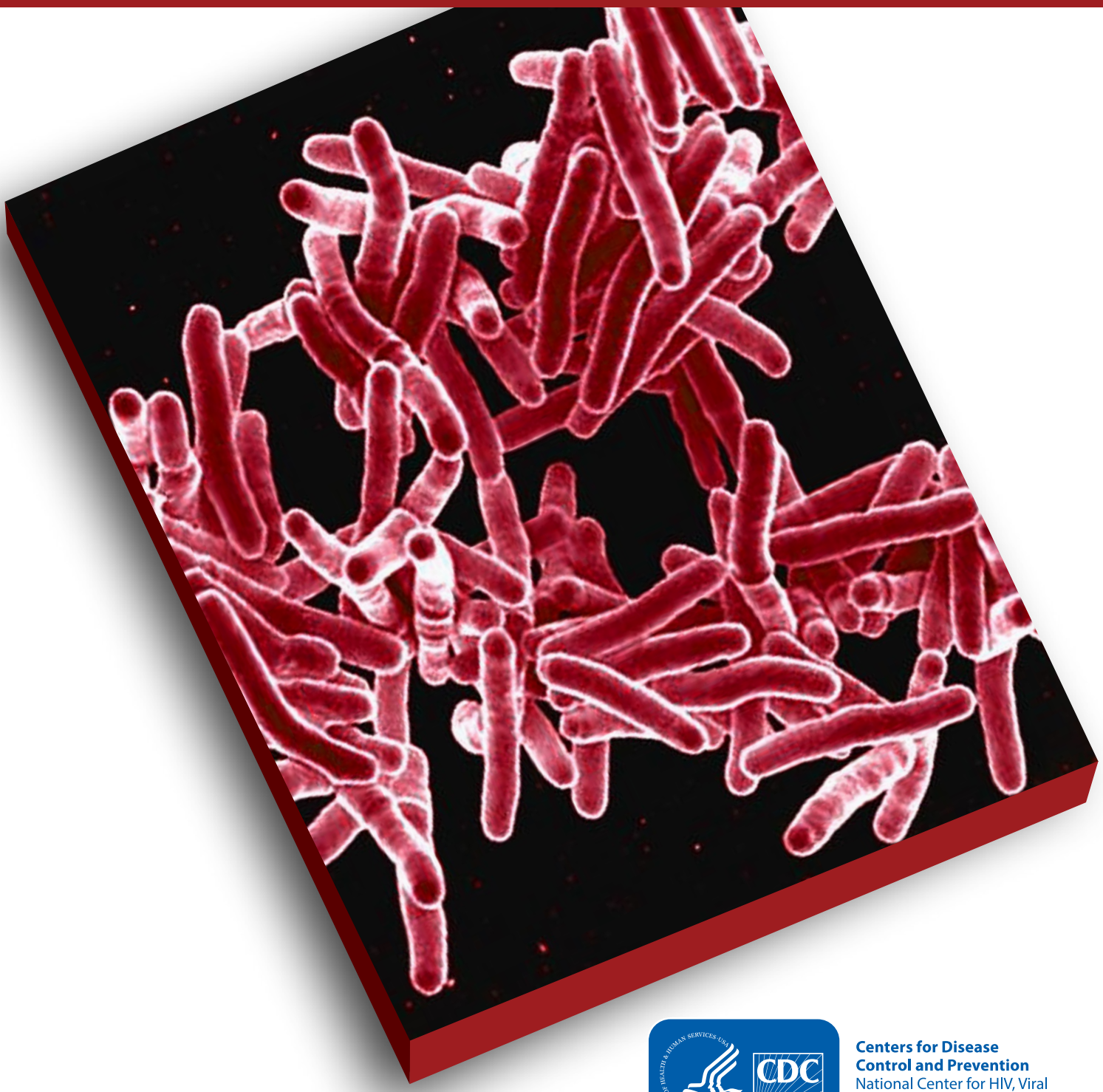
For accessible version: https://www.cdc.gov/tb/topic/laboratory/mpep/2021/march-mpepReport.htm.

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program
Report of Results
March 2021



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

CS 327352-A

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for Martch 2021 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 2021.

Report Content

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Note on Accessibility:
Find descriptions and explanations of figures in Appendix 1: Accessible Explanation of Figures on page 33.

Contents

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 or customized 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]. Recently, World Health Organization (WHO) published two technical reports investigating critical concentrations, by method, for INH, RMP, EMB, PZA and twelve second-line anti-tuberculosis drugs [2, 3]. Based on the systematic review data, recommendations were made for adjustments to critical concentrations for RMP, MOX, LEV, AMK and KAN for some methods

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Expected Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in March 2021 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 [4].

Table 1. Expected Growth-based Results for March 2021 Survey

Note—S=susceptible, R=resistant

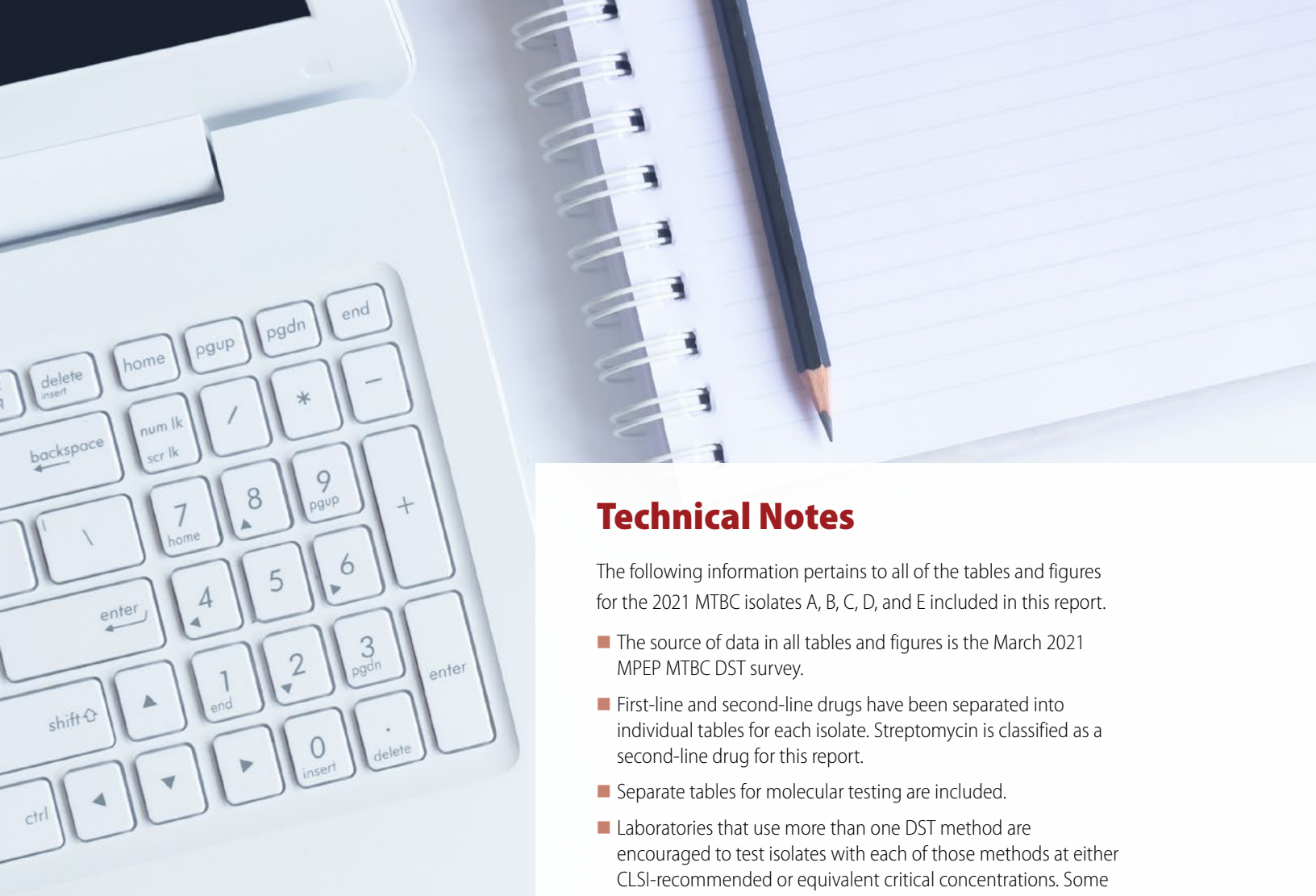
Isolate	RMP	INH	EMB	PZA	Second-line Drugs Resistant to:
2021A	R	S	S	S	
2021B	S	S	R	S	STR
2021C	S	S	S	R	
2021D	S	R	S	S	OFL, CIP, STR
2021E	S	R	S	S	

Table 2. Expected Molecular Results (Mutations Detected in Loci Associated with Resistance) for March 2021 Survey

Note—Empty cell=No mutation detected

Isolate	*rpoB*[¥]	*katG*	*ahpC*	*embB*	*pncA*	*gyrA*
2021A	Ser450Leu* (Ser531Leu)**					
2021B				Gln497Arg		
2021C					His57Asp	
2021D		Ser315Thr				Asp94Asn (90%[†]) Ala90Val (10%[†])
2021E		Partial deletion	C-72T			

[¥] Mutation is listed using both the *M. tuberculosis* and *E.coli* numbering system [5, 6]
**M. tuberculosis* numbering system used
***E. coli* numbering system used
[†] Results from whole genome sequencing. Percent indicates proportion of reads with noted mutation.



Technical Notes

The following information pertains to all of the tables and figures for the 2021 MTBC isolates A, B, C, D, and E included in this report.

- The source of data in all tables and figures is the March 2021 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 Sensititre system allows determination of a minimum inhibitory concentration (MIC) for each drug in the panel. Laboratories using this method may 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

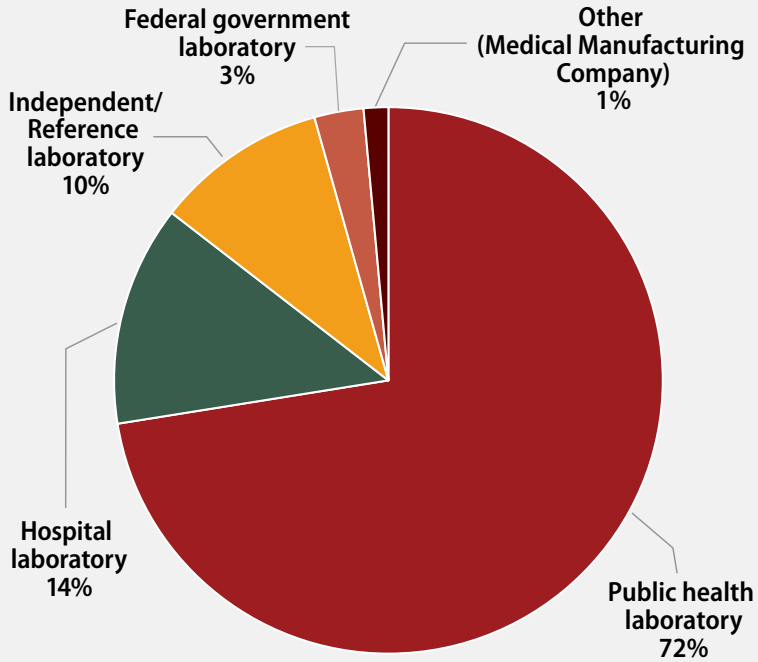
Primary Classification

This report contains DST results submitted to CDC by survey participants at 70 laboratories in 34 states.

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

- **50 (72%):** Public health 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)

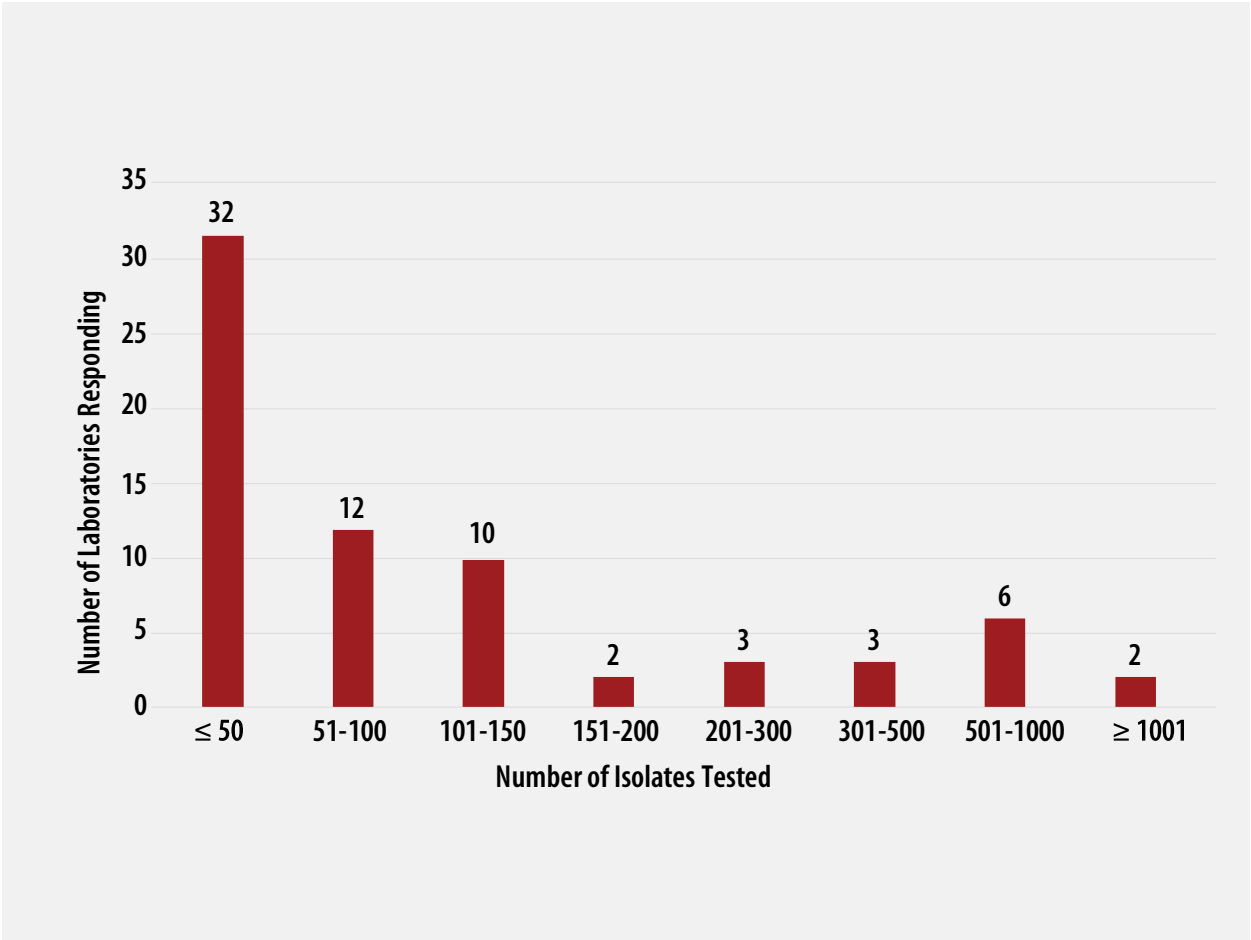
Figure 1. Primary Classification of Participating Laboratories, March 2021



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 70 participants in 2020 (excluding isolates used for quality control) is shown in Figure 2. In 2020, the counts ranged from 0 to 3,498 tests. Participants at 32 (46%) 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 [7].

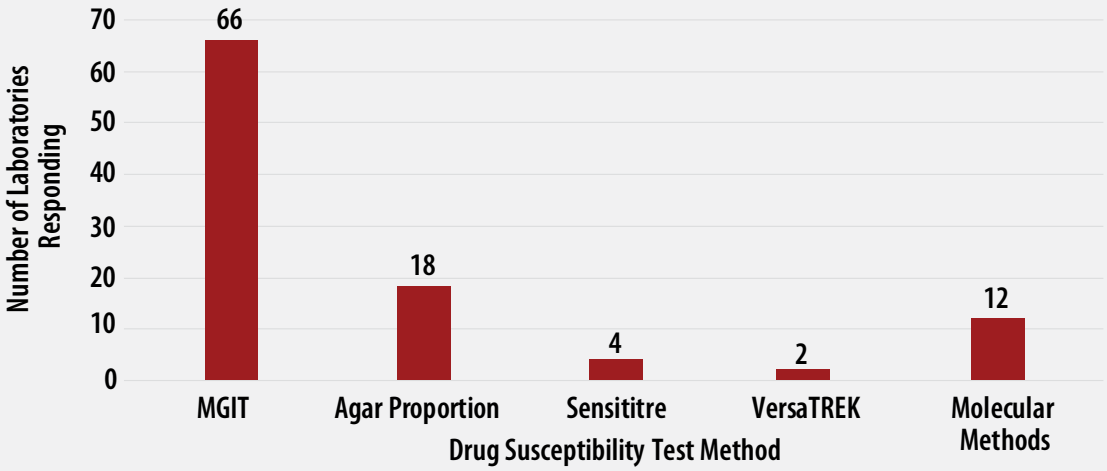
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=70)



MTBC DST Methods Used by Participants

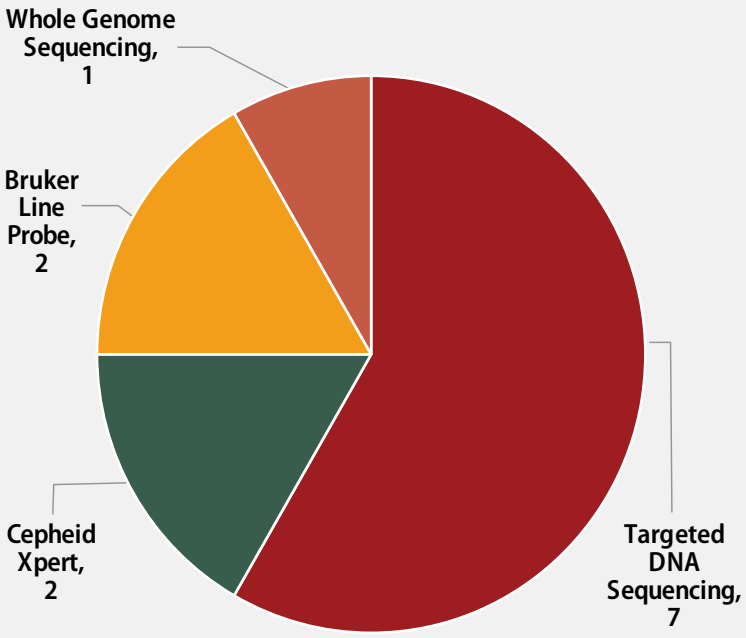
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Of participating laboratories, 43 (61%) reported results for only one method, 22 (32%) reported two methods, and 5 (7%) noted three susceptibility methods.

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



Twelve molecular methods reported by participants are shown in Figure 4. The method used most frequently by laboratories (7) was targeted DNA sequencing (58%), including pyrosequencing and Sanger sequencing. Two (17%) laboratories reported use of the Cepheid Xpert MTB/RIF assay, two (17%) reported results for line probe assays, Genotype MTBDR*plus* and MTBDRsl by Bruker, and one (8%) reported results from whole genome sequencing.

Figure 4. Molecular Method Reported (n=12)

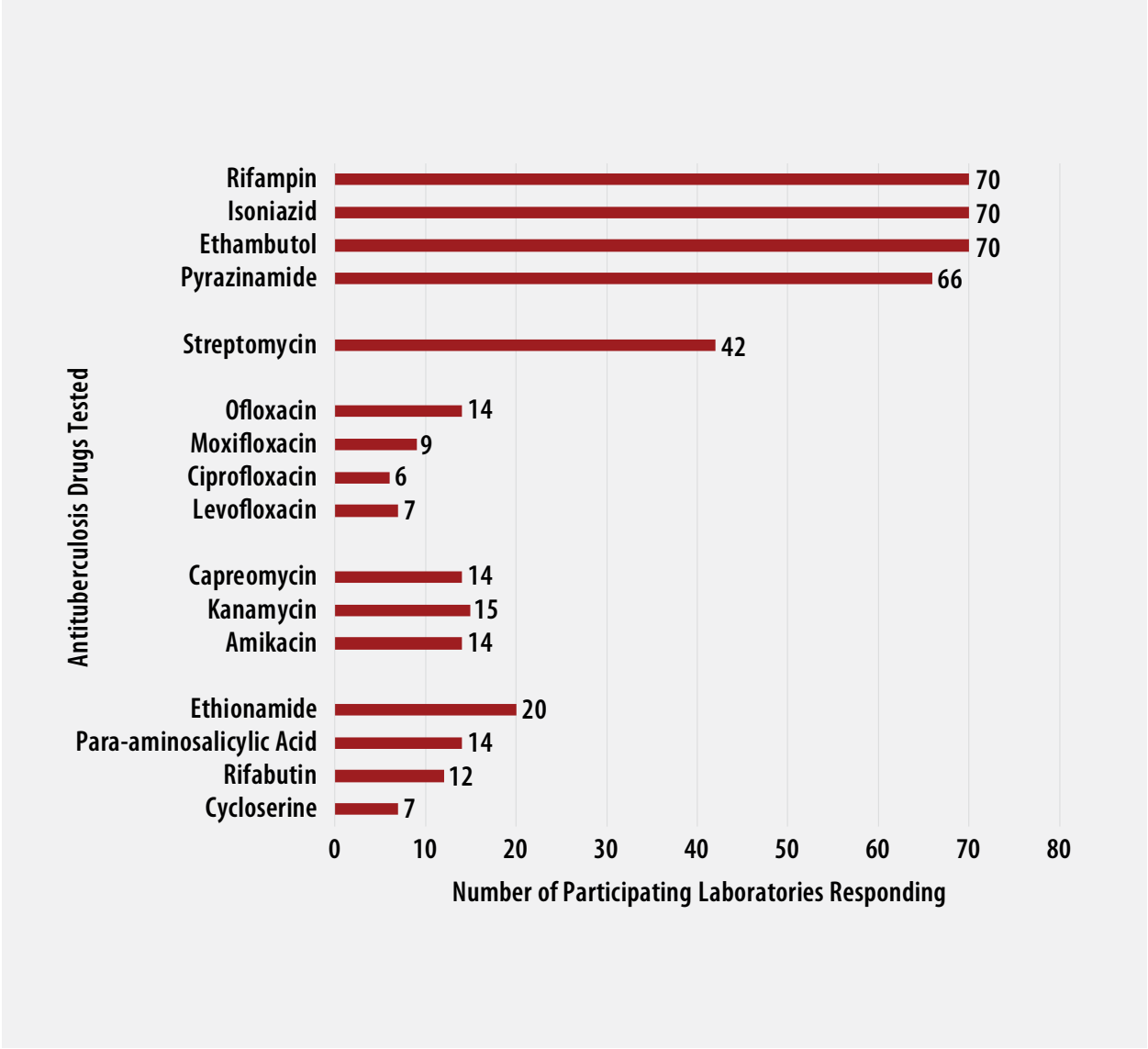


Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the March 2021 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. One laboratory performs molecular testing for PZA via sequencing of *pncA*, in place of growth-based DST.

For 23 laboratories reporting second-line drug results (with the exception of streptomycin), five (22%) 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 2021A

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 6-month regimen for the treatment of TB. RMP's mechanism of action is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [8]. 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 [9]. Mutations in codons 450, 445, and 435 (*E. coli* numbering system corresponding to 531, 526, and 516) are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance [8, 9]. 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* [10]. The Xpert MTB/RIF assay could generate results that falsely indicate resistance when compared to growth-based methods because of the presence of silent/synonymous mutations [11]. Sequencing of *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 2021A revealed a C>T point mutation in codon 450 (*E. coli* numbering 531) resulting in wild-type serine being replaced by leucine (Ser450Leu). Isolates with Ser450Leu (Ser531Leu in *E. coli* numbering system) mutations consistently test resistant to RMP in growth-based assays.

For Isolate 2021A, 85 results for RMP were reported. This isolate was reported as **resistant** 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% (3/3)** 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 six laboratories specifically noting the Ser450Leu 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* Ser450Leu mutation [12].

Among three methods, 12 results for RBT were reported for Isolate 2021A. This isolate was reported as **resistant** to RBT by method, as follows:

- **100% (7/7)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

Of the four molecular results reported specifically for RBT, two (50%) laboratories reported detection of a mutation.

Two of the laboratories performing Sensititre reported RBT MIC values as 0.25 µg/ml (n=1) and 4 µg/ml (n=1). Another laboratory reported a RBT MIC value as 8 µg/ml (n=1) but as no categorical interpretation was provided, the data were excluded from Table 9.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2021A are listed in Tables 3–10.

Table 3. Isolate 2021A—Participant Results for First-Line DST by AP

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

Table 4. Isolate 2021A—Participant Results for First-Line DST by MGIT

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

Table 5. Isolate 2021A—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	0	3	3
Isoniazid—Low	3	0	3
Isoniazid—High	2	0	2
Ethambutol	3	0	3

Table 6. Isolate 2021A—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 2021A—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	11	5	16
Ofloxacin	10	0	10
Ciprofloxacin	5	0	5
Levofloxacin	3	0	3
Moxifloxacin	4	0	4
Amikacin	9	0	9
Kanamycin	12	0	12
Capreomycin	11	0	11
Ethionamide	15	0	15
Rifabutin	0	7	7
Cycloserine	6	0	6
p-Aminosalicylic acid	11	0	11

Table 8. Isolate 2021A—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	8	22	30
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	4	0	4
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	0	3	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1

Table 9. Isolate 2021A—Participant Results for Second-Line DST by Sensititre

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

* One additional laboratory reported 'No Interpretation' for OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.

Table 10. Isolate 2021A—Participant Results for Molecular Testing

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

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

Isolate 2021B

Expected Result: Resistant to EMB at 5.0 µg/ml and STR at 2.0 µg/ml by agar proportion

Ethambutol

Ethambutol (EMB) is an important first-line drug for the treatment of TB and is used in combination with RMP, INH, 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 [8, 9]. EMB targets the arabinosyl transferases (*embCAB* operon), thereby inhibiting the biosynthesis of the cell wall components arabinogalactan and lipoarabinomannan [13].

Issues of false-susceptibility with some growth-based methods for EMB, particularly in broth-based media, have been reported and remain a potential concern. Probable causes include the bacteriostatic nature of the drug, reduced drug activity in culture, and an organism's MIC for EMB falling too close to the critical concentration tested [14-16].

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 [4, 13].

DNA sequence analysis of *embB* of Isolate 2021B revealed a A>G point mutation at codon 497 in the *embB* gene resulting in wild-type glutamine being replaced by arginine (Gln497Arg). Mutations in the 497 codon of *embB* have been associated with EMB resistance [4, 17].

For Isolate 2021B, 86 EMB results were reported. This isolate was reported **resistant** to EMB by method, as follows:

- **94% (16/17)** of the results when using AP
- **55% (35/64)** of the results when using MGIT
- **67% (2/3)** of the results when using Sensititre
- **50% (1/2)** of the results when using VersaTREK

Of the three molecular results reported for EMB, all (100%) laboratories reported detection of a mutation with two specifically noting the Gln497Arg mutation.

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

Streptoymycin

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 [8, 9]. In MTBC, the genetic basis of the majority of resistance to STR is usually due to mutations in *rrs* or *rpsL* [9, 18]. 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, 19].

DNA sequencing analysis did not reveal a mutation in *rrs* or *rpsL*; other mechanisms of resistance may exist.

Among three methods, 48 results for STR were reported for Isolate 2021B. This isolate was reported as **resistant** to STR by method, as follows:

- **81% (13/16)** of the results when using AP
- **90% (27/30)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Two of the laboratories performing Sensititre reported STR MIC values as 4.0 µg/ml (n=1) and 8.0 µg/ml (n=1). A third laboratory reported a STR MIC value as 4 µg/ml (n=1) and indicated borderline resistance

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2021B are listed in Tables 11–18.

Table 11. Isolate 2021B—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	17	0	17
Isoniazid—Low	14	3	17
Isoniazid—High	17	0	17
Ethambutol	1	16	17

Table 12. Isolate 2021B—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	63	1	64
Isoniazid—Low	62	2	64
Isoniazid—High	22	1	23
Ethambutol	29	35	64
Pyrazinamide	65	0	65

Table 13. Isolate 2021B—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	3	0	3
Isoniazid—Low	3	0	3
Isoniazid—High	2	0	2
Ethambutol	1	2	3

Table 14. Isolate 2021B—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	1	1	2
Pyrazinamide	1	0	1

Table 15. Isolate 2021B—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	3	13	16
Ofloxacin	10	0	10
Ciprofloxacin	5	0	5
Levofloxacin	3	0	3
Moxifloxacin	4	0	4
Amikacin	9	0	9
Kanamycin	12	0	12
Capreomycin	11	0	11
Ethionamide	16	0	16
Rifabutin	7	0	7
Cycloserine	6	0	6
p-Aminosalicylic acid	11	0	11

Table 16. Isolate 2021B—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	3	27	30
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	4	0	4
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 17. Isolate 2021B—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	0	2	2*
Ofloxacin	1	0	1†
Ciprofloxacin	0	0	0
Levofloxacin	0	0	0
Moxifloxacin	1	0	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†

* One additional laboratory reported 'Borderline' for STR by Sensititre.
† One additional laboratory reported 'No Interpretation' for OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.

Table 18. Isolate 2021B—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	2	6	8
Ethambutol	3	0	3
Pyrazinamide	0	2	2
Ofloxacin	1*	5	6
Ciprofloxacin	1*	5	6
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	0	4	4

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

Isolate 2021C

Expected Result: *Mycobacterium bovis*; Resistant to PZA at 100 µg/ml by MGIT

Pyrazinamide

Unlike *M. tuberculosis*, *M. bovis* has an inherent resistance to PZA caused by a characteristic single point mutation of C>G at nucleotide position 169 of the *pncA* gene resulting in aspartic acid replacing histidine at codon 57 (His57Asp). This substitution causes defective pyrazinamidase activity and confers natural PZA resistance in *M. bovis* strains, including BCG substrains [20, 21]. DNA sequence analysis of *pncA* in Isolate 2021C confirmed the His57Asp mutation.

Among two methods, 64 results for PZA were reported for Isolate 2021C. This isolate was reported as **resistant** to PZA by method, as follows:

- **95% (60/63)** of the results when using MGIT
- **100% (1/1)** of the results when using VersaTREK

Of the five molecular results reported for PZA, all (100%) laboratories reported detection of a mutation with four laboratories specifically noting the His57Asp mutation or *M.bovis*.

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2021C are listed in Tables 19–26.

One laboratory noted no growth for at least one antituberculosis drug tested for Isolate 2021C.

Table 19. Isolate 2021C—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	15	0	15
Isoniazid—Low	15	0	15
Isoniazid—High	15	0	15
Ethambutol	15	0	15

Table 20. Isolate 2021C—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	64	0	64
Isoniazid—Low	63	1	64
Isoniazid—High	23	1	24
Ethambutol	64	0	64
Pyrazinamide	3	60	63*

* One additional laboratory reported contaminated for PZA by MGIT.

Table 21. Isolate 2021C—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	3	0	3
Isoniazid—Low	3	0	3
Isoniazid—High	2	0	2
Ethambutol	3	0	3

Table 22. Isolate 2021C—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	0	1	1

Table 23. Isolate 2021C—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	14	0	14
Ofloxacin	9	0	9
Ciprofloxacin	3	0	3
Levofloxacin	3	0	3
Moxifloxacin	4	0	4
Amikacin	9	0	9
Kanamycin	10	0	10
Capreomycin	10	0	10
Ethionamide	10	3	13
Rifabutin	7	0	7
Cycloserine	5	0	5
p-Aminosalicylic acid	9	0	9

Table 24. Isolate 2021C—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	29	0	29
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	4	0	4
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 2021C—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	3	0	3
Ofloxacin	1	0	1*
Ciprofloxacin	0	0	0
Levofloxacin	1	0	1
Moxifloxacin	2	0	2*
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 'No Interpretation' for OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.

Table 26. Isolate 2021C—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	0	8	8
Ethambutol	0	3	3
Pyrazinamide	5	0	5
Ofloxacin	1*	5	6
Ciprofloxacin	1*	5	6
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	0	4	4

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

Isolate 2021D

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml, OFL at 2.0 µg/ml, and CIP 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 [4, 8]. 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 [4, 8, 9]. 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 [22, 23]. In MTBC, *ahpC* codes for an alkyl hydroperoxide reductase that is associated with resistance to reactive oxygen and reactive nitrogen intermediates; consequently, it is believed that mutations in the promoter region could be surrogate markers for INH resistance [8].

DNA sequence analysis of Isolate 2021D revealed a G>C 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 2021D, 85 INH results were reported for the critical concentration. This isolate was reported **resistant** to INH by method, as follows:

- **100% (17/17)** of the results when using AP
- **98% (63/64)** 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-five or 100% of results at the higher concentrations of INH were reported as resistant. Only 33 (52%) 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 eight molecular results reported for INH, all (100%) laboratories reported detection of a mutation with six laboratories specifically noting the Ser315Thr mutation.

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

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. FQ are an important class of drugs used to treat tuberculosis (TB) cases that are resistant to first-line drugs, yet this class of antibiotics also have become an important part of newer TB regimens [24, 25]. In the U.S., 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 [24, 26]. The primary mechanism of FQ action is the inhibition of DNA synthesis [18] 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 [4, 9, 18]. 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 [18].

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 [27, 28]. 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 (LEV), and moxifloxacin (MOX) at the defined critical concentrations [29, 30]. CLSI currently recommends testing LEV and/or MOX [1].

DNA sequencing of *gyrA* in Isolate 2021D detected a A>G point mutation in codon 94 of *gyrA* for 90% of alleles, resulting in wild-type aspartic acid being replaced with asparagine (Asp94Asn). A second *gyrA* mutation was detected in codon 90 for 10% of alleles, a C>T point mutation resulting in wild-type alanine being replaced with valine (Ala90Val). Both Asp94Asn and Ala90Val mutations have been associated with FQ resistance [4, 31]. Sequencing of *gyrB* for this isolate was wild-type (i.e., no mutations were detected).

Among three methods, 15 results for OFL were reported for Isolate 2021D. This isolate was reported as resistant to OFL by method, as follows:

- **90% (9/10)** 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 2021D; 100% (21/21) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other FQ 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% (3/3)** 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% (4/4)** 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; five laboratories specifically noted the Asp94Asn mutation.

Three laboratories performing Sensititre reported MIC values for FQ drugs; two of these did not report interpretations. Reported MIC values were as follows: OFL at 16 µg/ml (n=2); LEV at 8 µg/ml (n=1); and MOX at 4 µg/ml (n=1) and 8 µg/ml (n=1).

Streptoymycin

Among three methods, 48 results for STR were reported for Isolate 2021D. This isolate was reported as **resistant** to STR by method, as follows:

- **81% (13/16)** of the results when using AP
- **100% (29/29)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre

Three of the laboratories performing Sensititre reported STR MIC values as 4.0 µg/ml (n=2) and 8.0 µg/ml (n=1).

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2021D are listed in Tables 27–34.

Table 27. Isolate 2021D—Participant Results for First-Line DST by AP

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

Table 28. Isolate 2021D—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	63	1	64
Isoniazid—Low	1	63	64
Isoniazid—High	0	33	33
Ethambutol	63	1	64
Pyrazinamide	65	0	65

Table 29. Isolate 2021D—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	3	0	3
Isoniazid—Low	0	2	2
Isoniazid—High	0	3	3
Ethambutol	3	0	3

Table 30. Isolate 2021D—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 2021D—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	3	13	16
Ofloxacin	1	9	10
Ciprofloxacin	0	5	5
Levofloxacin	0	3	3
Moxifloxacin	0	4	4
Amikacin	9	0	9
Kanamycin	11	1	12
Capreomycin	11	0	11
Ethionamide	16	0	16
Rifabutin	7	0	7
Cycloserine	6	0	6
p-Aminosalicylic acid	11	0	11

Table 32. Isolate 2021D—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	0	29	29*
Ofloxacin	0	4	4
Ciprofloxacin	0	1	1
Levofloxacin	0	3	3
Moxifloxacin	0	4	4
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 33. Isolate 2021D—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	0	3	3
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 'No Interpretation' for OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.

Table 34. Isolate 2021D—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	3	3
Ofloxacin	7	0	7
Ciprofloxacin	7	0	7
Levofloxacin	6	0	6
Moxifloxacin	6	0	6
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	2	2
Rifabutin	0	4	4

Isolate 2021E

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

Isoniazid

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2021E revealed a partial *katG* deletion and a C>T point mutation at nucleotide position -72 of the intergenic region of *oxyR'-ahpC* (C-72T); *fabG1* and *inhA* were wild-type (i.e., no mutations were detected). As previously mentioned, changes in *katG* are generally associated with high-level resistance to INH.

For Isolate 2021E, 76 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (17/17)** of the results when using AP
- **98% (54/55)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Forty-eight (98%) results were reported as resistant at the higher concentrations of INH. Only 27 (49%) 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 eight molecular results reported for INH, two (25%) laboratories reported detection of a mutation with one laboratory specifically noting the *katG* deletion and *ahpC* C-72T mutation.

Three of the laboratories performing Sensititre reported INH MIC values as 4 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 2021E are listed in Tables 35–42.

Nine laboratories noted no growth for at least one antituberculosis drug tested for Isolate 2021E.

Table 35. Isolate 2021E—Participant Results for First-Line DST by AP

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

Table 36. Isolate 2021E—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	53	2	55
Isoniazid—Low	1	54	55
Isoniazid—High	1	26	27
Ethambutol	54	1	55
Pyrazinamide	60	2	62

Table 37. Isolate 2021E—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	3	0	3
Isoniazid—Low	0	2	2
Isoniazid—High	0	3	3
Ethambutol	3	0	3

Table 38. Isolate 2021E—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 39. Isolate 2021E—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	16	0	16
Ofloxacin	10	0	10
Ciprofloxacin	5	0	5
Levofloxacin	3	0	3
Moxifloxacin	4	0	4
Amikacin	9	0	9
Kanamycin	11	1	12
Capreomycin	11	0	11
Ethionamide	15	0	15
Rifabutin	7	0	7
Cycloserine	6	0	6
p-Aminosalicylic acid	11	0	11

Table 40. Isolate 2021E—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	27	0	27
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	4	0	4
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	2	1	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	1	1

Table 41. Isolate 2021E—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	3	0	3
Ofloxacin	1	0	1*
Ciprofloxacin	0	0	0
Levofloxacin	0	0	0
Moxifloxacin	1	0	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 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.

Table 42. Isolate 2021E—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	2	6	8
Ethambutol	0	3	3
Pyrazinamide	0	2	2
Ofloxacin	0	6	6
Ciprofloxacin	0	6	6
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	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.

†CLSI critical concentrations for RMP differ from revised WHO recommendation of 0.5 µg/ml published in 2021 [2].

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.

†CLSI critical concentration for AMK differ from revised WHO recommendation of 2.0 µg/ml published in 2018 [3].

¥CLSI critical concentration for KAN differ from revised WHO recommendation of 4.0 µg/ml for 7H10. WHO recommended the withdrawal of the current KAN critical concentration for 7H11 published in 2018 [3].

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.

†CLSI critical concentrations for RMP differ from revised WHO recommendation of 0.5 µg/ml published in 2021 [2].

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.

Revised WHO recommendations provide LEV and MOX critical concentrations for MGIT published in 2018 [3].

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Appendix 1: Accessible Explanations of Figures

Figure 1. The primary classification of the 70 laboratories participating in the March 2021 MPEP survey is shown in this pie chart. The largest slice, at 72%, represents 50 laboratories that have self-classified as a public health 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. (return to page 8)

Figure 2. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=70) in 2020 is displayed in this vertical bar graph. The vertical y–axis is the number of laboratories responding and ranges from 0 to 35 using increments of 5. Along the horizontal x-axis are eight vertical bars representing the number of isolates tested per year. From left to right, 32 laboratories tested less than or equal to 50 isolates per year; 12 laboratories tested between 51 to 100 isolates per year; 10 laboratories tested between 101 to 150 isolates per year; 2 laboratories tested between 151 to 200 isolates per year; 3 laboratories tested between 201 to 300 isolates per year; 3 laboratories tested between 301 to 500 isolates per year; 6 laboratories tested between 501 to 1000 isolates per year, and 2 laboratories tested greater than or equal to 1,001 isolates per year. (return to page 9)

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, 18 used agar proportion, 4 used Sensititre, 2 used VersaTREK, and 12 used molecular methods. (return to page 10)

Figure 4. The molecular methods used by MPEP participants (N=12) are displayed in this pie chart. The largest slice represents the 7 laboratories that perform targeted DNA sequencing. The next three slices represent 2 laboratories that use the Cepheid Xpert MTB/RIF assay, 2 laboratories that uses Bruker line probe assays, and 1 laboratory that uses whole genome sequencing. (return to page 10)

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; 42 laboratories tested streptomycin; 14 laboratories tested ofloxacin; 9 laboratories tested moxifloxacin; 6 laboratories tested ciprofloxacin; 7 laboratories tested levofloxacin; 14 laboratories tested capreomycin; 15 laboratories tested kanamycin; 14 laboratories tested amikacin; 20 laboratories tested ethionamide; 14 laboratories tested PAS; 12 laboratories tested rifabutin; and 7 laboratories tested cycloserine. (return to page 11)

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Publication date: October 2021