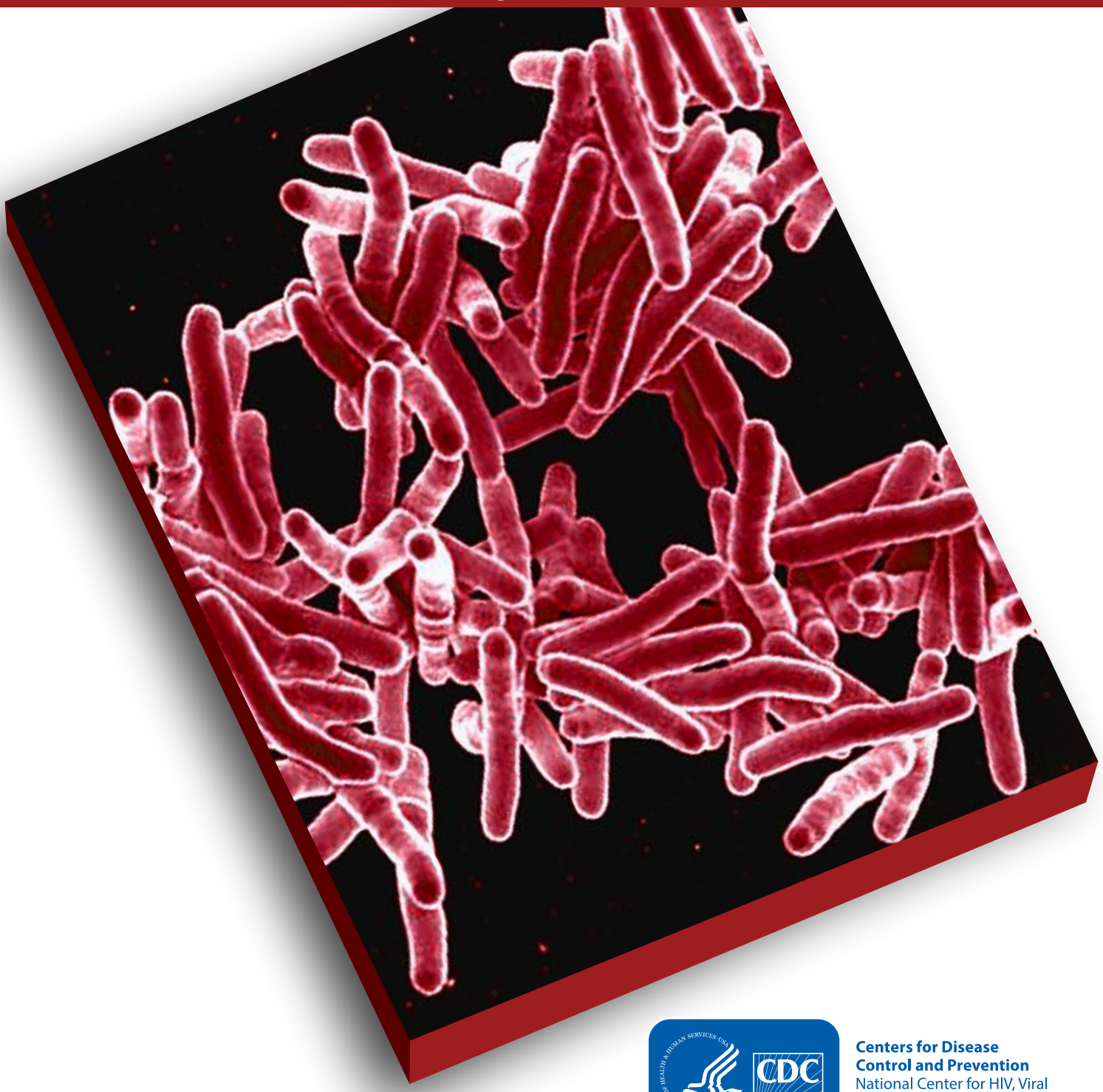
For accessible version: https://www.cdc.gov/tb/topic/laboratory/mpep/2021/aug-mpepReport.htm.

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program
Report of Results
August 2021



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

CS 330821-A

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for August 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 August 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 39.

Contents

Abbreviations and Acronyms

Acronym	Definition
AMK	amikacin
AP	agar proportion—performed on Middlebrook 7H10 or 7H11
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
LVX	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
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" and "M62: Performance Standards for Susceptibility Testing of Mycobacteria, *Nocardia* spp., and Other Aerobic Actinomycetes" [1, 2]. Additionally, 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 [3, 4]. Based on the systematic review data, recommendations were made for adjustments to critical concentrations for RMP, MOX, LVX, AMK, and KAN for some DST methods.

Expected Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in August 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 [5].

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

Note—S=susceptible, R=resistant

Isolate	RMP	INH	EMB	PZA	Second-line Drugs Resistant to:
2021F	S	S	S	S	OFL, CIP
2021G	S	R	S	S	STR, OFL, CIP
2021H	S	R	S	S	OFL, CIP, ETA
2021I	S	S	S	S*	OFL, CIP
2021J	S	R	S	S	OFL, CIP, ETA

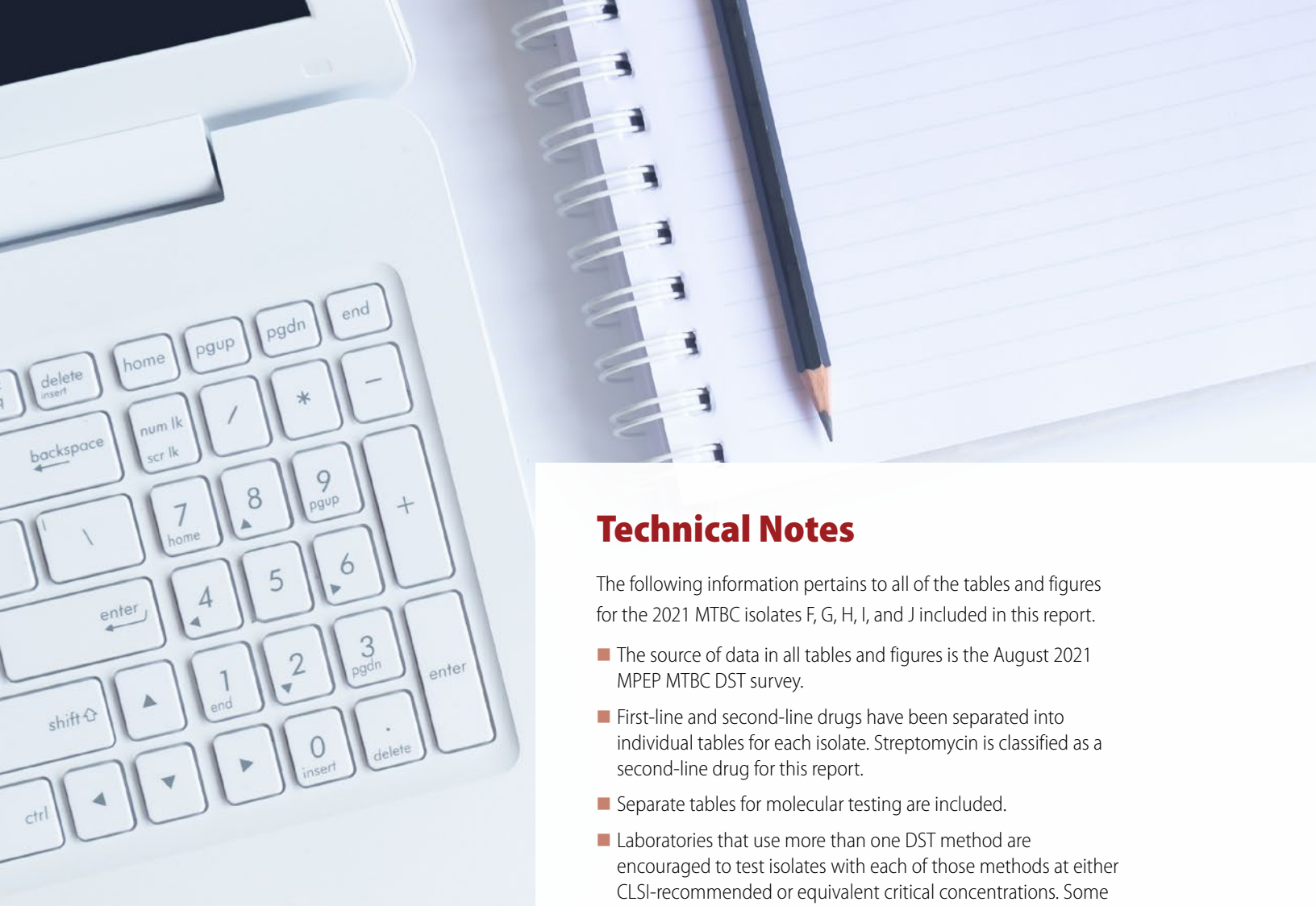
*80% consensus for a single categorical result for this drug of either susceptible or resistant was not achieved for this isolate among participating laboratories.

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

Note—Empty cell=No mutation detected

Isolate	*rpoB*[¥]	*katG*	*inhA*	*fabG1*	*gyrA*
2021F					Ala90Val
2021G		Asp94Asn			Asp94Gly
2021H	Arg447Arg* (Arg528Arg)[†]			Leu203Leu	Asp94Asn
2021I					Ser91Pro
2021J			C-15T		Ala90Val

[¥] Mutation is listed using both the *M. tuberculosis* and *E.coli* numbering system [6, 7]
* *M. tuberculosis* numbering system used
[†] *E. coli* numbering system used



Technical Notes

The following information pertains to all of the tables and figures for the 2021 MTBC isolates F, G, H, I, and J included in this report.

- The source of data in all tables and figures is the August 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 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

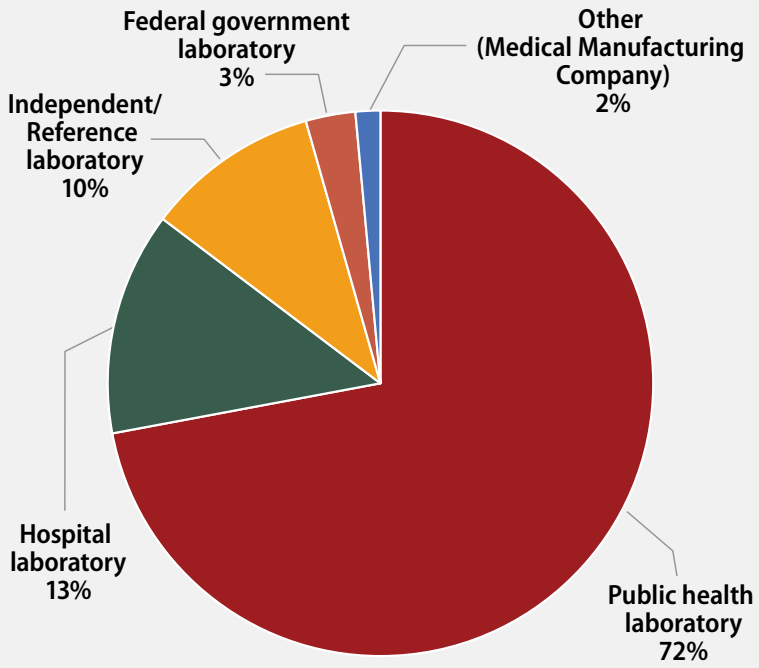
Primary Classification

This report contains DST results submitted to CDC by survey participants at 68 laboratories in 34 states.

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

- **49 (72%):** Public health laboratory (e.g., local, county, state)
- **9 (13%):** Hospital laboratory
- **7 (10%):** Independent/Reference laboratory (non-hospital based)
- **2 (3%):** Federal government laboratory
- **1 (2%):** Other (Medical Manufacturing Company)

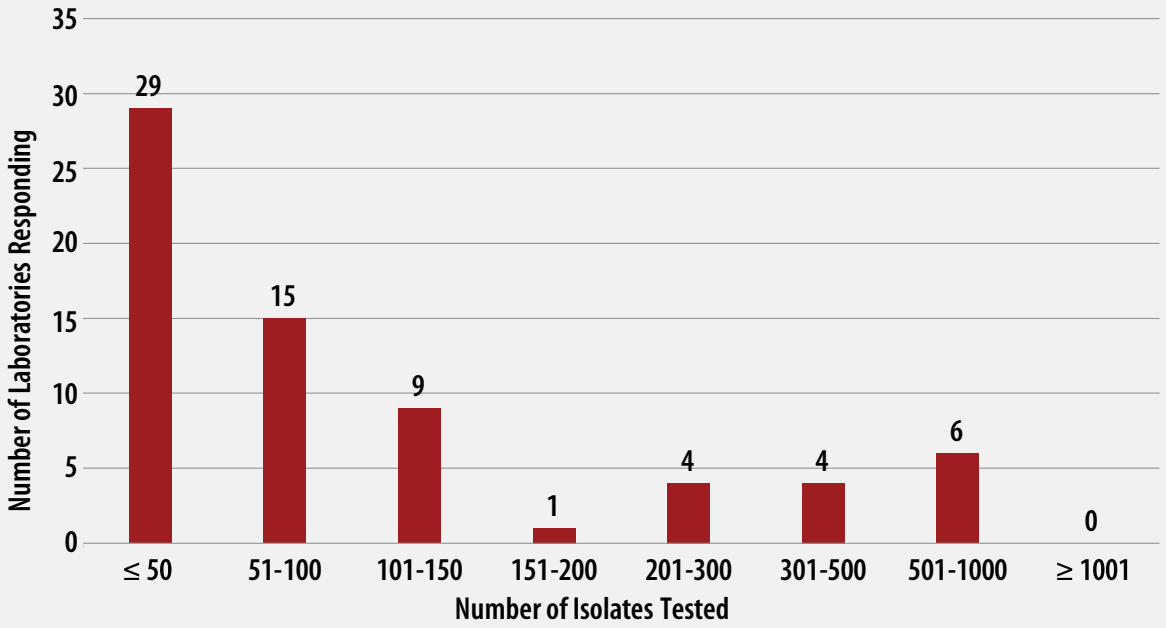
Figure 1. Primary Classification of Participating Laboratories, August 2021



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 68 participants in 2020 (excluding isolates used for quality control) is shown in Figure 2. In 2020, the counts ranged from 0 to 782 tests. Participants at 29 (42%) 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 [8].

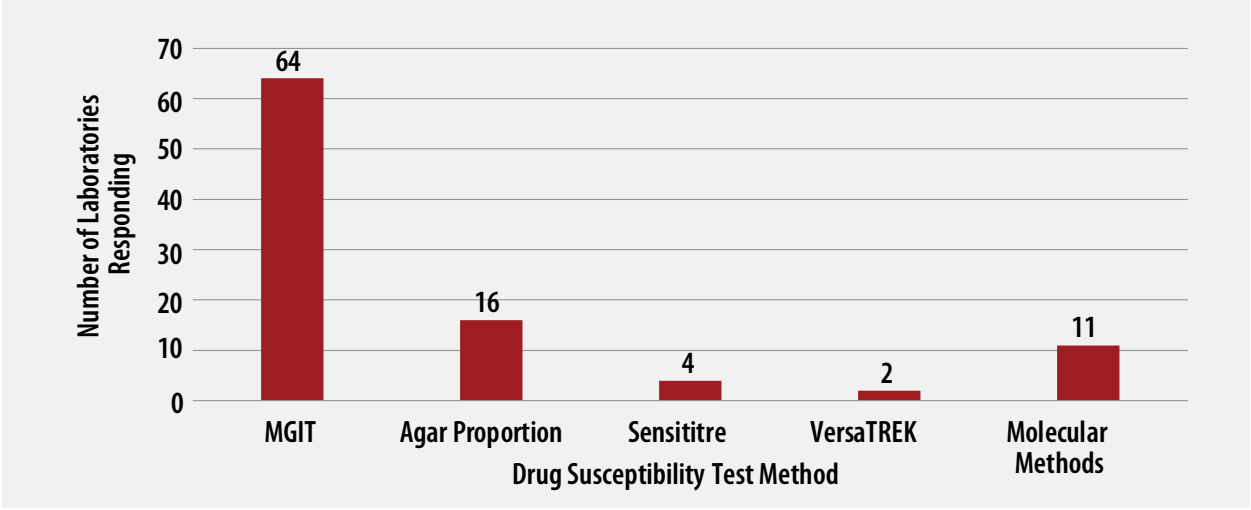
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=68)



MTBC DST Methods Used by Participants

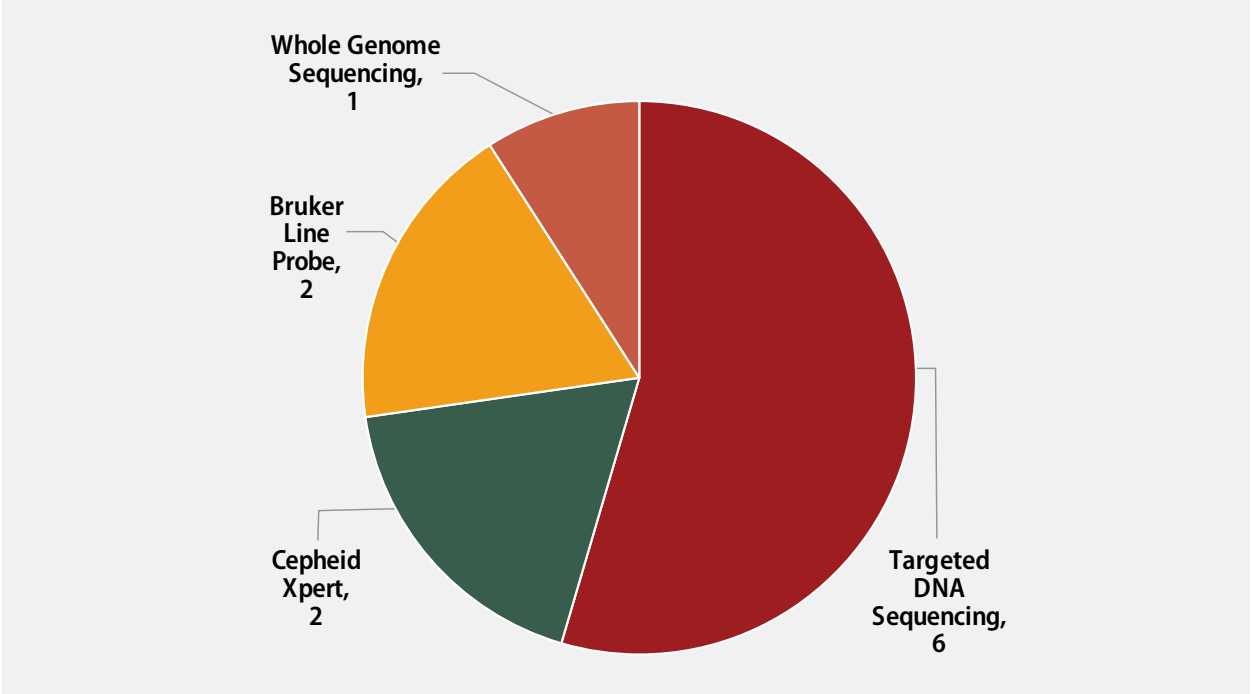
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Of participating laboratories, 43 (63%) reported results for only one method, 21 (31%) reported two methods, and 4 (6%) noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=97)



Molecular methods reported by participants are shown in Figure 4. The method used most frequently by six laboratories (55%) was targeted DNA sequencing, including pyrosequencing and Sanger sequencing. Two (18%) laboratories reported use of the Cepheid Xpert MTB/RIF assay, two (18%) reported results for line probe assays, Bruker Genotype MTBDR*plus* and MTBDR*sl*, and one (9%) reported results from whole genome sequencing.

Figure 4. Molecular Method Reported (n=11)

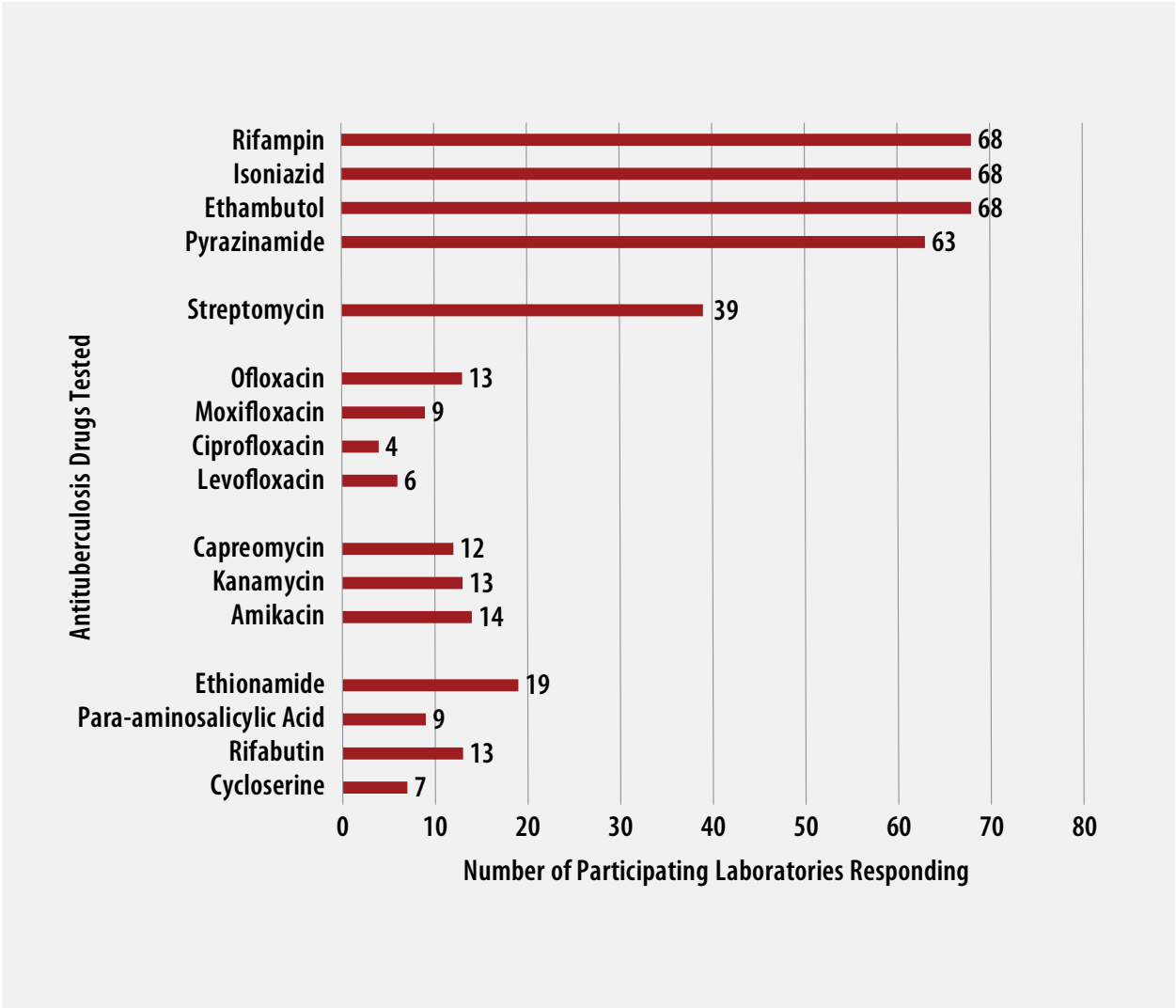


Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the August 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 63 (93%) 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 21 laboratories reporting second-line drug results (with the exception of streptomycin), five (22%) tested all three second-line injectable drugs (amikacin, kanamycin, and capreomycin) and at least one fluoroquinolone (ofloxacin, ciprofloxacin, levofloxacin, or moxifloxacin) needed to confidently define XDR TB. CDC has adopted a new hybrid definition of XDR that includes both the former classification (i.e., MDR with resistance to second-line injectable plus fluoroquinolone) or the revised WHO definition (i.e., MDR plus resistance to fluoroquinolone and either bedaquiline or linezolid) [9, 10].

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2021F

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

Ofloxacin and Ciprofloxacin

Fluoroquinolones (FQ) are one of the most commonly prescribed classes of antibiotic in the United States due to their activity against various types of bacteria. They are an important class of drugs used to treat tuberculosis (TB) resistant to first-line drugs but also have the potential to become an important part of new TB regimens including as part of treatment regimens for drug-susceptible TB [11, 12]. Prolonged treatment with a FQ (>10 days) before a diagnosis of TB is associated with a higher risk for FQ resistance and diagnostic delays [11, 13]. The primary mechanism of action of FQ is the inhibition of DNA synthesis [14] 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, known as the quinolone resistance determining region (QRDR), of the MTBC *gyrA* gene. Mutations in the QRDR, commonly occurring at codons 90, 91, and 94, prevent the drugs from effectively binding DNA gyrase [5, 14, 15]. 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 [14].

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 [16, 17]. This phenomenon is not limited to FQ but is commonly noted with this drug class.

Studies suggest that the level of resistance, as measured by MIC, to ofloxacin (OFL), ciprofloxacin (CIP), levofloxacin (LVX), and moxifloxacin (MOX) is dependent on the mutation and can vary among the FQs [18, 19]. CLSI and WHO currently recommends testing LVX and/or MOX, however, the preferred FQ to be tested is the one used in the treatment regimen [1, 9].

DNA sequencing of *gyrA* in Isolate 2021F detected a C>T point mutation in codon 90 of *gyrA* resulting in wild-type alanine being replaced with valine (Ala90Val). The Ala90Val mutation has been associated with FQ resistance [5, 20]. Sequencing of *gyrB* locus for this isolate revealed no mutations (i.e., wild-type sequence).

Among three growth-based methods, 12 results for OFL were reported for Isolate 2021F. This isolate was reported as **resistant** to OFL by method, as follows:

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

Participating laboratories also reported results for other FQ drugs (e.g., CIP, LVX, and MOX) for Isolate 2021F; 88% (14/16) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other FQ by method, as follows:

CIP

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

LVX

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

MOX

- **75% (3/4)** of the results when using AP
- **75% (3/4)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

A mutation in the *gyrA* gene was detected by all (100%) laboratories that reported molecular testing for FQ, with five laboratories noting the Ala90Val mutation.

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

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

Seven laboratories noted no growth for Isolate 2021F and did not report results for at least one antituberculosis drug tested.

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

Drug	Susceptible	Resistant	Total
Rifampin	15	0	15
Isoniazid—Low	13	2	15
Isoniazid—High	15	0	15
Ethambutol	15	0	15

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

Drug	Susceptible	Resistant	Total
Rifampin	53	0	53
Isoniazid—Low	46	6	52
Isoniazid—High	20	0	20
Ethambutol	53	0	53
Pyrazinamide	62	0	62

Table 5. Isolate 2021F—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 6. Isolate 2021F—Participant Results for First-Line DST by VersaTREK

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

Table 7. Isolate 2021F—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	13	0	13
Ofloxacin	0	7	7
Ciprofloxacin	0	2	2
Levofloxacin	0	2	2
Moxifloxacin	1	3	4
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	9	0	9
Ethionamide	12	1	13
Rifabutin	7	0	7
Cycloserine	4	1	5
p-Aminosalicylic acid	5	0	5

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

Drug	Susceptible	Resistant	Total
Streptomycin	27	0	27
Ofloxacin	0	3	3
Ciprofloxacin	0	0	0*
Levofloxacin	0	3	3
Moxifloxacin	1	3	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 CIP by MGIT.

Table 9. Isolate 2021F—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	0	2	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 LVX and MOX by Sensititre.

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

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	0	8	8
Ethambutol	0	4	4
Pyrazinamide	0	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	4	4
Rifabutin	0	4	4

Isolate 2021G

Expected Result: Resistant to INH at 0.2 µg/ml, OFL at 2.0 µg/ml, CIP at 2.0 µg/ml, and STR at 2.0 µg/ml by agar proportion

Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculosis drug and is a cornerstone of regimens used to treat TB disease and latent TB infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *katG* gene [5, 21]. 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 [5, 15, 21]. 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 *fabG1/inhA* promoter region, which are generally associated with low-level resistance to INH and are less frequent than *katG* mutations. It has been reported that approximately 10–15% of isolates found to be INH resistant have no mutations detected in either of these loci, however this percent is decreasing due to the more comprehensive nature of whole genome sequencing [22, 23]. 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 (Leu203Leu) has been associated with low-level INH resistance [24, 25].

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2021G detected a C>T point mutation at codon 94 in the *katG* locus resulting in wild-type aspartic acid being replaced with asparagine (Asp94Asn); *inhA*, *fabG1*, and *ahpC* were wild-type (i.e., no mutations were detected). The *katG* Asp94Asn mutation's role in INH resistance is not fully characterized at this time, but it appears to confer low-level resistance. For internal comparison purposes, this isolate was previously sent as MPEP Isolate 2019A and most participating laboratories reported resistance at the low concentration of INH.

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 2021G, 83 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **88% (14/16)** of the results when using AP
- **97% (60/62)** of the results when using MGIT
- **33% (1/3)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Four (8%) results were reported as resistant at the higher concentrations of INH. Only 32 (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 8 molecular results reported for INH, one (13%) laboratory reported detection of a mutation and specifically noted the *katG* Asp94Asn mutation.

Two of the laboratories performing Sensititre reported INH MIC values as ≤0.12 µg/ml and 0.12 µg/ml.

Ofloxacin and Ciprofloxacin

DNA sequencing of *gyrA* in Isolate 2021G detected an A>G point mutation in codon 94 of *gyrA* resulting in wild-type aspartic acid being replaced with glycine (Asp94Gly). The Asp94Gly mutation has been associated with FQ resistance [5, 20]. Sequencing of *gyrB* locus for this isolate revealed no mutations (i.e., wild-type sequence).

Among three methods, 13 results for OFL were reported for Isolate 2021G. This isolate was reported as **resistant** to OFL by method, as follows:

- **100% (8/8)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Participating laboratories also reported results for other FQ (e.g., CIP, LVX, and MOX) for Isolate 2021G; 100% (19/19) of results noted resistance. The isolate was reported **resistant** to three other FQ by method, as follows:

CIP

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

LVX

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

MOX

- **100% (4/4)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **100% (2/2)** 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 with five laboratories specifically noting the Asp94Gly mutation.

Three of the laboratories performing Sensititre reported MIC values for FQ drugs; one of these did not report interpretations. Reported MIC values were as follows: OFL at 16 µg/ml (n=2); MOX at 4 µg/ml (n=2) and 8 µg/ml (n=1); and LVX at 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 [15, 21]. In MTBC, the genetic basis of the majority of resistance to STR is usually due to mutations in *rrs* or *rpsL* [14, 15].

DNA sequencing of *rpsL* analysis revealed a A>G point mutation resulting in wild-type lysine being replaced by arginine (Lys43Arg). The Lys43Arg mutation has been associated with resistance to STR [26].

Among three methods, 45 results for STR were reported for Isolate 2021G. This isolate was reported as **resistant** to STR by method, as follows:

- **100% (14/14)** of the results when using AP
- **100% (28/28)** 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 ≥32 µg/ml (n=2) and 32 µg/ml (n=1).

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

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

Drug	Susceptible	Resistant	Total
Rifampin	16	0	16
Isoniazid—Low	2	14	16
Isoniazid—High	15	1	16
Ethambutol	16	0	16

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

Drug	Susceptible	Resistant	Total
Rifampin	62	0	62
Isoniazid—Low	2	60	62
Isoniazid—High	29	3	32
Ethambutol	62	0	62
Pyrazinamide	62	0	62

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

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

Table 14. Isolate 2021G—Participant Results for First-Line DST by VersaTREK

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

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

Drug	Susceptible	Resistant	Total
Streptomycin	0	14	14
Ofloxacin	0	8	8
Ciprofloxacin	0	3	3
Levofloxacin	0	3	3
Moxifloxacin	0	4	4
Amikacin	8	0	8
Kanamycin	9	0	9
Capreomycin	9	0	9
Ethionamide	12	2	14
Rifabutin	7	0	7
Cycloserine	5	0	5
p-Aminosalicylic acid	5	0	5

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

Drug	Susceptible	Resistant	Total
Streptomycin	0	28	28
Ofloxacin	0	3	3
Ciprofloxacin	0	1	1
Levofloxacin	0	3	3
Moxifloxacin	0	3	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 MOX by MGIT.

Table 17. Isolate 2021G—Participant Results for Second-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Streptomycin	0	3	3
Ofloxacin	0	2	2
Ciprofloxacin	0	0	0
Levofloxacin	0	0	0*
Moxifloxacin	0	2	2*
Amikacin	2	0	2*
Kanamycin	2	0	2
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 LVX, MOX, AMK, and CYC by Sensititre.

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

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	1	7	8
Ethambutol	0	4	4
Pyrazinamide	0	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	4	4
Rifabutin	0	4	4

Isolate 2021H

Expected Result: Resistant to INH at 0.2 µg/ml, OFL at 2.0 µg/ml, CIP at 2.0 µg/ml, and ETA at 5.0 µg/ml by agar proportion

Isoniazid

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2021H detected a G>A point mutation at codon 203 in the *fabG1* locus resulting in wild-type leucine being replaced by leucine (Leu203Leu); *katG*, *inhA*, and *ahpC* were wild-type (i.e., no mutations were detected).

Within *fabG1*, the silent/synonymous mutation (i.e., nucleotide change but no corresponding change in amino acid) Leu203Leu has been found to confer INH resistance through the formation of an alternative promoter, thereby increasing the transcriptional levels of *inhA* [25]. Although silent mutations were previously believed to not play a role in drug resistance, the Leu203Leu mutation demonstrates that silent mutations could be associated with resistance depending on the specific gene and the location of the mutation.

For Isolate 2021H, 81 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (15/15)** of the results when using AP
- **59% (36/61)** of the results when using MGIT
- **0% (0/3)** of the results when using Sensititre
- **50% (1/2)** of the results when using VersaTREK

No results were reported as resistant at the higher concentrations of INH. Only 27 (44%) 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, 3 (38%) laboratories reported detection of a mutation and specifically noted the Leu203Leu mutation.

Two of the laboratories performing Sensititre reported INH MIC values as 0.12 µg/ml (n=2).

Ofloxacin and Ciprofloxacin

DNA sequencing of *gyrA* in Isolate 2021H detected a G>A point mutation in codon 94 resulting in wild-type aspartic acid being replaced with asparagine (Asp94Asn). The Asp94Asn mutation has been associated with FQ resistance [5, 20]. Sequencing of *gyrB* locus for this isolate revealed no mutations (i.e., wild-type sequence).

Among three methods, 13 results for OFL were reported for Isolate 2021H. This isolate was reported as **resistant** to OFL by method, as follows:

- **100% (8/8)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Participating laboratories also reported results for other FQ (e.g., CIP, LVX, and MOX) for Isolate 2021H; 95% (19/20) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other FQ by method, as follows:

CIP

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

LVX

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

MOX

- **75% (3/4)** of the results when using AP
- **100% (4/4)** of the results when using MGIT
- **100% (2/2)** 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 with five laboratories specifically noting the Asp94Asn mutation.

Three of the laboratories performing Sensititre reported MIC values for FQ drugs; one of these did not report interpretations. Reported MIC values were as follows: OFL at 16 µg/ml (n=1) and 32 µg/ml (n=1); MOX at 8 µg/ml (n=3); and LVX at 8 µg/ml (n=1).

Ethionamide

Resistance to INH and ethionamide (ETA) can occur by mutations in the *fabG1–inhA* regulatory region, which are generally associated with low-level resistance to INH. Mutations in *ethA* also confer resistance to ETA, without concomitant resistance to INH [27]. Sequencing analysis of *ethA* did not detect a mutation and as previously noted, sequencing of the *inhA* gene revealed wild-type (i.e., no mutations were detected). The synonymous/silent mutation Leu203Leu was detected in the *fabG1* locus for Isolate 2021H. This mutation has been associated with ETA resistance [26].

Issues with reproducibility of DST results for ETA have been reported [28] and remain a potential concern.

For Isolate 2021H, 19 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **79% (11/14)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

Two of the laboratories performing Sensititre reported ETA MIC values as 5 µg/ml (n=1) and 10 µg/ml (n=1).

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 [21]. The primary mechanism of resistance is mutations within the 81-bp central region of the *rpoB* gene (i.e., rifampin resistance determining region or RRDR) that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase [15]. 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 [15, 21]. 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 [29]. 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 [30]. 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 2021H revealed a C>T point mutation in codon 447 (*E. coli* numbering 528) of the *rpoB* locus. However, this mutation does not result in an amino acid change; arginine remains arginine (Arg447Arg). The Arg447Arg synonymous (i.e., silent) mutation in *rpoB* is not considered clinically significant and isolates with this mutation reliably test as RMP-susceptible in growth-based systems. However, as noted above, the Xpert MTB/RIF assay could indicate RMP resistance for this isolate and sequencing of *rpoB* should be performed.

For Isolate 2021H, 82 results for RMP were reported. This isolate was reported as **susceptible** to RMP by method, as follows:

- **100% (15/15)** of the results when using AP
- **100% (62/62)** 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 ten molecular results reported for RMP, five (50%) laboratories reported mutation detected specifically noting the Arg447Arg silent mutation. Five laboratories reported mutation not detected, however this may be due to laboratory reporting practices when a silent mutation not associated with resistance is detected.

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

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

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

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

Drug	Susceptible	Resistant	Total
Rifampin	62	0	62
Isoniazid—Low	25	36	61*
Isoniazid—High	27	0	27
Ethambutol	62	0	62
Pyrazinamide	63	0	63

* One additional laboratory reported borderline for INH—Low by MGIT.

Table 21. Isolate 2021H—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	2	1	3

Table 22. Isolate 2021H—Participant Results for First-Line DST by VersaTREK

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

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

Drug	Susceptible	Resistant	Total
Streptomycin	13	0	13
Ofloxacin	0	8	8
Ciprofloxacin	0	3	3
Levofloxacin	0	3	3
Moxifloxacin	1	3	4
Amikacin	8	0	8
Kanamycin	9	0	9
Capreomycin	9	0	9
Ethionamide	3	11	14
Rifabutin	7	0	7
Cycloserine	4	1	5
p-Aminosalicylic acid	5	0	5

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

Drug	Susceptible	Resistant	Total
Streptomycin	28	0	28
Ofloxacin	0	3	3
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	0	3	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1

Table 25. Isolate 2021H—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	0	2	2*
Amikacin	3	0	3

Drug	Susceptible	Resistant	Total
Kanamycin	2	0	2
Capreomycin	1	0	1
Ethionamide	1	1	2
Rifabutin	3	0	3
Cycloserine	1	0	1*
p-Aminosalicylic acid	3	0	3

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

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

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	5	5	10
Isoniazid	3	5	8
Ethambutol	1	3	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	2	2	4
Rifabutin	1	3	4

Isolate 2021I

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

Ofloxacin and Ciprofloxacin

DNA sequencing of *gyrA* in Isolate 2021I revealed a T>C point mutation in codon 91 of *gyrA* resulting in wild-type serine being replaced with proline (Ser91Pro). The Ser91Pro mutation has been associated with FQ resistance [5, 20]. Sequencing of *gyrB* locus for this isolate revealed no mutations (i.e., wild-type sequence).

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

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

Participating laboratories also reported results for other FQ drugs (e.g., CIP, LVX, and MOX) for Isolate 2021I; 84% (16/19) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other FQ by method, as follows:

CIP

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

LVX

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

MOX

- **100% (4/4)** of the results when using AP
- **75% (3/4)** of the results when using MGIT
- **50% (1/2)** 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 with five laboratories specifically noting the Ser91Pro mutation.

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

Pyrazinamide

For Isolate 2021I, DNA sequencing of the *pncA* gene revealed the silent/synonymous mutation (i.e., nucleotide change but no corresponding change in amino acid) Ser65Ser that is not associated with resistance. There may be additional mechanisms of resistance to PZA besides nucleotide changes in the *pncA* gene that are still unknown [31]. Issues with false-resistance to PZA have been reported [32] and remain a potential concern.

Isolate 2021I was expected to be **susceptible** to PZA; however, of those testing PZA, **resistance** was reported by:

- **42% (25/60)** of the results when using MGIT
- **0% (0/1)** of the results when using VersaTREK

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

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

Drug	Susceptible	Resistant	Total
Rifampin	14	0	14
Isoniazid—Low	14	0	14
Isoniazid—High	14	0	14
Ethambutol	14	0	14

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

Drug	Susceptible	Resistant	Total
Rifampin	62	0	62
Isoniazid—Low	62	0	62
Isoniazid—High	23	0	23
Ethambutol	62	0	62
Pyrazinamide	35	25	60*

*Two additional laboratories reported borderline for PZA by MGIT

Table 29. Isolate 2021I—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 30. Isolate 2021I—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 31. Isolate 2021I—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	12	0	12
Ofloxacin	0	7	7
Ciprofloxacin	0	2	2
Levofloxacin	0	3	3
Moxifloxacin	0	4	4
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	9	0	9
Ethionamide	13	0	13
Rifabutin	7	0	7
Cycloserine	4	1	5
p-Aminosalicylic acid	5	0	5

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

Drug	Susceptible	Resistant	Total
Streptomycin	28	0	28
Ofloxacin	0	3	3
Ciprofloxacin	1	0	1
Levofloxacin	0	3	3
Moxifloxacin	1	3	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 33. Isolate 2021I—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

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

Table 34. Isolate 2021I—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	0	8	8
Ethambutol	0	4	4
Pyrazinamide	1*	2	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	4	4
Rifabutin	0	4	4

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

Isolate 2021J

Expected Result: Resistant to INH at 0.2 µg/ml, OFL at 2.0 µg/ml, CIP at 2.0 µg/ml, and ETA at 5.0 µg/ml by agar proportion

Isoniazid

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2021J revealed a C>T point mutation at nucleotide position -15 of the promoter region of the *inhA* gene (C-15T); *katG*, *fabG1*, and *ahpC* were wild-type (i.e., no mutations were detected). Mutations in the promoter region of the *inhA* gene are generally associated with low-level resistance to INH.

For Isolate 2021J, 82 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (16/16)** of the results when using AP
- **100% (62/62)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

One (2%) result was reported as resistant at the higher concentrations of INH. Only 33 (53%) 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 C-15T mutation.

One laboratory performing Sensititre reported INH MIC value as 0.25 µg/ml (n=1). Another laboratory reported an INH MIC value as 0.25 µg/ml (n=1) and indicated a result of borderline.

Ofloxacin and Ciprofloxacin

DNA sequencing of *gyrA* in Isolate 2021J detected a C>T point mutation in codon 90 of *gyrA* resulting in wild-type alanine being replaced with valine (Ala90Val). The Ala90Val mutation has been associated with FQ resistance [5, 20]. Sequencing of *gyrB* locus for this isolate revealed no mutations (i.e., wild-type sequence).

Although resistance was expected for all FQ drugs, variable results were reported by participants. The Ala90Val mutation has been associated with low-level FQ resistance, but the MIC for this isolate could be close to the critical concentration thereby impacting DST reproducibility [4].

Among three growth-based methods, 13 results for OFL were reported for Isolate 2021J. This isolate was reported as **resistant** to OFL by method, as follows:

- **100% (8/8)** of the results when using AP
- **67% (2/3)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Participating laboratories also reported results for other FQ drugs (e.g., CIP, LVX, and MOX) for Isolate 2021J; 84% (16/19) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other FQ by method, as follows:

CIP

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

LVX

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

MOX

- **100% (4/4)** of the results when using AP
- **75% (3/4)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre

A mutation in the *gyrA* gene was detected by most (86%) laboratories that reported molecular testing for FQ drugs. Five laboratories noted the Ala90Val mutation.

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

Ethionamide

As previously noted in Isolate 2021H, resistance to INH and ETA can occur by mutations in the promoter region of the *inhA* gene which are generally associated with low-level resistance to INH. A point mutation (C-15T) was detected in the promoter region of *inhA* for Isolate 2021J. This mutation has been associated with ETA resistance [26].

For Isolate 2021J, 19 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **71% (10/14)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Two of the laboratories performing Sensititre reported an ETA MIC value as 20 µg/ml (n=1) and 40 µg/ml (n=1).

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2021J are listed in Tables 35–42.

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

Drug	Susceptible	Resistant	Total
Rifampin	16	0	16
Isoniazid—Low	0	16	16
Isoniazid—High	15	1	16
Ethambutol	16	0	16

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

Drug	Susceptible	Resistant	Total
Rifampin	62	0	62
Isoniazid—Low	0	62	62
Isoniazid—High	33	0	33
Ethambutol	62	0	62
Pyrazinamide	62	0	62

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

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

*One additional laboratory reported borderline for INH—Low and INH—High by Sensititre.

Table 38. Isolate 2021J—Participant Results for First-Line DST by VersaTREK

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

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

Drug	Susceptible	Resistant	Total
Streptomycin	14	0	14
Ofloxacin	0	8	8
Ciprofloxacin	0	3	3
Levofloxacin	0	3	3
Moxifloxacin	0	4	4
Amikacin	8	0	8
Kanamycin	9	0	9
Capreomycin	9	0	9
Ethionamide	4	10	14
Rifabutin	7	0	7
Cycloserine	4	1	5
p-Aminosalicylic acid	5	0	5

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

Drug	Susceptible	Resistant	Total
Streptomycin	28	0	28
Ofloxacin	1	2	3
Ciprofloxacin	1	0	1
Levofloxacin	0	2	2*
Moxifloxacin	1	3	4
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	0	3	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1

*One additional laboratory reported borderline for LVX by MGIT.

Table 41. Isolate 2021J—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	0	2	2
Rifabutin	3	0	3
Cycloserine	2	0	2
p-Aminosalicylic acid	3	0	3

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

Table 42. Isolate 2021J—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	1*	2	3
Ofloxacin	6	1	7
Ciprofloxacin	6	1	7
Levofloxacin	5	1	6
Moxifloxacin	5	1	6
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	4	0	4
Rifabutin	0	4	4

**This laboratory noted the detection of a mutation not associated with PZA 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-A2 document [1]

*The higher concentration of INH should be tested as second-line drug 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 [1, 3].

Second-line Drugs	7H10 agar	7H11 agar
Streptomycin	2.0	2.0
Amikacin	1.0	Not determined*
Capreomycin	0.5	0.5
Kanamycin	4.0†	Not determined*
Levofloxacin	10.0†	10.0¥
Moxifloxacin	5.0†	6.0¥
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.

†CLSI critical concentrations differ from revised WHO recommendations published in 2018 [1, 4].

- For AMK, the WHO recommended critical concentration for 7H10 agar is 2.0 µg/ml
- For CAP, the WHO recommended critical concentration for 7H10 agar is 4.0 µg/ml and 'Not determined' for 7H11 agar
- For KAN, the WHO recommended critical concentration for 7H10 agar is 4.0 µg/ml.

¥WHO has withdrawn the recommended critical concentrations for CAP and KAN for 7H11 agar and PAS for 7H10 and 7H11.[4].

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 [3].

Second-line Drug	MGIT
Streptomycin	1.0 (and 4.0*)
Levofloxacin	1.0†
Moxifloxacin	0.25
Amikacin	1.0
Capreomycin	2.5
Kanamycin	2.5
Ethionamide	5.0
p-Aminosalicylic acid	Not recommended†

NOTE—Critical concentrations as indicated in WHO 2018 Technical Report on critical concentrations unless noted otherwise [4]. Data for second-line critical concentrations not available for VersaTREK

*Critical concentration as indicated in applicable manufacturer package insert. The higher concentration of STR should be tested after resistance at the critical concentration is detected.

†WHO critical concentrations differ from CLSI M62 recommendations published in 2018 [2, 4].

- For LVX, the CLSI recommended critical concentration for MGIT is 1.5 µg/ml
- For PAS, the CLSI recommended critical concentration for MGIT is 4.0 µg/ml

References

1. CLSI, *Susceptibility Testing of Mycobacteria, Nocardiae spp., and Other Aerobic Actinomycetes*, in *3rd Ed. CLSI Standard M24*. 2018, Clinical and Laboratory Standards Institute: Wayne, PA.
2. CLSI, *Performance Standards for Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes*, in *1st Ed. CLSI supplement M62*. 2018, Clinical and Laboratory Standards Institute: Wayne, PA.
3. World Health Organization, *Technical Report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine)*. 2021: Geneva.
4. World Health Organization, *Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis*. 2018: Geneva.
5. 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.
6. Andre, E., et al., *Consensus numbering system for the rifampicin resistance-associated rpoB gene mutations in pathogenic mycobacteria*. Clin Microbiol Infect, 2017. **23**(3): p. 167-172.
7. APHL, *Issues in Mycobacterium tuberculosis complex (MTBC) Drug Susceptibility Testing: Rifampin (RIF), in APHL Issues in Brief: Infectious Diseases*. 2019, Association of Public Health Laboratories: Washington, D.C.
8. APHL, *TB Drug Susceptibility Testing Expert Panel Meeting Summary Report*. 2007, Association of Public Health Laboratories: Washington, D.C.
9. World Health Organization, *Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis, 27-29 October 2020*. 2021, World Health Organization: Geneva.
10. CDC Division of Tuberculosis Elimination, *Dear Colleague Letter: Surveillance definitions for extensively drug resistant (XDR) and pre-XDR tuberculosis*. 2022.
11. 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.
12. Carr W, K.E., Starks A, Goswami N, Allen L, Winston C, *Interim Guidance: 4-Month Rifapentine-Moxifloxacin Regimen for the Treatment of Drug-Susceptible Pulmonary Tuberculosis—United States, 2022*. MMWR Morb Mortal Wkly Rep, 2022. **71**(8): p. 285-289.
13. 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.
14. 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.
15. Zhang, Y. and W.W. Yew, *Mechanisms of drug resistance in Mycobacterium tuberculosis*. Int J Tuberc Lung Dis, 2009. **13**(11): p. 1320-30.
16. 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.
17. Rinder, H., K.T. Mieskes, and T. Loscher, *Heteroresistance in Mycobacterium tuberculosis*. Int J Tuberc Lung Dis, 2001. **5**(4): p. 339-45.
18. Willby, M., et al., *Correlation between GyrA substitutions and ofloxacin, levofloxacin, and moxifloxacin cross-resistance in Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2015. **59**(9): p. 5427-34.
19. 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.
20. 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.
21. 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.
22. Seifert, M., et al., *Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review*. PLoS One, 2015. (3): p. e0119628.

23. Kandler, J.L., et al., *Validation of Novel Mycobacterium tuberculosis Isoniazid Resistance Mutations Not Detectable by Common Molecular Tests*. Antimicrob Agents Chemother, 2018. **62**(10).
24. 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.
25. Ando, H., et al., *A silent mutation in mabA confers isoniazid resistance on Mycobacterium tuberculosis*. Mol Microbiol, 2014. **91**(3): p. 538-47.
26. *Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance.* 2021, World Health Organization: Geneva.
27. Morlock, G.P., et al., *ethA, inhA, and katG loci of ethionamide-resistant clinical Mycobacterium tuberculosis isolates*. Antimicrob Agents Chemother, 2003. **47**(12): p. 3799-805.
28. Varma-Basil, M. and R. Prasad, *Dilemmas with ethionamide susceptibility testing of Mycobacterium tuberculosis: A microbiologist & physician's nightmare*. Indian J Med Res, 2015. **142**(5): p. 512-4.
29. Centers for Disease Control and Prevention, *Availability of an assay for detecting Mycobacterium tuberculosis, including rifampin-resistant strains, and considerations for its use—United States, 2013*. MMWR Morb Mortal Wkly Rep, 2013. **62**(41): p. 821-7.
30. Van Deun, A., et al., *Rifampin drug resistance tests for tuberculosis: challenging the gold standard*. J Clin Microbiol, 2013. **51**(8): p. 2633-40.
31. Ramirez-Busby, S.M. and F. Valafar, *Systematic Review of Mutations in Pyrazinamidase Associated with Pyrazinamide Resistance in Mycobacterium tuberculosis Clinical Isolates*. Antimicrob Agents Chemother, 2015. **59**(9): p. 5267-77.
32. Chedore, P., et al., *Potential for erroneous results indicating resistance when using the Bactec MGIT 960 system for testing susceptibility of Mycobacterium tuberculosis to pyrazinamide*. J Clin Microbiol, 2010. **48**(1): p. 300-1.

Appendix 1: Accessible Explanations of Figures

Figure 1. The primary classification of the 68 laboratories participating in the August 2021 MPEP survey is shown in this pie chart. The largest slice represents 50 laboratories, or 72% of 68 that have self-classified as a health department laboratory. The next major slice signifies 10 laboratories, or 13% of 68 that self-classified as hospital laboratories. The remaining three slices of the pie chart represent 7, or 10% of 68 that self-classified as independent laboratories, 2, or 3% of 68 that self-classified as federal government laboratories, and 1 laboratory, or 1% of 68 that self-classified as a medical manufacturer. (return to page 8)

Figure 2. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=68) 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, 29 laboratories tested less than or equal to 50 isolates per year; 15 laboratories tested between 51 to 100 isolates per year; 9 laboratories tested between 101 to 150 isolates per year; 1 laboratory tested between 151 to 200 isolates per year; 4 laboratories tested between 201 to 300 isolates per year; 4 laboratories tested between 301 to 500 isolates per year; 6 laboratories tested between 501 to 1000 isolates per year, and 0 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=97) 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: 64 used MGIT, 16 used agar proportion, 4 used Sensititre, 2 used VersaTREK, and 11 used molecular methods. (return to page 10)

Figure 4. The molecular methods used by MPEP participants (N=11) are displayed in this pie chart. The largest slice represents the 6 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. 68 laboratories tested rifampin; 68 laboratories tested isoniazid; 68 laboratories tested ethambutol; 63 laboratories tested pyrazinamide; 39 laboratories tested streptomycin; 13 laboratories tested ofloxacin; 9 laboratories tested moxifloxacin; 4 laboratories tested ciprofloxacin; 6 laboratories tested levofloxacin; 13 laboratories tested capreomycin; 12 laboratories tested kanamycin; 14 laboratories tested amikacin; 19 laboratories tested ethionamide; 9 laboratories tested PAS; 13 laboratories tested rifabutin; and 7 laboratories tested cycloserine. (return to page 11)

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