Centers for Disease Control and Prevention
Model Performance Evaluation Program

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Report of Results
August 2016

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

CS 273955-A

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for August 2016 Samples Survey

Purpose

The purpose of this report is to present results of the U.S. Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for Mycobacterium tuberculosis complex (MTBC) drug susceptibility testing survey sent to participants in August 2016.

Report Content

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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

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in August 2016 are shown in the tables below. Although CDC recommends broth-based methods for routine first-line DST of MTBC isolates, the results obtained by the reference agar proportion method (except for pyrazinamide, in which MGIT was performed) are shown in Table 1. Molecular results obtained by using DNA sequencing are listed in Table 2 [2].

Table 1. Expected Growth-based Results for August 2016 Survey

Growth-based Results					
	First-Line Drugs				Second-Line Drugs
	RMP	INH	EMB	PZA	Resistant to:
2016F	S	S	S	S	AMK, KAN, CAP
2016G	R	S	S	S	
2016H	S	S	S	S	
2016I	S	R	S	S	ETA
2016J	S	R	S	S	STR, ETA

Note—S=susceptible, R=resistant

Table 2. Expected Molecular Results for August 2016 Survey

Mutations Detected in Loci Associated with Resistance			
	rpoB	*katG*	*rrs*
2016F			A1401G
2016G	His526Tyr		
2016H			
2016I		Ser315Thr	
2016J		Ser315Thr	

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 2016 MTBC isolates F, G, H, I, and J in this report.

- The source of data in all tables and figures is the August 2016 MPEP MTBC DST survey.
- The number of reported results (S represents susceptible and R represents resistant) for each drug are indicated in each table.
- 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.
- 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 79 (the number of participating laboratories). This report contains all results reported by participating laboratories.
- Critical concentrations of antituberculosis drugs used for each DST method are listed 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.
- For 30 laboratories reporting second-line drug results (with the exception of streptomycin), nine (30%) 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

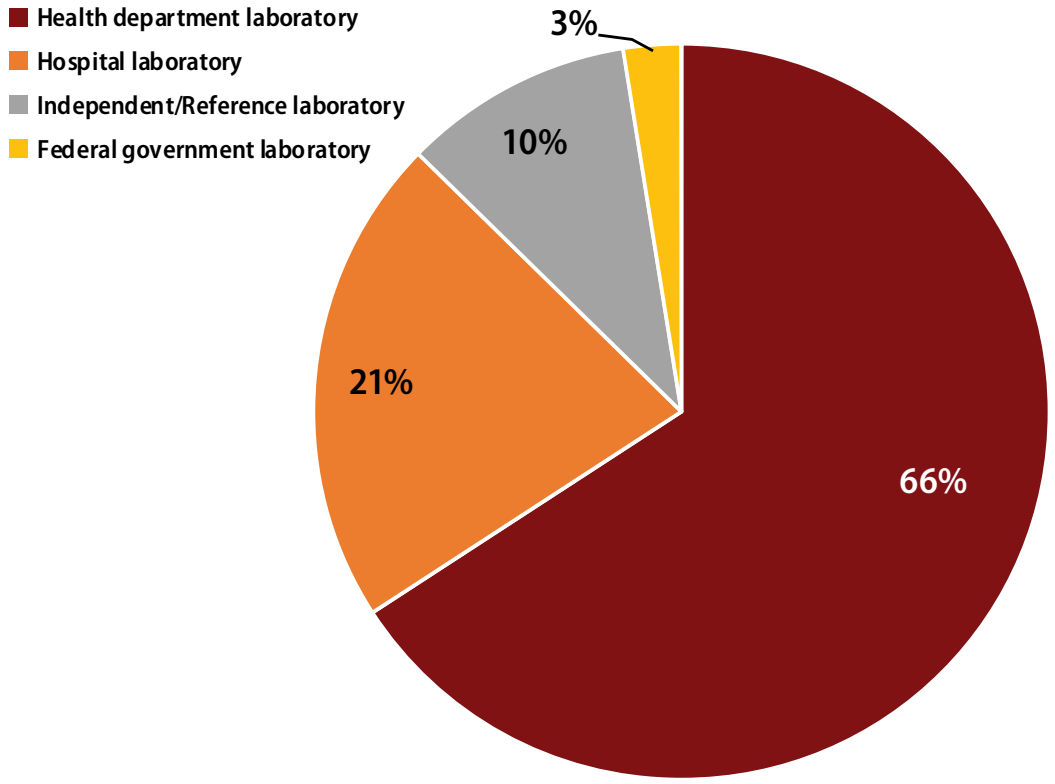
Primary Classification

This report contains DST results submitted to CDC by survey participants at 79 laboratories in 37 states.

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

- **52 (66%):** Health department laboratory (e.g., local, county, state)
- **17 (21%):** Hospital laboratory
- **8 (10%):** Independent / Reference laboratory (non-hospital based)
- **2 (3%):** Federal government laboratory

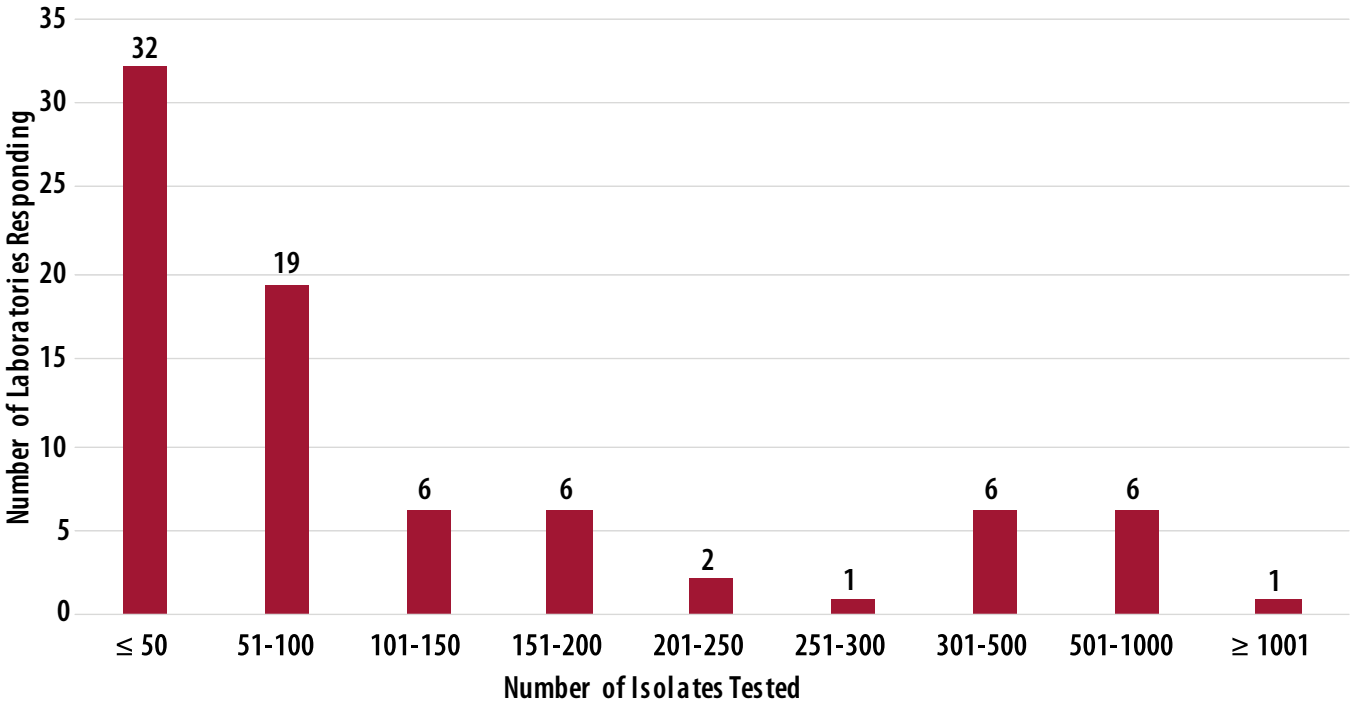
Figure 1. Primary Classification of Participating Laboratories, August 2016



Annual Number of *Mycobacterium tuberculosis* Complex Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 79 participants in 2015 (excluding isolates used for quality control) is shown in Figure 2. In 2015, the counts ranged from 0 to 1416 tests. Participants at 32 (41%) 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].

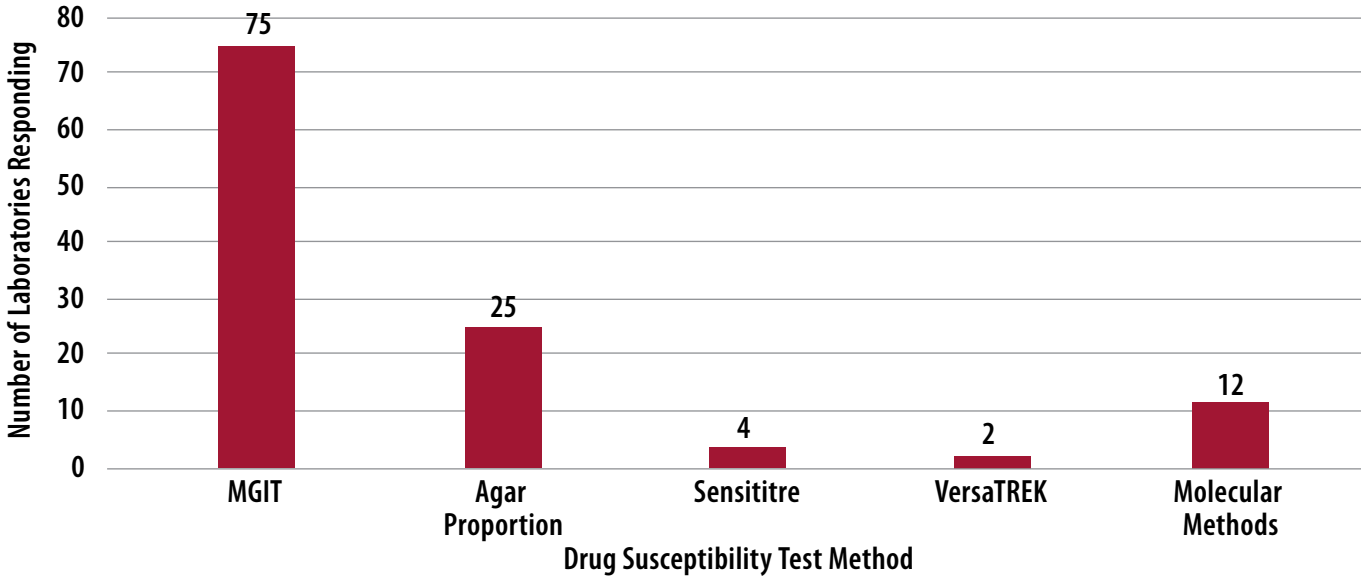
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=79)



Mycobacterium tuberculosis Complex Drug Susceptibility Test Methods Used by Participants

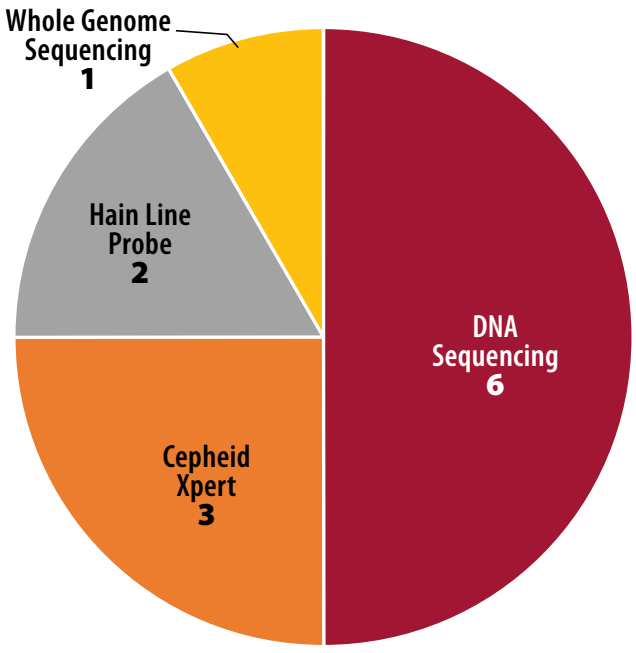
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 44 (57%) laboratories reported results for only one method, 31 laboratories reported two methods, and four laboratories noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=118)



Molecular methods reported by ten participants are shown in Figure 4. The method used most frequently by laboratories was DNA sequencing (50%), including pyrosequencing and Sanger sequencing. Three laboratories reported results for the Cepheid Xpert MTB/RIF assay, two reported use of the line probe assays Genotype MTBDRplus and MTBDRsl by Hain Lifescience, and one reported results from whole genome sequencing.

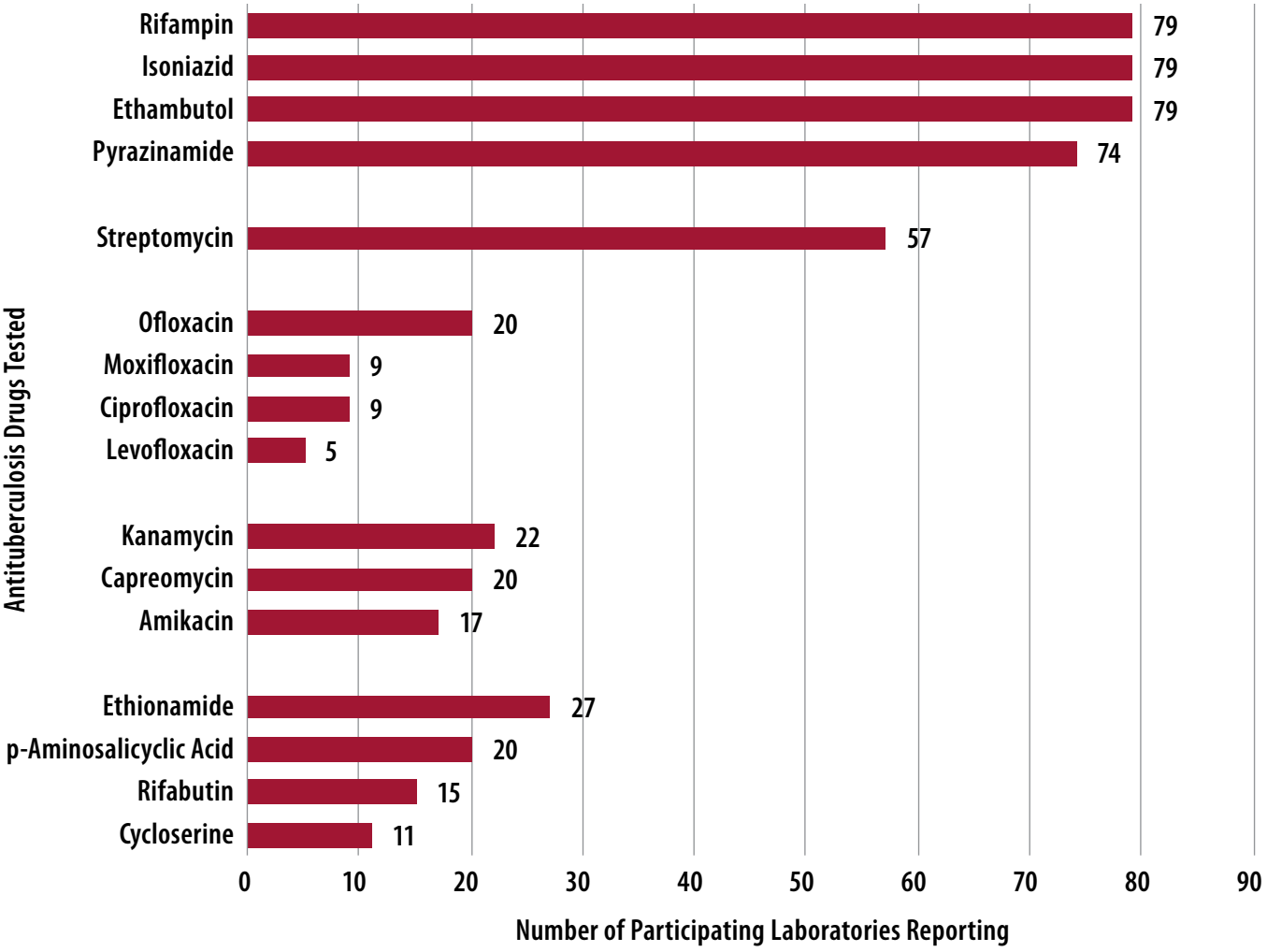
Figure 4. Molecular Method Reported (n=12)



Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the August 2016 survey is shown in Figure 5. 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 74 (94%) of the participants also reported results for PZA. The number of laboratories testing second-line drugs has stayed relatively stable since the May 2014 survey despite the overall decrease in participating laboratories.

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2016F

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 Injectables

The second-line injectable drugs include a cyclic-peptide antibiotic, capreomycin (CAP), and two aminoglycoside antibiotics, kanamycin (KAN) and amikacin (AMK). All three drugs inhibit protein synthesis and the primary mechanisms of resistance occur due to mutations in the following genes: *rrs* for AMK; *rrs* and *eis* for KAN; and *rrs* and *tlyA* for CAP [8]. Since these drugs share a molecular target and bind at similar locations, cross-resistance has frequently been observed for mutations in the *rrs* that codes for 16S rRNA [2, 12]. The most common rrs mutation for cross-resistance to all three drugs is the A1401G point mutation [12].

Isolate 2016F was resistant to all of the second-line injectable drugs (AMK, KAN, and CAP) by the AP method and DNA sequence analysis of *rrs* revealed the A1401G mutation.

For Isolate 2016F, 56 results were reported for AMK, KAN, and CAP. The isolate was reported **resistant** to the three second-line injectables by method, as follows:

Amikacin

- **100% (12/12)** of the results when using AP
- **100% (2/2)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Capreomycin

- **81% (13/16)** of the results when using AP
- **100% (3/3)** of the results when using MGIT

Kanamycin

- **89% (16/18)** of the results when using AP
- **100% (1/1)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

This A1401G mutation in the *rrs* gene was detected by five (100%) laboratories that reported molecular testing for AMK and KAN and four (80%) of the laboratories that reported molecular results for CAP.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2016F are listed in Tables, 3, 4, and 5.

Table 3. Isolate 2016F—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	20	0	20	72	1	73	4	0	4	2	0	2
Isoniazid-Low	21	0	21	73	0	73	4	0	4	2	0	2
Isoniazid-High	21	0	21	22	0	22	4	0	4	2	0	2
Ethambutol	20	1	21	73	0	73	4	0	4	2	0	2
Pyrazinamide				73	0	73				1	0	1

Note—S=susceptible, R=resistant

Table 4. Isolate 2016F—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	22	0	22	44	0	44	3	0	3
Ofloxacin	14	0	14	3	0	3	2	0	2
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	2	0	2
Amikacin	0	12	12	0	2	2	0	2	2
Kanamycin	2	16	18	0	1	1	0	2	2
Capreomycin	3	13	16	0	3	3			
Ethionamide	20	0	20	3	0	3	3	0	3
Rifabutin	8	0	8	3	0	3	3	0	3
Cycloserine	8	1	9				2	0	2
***p*-Aminosalicylic acid**	16	0	16				3	0	3

Note—S=susceptible, R=resistant

Table 5. Isolate 2016F—Participant Results for Molecular Testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	0	9	9
Ethambutol	0	5	5
Pyrazinamide	0	3	3
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	5	0	5
Kanamycin	5	0	5
Capreomycin	4	1	5
Ethionamide	0	2	2
Rifabutin	0	2	2

Isolate 2016G

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

Rifampin

Rifampin (RMP) is a bactericidal drug used as part of a standard first-line regimen for the treatment of TB. RMP's mechanism of action is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [4]. The primary mechanism of resistance is a mutation within the 81-bp central region of the *rpo*B gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase [5]. Mutations in codons 531, 526, and 516 (*E. coli* numbering system corresponding to 450, 445, and 435 in MTBC) are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance [4, 5]. The activity of RMP on isolates with *rpoB* mutations 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 be confirmed by DNA sequencing of *rpoB* [13]. The Xpert MTB/RIF assay could generate results that falsely indicate resistance when compared to growth-based methods because of the presence of silent/synonymous mutations [14]. Sequencing of *rpoB* will allow for clarification of the result and understanding of possible discordance between rapid molecular and growth-based testing results.

DNA sequence analysis of *rpoB* in Isolate 2016G revealed a C>G point mutation in codon 526 resulting in wild-type histidine being replaced by tyrosine (His526Tyr). Isolates with His526Tyr mutations consistently test resistant to RMP in growth-based assays.

Among four methods, 99 results for RMP were reported for Isolate 2016G. This isolate was reported as **resistant** to RMP by method, as follows:

- **100% (21/21)** of the results when using AP
- **100% (72/72)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

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

Pyrazinamide

Pyrazinamide (PZA) is an important first-line drug for treatment of TB and is used with INH and RMP. The addition of this drug shortens TB treatment from the previous 9–12 months to 6 months because it kills a population of persistent bacilli in acidic pH environments within the lesions that are not killed by other drugs. PZA-resistant MTBC strains lose pyrazinamidase activity and resistance to PZA is usually caused by nucleotide changes scattered throughout the *pncA* gene. There may be additional mechanisms of resistance to PZA that are still unknown[15], but issues with false resistance to PZA have been reported as well [16] and remain a potential concern.

For Isolate 2016G, DNA sequencing of the *pncA* gene did not reveal a mutation.

Isolate 2016G was expected to be susceptible to PZA; however, of those testing PZA, resistance was reported by:

- **25% (18/72)** of the results when using MGIT
- **0% (0/1)** of the results when using VersaTREK

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2016G are listed in Tables 6, 7, and 8.

Table 6. Isolate 2016G—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	21	21*	0	72	72†	0	4	4	0	2	2
Isoniazid–Low	22	0	22*	72	0	72†	4	0	4	2	0	2
Isoniazid–High	22	0	22*	23	0	23†	4	0	4	2	0	2
Ethambutol	22	0	22*	72	0	72†	3	1	4	2	0	2
Pyrazinamide				54	18	72†				1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for RMP, INH, and EMB by AP.

† In addition, one laboratory reported contamination for RMP, INH, EMB, and PZA by MGIT.

Table 7. Isolate 2016G—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*	43	2	45†	3	0	3
Ofloxacin	14	0	14*	3	0	3	2	0	2
Ciprofloxacin	7	0	7*	1	0	1			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	4	0	4	2	0	2
Amikacin	11	0	11*	3	0	3	3	0	3
Kanamycin	18	0	18*	1	0	1	2	0	2
Capreomycin	15	0	15*	4	0	4	1	0	1
Ethionamide	20	0	20*	4	0	4	3	0	3
Rifabutin	0	7	7*	0	3	3	0	3	3
Cycloserine	8	1	9				2	0	2
***p*-Aminosalicylic acid**	16	0	16*				3	0	3

Note—S=susceptible, R=resistant

*In addition, one laboratory reported no growth for STR, OFL, CIP, AMK, KAN, CAP, ETA, RBT, and PAS by AP.

†In addition, one laboratory reported no growth for STR by MGIT.

Table 8. Isolate 2016G—Participant Results for Molecular Testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	12	0	12
Isoniazid	0	9	9
Ethambutol	0	5	5
Pyrazinamide	0	3	3
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	5	5
Kanamycin	0	5	5
Capreomycin	0	5	5
Ethionamide	0	2	2
Rifabutin	2	0	2

Isolate 2016H

Expected Result: Susceptible to all first- and second-line drugs by agar proportion

Isolate 2016H is susceptible to all first- and second-line drugs.

Most (98%) results were reported susceptible for this isolate across all methods.

Two laboratories reported the detection of a neutral mutation for EMB; no other mutations were detected using molecular methods.

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2016H are listed in Tables 9, 10, and 11.

Table 9. Isolate 2016H—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	20	0	20	73	0	73	4	0	4	2	0	2
Isoniazid–Low	20	0	20	72	1	73	4	0	4	2	0	2
Isoniazid–High	20	0	20	22	0	22	4	0	4	2	0	2
Ethambutol	21	0	21	71	2	73	4	0	4	2	0	2
Pyrazinamide				70	3	73				1	0	1

Note—S=susceptible, R=resistant

Table 10. Isolate 2016H—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	22	0	22	43	1	44	3	0	3
Ofloxacin	14	0	14	3	0	3	2	0	2
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	2	0	2
Amikacin	12	0	12	2	0	2	3	0	3
Kanamycin	18	0	18	1	0	1	2	0	2
Capreomycin	16	0	16	3	0	3	1	0	1
Ethionamide	14	5	19	3	0	3	1	2	3
Rifabutin	8	0	8	3	0	3	3	0	3
Cycloserine	9	0	9				2	0	2
p-Aminosalicylic acid	15	1	16				3	0	3

Note—S=susceptible, R=resistant

Table 11. Isolate 2016H—Participant Results for Molecular Testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	0	11	11
Isoniazid	0	9	9
Ethambutol	2	3	5
Pyrazinamide	0	3	3
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	5	5
Kanamycin	0	5	5
Capreomycin	0	5	5
Ethionamide	0	2	2
Rifabutin	0	2	2

Isolate 2016I

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml and ETA at 5.0 µg/ml by agar proportion

Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculosis drug and is a cornerstone of regimens used to treat tuberculosis (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 (encoded by the inhA gene); this binding inhibits cell wall mycolic acid biosynthesis. There are two mechanisms that account for the majority of INH resistance [2, 4, 5]. 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. Approximately 10–15% of isolates found to be INH resistant have no mutations detected in either of these loci. Numerous loci have been investigated to identify additional genes correlated with INH resistance. The *fabG1* (also known as *mabA*) gene, like *inhA*, is involved in mycolic acid biosynthesis and at least one mutation in this region has been associated with low-level INH resistance [6, 7]. In MTBC, *ahpC* codes for an alkyl hydroperoxide reductase that is associated with resistance to reactive oxygen and reactive nitrogen intermediates; consequently it was initially believed that mutations in the promoter region could be surrogate markers for INH resistance [4].

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2016I revealed a T>A point mutation at codon 315 in the *katG* locus resulting in wild-type serine being replaced by threonine (Ser315Thr); *inhA*, *fabG1* and *ahpC* were wild-type (i.e., no mutations were detected).

The recommended critical concentration and additional higher concentrations for testing INH using the AP method are 0.2 µg/ml and 1.0 µg/ml, respectively. The equivalent concentrations for MGIT and VersaTREK are 0.1 µg/ml and 0.4 µg/ml [1].

For Isolate 2016I, 99 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (21/21)** of the results when using AP
- **100% (72/72)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Sixty-three (98%) results were reported as resistant at the higher concentrations of INH.

For the nine molecular results reported for INH, all (100%) detected a mutation.

Ethionamide

Ethionamide (ETA) is a structural analog of INH. ETA, like INH, targets *inhA*, an enzyme involved in mycolic acid biosynthesis [10]. Resistance to INH and ETA can occur by mutations in the promoter region of the *inhA* gene which are generally associated with low-level resistance to INH. Mutations in *ethA* also confer resistance to ETA, without concomitant resistance to INH [10].

Sequencing of the *inhA* gene revealed wild-type (i.e., no mutations were detected) for Isolate 2016I and sequencing analysis of *ethA* was not performed.

Issues with reproducibility of DST results for ETA have been reported [11] and remain a potential concern.

For Isolate 2016I, 27 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **5% (1/21)** of the results when using AP
- **0% (0/3)** of the results when using MGIT
- **0% (0/3)** of the results when using Sensititre

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2016I are listed in Tables 12, 13, and 14.

Table 12. Isolate 2016I—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	21	0	21*	72	0	72†	4	0	4	2	0	2
Isoniazid–Low	0	21	21*	0	72	72†	0	4	4	0	2	2
Isoniazid–High	1	20	21*	0	37	37†	0	4	4	0	2	2
Ethambutol	22	0	22*	72	0	72†	4	0	4	2	0	2
Pyrazinamide				72	1	73†				1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for RMP, INH, and EMB by AP.

† In addition, one laboratory reported contaminated for RMP, INH, EMB, and PZA by MGIT.

Table 13. Isolate 2016I—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*	43	0	43†	3	0	3
Ofloxacin	15	0	15	4	0	4	2	0	2
Ciprofloxacin	8	0	8	1	0	1			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	3	0	3
Amikacin	12	0	12	2	0	2	3	0	3
Kanamycin	19	0	19	1	0	1	2	0	2
Capreomycin	16	0	16	3	0	3	1	0	1
Ethionamide	20	1	21	3	0	3	3	0	3
Rifabutin	8	0	8	3	0	3	3	0	3
Cycloserine	9	0	9				2	0	2
***p*-Aminosalicylic acid**	17	0	17				3	0	3

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for STR by AP.

† In addition, one laboratory reported contaminated for STR by MGIT

Table 14. Isolate 2016I—Participant Results for Molecular Testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	0	11	11
Isoniazid	9	0	9
Ethambutol	2	3	5
Pyrazinamide	0	3	3
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	5	5
Kanamycin	0	5	5
Capreomycin	0	5	5
Ethionamide	1	1	2
Rifabutin	0	2	2

Isolate 2016J

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml, STR at 2.0 µg/ml, and ETA at 5.0 µg/ml by agar proportion

Isoniazid

As previously noted, resistance to INH most commonly occurs due to mutations in the *katG* gene or the promoter region of the *inhA* gene, however, mutations in *fabG1* can also cause resistance. DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2016J revealed a T>A point mutation at codon 315 in the *katG* locus resulting in wild-type serine being replaced by threonine (Ser315Thr); *inhA*, *fabG1* and *ahpC* were wild-type (i.e., no mutations were detected).

The recommended critical concentration and additional higher concentrations for testing INH using the AP method are 0.2 µg/ml and 1.0 µg/ml, respectively. The equivalent concentrations for MGIT and VersaTREK are 0.1 µg/ml and 0.4 µg/ml [1].

For Isolate 2016J, 101 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (22/22)** of the results when using AP
- **100% (73/73)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Sixty-five (100%) results were reported as resistant at the higher concentrations of INH.

For the nine molecular results reported for INH, all (100%) detected a mutation.

Streptomycin

Streptomycin (STR) belongs to the aminoglycoside class of drugs and its primary mechanism of action is to inhibit protein synthesis by preventing the initiation of translation by binding to the 16s rRNA[4, 5]. In MTBC, the genetic basis of the majority of resistance to STR is usually due to mutations in *rrs* or *rpsL*[5, 8]. CLSI recommended testing STR as a second-line drug based on American Thoracic Society's categorization of STR as a second-line drug for treatment due to increased resistance in many parts of the world [1, 9].

Among three methods, 71 results for STR were reported for Isolate 2016J. This isolate was reported as **resistant** to STR by method, as follows:

- **100% (24/24)** of the results when using AP
- **98% (43/44)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre

Ethionamide

As previously noted, resistance to INH and ETA can occur by mutations in the promoter region of the *inhA* gene which are generally associated with low-level resistance to INH. Sequencing of the *inhA* gene revealed wild-type (i.e., no mutations were detected) for Isolate 2016J and sequencing analysis of *ethA* was not performed.

Issues with reproducibility of DST results for ETA have been reported [11] and remain a potential concern.

For Isolate 2016J, 27 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **76% (16/21)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **67% (2/3)** of the results when using Sensititre

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2016J are listed in Tables 15, 16, and 17.

Table 15. Isolate 2016J—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	73	0	73	4	0	4	2	0	2
Isoniazid–Low	0	22	22	0	73	73	0	4	4	0	2	2
Isoniazid–High	0	22	22	0	37	37	0	4	4	0	2	2
Ethambutol	22	1	23	70	2	72	4	0	4	2	0	2
Pyrazinamide				73	0	73				1	0	1

Note—S=susceptible, R=resistant

Table 16. Isolate 2016J—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	0	24	24	1	43	44	0	3	3
Ofloxacin	15	0	15	4	0	4	2	0	2
Ciprofloxacin	8	0	8	1	0	1			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	3	0	3
Amikacin	12	0	12	2	0	2	3	0	3
Kanamycin	19	0	19	1	0	1	2	0	2
Capreomycin	16	0	16	3	0	3	1	0	1
Ethionamide	5	16	21	0	3	3	1	2	3
Rifabutin	9	0	9	3	0	3	3	0	3
Cycloserine	8	1	9				1	0	1
***p*-Aminosalicylic acid**	17	0	17				3	0	3

Note—S=susceptible, R=resistant

Table 17. Isolate 2016J—Participant Results for Molecular Testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	0	11	11
Isoniazid	9	0	9
Ethambutol	0	5	5
Pyrazinamide	0	3	3
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	5	5
Kanamycin	0	5	5
Capreomycin	0	5	5
Ethionamide	1	1	2
Rifabutin	0	2	2

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 Drugs		
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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