

Centers for Disease Control and Prevention
Model Performance Evaluation Program

***Mycobacterium tuberculosis* Complex Drug Susceptibility Testing Program**

Report of Results
November 2015
Performance Evaluation Survey

National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
Division of Tuberculosis Elimination



***Mycobacterium tuberculosis* Complex Drug Susceptibility Testing Report for November 2015 Survey**

Purpose The purpose of this report is to present the results of the U.S. Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for *Mycobacterium tuberculosis* complex drug susceptibility testing survey sent to participants in November 2015.

Report Content The material in this report was developed and prepared by

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

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

Contact Information Comments and inquiries regarding this report should be directed to
TBMPEP@cdc.gov
404-639-4013

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services.

Table of Contents

Mycobacterium tuberculosis Complex Drug Susceptibility Testing MPEP Report for November 2015 Survey

Introduction: Overview of MPEP Final Report	4
Expected Susceptibility Testing Results	4
Abbreviations and Acronyms	5
Technical Notes	6
Descriptive Information about Participating Laboratories	
Primary Classification	7
Annual Number of <i>M. tuberculosis</i> Complex Drug Susceptibility Tests Performed	8
<i>M. tuberculosis</i> Complex Drug Susceptibility Testing Methods Used	9
Antituberculous Drugs Tested by Participants	10
Detailed Information for Each Isolate	
Isolate 2015F	11
Isolate 2015G	15
Isolate 2015H	18
Isolate 2015I	21
Isolate 2015J	23
Equivalent Critical Concentrations	25
References	26

Introduction: Overview of MPEP Final Report

The Model Performance Evaluation Program (MPEP) is an educational self-assessment tool in which five isolates of *M. tuberculosis* complex (MTBC) are sent to participating laboratories biannually for staff to monitor their ability to determine drug resistance among the isolates. It is not a formal, graded proficiency testing program. This report includes results for a subset of laboratories performing drug susceptibility 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 approved standards, participants should refer to consensus documents published by the Clinical and Laboratory Standards Institute (CLSI), “Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard,” M24-A2 [1].

Expected Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in November 2015 are shown in the tables below. Although CDC recommends broth-based methods for routine first-line DST of MTBC isolates, Table 1 provides the results obtained by the reference agar proportion method, except for pyrazinamide, in which MGIT was performed. Table 2 provides molecular results obtained by using DNA sequencing [2].

Table 1. Expected Growth-based Results for November 2015 Survey

Growth-based Results					
	First-Line Drugs				Second-Line Drugs
	RMP	INH	EMB	PZA	Resistant to:
2015F	S	R	R	S	STR, ETA
2015G	S	S	S	S	OFL
2015H	R	S	S	S	
2015I	S	S	S	S	AMK, KAN, CAP
2015J	S	S	S	S	OFL

Note—S=susceptible, R=resistant

Table 2. Expected Molecular Results for November 2015 Survey

Mutations Detected in Loci Associated with Resistance							
	<i>rpoB</i>	<i>katG</i>	<i>inhA</i>	<i>embB</i>	<i>pncA</i>	<i>gyrA</i>	<i>rrs</i>
2015F		Ser315Thr	C-15T	Met306Ile			
2015G					Ser65Ser	Ala90Val	
2015H	His526Tyr						
2015I							A1401G
2015J					Ser65Ser	Ser91Pro & Asp94Asn	

Abbreviations and Acronyms

AMK	amikacin
AP	agar proportion — performed on Middlebrook 7H10 or 7H11
bp	base pair
CAP	capreomycin
CDC	U.S. Centers for Disease Control and Prevention
CIP	ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CYS	cycloserine
DNA	deoxyribonucleic acid
DST	drug susceptibility testing
EMB	ethambutol
ETA	ethionamide
HMO	Health Maintenance Organization
INH	isoniazid
KAN	kanamycin
LEV	levofloxacin
MDR	multidrug resistant
MGIT	BACTEC MGIT 960 – Mycobacteria Growth Indicator Tube
MIC	minimum inhibitory concentration
MOX	moxifloxacin
MPEP	Model Performance Evaluation Program
MTBC	<i>Mycobacterium tuberculosis</i> complex
PAS	<i>p</i> -aminosalicylic acid
PZA	pyrazinamide
OFL	ofloxacin
R	resistant
RBT	rifabutin
RMP	rifampin
RNA	ribonucleic acid
S	susceptible
Sensititre	Thermo Scientific Sensititre <i>Mycobacterium tuberculosis</i> MIC plate
STR	streptomycin
TB	tuberculosis
VersaTREK	Thermo Scientific VersaTREK Myco susceptibility
XDR	extensively drug resistant

Technical Notes

The following information pertains to all of the tables and figures for the 2015 MPEP MTBC isolates F, G, H, I, and J in this report.

- The source of data in all tables and figures is the November 2015 MPEP MTBC DST survey.
- The tables indicate the number of reported results (S represents susceptible and R represents resistant) for each drug.
- 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 82 (the number of participating laboratories). This report contains all results reported by participating laboratories.
- Critical concentrations of antituberculosis drugs used for each DST method are listed at the end of this report.
- The Trek Sensititre system allows determination of a minimum inhibitory concentration (MIC) for each drug in the panel. Laboratories using this method must establish breakpoints to provide a categorical interpretation of S or R.
- Of the 31 laboratories reporting second-line drug results (with the exception of streptomycin), nine (29%) tested all three second-line injectable drugs and at least one fluoroquinolone needed to confidently define XDR TB. The second-line injectable drugs are amikacin, kanamycin, and capreomycin. Fluoroquinolones include ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin.

Descriptive Information about Participant Laboratories

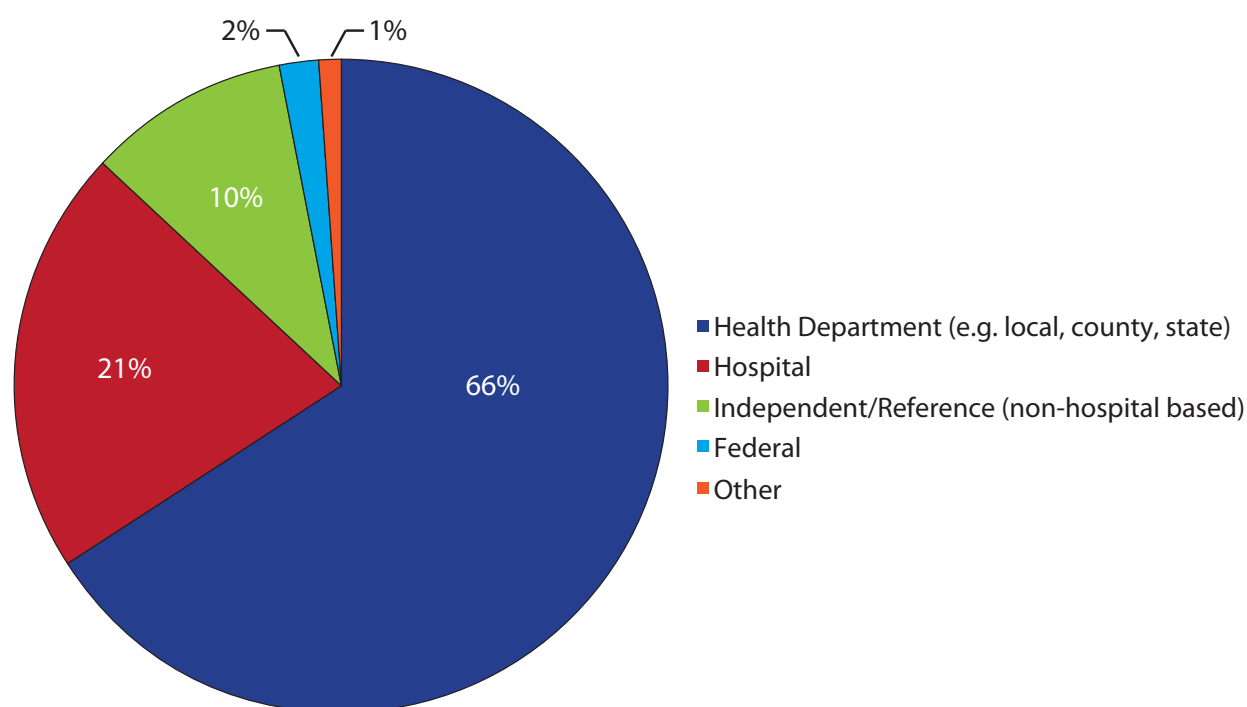
Primary Classification

This report contains DST results submitted to CDC by survey participants at 82 laboratories in 37 states.

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

- 54 (66%): Health department laboratory (e.g., local, county, state)
- 17 (21%): Hospital laboratory
- 8 (10%): Independent/Reference laboratory (non-hospital based)
- 2 (2%): Federal government laboratory
- 1 (1%): Other (quality control manufacturer)

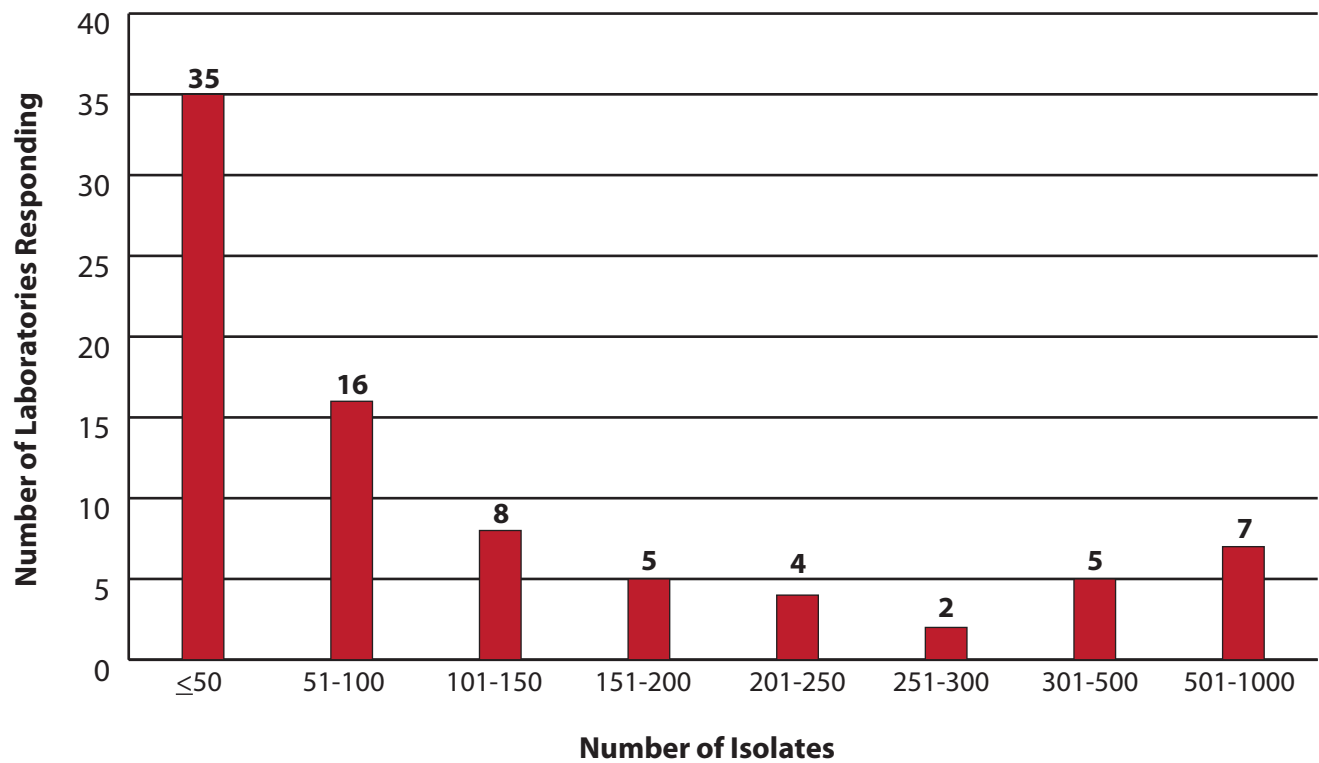
Figure 1. Primary Classification of Participating Laboratories, November 2015



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 82 participants in 2014 (excluding isolates used for quality control) is shown in Figure 2. In 2014, the counts ranged from 0 to 864 tests and participants at 35 (43%) laboratories reported testing 50 or fewer DST isolates per year. Laboratories with low MTBC DST volumes are encouraged to consider referral of testing because of concerns about maintaining proficiency [3].

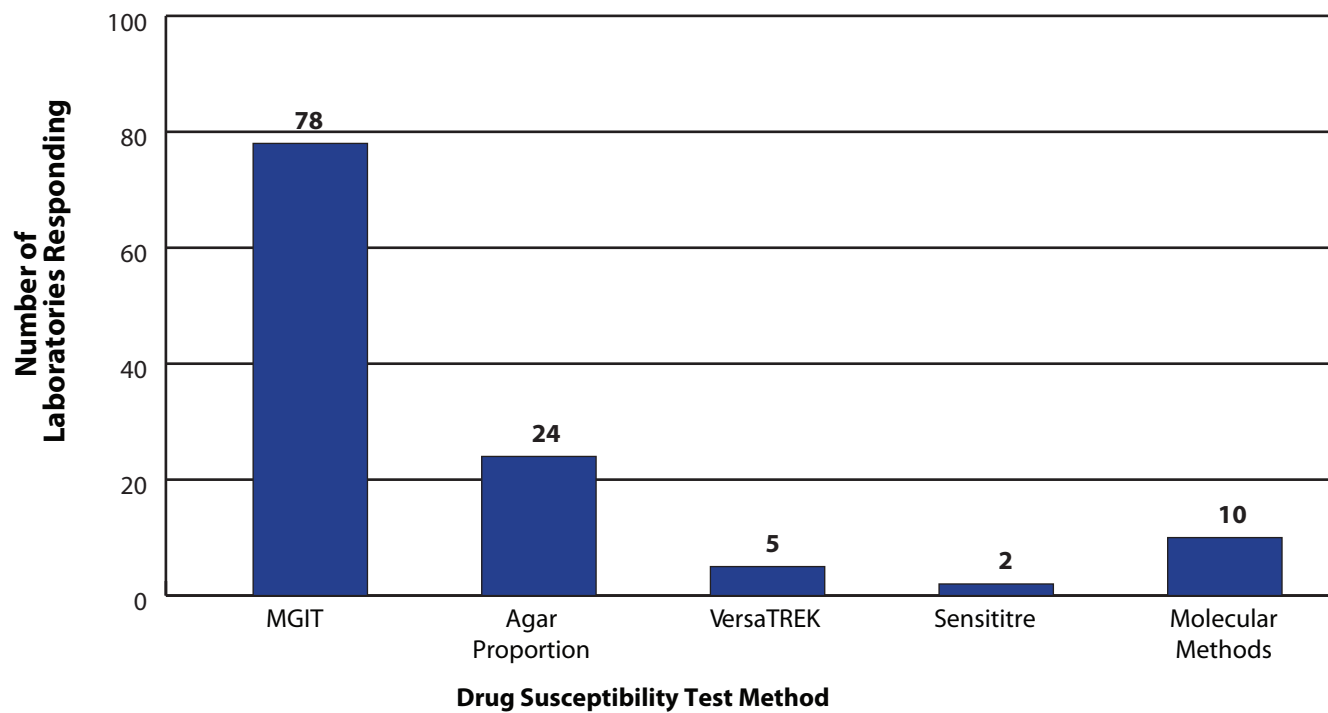
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=82)



MTBC DST Methods Used by Participants

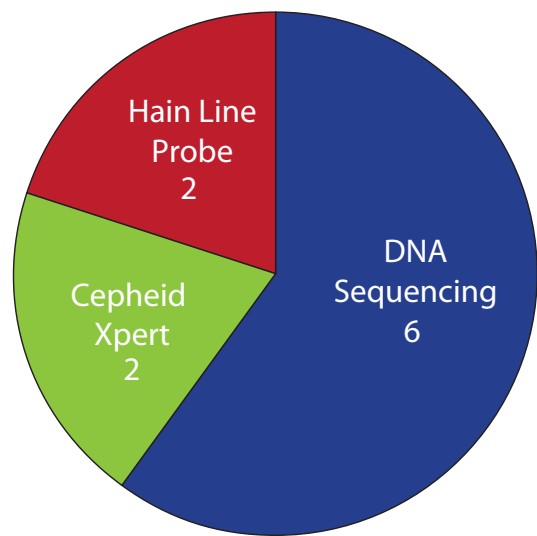
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 49 (60%) laboratories reported results for only one method, 29 laboratories reported two methods, and four laboratories noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=119)



Molecular methods reported by participants are shown in Figure 4. The method used most frequently by laboratories was DNA sequencing (60%), including pyrosequencing and Sanger sequencing. Two laboratories reported results for the Cepheid Xpert MTB/RIF assay and two reported use of the line probe assays Genotype MTBDR*plus* and MTBDR*s*/ by Hain Lifescience.

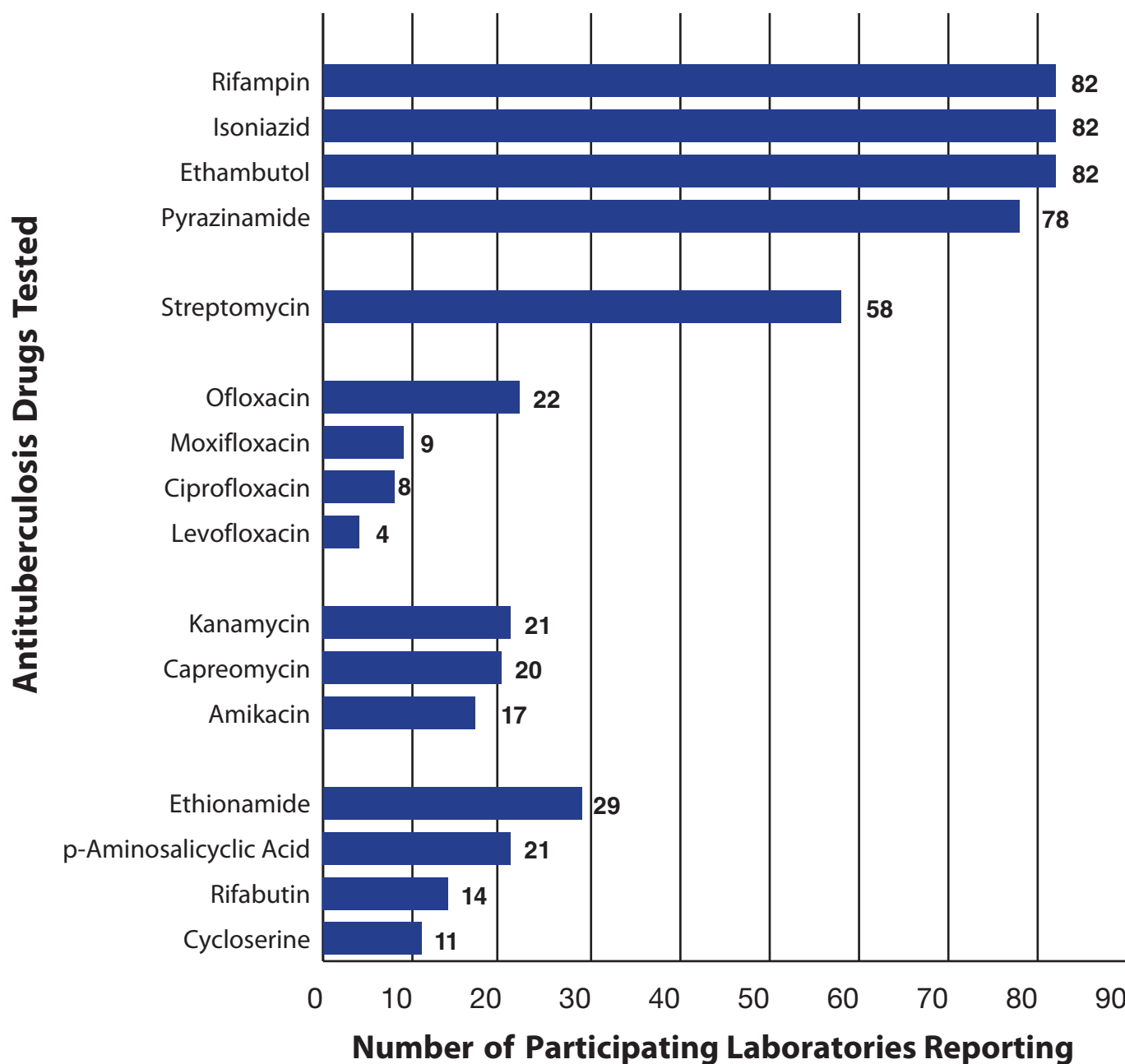
Figure 4. Molecular Method Reported (n=10)



Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in November 2015 is shown in Figure 5. CLSI recommends testing a full panel of first-line drugs (rifampin [RMP], isoniazid [INH], ethambutol [EMB], and pyrazinamide [PZA])[1], because it represents a combination of tests that provides the clinician with comprehensive information related to the four-drug antituberculosis therapy currently recommended for most patients in the United States. All participants reported results for three of the first-line drugs—RMP, INH, and EMB—and 78 (95%) of the participants also reported results for PZA. There has been a slight increase in the number of laboratories testing second-line drugs since the May 2014 survey. The number of laboratories performing Sensititre, which includes second-line drugs, has also increased; however, the overall increase in second-line testing cannot only be attributable to use of this test.

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2015F

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml, EMB at 5.0 µg/ml, STR at 2.0 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 tuberculosis (TB) disease and latent infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *katG* gene [2, 4]. The target of activated INH is enoyl-acyl-carrier protein reductase (encoded by the *inhA* gene); this binding inhibits cell wall mycolic acid biosynthesis. There are two mechanisms that account for the majority of INH resistance [2, 4, 5]. The most common mechanism, mutations in *katG*, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inhA* gene, which are generally associated with low-level resistance to INH and are less frequent than *katG* mutations. Approximately 10–15% of isolates found to be INH resistant have no mutations detected in either of these loci. Numerous loci have been investigated to identify additional genes correlated with INH resistance. The *fabG1* (also known as *mabA*) gene, like *inhA*, is involved in mycolic acid biosynthesis and at least one mutation in this region has been associated with low-level INH resistance [6, 7]. In MTBC, *ahpC* codes for an alkyl hydroperoxide reductase that is associated with resistance to reactive oxygen and reactive nitrogen intermediates; consequently it was initially believed that mutations in the promoter region could be surrogate markers for INH resistance [4].

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2015F revealed a T>A point mutation at codon 315 in the *katG* locus resulting in wild-type serine being replaced by threonine (Ser315Thr). Sequencing also revealed a C>T point mutation at nucleotide position -15 of the promoter region of the *inhA* gene (C-15T). *fabG1* and *ahpC* were wild-type (i.e., no mutations were detected).

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

For Isolate 2015F, 105 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- 100% (23/23) of the results when using AP
- 100% (75/75) of the results when using MGIT
- 100% (5/5) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Sixty-nine (100%) results were reported as **resistant** at the higher concentrations of INH.

Of the seven molecular results reported for INH, all (100%) detected a mutation; four of these laboratories noted mutations in both *katG* and *inhA*.

Ethambutol

Ethambutol (EMB) is an important first-line drug for the treatment of TB and is used in combination with INH, RMP, and PZA to prevent emergence of drug resistance. EMB is a bacteriostatic agent that is active against growing bacilli and has no effect on non-replicating bacilli [4, 5]. EMB targets the arabinosyl transferases (*embCAB* operon), thereby inhibiting the biosynthesis of the cell wall components arabinogalactan and lipoarabinomannan [8].

Sequence analysis of EMB-resistant clinical isolates has shown that EMB resistance is associated primarily with missense (non-synonymous) mutations within the EMB resistance determining region of the gene *embB* at codons 306, 406, and 497 [2, 8]. False susceptibility with some growth-based methods for EMB have been reported [9, 10].

DNA sequence analysis of *embB* of Isolate 2015F revealed a G>A point mutation at codon 306 in the *embB* gene resulting in wild-type methionine being replaced by isoleucine (Met306Ile).

For Isolate 2015F, 102 EMB results were reported. This isolate was reported **resistant** to EMB by method, as follows:

- 83% (20/24) of the results when using AP
- 43% (31/72) of the results when using MGIT
- 75% (3/4) of the results when using Sensititre
- 50% (1/2) of the results when using VersaTREK

All three (100%) of the laboratories reporting molecular results for EMB detected a mutation.

Streptomycin

Streptomycin (STR) belongs to the aminoglycoside class of drugs and its primary mechanism of action is to inhibit protein synthesis by preventing the initiation of translation by binding to the 16s rRNA[4, 5]. In MTBC, the genetic basis of the majority of resistance to STR is usually due to mutations in *rrs* or *rpsL*[5, 11]. CLSI recommended testing STR as a second-line drug based on American Thoracic Society's categorization of STR as a second-line drug for treatment due to increased resistance in many parts of the world [1, 12].

Among three methods, 73 results for STR were reported for Isolate 2015F. This isolate was reported as **resistant** to STR by method, as follows:

- 100% (23/23) of the results when using AP
- 100% (46/46) of the results when using MGIT
- 100% (4/4) of the results when using Sensititre

Ethionamide

Ethionamide (ETA) is a structural analog of INH. ETA, like INH, targets *inhA*, an enzyme involved in mycolic acid biosynthesis [13]. 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. Mutations in *ethA* also confer resistance to ETA, without concomitant resistance to INH [13].

As noted above, a C>T point mutation was detected in the *inhA* gene (C-15T) for Isolate 2015F.

For Isolate 2015F, 29 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- 73% (16/22) of the results when using AP
- 100% (3/3) of the results when using MGIT
- 75% (3/4) of the results when using Sensititre

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2015F are listed in Tables, 3, 4, and 5.

Table 3. Isolate 2015F—Participant Results for First-Line DST

Results by Method for First-Line Drugs												
Drug	AP			MGIT			Sensititre			VersaTREK		
	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	23	0	23	75	0	75	5	0	5	2	0	2
Isoniazid-Low	0	23	23	0	75	75	0	5	5	0	2	2
Isoniazid-High	0	23	23	0	39	39	0	5	5	0	2	2
Ethambutol	4	20	24	41	31	72*	1	3	4	1	1	2
Pyrazinamide				77	0	77				1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for EMB by MGIT.

Table 4. Isolate 2015F—Participant Results for First-Line DST

Results by Method for Second-Line Drugs									
Drug	AP			MGIT			Sensititre		
	S	R	Total	S	R	Total	S	R	Total
Streptomycin	0	23	23	0	46	46	0	4	4
Ofloxacin	17	0	17	3	0	3	3	0	3
Ciprofloxacin	9	0	9						
Levofloxacin	2	0	2	1	0	1	1	0	1
Moxifloxacin	4	0	4	2	0	2	3	0	3
Amikacin	12	0	12	2	0	2	4	0	4
Kanamycin	18	1	19				3	0	3
Capreomycin	15	1	16	3	0	3	1	0	1
Ethionamide	6	16	22	0	3	3	1	3	4
Rifabutin	9	0	9	1	0	1	4	0	4
Cycloserine	9	0	9				2	0	2
<i>p</i>-Aminosalicylic acid	17	1	18				3	0	3

Note—S=susceptible, R=resistant

Table 5. Isolate 2015F—Participant Results for Molecular Testing

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

* Four laboratories noted that mutations were detected in both katG and inhA.

Isolate 2015G

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

Ofloxacin

Fluoroquinolones (FQ) are one of the most commonly prescribed classes of antibiotic in the United States due to their activity against various types of bacteria. They are an important class of drugs used to treat TB resistant to first-line drugs but also have the potential to become an important part of new TB regimens [14]. In the United States, resistance to FQ is relatively uncommon in strains of MTBC susceptible to first-line drugs, however prolonged treatment with a FQ (>10 days) before a diagnosis of TB is associated with a higher risk for FQ resistance and diagnostic delays [14, 15]. The primary mechanism of action of FQ is the inhibition of DNA synthesis [11] by inhibiting DNA gyrase. The enzyme DNA gyrase generates the activity for cleaving and resealing double-stranded DNA. This action is necessary for DNA replication, transcription, and recombination.

Resistance to FQ has mainly been attributed to point mutations in a 21-bp region of the MTBC *gyrA* gene, often called the quinolone resistance determining region (QRDR). These mutations, commonly occurring at codons 90, 91, and 94, prevent the drugs from effectively binding DNA gyrase [2, 5, 11]. 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 [11].

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 loci 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 class of drugs.

As newer FQ are assessed for use as antituberculosis drugs, it is important to determine cross-resistance between these and older FQ that are tested in growth-based DST methods. Studies suggest that there may not be full cross-resistance between ofloxacin (OFL), ciprofloxacin (CIP), levofloxacin (LVX), and moxifloxacin (MOX) at the defined critical concentrations and that low- and high-level resistance, as seen with INH, may be applicable to FQ as well, particularly MOX [18, 19].

DNA sequence of *gyrA* in Isolate 2015G revealed a C>T point mutation in codon 90 of *gyrA* resulting in wild-type alanine being replaced with valine (Ala90Val). Sequencing of *gyrB* was wild-type (i.e., no mutations were detected).

Among three methods, 22 results for OFL were reported for Isolate 2015G. This isolate was reported as **resistant** to OFL by method, as follows:

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

Participating laboratories also reported results for other FQ drugs (i.e., CIP, LVF, and MOX) for Isolate 2015G; 100% (19/19) of results noted resistance to these additional FQ.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2015G are listed in Tables 6, 7, and 8.

Table 6. Isolate 2015G—Participant Results for First-Line DST

Results by Method for First-Line Drugs												
Drug	AP			MGIT			Sensitre			VersaTREK		
	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	22	0	22	75	0	75	5	0	5	2	0	2
Isoniazid–Low	22	0	22	75	0	75	4	1	5	2	0	2
Isoniazid–High	22	0	22	24	0	24	4	1	5	2	0	2
Ethambutol	22	0	22	74	1	75	5	0	5	2	0	2
Pyrazinamide				77	0	77				1	0	1

Note—S=susceptible, R=resistant

Table 7. Isolate 2015G—Participant Results for Second-Line DST

Results by Method for Second-Line Drugs									
Drug	AP			MGIT			Sensitre		
	S	R	Total	S	R	Total	S	R	Total
Streptomycin	22	0	22	46	0	46	4	0	4
Ofloxacin	0	16	16	0	3	3	0	3	3
Ciprofloxacin	0	8	8						
Levofloxacin	0	2	2	0	1	1			
Moxifloxacin	0	3	3*	0	2	2	0	3	3
Amikacin	12	0	12	2	0	2	4	0	4
Kanamycin	17	1	18				3	0	3
Capreomycin	15	1	16	3	0	3			
Ethionamide	22	0	22	3	0	3	3	1	4
Rifabutin	9	0	9	1	0	1	4	0	4
Cycloserine	8	1	9				2	0	2
p-Aminosalicylic acid	17	0	17				3	0	3

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for MOX by AP.

Table 8. Isolate 2015G—Participant Results for Molecular Testing

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

* One laboratory noted the mutation detected was a silent mutation for PZA.

Isolate 2015H

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

Rifampin

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

CDC has recommended that RMP resistance detected by the Xpert MTB/RIF assay should be confirmed by DNA sequencing of *rpoB* [20]. 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 [21]. Sequencing of *rpoB* will allow for clarification of the result and understanding of possible discordance between the rapid molecular and the growth-based testing results.

DNA sequence analysis of *rpoB* in Isolate 2015H revealed a C>G point mutation in codon 526 resulting in wild-type histidine being replaced by tyrosine (His526Tyr). Isolates with His526Tyr mutations consistently test resistant to RMP in growth-based assays.

Among four methods, 103 results for RMP were reported for Isolate 2015H. This isolate was reported as **resistant** to RMP by method, as follows:

- 100% (22/22) of the results when using AP
- 100% (74/74) of the results when using MGIT
- 100% (5/5) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

All ten (100%) of the molecular results reported for RMP noted that a mutation was detected.

Pyrazinamide

Pyrazinamide (PZA) is an important first-line drug for treatment of TB and is used with INH and RMP. 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. PZA-resistant MTBC 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 [22], but issues with false resistance to PZA have been reported as well [23] and remain a potential concern. For Isolate 2015H, DNA sequencing of the *pncA* gene did not reveal a mutation.

Isolate 2015H was expected to be susceptible to PZA. However, of those testing PZA, **resistance** was reported by:

- 53% (41/77) of the results when using MGIT
- 0% (0/1) of the results when using VersaTREK

Two laboratories reported results for molecular methods; neither detected a mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2015H are listed in Tables 9, 10, and 11.

Table 9. Isolate 2015H—Participant Results for First-Line DST

Results by Method for First-Line Drugs												
Drug	AP			MGIT			Sensititre			VersaTREK		
	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	0	22	22*	0	74	74	0	5	5	0	2	2
Isoniazid–Low	22	0	22*	75	0	75	5	0	5	2	0	2
Isoniazid–High	22	0	22*	24	0	24	5	0	5	2	0	2
Ethambutol	23	0	23*	74	1	75	5	0	5	2	0	2
Pyrazinamide				36	41	77				1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for RMP, INH, and EMB by AP.

Table 10. Isolate 2015H—Participant Results for Second-Line DST

Results by Method for Second-Line Drugs									
Drug	AP			MGIT			Sensititre		
	S	R	Total	S	R	Total	S	R	Total
Streptomycin	21	1	22*	46	0	46	4	0	4
Ofloxacin	16	0	16*	3	0	3	3	0	3
Ciprofloxacin	8	0	8*						
Levofloxacin	2	0	2	1	0	1	1	0	1
Moxifloxacin	4	0	4	2	0	2	3	0	3
Amikacin	11	0	11*	2	0	2	4	0	4
Kanamycin	17	1	18*				3	0	3
Capreomycin	14	1	15*	3	0	3	1	0	1
Ethionamide	22	0	22*	3	0	3	4	0	4
Rifabutin	1	7	8*	0	1	1	0	4	4
Cycloserine	8	1	9				2	0	2
p-Aminosalicylic acid	17	0	17*				4	0	4

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for STR, OFL, CIP, AMK, KAN, CAP, ETA, RBT, and PAS by AP.

Table 11. Isolate 2015H—Participant Results for Molecular Testing

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

Isolate 2015I

Expected Result: Resistant to AMK at 4.0 µg/ml, CAP at 10.0 µg/ml, and KAN at 5.0 µg/ml by agar proportion

Second-line injectable drugs

The second-line injectable drugs include a cyclic-peptide antibiotic, capreomycin (CAP), and two aminoglycoside antibiotics, kanamycin (KAN) and amikacin (AMK). All three drugs inhibit protein synthesis and the primary mechanisms of resistance occur due to mutations in the genes as follows: *rrs* for AMK; *rrs* and *eis* for KAN; and *rrs* and *tlyA* for CAP [11]. Since these drugs share a molecular target and bind at similar locations, cross-resistance has frequently been detected [2, 24]. The most common mechanism of cross-resistance to all three drugs is due to an A1401G point mutation in the *rrs* gene coding for 16S rRNA [24].

Isolate 2015I was resistant to all of the second-line injectable drugs (AMK, KAN, and CAP) by the AP method and DNA sequence analysis of the *rrs* gene revealed an A1401G mutation.

For Isolate 2015I, 54 results were reported for AMK, KAN, and CAP. The isolate was reported **resistant** to the three second-line injectables by method, as follows:

- 91% (39/43) of the results when using AP
- 100% (5/5) of the results when using MGIT
- 100% (6/6) of the results when using Sensititre

This A1401G mutation in the *rrs* gene was detected by the three laboratories that reported molecular testing for the second-line injectable drugs.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2015I are listed in Tables 12, 13, and 14.

Table 12. Isolate 2015I—Participant Results for First-Line DST

Results by Method for First-Line Drugs												
Drug	AP			MGIT			Sensititre			VersaTREK		
	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	21	0	21*	75	0	75	5	0	5	2	0	2
Isoniazid–Low	21	0	21*	75	0	75	5	0	5	2	0	2
Isoniazid–High	21	0	21*	24	0	24	5	0	5	2	0	2
Ethambutol	22	0	22*	74	1	75	5	0	5	2	0	2
Pyrazinamide				75	2	77				1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for RMP, INH, and EMB by AP.

Table 13. Isolate 2015I—Participant Results for Second-Line DST

Results by Method for Second-Line Drugs									
Drug	AP			MGIT			Sensititre		
	S	R	Total	S	R	Total	S	R	Total
Streptomycin	20	1	21*	45	1	46	4	0	4
Ofloxacin	15	0	15*	3	0	3	3	0	3
Ciprofloxacin	7	0	7*						
Levofloxacin	2	0	2	1	0	1	1	0	1
Moxifloxacin	4	0	4	2	0	2	3	0	3
Amikacin	1	10	11*	0	2	2	0	3	3
Kanamycin	2	15	17*				0	3	3
Capreomycin	1	14	15*	0	3	3			
Ethionamide	21	0	21*	3	0	3	4	0	4
Rifabutin	8	0	8*	1	0	1	4	0	4
Cycloserine	8	1	9				2	0	2
p-Aminosalicylic acid	16	0	16*				4	0	4

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for STR, OFL, CIP, AMK, KAN, CAP, ETA, RBT, and PAS by AP.

Table 14. Isolate 2015I—Participant Results for Molecular Testing

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

Isolate 2015J

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

Ofloxacin

DNA sequence of *gyrA* in Isolate 2015J 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 [2, 25]. DNA sequencing of *gyrA* also revealed a G>A point mutation in codon 94 resulting in wild-type aspartate being replaced with asparagine (Asp94Asn). Sequencing of *gyrB* was wild-type (i.e., no mutations were detected).

Among three methods, 22 results for OFL were reported for Isolate 2015J. This isolate was reported as resistant to OFL by method, as follows:

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

Participating laboratories also reported results for other FQ drugs (i.e., CIP, LEV, and MOX) for Isolate 2015J; 100% (19/19) of results noted resistance to these additional FQ.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2015J are listed in Tables 15, 16, and 17.

Table 15. Isolate 2015J—Participant Results for First-Line DST

Drug	Results by Method for First-Line Drugs											
	AP			MGIT			Sensititre			VersaTREK		
	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	22	0	22	75	0	75	5	0	5	2	0	2
Isoniazid–Low	22	0	22	75	0	75	5	0	5	2	0	2
Isoniazid–High	22	0	22	24	1	25	5	0	5	2	0	2
Ethambutol	23	0	23	74	1	75	5	0	5	2	0	2
Pyrazinamide				77	0	77				1	0	1

Note—S=susceptible, R=resistant

Table 16. Isolate 2015J—Participant Results for Second-Line DST

Results by Method for Second-Line Drugs									
Drug	AP			MGIT			Sensititre		
	S	R	Total	S	R	Total	S	R	Total
Streptomycin	22	0	22	46	0	46	4	0	4
Ofloxacin	0	16	16	0	3	3	0	3	3
Ciprofloxacin	0	8	8						
Levofloxacin	0	2	2	0	1	1			
Moxifloxacin	0	3	3*	0	2	2	0	3	3
Amikacin	12	0	12	2	0	2	4	0	4
Kanamycin	17	1	18				3	0	3
Capreomycin	15	1	16	3	0	3	1	0	1
Ethionamide	22	0	22	3	0	3	4	0	4
Rifabutin	9	0	9	1	0	1	4	0	4
Cycloserine	8	1	9				2	0	2
p-Aminosalicylic acid	17	0	17				4	0	4

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for MOX by AP.

Table 17. Isolate 2015J—Participant Results for Molecular Testing

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

* One laboratory noted the mutation detected was a silent mutation for PZA

Equivalent Critical Concentrations

(Concentrations listed as µg/ml)

Agar Proportion

	7H10 agar	7H11 agar
First-line Drugs		
Isoniazid	0.2 and 1.0*	0.2 and 1.0*
Rifampin	1.0	1.0
Ethambutol	5.0 and 10.0*	7.5
Pyrazinamide	Not recommended	Not recommended
Second-line Drugs		
Streptomycin	2.0 and 10.0	2.0 and 10.0
Amikacin	4.0	-†
Capreomycin	10.0	10.0
Kanamycin	5.0	6.0
Levofloxacin	1.0	-†
Moxifloxacin	0.5	0.5
Ofloxacin	2.0	2.0
Ethionamide	5.0	10.0
Rifabutin	0.5	0.5
<i>p</i> -Aminosalicylic acid	2.0	8.0

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

* The higher concentration of INH and EMB should be tested as second-line drugs after resistance at the critical concentration is detected.

† Breakpoints for establishing susceptibility have not been determined

Broth Based Media

	MGIT	VersaTREK
First-line Drugs		
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
Second-line Drug		
Streptomycin	1.0 (and 4.0*)	

NOTE: Critical concentrations as indicated in applicable manufacturer package inserts

* The higher concentration of INH, EMB, and STR should be tested after resistance at the critical concentration is detected.

References

1. CLSI, *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard - Second Edition in CLSI Document M24 A-2*. 2011, Clinical and Laboratory Standards Institute: Wayne, PA.
2. Campbell, P.J., et al., *Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2011. 55(5): p. 2032-41.
3. APHL, *TB Drug Susceptibility Testing Expert Panel Meeting Summary Report*. 2007, Association of Public Health Laboratories: Washington, D.C.
4. Almeida Da Silva, P.E. and J.C. Palomino, *Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs*. J Antimicrob Chemother, 2011. 66(7): p. 1417-30.
5. Zhang, Y. and W.W. Yew, *Mechanisms of drug resistance in Mycobacterium tuberculosis*. Int J Tuberc Lung Dis, 2009. 13(11): p. 1320-30.
6. Ramaswamy, S.V., et al., *Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2003. 47(4): p. 1241-50.
7. Ando, H., et al., *A silent mutation in mabA confers isoniazid resistance on Mycobacterium tuberculosis*. Mol Microbiol, 2014. 91(3): p. 538-47.
8. Starks, A.M., et al., *Mutations at embB codon 306 are an important molecular indicator of ethambutol resistance in Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2009. 53(3): p. 1061-6.
9. Angra, P.K., et al., *Performance of tuberculosis drug susceptibility testing in U.S. laboratories from 1994 to 2008*. J Clin Microbiol, 2012. 50(4): p. 1233-9.
10. Madison, B., et al., *Multicenter evaluation of ethambutol susceptibility testing of mycobacterium tuberculosis by agar proportion and radiometric methods*. J Clin Microbiol, 2002. 40(11): p. 3976-9.
11. 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.
12. Centers for Disease Control and Prevention, *Treatment of Tuberculosis, American Thoracic Society, CDC, and Infectious Diseases Society of America*. 2003, MMWR. p. 4,11,19-20.
13. 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.
14. 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.
15. 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.
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. *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.
21. Van Deun, A., et al., *Rifampin drug resistance tests for tuberculosis: challenging the gold standard*. J Clin Microbiol, 2013. 51(8): p. 2633-40.
22. 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.
23. 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.
24. Maus, C.E., B.B. Plikaytis, and T.M. Shinnick, *Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2005. 49(8): p. 3192-7.
25. 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.

