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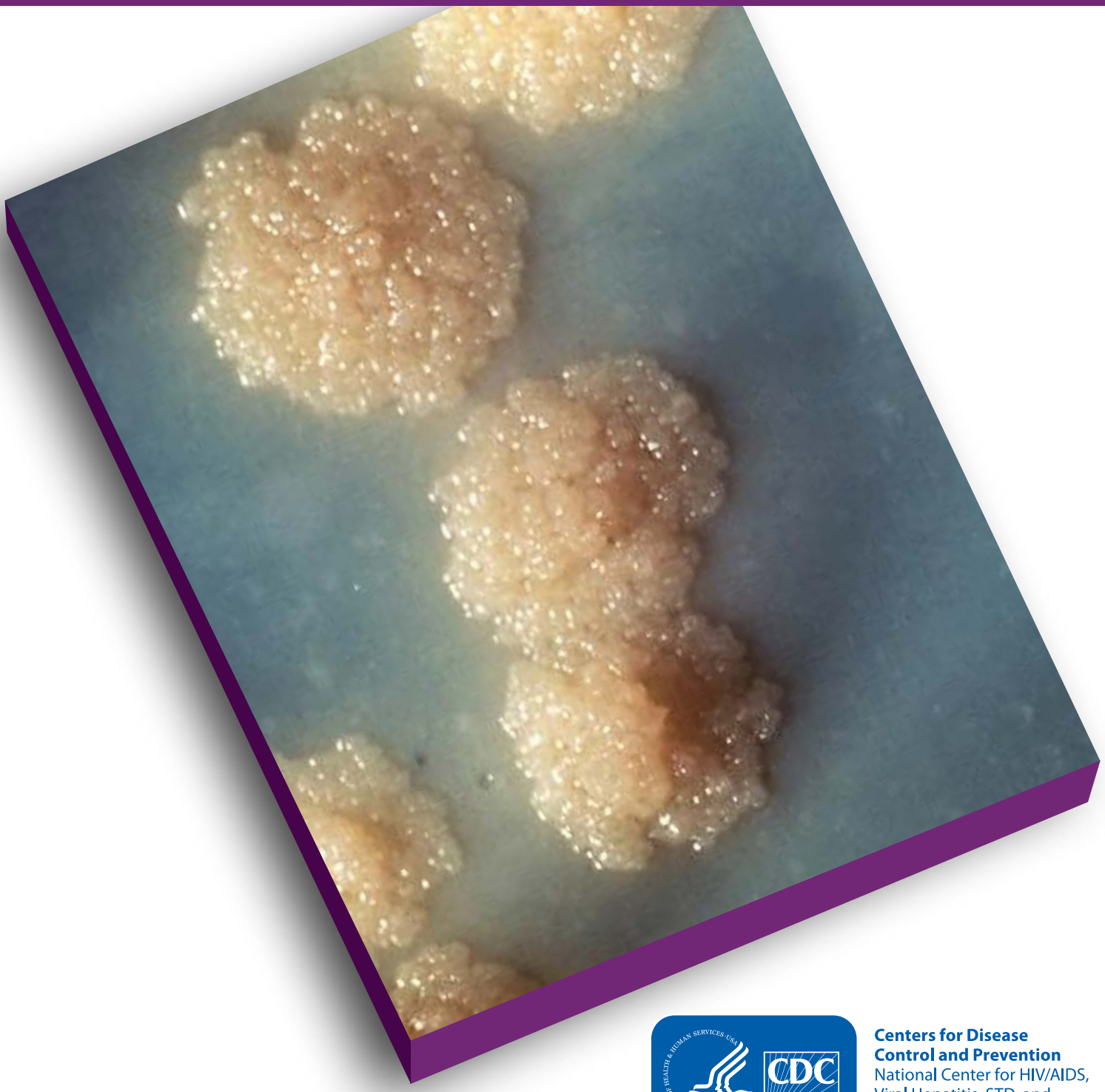
Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program
Report of Results
February 2020



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 February 2020 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 February 2020.

Report Content

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

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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
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 MYCOTB AST 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. The associated 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 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 February 2020 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. Minimum inhibitory concentration testing result for rifampin was also considered for Isolate 2020E. Molecular results obtained by DNA sequencing are listed in Table 2 [2].

Table 1. Expected Growth-based Results for February 2020 Survey

Note—S=susceptible, R=resistant , V=variable

Isolate	RMP	INH	EMB	PZA	Second-line Drugs Resistant to:
2020A	R	S	S	S	STR
2020B	S	R	R+	S	
2020C	S	S	S	S	
2020D	R	S	S	S	
2020E	V*	S	S	S	

* Isolate has mutation that may result in variable results by growth-based methods. 80% consensus for a single categorical result of either susceptible or resistant was not achieved for this isolate among participating laboratories.
+ Although EMB resistance was expected, >80% of participating laboratories reported susceptible. This may be due to the presence of a mutation with reported variable resistance in growth-based methods due to an MIC close to the critical concentration.

Table 2. Expected Molecular Results (Mutations Detected in Loci Associated with Resistance) for February 2020

Note—Empty cell=No mutation detected

Isolate	*rpoB**	*katG*	*ahpC*	*embB*	*pncA*
2020A	Ser531Leu				
2020B		Ser315Thr	G-88A	Met306Ile	
2020C	Leu511Val				
2020D	Val146Phe+				Thr135Ala
2020E	Ser522Gln¥				

* E.coli numbering system used
+ May also be indicated as Val176Phe
¥ Mutation may result in variable results by growth-based methods

Technical Notes

The following information pertains to all of the tables and figures for the 2020 MTBC isolates A, B, C, D, and E, included in this report.

- The source of data in all tables and figures is the February 2020 MPEP MTBC DST survey.
- 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 the number of participating laboratories. This report contains all results reported by participating laboratories.
- 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 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

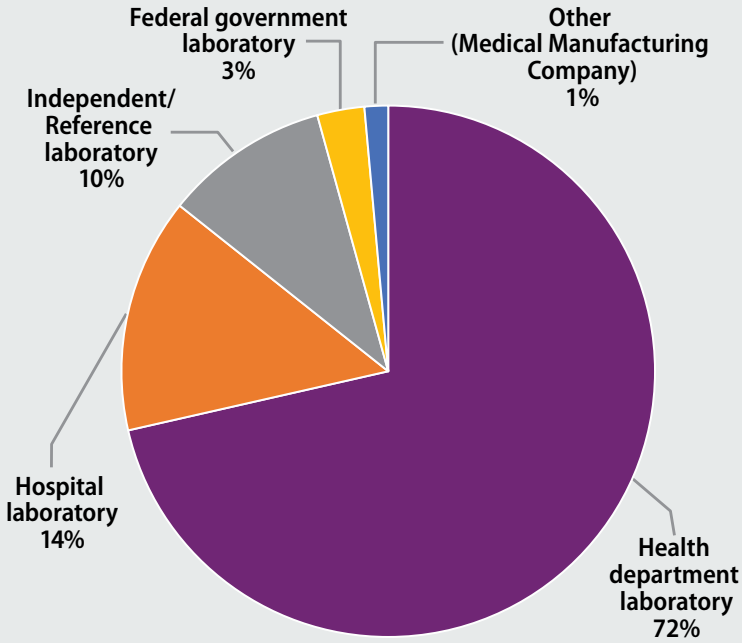
Primary Classification

This report contains DST results submitted to CDC by survey participants at 70 laboratories in 35 states.

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

- **50 (72%)**: Health department laboratory (e.g., local, county, state)
- **10 (14%)**: Hospital laboratory
- **7 (10%)**: Independent/Reference laboratory (non-hospital based)
- **2 (3%)**: Federal government laboratory
- **1 (1%)**: Other (Medical Manufacturing Company)

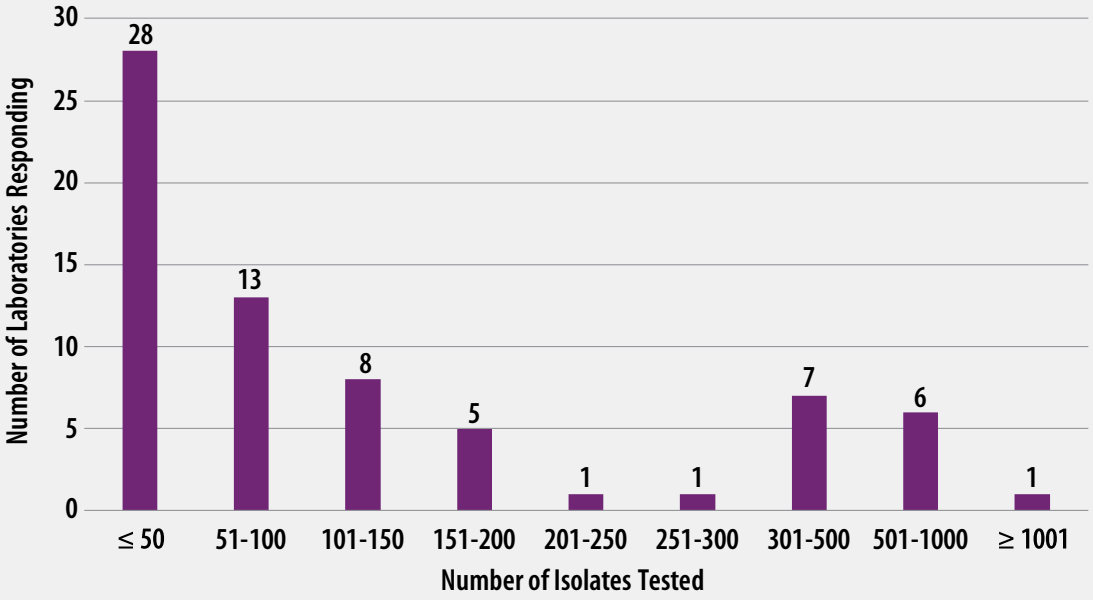
Figure 1. Primary Classification of Participating Laboratories, February 2020



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 70 participants in 2019 (excluding isolates used for quality control) is shown in Figure 2. In 2019, the counts ranged from 0 to 1,039 tests. Participants at 28 (40%) 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].

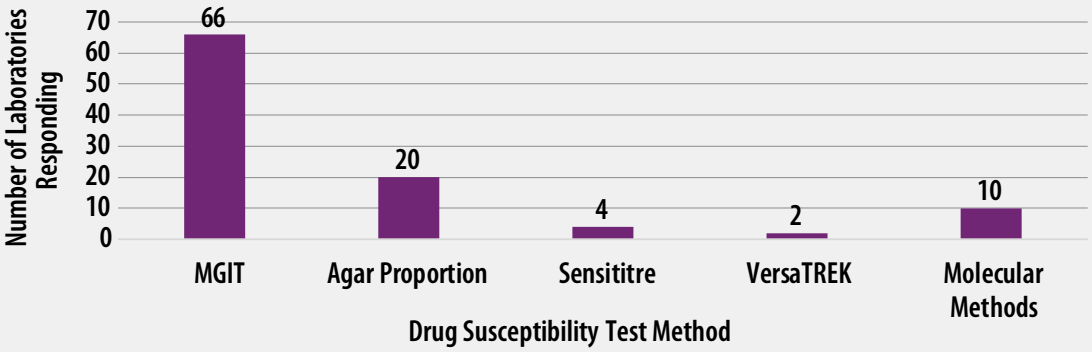
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=70)



MTBC DST Methods Used by Participants

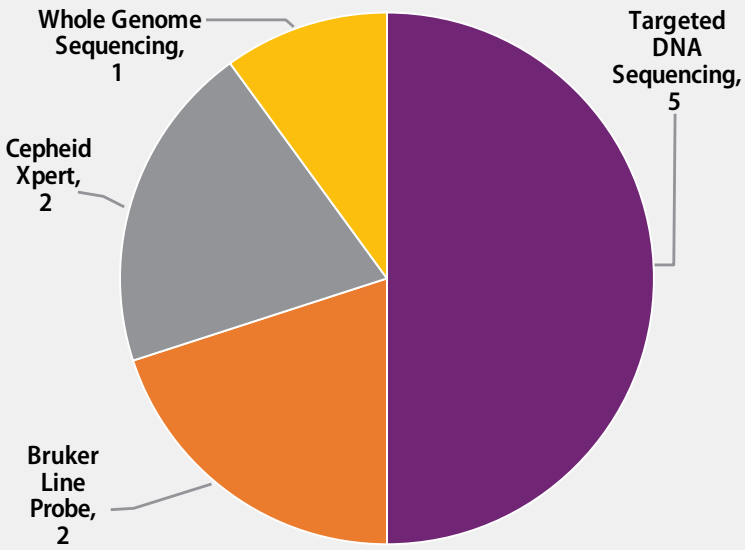
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 42 (60%) laboratories reported results for only one method, 24 (34%) laboratories reported two methods, and 4 (6%) laboratories noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=102)



Molecular methods reported by 10 participants are shown in Figure 4. The method used most frequently by laboratories (5) was targeted DNA sequencing (50%), including pyrosequencing and Sanger sequencing. Two (20%) laboratories reported use of line probe assays, Genotype MTBDR*plus* and MTBDR*sl* by Bruker, two (20%) reported results for the Cepheid Xpert MTB/RIF assay, and one (10%) reported results from whole genome sequencing.

Figure 4. Molecular Method Reported (n=10)

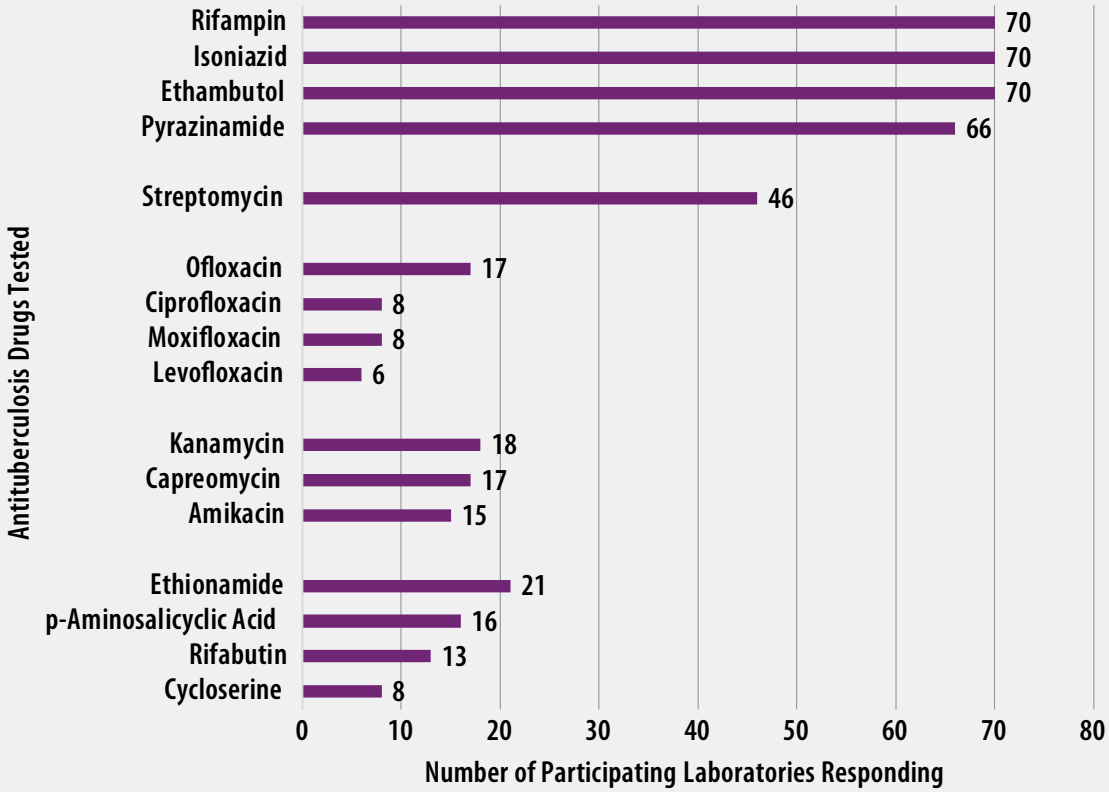


Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the February 2020 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 66 (94%) also reported results for PZA by growth-based DST methods.

For 24 laboratories reporting second-line drug results (with the exception of streptomycin), eight (33%) tested all three second-line injectable drugs and at least one fluoroquinolone needed to confidently define XDR TB. The second-line injectable drugs are amikacin, kanamycin and capreomycin. Fluoroquinolones include ofloxacin, ciprofloxacin, levofloxacin and moxifloxacin.

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2020A

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

Rifampin

Rifampin (RMP) is a bactericidal drug used as part of a standard first-line regimen for the treatment of TB. RMP's mechanism of action is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [4]. The primary mechanism of resistance is a mutation within the 81-bp central region of the *rpoB* gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase [5]. Mutations in codons 531, 526 and 516 (*E. coli* numbering system corresponding to 450, 445 and 435 in MTBC) are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance [4, 5]. The activity of RMP on isolates with *rpoB* mutations depends on both the mutation position and the type of amino acid change.

CDC has recommended that RMP resistance detected by the Xpert MTB/RIF assay be confirmed by DNA sequencing of *rpoB* [6]. The Xpert MTB/RIF assay could generate results that falsely indicate resistance when compared to growth-based methods because of the presence of silent/synonymous mutations [7]. Sequencing of *rpoB* will allow for clarification of the result and understanding of possible discordance between rapid molecular and growth-based testing results.

DNA sequence analysis of *rpoB* in Isolate 2020 revealed a C>T point mutation in codon 531 resulting in wild-type serine being replaced by leucine (Ser531Leu). Isolates with Ser531Leu (Ser450Leu in MTBC numbering system) mutations consistently test resistant to RMP in growth-based assays.

Among four methods, 84 results for RMP were reported for Isolate 2020A. This isolate was reported as **resistant** to RMP by method, as follows:

- 100% (17/17) of the results when using AP
- 97% (59/61) of the results when using MGIT
- 100% (4/4) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Of the 10 molecular results reported for RMP, all (100%) laboratories reported detection of a mutation with 4 laboratories specifically noting the Ser531Leu mutation.

Three of the laboratories performing Sensititre reported RMP MIC values as 16 µg/ml (n=1) and >16 µg/ml (n=2).

Rifabutin

Participant results are consistent with rifabutin (RBT) results based on the presence of the *rpoB* Ser531Leu mutation[8].

Among three methods, 12 results for RBT were reported for Isolate 2020A. This isolate was reported as **resistant** to RBT by method, as follows:

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

Three of the laboratories performing Sensititre reported RBT MIC values as 2 µg/ml (n=2) and 8 µg/ml (n=1).

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, 9]. 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, 10].

Among three methods, 51 results for STR were reported for Isolate 2020A. This isolate was reported as resistant to STR by method, as follows:

- 24% (4/17) of the results when using AP
- 77% (24/31) of the results when using MGIT
- 0% (0/3) of the results when using Sensititre

Three of the laboratories performing Sensititre reported STR MIC values as 0.5 µg/ml, 1 µg/ml and 2 µg/ml.

Complete first-line DST, second-line DST and molecular results submitted by all participants for Isolate 2020A are listed in Tables 3–10.

Two laboratories noted no growth for at least one antituberculosis drug tested for Isolate 2020A.

Table 3. Isolate 2020A—Participant Results for First-Line DST by AP

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

Table 4. Isolate 2020A—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	2	59	61
Isoniazid—Low	59	2	61
Isoniazid—High	23	0	23
Ethambutol	61	0	61
Pyrazinamide	64	0	64

Table 5. Isolate 2020A—Participant Results for First-Line DST by Sensititre

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

Table 6. Isolate 2020A—Participant Results for First-Line DST by VersaTREK

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

Table 7. Isolate 2020A—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	13	4	17
Ofloxacin	10	0	10
Ciprofloxacin	7	0	7
Levofloxacin	2	0	2
Moxifloxacin	2	0	2
Amikacin	7	0	7
Kanamycin	13	0	13
Capreomycin	13	0	13
Ethionamide	14	0	14
Rifabutin	0	7	7
Cycloserine	6	0	6
p-Aminosalicylic acid	10	0	10

Table 8. Isolate 2020A—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	7	24	31
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	0	2	2
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1

Table 9. Isolate 2020A—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	0	3	3
Cycloserine	2	0	2
p-Aminosalicylic acid	3	0	3

* One additional laboratory reported 'No Interpretation' for MOX by Sensititre.

Table 10. Isolate 2020A—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	10	0	10
Isoniazid	0	7	7
Ethambutol	0	3	3
Pyrazinamide	0	2	2
Ofloxacin	1*	4	5
Ciprofloxacin	1*	4	5
Levofloxacin	1*	5	6
Moxifloxacin	1*	5	6
Amikacin	0	5	5
Kanamycin	0	5	5
Capreomycin	0	4	4
Ethionamide	0	2	2
Rifabutin	4	0	4

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

Isolate 2020B

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

Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculosis drug and is a cornerstone of regimens used to treat TB disease and latent TB infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *katG* gene [2, 4]. The target of activated INH is enoyl-acyl-carrier protein reductase (encoded by the *inhA* gene); this binding inhibits cell wall mycolic acid biosynthesis. There are two mechanisms that account for the majority of INH resistance [2, 4, 5]. The most common mechanism, mutations in *katG*, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inhA* gene, which are generally associated with low-level resistance to INH and are less frequent than *katG* mutations. Approximately 10–15% of isolates found to be INH resistant have no mutations detected in either of these loci. Numerous loci have been investigated to identify additional genes correlated with INH resistance. The *fabG1* (also known as *mabA*) gene, like *inhA*, is involved in mycolic acid biosynthesis and at least one mutation in this region has been associated with low-level INH resistance [11, 12]. In MTBC, *ahpC* codes for an alkyl hydroperoxide reductase that is associated with resistance to reactive oxygen and reactive nitrogen intermediates; consequently, it was initially believed that mutations in the promoter region could be surrogate markers for INH resistance [4].

DNA sequence analysis of *inhA*, *katG*, *fabG1* and *ahpC* of Isolate 2020B detected a C>G point mutation at codon 315 in the *katG* locus resulting in wild-type serine being replaced by threonine (Ser315Thr) and a G>A point mutation at nucleotide position -88 of the intergenic region of *oxyR'-ahpC* (G-88A); *inhA* and *fabG1* 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 2020B, 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
- 98% (62/63) 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 (98%) results were reported as **resistant** at the higher concentrations of INH. Only 35 laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a different method.

Of the 7 molecular results reported for INH, all (100%) laboratories reported detection of a mutation with 4 laboratories specifically noting the Ser315Thr mutation.

Three of the laboratories performing Sensititre reported INH MIC values as 4 µg/ml (n=2) and >4 µg/ml (n=1).

Ethambutol

Ethambutol (EMB) is an important first-line drug for the treatment of TB and is used in combination with INH, RMP and PZA to prevent emergence of drug resistance. EMB is a bacteriostatic agent that is active against growing bacilli and has no effect on non-replicating bacilli [4, 5]. EMB targets the arabinosyl transferases (*embCAB* operon), thereby inhibiting the biosynthesis of the cell wall components arabinogalactan and lipoarabinomannan [13].

Sequence analysis of EMB-resistant clinical isolates has shown that EMB resistance is associated primarily with missense (non-synonymous) mutations within the EMB resistance determining region of the gene *embB* at codons 306, 406 and 497 [2, 13]. False susceptibility with some growth–based methods for EMB have been reported [14, 15].

DNA sequence analysis of *embB* of Isolate 2020B revealed a G>A point mutation at codon 306 in the *embB* gene resulting in wild-

type methionine being replaced by isoleucine (Met306Ile). While certain *embB* mutations at the 306 codon, such as Met306Val and Met306Leu, are associated with EMB resistance, isolates with Met306Ile have been reported to show variable resistance [2]. This may be due to an increased MIC close to the critical concentration tested.

For Isolate 2020B, 86 EMB results were reported. This isolate was reported resistant to EMB by method, as follows:

- 40% (8/20) of the results when using AP
- 2% (1/61) of the results when using MGIT
- 33% (1/3) of the results when using Sensititre
- 0% (0/2) of the results when using VersaTREK

Of the 3 molecular results reported for EMB, all (100%) laboratories reported detection of a mutation and specifically noted the Met306Ile mutation.

Three of the laboratories performing Sensititre reported EMB MIC values as 2 µg/ml (n=1) and 4 µg/ml (n=2).

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2020B are listed in Tables 11–18.

Table 11. Isolate 2020B—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	12	8	20

Table 12. Isolate 2020B—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	63	0	63
Isoniazid—Low	1	62	63
Isoniazid—High	1	35	36
Ethambutol	60	1	61*
Pyrazinamide	64	0	64*

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

Table 13. Isolate 2020B—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	2	1	3*

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

Table 14. Isolate 2020B—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 2020B—Participant Results for Second-Line DST by AP

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

Table 16. Isolate 2020B—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	31	0	31*
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1

* One additional laboratory reported borderline for STR by MGIT.

Table 17. Isolate 2020B—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	3	0	3
Capreomycin	1	0	1
Ethionamide	2	0	2
Rifabutin	3	0	3
Cycloserine	1	0	1*
p-Aminosalicylic acid	3	0	3

* One additional laboratory reported 'No Interpretation' for MOX and CYC by Sensititre.

Table 18. Isolate 2020B—Participant Results for Molecular Testing

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

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

Isolate 2020C

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

Rifampin

DNA sequence analysis of *rpoB* in Isolate 2020C revealed a C>G point mutation in codon 511 resulting in wild-type leucine being replaced by valine (Leu511Val). The effects of a Leu511Val (Leu430Val in MTBC numbering system) mutation on rifampin susceptibility are currently unknown.

Among four methods, 86 results for RMP were reported for Isolate 2020C. This isolate was reported as **susceptible** to RMP by method, as follows:

- 100% (17/17) of the results when using AP
- 100% (63/63) of the results when using MGIT
- 100% (4/4) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Of the 8 molecular results reported for RMP, 6 (75%) laboratories reported detection of a mutation with 3 laboratories specifically noting the Leu511Val mutation.

Three of the laboratories performing Sensititre reported RMP MIC values as ≤0.12 µg/ml (n=2) and 0.25 µg/ml (n=1).

Complete first-line DST, second-line DST and molecular results submitted by all participant for Isolate 2020C are listed in Tables 19–26.

Table 19. Isolate 2020C—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	17	0	17

Table 20. Isolate 2020C—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	63	0	63
Isoniazid—Low	62	1	63
Isoniazid—High	22	1	23
Ethambutol	63	0	63
Pyrazinamide	63	2	65

Table 21. Isolate 2020C—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 2020C—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 2020C—Participant Results for Second-Line DST by AP

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

Table 24. Isolate 2020C—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	33	0	33
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1

Table 25. Isolate 2020C—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	3	0	3
Ofloxacin	2	0	2
Ciprofloxacin	0	0	0
Levofloxacin	1	0	1
Moxifloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	1	1	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 2020C—Participant Results for Molecular Testing

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

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

Isolate 2020D

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

Rifampin

DNA sequence analysis of *rpoB* in Isolate 2020D revealed a G>T point mutation in codon 146 of *rpoB* (*E. coli* numbering system and also sometimes indicated as codon 176—*M. tuberculosis* codon 170) resulting in wild-type valine being replaced by phenylalanine (Val146Phe or Val176Phe). Isolates with Val146Phe mutation have been shown to confer resistance [16].

Among four methods, 65 results for RMP were reported for Isolate 2020D; 17 laboratories reported no growth due to growth issues during testing (15 using MGIT and 2 using AP). This isolate was reported as **resistant** to RMP by method, as follows:

- 94% (16/17) of the results when using AP
- 90% (38/42) of the results when using MGIT
- 100% (4/4) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Of the 10 molecular results reported for RMP, 2 (20%) laboratories reported detection of a mutation with 1 laboratory specifically noting the Val146Phe mutation.

Three of the laboratories performing Sensititre reported RMP MIC values as 16 µg/ml (n=1) and >16 µg/ml (n=2).

Pyrazinamide

Isolate 2020D was expected to be susceptible to PZA. DNA sequence analysis of *pncA* in Isolate 2020D revealed a T>C point mutation in codon 135 resulting in wild-type threonine being replaced by alanine (Thr135Ala). Mutations in *pncA* gene are typically associated with PZA resistance, however it has been reported that not all *pncA* mutations confer resistance [9, 17]. The effects of a Thr135Ala mutation on PZA susceptibility are currently unknown.

Of the 58 laboratories reporting results for PZA for Isolate 2020D, **susceptible** was reported by:

- 98% (55/56) of the results when using MGIT
- 100% (1/1) of the results when using VersaTREK

Of the 2 molecular results reported for PZA, both (100%) laboratories reported detection of a mutation, specifically noting the Thr135Ala mutation.

Complete first-line DST, second-line DST and molecular results submitted by all participants for Isolate 2020D are listed in Tables 27–34.

Seventeen laboratories noted no growth and one laboratory noted contamination for at least one antituberculosis drug tested for Isolate 2020D

Table 27. Isolate 2020D—Participant Results for First-Line DST by AP

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

Table 28. Isolate 2020D—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	4	38	42
Isoniazid—Low	42	1	43
Isoniazid—High	17	0	17
Ethambutol	43	0	43
Pyrazinamide	55	1	56

Table 29. Isolate 2020D—Participant Results for First-Line DST by Sensititre

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

Table 30. Isolate 2020D—Participant Results for First-Line DST by VersaTREK

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

Table 31. Isolate 2020D—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	15	2	17
Ofloxacin	11	0	11
Ciprofloxacin	6	0	6
Levofloxacin	2	0	2
Moxifloxacin	2	0	2
Amikacin	8	0	8
Kanamycin	13	0	13
Capreomycin	12	0	12
Ethionamide	14	0	14
Rifabutin	3	4	7
Cycloserine	5	0	5
p-Aminosalicylic acid	10	0	10

Table 32. Isolate 2020D—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	21	2	23
Ofloxacin	2	0	2
Ciprofloxacin	0	0	0
Levofloxacin	3	0	3
Moxifloxacin	2	0	2
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	1	1	2
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1

Table 33. Isolate 2020D—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	1	2	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	0	3	3
Cycloserine	2	0	2
p-Aminosalicylic acid	3	0	3

* One additional laboratory reported 'No Interpretation' for MOX by Sensititre.

Table 34. Isolate 2020D—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	2	8	10
Isoniazid	0	7	7
Ethambutol	0	3	3
Pyrazinamide	2	0	2
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	6	6
Moxifloxacin	0	6	6
Amikacin	0	5	5
Kanamycin	0	5	5
Capreomycin	0	4	4
Ethionamide	0	2	2
Rifabutin	1	3	4

Isolate 2020E

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

Rifampin

As noted in Isolate 2020A, the most commonly encountered mutation in *rpoB*, Ser531Leu, and additional mutations in codons 526 and 516 have generally been reported to confer high-level RMP resistance (i.e., minimum inhibitory concentration [MIC] is much higher than the critical concentration). However, some mutations have been associated with low-level, yet probably clinically relevant, RMP resistance [7, 18, 19]. Low-level RMP resistance can be operationally defined as the presence of a mutation that increases the RMP MIC above the MIC seen in RMP-susceptible isolates that do not have a detectable *rpoB* mutation (i.e., wildtype). However, isolates with mutations conferring low-level RMP resistance may test as susceptible with growth-based drug susceptibility methods. The clinical impact of these *rpoB* mutations, sometimes referred to as "disputed" mutations, will depend on the frequency of their occurrence, which may vary from one setting to another [7, 20]. The diminished RMP activity suggests that clinical outcome in patients being treated with RMP-based standard therapy could be impacted[21-23].

DNA sequence analysis of *rpoB* in Isolate 2020E revealed T>C and C>A point mutations in codon 522 of *rpoB* resulting in wild-type serine being replaced by glutamine (Ser522Gln) (Ser441Gln in MTBC numbering system).

Isolate 2020E was expected to exhibit variable results. Little is known regarding this mutation and its effects in different testing methods; it appears to act like a mutation associated with low-level rifampin resistance, but more research of the Ser522Gln mutation is needed.

Among four methods, 85 results for RMP were reported for Isolate 2020E. This isolate was reported as **resistant** to RMP by method, as follows:

- 89% (16/18) of the results when using AP
- 36% (22/61) of the results when using MGIT
- 100% (4/4) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Of the 10 molecular results reported for RMP, all (100%) laboratories reported detection of a mutation with 2 laboratories specifically noting the Ser522Gln mutation.

Three of the laboratories performing Sensititre reported RMP MIC values as 8 µg/ml (n=2) and >16 µg/ml (n=1).

Complete first-line DST, second-line DST and molecular results submitted by all participants for Isolate 2020E are listed in Tables 35–42.

Table 35. Isolate 2020E—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	2	16	18
Isoniazid—Low	17	1	18
Isoniazid—High	18	0	18
Ethambutol	19	0	19

Table 36. Isolate 2020E—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	39	22	61
Isoniazid—Low	62	0	62
Isoniazid—High	23	0	23
Ethambutol	62	0	62
Pyrazinamide	65	0	65

Table 37. Isolate 2020E—Participant Results for First-Line DST by Sensititre

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

Table 38. Isolate 2020E—Participant Results for First-Line DST by VersaTREK

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

Table 39. Isolate 2020E—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	18	0	18
Ofloxacin	12	0	12
Ciprofloxacin	7	0	7
Levofloxacin	2	0	2
Moxifloxacin	2	0	2
Amikacin	9	0	9
Kanamycin	14	0	14
Capreomycin	13	0	13
Ethionamide	16	0	16
Rifabutin	6	1	7
Cycloserine	6	0	6
p-Aminosalicylic acid	12	0	12

Table 40. Isolate 2020E—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	32	0	32
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1

Table 41. Isolate 2020E—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

* One additional laboratory reported 'No Interpretation' for MOX by Sensititre.

Table 42. Isolate 2020E—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	10	0	10
Isoniazid	0	7	7
Ethambutol	0	3	3
Pyrazinamide	0	2	2
Ofloxacin	1*	4	5
Ciprofloxacin	1*	4	5
Levofloxacin	1*	5	6
Moxifloxacin	1*	5	6
Amikacin	0	5	5
Kanamycin	0	5	5
Capreomycin	0	4	4
Ethionamide	0	2	2
Rifabutin	1	3	4

* This laboratory noted the detection of 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	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 70 laboratories participating in the February 2020 MPEP survey is shown in this pie chart. The largest slice, at 72%, represents 50 laboratories that have self-classified as a health department laboratory. The next major slice signifies 10 hospital laboratories. The remaining three slices of the pie chart represent 7 independent laboratories, 2 federal government laboratories, and 1 laboratory self-identified as a medical manufacturer. (page 7)

Figure 2. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=70) in 2019 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; 13 laboratories tested between 51 to 100 isolates per year; 8 laboratories tested between 101 to 150 isolates per year; 5 laboratories tested between 151 to 200 isolates per year; 1 laboratory tested between 201 to 250 isolates per year; 1 laboratory tested between 251 to 300 isolates per year; 7 laboratories tested between 301 to 500 isolates per year; 6 laboratories tested between 501 to 1000 isolates per year, and 1 laboratory tested greater than or equal to 1001 isolates per year. (page 8)

Figure 3. The drug susceptibility testing methods used by MPEP participants (N=102) is displayed in this vertical bar graph. The vertical y-axis is the number of laboratories reporting with ranges from 0 to 70, by increments of 10, and the horizontal x- axis lists the susceptibility testing methods. Each bar represents the number of reporting laboratories performing a particular drug susceptibility test method. From left to right: 66 used MGIT, 20 used agar proportion, 4 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 Bruker line probe assays, 2 laboratories that use the Cepheid Xpert MTB/RIF assay, 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. 70 laboratories tested rifampin; 70 laboratories tested isoniazid; 70 laboratories tested ethambutol; 66 laboratories tested pyrazinamide; 46 laboratories tested streptomycin; 17 laboratories tested ofloxacin; 8 laboratories tested ciprofloxacin; 8 laboratories tested moxifloxacin; 6 laboratories tested levofloxacin; 18 laboratories tested kanamycin; 17 laboratories tested capreomycin; 15 laboratories tested amikacin; 21 laboratories tested ethionamide; 16 laboratories tested PAS; 13 laboratories tested rifabutin; and 8 laboratories tested cycloserine. (page 10)

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