For accessible version go to https://www.cdc.gov/tb/topic/laboratory/mpep/2022/aug-mpepReport.htm.

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program
Report of Results
August 2022

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

CS 341552-A

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for August 2022 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 2022.

Report Content

The material in this report was developed and prepared by:

Cortney Stafford, MPH, MT (ASCP), Health Scientist, Laboratory Capacity Team, Laboratory Branch (LB), Division of TB Elimination (DTBE), National Center for HIV, Viral Hepatitis, STD, and TB Prevention (NCHHSTP), CDC

Acknowledged contributors from NCHHSTP, DTBE, and LB: Lois Diem, Stephanie Johnston, Atanaska Marinova-Petkova, Wan Moon, James Posey, and Angela Starks

Contact Information

Comments and inquiries regarding this report should be directed to:

TBMPEP@cdc.gov
404-639-4013
CDC TB MPEP Website

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	fluoroquinolone
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 "M24S: Performance Standards for Susceptibility Testing of Mycobacteria, *Nocardia* spp., and Other Aerobic Actinomycetes" [1-3]. Additionally, the World Health Organization (WHO) published two technical reports investigating critical concentrations, by method, for INH, RMP, EMB, PZA and second-line anti-tuberculosis drugs [4, 5].

Expected Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in August 2022 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 whole genome sequencing are listed in Table 2 [6].

Table 1. Expected Growth-based Results for August 2022 Survey

Note—S=susceptible, R=resistant

Isolate	RMP	INH	EMB	PZA	Second-line Drug Resistances:
2022F	S	S	S	S	
2022G	S	S	S	R*	
2022H	S	S	S	S	
2022I	S	R	S	S	ETA
2022J	S	R	S	S	

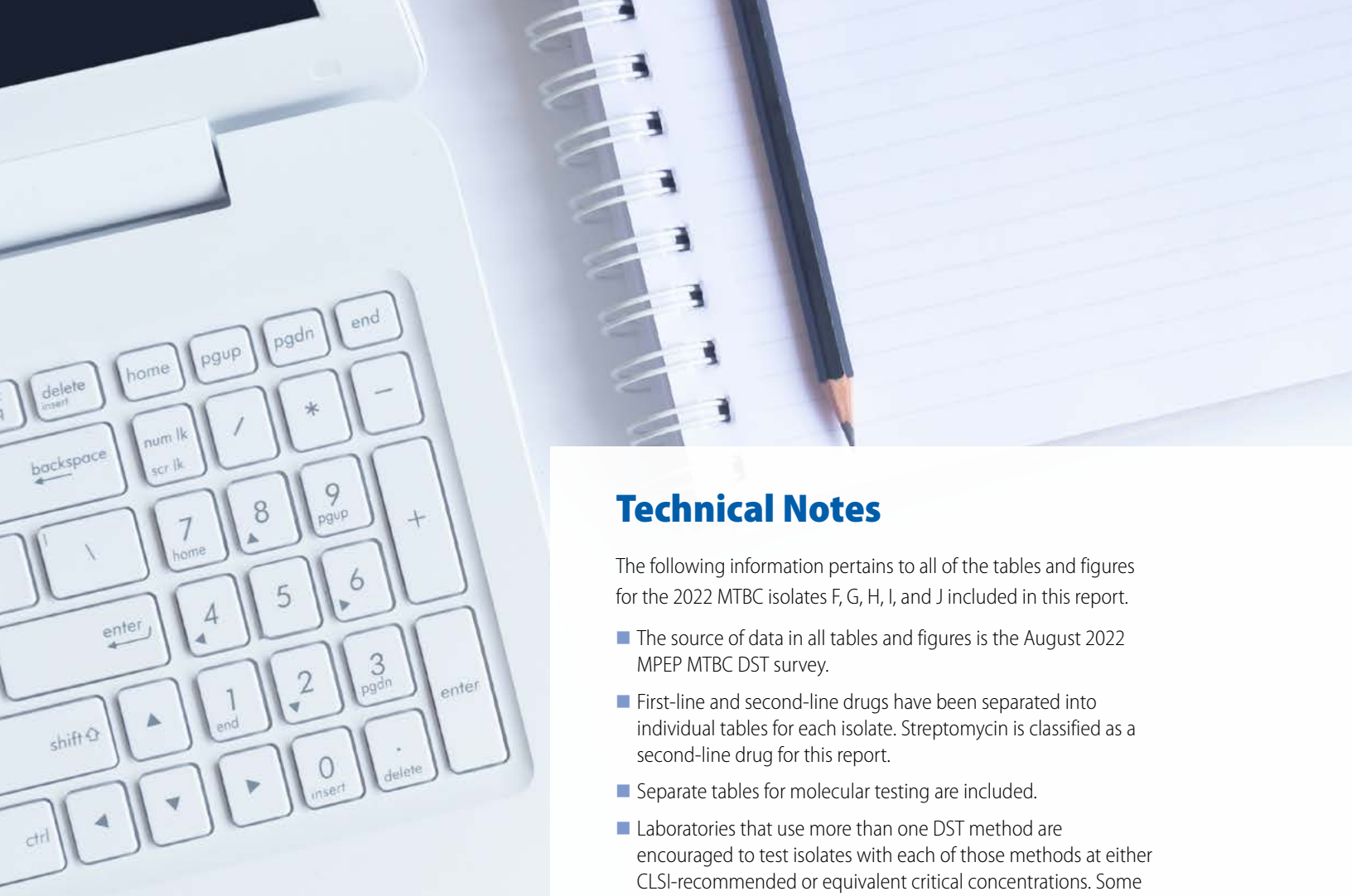
*80% consensus for a single categorical result across all methods reported for this drug of either susceptible or resistant was not achieved for this isolate among participating laboratories.

Table 2. Expected Molecular Results (Mutations Detected in Loci Associated with Resistance) for August 2022 Survey

Note—Empty cell=No mutation detected

Isolate	*rpoB*[¥]	*katG*	*inhA*	*pncA*	*ethA*
2022F	Phe433Phe* (Phe514Phe)[†]				
2022G				His82Asp	
2022H	Leu430Pro* (Leu511Pro)[†]				
2022I			C-15T		
2022J		Deletion			Ser266Arg[§]

[¥] Mutation is listed using both the *M. tuberculosis* and *E.coli* numbering system [7,8]
* *M. tuberculosis* numbering system used
[†] *E. coli* numbering system used
[§] Mutation not associated with resistance



Technical Notes

The following information pertains to all of the tables and figures for the 2022 MTBC isolates F, G, H, I, and J included in this report.

- The source of data in all tables and figures is the August 2022 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.
- VersaTREK™ tables are not included in this report since results were not received for this method for the August MPEP MTBC DST survey.

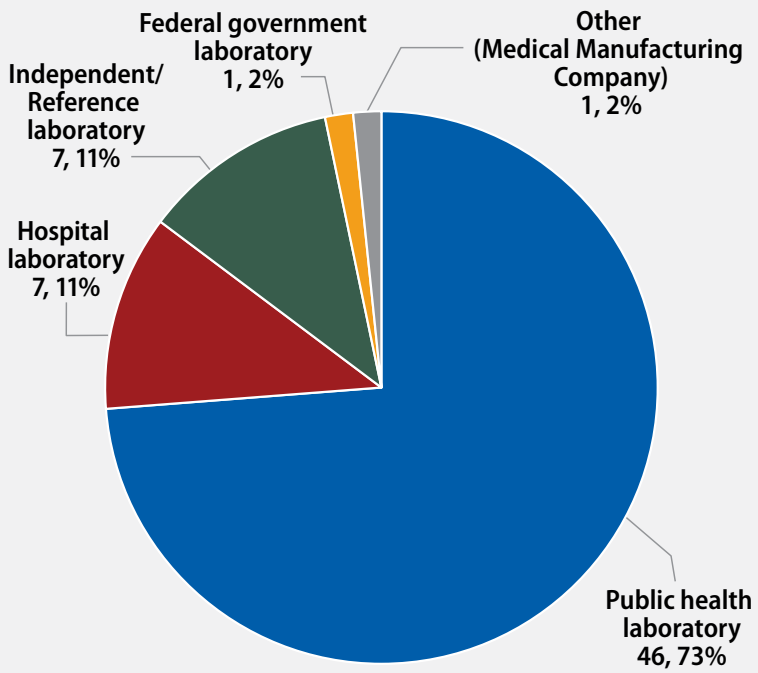
Descriptive Information about Participant Laboratories

Primary Classification

This report contains DST results submitted to CDC by survey participants at 61 laboratories in 32 states.

The participants were asked to indicate the primary classification of their laboratory (Figure 1).

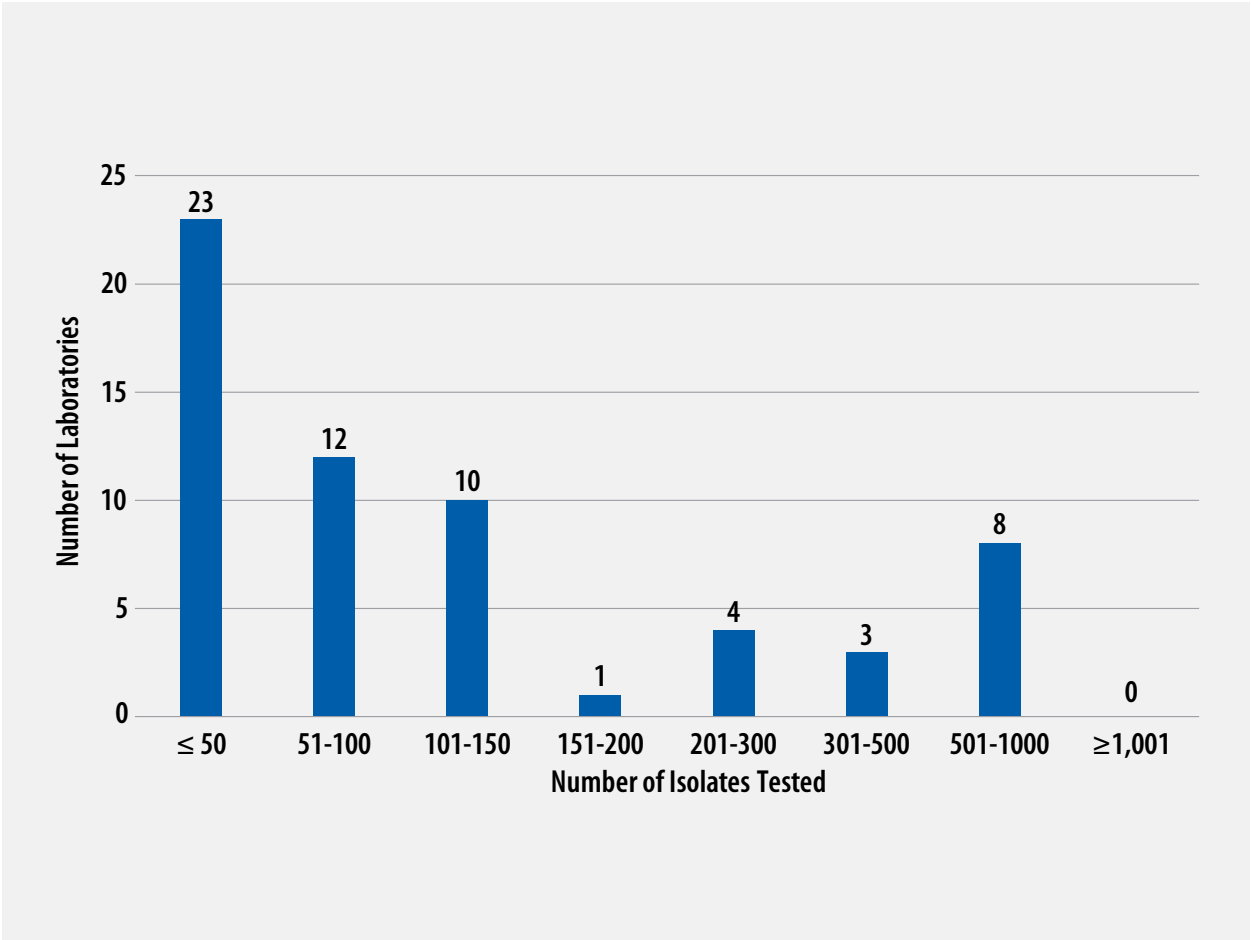
Figure 1. Primary Classification of Participating Laboratories, August 2022



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 61 participants in 2021 (excluding isolates used for quality control) is shown in Figure 2. In 2021, the counts ranged from 0 to 860 tests. Participants at 23 (38%) 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 [9].

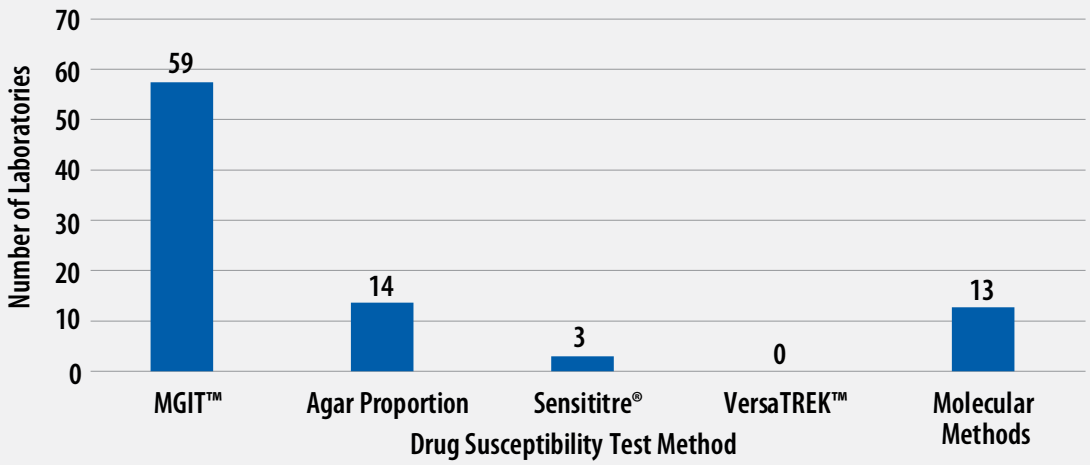
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=61)



MTBC DST Methods Performed by Participants

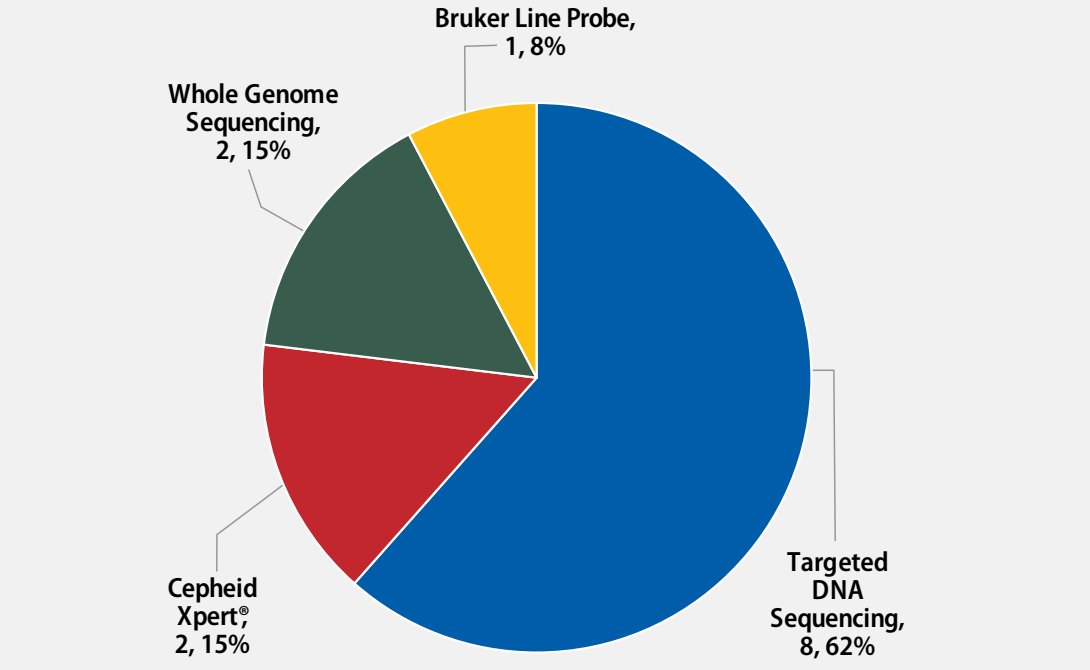
The DST methods that were performed by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Of participating laboratories, 39 (64%) reported results for only one method, 19 (31%) reported two methods, and 3 (5%) noted three susceptibility methods. Fifty-nine (66%) participating laboratories indicated use of MGIT.

Figure 3. MTBC Drug Susceptibility Test Method Performed by Participants (n=89)



Molecular methods reported by participants are shown in Figure 4. The method performed most frequently (62%) was targeted DNA sequencing.

Figure 4. Molecular Method Reported (n=13)

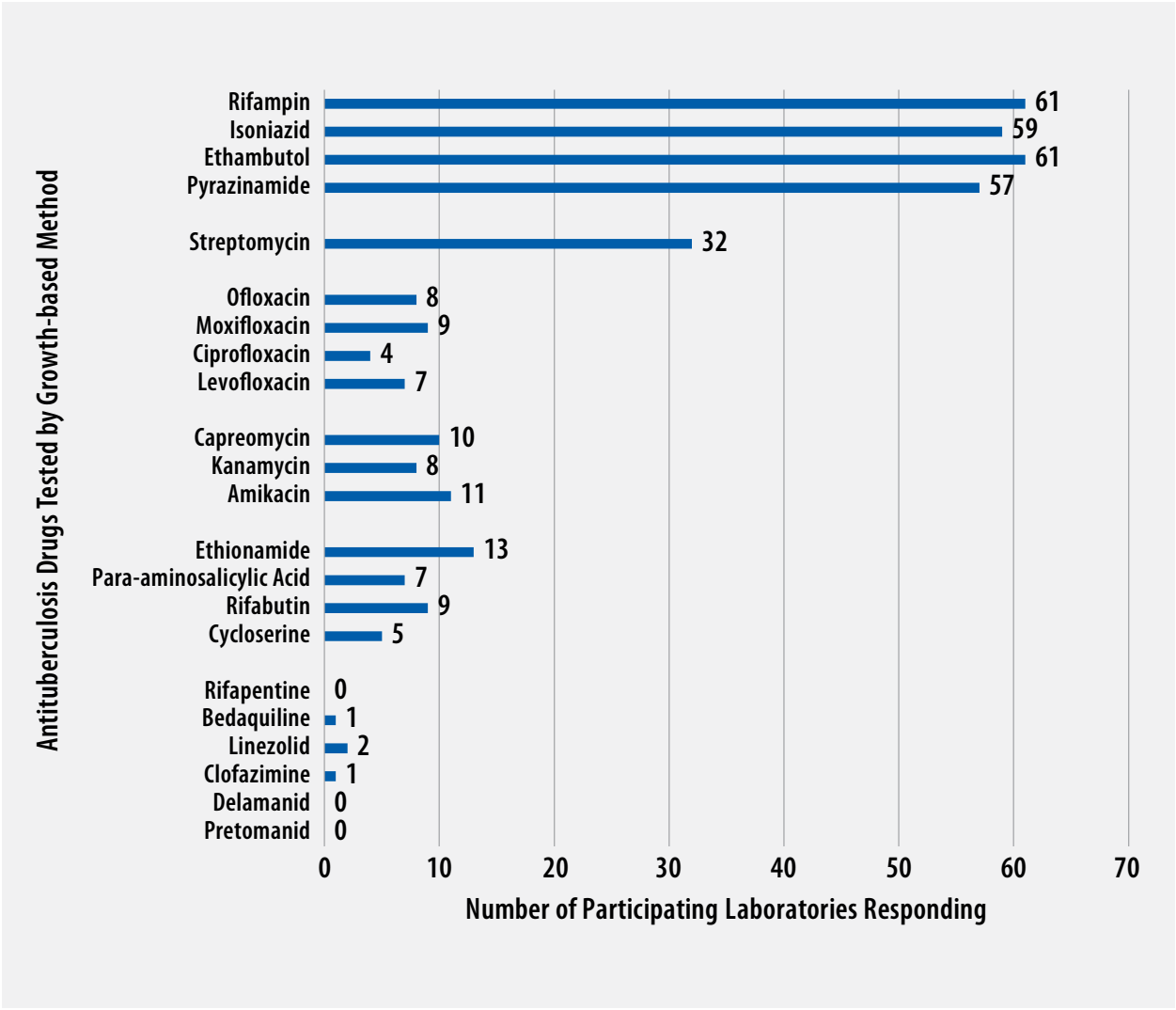


Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the August 2022 survey is presented in Figure 5. CLSI recommends testing a full panel of first-line drugs (rifampin [RMP], isoniazid [INH], *eth*Ambutol [EMB], and pyrazinamide [PZA])[1] because it represents a combination of tests that provides the clinician with comprehensive information related to the 6- or 9-month four-drug RIPE TB treatment regimen used for many patients. Laboratories should consider the addition of fluoroquinolones to their testing panel as CDC recommends susceptibility testing for fluoroquinolones (e.g., moxifloxacin) with use of the alternate 4-month rifapentine-moxifloxacin treatment regimen; RMP may be used as a proxy for rifapentine [10].

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) [11, 12]. Eighteen laboratories reported second-line drug results other than streptomycin. Four (22%) of these laboratories 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 the former classification of XDR TB. Two laboratories tested at least one fluoroquinolone and either bedaquiline or linezolid to define the WHO's revised XDR TB definition.

Figure 5. Antituberculosis Drugs Tested by Growth-based Method by Participants



Isolate 2022F

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

Rifampin

Rifampin (RMP) is a bactericidal drug used as part of a standard first-line regimen for the treatment of TB. RMP's mechanism of action is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [13]. 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 [14]. 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 [13, 14]. 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 [15]. 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[16]. Sequencing of *rpoB* allows 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 2022F revealed a C>T point mutation in codon 433 (Phe433Phe). This mutation does not result in an amino acid change; phenylalanine remains phenylalanine (Phe433Phe). The Phe433Phe 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 2022F, 71 results for RMP were reported. This isolate was reported **susceptible** to RMP by method, as follows:

- **100% (11/11)** of the results when using AP
- **100% (57/57)** of the results when using MGIT™
- **100% (3/3)** of the results when using Sensititre®

Of the 10 molecular results reported for RMP, 8 (80%) laboratories reported detection of a mutation with six laboratories specifically noting the Phe433Phe mutation.

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

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2022F are listed in Tables 3–9.

One laboratory noted contaminated/no growth for Isolate 2022F and did not report results for at least one antituberculosis drug tested.

Table 3. Isolate 2022F—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	11	0	11
Isoniazid—Low	10	0	10
Isoniazid—High	10	0	10
Ethambutol	11	0	11

Table 4. Isolate 2022F—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	57	0	57
Isoniazid—Low	56	1	57
Isoniazid—High	22	0	22
Ethambutol	57	0	57
Pyrazinamide	57	0	57

Table 5. Isolate 2022F—Participant Results for First-Line DST by Sensititre®

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

*One additional laboratory reported susceptible for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

Table 6. Isolate 2022F—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	9	1	10
Ofloxacin	4	0	4
Ciprofloxacin	3	0	3
Moxifloxacin	3	0	3
Levofloxacin	3	0	3
Amikacin	7	0	7
Kanamycin	5	0	5
Capreomycin	7	0	7
Ethionamide	10	0	10
Rifabutin	5	0	5
Cycloserine	4	0	4
p-Aminosalicylic acid	5	0	5
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

Table 7. Isolate 2022F—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	22	2	24
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	4	0	4
Levofloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	0	1	1
Capreomycin	2	0	2
Ethionamide	2	0	2
Rifabutin	2	0	2
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	1	0	1
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 8. Isolate 2022F—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	2	0	2
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	1	0	1
Levofloxacin	0	0	0
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
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

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

Table 9. Isolate 2022F—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	8	2	10
Isoniazid	0	8	8
Ethambutol	0	5	5
Pyrazinamide	0	5	5
Streptomycin	0	3	3
Ofloxacin	0	7	7
Ciprofloxacin	0	7	7
Moxifloxacin	0	6	6
Levofloxacin	0	6	6
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	4	4
Cycloserine	0	1	1
p-Aminosalicylic acid	0	1	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

Isolate 2022G

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

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

Pyrazinamide

Pyrazinamide (PZA) is an important first-line drug for treatment of TB and is used with INH and RIF. The addition of this drug shortens TB treatment from the previous 9–12 months to 6 months because it kills a population of persistent bacilli in acidic pH environments within the lesions that are not killed by other drugs [17]. PZA is a prodrug that requires conversion to its active form, pyrazinoic acid, by the pyrazinamidase encoded by the *pncA* gene of *M. tuberculosis*. PZA-resistant *M. tuberculosis* strains lose pyrazinamidase activity, and resistance to PZA is usually caused by nucleotide changes scattered throughout the *pncA* gene. However, there may be additional mechanisms of resistance to PZA that are still unknown [18].

DNA sequence analysis of *pncA* in Isolate 2022G revealed a C>G point mutation in codon 82 resulting in wild-type histidine being replaced by aspartate (His82Asp). The His82Asp mutation is thought to confer PZA resistance.

Among those performing MGIT, 54 results for PZA were reported for Isolate 2022G. This isolate was reported as **resistant** to PZA by method, as follows:

- **74% (42/54)** of the results when using MGIT™

Of the 5 molecular results reported for PZA, all (100%) laboratories reported detection of a mutation, with 4 specifically noting the His82Asp mutation.

For internal comparison purposes, this isolate was previously sent as MPEP 2019G where 85% (56/66) of MGIT™ results and 0% (0/1) of VersaTREK™ results were reported as resistant.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2022G are listed in Tables 10–16.

Two laboratories noted contaminated/no growth for Isolate 2022G and did not report results for at least one antituberculosis drug tested.

Table 10. Isolate 2022G—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	10	0	10
Isoniazid—Low	9	0	9
Isoniazid—High	9	0	9
Ethambutol	10	0	10

Table 11. Isolate 2022G—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	56	1	57
Isoniazid—Low	57	0	57
Isoniazid—High	22	0	22
Ethambutol	57	0	57
Pyrazinamide	12	42	54*

*One additional laboratory reported intermediate and one additional laboratory reported no interpretation for PZA by MGIT™.

Table 12. Isolate 2022G—Participant Results for First-Line DST by Sensititre®

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

*One additional laboratory reported susceptible for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

Table 13. Isolate 2022G—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	9	0	9
Ofloxacin	4	0	4
Ciprofloxacin	3	0	3
Moxifloxacin	3	0	3
Levofloxacin	3	0	3
Amikacin	7	0	7
Kanamycin	5	0	5
Capreomycin	7	0	7
Ethionamide	9	1	10
Rifabutin	5	0	5
Cycloserine	4	0	4
p-Aminosalicylic acid	5	0	5
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

Table 14. Isolate 2022G—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	24	0	24
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	5	0	5
Levofloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	0	2
Ethionamide	2	0	2
Rifabutin	2	0	2
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	1	0	1
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 15. Isolate 2022G—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	2	0	2
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	1	0	1*
Levofloxacin	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
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

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

Table 16. Isolate 2022G—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	0	10	10
Isoniazid	0	8	8
Ethambutol	0	5	5
Pyrazinamide	5	0	5
Streptomycin	0	3	3
Ofloxacin	0	7	7
Ciprofloxacin	0	7	7
Moxifloxacin	0	6	6
Levofloxacin	0	6	6
Amikacin	0	7	7
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	4	4
Cycloserine	0	1	1
p-Aminosalicylic acid	0	1	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

Isolate 2022H

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

Rifampin

DNA sequence analysis of *rpoB* in Isolate 2022H revealed a T>C point mutation resulting in wild-type leucine being replaced by proline in MTB codon 430 (Leu430Pro). Isolates with Leu430Pro (Leu511Pro in E. coli numbering system) mutations are associated with low-level RMP resistance and can test as susceptible in growth-based assays [19-21].

For Isolate 2022H, 67 results for RMP were reported. This isolate was reported **susceptible** to RMP by method, as follows:

- **100% (9/9)** of the results when using AP
- **98% (54/55)** of the results when using MGIT™
- **100% (3/3)** of the results when using Sensititre®

Of the 10 molecular results reported for RMP, 9 (90%) laboratories reported detection of a mutation in *rpoB*. Seven laboratories specifically noted the Leu430Pro mutation.

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

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2022H are listed in Tables 17–23.

Three laboratories noted contaminated/no growth for Isolate 2022H and did not report results for at least one antituberculosis drug tested.

Table 17. Isolate 2022H—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	9	0	9
Isoniazid—Low	7	1	8
Isoniazid—High	8	0	8
Ethambutol	8	1	9

Table 18. Isolate 2022H—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	54	1	55
Isoniazid—Low	50	4	54
Isoniazid—High	22	1	23
Ethambutol	48	5	53*
Pyrazinamide	55	0	55

* One additional laboratory reported intermediate for EMB by MGIT™.

Table 19. Isolate 2022H—Participant Results for First-Line DST by Sensititre®

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

* One additional laboratory reported Susceptible for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

Table 20. Isolate 2022H—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	8	0	8
Ofloxacin	4	0	4
Ciprofloxacin	2	0	2
Moxifloxacin	3	0	3
Levofloxacin	3	0	3
Amikacin	6	0	6
Kanamycin	4	0	4
Capreomycin	5	1	6
Ethionamide	8	1	9
Rifabutin	4	0	4
Cycloserine	4	0	4
p-Aminosalicylic acid	4	0	4
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

Table 21. Isolate 2022H—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	23	0	23
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	4	0	4
Levofloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	0	2
Ethionamide	2	0	2
Rifabutin	2	0	2
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	1	0	1
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 22. Isolate 2022H—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	2	0	2
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	1	0	1*
Levofloxacin	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
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

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

Table 23. Isolate 2022H—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	9	1	10
Isoniazid	0	8	8
Ethambutol	1	4	5
Pyrazinamide	0	5	5
Streptomycin	0	3	3
Ofloxacin	1*	6	7
Ciprofloxacin	1*	6	7
Moxifloxacin	1*	5	6
Levofloxacin	1*	5	6
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	4	4
Cycloserine	0	1	1
p-Aminosalicylic acid	0	1	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

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

Isolate 2022I

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

Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculosis drug and is a cornerstone of regimens used to treat TB disease and latent TB infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *katG* gene [6, 13]. The target of activated INH is enoyl-acyl-carrier protein reductase (encoded by the *inhA* gene); this binding inhibits cell wall mycolic acid biosynthesis. There are two mechanisms that account for the majority of INH resistance [6, 13, 22]. 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 [23, 24]. 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 [21, 25, 26].

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2022I 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). The C-15T mutation has been associated with low-level INH resistance [21, 27].

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 2022I, 69 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **91% (10/11)** of the results when using AP
- **100% (57/57)** of the results when using MGIT™
- **0% (0/1)** of the results when using Sensititre®

Two (4%) results were reported resistant at the higher concentrations of INH. Only 32 (56%) 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, 7 (88%) laboratories reported detection of a mutation with all specifically noting the *inhA* C-15T mutation.

One of the laboratories performing Sensititre® reported INH MIC value as 0.25 µg/ml (n=1). Another laboratory reported INH MIC value as 0.25 µg/ml (n=1) and noted 'Intermediate'. A third laboratory reported INH MIC value as 0.25 µg/ml (n=1) and noted 'Resistant' but since an interpretation was not indicated for INH—Low and INH—High, the result was excluded from Table 26.

For internal comparison purposes, this isolate was previously sent as MPEP 2018E where 94% (17/18) of AP results, 100% (72/72) of MGIT™ results, 100% (4/4) of Sensititre® results, and 100% (1/1) of VersaTREK™ results were reported as resistant.

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 but sequencing of the promoter region of the *inhA* gene revealed a C>T point mutation at nucleotide position -15 (C-15T). This mutation has been associated with ETA resistance [21, 27].

For Isolate 2022I, 11 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **63% (5/8)** of the results when using AP
- **100% (2/2)** of the results when using MGIT™
- **100% (1/1)** of the results when using Sensititre®

One of the laboratories performing Sensititre® reported ETA MIC value as 20 µg/ml (n=1).

For internal comparison purposes, this isolate was previously sent as MPEP 2018E where 65% (11/17) of AP results, 100% (3/3) of MGIT™ results, and 50% (1/2) of Sensititre® results were reported as resistant.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2022I are listed in Tables 24–30.

One laboratory noted contaminated/no growth for Isolate 2022I and did not report results for at least one antituberculosis drug tested.

Table 24. Isolate 2022I—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	11	0	11
Isoniazid—Low	1	10	11
Isoniazid—High	10	0	10
Ethambutol	11	0	11

Table 25. Isolate 2022I—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	57	0	57
Isoniazid—Low	0	57	57
Isoniazid—High	30	2	32*
Ethambutol	56	1	57
Pyrazinamide	56	1	57

* One additional laboratory reported No Interpretation for RMP by MGIT™.

Table 26. Isolate 2022I—Participant Results for First-Line DST by Sensititre®

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

* One additional laboratory reported resistant for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

† One additional laboratory reported intermediate for INH—High by Sensititre®.

Table 27. Isolate 2022I—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	10	0	10
Ofloxacin	5	0	5
Ciprofloxacin	3	0	3
Moxifloxacin	3	0	3
Levofloxacin	3	0	3
Amikacin	7	0	7
Kanamycin	5	0	5
Capreomycin	6	0	6
Ethionamide	3	5	8*
Rifabutin	4	0	4
Cycloserine	3	0	3
p-Aminosalicylic acid	5	0	5
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

* One additional laboratory reported No Interpretation for ETA by AP.

Table 28. Isolate 2022I—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	23	1	24
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	5	0	5
Levofloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	0	2
Ethionamide	0	2	2
Rifabutin	2	0	2
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	1	0	1
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 29. Isolate 2022I—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	2	0	2
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	1	0	1*
Levofloxacin	0	0	0*
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	1	0	1
Ethionamide	0	1	1
Rifabutin	2	0	2
Cycloserine	0	0	0*
p-Aminosalicylic acid	2	0	2
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

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

Table 30. Isolate 2022I—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	0	10	10
Isoniazid	7	1	8
Ethambutol	0	5	5
Pyrazinamide	1	4	5
Streptomycin	0	3	3
Ofloxacin	1*	6	7
Ciprofloxacin	1*	6	7
Moxifloxacin	1*	5	6
Levofloxacin	1*	5	6
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	3†	1	4
Cycloserine	0	1	1
p-Aminosalicylic acid	0	1	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

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

† These laboratories noted the detection of the *inhA* mutation associated with ETA resistance.

Isolate 2022J

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

Isoniazid

As previously noted, resistance to INH most commonly occurs due to mutations in the *katG* gene or the promoter region of the *inhA* gene; however, mutations in *fabG1* can also cause resistance with the role of mutations in *ahpC* remaining less clear. DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2022J revealed a deletion in the *katG* locus; *inhA*, *fabG1*, and *ahpC* were wild-type (i.e., no mutations were detected).

For Isolate 2022J, 68 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (11/11)** of the results when using AP
- **100% (57/57)** of the results when using MGIT™

Forty-two or 98% of results at the higher concentrations of INH were reported as resistant. Only 32 (56%) 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 7 molecular results reported for INH, 4 (100%) laboratories reported detection of a mutation with all laboratories specifically noting the *katG* deletion/mutation.

Two of the laboratories performing Sensititre® reported INH MIC values as 4 µg/ml (n=2). A third laboratory reported INH MIC value as 4 µg/ml (n=1) and noted 'Resistant' but since an interpretation was not indicated for INH—Low and INH—High, the result was excluded from Table 33.

For internal comparison purposes, this isolate was previously sent as MPEP 2018C where 100% (97/97) of results were reported as resistant.

Ethionamide

As previously noted, resistance to ETA is commonly due to mutations in the *ethA* gene or mutations in *fabG1* or *inhA* resulting in cross-resistance with INH.

DNA sequencing analysis revealed a G>C point mutation in codon 266 of *ethA* gene resulting in wild-type serine being replaced by arginine (Ser266Arg); *inhA* and *fabG1* were wild-type (i.e., no mutations were detected). The Ser266Arg mutation is not associated with resistance.

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

For Isolate 2022J, 13 ETA results were reported. This isolate was reported **susceptible** to ETA by method, as follows:

- **80% (8/10)** of the results when using AP
- **100% (2/2)** of the results when using MGIT™
- **100% (1/1)** of the results when using Sensititre®

Of the 4 molecular results reported for ETA, no (0%) laboratories reported detection of a mutation.

One of the laboratories performing Sensititre® reported an ETA MIC value as 0.6 µg/ml (n=1).

For internal comparison purposes, this isolate was previously sent as MPEP 2018C where 100% (23/23) of results were reported as susceptible.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2022J are listed in Tables 31–37.

Table 31. Isolate 2022J—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	12	0	12
Isoniazid—Low	0	11	11
Isoniazid—High	0	10	10
Ethambutol	12	0	12

Table 32. Isolate 2022J—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	57	0	57
Isoniazid—Low	0	57	57
Isoniazid—High	1	31	32
Ethambutol	57	0	57
Pyrazinamide	57	0	57

Table 33. Isolate 2022J—Participant Results for First-Line DST by Sensititre®

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

*One additional laboratory reported resistant for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

Table 34. Isolate 2022J—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	11	0	11
Ofloxacin	6	0	6
Ciprofloxacin	4	0	4
Moxifloxacin	3	0	3
Levofloxacin	3	0	3
Amikacin	7	0	7
Kanamycin	5	1	6
Capreomycin	6	1	7
Ethionamide	8	2	10
Rifabutin	4	1	5
Cycloserine	3	1	4
p-Aminosalicylic acid	5	0	5
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

Table 35. Isolate 2022J—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	23	1	23
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	5	0	5
Levofloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	0	2
Ethionamide	2	0	2
Rifabutin	2	0	2
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	1	0	1
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 36. Isolate 2022J—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	2	0	2
Ofloxacin	1	0	1
Ciprofloxacin	0	0	0
Moxifloxacin	2	0	2
Levofloxacin	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
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

Table 37. Isolate 2022J—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	0	10	10
Isoniazid	4	3	7*
Ethambutol	0	5	5
Pyrazinamide	0	5	5
Streptomycin	0	3	3
Ofloxacin	1†	6	7
Ciprofloxacin	1†	6	7
Moxifloxacin	1†	5	6
Levofloxacin	1†	5	6
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	4	4
Cycloserine	0	1	1
p-Aminosalicylic acid	1	0	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

* One additional laboratory reported 'no result' for INH.

† This laboratory noted the detection of a mutation not associated with FQ 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, 29].

Second-line Drugs	7H10 agar	7H11 agar
Streptomycin	2.0	2.0
Levofloxacin	1.0	Not determined*
Moxifloxacin	0.5	0.5
Amikacin	4.0†	Not determined*
Capreomycin	10.0†	10.0¥
Kanamycin	5.0†	6.0¥
Ethionamide	5.0	10.0
Rifabutin	0.5	0.5
p-Aminosalicylic acid	2.0¥	8.0¥
Rifapentine	Not determined*	Not determined*
Bedaquiline	Not determined*	0.25‡
Linezolid	1.0‡	1.0‡
Clofazimine	Not determined*	Not determined*
Delamanid	Not determined*	0.016‡
Pretomanid	Not determined*	Not determined*

NOTE—Critical concentrations as indicated in CLSI M24-A2 document [1]

* Breakpoints for establishing susceptibility have not been 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].

‡ Critical concentrations as indicated in WHO 2018 Technical Report on critical concentrations [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 [29].

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†
Rifapentine	Not determined
Bedaquiline	1.0
Linezolid	1.0
Clofazimine	1.0
Delamanid	0.06
Pretomanid	Not determined

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 2023 [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

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For more information please contact
Centers for Disease Control and Prevention
1600 Clifton Road NE, Atlanta, GA 33029-4027
Telephone: 1-800-CDC-INFO (232-4636)
MPEP Telephone: 404-639-4013
MPEP Email: TBMPEP@cdc.gov
MPEP Web: www.cdc.gov/tb/topic/laboratory/mpep/default.htm
Publication date: November 2022