Centers for Disease Control and Prevention

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program

Report of Results
February 2018

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

CS 295068-A

Mycobacterium tuberculosis Complex Drug Susceptibility Testing MPEP Report for February 2018 Samples Survey

Explanations of figures for accessibility is found in Appendix 1: Accessible Explanation of Figures on page 36.

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 February 2018.

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 Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in February 2018 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 DNA sequencing are listed in Table 2 [2].

Table 1. Expected Growth-based Results for February 2018 Survey

Note—S=susceptible, R=resistant

Isolate	RMP	INH	EMB	PZA	Second-line Drugs Resistant to:
2018A	S	R	S	S	
2018B	S	R	S	S	OFL, ETA
2018C	S	R	S	S	
2018D	S	R	S	S	ETA
2018E	S	R	S	S	ETA

Table 2. Expected Molecular Results (Mutations Detected in Loci Associated with Resistance) for February 2018 Survey

Isolate	*rpoB*	*katG*	*inhA*	*fabG1*	*gyrB*
2018A	None Detected	Ser315Thr	None Detected	None Detected	None Detected
2018B	Phe514Phe	None Detected	C-15T	None Detected	Arg485Cys
2018C	None Detected	(Gene deleted)	None Detected	None Detected	None Detected
2018D	Arg528Arg	None Detected	None Detected	Leu203Leu	None Detected
2018E	None Detected	None Detected	C-15T	None Detected	None Detected

Abbreviations and Acronyms

Abbreviations & Acronyms	Definition
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*
nt	nucleotide
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 2018 MTBC isolates A, B, C, D, and E in this report.

- The source of data in all tables and figures is the February 2018 MPEP MTBC DST survey.
- The number of reported results for each drug are indicated in each table.
- First-line and second-line drugs have been separated for each isolate. Streptomycin is classified as a second-line drug for this report.
- Separate tables for molecular testing are included.
- For 508 Compliance, individual tables have been created for each method by isolate. Please ensure that data are compared across methods as well as within.
- 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 75 (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 susceptible or resistant.
- For 27 laboratories reporting second-line drug results (with the exception of streptomycin), nine (33%) 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.
- For participant result tables for first- and second-line DST that have drug-method totals equal to 0, results were not received or the test was not performed.

Descriptive Information about Participant Laboratories

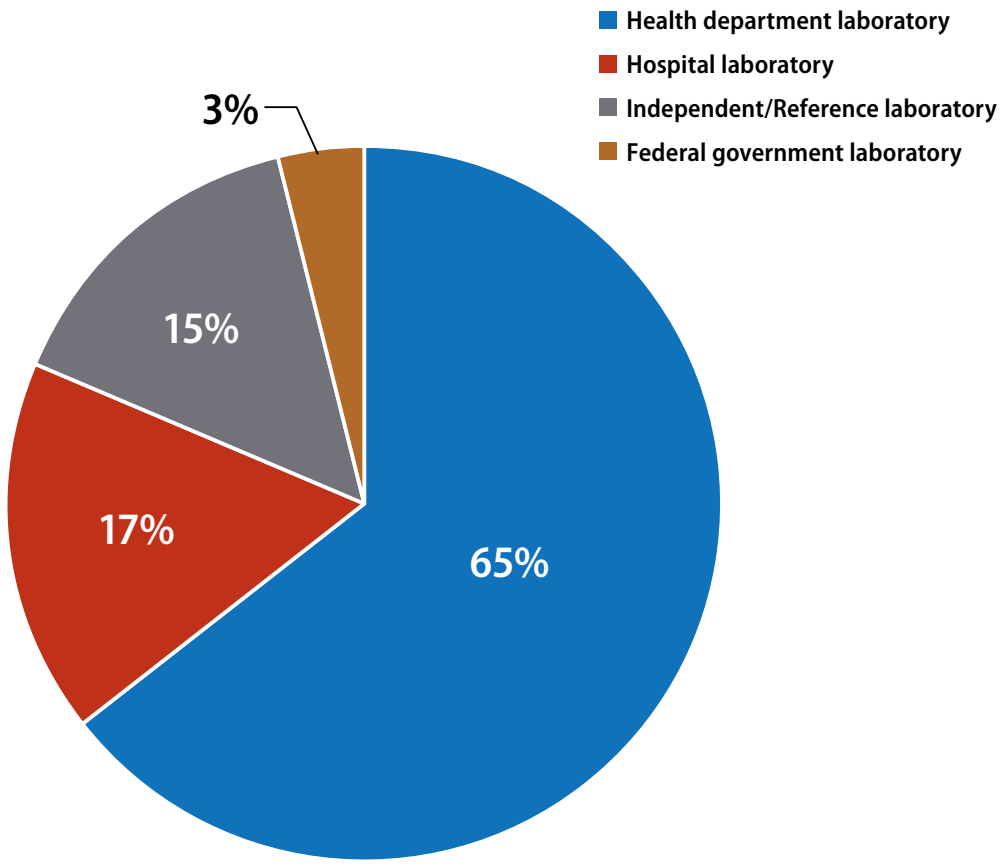
Primary Classification

This report contains DST results submitted to CDC by survey participants at 75 laboratories in 35 states.

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

- **49 (65%):** Health department laboratory (e.g., local, county, state)
- **13 (17%):** Hospital laboratory
- **11 (15%):** Independent/Reference laboratory (non-hospital based)
- **2 (3%):** Federal government laboratory

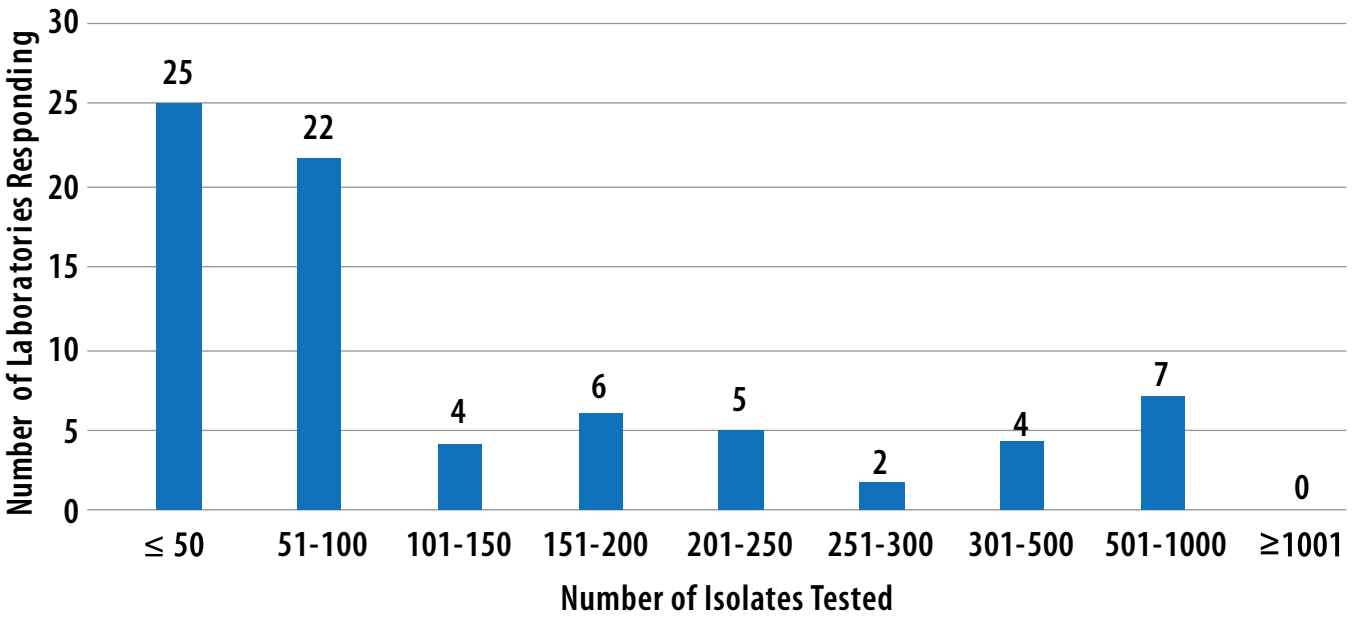
Figure 1. Primary Classification of Participating Laboratories, February 2018



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 75 participants in 2017 (excluding isolates used for quality control) is shown in Figure 2. In 2017, the counts ranged from 0 to 926 tests. Participants at 25 (33%) 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].

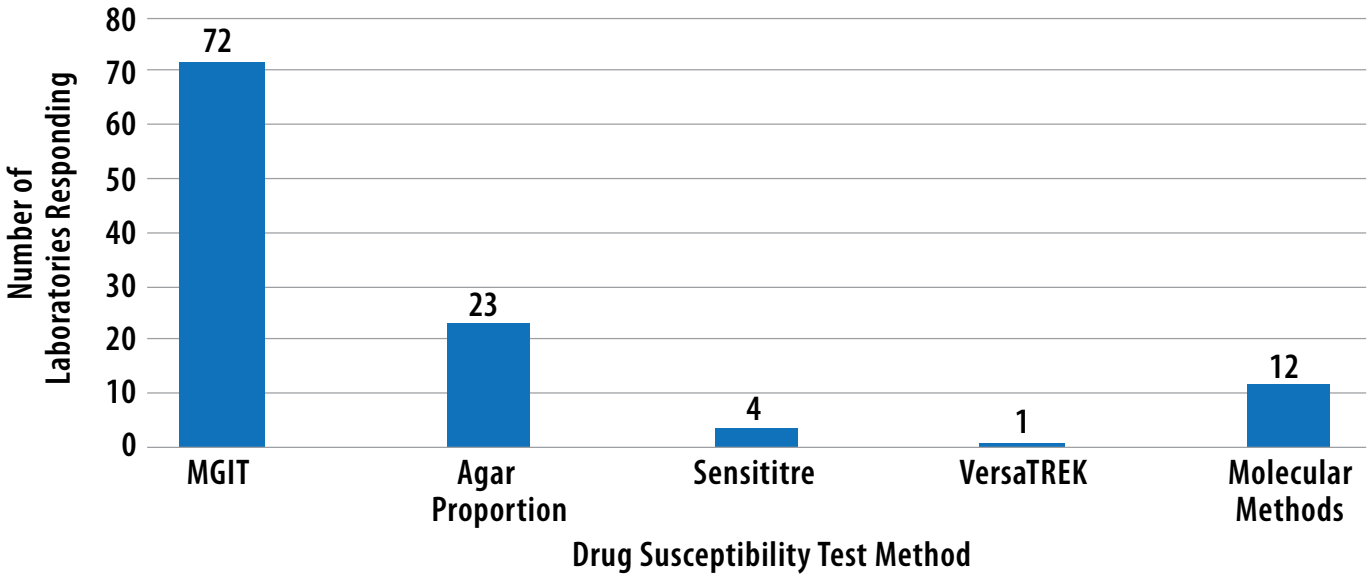
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=75)



MTBC DST Methods Used by Participants

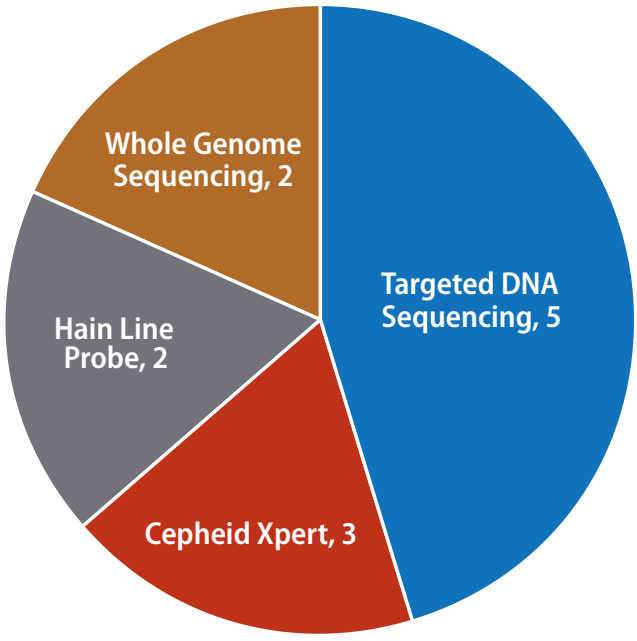
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 44 (59%) laboratories reported results for only one method, 25 laboratories reported two methods, and six laboratories noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=112)



Molecular methods reported by twelve participants are shown in Figure 4. The method used most frequently by laboratories was targeted DNA sequencing (45%), 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 MTBDR*plus* and MTBDR*sl* by Hain Lifescience, and two reported results from whole genome sequencing.

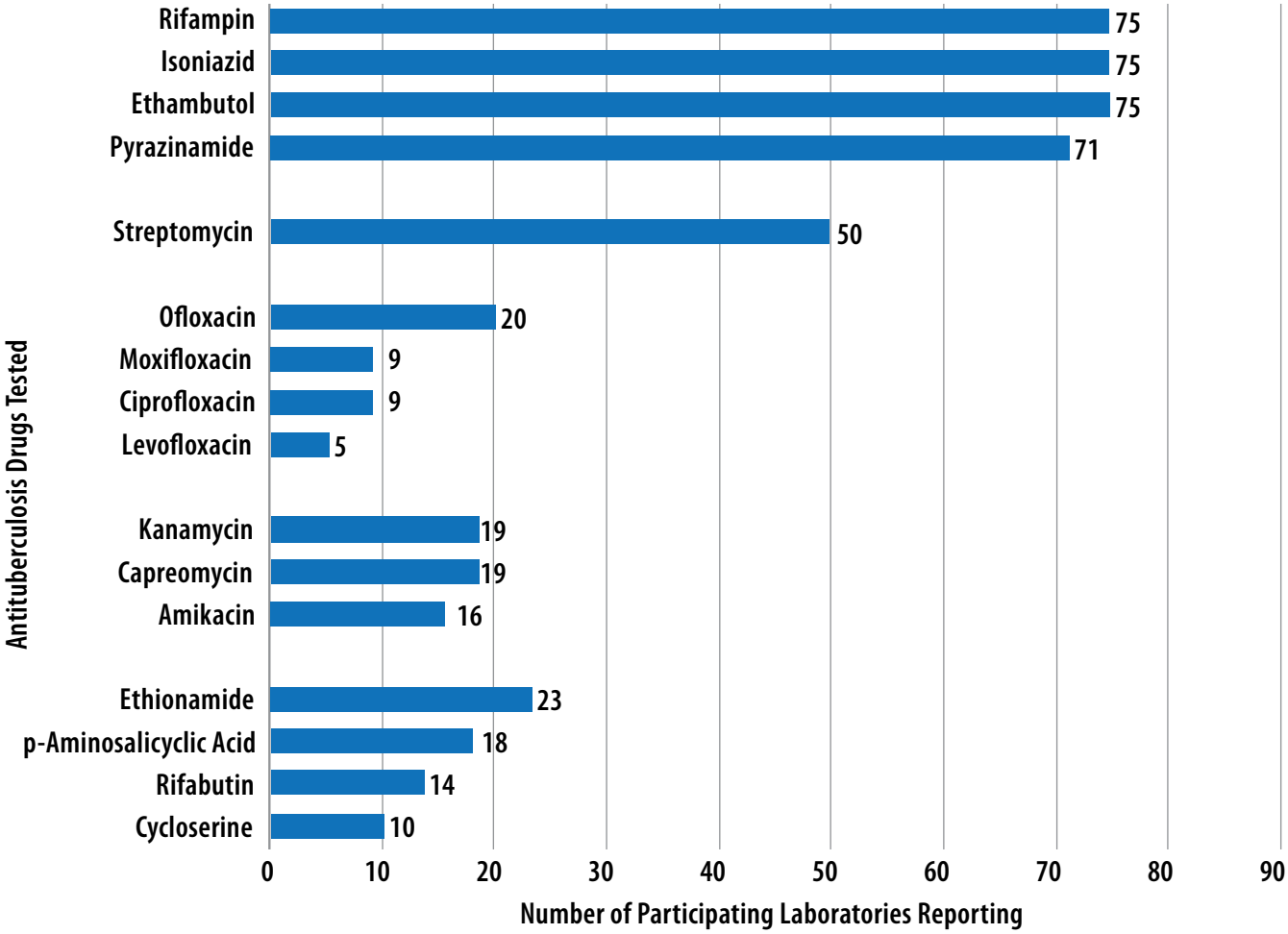
Figure 4. Molecular Method Reported (n=12)



Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the February 2018 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. All participants reported results for three of the first-line drugs (RMP, INH, and EMB) and 71 (95%) also reported results for PZA by growth-based DST methods.

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2018A

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 and is a cornerstone of regimens used to treat TB disease and latent infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *kat*G gene [2, 4]. The target of activated INH is enoyl-acyl-carrier protein reductase (encoded by the *inh*A 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 *kat*G, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inh*A gene, which are generally associated with low-level resistance to INH and are less frequent than *kat*G 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 *fab*G1 (also known as *mab*A) gene, like *inh*A, 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, *ahp*C 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 *inh*A, *kat*G, *fab*G1, and *ahp*C of Isolate 2018A revealed a T>A point mutation at codon 315 in the *kat*G locus resulting in wild-type serine being replaced by threonine (Ser315Thr); *inh*A, *fab*G1, and *ahp*C 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 2018A, 98 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (21/21)** of the results when using AP
- **99% (71/72)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (1/1)** of the results when using VersaTREK

Sixty-four (98%) results were reported as **resistant** at the higher concentrations of INH. Only 39 laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a second DST method.

Of the 9 molecular results reported for INH, all (100%) laboratories reported detection of a mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2018A are listed in Tables 3–10.

One laboratory noted contamination for at least one antituberculosis drug tested for Isolate 2018A.

Table 3. Isolate 2018A—Participant Results for First-Line DST by AP

*One additional laboratory reported borderline for EMB by AP.

Drug	Susceptible	Resistant	Total
Rifampin	21	0	21
Isoniazid—Low	0	21	21
Isoniazid—High	0	21	21
Ethambutol	20	1	21*
Pyrazinamide	0	0	0

Table 4. Isolate 2018A—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	72	0	72
Isoniazid—Low	1	71	72
Isoniazid—High	1	38	39
Ethambutol	72	0	72
Pyrazinamide	71	1	72

Table 5. Isolate 2018A—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	0	4	4
Isoniazid—High	0	4	4
Ethambutol	4	0	4
Pyrazinamide	0	0	0

Table 6. Isolate 2018A—Participant Results for First-Line DST by VersaTREK

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	0	1	1
Isoniazid—High	0	1	1
Ethambutol	1	0	1
Pyrazinamide	0	0	0

Table 7. Isolate 2018A—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	6	14	20
Ofloxacin	14	0	14
Ciprofloxacin	8	0	8
Levofloxacin	1	0	1
Moxifloxacin	3	0	3
Amikacin	11	0	11
Kanamycin	16	0	16
Capreomycin	14	0	14
Ethionamide	4	14	18
Rifabutin	8	0	8
Cycloserine	8	0	8
***p*-Aminosalicylic acid**	15	0	15

Table 8. Isolate 2018A—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	23	12	35
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	0	3	3
Rifabutin	3	0	3
Cycloserine	0	0	0
***p*-Aminosalicylic acid**	0	0	0

Table 9. Isolate 2018A—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	0	2	2
Ofloxacin	2	0	2
Ciprofloxacin	0	0	0
Levofloxacin	1	0	1
Moxifloxacin	2	0	2
Amikacin	3	0	3
Kanamycin	2	0	2
Capreomycin	1	0	1
Ethionamide	1	1	2
Rifabutin	3	0	3
Cycloserine	2	0	2
***p*-Aminosalicylic acid**	3	0	3

Table 10. Isolate 2018A—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	9	0	9
Ethambutol	0	6	6
Pyrazinamide	0	4	4
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	4	4
Ethionamide	1	2	3
Rifabutin	0	3	3

Isolate 2018B

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

Isoniazid

DNA sequence analysis of *inh*A, *kat*G, *fab*G1, and *ahp*C of Isolate 2018B revealed a C>T point mutation at nucleotide position -15 of the promoter region of the *inh*A gene (C-15T); *kat*G, *fab*G1 and *ahp*C were wild-type (i.e., no mutations were detected). Mutations in the promoter region of the *inh*A gene are generally associated with low-level resistance to INH.

For Isolate 2018B, 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% (1/1)** of the results when using VersaTREK

One (2%) result was reported as **resistant** at the higher concentrations of INH. Only 39 laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a second DST method.

Of the 9 molecular results reported for INH, 8 (89%) laboratories reported detection of a mutation.

Ethionamide

Ethionamide (ETA) is a structural analog of INH. ETA, like INH, targets *inh*A, an enzyme involved in mycolic acid biosynthesis [8]. Resistance to INH and ETA can occur by mutations in the promoter region of the *inh*A gene which are generally associated with low-level resistance to INH. Mutations in *eth*A also confer resistance to ETA, without concomitant resistance to INH [8].

As noted above, a C>T point mutation was detected in the *inh*A gene (C-15T) for Isolate 2018B.

Issues with reproducibility of DST results for ETA have been reported [9] and remain a potential concern.

For Isolate 2018B, 23 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **89% (16/18)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

Of the three molecular results reported for ETA, all (100%) reported Mutation Detected.

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 *rpo*B 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 *rpo*B [10]. 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 [11]. Sequencing of *rpo*B will allow for clarification of the result and understanding of possible discordance between rapid molecular and growth-based testing results.

DNA sequence analysis of *rpo*B in Isolate 2018B revealed a C>T point mutation in codon 514 of the *rpo*B locus. However, this mutation does not result in an amino acid change; phenylalanine remains phenylalanine (Phe514Phe). This synonymous (i.e., silent) mutation in *rpo*B is not considered clinically significant and isolates with this mutation reliably test as RMP-susceptible in growth-based systems. The Xpert MTB/RIF will generate a report of RMP resistance detected for isolates with this mutation. Sequencing of *rpo*B will allow for clarifying the result and understanding discordance between the Xpert result and results from growth-based testing.

Among four methods, 98 results for RMP were reported for Isolate 2018B. This isolate was reported as **susceptible** to RMP by method, as follows:

- **100% (21/21)** of the results when using AP
- **99% (71/72)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (1/1)** of the results when using VersaTREK

Seven (58%) of the molecular results reported for RMP noted that a mutation was detected; five of which noted the silent mutation Phe514Phe. Five laboratories reported Mutation Not Detected, however this may be due to the detection of a silent mutation not associated with resistance.

Ofloxacin

Fluoroquinolones (FQ) are one of the most commonly prescribed classes of antibiotic in the United States due to their activity against various types of bacteria. They are an important class of drugs used to treat tuberculosis (TB) resistant to first-line drugs but also have the potential to become an important part of new TB regimens [12]. In the United States, resistance to FQ is relatively uncommon in strains of MTBC susceptible to first-line drugs, however prolonged treatment with a FQ (>10 days) before a diagnosis of TB is associated with a higher risk for FQ resistance and diagnostic delays [12, 13]. The primary mechanism of action of FQ is the inhibition of DNA synthesis [14] by inhibiting DNA gyrase. The enzyme DNA gyrase generates the activity for cleaving and resealing double-stranded DNA. This action is necessary for DNA replication, transcription, and recombination.

Resistance to FQ has mainly been attributed to point mutations in a 21-bp region of the MTBC *gyr*A gene, often called the quinolone resistance determining region (QRDR). These mutations, commonly occurring at codons 90, 91, and 94, prevent the drugs from effectively binding DNA gyrase [2, 5, 14]. Mutations in the *gyr*B gene have been noted with varying rates of resistance, but high-level resistance is less common without a concurrent *gyr*A mutation [14].

Heteroresistance is the result of varying levels of resistance within a population of MTBC due to the presence of sub-populations with differing nucleotides at a locus associated with drug resistance, resulting in both drug-resistant and drug-susceptible organisms [15, 16]. This phenomenon is not limited to FQ but is commonly noted with this class of drugs.

As newer FQ are assessed for use as antituberculosis drugs, it is important to determine cross-resistance between these and older FQ that are tested in growth-based DST methods. Studies suggest that there may not be full cross-resistance between ofloxacin (OFL), ciprofloxacin (CIP), levofloxacin (LEV), and moxifloxacin (MOX) at the defined critical concentrations and that low- and high-level resistance, as seen with INH, may be applicable to FQ as well, particularly MOX [17, 18].

DNA sequencing of *gyr*A was wild-type (i.e., no mutations were detected) but sequencing of *gyr*B in Isolate 2018B revealed a CGT>TGT point mutation in codon 485 of *gyr*B resulting in wild-type aspargine being replaced with cystine (Asp485Cys). The effects of this specific mutation on FQ resistance are not completely defined [19, 20].

Among three methods, 20 results for OFL were reported for Isolate 2018B. This isolate was reported as **resistant** to OFL by method, as follows

- **71% (10/14)** of the results when using AP
- **100% (4/4)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Participating laboratories also reported results for other FQ drugs (i.e., CIP, LEV, and MOX) for Isolate 2018B; 90% (18/20) of results noted **resistance** to these additional FQ. The isolate was reported **resistant** to three other fluoroquinolones by method, as follows:

Ciprofloxacin

- **88% (7/8)** of the results when using AP
- **100% (1/1)** of the results when using MGIT

Levofloxacin

- **100% (1/1)** of the results when using AP
- **100% (3/3)** of the results when using MGIT

Moxifloxacin

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

The mutation in the *gyr*B gene was detected by three (60%) laboratories that reported molecular testing for FQ drugs.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2018B are listed in Tables 11–18.

Table 11. Isolate 2018B—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	21	0	21
Isoniazid—Low	0	21	21
Isoniazid—High	21	0	21
Ethambutol	21	1	22
Pyrazinamide	0	0	0

Table 12. Isolate 2018B—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	71	1	72
Isoniazid—Low	0	72	72
Isoniazid—High	39	0	39
Ethambutol	72	0	72
Pyrazinamide	72	0	72

Table 13. Isolate 2018B—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	0	4	4
Isoniazid—High	3	1	4
Ethambutol	3	1	4
Pyrazinamide	0	0	0

Table 14. Isolate 2018B—Participant Results for First-Line DST by VersaTREK

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	0	1	1
Isoniazid—High	1	0	1
Ethambutol	1	0	1
Pyrazinamide	0	0	0

Table 15. Isolate 2018B—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	14	6	20
Ofloxacin	4	10	14
Ciprofloxacin	1	7	8
Levofloxacin	0	1	1
Moxifloxacin	1	2	3
Amikacin	11	0	11
Kanamycin	16	0	16
Capreomycin	15	0	15
Ethionamide	2	16	18
Rifabutin	8	0	8
Cycloserine	8	0	8
***p*-Aminosalicylic acid**	14	1	15

Table 16. Isolate 2018B—Participant Results for Second-Line DST by MGIT

* One additional laboratory reported borderline for MOX by MGIT.

Drug	Susceptible	Resistant	Total
Streptomycin	1	34	35
Ofloxacin	0	4	4
Ciprofloxacin	0	1	1
Levofloxacin	0	3	3
Moxifloxacin	0	2	2*
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	0	3	3
Rifabutin	3	0	3
Cycloserine	0	0	0
***p*-Aminosalicylic acid**	0	0	0

Table 17. Isolate 2018B—Participant Results for Second-Line DST by Sensititre

* One additional laboratory reported borderline for STR by Sensititre..

Drug	Susceptible	Resistant	Total
Streptomycin	1	1	2*
Ofloxacin	0	2	2
Ciprofloxacin	0	0	0
Levofloxacin	0	0	0
Moxifloxacin	0	2	2
Amikacin	3	0	3
Kanamycin	2	0	2
Capreomycin	2	0	2
Ethionamide	1	1	2
Rifabutin	3	0	3
Cycloserine	2	0	2
***p*-Aminosalicylic acid**	3	0	3

Table 18. Isolate 2018B—Participant Results for Molecular Testing

* Five of these laboratories noted the detection of a synonymous mutation Phe514Phe.

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	7*	5	12
Isoniazid	8	1	9
Ethambutol	0	6	6
Pyrazinamide	0	4	4
Ofloxacin	3	2	5
Ciprofloxacin	3	2	5
Levofloxacin	2	2	4
Moxifloxacin	2	2	4
Amikacin	0	5	5
Kanamycin	0	5	5
Capreomycin	0	4	4
Ethionamide	3	0	3
Rifabutin	1	2	3

Isolate 2018C

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

Isoniazid

DNA sequence analysis revealed a deletion of the entire *kat*G gene; *inh*A, *fab*G1, and *ahp*C were wild-type (i.e., no mutations were detected). Deletion of *kat*G has been associated with high-level INH resistance and the loss of catalase activity may be related to loss of virulence [14].

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 2018C, 97 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% (71/71)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (1/1)** of the results when using VersaTREK

Sixty-five (100%) results were reported as resistant at the higher concentrations of INH. Only 39 laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a second DST method.

Of the 7 molecular results reported for INH, 6 (86%) laboratories reported detection of a mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2018C are listed in Tables 19–26.

Two laboratories noted contamination for at least one antituberculosis drug tested for Isolate 2018C.

Table 19. Isolate 2018C—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	21	0	21
Isoniazid—Low	0	21	21
Isoniazid—High	0	21	21
Ethambutol	22	0	22
Pyrazinamide	0	0	0

Table 20. Isolate 2018C—Participant Results for First-Line DST by MGIT

* One additional laboratory reported borderline for PZA by MGIT.

Drug	Susceptible	Resistant	Total
Rifampin	72	0	72
Isoniazid—Low	0	71	71
Isoniazid—High	0	39	39
Ethambutol	71	0	71
Pyrazinamide	69	1	70*

Table 21. Isolate 2018C—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	0	4	4
Isoniazid—High	0	4	4
Ethambutol	4	0	4
Pyrazinamide	0	0	0

Table 22. Isolate 2018C—Participant Results for First-Line DST by VersaTREK

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	0	1	1
Isoniazid—High	0	1	1
Ethambutol	1	0	1
Pyrazinamide	0	0	0

Table 23. Isolate 2018C—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	20	0	20
Ofloxacin	14	0	14
Ciprofloxacin	8	0	8
Levofloxacin	1	0	1
Moxifloxacin	3	0	3
Amikacin	11	0	11
Kanamycin	16	0	16
Capreomycin	15	0	15
Ethionamide	18	0	18
Rifabutin	8	0	8
Cycloserine	8	0	8
***p*-Aminosalicylic acid**	15	0	15

Table 24. Isolate 2018C—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	35	0	35
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
***p*-Aminosalicylic acid**	0	0	0

Table 25. Isolate 2018C—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	3	0	3
Ofloxacin	2	0	2
Ciprofloxacin	0	0	0
Levofloxacin	1	0	1
Moxifloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	2	0	2
Capreomycin	1	0	1
Ethionamide	2	0	2
Rifabutin	3	0	3
Cycloserine	2	0	2
***p*-Aminosalicylic acid**	3	0	3

Table 26. Isolate 2018C—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	6	1	7
Ethambutol	0	7	7
Pyrazinamide	0	5	5
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	3	3
Rifabutin	0	3	3

Isolate 2018D

Expected Result: Resistant to INH at 0.2 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 *kat*G gene or the promoter region of the *inh*A gene, however, mutations in *fab*G1 and *ahp*C can also cause resistance. DNA sequence analysis of *inh*A, *kat*G, *fab*G1, and *ahp*C for Isolate 2018D revealed a G>A point mutation at codon 203 of *fab*G1 resulting in the synonymous/silent mutation Leu203Leu; *inh*A, *kat*G, and *ahp*C were wild-type (i.e., no mutations were detected).

Within *fab*G1, the silent/synonymous mutation (i.e., nucleotide change but no corresponding change in amino acid) Leu203Leu has been found to confer INH resistance through the formation of an alternative promoter, thereby increasing the transcriptional levels of *inh*A [7]. Although silent mutations were previously believed to not play a role in drug resistance, the Leu203Leu mutation demonstrates that silent mutations could be associated with resistance depending on the specific gene and the location of the mutation.

For Isolate 2018D, 93 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (17/17)** of the results when using AP
- **17% (12/71)** of the results when using MGIT
- **0% (0/4)** of the results when using Sensititre
- **100% (1/1)** of the results when using VersaTREK

No laboratories reported **resistant** at the higher concentrations of INH. Only 28 laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a second DST method.

Of the 9 molecular results reported for INH, 4 (44%) laboratories reported detection of a mutation.

Ethionamide

Resistance to INH and ETA can occur by mutations in the *fab*G1–*inh*A regulatory region, which are generally associated with low-level resistance to INH. Mutations in *eth*A also confer resistance to ETA, without concomitant resistance to INH [8].

Sequencing analysis of *eth*A was not performed and as previously noted, sequencing of the *inh*A gene revealed wild-type (i.e., no mutations were detected). The synonymous/silent mutation Leu203Leu was detected in the *fab*G1 locus for Isolate 2018D.

For Isolate 2018D, 21 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **81% (13/16)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

Rifampin

DNA sequence analysis of *rpo*B in Isolate 2018D revealed a C>T point mutation in codon 528 of the *rpo*B locus. However, this mutation does not result in an amino acid change; arginine remains arginine (Arg528Arg). Unlike the *fab*G1 silent mutation in this isolate that was associated with INH resistance, the Arg528Arg synonymous (i.e., silent) mutation in *rpo*B is not considered clinically significant and isolates with this mutation reliably test as RMP-susceptible in growth-based systems.

The Xpert MTB/RIF could generate a report of RMP resistance detected for isolates with this mutation. Sequencing of *rpo*B will allow for clarifying the result and understanding discordance between the Xpert result and those from growth-based testing.

Among four methods, 94 results for RMP were reported for Isolate 2018D. This isolate was reported as **susceptible** to RMP by method, as follows:

- **100% (18/18)** of the results when using AP
- **100% (71/71)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (1/1)** of the results when using VersaTREK

Of the eleven molecular results reported for RMP, 2 (18%) reported Mutation Detected; these 2 laboratories noted that a silent mutation was detected as a comment. Five laboratories reported Mutation Not Detected, however this may be due to the detection of a silent mutation not associated with resistance.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2018D are listed in Tables 27–34.

One laboratory noted contamination for at least one antituberculosis drug tested for Isolate 2018D.

Table 27. Isolate 2018D—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	18	0	18
Isoniazid—Low	0	17	17
Isoniazid—High	18	0	18
Ethambutol	18	1	19
Pyrazinamide	0	0	0

Table 28. Isolate 2018D—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	71	0	71
Isoniazid—Low	59	12	71
Isoniazid—High	28	0	28
Ethambutol	71	0	71
Pyrazinamide	72	0	72

Table 29. Isolate 2018D—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	4	0	4
Isoniazid—High	4	0	4
Ethambutol	4	0	4
Pyrazinamide	0	0	0

Table 30. Isolate 2018D—Participant Results for First-Line DST by VersaTREK

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	0	1	1
Isoniazid—High	1	0	1
Ethambutol	1	0	1
Pyrazinamide	0	0	0

Table 31. Isolate 2018D—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	17	0	17
Ofloxacin	12	0	12
Ciprofloxacin	7	0	7
Levofloxacin	1	0	1
Moxifloxacin	3	0	3
Amikacin	10	0	10
Kanamycin	14	0	14
Capreomycin	15	0	15
Ethionamide	3	13	16
Rifabutin	8	0	8
Cycloserine	8	0	8
***p*-Aminosalicylic acid**	13	0	13

Table 32. Isolate 2018D—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	34	0	34
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	0	3	3
Rifabutin	3	0	3
Cycloserine	0	0	0
***p*-Aminosalicylic acid**	0	0	0

Table 33. Isolate 2018D—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	2	1	3
Ofloxacin	2	0	2
Ciprofloxacin	0	0	0
Levofloxacin	1	0	1
Moxifloxacin	2	0	2
Amikacin	3	0	3
Kanamycin	2	0	2
Capreomycin	1	0	1
Ethionamide	1	1	2
Rifabutin	3	0	3
Cycloserine	1	0	1
***p*-Aminosalicylic acid**	3	0	3

Table 34. Isolate 2018D—Participant Results for Molecular Testing

* These two laboratories noted the detection of a synonymous mutation Arg528Arg.

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

Isolate 2018E

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

Isoniazid

DNA sequence analysis of *inh*A, *kat*G, *fab*G1, and *ahp*C of Isolate 2018E revealed a C>T point mutation at nucleotide position -15 of the promoter region of the *inh*A gene (C-15T); *kat*G, *fab*G1 and *ahp*C were wild-type (i.e., no mutations were detected). Mutations in the promoter region of the *inh*A gene are generally associated with low-level resistance to INH.

For Isolate 2018E, 95 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **94% (17/18)** 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% (1/1)** of the results when using VersaTREK

Four (6%) results were reported as resistant at the higher concentrations of INH. Only 40 laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a second DST method.

Of the 9 molecular results reported for INH, all (100%) laboratories reported detection of a mutation.

Ethionamide

As previously noted in Isolate 2018B, resistance to INH and ETA can occur by mutations in the promoter region of the *inh*A gene which are generally associated with low-level resistance to INH. A point mutation (C-15T) was detected in the promoter region for Isolate 2018E.

For Isolate 2018E, 22 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **65% (11/17)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

Of the three molecular results reported for ETA, all (100%) reported Mutation Detected.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2018E are listed in Tables 35–42.

Two laboratories noted no growth for at least one antituberculosis drug tested for Isolate 2018E.

Table 35. Isolate 2018E—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	18	0	18
Isoniazid—Low	1	17	18
Isoniazid—High	17	1	18
Ethambutol	19	0	19
Pyrazinamide	0	0	0

Table 36. Isolate 2018E—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	72	0	72
Isoniazid—Low	0	72	72
Isoniazid—High	39	1	40
Ethambutol	72	0	72
Pyrazinamide	72	0	72

Table 37. Isolate 2018E—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	0	4	4
Isoniazid—High	2	2	4
Ethambutol	4	0	4
Pyrazinamide	0	0	0

Table 38. Isolate 2018E—Participant Results for First-Line DST by VersaTREK

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	0	1	1
Isoniazid—High	1	0	1
Ethambutol	1	0	1
Pyrazinamide	0	0	0

Table 39. Isolate 2018E—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	19	0	19
Ofloxacin	12	0	12
Ciprofloxacin	8	0	8
Levofloxacin	1	0	1
Moxifloxacin	3	0	3
Amikacin	10	0	10
Kanamycin	16	0	16
Capreomycin	15	0	15
Ethionamide	6	11	17
Rifabutin	8	0	8
Cycloserine	8	0	8
***p*-Aminosalicylic acid**	15	0	15

Table 40. Isolate 2018E—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	35	0	35
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	0	3	3
Rifabutin	3	0	3
Cycloserine	0	0	0
***p*-Aminosalicylic acid**	0	0	0

Table 41. Isolate 2018E—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	3	0	3
Ofloxacin	2	0	2
Ciprofloxacin	0	0	0
Levofloxacin	1	0	1
Moxifloxacin	2	0	2
Amikacin	3	0	3
Kanamycin	2	0	2
Capreomycin	1	0	1
Ethionamide	1	1	2
Rifabutin	3	0	3
Cycloserine	2	0	2
***p*-Aminosalicylic acid**	3	0	3

Table 42. Isolate 2018E—Participant Results for Molecular Testing

* These two laboratories noted the detection of a synonymous mutation Arg528Arg.
† These two laboratories noted the detection of a synonymous mutation Ser65Ser.

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	2*	9	11
Isoniazid	9	0	9
Ethambutol	0	6	6
Pyrazinamide	2†	2	4
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	3	0	3
Rifabutin	0	3	3

Equivalent Critical Concentrations

(Concentrations listed as µg/ml)

Agar Proportion

NOTE—For First-Line and Second-Line Drugs: Critical concentrations as indicated in CLSI M24-A2 document [1]

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

First-Line Drugs	7H10 agar	7H11 agar
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

*For Second-Line Drugs: Breakpoints for establishing susceptibility have not been determined.

Second-Line Drugs	7H10 agar	7H11 agar
Streptomycin	2.0 and 10.0	2.0 and 10.0
Amikacin	4.0	Not determined*
Capreomycin	10.0	10.0
Kanamycin	5.0	6.0
Levofloxacin	1.0	Not determined*
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

Broth Based Media

NOTE—For First-Line and Second-Line Drugs: Critical concentrations as indicated in applicable manufacturer package inserts.

*For First-Line Drugs: The higher concentration of INH and EMB should be tested after resistance at the critical concentration is detected.

First-Line Drugs	MGIT	VersaTREK
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

*For Second-Line Drugs: The higher concentration of STR should be tested after resistance at the critical concentration is detected.

Second-Line Drugs	MGIT	VersaTREK
Streptomycin	1.0 (and 4.0*)	Not available

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Appendix 1: Accessible Explanations of Figures

Figure 1. The primary classification of the 75 laboratories participating in the February 2018 MPEP survey is show in this pie chart. The largest slice, at 65%, represents 49 laboratories that have self-classified as a health department laboratory. The next major slice signifies 13 hospital laboratories. The remaining two slices of the pie chart represent 11 independent laboratories and 2 federal government laboratories.

Figure 2. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=75) in 2017 is displayed in this vertical bar graph. The vertical y–axis is the number of laboratories responding and ranges from 0 to 30 using increments of 5. Along the horizontal x-axis are nine vertical bars representing the number of isolates tested per year. From left to right, 25 laboratories tested less than or equal to 50 isolates per year; 22 laboratories tested between 51 to 100 isolates per year; 4 laboratories tested between 101 to 150 isolates per year; 6 laboratories tested between 151 to 200 isolates per year; 5 laboratories tested between 201 to 250 isolates per year; 2 laboratories tested between 251 to 300 isolates per year; 4 laboratories tested between 301 to 500 isolates per year; 7 laboratories tested between 501 to 1000 isolates per year, and 0 laboratories tested greater than or equal to 1001 isolates per year.

Figure 3. The drug susceptibility testing methods used by MPEP participants (N=112) is displayed in this vertical bar graph. The vertical y-axis is the number of laboratories reporting with ranges from 0 to 80, by increments of 10, and the horizontal x- axis lists the susceptibility testing methods. Each bar represents the number of reporting laboratories performing a particular drug susceptibility test method. From left to right: 72 used MGIT, 23 used agar proportion, 4 used Sensititre, 1 used VersaTREK, and 12 used molecular methods.

Figure 4. The molecular methods used by MPEP participants (N=12) are displayed in this pie chart. The largest slice represents the 5 laboratories that perform targeted DNA sequencing. The next three slices represent 3 laboratories that use the Cepheid Xpert MTB/RIF assay, 2 laboratories that use Hain line probe assays, and 2 laboratories that use whole genome sequencing.

Figure 5. The antituberculosis drugs tested by MPEP participants is displayed in a horizontal bar graph. The vertical y -axis contains a list of each drug tested and the horizontal x-axis contains the number of laboratories with ranges from 0 to 90, by increments of 10. There are 16 horizontal bars with each bar representing the number of laboratories reporting a result for a particular drug for susceptibility testing. 75 laboratories tested rifampin; 75 laboratories tested isoniazid; 75 laboratories tested ethambutol; 71 laboratories tested pyrazinamide; 50 laboratories tested streptomycin; 20 laboratories tested ofloxacin; 9 laboratories tested moxifloxacin; 9 laboratories tested ciprofloxacin; 5 laboratories tested levofloxacin; 19 laboratories tested kanamycin; 19 laboratories tested capreomycin; 16 laboratories tested amikacin; 23 laboratories tested ethionamide; 18 laboratories tested PAS; 14 laboratories tested rifabutin; and 10 laboratories tested cycloserine.

Notes:

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