Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program
Report of Results, August 2018

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 August 2018 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 2018.

Report Content

The material in this report was developed and prepared by:

Cortney Stafford, MPH, MT (ASCP), Health Scientist, Laboratory Capacity Team, NCHHSTP, DTBE, LB

Acknowledged NCHHSTP, DTBE, LB contributors:
Beverly Metchock, Stephanie Johnston, Lois Diem, Mitchell Yakrus, and Angela Starks

Contact Information

Comments and inquiries regarding this report should be directed to:

TBMPEP@cdc.gov
404-639-4013
https://www.cdc.gov/tb/topic/laboratory/mpep/default.htm

Explanations of figures for accessibility is found in Appendix 1: Accessible Explanation of Figures on page 31.

Contents

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for August 2018 Survey . . . 2
- Purpose . . . 2
- Report Content . . . 2
- Contact Information . . . 2

Abbreviations and Acronyms . . . 4

Introduction: Overview of MPEP Final Report . . . 5

Expected Drug Susceptibility Testing Results . . . 5

Technical Notes . . . 6

Descriptive Information about Participant Laboratories . . . 7
- Primary Classification . . . 7
- Annual Number of MTBC Drug Susceptibility Tests Performed . . . 8
- MTBC DST Methods Used by Participants . . . 9
- Antituberculosis Drugs Tested by Participants . . . 10

Isolate 2018F . . . 11

Isolate 2018G . . . 15

Isolate 2018H . . . 19

Isolate 2018I . . . 22

Isolate 2018J . . . 25

Equivalent Critical Concentrations . . . 29
- Agar Proportion . . . 29
- Broth Based Media . . . 29

References . . . 30

Appendix 1: Accessible Explanations of Figures . . . 31

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
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
WHO	World Health Organization
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 testing (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].

WHO recently released a technical report describing a review of different critical concentrations used for phenotypic DST and provided a list of revised or newly established critical concentrations for antituberculosis drugs used for the treatment of drug-resistant TB. http://www.who.int/tb/publications/2018/WHO_technical_report_concentrations_TB_drug_susceptibility/en/

Expected Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in August 2018 are shown in the tables below. Although CDC recommends broth-based methods for routine first-line DST of MTBC isolates, the results obtained by the reference agar proportion method (except for pyrazinamide, in which MGIT was performed) are shown in Table 1. Molecular results obtained by DNA sequencing are listed in Table 2 [2].

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

Note—S=susceptible, R=resistant, Empty cell=None detected

Isolate	RMP	INH	EMB	PZA	Second-line Drugs Resistant to:
2018F	S	R	S	S	STR
2018G	S	S	S	S	OFL, CIP
2018H	S	S	S	S	OFL, CIP
2018I	S	R	S	S	
2018J	S	S	S	S	

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

Empty cell=None detected

Isolate	*rpoB*	*inhA*	*katG*	*fabG1*	*gyrA*
2018F			Ser315Thr		
2018G					Ser91Pro & Asp94Asn
2018H					Ser91Pro & Asp94Asn
2018I			Ser315Thr		
2018J					

Technical Notes

The following information pertains to all of the tables and figures for the 2018 MTBC isolates F, G, H, I, and J in this report.

- The source of data in all tables and figures is the August 2018 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 25 laboratories reporting second-line drug results (with the exception of streptomycin), seven (28%) tested all three second-line injectable drugs and at least one fluoroquinolone needed to confidently define XDR tuberculosis (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

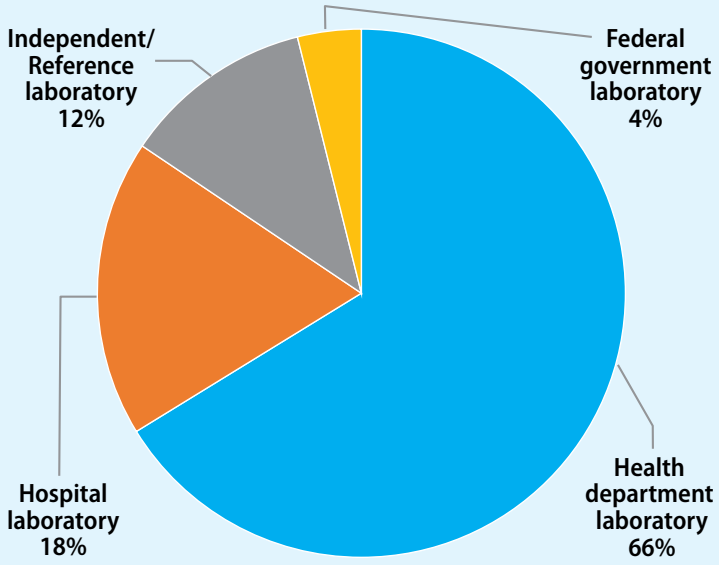
Primary Classification

This report contains DST results submitted to CDC by survey participants at 77 laboratories in 34 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)
- **14 (18%)**: Hospital laboratory
- **9 (12%)**: Independent/Reference laboratory (non-hospital based)
- **3 (4%)**: Federal government laboratory

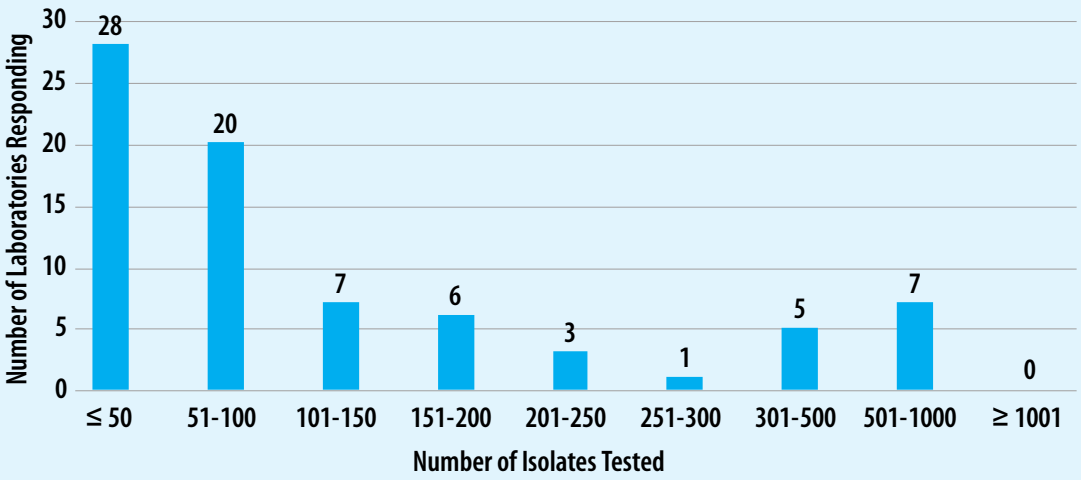
Figure 1. Primary Classification of Participating Laboratories, August 2018



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 77 participants in 2017 (excluding isolates used for quality control) is shown in Figure 2. In 2017, the counts ranged from 0 to 926 tests. Participants at 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].

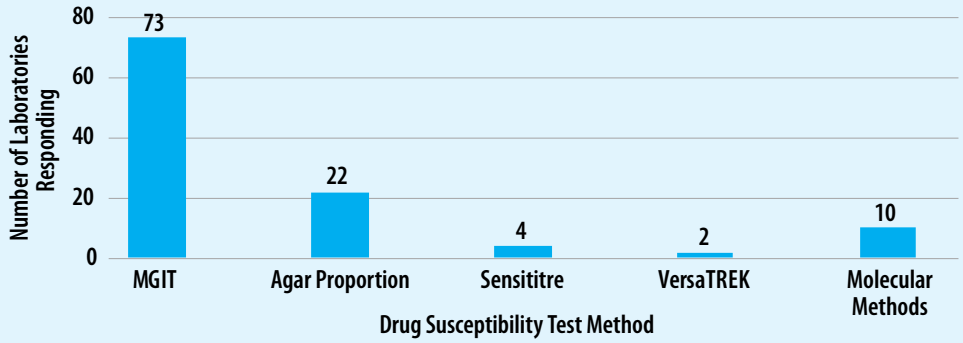
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=77)



MTBC DST Methods Used by Participants

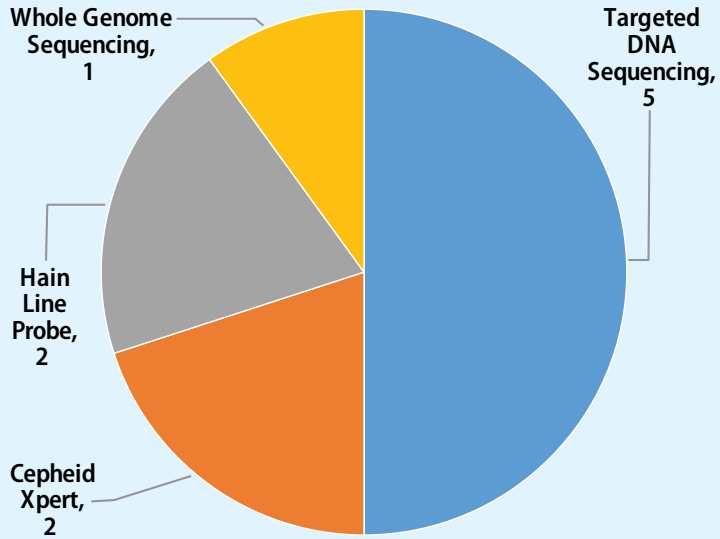
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 46 (59%) laboratories reported results for only one method, 28 laboratories reported two methods, and three laboratories noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=111)



Molecular methods reported by ten 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 the line probe assays Genotype MTBDRplus and MTBDRsl by Hain Lifescience, and one reported results from whole genome sequencing.

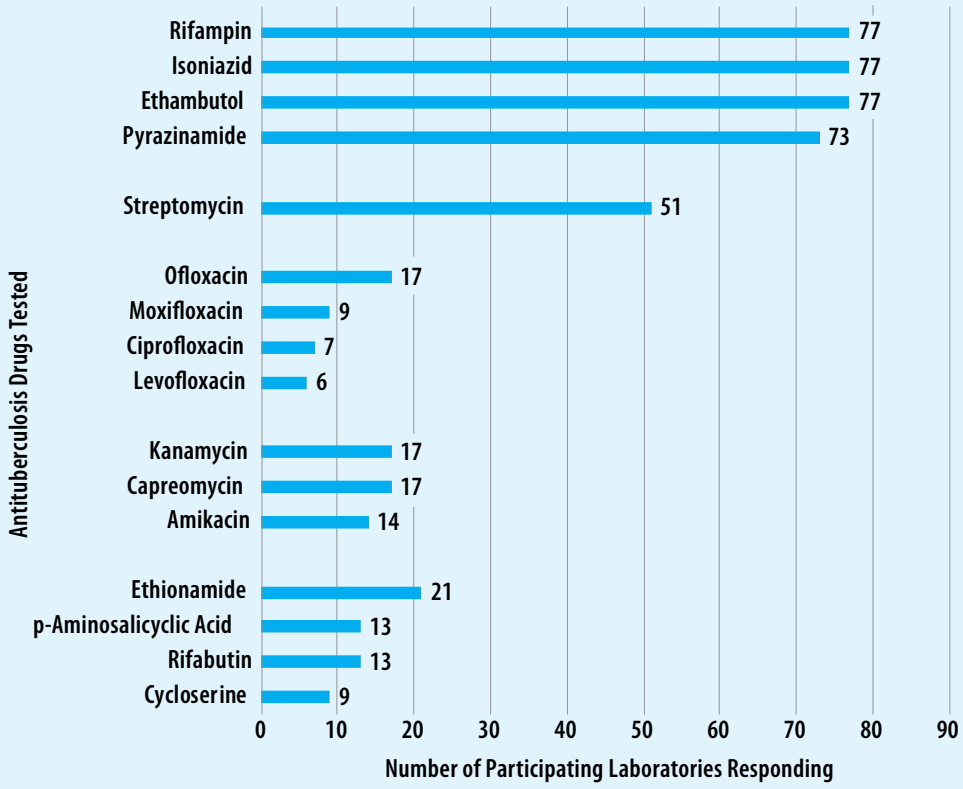
Figure 4. Molecular Method Reported (n=10)



Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the August 2018 survey is shown in Figure 5. CLSI recommends testing a full panel of first-line drugs (rifampin [RMP], isoniazid [INH], ethambutol [EMB], and pyrazinamide [PZA])[1], because it represents a combination of tests that provides the clinician with comprehensive information related to the four-drug antituberculosis therapy currently recommended for most patients. All participants reported results for three of the first-line drugs (RMP, INH, and EMB) and 73 (95%) also reported results for PZA by growth-based DST methods.

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2018F

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 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 infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *kat*G gene [2, 4]. The target of activated INH is enoyl-acyl-carrier protein reductase (encoded by the *inh*A gene); this binding inhibits cell wall mycolic acid biosynthesis. There are two mechanisms that account for the majority of INH resistance [2, 4, 5]. The most common mechanism, mutations in *kat*G, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inh*A gene, which are generally associated with low-level resistance to INH and are less frequent than *kat*G mutations. Approximately 10–15% of isolates found to be INH resistant have no mutations detected in either of these loci. Numerous loci have been investigated to identify additional genes correlated with INH resistance. The *fab*G1 (also known as *mab*A) gene, like *inh*A, is involved in mycolic acid biosynthesis and at least one mutation in this region has been associated with low-level INH resistance [6, 7]. In MTBC, *ahp*C codes for an alkyl hydroperoxide reductase that is associated with resistance to reactive oxygen and reactive nitrogen intermediates; consequently it was initially believed that mutations in the promoter region could be surrogate markers for INH resistance [4].

DNA sequence analysis of *inh*A, *kat*G, *fab*G1, and *ahp*C of Isolate 2018F revealed a T>A point mutation at codon 315 in the *kat*G locus resulting in wild-type serine being replaced by threonine (Ser315Thr); *inh*A, *fab*G1, and *ahp*C were wild-type (i.e., no mutations were detected).

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

For Isolate 2018F, 97 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% (72/72)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Sixty-five (100%) results were reported as **resistant** at the higher concentrations of INH. Only 40 of 72 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.

Streptomycin

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

Among three methods, 58 results for STR were reported for Isolate 2018F. This isolate was reported as **resistant** to STR by method, as follows:

- **100% (19/19)** of the results when using AP
- **100% (36/36)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre

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

Table 3. Isolate 2018F—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	19	2	21

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

Drug	Susceptible	Resistant	Total
Rifampin	72	0	72
Isoniazid—Low	0	72	72
Isoniazid—High	0	40	40
Ethambutol	71	1	72
Pyrazinamide	69	3	72

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

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

* One additional laboratory reported borderline for EMB by Sensititre.

Table 6. Isolate 2018F—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 7. Isolate 2018F—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	0	19	19
Ofloxacin	12	0	12
Ciprofloxacin	6	0	6
Levofloxacin	2	0	2
Moxifloxacin	4	0	4
Amikacin	9	0	9
Kanamycin	14	0	14
Capreomycin	13	0	13
Ethionamide	3	13	16
Rifabutin	7	0	7
Cycloserine	7	0	7
p-Aminosalicylic acid	10	0	10

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

Drug	Susceptible	Resistant	Total
Streptomycin	0	36	36
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	0	3	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0

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

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

* One additional laboratory reported borderline for ETA by Sensititre.

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

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

* This laboratory noted the detection of a gyrA mutation not associated with fluoroquinolone resistance.

Isolate 2018G

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 TB resistant to first-line drugs but also have the potential to become an important part of new TB regimens [10]. 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 [10, 11]. The primary mechanism of action of FQ is the inhibition of DNA synthesis [8] by inhibiting DNA gyrase. The enzyme DNA gyrase generates the activity for cleaving and resealing double-stranded DNA. This action is necessary for DNA replication, transcription, and recombination.

Resistance to FQ has mainly been attributed to point mutations in a 21-bp region of the MTBC *gyr*A gene, often called the quinolone resistance determining region (QRDR). These mutations, commonly occurring at codons 90, 91, and 94, prevent the drugs from effectively binding DNA gyrase [2, 5, 8]. 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 [8].

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 [12, 13]. 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 [14, 15].

DNA sequencing of *gyr*A in Isolate 2018G revealed a T>C point mutation in codon 91 of *gyr*A resulting in wild-type serine being replaced with proline (Ser91Pro). The Ser91Pro mutation has been associated with FQ resistance [2, 16]. 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, 17 results for OFL were reported for Isolate 2018G. This isolate was reported as **resistant** to OFL by method, as follows:

- **91% (10/11)** 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 2018G; 90% (18/20) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other fluoroquinolones by method, as follows:

Ciprofloxacin

- **100% (5/5)** of the results when using AP
- **100% (1/1)** of the results when using MGIT

Levofloxacin

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

Moxifloxacin

- **100% (4/4)** of the results when using AP
- **67% (2/3)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

A mutation in the *gyr*A gene was 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 2018G are listed in Tables 11–18.

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

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

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

Drug	Susceptible	Resistant	Total
Rifampin	72	0	72
Isoniazid—Low	72	0	72
Isoniazid—High	25	0	25
Ethambutol	72	0	72
Pyrazinamide	71	1	72

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

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

Table 14. Isolate 2018G—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 15. Isolate 2018G—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	17	0	17
Ofloxacin	1	10	11
Ciprofloxacin	0	5	5
Levofloxacin	0	2	2
Moxifloxacin	0	4	4
Amikacin	9	0	9
Kanamycin	13	0	13
Capreomycin	13	0	13
Ethionamide	15	0	15
Rifabutin	7	0	7
Cycloserine	7	0	7
p-Aminosalicylic acid	9	0	9

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

Drug	Susceptible	Resistant	Total
Streptomycin	37	0	37
Ofloxacin	0	4	4
Ciprofloxacin	0	1	1
Levofloxacin	0	3	3
Moxifloxacin	1	2	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 2018G—Participant Results for Second-Line DST by Sensititre

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

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

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

* This laboratory noted the detection of a synonymous mutation Ser65Ser in *pncA*.

Isolate 2018H

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

Ofloxacin

DNA sequencing of *gyr*A in Isolate 2018H revealed a T>C point mutation in codon 91 of *gyr*A resulting in wild-type serine being replaced with proline (Ser91Pro). The Ser91Pro mutation has been associated with FQ resistance [2, 16]. Like 2018G, 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, 17 results for OFL were reported for Isolate 2018H. This isolate was reported as **resistant** to OFL by method, as follows:

- **91% (10/11)** 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 2018H; 95% (18/19) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other fluoroquinolones by method, as follows:

Ciprofloxacin

- **100% (5/5)** of the results when using AP
- **100% (1/1)** of the results when using MGIT

Levofloxacin

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

Moxifloxacin

- **100% (4/4)** of the results when using AP
- **100% (2/2)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

A mutation in the *gyr*A gene was 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 participant for Isolate 2018H are listed in Tables 19–26.

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

Drug	Susceptible	Resistant	Total
Rifampin	17	0	17
Isoniazid—Low	16	1	17
Isoniazid—High	17	0	17
Ethambutol	18	0	18

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

Drug	Susceptible	Resistant	Total
Rifampin	72	0	72
Isoniazid—Low	71	1	72
Isoniazid—High	26	0	26
Ethambutol	72	0	72
Pyrazinamide	71	1	72

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

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

Table 22. Isolate 2018H—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 2018H—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	17	0	17
Ofloxacin	1	10	11
Ciprofloxacin	0	5	5
Levofloxacin	0	2	2
Moxifloxacin	0	4	4
Amikacin	9	0	9
Kanamycin	13	0	13
Capreomycin	13	0	13
Ethionamide	15	0	15
Rifabutin	7	0	7
Cycloserine	7	0	7
p-Aminosalicylic acid	9	0	9

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

Drug	Susceptible	Resistant	Total
Streptomycin	36	0	36
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 25. Isolate 2018H—Participant Results for Second-Line DST by Sensititre

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

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

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

* This laboratory noted the detection of a synonymous mutation Ser65Ser in *pnc*A.

Isolate 2018I

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

Isoniazid Pyrazinamide

As previously noted, resistance to INH most commonly occurs due to mutations in the *kat*G gene or the promoter region of the *inh*A gene, however, mutations in *fab*G1 and *ahp*C can also cause resistance. Like 2018F, DNA sequence analysis of *inh*A, *kat*G, *fab*G1, and *ahp*C of Isolate 2018I also revealed a T>A point mutation at codon 315 in the *kat*G locus resulting in wild-type serine being replaced by threonine (Ser315Thr); *inh*A, *fab*G1, and *ahp*C were wild-type (i.e., no mutations were detected).

For Isolate 2018I, 97 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (19/19)** of the results when using AP
- **99% (71/72)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Sixty-four of 65 (98%) results were reported as **resistant** at the higher concentrations of INH. Only 40 of 72 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.

Pyrazinamide

Isolate 2018I was expected to be susceptible to PZA, however a number of laboratories (13) indicated PZA resistance by MGIT. No mutation was reported for *pnc*A by those performing molecular testing.

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

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

Table 27. Isolate 2018I—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	21	0	21

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

Drug	Susceptible	Resistant	Total
Rifampin	72	0	72
Isoniazid—Low	1	71	72
Isoniazid—High	0	40	40
Ethambutol	72	0	72
Pyrazinamide	57	13	70*

* One additional laboratory reported borderline for PZA by MGIT.

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

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

Table 30. Isolate 2018I—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 2018I—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	19	0	19
Ofloxacin	12	0	12
Ciprofloxacin	6	0	6
Levofloxacin	2	0	2
Moxifloxacin	4	0	4
Amikacin	9	0	9
Kanamycin	14	0	14
Capreomycin	13	0	13
Ethionamide	14	2	16
Rifabutin	7	0	7
Cycloserine	7	0	7
p-Aminosalicylic acid	10	0	10

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

Drug	Susceptible	Resistant	Total
Streptomycin	37	0	37
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0

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

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

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

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

*Two laboratories noted the detection of an *emb*B mutation, one noting it was not associated with EMB resistance.

† This laboratory noted the detection of a *gyr*A mutation not associated with fluoroquinolone resistance.

Isolate 2018J

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

Isolate 2018J is susceptible to all first- and second-line drugs. Most (97%) results were reported susceptible for this isolate across all methods; however, unexpected resistance was noted by a number of laboratories for streptomycin and the second-line injectables.

Streptomycin

Among three methods, 55 results for STR were reported for Isolate 2018J. This isolate was reported as **resistant** to STR by method, as follows:

- **35% (6/17)** of the results when using AP
- **0% (0/37)** of the results when using MGIT
- **0% (0/1)** 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 [8]. No mutation was detected after DNA sequence analysis of *rrs*, *eis*, and *tly*A in Isolate 2018J.

For Isolate 2018J, 46 results were reported for AMK, KAN, and CAP. The isolate was reported **resistant** to the three second-line injectables by method, as follows:

Amikacin

- **11% (1/9)** of the results when using AP
- **0% (0/2)** of the results when using MGIT
- **33% (1/3)** of the results when using Sensititre

Kanamycin

- **23% (3/13)** of the results when using AP
- **0% (0/1)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

Capreomycin

- **54% (7/13)** of the results when using AP
- **33% (1/3)** of the results when using MGIT

None (0%) of the laboratories reporting molecular testing for AMK, KAN, and CAP reported detection of a mutation.

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

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

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

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

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

Drug	Susceptible	Resistant	Total
Rifampin	72	0	72
Isoniazid—Low	71	0	71
Isoniazid—High	26	0	26
Ethambutol	72	0	72
Pyrazinamide	72	0	72

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

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

Table 38. Isolate 2018J—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 2018J—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	11	6	17
Ofloxacin	11	0	11
Ciprofloxacin	5	0	5
Levofloxacin	2	0	2
Moxifloxacin	4	0	4
Amikacin	8	1	9
Kanamycin	10	3	13
Capreomycin	6	7	13
Ethionamide	15	0	15
Rifabutin	7	0	7
Cycloserine	7	0	7
p-Aminosalicylic acid	9	0	9

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

Drug	Susceptible	Resistant	Total
Streptomycin	37	0	37
Ofloxacin	3	0	3
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	1	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0

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

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

*Two additional laboratories reported borderline for STR by Sensititre.

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

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

*This laboratory noted the detection of a synonymous mutation Pro241Pro in *kat*G.

† This laboratory noted the detection of a *gyr*A mutation not associated with fluoroquinolone resistance.

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 STR 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 INH and EMB should be tested after resistance at the critical concentration is detected.

References

1. CLSI, *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard—Second Edition in CLSI Document M24 A-2*. 2011, Clinical and Laboratory Standards Institute: Wayne, PA.
2. Campbell, P.J., et al., *Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2011. **55**(5): p. 2032-41.
3. APHL, *TB Drug Susceptibility Testing Expert Panel Meeting Summary Report*. 2007, Association of Public Health Laboratories: Washington, D.C.
4. Almeida Da Silva, P.E. and J.C. Palomino, *Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs*. J Antimicrob Chemother, 2011. **66**(7): p. 1417-30.
5. Zhang, Y. and W.W. Yew, *Mechanisms of drug resistance in Mycobacterium tuberculosis*. Int J Tuberc Lung Dis, 2009. **13**(11): p. 1320-30.
6. Ramaswamy, S.V., et al., *Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2003. **47**(4): p. 1241-50.
7. Ando, H., et al., *A silent mutation in mabA confers isoniazid resistance on Mycobacterium tuberculosis*. Mol Microbiol, 2014. **91**(3): p. 538-47.
8. Zhang, Y. and W.W. Yew, *Mechanisms of drug resistance in Mycobacterium tuberculosis: update 2015*. Int J Tuberc Lung Dis, 2015. **19**(11): p. 1276-89.
9. Centers for Disease Control and Prevention, *Treatment of Tuberculosis, American Thoracic Society, CDC, and Infectious Diseases Society of America*. 2003, MMWR. p. 4,11,19-20.
10. Devasia, R.A., et al., *Fluoroquinolone resistance in Mycobacterium tuberculosis: the effect of duration and timing of fluoroquinolone exposure*. Am J Respir Crit Care Med, 2009. **180**(4): p. 365-70.
11. Chen, T.C., et al., *Fluoroquinolones are associated with delayed treatment and resistance in tuberculosis: a systematic review and meta-analysis*. Int J Infect Dis, 2011. **15**(3): p. e211-6.
12. Eilertson, B., et al., *High proportion of heteroresistance in gyrA and gyrB in fluoroquinolone-resistant Mycobacterium tuberculosis clinical isolates*. Antimicrob Agents Chemother, 2014. **58**(6): p. 3270-5.
13. Rinder, H., K.T. Mieskes, and T. Loscher, *Heteroresistance in Mycobacterium tuberculosis*. Int J Tuberc Lung Dis, 2001. **5**(4): p. 339-45.
14. Willby, M., et al., *Correlation between* Gyr*A substitutions and ofloxacin, levofloxacin, and moxifloxacin cross-resistance in Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2015. **59**(9): p. 5427-34.
15. Kam, K.M., et al., *Stepwise decrease in moxifloxacin susceptibility amongst clinical isolates of multidrug-resistant Mycobacterium tuberculosis: correlation with ofloxacin susceptibility*. Microb Drug Resist, 2006. **12**(1): p. 7-11.
16. Maruri, F., et al., *A systematic review of gyrase mutations associated with fluoroquinolone-resistant Mycobacterium tuberculosis and a proposed gyrase numbering system*. Journal of Antimicrobial Chemotherapy, 2012. **67**(4): p. 819-831.

Appendix 1: Accessible Explanations of Figures

Figure 1. The primary classification of the 77 laboratories participating in the August 2018 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 14 hospital laboratories. The remaining two slices of the pie chart represent 9 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 2017 is displayed in this vertical bar graph. The vertical y–axis is the number of laboratories responding and ranges from 0 to 30 using increments of 5. Along the horizontal x-axis are nine vertical bars representing the number of isolates tested per year. From left to right, 28 laboratories tested less than or equal to 50 isolates per year; 20 laboratories tested between 51 to 100 isolates per year; 7 laboratories tested between 101 to 150 isolates per year; 6 laboratories tested between 151 to 200 isolates per year; 3 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; 7 laboratories tested between 501 to 1000 isolates per year, and 0 laboratories tested greater than or equal to 1001 isolates per year.

Figure 3. The drug susceptibility testing methods used by MPEP participants (N=111) are 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: 73 used MGIT, 22 used agar proportion, 4 used Sensititre, 2 used VersaTREK, and 10 used molecular methods.

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 Hain line probe assays, and 1 laboratory that uses whole genome sequencing.

Figure 5. The antituberculosis drugs tested by MPEP participants are 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; 73 laboratories tested pyrazinamide; 51 laboratories tested streptomycin; 17 laboratories tested ofloxacin; 9 laboratories tested moxifloxacin; 7 laboratories tested ciprofloxacin; 6 laboratories tested levofloxacin; 17 laboratories tested kanamycin; 17 laboratories tested capreomycin; 14 laboratories tested amikacin; 21 laboratories tested ethionamide; 13 laboratories tested PAS; 13 laboratories tested rifabutin; and 9 laboratories tested cycloserine.

Notes:

For more information please contact
Centers for Disease Control and Prevention
1600 Clifton Road NE, Atlanta, GA 33029-4027
Telephone: 1-800-CDC-INFO (232-4636)
MPEP Telephone: 404-639-4013
MPEP Email: TBMPEP@cdc.gov
MPEP Web: www.cdc.gov/tb/topic/laboratory/mpep/default.htm
Publication date: February 2019

CS 300584-A