# *Mycobacterium tuberculosis* Complex Drug Susceptibility Testing Program

## Model Performance Evaluation Program
## Report of Results
## March 2019

**Centers for Disease Control and Prevention**
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention

# *Mycobacterium tuberculosis* Complex Drug Susceptibility Testing Report for March 2019 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 March 2019.

## Report Content

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**Note on Accessibility:**
Find descriptions and explanations of figures in Appendix 1: Accessible Explanation of Figures on page 32.

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## Abbreviations and Acronyms

| Acronym | 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 |
| FQ | fluoroquinolones |
| 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 |

# 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), "M24: Susceptibility Testing of Mycobacteria, *Nocardiae* spp., and Other Aerobic Actinomycetes" [1]

# Expected Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in March 2019 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 March 2019 Survey

Note—S=susceptible, R=resistant

| Isolate | RMP | INH | EMB | PZA | Second-line Drugs Resistant to: |
|---|---|---|---|---|---|
| 2019A | S | S | S | S | STR, OFL, CIP |
| 2019B | S | R | S | S | STR, OFL, CIP |
| 2019C* | S | S | S | S | STR, OFL, CIP, AMK, KAN, CAP |
| 2019D | S | R | S | S | STR, OFL, CIP |
| 2019E | S | S | S | S | OFL, CIP |

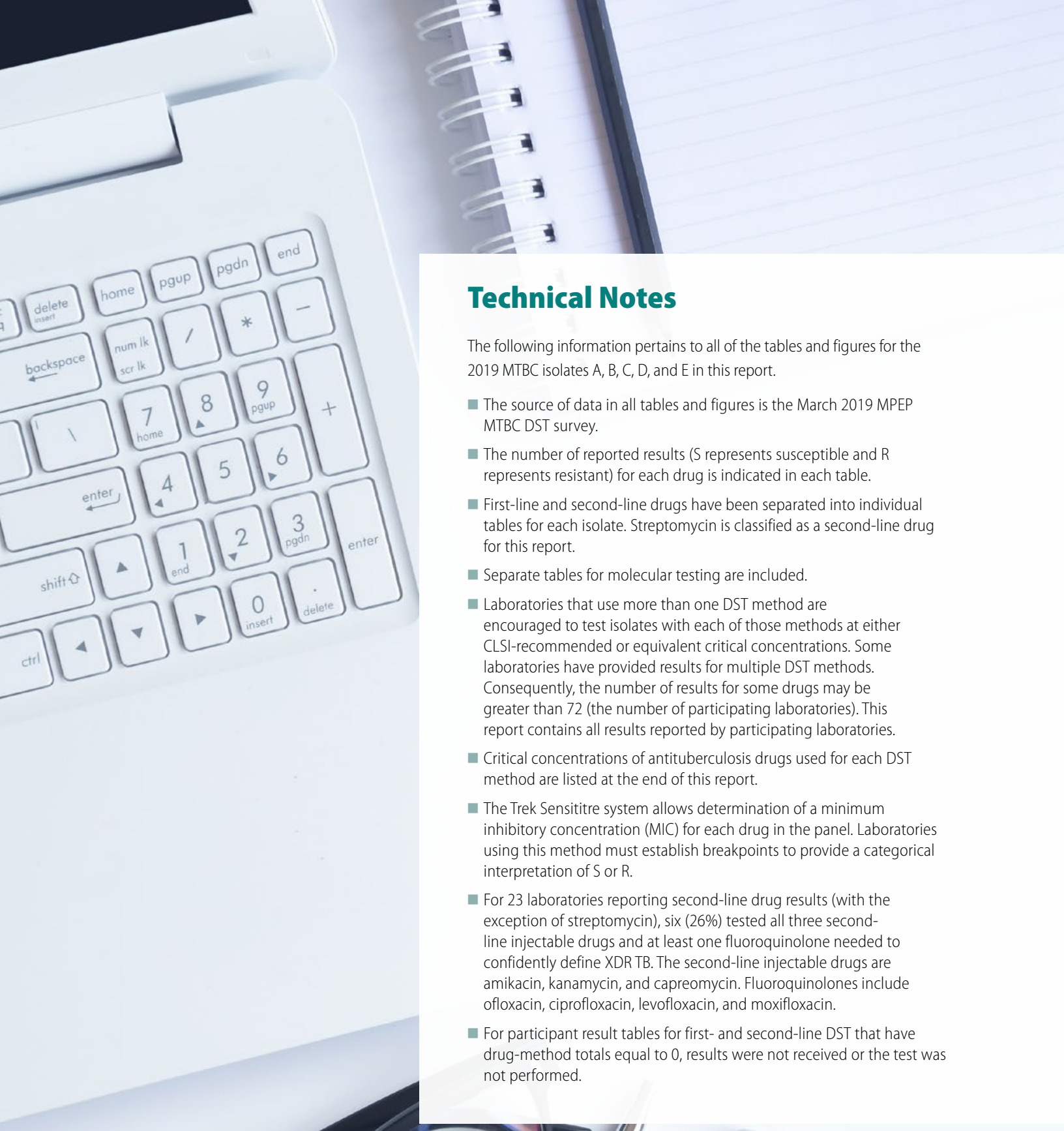
*Although resistance was expected for all fluoroquinolones, variable results were reported by participants; the reason for this variability is unknown.

### Table 2. Expected Molecular Results (Mutations Detected in Loci Associated with Resistance) for March 2019 Survey

Note—Empty cell=No mutation detected

| Isolate | *katG* | *gyrA* | *rrs* |
|---|---|---|---|
| 2019A | | Asp94Gly | |
| 2019B | Ser315Thr | Asp94Asn | |
| 2019C | | Ala90Val | A1401G |
| 2019D | Ser315Thr | Asp94† | |
| 2019E | | Asp94† | |

†Specific amino acid change(s) could not be determined due to the detection of multiple nucleotide changes within *gyrA* codon 94, indicating heteroresistance.



## Technical Notes

The following information pertains to all of the tables and figures for the 2019 MTBC isolates A, B, C, D, and E in this report.

- The source of data in all tables and figures is the March 2019 MPEP MTBC DST survey.
- The number of reported results (S represents susceptible and R represents resistant) for each drug is 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 72 (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 23 laboratories reporting second-line drug results (with the exception of streptomycin), six (26%) 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

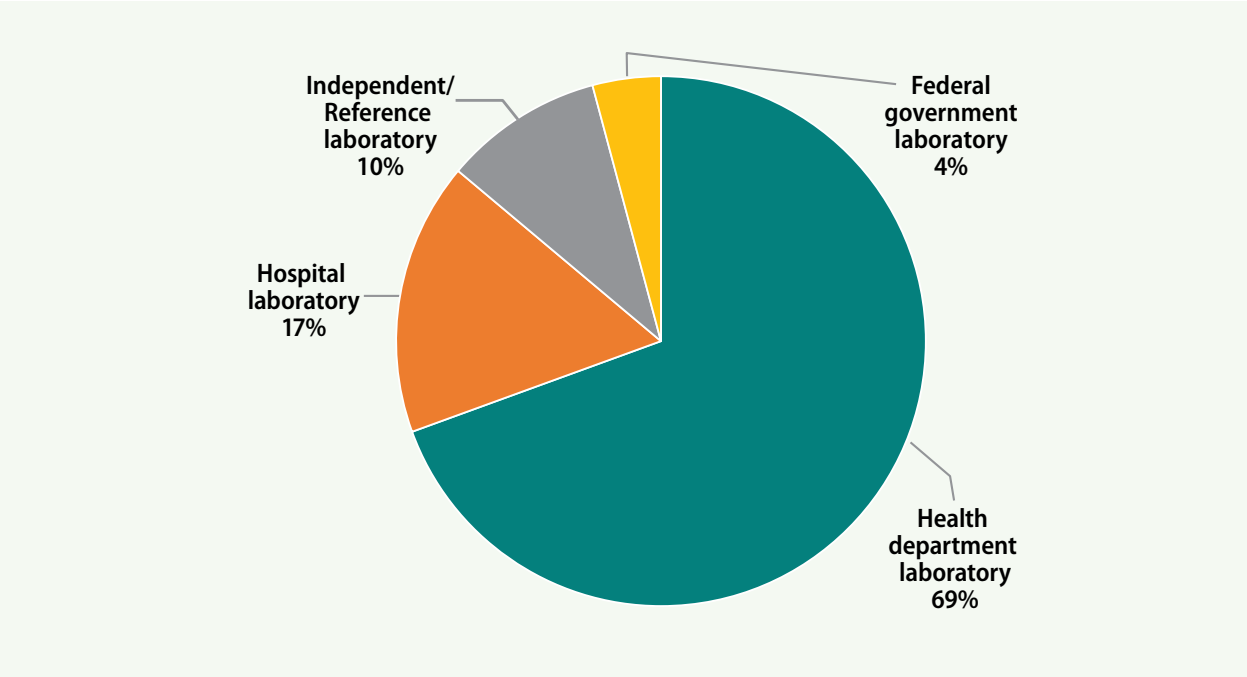
## Primary Classification

This report contains DST results submitted to CDC by survey participants at 72 laboratories in 32 states.

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

- **50 (69%): Health department laboratory (e.g., local, county, state)**
- **12 (17%): Hospital laboratory**
- **7 (10%): Independent/Reference laboratory (non-hospital based)**
- **3 (4%): Federal government laboratory**

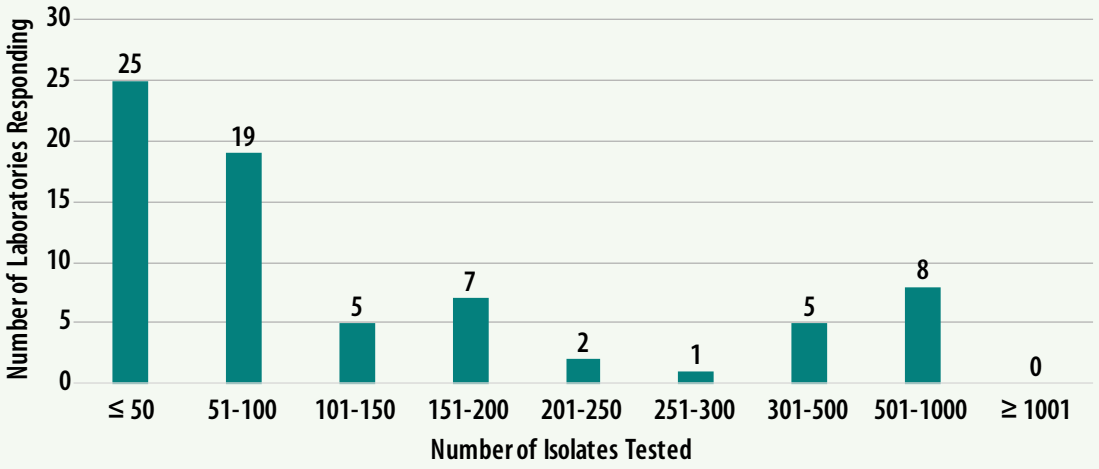
**Figure 1. Primary Classification of Participating Laboratories, March 2019**



## Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 72 participants in 2018 (excluding isolates used for quality control) is shown in Figure 2. In 2018, the counts ranged from 0 to 948 tests. Participants at 25 (35%) 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].

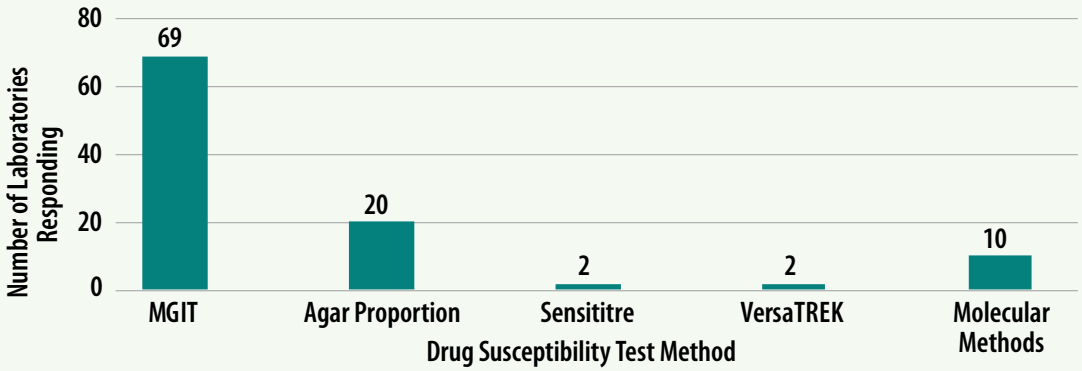
**Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=72)**



## MTBC DST Methods Used by Participants

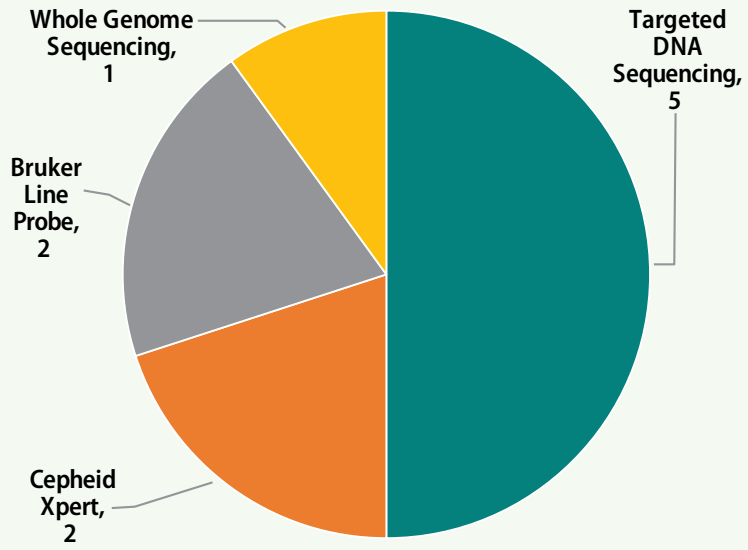
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 45 (63%) laboratories reported results for only one method, 25 laboratories reported two methods, and 2 laboratories noted three susceptibility methods.

### Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=103)




Molecular methods reported by twelve participants are shown in Figure 4. The method used most frequently by laboratories was targeted DNA sequencing (50%), including pyrosequencing and Sanger sequencing. Two laboratories reported results for the Cepheid Xpert MTB/RIF assay, two reported use of line probe assays Genotype MTBDR*plus* and MTBDR*s*/ by Bruker, and one reported results from whole genome sequencing.

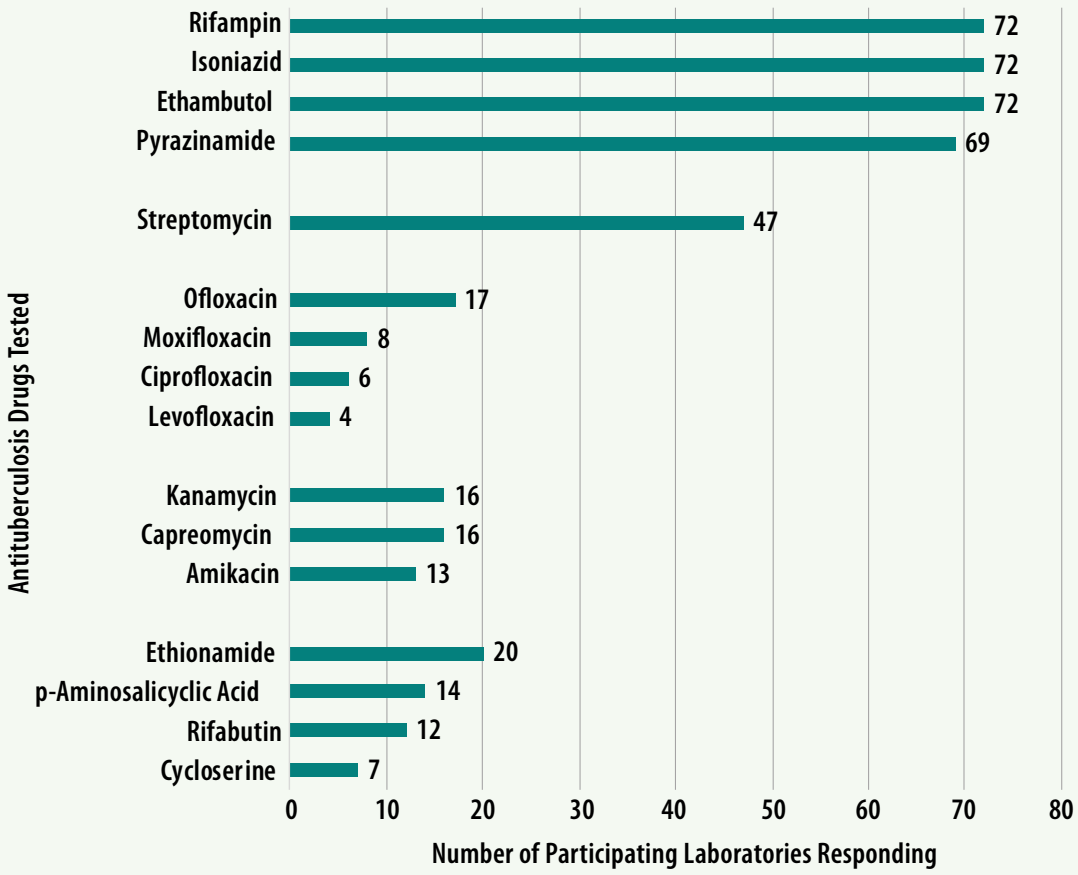
### Figure 4. Molecular Method Reported (n=10)



## Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the March 2019 survey is presented 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 69 (96%) also reported results for PZA by growth-based DST methods.

### Figure 5. Antituberculosis Drugs Tested by Participants



# Isolate 2019A

Expected Result: Resistant to OFL at 2.0 µg/ml, CIP at 2.0 µg/ml, and STR at 2.0 µg/ml by agar proportion

## Ofloxacin and Ciprofloxacin

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 *gyrA* 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 *gyrB* gene have been noted with varying rates of resistance, but high-level resistance is less common without a concurrent *gyrA* 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.

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 [10, 11]. CLSI currently recommends testing LVX and/or MOX, but the FQ to be tested should be based on consultation with TB Programs and physicians treating drug resistant TB [1].

DNA sequencing of *gyrA* in Isolate 2019A detected an A>G point mutation in codon 94 of *gyrA* resulting in wild-type aspartic acid being replaced with glycine (Asp94Gly). The Asp94Gly mutation has been associated with FQ resistance [2, 12]. Sequencing of *gyrB* was wild-type (i.e., no mutations were detected).

Among three growth-based methods, 17 results for OFL were reported for Isolate 2019A. 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% (1/1)** of the results when using Sensititre

Participating laboratories also reported results for other FQ drugs (e.g., CIP, LVF, and MOX) for Isolate 2019A; 100% (17/17) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other FQ by method, as follows:

**Ciprofloxacin**

- **100% (7/7)** 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% (2/2)** of the results when using AP
- **100% (2/2)** of the results when using MGIT
- **100% (1/1)** of the results when using Sensititre

A mutation in the *gyrA* gene was detected by all (100%) laboratories that reported molecular testing for FQ drugs.

The two laboratories performing Sensititre reported MIC values for FQ drugs; one of these did not report interpretations. Reported MIC values were as follows: OFL at 16 µg/ml; LFV at 8 µg/ml; and MOX at 4 µg/ml and 8 µg/ml.

### 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 *rpsL* [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, 14].

Among three methods, 55 results for STR were reported for Isolate 2019A. This isolate was reported as **resistant** to STR by method, as follows:

- **100% (19/19)** of the results when using AP
- **100% (34/34)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Both laboratories performing Sensititre reported STR MIC values as >32 µg/ml.

### Isoniazid

Isolate 2019A was expected to be susceptible to isoniazid (INH), however a number of reporting laboratories indicated low-level INH resistance.

For Isolate 2019A, 88 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **84% (16/19)** of the results when using AP
- **94% (61/65)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Four (7%) results were reported as **resistant** at the higher concentrations of INH. One laboratory noted an Asp94Asn mutation in *katG* for this isolate by molecular testing, but its role in INH resistance is unknown.

*Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2019A are listed in Tables 3–10.*

### Table 3. Isolate 2019A—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 19 | 0 | 19 |
| Isoniazid—Low | 3 | 16 | 19 |
| Isoniazid—High | 18 | 1 | 19 |
| Ethambutol | 20 | 0 | 20 |

### Table 4. Isolate 2019A—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 66 | 0 | 66 |
| Isoniazid—Low | 4 | 61 | 65* |
| Isoniazid—High | 35 | 2 | 37 |
| Ethambutol | 66 | 0 | 66 |
| Pyrazinamide | 66 | 1 | 67* |

* One additional laboratory reported contaminated for INH and borderline for PZA by MGIT.

## Table 5. Isolate 2019A—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 1 | 1 | 2 |
| Isoniazid—High | 1 | 1 | 2 |
| Ethambutol | 2 | 0 | 2 |

* One additional laboratory reported borderline for EMB by Sensititre.

## Table 6. Isolate 2019A—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 1 | 1 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

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

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 0 | 19 | 19 |
| Ofloxacin | 0 | 12 | 12 |
| Ciprofloxacin | 0 | 7 | 7 |
| Levofloxacin | 0 | 1 | 1 |
| Moxifloxacin | 0 | 2 | 2 |
| Amikacin | 9 | 0 | 9 |
| Kanamycin | 14 | 0 | 14 |
| Capreomycin | 12 | 0 | 12 |
| Ethionamide | 15 | 1 | 16 |
| Rifabutin | 7 | 0 | 7 |
| Cycloserine | 6 | 0 | 6 |
| *p*-Aminosalicylic acid | 12 | 0 | 12 |

## Table 8. Isolate 2019A—Participant Results for Second-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 0 | 34 | 34 |
| 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 | 3 | 0 | 3 |
| Rifabutin | 3 | 0 | 3 |
| Cycloserine | 0 | 0 | 0 |
| *p*-Aminosalicylic acid | 0 | 0 | 0 |

* One additional laboratory reported borderline for MOX by MGIT.

## Table 9. Isolate 2019A—Participant Results for Second-Line DST by Sensititre

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

## Table 10. Isolate 2019A—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| Rifampin | 0 | 10 | 10 |
| Isoniazid | 1 | 7 | 8 |
| Ethambutol | 0 | 4 | 4 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 4 | 0 | 4 |
| Ciprofloxacin | 4 | 0 | 4 |
| Levofloxacin | 6 | 0 | 6 |
| Moxifloxacin | 6 | 0 | 6 |
| Amikacin | 0 | 5 | 5 |
| Kanamycin | 0 | 5 | 5 |
| Capreomycin | 0 | 4 | 4 |
| Ethionamide | 0 | 2 | 2 |
| Rifabutin | 0 | 4 | 4 |

# Isolate 2019B

## Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml, OFL at 2.0 µg/ml, CIP at 2.0 µg/ml, and STR 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 TB infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *katG* gene [2, 13]. 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, 7, 13]. 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 [15, 16]. 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 [13].

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2019B detected 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 2019B, 88 INH results were reported. This isolate was reported resistant to INH by method, as follows:

- **100% (19/19)** of the results when using AP
- **100% (65/65)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Sixty (98%) results were reported as **resistant** at the higher concentrations of INH. Only 38 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 8 molecular results reported for INH, all (100%) laboratories reported detection of a mutation with 6 laboratories specifically noting the Ser315Thr mutation.

The two laboratories performing Sensititre reported INH MIC values as 2 µg/ml and 4 µg/ml.

### Ofloxacin and Ciprofloxacin

DNA sequencing of *gyrA* in Isolate 2019B detected a G>A point mutation in codon 94 resulting in wild-type aspartic acid being replaced with asparagine (Asp94Asn). The Asp94Asn mutation has been associated with FQ resistance [2, 12]. Sequencing of *gyrB* was wild-type (i.e., no mutations were detected).

Among three methods, 17 results for OFL were reported for Isolate 2019B. 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% (1/1)** of the results when using Sensititre

Participating laboratories also reported results for other FQ drugs (e.g., CIP, LVF, and MOX) for Isolate 2019B; 100% (18/18) of results noted resistance to these additional FQ. The isolate was reported resistant to three other FQ by method, as follows:

**Ciprofloxacin**

- **100% (7/7)** 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% (2/2)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **100% (1/1)** of the results when using Sensititre

A mutation in the *gyrA* gene was detected by all (100%) laboratories that reported molecular testing for FQ drugs.

The two laboratories performing Sensititre reported MIC values for FQ drugs; one of these did not report interpretations. Reported MIC were as follows: OFL at 16 µg/ml; LFV at 8 µg/ml; and both results for MOX at 4 µg/ml.

## Streptomycin

Among three methods, 54 results for STR were reported for Isolate 2019B. This isolate was reported as resistant to STR by method, as follows:

- **79% (15/19)** of the results when using AP
- **91% (31/34)** of the results when using MGIT
- **0% (0/1)** of the results when using Sensititre

The two laboratories performing Sensititre reported STR MIC values as 2 µg/ml and 4 µg/ml.

*Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2019B are listed in Tables 11–18.*

### Table 11. Isolate 2019B—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 19 | 0 | 19 |
| Isoniazid—Low | 0 | 19 | 19 |
| Isoniazid—High | 0 | 19 | 19 |
| Ethambutol | 20 | 0 | 20 |

### Table 12. Isolate 2019B—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 66 | 0 | 66 |
| Isoniazid—Low | 0 | 65 | 65 |
| Isoniazid—High | 1 | 37 | 38 |
| Ethambutol | 65 | 1 | 66 |
| Pyrazinamide | 65 | 3 | 68 |

### Table 13. Isolate 2019B—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 0 | 2 | 2 |
| Ethambutol | 2 | 0 | 2 |

### Table 14. Isolate 2019B—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 0 | 2 | 2 |
| Ethambutol | 2 | 0 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

### Table 15. Isolate 2019B—Participant Results for Second-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 4 | 15 | 19 |
| Ofloxacin | 0 | 12 | 12 |
| Ciprofloxacin | 0 | 7 | 7 |
| Levofloxacin | 0 | 1 | 1 |
| Moxifloxacin | 0 | 2 | 2 |
| Amikacin | 9 | 0 | 9 |
| Kanamycin | 14 | 0 | 14 |
| Capreomycin | 12 | 0 | 12 |
| Ethionamide | 14 | 2 | 16 |
| Rifabutin | 7 | 0 | 7 |
| Cycloserine | 6 | 0 | 6 |
| *p*-Aminosalicylic acid | 12 | 0 | 12 |

### Table 16. Isolate 2019B—Participant Results for Second-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 3 | 31 | 34 |
| Ofloxacin | 0 | 4 | 4 |
| Ciprofloxacin | 0 | 1 | 1 |
| Levofloxacin | 0 | 3 | 3 |
| Moxifloxacin | 0 | 3 | 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 17. Isolate 2019B—Participant Results for Second-Line DST by Sensititre

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

* One additional laboratory reported borderline for STR by Sensititre.

## Table 18. Isolate 2019B—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| Rifampin | 0 | 10 | 10 |
| Isoniazid | 8 | 0 | 8 |
| Ethambutol | 0 | 4 | 4 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 4 | 0 | 4 |
| Ciprofloxacin | 4 | 0 | 4 |
| Levofloxacin | 6 | 0 | 6 |
| Moxifloxacin | 6 | 0 | 6 |
| Amikacin | 1 | 4 | 5 |
| Kanamycin | 1 | 4 | 5 |
| Capreomycin | 1 | 3 | 4 |
| Ethionamide | 0 | 2 | 2 |
| Rifabutin | 0 | 4 | 4 |

# Isolate 2019C

Expected Result: Resistant to OFL at 2.0 µg/ml, CIP at 2.0 µg/ml, STR at 2.0 µg/ml, AMK at 4.0 µg/ml, KAN at 5.0 µg/ml, and CAP at 10.0 µg/ml by agar proportion

## Ofloxacin and Ciprofloxacin

DNA sequencing of *gyrA* in Isolate 2019C detected a C>T point mutation in codon 90 of *gyrA* resulting in wild-type alanine being replaced with valine (Ala90Val). The Ala90Val mutation has been associated with FQ resistance [2, 12]. Sequencing of *gyrB* for this isolate was wild-type (i.e., no mutations were detected).

Although resistance was expected for all FQ drugs, variable results were reported by participants; the reason for this variability is unknown. The Ala90Val mutation has been associated with low-level FQ resistance but the MIC for this isolate could be close to the critical concentration thereby impacting DST reproducibility [17].

Among three methods, 16 results for OFL were reported for Isolate 2019C. This isolate was reported as **resistant** to OFL by method, as follows:

- **75% (9/12)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **100% (1/1)** of the results when using Sensititre

Participating laboratories also reported results for other FQ drugs (e.g., CIP, LVF, and MOX) for Isolate 2019C; 40% (6/15) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other FQ by method, as follows:

**Ciprofloxacin**

- **14% (1/7)** of the results when using AP

**Levofloxacin**

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

**Moxifloxacin**

- **50% (1/2)** of the results when using AP
- **100% (2/2)** of the results when using MGIT
- **0% (0/1)** of the results when using Sensititre

A mutation in the *gyrA* gene was detected by all (100%) laboratories that reported molecular testing for FQ drugs.

The two laboratories performing Sensititre reported MIC values for FQ drugs; one of these did not report interpretations. Reported MIC values were as follows: OFL at 8 µg/ml; LFV at 4 µg/ml; and both results for MOX were 2 µg/ml.

## Streptomycin

Among three methods, 55 results for STR were reported for Isolate 2019C. This isolate was reported as **resistant** to STR by method, as follows:

- **100% (19/19)** of the results when using AP
- **100% (34/34)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Both laboratories performing Sensititre reported STR MIC values as >32 µg/ml.

## 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 [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, 18]. The most common *rrs* mutation for cross-resistance to all three drugs is the A1401G point mutation [18].

For Isolate 2019C, 42 results were reported for AMK, KAN, and CAP. The isolate was reported **resistant** to the three second-line injectables by method, as follows:

**Amikacin**

- **89% (8/9)** of the results when using AP
- **100% (2/2)** of the results when using MGIT
- **100% (1/1)** of the results when using Sensititre

**Kanamycin**

- **100% (14/14)** of the results when using AP
- **100% (1/1)** of the results when using MGIT
- **100% (1/1)** of the results when using Sensititre

**Capreomycin**

- **64% (7/11)** of the results when using AP
- **67% (2/3)** of the results when using MGIT

The mutation in the *rrs* gene was detected by three (60%) laboratories that reported molecular testing for AMK and KAN, and CAP, with two laboratories specifically noting it was the A1401G mutation.

Laboratories performing Sensititre reported MIC values for second-line injectable drugs. One laboratory did not report interpretations. Reported MIC values were as follows: KAN at >40 µg/ml; CAP at 10 µg/ml; and both results for AMK were >16 µg/ml.

## Ethionamide

Isolate 2019C was expected to be susceptible to ethionamide (ETA), however the majority of reporting laboratories (89%) indicated ETA resistance. One laboratory noted an *ethA* deletion for this isolate by molecular testing.

Among three methods, 18 results for ETA were reported for Isolate 2019C. This isolate was reported as **resistant** to ETA by method, as follows:

- **93% (13/14)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **0% (0/1)** of the results when using Sensititre

*Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2019C are listed in Tables 19–26.*

*One laboratory noted no growth for at least one antituberculosis drug tested for Isolate 2019C.*

### Table 19. Isolate 2019C—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 19 | 0 | 19 |
| Isoniazid—Low | 19 | 0 | 19 |
| Isoniazid—High | 19 | 0 | 19 |
| Ethambutol | 17 | 3 | 20 |

## Table 20. Isolate 2019C—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 66 | 0 | 66 |
| Isoniazid—Low | 66 | 0 | 66 |
| Isoniazid—High | 26 | 0 | 26 |
| Ethambutol | 66 | 0 | 66 |
| Pyrazinamide | 67 | 1 | 68 |

## Table 21. Isolate 2019C—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 2 | 0 | 2 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 1 | 1 | 2 |

## Table 22. Isolate 2019C—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 2 | 0 | 2 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 2 | 0 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

## Table 23. Isolate 2019C—Participant Results for Second-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 0 | 19 | 19 |
| Ofloxacin | 3 | 9 | 12 |
| Ciprofloxacin | 6 | 1 | 7 |
| Levofloxacin | 0 | 1 | 1 |
| Moxifloxacin | 1 | 1 | 2 |
| Amikacin | 1 | 8 | 9 |
| Kanamycin | 0 | 14 | 14 |
| Capreomycin | 4 | 7 | 11* |
| Ethionamide | 1 | 13 | 14* |
| Rifabutin | 7 | 0 | 7 |
| Cycloserine | 6 | 0 | 6 |
| *p*-Aminosalicylic acid | 11 | 1 | 12 |

* One additional laboratory reported borderline for CAP and ETA by AP.

## Table 24. Isolate 2019C—Participant Results for Second-Line DST by MGIT

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

## Table 25. Isolate 2019C—Participant Results for Second-Line DST by Sensititre

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

## Table 26. Isolate 2019C—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| Rifampin | 0 | 10 | 10 |
| Isoniazid | 0 | 8 | 8 |
| Ethambutol | 0 | 4 | 4 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 4 | 0 | 4 |
| Ciprofloxacin | 4 | 0 | 4 |
| Levofloxacin | 6 | 0 | 6 |
| Moxifloxacin | 6 | 0 | 6 |
| Amikacin | 2 | 3 | 5 |
| Kanamycin | 2 | 3 | 5 |
| Capreomycin | 2 | 2 | 4 |
| Ethionamide | 1* | 1 | 2 |
| Rifabutin | 0 | 4 | 4 |

* This laboratory noted the detection of an *ethA* deletion.

# Isolate 2019D

## Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml, OFL at 2.0 µg/ml, CIP at 2.0 µg/ml, and STR at 2.0 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* and *ahpC* can also cause resistance. Like 2019B, DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2019D also 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).

For Isolate 2019D, 88 INH results were reported. This isolate was reported resistant to INH by method, as follows:

- **100% (19/19)** of the results when using AP
- **100% (65/65)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Fifty-seven (97%) results were reported as resistant at the higher concentrations of INH. Only 38 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 8 molecular results reported for INH, all (100%) laboratories reported detection of a mutation with 6 laboratories specifically noting the Ser315Thr mutation.

Both laboratories performing Sensititre reported INH MIC values as 2 µg/ml.

### Ofloxacin and Ciprofloxacin

Unlike the FQ resistance seen with Isolates 2019A, 2019B, and 2019C, heteroresistance was observed for OFL with Isolate 2019D. 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 sequencing of *gyrA* in Isolate 2019D detected a variety of point mutations at codon 94 resulting in wild-type aspartic acid being replaced with other amino acids. Mutations at codon 94 have been associated with FQ resistance [2, 12]. Sequencing of *gyrB* was wild-type (i.e., no mutations were detected).

Among three methods, 17 results for OFL were reported for Isolate 2019D. 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% (1/1)** of the results when using Sensititre

Participating laboratories also reported results for other FQ drugs (e.g., CIP, LVF, and MOX) for Isolate 2019D; 100% (15/15) of results noted resistance to these additional FQ. The isolate was reported resistant to three other FQ by method, as follows:

**Ciprofloxacin**

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

**Levofloxacin**

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

**Moxifloxacin**

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

A mutation in the *gyrA* gene was detected by all (100%) laboratories that reported molecular testing for FQ drugs.

The two laboratories performing Sensititre reported MIC values for FQ drugs; one of these did not report interpretations. Reported MIC values were as follows: OFL at 16 µg/ml; LFV at 8 µg/ml; and both results for MOX were 8 µg/ml.

## Streptomycin

Among three methods, 55 results for STR were reported for Isolate 2019D. This isolate was reported as resistant to STR by method, as follows:

- **79% (15/19)** of the results when using AP
- **97% (33/34)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

The two laboratories performing Sensititre reported STR MIC values as 8 µg/ml and 16 µg/ml.

*Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2019D are listed in Tables 27–34.*

*One laboratory noted no growth for at least one antituberculosis drug tested for Isolate 2019D.*

### Table 27. Isolate 2019D—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 19 | 0 | 19 |
| Isoniazid—Low | 0 | 19 | 19 |
| Isoniazid—High | 1 | 18 | 19 |
| Ethambutol | 20 | 0 | 20 |

### Table 28. Isolate 2019D—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 65 | 0 | 65 |
| Isoniazid—Low | 0 | 65 | 65 |
| Isoniazid—High | 1 | 37 | 38 |
| Ethambutol | 65 | 0 | 65 |
| Pyrazinamide | 64 | 3 | 67 |

### Table 29. Isolate 2019D—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 0 | 2 | 2 |
| Ethambutol | 2 | 0 | 2 |

## Table 30. Isolate 2019D—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 0 | 2 | 2 |
| Ethambutol | 2 | 0 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

## Table 31. Isolate 2019D—Participant Results for Second-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 4 | 15 | 19 |
| Ofloxacin | 0 | 12 | 12 |
| Ciprofloxacin | 0 | 7 | 7 |
| Levofloxacin | 0 | 0 | 0 |
| Moxifloxacin | 0 | 1 | 1 |
| Amikacin | 9 | 0 | 9 |
| Kanamycin | 14 | 0 | 14 |
| Capreomycin | 12 | 0 | 12 |
| Ethionamide | 15 | 0 | 15 |
| Rifabutin | 7 | 0 | 7 |
| Cycloserine | 6 | 0 | 6 |
| *p*-Aminosalicylic acid | 12 | 0 | 12 |

## Table 32. Isolate 2019D—Participant Results for Second-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 1 | 33 | 34 |
| 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 | 3 | 0 | 3 |
| Rifabutin | 3 | 0 | 3 |
| Cycloserine | 0 | 0 | 0 |
| *p*-Aminosalicylic acid | 0 | 0 | 0 |

* One additional laboratory reported borderline for MOX by MGIT.

## Table 33. Isolate 2019D—Participant Results for Second-Line DST by Sensititre

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

## Table 34. Isolate 2019D—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| Rifampin | 0 | 10 | 10 |
| Isoniazid | 8 | 0 | 8 |
| Ethambutol | 0 | 4 | 4 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 4 | 0 | 4 |
| Ciprofloxacin | 4 | 0 | 4 |
| Levofloxacin | 4 | 0 | 4 |
| Moxifloxacin | 4 | 0 | 4 |
| Amikacin | 0 | 5 | 5 |
| Kanamycin | 0 | 5 | 5 |
| Capreomycin | 0 | 4 | 4 |
| Ethionamide | 0 | 2 | 2 |
| Rifabutin | 0 | 4 | 4 |

# Isolate 2019E

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

## Ofloxacin and Ciprofloxacin

Similar to Isolate 2019D, heteroresistance was observed for OFL with Isolate 2019E. DNA sequencing of *gyrA* in Isolate 2019E also detected a variety of point mutations at codon 94 resulting in wild-type aspartic acid being replaced with other amino acids. Mutations at codon 94 have been associated with FQ resistance [2, 12]. Sequencing of *gyrB* was wild-type (i.e., no mutations were detected).

Among three methods, 15 results for OFL were reported for Isolate 2019E. This isolate was reported as resistant to OFL by method, as follows:

- **100% (10/10)** of the results when using AP
- **100% (4/4)** of the results when using MGIT
- **100% (1/1)** of the results when using Sensititre

Participating laboratories also reported results for other FQ drugs (e.g., CIP, LVF, and MOX) for Isolate 2019E; 94% (16/17) of results noted resistance to these additional FQ. The isolate was reported resistant to three other FQ 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**

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

A mutation in the *gyrA* gene was detected by all (100%) laboratories that reported molecular testing for FQ drugs.

The two laboratories performing Sensititre reported MIC values for FQ drugs; one of these did not report interpretations. Reported MIC values were as follows: OFL at 32 µg/ml; LFV at 8 µg/ml; and MOX was 8 µg/ml and >8 µg/ml.

*Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2019E are listed in Tables 35–42.*

### Table 35. Isolate 2019E—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 16 | 0 | 16 |
| Isoniazid—Low | 16 | 0 | 16 |
| Isoniazid—High | 16 | 0 | 16 |
| Ethambutol | 17 | 0 | 17 |

## Table 36. Isolate 2019E—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 66 | 0 | 66 |
| Isoniazid—Low | 65 | 1 | 66 |
| Isoniazid—High | 26 | 0 | 26 |
| Ethambutol | 66 | 0 | 66 |
| Pyrazinamide | 67 | 0 | 67 |

## Table 37. Isolate 2019E—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 2 | 0 | 2 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 2 | 0 | 2 |

## Table 38. Isolate 2019E—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 2 | 0 | 2 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 2 | 0 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

## Table 39. Isolate 2019E—Participant Results for Second-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 16 | 0 | 16 |
| Ofloxacin | 0 | 10 | 10 |
| Ciprofloxacin | 0 | 6 | 6 |
| Levofloxacin | 0 | 1 | 1 |
| Moxifloxacin | 1 | 1 | 2 |
| Amikacin | 8 | 0 | 8 |
| Kanamycin | 13 | 0 | 13 |
| Capreomycin | 12 | 0 | 12 |
| Ethionamide | 11 | 1 | 12* |
| Rifabutin | 7 | 0 | 7 |
| Cycloserine | 6 | 0 | 6 |
| *p*-Aminosalicylic acid | 10 | 0 | 10 |

* One additional laboratory reported borderline for ETA by AP.

## Table 40. Isolate 2019E—Participant Results for Second-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 34 | 0 | 34 |
| Ofloxacin | 0 | 4 | 4 |
| Ciprofloxacin | 0 | 1 | 1 |
| Levofloxacin | 0 | 3 | 3 |
| Moxifloxacin | 0 | 3 | 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 41. Isolate 2019E—Participant Results for Second-Line DST by Sensititre

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

## Table 42. Isolate 2019E—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| Rifampin | 0 | 10 | 10 |
| Isoniazid | 0 | 7 | 7 |
| Ethambutol | 0 | 4 | 4 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 4 | 0 | 4 |
| Ciprofloxacin | 4 | 0 | 4 |
| Levofloxacin | 6 | 0 | 6 |
| Moxifloxacin | 6 | 0 | 6 |
| Amikacin | 0 | 5 | 5 |
| Kanamycin | 0 | 5 | 5 |
| Capreomycin | 0 | 4 | 4 |
| Ethionamide | 0 | 2 | 2 |
| Rifabutin | 0 | 4 | 4 |

# 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 | 7.5 |
| Pyrazinamide | Not recommended | Not recommended |

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

*The higher concentration of INH 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 | 2.0 |
| 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 |
| 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 Drug | 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 72 laboratories participating in the March 2019 MPEP survey is show in this pie chart.** The largest slice, at 69%, represents 50 laboratories that have self-classified as a health department laboratory. The next major slice signifies 12 hospital laboratories. The remaining two slices of the pie chart represent 7 independent laboratories and 3 federal government laboratories. (page 7)

**Figure 2. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=72) in 2018 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; 19 laboratories tested between 51 to 100 isolates per year; 5 laboratories tested between 101 to 150 isolates per year; 7 laboratories tested between 151 to 200 isolates per year; 2 laboratories tested between 201 to 250 isolates per year; 1 laboratory tested between 251 to 300 isolates per year; 5 laboratories tested between 301 to 500 isolates per year; 8 laboratories tested between 501 to 1000 isolates per year, and 0 laboratories tested greater than or equal to 1001 isolates per year. (page 8)

**Figure 3. The drug susceptibility testing methods used by MPEP participants (N=103) 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 20, 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: 69 used MGIT, 20 used agar proportion, 2 used Sensititre, 2 used VersaTREK, and 10 used molecular methods. (page 9)

**Figure 4. The molecular methods used by MPEP participants (N=10) are 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 MTB/RIF assay, 2 laboratories that use Bruker line probe assays, and 1 laboratory that uses whole genome sequencing. (page 9)

**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 80, 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. 72 laboratories tested rifampin; 72 laboratories tested isoniazid; 72 laboratories tested ethambutol; 69 laboratories tested pyrazinamide; 47 laboratories tested streptomycin; 17 laboratories tested ofloxacin; 8 laboratories tested moxifloxacin; 6 laboratories tested ciprofloxacin; 4 laboratories tested levofloxacin; 16 laboratories tested kanamycin; 16 laboratories tested capreomycin; 13 laboratories tested amikacin; 20 laboratories tested ethionamide; 14 laboratories tested PAS; 12 laboratories tested rifabutin; and 7 laboratories tested cycloserine. (page 10)

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