDC recommends broth-based methods for routine first-line DST of MTBC isolates, Table 1 provides the results obtained by the reference agar proportion method, except for pyrazinamide, where MGIT was the testing method. Table 2 provides molecular results obtained by using DNA sequencing [2].

Table 1. Expected Conventional Results for November 2014 Survey

Growth-based Results					
	First-Line Drugs				**Second-Line Drugs**
	INH	RMP	EMB	PZA	Resistant to:
2014F	S	R	S	S	
2014G	S	S	S	S	OFL
2014H	S	S	S	S	OFL
2014I	R	S	S	S	
2014J	S	S	S	S	AMK, KAN, CAP

Note—S=susceptible, R=resistant

Table 2. Expected Molecular Results for November 2014 Survey

Mutations Detected				
	First-Line Drugs		**Second-Line Drugs**	
MPEP Isolate	*katG*	*rpoB*	*gyrA*	*rrs*
2014F		His526Tyr		
2014G			Ala90Val	
2014H			Ser91Pro	
2014I	Ser315Thr	His526Asn*		
2014J				A1401G

* Disputed mutation that may result in variable results by growth-based methods

Abbreviations and Acronyms

AMK	amikacin
AP	agar proportion — performed on Middlebrook 7H10 or 7H11
bp	base pair
CAP	capreomycin
CDC	U.S. Centers for Disease Control and Prevention
CIP	ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CYS	cycloserine
DNA	deoxyribonucleic acid
DST	drug susceptibility testing
EMB	ethambutol
ETA	ethionamide
HMO	Health Maintenance Organization
INH	isoniazid
KAN	kanamycin
LEV	levofloxacin
MDR	multidrug resistant
MGIT	BACTEC MGIT 960 – Mycobacteria Growth Indicator Tube
MIC	minimum inhibitory concentration
MOX	moxifloxacin
MPEP	Model Performance Evaluation Program
MTBC	*Mycobacterium tuberculosis* complex
PAS	p-aminosalicylic acid
PZA	pyrazinamide
OFL	ofloxacin
R	resistant
RBT	rifabutin
RMP	rifampin
RNA	ribonucleic acid
S	susceptible
Sensititre	Thermo Scientific Sensititre *Mycobacterium tuberculosis* MIC plate
STR	streptomycin
TB	tuberculosis
VersaTREK	Thermo Scientific VersaTREK Myco susceptibility
XDR	extensively drug resistant

Technical Notes

The following information pertains to all of the tables and figures for the 2014 MTBC isolates F, G, H, I, and J in this report.

- The source of data in all tables and figures is from the November 2014 MPEP MTBC DST survey, with the exception of Figure 5 that compares data from the November 2014 MPEP survey and the May 2014 MPEP survey.

- The tables indicate the number of reported results (S represents susceptible and R represents resistant) for each drug.

- First-line and second-line drugs have been separated into individual tables for each isolate. Streptomycin is classified as a second-line drug for this report.

- Separate tables for molecular testing are included where data are of note.

- Laboratories that use more than one DST method are encouraged to test isolates with each of those methods at either CLSI-recommended or equivalent critical concentrations. Some laboratories have provided results for multiple DST methods. Consequently, the number of results for some drugs may be greater than 87 (the number of participating laboratories). This report contains all results reported by participating laboratories.

- As a reference, a list of critical concentrations for antituberculosis drugs, by method, can be found at the end of this report.

- The Trek Sensititre system allows determination of a minimum inhibitory concentration (MIC) for each drug in the panel. Laboratories using this method must establish breakpoints to provide a categorical interpretation of S or R.

- Of the 33 laboratories reporting second-line drug results (with the exception of streptomycin), only 7 (21%) tested all three second-line injectable drugs and at least one fluoroquinolone needed to confidently define XDR TB. The second-line injectable drugs are amikacin, kanamycin, and capreomycin. Fluoroquinolones include ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin.

Descriptive Information about Participant Laboratories

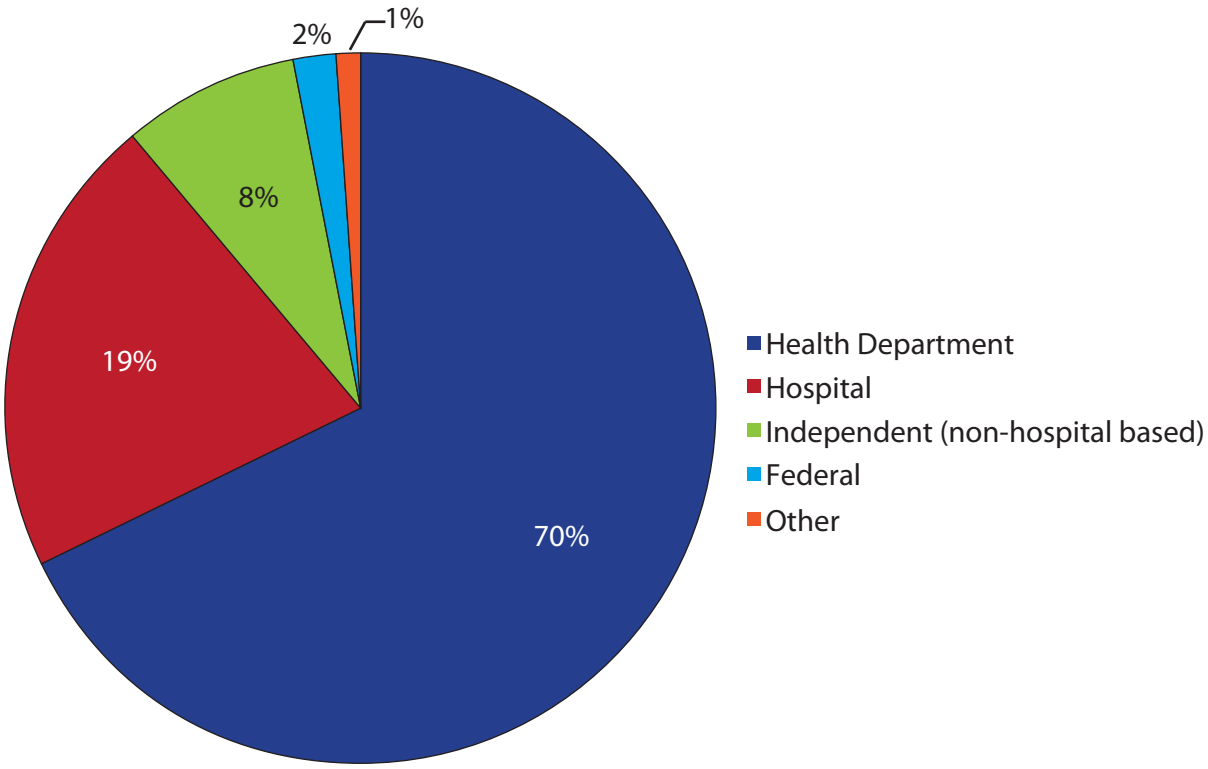
Primary Classification

This report contains the DST results submitted to CDC by survey participants at 87 laboratories in 41 states.

The participants were asked to indicate the primary classification of their laboratory (Figure 1). MPEP participants self-classified as

- 61 (70%): Health department (city, country, state, regional, or district laboratory)
- 16 (19%): Hospital laboratory
- 7 (8%): Independent (e.g., commercial, commercial manufacturer of reagents, reference laboratory [non-governmental affiliated])
- 2 (2%): Federal government laboratory
- 1 (1%): Other (quality control manufacturer)

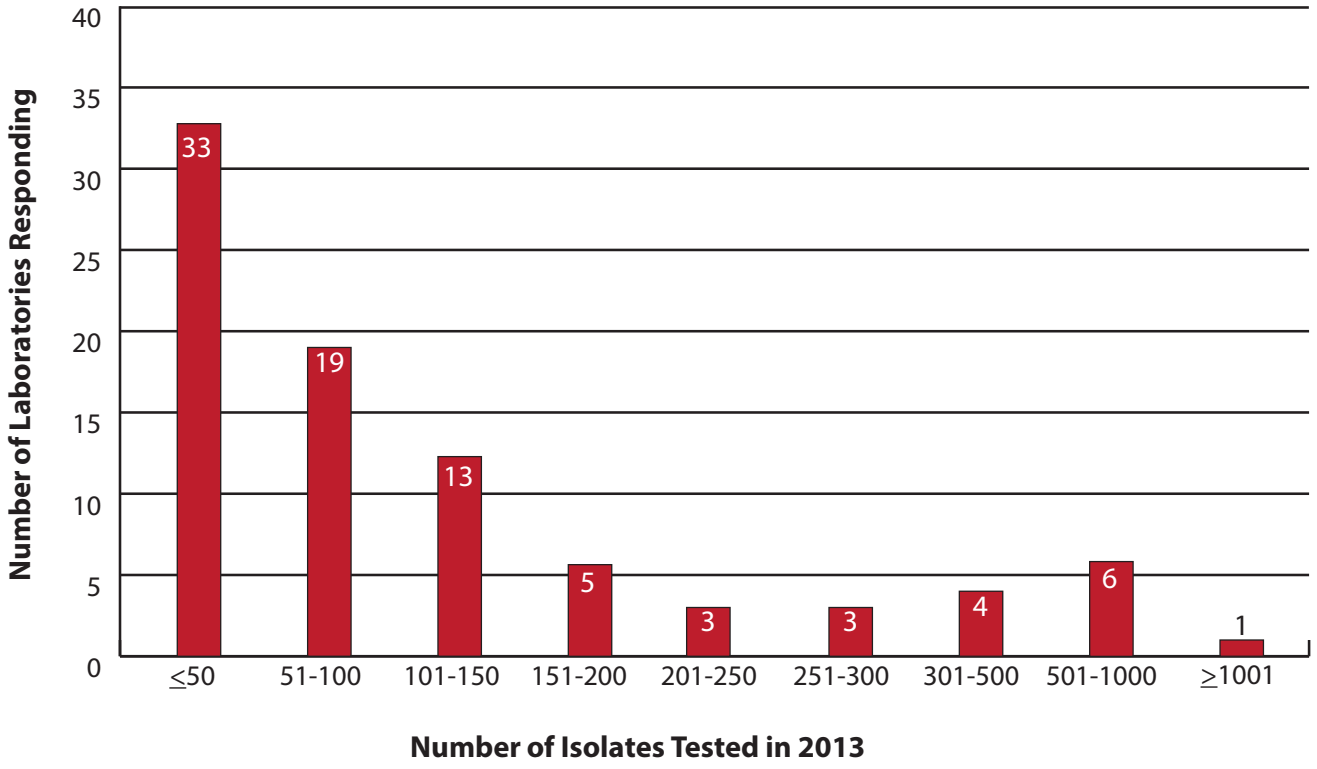
Figure 1. Primary Classification of Participating Laboratories



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 87 participants in 2013 (excluding isolates used for quality control) is shown in Figure 2. In 2013, the counts ranged from 0 to 1234 tests and participants at 33 (38%) laboratories reported testing 50 or fewer DST isolates per year. Laboratories with low MTBC DST volumes are encouraged to consider referral of testing because of concerns about maintaining proficiency [3].

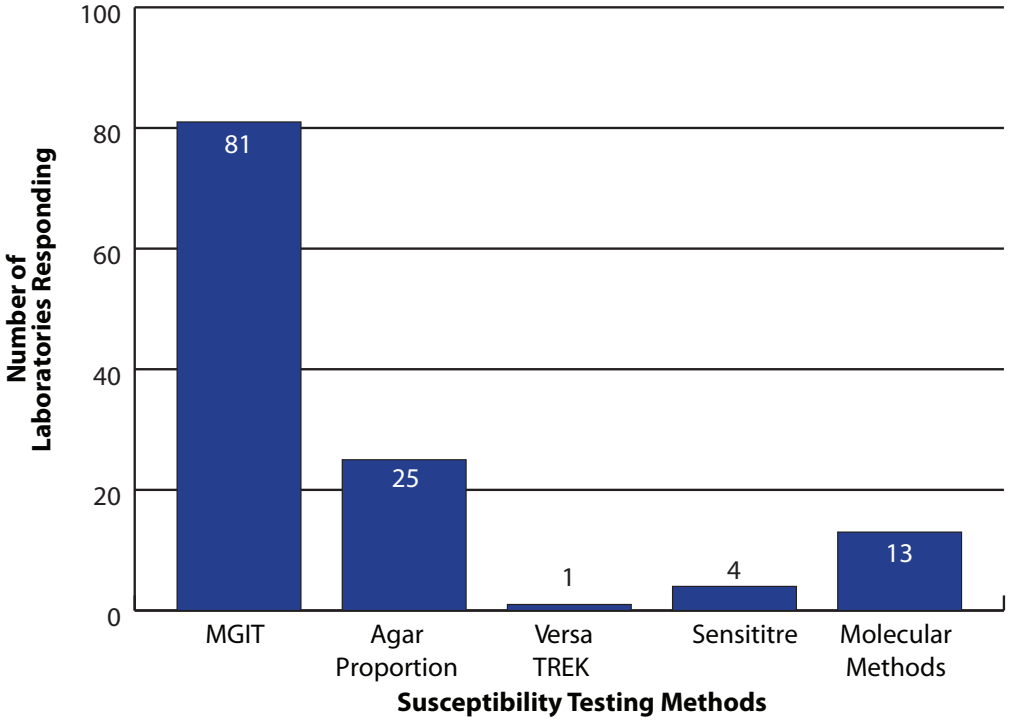
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in 2013 (n=87)



MTBC DST Methods Used by Participants

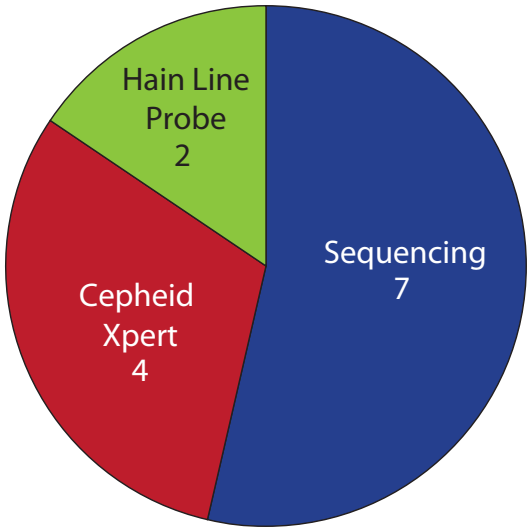
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 55 (63%) laboratories reported only one method, 28 laboratories reported two methods, and 3 laboratories noted three susceptibility methods. One laboratory noted four susceptibility methods, including multiple molecular methods.

Figure 3. MTBC Susceptibility Test Method Used by Participants (n=124)



The summary of molecular methods reported is shown in Figure 4. The method used by most laboratories indicating molecular methods was DNA sequencing (54%), including pyrosequencing and Sanger sequencing. Four laboratories reported results for the Cepheid Xpert MTB/RIF assay and two laboratories used the line probe assays Genotype MTBDRplus and MTBDRsl by Hain Lifescience.

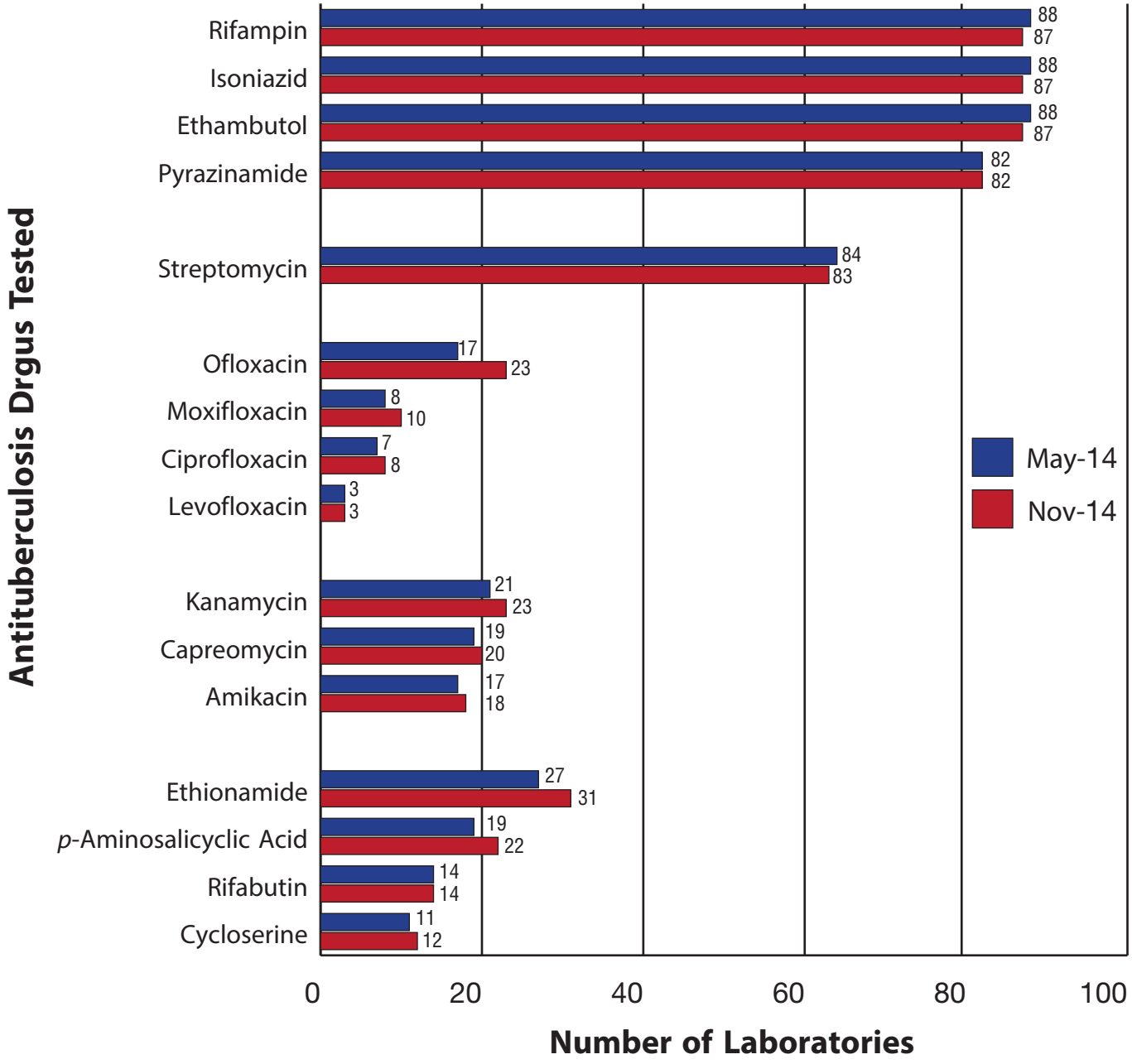
Figure 4. Molecular Method Used (n=13)



Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in November 2014 is shown in Figure 5. The number of antituberculosis drugs tested by participants in May 2014 is shown for comparison. CLSI recommends testing a full panel of first-line drugs (rifampin [RMP], isoniazid [INH], ethambutol [EMB], and pyrazinamide [PZA])[1], because it represents a combination of tests that provides the clinician with comprehensive information related to the four-drug antituberculosis therapy currently recommended for most patients in the United States. All participants reported results for three of the first-line drugs—RMP, INH, and EMB—and 82 (94%) of the participants also reported results for PZA. There has been a slight increase in the number of laboratories testing second-line drugs since the May 2014 survey. The number of laboratories performing Sensititre, which includes second-line drugs, has also increased; however, the overall increase in second-line testing cannot only be attributable to use of this test.

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2014F

Expected Result: Resistant to RMP at 1.0 µg/ml by agar proportion

Rifampin

Rifampin (RMP) is a bactericidal drug used for the treatment of tuberculosis caused by organisms known or presumed to be susceptible to this drug. RMP's mechanism of action is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [4]. More than 96% of RMP-resistant isolates contain a mutation in the 81-bp central region of the *rpoB* gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase. The activity of RMP on RMP-resistant isolates depends on both the mutation position and the type of amino acid change.

CDC has recommended that RMP resistance detected by the Xpert MTB/RIF assay should be confirmed by DNA sequencing of genetic loci associated with RMP resistance (i.e., *rpoB*) [5]. The Xpert MTB/RIF assay may generate results that falsely indicate resistance when compared to growth-based methods because of the presence of silent mutations (i.e., nucleotide change but no corresponding change in amino acid) [6]. Sequencing of *rpoB* will allow for clarifying the result and understanding possible discordance between the rapid molecular and the growth-based testing results.

DNA sequence analysis of *rpoB* in Isolate 2014F revealed a C>T point mutation in codon 526 resulting in histidine being replaced by tyrosine (His526Tyr). Isolates with His526Tyr mutations consistently test as resistant to RMP in growth-based assays.

Among four methods, 109 results for RMP were reported for Isolate 2014F. This isolate was reported as **resistant** to RMP by method, as follows

- 100% (24/24) of the results when using AP;
- 100% (80/80) of the results when using MGIT;
- 100% (4/4) of the results when using Sensititre; and
- 100% (1/1) of the results when using VersaTREK.

All eleven (100%) of the molecular results reported for RMP noted that a mutation was detected.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2014F are listed in Tables, 3, 4, and 5.

Table 3. Isolate 2014F—Participant results for first-line DST

Results by Method for First-Line Drugs												
	AP			MGIT			Sensititre			VersaTREK		
Drug	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	0	24	24	0	80	80	0	4	4	0	1	1
Isoniazid-Low	23	0	23	80	0	80	4	0	4	1	0	1
Isoniazid-High	22	0	22	29	0	29	4	0	4	1	0	1
Ethambutol	24	0	24	80	0	80	4	0	4	1	0	1
Pyrazinamide				77	4	81				1	0	1

Note—S=susceptible, R=resistant

Table 4. Isolate 2014F—Participant results for second-line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	S	R	Total	S	R	Total	S	R	Total
Streptomycin	23	0	23	49	0	49*	4	0	4
Ofloxacin	16	0	16	3	0	3	4	0	4
Ciprofloxacin	7	0	7	2	0	2			
Levofloxacin	1	0	1	2	0	2			
Moxifloxacin	3	0	3	4	0	4	3	0	3
Amikacin	12	0	12	3	0	3	4	0	4
Kanamycin	18	0	18	1	0	1	4	0	4
Capreomycin	16	0	16	5	0	5			
Ethionamide	23	0	23	5	0	5	4	0	4
Rifabutin	2	6	8	0	2	2	0	4	4
Cycloserine	9	0	9	1	0	1	3	0	3
p-Aminosalicylic acid	18	0	18	1	0	1	4	0	4

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for STR by MGIT.

Table 5. Isolate 2014F—Participant results for molecular testing

	Molecular Testing		
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	11	0	11
Isoniazid	0	7	7
Ethambutol	0	2	2
Pyrazinamide	0	3	3
Ofloxacin	0	3	3
Ciprofloxacin	0	3	3
Levofloxacin	0	3	3
Moxifloxacin	0	3	3
Amikacin	0	3	3
Kanamycin	0	3	3
Capreomycin	0	3	3
Ethionamide	0	1	1
Rifabutin	1	0	1

Isolate 2014G

Expected Result: Resistant to OFL at 2.0 µg/ml by agar proportion

Ofloxacin

Fluoroquinolones (FQ) are the most commonly prescribed antibiotic class in the United States due to their activity against various types of bacteria. Currently, they are an important class of drugs used to treat tuberculosis resistant to first-line drugs but have the potential to become part of future first-line regimens [7]. In the United States, resistance to FQ is relatively uncommon in strains of *M. tuberculosis* susceptible to first-line drugs, but treatment with a FQ before diagnosis with tuberculosis is associated with a high risk of FQ resistance and diagnostic delays [7, 8].

Resistance to FQ has been mainly attributed to mutations in a 21-bp region of the *M. tuberculosis gyrA* gene, often called the quinolone resistance determining region (QRDR), particularly at codons 90 and 94 [2, 9]. DNA sequence of *gyrA* in Isolate 2014G revealed a C>T point mutation in codon 90 of *gyrA* resulting in alanine being replaced with valine (Ala90Val).

Among three methods, 21 results for OFL were reported for Isolate 2014G. This isolate was reported as **resistant** to OFL by method, as follows

- 100% (14/14) of the results when using AP;
- 100% (3/3) of the results when using MGIT; and
- 100% (4/4) of the results when using Sensititre.

Participating laboratories also reported results for other FQ drugs (i.e., ciprofloxacin, levofloxacin, and moxifloxacin) for Isolate 2014G; 95% (18/19) of results noted resistance to these additional FQ.

The Ala90Val mutation in the *gyrA* gene was detected by the three laboratories that reported molecular testing for FQ.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2014G are listed in Tables 6, 7, and 8.

Table 6. Isolate 2014G—Participant results for first-line DST

Results by Method for First-Line Drugs												
	AP			MGIT			Sensititre			VersaTREK		
Drug	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	22	0	22	80	0	80	4	0	4	0	1	1
Isoniazid–Low	21	0	21	80	0	80	4	0	4	1	0	1
Isoniazid–High	19	1	20	28	0	28	4	0	4	1	0	1
Ethambutol	22	0	22	80	0	80	4	0	4	1	0	1
Pyrazinamide				81	0	81				1	0	1

Note—S=susceptible, R=resistant

Table 7. Isolate 2014G—Participant results for second-line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	S	R	Total	S	R	Total	S	R	Total
Streptomycin	20	0	20	50	0	50	4	0	4
Ofloxacin	0	14	14	0	3	3	0	4	4
Ciprofloxacin	0	5	5	0	2	2			
Levofloxacin	0	1	1	0	2	2			
Moxifloxacin	0	3	3	1	2	3	0	3	3
Amikacin	11	0	11	3	0	3	4	0	4
Kanamycin	17	0	17				4	0	4
Capreomycin	15	0	15	4	0	4			
Ethionamide	20	0	20	4	0	4	4	0	4
Rifabutin	8	0	8	2	0	2	4	0	4
Cycloserine	8	0	8	1	0	1	3	0	3
p-Aminosalicylic acid	15	0	15	1	0	1	4	0	4

Note—S=susceptible, R=resistant

Table 8. Isolate 2014G—Participant results for molecular testing

	Molecular Testing		
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	0	10	10
Isoniazid	0	7	7
Ethambutol	0	2	2
Pyrazinamide	1*	2	3
Ofloxacin	3	0	3
Ciprofloxacin	3	0	3
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	0	3	3
Kanamycin	0	3	3
Capreomycin	0	3	3
Ethionamide	0	1	1
Rifabutin	0	1	1

* One laboratories noted the mutation detected was a silent mutation

Isolate 2014H

Expected Result: Resistant to OFL at 2.0 µg/ml by agar proportion

Ofloxacin

DNA sequence of *gyrA* in Isolate 2014H revealed a T>C point mutation in codon 91 of *gyrA* resulting in serine being replaced with proline (Ser91Pro). The Ser91Pro mutation has been associated with FQ resistance [2, 10].

Among three methods, 21 results for OFL were reported for Isolate 2014H. This isolate was reported as **resistant** to OFL by method, as follows

- 100% (14/14) of the results when using AP;
- 100% (3/3) of the results when using MGIT; and
- 100% (4/4) of the results when using Sensititre.

Participating laboratories reported results for other FQ drugs (i.e., ciprofloxacin, levofloxacin, and moxifloxacin) for Isolate 2014H as well. 100% (19/19) resistance was noted across methods to these additional FQ.

The Ser91Pro mutation in the *gyrA* gene was detected by the three laboratories that reported molecular testing for FQ.

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2014H are listed in Tables 9, 10, and 11.

Table 9. Isolate 2014H—Participant results for first-line DST

Results by Method for First-Line Drugs												
	AP			MGIT			Sensititre			VersaTREK		
Drug	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Rifampin	22	0	22	80	0	80	4	0	4	1	0	1
Isoniazid–Low	21	0	21	79	1	80	3	1	4	1	0	1
Isoniazid–High	19	1	20	28	0	28	3	1	4	1	0	1
Ethambutol	22	0	22	80	0	80	4	0	4	1	0	1
Pyrazinamide				80	1	81				1	0	1

Note—S=susceptible, R=resistant

Table 10. Isolate 2014H—Participant results for second-line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	S	R	Total	S	R	Total	S	R	Total
Streptomycin	20	0	20	49	0	49	4	0	4
Ofloxacin	0	14	14	0	3	3	0	4	4
Ciprofloxacin	0	5	5	0	2	2			
Levofloxacin	0	1	1	0	2	2			
Moxifloxacin	0	3	3	0	3	3	0	3	3
Amikacin	11	0	11	3	0	3	4	0	4
Kanamycin	17	0	17				4	0	4
Capreomycin	15	0	15	4	0	4			
Ethionamide	20	0	20	4	0	4	4	0	4
Rifabutin	8	0	8	3	0	3	4	0	4
Cycloserine	8	0	8	1	0	1	3	0	3
p-Aminosalicylic acid	14	1	15	1	0	1	4	0	4

Note—S=susceptible, R=resistant

Table 11. Isolate 2014H—Participant results for molecular testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	0	7	7
Ethambutol	0	2	2
Pyrazinamide	1*	2	3
Ofloxacin	3	0	3
Ciprofloxacin	3	0	3
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	0	3	3
Kanamycin	0	3	3
Capreomycin	0	3	3
Ethionamide	0	1	1
Rifabutin	0	1	1

* One laboratory noted the mutation detected was a silent mutation

Isolate 2014I

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml by agar proportion

Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculosis drug. It is a cornerstone of regimens used to treat both TB disease and latent infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *katG* gene [2, 4]. The target of activated INH is enoyl-acyl-carrier protein reductase (*inhA*) which is required for mycolic acid biosynthesis. The two mechanisms accounting for the majority of INH resistance occur in these two loci [2, 4, 9]. The most common mechanism, mutations in *katG*, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inhA* gene which are generally associated with low-level resistance to INH and are less frequent than *katG* mutations. DNA sequence analysis of *inhA* and *katG* of Isolate 2014I revealed a T>A point mutation at codon 315 in the *katG* locus resulting in serine being replaced by threonine (Ser315Thr); *inhA* was wild-type (i.e., no mutations were detected).

The recommended critical concentration and additional higher concentrations for testing INH using the AP method are, respectively, 0.2 µg/ml and 1.0 µg/ml. The equivalent concentrations for MGIT and VersaTREK are 0.1 µg/ml and 0.4 µg/ml.

For Isolate 2014I, 109 INH results were reported for the critical concentration. This isolate was reported **resistant** to INH at the critical concentration by method, as follows

- 100% (24/24) of the results when using AP;
- 100% (80/80) of the results when using MGIT;
- 100% (4/4) of the results when using Sensititre; and
- 100% (1/1) of the results when using VersaTREK.

Seventy-two (99%) results were reported as **resistant** at the higher concentrations of INH as well.

Eight (100%) laboratories reported the detection of a mutation for INH by molecular testing.

Rifampin

DNA sequence analysis of *rpoB* in Isolate 2014I revealed a C>A point mutation in codon 526 resulting in histidine being replaced by asparagine (His526Asn). Unlike the His526Tyr mutation detected in Isolate 2014F that consistently tests as resistant in growth-based methods, isolates with the His526Asn mutation are associated with low-level RMP resistance and often test as susceptible in growth-based assays [11, 12]. Low-level resistance can be operationally defined as the presence of a mutation which increases the RMP MIC above the MIC seen in RMP-susceptible isolates that do not have a detectable mutation in *rpoB*. The clinical impact of these disputed mutations will depend on the frequency of their occurrence, which may vary from one setting to another [13]. However, the diminished RMP activity suggests that clinical outcome in patients being treated with RMP-based standard therapy could be impacted. This is an area that needs continued investigation through clinical studies.

For Isolate 2014I, 109 RMP results were reported. The isolate was reported **susceptible** to RMP by method, as follows

- 100% (24/24) of the results when using AP;
- 100% (80/80) of the results when using MGIT;
- 100% (4/4) of the results when using Sensititre; and
- 100% (1/1) of the results when using VersaTREK.

Of the 11 results reported for RMP molecular testing, 91% (10/11) reported that a mutation was detected for RMP.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2014I are listed in Tables 12, 13, and 14.

Table 12. Isolate 2014I—Participant results for first-line DST

Results by Method for First-Line Drugs											
	AP			MGIT			Sensititre			VersaTREK		
Drug	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	24	0	24	80	0	80	4	0	4	1	0	1
Isoniazid–Low	0	24	24	0	80	80	0	4	4	0	1	1
Isoniazid–High	0	24	24	1	43	44	0	4	4	0	1	1
Ethambutol	24	0	24	80	0	80	4	0	4	1	0	1
Pyrazinamide				78	2	80				1	0	1

Note—S=susceptible, R=resistant

Table 13. Isolate 2014I—Participant results for second-line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	S	R	Total	S	R	Total	S	R	Total
Streptomycin	23	0	23	51	0	51	4	0	4
Ofloxacin	16	0	16	3	0	3	3	1	4
Ciprofloxacin	7	0	7	2	0	2			
Levofloxacin	1	0	1	2	0	2			
Moxifloxacin	3	0	3	4	0	4	3	0	3
Amikacin	12	0	12	3	0	3	4	0	4
Kanamycin	18	0	18	1	0	1	4	0	4
Capreomycin	16	0	16	5	0	5			
Ethionamide	23	0	23	5	0	5	4	0	4
Rifabutin	8	0	8	2	0	2	4	0	4
Cycloserine	8	1	9	0	1	1	3	0	3
p-Aminosalicylic acid	18	0	18	1	0	1	4	0	4

Note—S=susceptible, R=resistant

Table 14. Isolate 2014I—Participant results for molecular testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	10	1	11
Isoniazid	8	0	8
Ethambutol	0	2	2
Pyrazinamide	0	3	3
Ofloxacin	0	3	3
Ciprofloxacin	0	3	3
Levofloxacin	0	3	3
Moxifloxacin	0	3	3
Amikacin	0	3	3
Kanamycin	0	3	3
Capreomycin	0	3	3
Ethionamide	0	1	1
Rifabutin	1	0	1

Isolate 2014J

Expected Result: Resistant to AMK at 4.0 µg/ml, CAP at 10.0 µg/ml, and KAN at 5.0 µg/ml by agar proportion

Second-line injectable drugs

The second-line injectable drugs include a cyclic-peptide antibiotic, capreomycin (CAP), and two aminoglycoside antibiotics, kanamycin (KAN) and amikacin (AMK). All three drugs affect protein translation, share a molecular target, and bind at similar locations. As a result, cross-resistance has frequently been detected [2, 14]. The most common mechanism of cross-resistance to all three drugs is an A1401G mutation in the *rrs* gene coding for 16S rRNA [14]. Isolate 2014J was resistant to all of the second-line injectable drugs (AMK, KAN, and CAP) by the AP method and DNA sequence analysis of the *rrs* gene revealed the A1401G mutation.

For Isolate 2014J, 58 results were reported for AMK, KAN, and CAP. The isolate was reported **resistant** to all three second-line injectables by method, as follows

- 95% (41/43) of the results when using AP;
- 100% (7/7) of the results when using MGIT; and
- 100% (8/8) of the results when using Sensititre.

This A1401G mutation in the *rrs* gene was detected by the three laboratories that reported molecular testing for the second-line injectable drugs.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2014J are listed in Tables 15, 16, and 17.

Table 15. Isolate 2014J—Participant results for first-line DST

Results by Method for First-Line Drugs												
	AP			MGIT			Sensititre			VersaTREK		
Drug	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	22	0	22	80	0	80	4	0	4	1	0	1
Isoniazid–Low	21	0	21	79	1	80	4	0	4	1	0	1
Isoniazid–High	20	0	20	29	0	29	4	0	4	1	0	1
Ethambutol	21	1	22	80	0	80	4	0	4	1	0	1
Pyrazinamide				80	0	80				1	0	1

Note—S=susceptible, R=resistant

Table 16. Isolate 2014J—Participant results for second-line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	S	R	Total	S	R	Total	S	R	Total
Streptomycin	20	0	20	50	0	50	4	0	4
Ofloxacin	14	0	14	3	0	3	3	1	4
Ciprofloxacin	5	0	5	2	0	2			
Levofloxacin	1	0	1	2	0	2			
Moxifloxacin	3	0	3	3	0	3	3	0	3
Amikacin	0	11	11	0	3	3	0	4	4
Kanamycin	2	15	17				0	4	4
Capreomycin	0	15	15	0	4	4			
Ethionamide	20	0	20	4	0	4	4	0	4
Rifabutin	8	0	8	2	0	2	4	0	4
Cycloserine	8	0	8	1	0	1	3	0	3
p-Aminosalicylic acid	15	0	15	1	0	1	4	0	4

Note—S=susceptible, R=resistant

Table 17. Isolate 2014J—Participant results for molecular testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	0	7	7
Ethambutol	0	2	2
Pyrazinamide	0	3	3
Ofloxacin	0	3	3
Ciprofloxacin	0	3	3
Levofloxacin	0	3	3
Moxifloxacin	0	3	3
Amikacin	3	0	3
Kanamycin	3	0	3
Capreomycin	3	0	3
Ethionamide	0	1	1
Rifabutin	0	1	1

Equivalent Critical Concentrations

(Concentrations listed as µg/ml)

Agar Proportion

	7H10 agar	7H11 agar
First-line Drugs		
Isoniazid	0.2 and 1.0*	0.2 and 1.0*
Rifampin	1.0	1.0
Ethambutol	5.0 and 10.0*	7.5
Pyrazinamide	Not recommended	Not recommended
Second-line Drugs		
Streptomycin	2.0 and 10.0	2.0 and 10.0
Amikacin	4.0	-†
Capreomycin	10.0	10.0
Kanamycin	5.0	6.0
Levofloxacin	1.0	-†
Moxifloxacin	0.5	0.5
Ofloxacin	2.0	2.0
Ethionamide	5.0	10.0
Rifabutin	0.5	0.5
p-Aminosalicylic acid	2.0	8.0

NOTE: Critical concentrations as indicated in CLSI M24-A2 document [1]

* The higher concentration of INH and EMB should be tested as second-line drugs after resistance at the critical concentration is detected.

† Breakpoints for establishing susceptibility have not be determined

Broth Based Media

	MGIT	VersaTREK
First-line Drugs		
Isoniazid	0.1 (and 0.4*)	0.1 (and 0.4*)
Rifampin	1.0	1.0
Ethambutol	5.0	5.0 (and 8.0*)
Pyrazinamide	100.0	300.0
Second-line Drug		
Streptomycin	1.0 (and 4.0*)	

NOTE: Critical concentrations as indicated in applicable manufacturer package inserts

* The higher concentration of INH, EMB, and STR should be tested after resistance at the critical concentration is detected.

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