Centers for Disease Control and Prevention

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program
Report of Results
August 2017

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

CS 289706-A

Mycobacterium tuberculosis Complex Drug Susceptibility Testing MPEP Report for August 2017 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 2017.

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 August 2017 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 August 2017 Survey

Growth-based Results					
	First-Line Drugs				Second-Line Drugs
Isolate	RMP	INH	EMB	PZA	Resistant to:
2017F	S	S	S	S	OFL, CIP
2017G	S	R	S	S	STR, AMK, KAN, CAP, ETA, OFL (heteroresistant)
2017H	S	S	S	S	AMK, KAN, CAP
2017I	S	S	S	S	CAP
2017J	S	S	S	S	STR

Note—S=susceptible, R=resistant

Table 2. Expected Molecular Results for August 2017 Survey

Mutations Detected in Loci Associated with Resistance			
Isolate	*gyrA*	*rrs*	*tlyA*
2017F	Ser91Pro & Asp94Asn	None detected	None detected
2017G	Ala90Val	A1401G	None detected
2017H	None detected	A1401G	None detected
2017I	None detected	None detected	Frameshift (G inserted after nt396)
2017J	None detected	None detected	None detected

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
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 2017 MTBC isolates F, G, H, I, and J in this report.

- The source of data in all tables and figures is the August 2017 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 77 (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 26 laboratories reporting second-line drug results (with the exception of streptomycin), eight (31%) 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

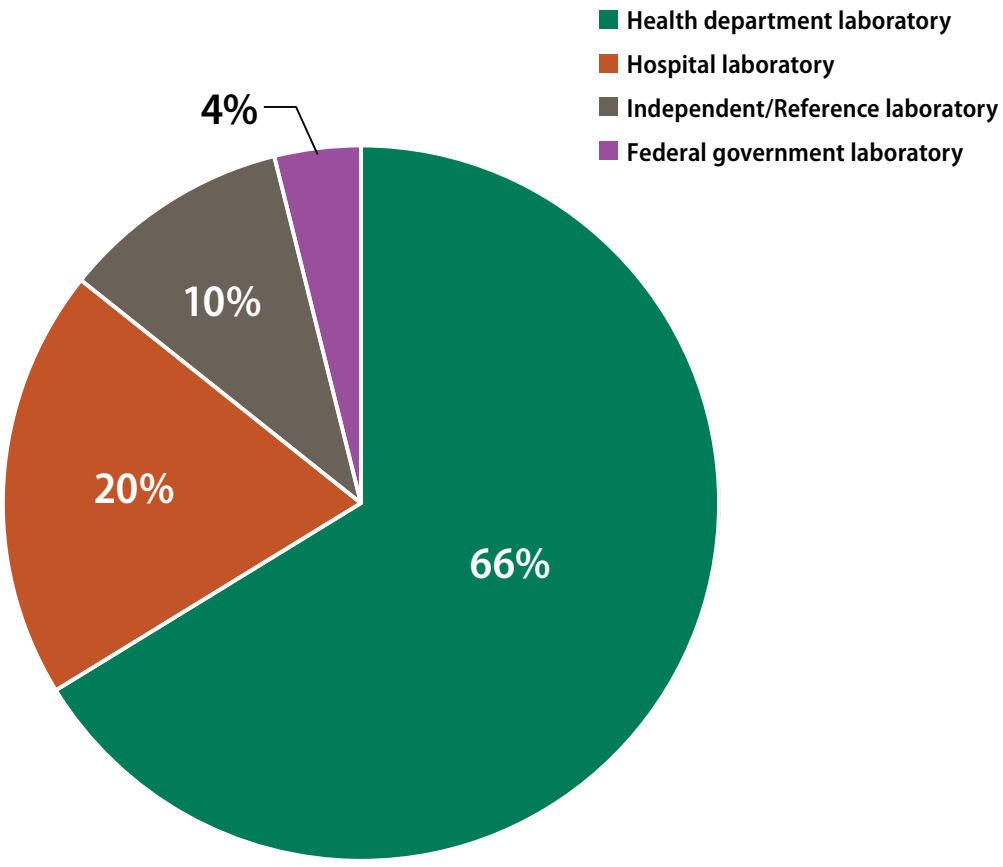
This report contains DST results submitted to CDC by survey participants at 77 laboratories in 36 states.

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

- **51 (66%):** Health department laboratory (e.g., local, county, state)
- **15 (20%):** Hospital laboratory
- **8 (10%):** Independent/Reference laboratory (non-hospital based)
- **3 (4%):** Federal government laboratory

Figure 1. Primary Classification of Participating Laboratories, August 2017

Accessible information for all figures is located in Appendix 1, page 28.

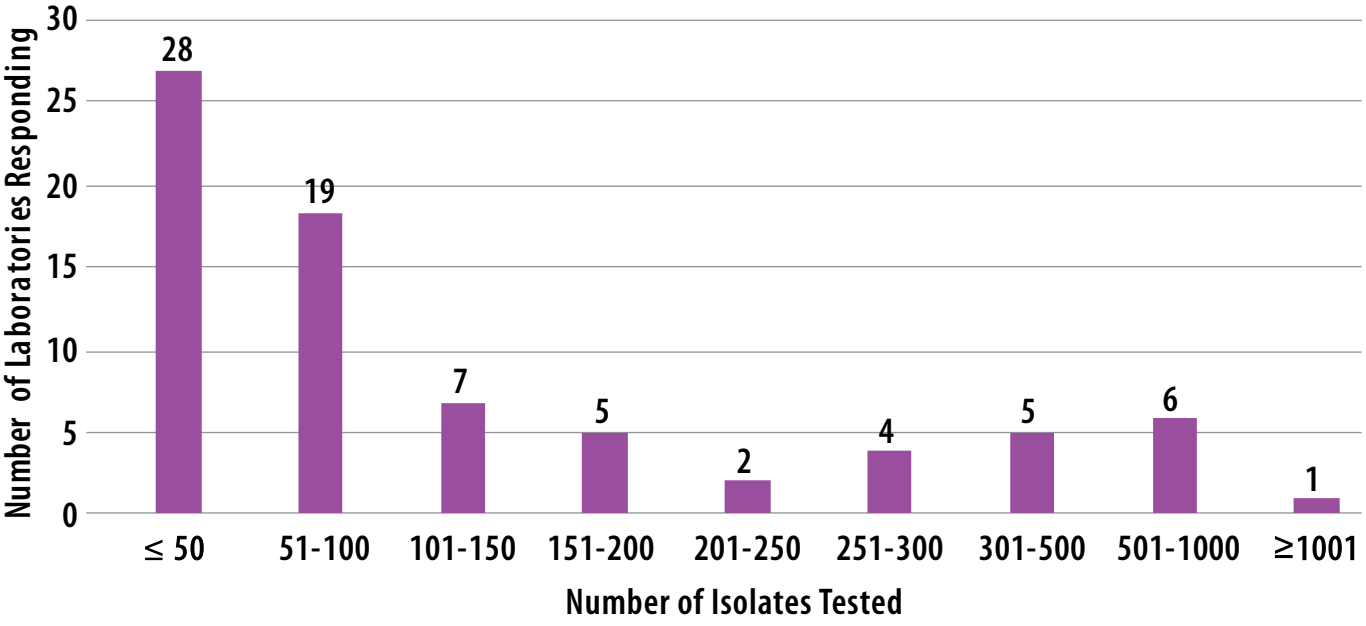


Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 77 participants in 2016 (excluding isolates used for quality control) is shown in Figure 2. In 2016, the counts ranged from 0 to 1,119 tests. Participants at 28 (36%) 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=77)

Accessible information for all figures is located in Appendix 1, page 28.

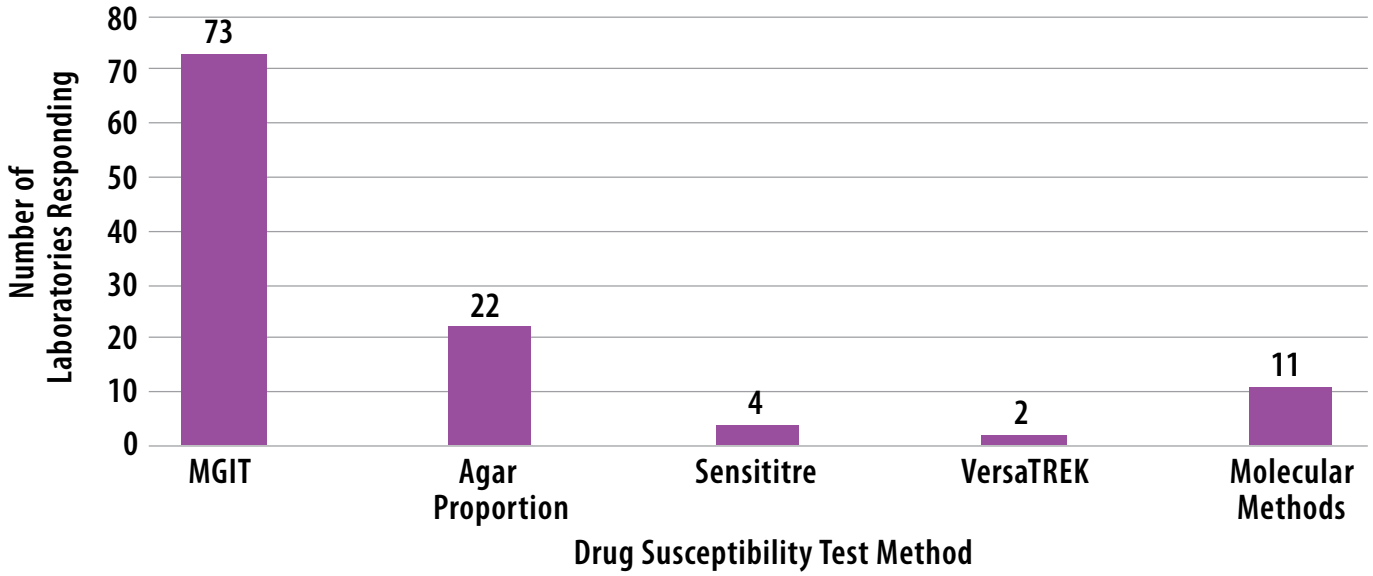


MTBC DST Methods Used by Participants

The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 46 (60%) laboratories reported results for only one method, 27 laboratories reported two methods, and four laboratories noted three susceptibility methods.

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

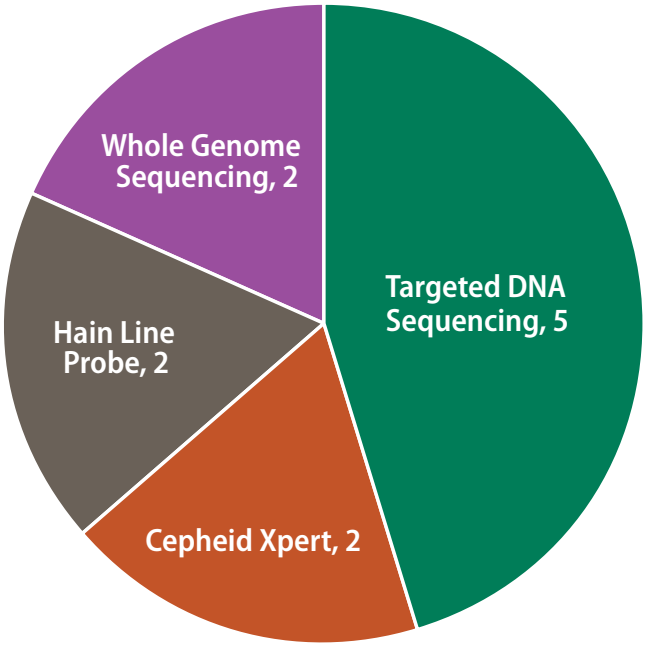
Accessible information for all figures is located in Appendix 1, page 28.



Molecular methods reported by eleven participants are shown in Figure 4. The method used most frequently by laboratories was targeted DNA sequencing (45%), including pyrosequencing and Sanger sequencing. Two 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=11)

Accessible information for all figures is located in Appendix 1, page 28.

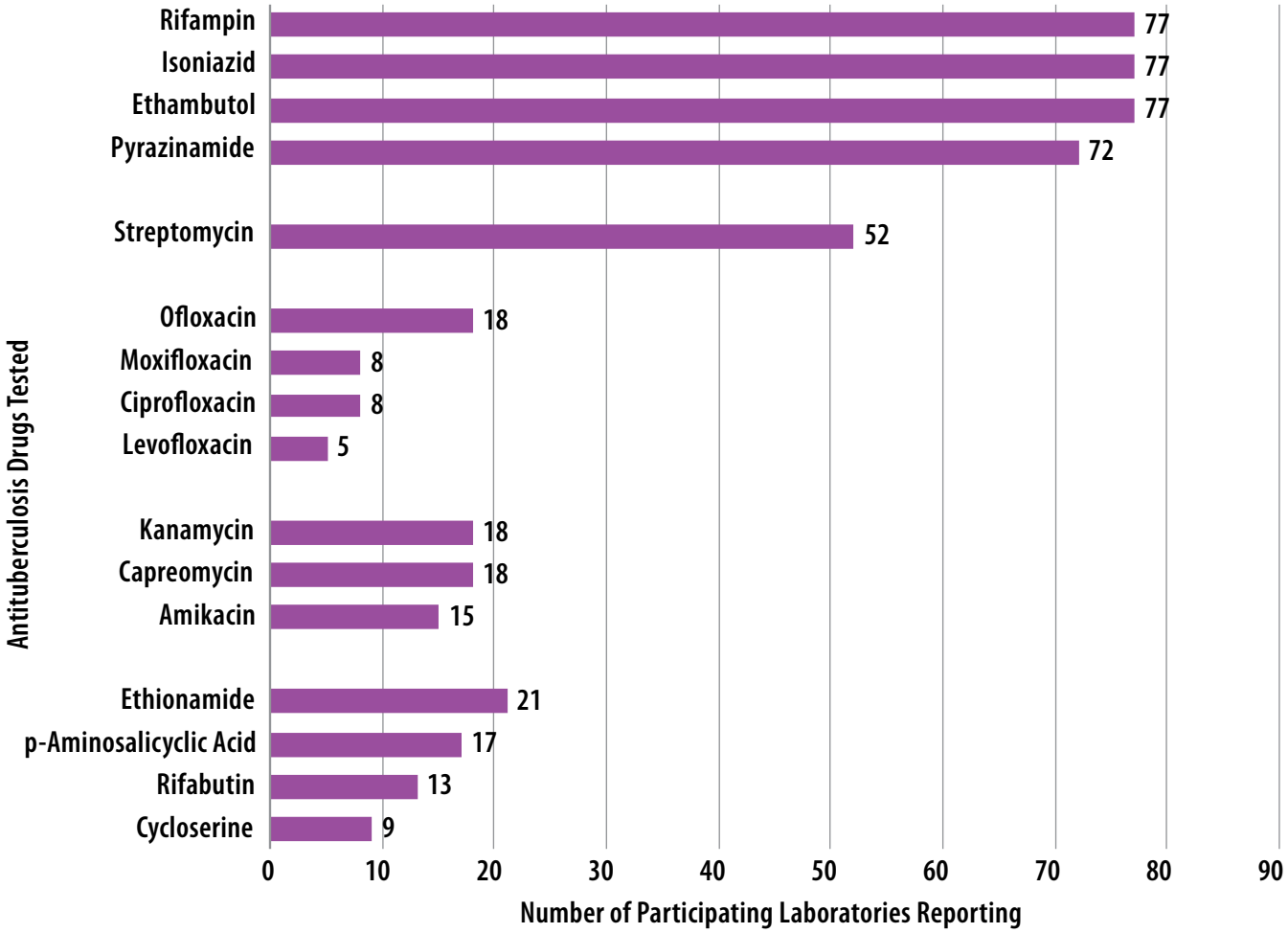


Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the August 2017 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 72 (94%) also reported results for PZA.

Figure 5. Antituberculosis Drugs Tested by Participants

Accessible information for all figures is located in Appendix 1, page 28.



Isolate 2017F

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

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 [4]. 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 [4, 5]. The primary mechanism of action of FQ is the inhibition of DNA synthesis [6] 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, 6, 7]. 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 [6].

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 [8, 9]. 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 (LVX), 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 [10, 11].

DNA sequencing of *gyr*A in Isolate 2017F revealed a T>C point mutation in codon 91 of gyrA resulting in wild-type serine being replaced with proline (Ser91Pro). The Ser91Pro mutation has been associated with FQ resistance [2, 12]. DNA sequencing also revealed a G>A point mutation in codon 94 resulting in wild-type aspartic acid being replaced with asparagine (Asp94Asn). Sequencing of *gyr*B was wild-type (i.e., no mutations were detected).

Among three methods, 18 results for OFL were reported for Isolate 2017F. This isolate was reported as **resistant** to OFL by method, as follows:

- **100% (12/12)** 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, LVF, and MOX) for Isolate 2017F; 100% (18/18) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other fluoroquinolones by method, as follows:

Ciprofloxacin

- **100% (6/6)** 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

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

Mutations in the *gyr*A gene were detected by all (100%) laboratories that reported molecular testing for FQ drugs.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2017F are listed in Tables, 3, 4, and 5.

Table 3. Isolate 2017F—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	18	0	18	71	0	71	4	0	4	2	0	2
Isoniazid—Low	18	0	18	71	0	71	4	0	4	2	0	2
Isoniazid—High	18	0	18	26	0	26	4	0	4	2	0	2
Ethambutol	18	0	18	71	0	71	4	0	4	2	0	2
Pyrazinamide	0	0	0	64	5	69*	0	0	0	1	0	1

Note—S=susceptible, R=resistant

*One additional laboratory reported borderline for PZA by MGIT.

Table 4. Isolate 2017F—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	18	0	18	37	0	37	3	0	3
Ofloxacin	0	12	12	0	4	4	0	2	2
Ciprofloxacin	0	6	6	0	1	1	0	0	0
Levofloxacin	0	1	1	0	3	3	0	0	0
Moxifloxacin	0	3	3	0	2	2*	0	2	2
Amikacin	10	0	10	2	0	2	3	0	3
Kanamycin	14	0	14	1	0	1	2	0	2
Capreomycin	14	0	14	3	0	3	1	0	1
Ethionamide	15	0	15	2	1	3	2	0	2
Rifabutin	7	0	7	3	0	3	3	0	3
Cycloserine	7	0	7	0	0	0	2	0	2
***p*-Aminosalicylic acid**	13	0	13	0	0	0	3	0	3

Note—S=susceptible, R=resistant

*One additional laboratory reported borderline for MOX by MGIT.

Table 5. Isolate 2017F—Participant Results for Molecular Testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	0	11	11
Isoniazid	0	9	9
Ethambutol	0	6	6
Pyrazinamide	2*	2	4
Ofloxacin	5	0	5
Ciprofloxacin	5	0	5
Levofloxacin	4	0	4
Moxifloxacin	4	0	4
Amikacin	0	5	5
Kanamycin	0	5	5
Capreomycin	0	4	4
Ethionamide	0	3	3
Rifabutin	0	3	3

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

Isolate 2017G

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml, STR at 2.0 µg/ml, AMK at 4.0 µg/ml, CAP at 10.0 µg/ml, KAN at 5.0 µg/ml, ETA at 5.0 µg/ml, and OFL at 2.0 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, 13]. 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, 7, 13]. 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 [14, 15]. 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 [13].

DNA sequence analysis of *inh*A, *kat*G, *fab*G1, and *ahp*C of Isolate 2017G were wild-type (i.e., no mutations were detected). As noted above, approximately 10–15% of isolates found to be INH resistant do not have mutations in the most common loci associated with drug resistance.

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 2017G, 98 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (20/20)** of the results when using AP
- **97% (70/72)** 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 (94%) results were reported as resistant at the higher concentrations of INH. Only 41 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 nine molecular results reported for INH, one (11%) laboratory reported mutation detected in the *kat*G gene (noted as Asn138Asp).

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[7, 13]. In MTBC, the genetic basis of the majority of resistance to STR is usually due to mutations in *rrs* or *rps*L[6, 7]. 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, 16].

Among three methods, 61 results for STR were reported for Isolate 2017G. This isolate was reported as **resistant** to STR by method, as follows:

- **100% (20/20)** of the results when using AP
- **97% (37/38)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre

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 *tly*A for CAP [6]. 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, 17]. The most common *rrs* mutation for cross-resistance to all three drugs is the A1401G point mutation [17].

For Isolate 2017G, 51 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% (10/10)** of the results when using AP
- **100% (2/2)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Kanamycin

- **100% (15/15)** of the results when using AP
- **100% (1/1)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Capreomycin

- **57% (8/14)** of the results when using AP
- **75% (3/4)** of the results when using MGIT
- **0% (0/1)** of the results when using Sensititre

The mutation in the *rrs* gene was detected by all (100%) laboratories that reported molecular testing for AMK, KAN, and CAP, with three laboratories specifically noting it was the A1401G mutation.

Ethionamide

Ethionamide (ETA) is a structural analog of INH. ETA, like INH, targets *inh*A, an enzyme involved in mycolic acid biosynthesis [18]. 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 [18].

Sequencing analysis of *eth*A was not performed and, as noted above, sequencing of the *inh*A gene revealed wild-type (i.e., no mutations were detected) for Isolate 2017G.

Issues with reproducibility of DST results for ETA have been reported [19] and remain a potential concern.

For Isolate 2017G, 21 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **94% (15/16)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

Of the three molecular results reported for ETA, two (67%) reported mutation detected, specifically noting it was a deletion mutation.

Ofloxacin

Unlike the FQ resistance seen with Isolate 2017F, heteroresistance was observed for OFL with Isolate 2017G. 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 loci associated with drug resistance, resulting in both drug-resistant and drug-susceptible organisms [8, 9].

DNA sequence of *gyr*A in Isolate 2017G revealed a C>T point mutation in codon 90 of *gyr*A resulting in wild-type alanine being replaced with valine (Ala90Val). The Ala90Val mutation has been associated with FQ resistance [2, 12]. Sequencing of *gyr*B was wild-type (i.e., no mutations were detected).

Among three methods, 19 results for OFL were reported for Isolate 2017G. This isolate was reported as **resistant** to OFL by method, as follows:

- **85% (11/13)** 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, LVF, and MOX) for Isolate 2017G; 53% (9/17) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other fluoroquinolones by method, as follows:

Ciprofloxacin
- **43% (3/7)** of the results when using AP

Moxifloxacin
- **50% (1/2)**of the results when using AP
- **67% (2/3)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

Levofloxacin
- **100% (1/1)** of the results when using MGIT
- **50% (1/2)** of the results when using MGIT

The mutation in *gyr*A was detected by all (100%) laboratories that reported molecular testing for FQ drugs; three laboratories specifically noted the Ala90Val mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2017G are listed in Tables 6, 7, and 8.

Table 6. Isolate 2017G—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	71	0	71	4	0	4	2	0	2
Isoniazid—Low	0	20	20	2	70	72	0	4	4	0	2	2
Isoniazid—High	1	19	20	1	40	41	2	2	4	0	2	2
Ethambutol	19	1	20	70	1	71	4	0	4	2	0	2
Pyrazinamide	0	0	0	70	1	71	0	0	0	1	0	1

Note—S=susceptible, R=resistant

Table 7. Isolate 2017G—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	20	20	1	37	38	0	3	3
Ofloxacin	2	11	13	0	4	4	0	2	2
Ciprofloxacin	4	3	7	0	0	0*	0	0	0
Levofloxacin	0	1	1	1	1	2*	0	0	0
Moxifloxacin	1	1	2	1	2	3	1	1	2
Amikacin	0	10	10	0	2	2	0	2	2
Kanamycin	0	15	15	0	1	1	0	2	2
Capreomycin	6	8	14	1	3	4	1	0	1
Ethionamide	1	15	16	0	3	3	1	1	2
Rifabutin	7	0	7	3	0	3	3	0	3
Cycloserine	6	2	8	0	0	0	1	0	1
***p*-Aminosalicylic acid**	13	1	14	0	0	0	3	0	3

Note—S=susceptible, R=resistant

*One additional laboratory reported borderline for CIP and LEV by MGIT.

Table 8. Isolate 2017G—Participant Results for Molecular Testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	1	8	9
Ethambutol	0	6	6
Pyrazinamide	0	4	4
Ofloxacin	5	0	5
Ciprofloxacin	5	0	5
Levofloxacin	4	0	4
Moxifloxacin	4	0	4
Amikacin	5	0	5
Kanamycin	5	0	5
Capreomycin	4	0	4
Ethionamide	2	1	3
Rifabutin	0	3	3

Isolate 2017H

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

As previously noted, the most common mechanism of resistance to all three second-line injectables is the A1401G mutation in the *rrs* gene. DNA sequence analysis of *rrs* of Isolate 2017H revealed the A1401G mutation.

For Isolate 2017H, 47 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% (10/10)** of the results when using AP
- **100% (2/2)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Kanamycin
- **100% (13/13)** of the results when using AP
- **100% (1/1)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Capreomycin
- **93% (13/14)** of the results when using AP
- **100% (3/3)** of the results when using MGIT

The mutation in the *rrs* gene was detected by all (100%) laboratories that reported molecular testing for AMK, KAN, and CAP. Three laboratories specifically noted detection of the A1401G mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2017H are listed in Tables 9, 10, and 11.

One laboratory noted contamination for at least one antituberculosis drug tested for Isolate 2017H.

Table 9. Isolate 2017H—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	18	0	18	70	0	70	4	0	4	2	0	2
Isoniazid—Low	18	0	18	70	0	70	4	0	4	2	0	2
Isoniazid—High	18	0	18	24	0	24	4	0	4	2	0	2
Ethambutol	18	0	18	70	0	70	4	0	4	2	0	2
Pyrazinamide	0	0	0	71	0	71	0	0	0	1	0	1

Note—S=susceptible, R=resistant

Table 10. Isolate 2017H—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	18	0	18	36	0	36	3	0	3
Ofloxacin	12	0	12	4	0	4	1	0	1*
Ciprofloxacin	6	0	6	1	0	1	0	0	0
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	1	0	1*
Amikacin	0	10	10	0	2	2	0	2	2
Kanamycin	0	13	13†	0	1	1	0	2	2
Capreomycin	1	13	14	0	3	3	0	0	0
Ethionamide	15	0	15	3	0	3	2	0	2
Rifabutin	7	0	7	3	0	3	3	0	3
Cycloserine	8	0	8	0	0	0	0	0	0*
***p*-Aminosalicylic acid**	13	0	13	0	0	0	3	0	3

Note—S=susceptible, R=resistant

*One additional laboratory reported borderline for OFL, MOX, and CYC by Sensititre.

†One additional laboratory reported borderline for KAN by AP.

Table 11. Isolate 2017H—Participant Results for Molecular Testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	0	9	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	5	0	5
Kanamycin	5	0	5
Capreomycin	4	0	4
Ethionamide	0	3	3
Rifabutin	0	3	3

Isolate 2017I

Expected Result: Resistant to CAP at 10.0 µg/ml by agar proportion

Second-line Injectables

As previously noted, the primary mechanisms of resistance for CAP occur due to mutations in *rrs* and *tly*A [6]. Resistance to CAP, but not KAN or AMK, is specifically associated with mutations in the open reading frame of the *tly*A gene which results in reduction of methylation and reduced ability for CAP to interact with its target [2, 17]. DNA sequence analysis of *tly*A of Isolate 2017I revealed a frameshift mutation due to a G nucleotide insertion after position 396. Sequencing of *rrs* was wild-type (i.e., no mutations were detected).

For Isolate 2017I, 17 results were reported for CAP. The isolate was reported **resistant** to CAP by method, as follows:

- **79% (11/14)** of the results when using AP
- **100% (3/3)** of the results when using MGIT

This frameshift mutation in the *tly*A gene was detected by only one (20%) laboratory that reported molecular testing for CAP.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2017I are listed in Tables 12, 13, and 14.

Table 12. Isolate 2017I—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	18	0	18	71	0	71	4	0	4	2	0	2
Isoniazid—Low	18	0	18	71	0	71	4	0	4	2	0	2
Isoniazid—High	18	0	18	25	0	25	4	0	4	2	0	2
Ethambutol	18	0	18	71	0	71	4	0	4	2	0	2
Pyrazinamide	0	0	0	63	7	70*	0	0	0	1	0	1

Note—S=susceptible, R=resistant

*One additional laboratory reported borderline for PZA by MGIT.

Table 13. Isolate 2017I—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	18	0	18	37	0	37	3	0	3
Ofloxacin	12	0	12	4	0	4	2	0	2
Ciprofloxacin	6	0	6	1	0	1	0	0	0
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	1	0	1*
Amikacin	10	0	10	2	0	2	3	0	3
Kanamycin	14	0	14	1	0	1	2	0	2
Capreomycin	3	11	14	0	3	3	0	0	0
Ethionamide	15	0	15	1	2	3	2	0	2
Rifabutin	7	0	7	3	0	3	3	0	3
Cycloserine	8	0	8	0	0	0	1	0	1
***p*-Aminosalicylic acid**	13	0	13	0	0	0	3	0	3

Note—S=susceptible, R=resistant
*One additional laboratory reported borderline for MOX by Sensititre.

Table 14. Isolate 2017I—Participant Results for Molecular Testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	0	9	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	1	4	5
Ethionamide	0	3	3
Rifabutin	0	3	3

Isolate 2017J

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

Streptomycin

Among three methods, 59 results for STR were reported for Isolate 2017J. This isolate was reported as **resistant** to STR by method, as follows:

- **100% (20/20)** of the results when using AP
- **100% (36/36)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre

Pyrazinamide

Pyrazinamide (PZA) is an important first-line drug for treatment of TB and is used with INH and rifampin. 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. Resistance to PZA is usually caused by nucleotide changes scattered throughout the *pnc*A gene. There may be additional mechanisms of resistance to PZA that are still unknown[20], but issues with false resistance to PZA have been reported as well [21] and remain a potential concern.

For Isolate 2017J, DNA sequencing of the *pnc*A gene did not reveal a mutation.

Isolate 2017J was expected to be susceptible to PZA; however, of those testing PZA, resistance was reported by:

- **35% (24/69)** 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 2017J are listed in Tables 15, 16, and 17.

Two laboratories noted contamination for at least one antituberculosis drug tested for Isolate 2017J.

Table 15. Isolate 2017J—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	70	0	70	4	0	4	2	0	2
Isoniazid—Low	20	0	20	69	1	70	4	0	4	2	0	2
Isoniazid—High	20	0	20	25	0	25	4	0	4	2	0	2
Ethambutol	20	0	20	70	0	70	4	0	4	2	0	2
Pyrazinamide	0	0	0	45	24	69	0	0	0	1	0	1

Note—S=susceptible, R=resistant

Table 16. Isolate 2017J—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	20	20	0	36	36	0	3	3
Ofloxacin	13	0	13	4	0	4	1	0	1*
Ciprofloxacin	6	0	6	1	0	1	0	0	0
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	1	0	1*
Amikacin	9	0	9	2	0	2	3	0	3
Kanamycin	15	0	15	1	0	1	2	0	2
Capreomycin	13	0	13	3	0	3	1	0	1
Ethionamide	13	1	14†	3	0	3	2	0	2
Rifabutin	7	0	7	3	0	3	3	0	3
Cycloserine	7	0	7	0	0	0	2	0	2
***p*-Aminosalicylic acid**	13	0	13	0	0	0	3	0	3

Note—S=susceptible, R=resistant

*One additional laboratory reported borderline for OFL and MOX by Sensititre.

†One additional laboratory reported borderline for ETA by AP.

Table 17. Isolate 2017J—Participant Results for Molecular Testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	0	11	11
Isoniazid	0	9	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	0	3	3
Rifabutin	0	3	3

Equivalent Critical Concentrations (Concentrations listed as µg/ml)

Agar Proportion

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

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.

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

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

*Breakpoints for establishing susceptibility have not be determined.

Broth Based Media

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

NOTE—Critical concentrations as indicated in applicable manufacturer package inserts

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

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

NOTE—Critical concentrations as indicated in applicable manufacturer package inserts

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

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Appendix 1: Accessible Explanations of Figures

Figure 1. The primary classification of the 77 laboratories participating in the August 2017 MPEP survey is shown in this pie chart. The largest slice, at 66%, represents 51 laboratories that have self-classified as a health department laboratory. The next major slice signifies 15 hospital laboratories. The remaining two slices of the pie chart represent 8 independent laboratories and 3 federal government laboratories.

Figure 2. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=77) in 2016 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, 28 laboratories tested less than or equal to 50 isolates per year; 19 laboratories tested between 51 to 100 isolates per year; 7 laboratories tested between 101 to 150 isolates per year; 5 laboratories tested between 151 to 200 isolates per year; 2 laboratories tested between 201 to 250 isolates per year; 4 laboratories tested between 251 to 300 isolates per year; 5 laboratories tested between 301 to 500 isolates per year; 6 laboratories tested between 501 to 1000 isolates per year, and 1 laboratory 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: 73 used MGIT, 22 used agar proportion, 4 used Sensititre, 2 used VersaTREK, and 11 used molecular methods.

Figure 4. The molecular methods used by MPEP participants (N=11) is displayed in this pie chart. The largest slice represents the 5 laboratories that perform targeted DNA sequencing. The next three slices represent 2 laboratories that use the Cepheid Xpert TB/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. 77 laboratories tested rifampin; 77 laboratories tested isoniazid; 77 laboratories tested ethambutol; 72 laboratories tested pyrazinamide; 52 laboratories tested streptomycin; 18 laboratories tested ofloxacin; 8 laboratories tested moxifloxacin; 8 laboratories tested ciprofloxacin; 5 laboratories tested levofloxacin; 18 laboratories tested kanamycin; 18 laboratories tested capreomycin; 15 laboratories tested amikacin; 21 laboratories tested ethionamide; 17 laboratories tested PAS; 13 laboratories tested rifabutin; and 9 laboratories tested cycloserine.

Notes:

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