Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Evaluation of a rapid molecular drug susceptibility test for tuberculosis

SUPPLEMENTARY APPENDIX
Table of Contents

Table S1. Study eligibility criteria.

Inclusion Criteria
Age ≥ 19 years (age of majority) if enrolled in South Korea; age ≥ 18 years (age of majority) if enrolled in China
Clinical signs and/or symptoms suggestive of pulmonary tuberculosis
Provision of informed consent
Meets one of the following criteria: A. Suspected or confirmed new pulmonary tuberculosis case who has received anti-tuberculosis drugs for less than 3 (three) days (target enrollment for Group A is approximately 50 participants). [case detection group] B. Confirmed pulmonary tuberculosis with documented rifampin resistance, who has received anti-tuberculosis drugs for 31 days or less [drug resistance risk group] C. History of prior tuberculosis PLUS ongoing signs and/or symptoms of pulmonary tuberculosis PLUS suspected drug resistance [drug resistance risk group].
Exclusion Criterion
Inability to provide a sputum specimen

Note: individuals were prospectively enrolled into either the case detection group or the drug resistance risk group. In the context of the known limitation of molecular tests that detect DNA to distinguish between viable and nonviable bacilli, prospective enrollment into a case detection group defined the set of participants in whom diagnostic accuracy of the investigational assay and the Xpert MTB/RIF assay for tuberculosis case detection was analyzed.

Table S2. Nucleic acid sequences of primers used for DNA sequencing.

Target	PCR Primers (5'-3')	Sequencing Primers (5'-3')
katG	GGTGCAGATGGGGCTGATCT TACCAGGCCTTGGCGAACTC	CAT GAA CGA CGT CGA AAC AG CAA TTC CTC GGG GTG TTC CA
inhA promoter	CGACATACCTGCTGCGCAAT TGCTCTTCTACCGCCGTGAA	CCT CGC TGC CCA GAA AGG GA ATC CCC CGG TTT CCT CCG GT
gyrA	GACGCGAAAGTCGTTGTGAA GGCCGTCGTAGTTAGGGATG	CAG CTA CAT CGA CTA TGC GA GGG CTT CGG TGT ACC TCA T
gyrB		CCA CCG ACA TCG GTG GAT T CTG CCA CTT GAG TTT GTA CA
rrs	GGGTCAACTCGGAGGAAGGT GTTGCCTCAGGACCCAACAG	GGG CTT CAC ACA TGC TAC AA ACA GAC AAG AAC CCC TCA CG
eis promoter	GGACCGGTACTTGCTCTGCA ACCGTCAGCTCATGCAAGGT	CGT AAC GTC ACG GCG AAA T ACC GCG ACG AAA CTG AGA C

Table S3. *M. tuberculosis* mutations as determined by DNA sequencing, and their detection by the investigational assay, overall and by enrollment site (DST main analysis population)[1]

Mutation	Number of isolates in which the mutation was identified by sequencing, for the DST main analysis population overall and by enrollment site			n (% of overall with determinate assay results) identified as having mutant Tm(s) by investigational assay
	China n (% of 210 enrollees)	South Korea n (% of 98 enrollees)	**Overall n (% of 308 enrollees)**	
katG				
S315T	69 (32.9)	48 (49.0)	117 (38.0)	113/116 (97.4)
S315N	4 (1.9)	1 (1.0)	5 (1.6)	5/5 (100)
S315R	0 (0)	1 (1.0)	1 (0.3)	1/1 (100)
***inhA* promoter**				
T(-8)C	2 (1.0)	1 (1.0)	3 (1.0)	3/3 (100)
T(-8)A	1 (0.5)	0 (0)	1 (0.3)	1/1 (100)
C(-15)T	21 (10.0)	9 (9.2)	30 (9.7)	29/30 (96.7)
gyrA[2]				
D94G	17 (8.1)	21 (21.4)	38 (12.3)	37/38 (97.4)
A90V	12 (5.7)	7 (7.1)	19 (6.2)	18/19 (94.7)
D94A	5 (2.4)	3 (3.1)	8 (2.6)	8/8 (100)
D94N	3 (1.4)	4 (4.1)	7 (2.3)	7/7 (100)
D94Y	2 (1.0)	1 (1.0)	3 (1.0)	3/3 (100)
S91P	4	2 (2.0)	6 (1.9)	6/6 (100)
G88C	0 (0)	1 (1.0)	1 (0.3)	1/1 (100)
D94C (GAC/TGC)[3]	1 (0.5)	0 (0)	1 (0.3)	0/1 (0)
D94S (GAC/AGC)[3]	1 (0.5)	0 (0)	1 (0.3)	1/1 (100)
D94G and A90V	0 (0)	1 (1.0)	1 (0.3)	0/1 (0)
D94Y and A90V	1 (0.5)	0 (0)	1 (0.3)	1/1 (100)
D94N and A90V	1 (0.5)	0 (0)	1 (0.3)	1/1 (100)
D94A and A90V	0 (0)	1 (1.0)	1 (0.3)	1/1 (100)
S91P and A90V	2 (1.0)	0 (0)	2 (0.6)	2/2 (100)
S91P and D94A	0 (0)	1 (1.0)	1 (0.3)	1/1 (100)
D94Y and D94N	1 (0.5)	0 (0)	1 (0.3)	1/1 (100)
gyrB[4]				
D500H	0 (0)	1 (1.0)	1 (0.3)	1/1 (100)
D500N	0 (0)	2 (2.0)	2 (0.6)	2/2 (100)
D500G	0 (0)	1 (1.0)	1 (0.3)	0/1 (0)
N538T	0 (0)	1 (1.0)	1 (0.3)	1/1 (100)
E540A	0 (0)	1 (1.0)	1 (0.3)	0/1 (0)
E540D	1 (0.5)	0 (0)	1 (0.3)	1/1 (100)
rrs[5]				
A1401G	18 (8.6)	15 (15.3)	33 (10.7)	30/31 (96.8)
***eis* promoter**[6]				
G(-10)A	3 (1.4)	1 (1.0)	4 (1.3)	3/4 (75.0)
C(-14)T	2 (1.0)	2 (2.0)	4 (1.3)	3/4 (75.0)
G(-37)T	1 (0.5)	3 (3.1)	4 (1.3)	3/3 (100)

Note: there are no known silent mutations that occur within the gene regions tested by the investigational assay for resistance. In our study, no silent mutations in these regions were detected either by the investigational assay or by sequencing.

Table S4. Operational characteristics of the investigational assay (DST main analysis population, n=308)

Investigational assay result	Number of participants	Interpretation of investigational assay result	Phenotypic DST result	Sequencing result
'Invalid'	2 (0.6%)	Assay failure. No signals obtained from assay internal control or *M. tuberculosis* gene targets	INH-R, KAN-R, AMK-R (n=1)	*katG* S315T and *rrs* A1401G
			No resistance to tested drugs (n=1)	Wild-type at all tested loci
'MTB not detected'	2 (0.6%)	Investigational assay did not detect *M. tuberculosis*, and therefore provided no information about resistance	No resistance to tested drugs	Wild-type at all tested loci
Indeterminate *katG*	1 (0.3%)	No information about isoniazid resistance	INH-R	Not done
Indeterminate *gyrA*	1 (0.3%)	No information about fluoroquinolone resistance	MXF 0.5-S, OFL-S	*gyrA* wild-type
Indeterminate *gyrB*	6 (1.9%)	Fluoroquinolone-resistant based on detection of mutant Tm for *gyrA* (n=2)	MXF 0.5-R, MXF 2.0-S, and OFL-R (n=1) MXF 0.5-R, MXF 2.0-R, and OFL-R (n=1)	*gyrB* wild-type
		Fluoroquinolone-susceptible based on detection of wild-type Tm for *gyrA* (n=3)	MXF 0.5-S, MXF 2.0-S, and OFL-S (n=3)	*gyrB* wild-type
		No information about fluoroquinolone resistance based on indeterminate results for *gyrA* and *gyrB*	MXF 0.5-S, MXF 2.0-S, and OFL-S (n=1)	*gyrB* wild-type
Indeterminate *rrs*	6 (1.9%)	No information about resistance to either KAN or AMK	KAN-S and AMK-S (n=5)	*rrs* wild-type
			KAN-R and AMK-R (n=1)	*rrs* A1401G
Indeterminate *eis* promoter	4 (1.3%)	KAN-S based on detection of wild-type Tm for *rrs*	KAN-S (n=2)	*eis* wild-type
		KAN-S based on detection of wild-type Tm for *rrs*	KAN-R (n=1)	*eis* G(-37)T
		No information about KAN resistance based on indeterminate results for *rrs* and *eis* promoter	KAN-R (n=1)	*eis* wild-type

Abbreviations: INH, isoniazid; KAN, kanamycin; AMK, amikacin; OFL, ofloxacin; MXF, moxifloxacin; R, resistant; S, susceptible

Table S5. Sensitivity and specificity of the investigational assay compared against DNA sequencing as the reference standard, by genetic target (DST main analysis population)[1]

Genetic target	DNA Seq / Investigational Assay	Mutation (# with heteroresistance detected by sequencing)	No mutation	Sensitivity % (95% CI) n/n	Specificity % (95% CI) n/n
katG[2]	Mutation	119 (0)	0	**97.5 (93.0-99.5) 119/122**	**100 (98.0-100) 181/181**
	No mutation	3 (2)	181		
***inhA* promoter**	Mutation	33 (1)	0	**97.1 (84.7-99.9) 33/34**	**100 (98.6-100) 270/270**
	No mutation	1 (1)	270		
gyrA[3]	Mutation	88 (10)	0	**95.7 (89.2-98.8) 88/92**	**100 (98.3-100) 211/211**
	No mutation	4 (4)	211		
gyrB[4]	Mutation	5 (1)	2	**71.4 (29.0-96.3) 5/7**	**99.3 (97.4-99.9) 289/291**
	No mutation	2 (2)	289		
rrs[5]	Mutation	30 (4)	0	**96.8 (83.3-99.9) 30/31**	**100 (98.6-100) 267/267**
	No mutation	1 (0)	267		
***eis* promoter**[6]	Mutation	9 (0)	1	**81.8 (48.2-97.7) 9/11**	**99.7 (98.1-100.0) 288/289**
	No mutation	2 (1)	288		

Notes

[1] there are no known silent mutations that occur within the gene regions tested by the investigational assay for resistance. In our study, no silent mutations in these regions were detected either by the investigational assay or by sequencing.

[2] one specimen excluded due to indeterminate investigational assay result for *katG*

[3] one specimen excluded due to indeterminate investigational assay result for *gyrA*

[4] six specimens excluded due to indeterminate investigational assay result for *gyrB*

[5] six specimens excluded due to indeterminate investigational assay result for *rrs*

[6] four specimens excluded due to indeterminate investigational assay result for *eis* promoter

Table S6. Characterization of *M. tuberculosis* populations for which heteroresistance was detected by DNA sequencing and/or the investigational assay (DST main analysis population)

Target	DNA Sequencing		Investigational assay result	Investigational assay & sequencing performed from same sputum (S1)?
	Mutation	Sequencing result		
katG	S315T	HR	Wild-type	Yes
katG	S315T	HR	Wild-type	Yes
inhA	C(-15)T	HR	HR	Yes
inhA	C(-15)T	resistant	HR	Yes
inhA	C(-15)T	HR	Wild-type	Yes
gyrA	A90V	resistant	HR	Yes
gyrA	A90V	HR	HR	Yes
gyrA	A90V	HR	Wild-type	No
gyrA	D94G	resistant	HR	Yes
gyrA	D94G	HR	HR	Yes
gyrA	D94G	HR	HR	No
gyrA	D94G	HR	Wild-type	Yes
gyrA	D94A	HR	resistant	Yes
gyrA	D94N	HR	HR	Yes
gyrA	D94S (GAC/AGC)	HR	HR	Yes
gyrA	D94C (GAC/TGC)	HR	Wild-type	Yes
gyrA	A90V; D94A	HR	HR	No
gyrA	A90V; S91P	HR	HR	Yes
gyrA	A90V; D94N	HR	resistant	Yes
gyrA	A90V; D94Y	HR	resistant	Yes
gyrA	A90V; D94G	HR	Wild-type	No
gyrB	E540A	HR	Wild-type	No
gyrB	D500G	HR	Wild-type	Yes
gyrB	E540D	HR	resistant	Yes
rrs	A1401G	HR	resistant	Yes
rrs	A1401G	HR	HR	Yes
rrs	A1401G	HR	HR	Yes
rrs	A1401G	HR	HR	Yes
eis	C(-14)T	HR	Wild-type	Yes

Abbreviation: HR, heteroresistant

Table S7A. Sensitivity and specificity of the investigational assay compared against phenotypic drug susceptibility testing as the reference standard, by drug (reflex test analysis population)[1]

Drug	MGIT DST / Investigational Assay	Resistant	Susceptible	Sensitivity % (95% CI) n/n	Specificity % (95% CI) n/n
Isoniazid[2]	Resistant	125	1	**86.2 (79.5-91.4) 125/145**	**88.9 (51.8-99.7) 8/9**
	Susceptible	20	8		
ofloxacin[3]	Resistant	77	7	**92.8 (84.9-97.3) 77/83**	**90.1 (80.7-95.9) 64/71**
	Susceptible	6	64		
moxifloxacin 0.5 µg/mL[3,4]	Resistant	72	11	**90.0 (81.2-95.6) 72/80**	**84.9 (74.6-92.2) 62/73**
	Susceptible	8	62		
moxifloxacin 2.0 µg/mL[3]	Resistant	47	37	**97.9 (88.9-100) 47/48**	**65.1 (55.2-74.1) 69/106**
	Susceptible	1	69		
kanamycin[5]	Resistant	35	4	**81.4 (66.6-91.6) 35/43**	**96.4 (91.0-99.0) 106/110**
	Susceptible	8	106		
amikacin[5]	Resistant	29	1	**76.3 (59.8-88.6) 29/38**	**99.1 (95.3-100) 114/115**
	Susceptible	9	114		

Notes

[1] one specimen excluded due to invalid investigational assay result

[2] one specimen excluded due to indeterminate investigational assay result for *katG*

[3] one specimen excluded due to indeterminate investigational assay result for *gyrA*

[4] one specimen excluded due to indeterminate moxifloxacin phenotypic DST result

[5] two specimens excluded due to indeterminate investigational assay result for *rrs*

Table S7B. Sensitivity and specificity of the investigational assay compared against DNA sequencing as the reference standard, by drug (reflex test analysis population)[1]

Drug	DNA Seq / Investigational Assay	Mutation	No mutation	Sensitivity % (95% CI) n/n	Specificity % (95% CI) n/n
isoniazid[2]	Mutation	126	0	**98.4 (94.5-99.8) 126/128**	**100 (86.8-100) 26/26**
	No mutation	2	26		
fluoro-quinolones[3]	Mutation	84	0	**98.8 (93.6-100) 84/85**	**100 (94.8-100) 69/69**
	No mutation	1	69		
kanamycin[4]	Mutation	38	1	**95.0 (83.1-99.4) 38/40**	**99.1 (95.2-100) 112/113**
	No mutation	2	112		
amikacin[4]	Mutation	30	0	**100 (88.4-100) 30/30**	**100 (97.1-100) 123/123**
	No mutation	0	123		

Notes

[1] one specimen excluded due to invalid investigational assay result

[2] one specimen excluded due to indeterminate investigational assay result for *katG*

[3] one specimen excluded due to indeterminate investigational assay result for *gyrA*

[4] two specimens excluded due to indeterminate investigational assay result for *rrs*

Table S7C. Sensitivity and specificity of the investigational assay compared against DNA sequencing as the reference standard, by genetic target (Reflex test analysis population)[1]

Genetic target	DNA Seq / Investigational Assay	Mutation (# with heteroresistance detected by sequencing)	No mutation	Sensitivity % (95% CI) n/n	Specificity % (95% CI) n/n
katG[2]	Mutation	101 (0)	0	**98.1 (93.1-99.8) 101/103**	**100 (93.0-100) 51/51**
	No mutation	2 (1)	51		
***inhA* promoter**	Mutation	26 (0)	0	**96.3 (81.0-99.9) 26/27**	**100 (97.2-100) 128/128**
	No mutation	1 (1)	128		
gyrA[3]	Mutation	81 (10)	0	**98.8 (93.4-100) 81/82**	**100 (95.0-100) 72/72**
	No mutation	1 (1)	72		
gyrB[4]	Mutation	4 (1)	2	**66.7 (22.2-95.7) 4/6**	**98.6 (95.1-99.8) 144/146**
	No mutation	2 (2)	144		
rrs[5]	Mutation	30 (4)	0	**100 (88.4-100) 30/30**	**100 (97.1-100) 123/123**
	No mutation	0	123		
***eis* promoter**[5]	Mutation	9 (0)	1	**81.8 (48.2-97.7) 9/11**	**99.3 (96.1-100) 141/142**
	No mutation	2 (1)	141		

Notes

[1] one specimen excluded due to invalid investigational assay result

[2] one specimen excluded due to indeterminate investigational assay result for *katG*

[3] one specimen excluded due to indeterminate investigational assay result for *gyrA*

[4] three specimens excluded due to indeterminate investigational assay result for *gyrB*

[5] two specimens excluded due to indeterminate investigational assay result for *rrs*

[6] two specimens excluded due to indeterminate investigational assay result for *eis* promoter

Table S8. Sensitivity and specificity of the investigational assay, by sputum smear microscopy status (DST main analysis population)

	Phenotypic DST						DNA Sequencing			
	INH	OFL	MXF 0.5 µg/mL	MXF 2.0 µg/mL	KAN	AMK	INH	MXF/ OFL	KAN	AMK
Smear-negative specimens										
Sensitivity										
no./total	17/20	14/15	12/13	6/7	3/5	2/3	18/18	14/14	4/4	2/2
(%)	(85.0)	(93.3)	(92.3)	(85.7)	(60.0)	(66.7)	(100)	(100)	(100)	(100)
95% CI	62.1-96.8	68.1-99.8	63.9-99.8	42.1-99.6	14.7-94.7	9.4-99.2	81.5-100	76.8-100	39.8-100	15.8-100
Specificity										
no./total	17/18	23/23	23/25	23/31	29/30	32/32	20/20	24/24	31/31	33/33
(%)	(94.4)	(100)	(92.0)	(74.2)	(96.7)	(100)	(100)	(100)	(100)	(100)
95% CI	72.7-99.9	85.2-100	74.0-99.0	55.4-88.1	82.8-99.9	89.1-100	83.2-100	85.8-100	88.8-100	89.4-100
Smear-positive specimens										
Sensitivity										
no./total	133/160	70/80	76/86	45/46	32/44	27/38	133/136	77/81	34/37	28/29
(%)	(83.1)	(87.5)	(88.4)	(97.8)	(72.7)	(71.1)	(97.8)	(95.1)	(91.9)	(96.6)
95% CI	76.4-88.6	78.2-93.8	79.7-94.3	88.5-100	57.2-85.0	54.1-84.6	93.7-99.5	87.8-98.6	78.1-98.3	82.2-99.9
Specificity										
no./total	105/105	178/185	177/187	187/219	216/219	224/225	129/129	184/184	225/226	234/234
(%)	(100)	(96.2)	(94.7)	(85.4)	(98.6)	(99.6)	(100)	(100)	(99.6)	(100)
95% CI	96.6-100	92.4-98.5	90.4-97.4	80.0-89.8	96.1-99.7	97.6-100	97.2-100	98.0-100	97.6-100	98.4-100
Total										
Sensitivity										
no./total	150/180	84/95	78/89	51/53	35/49	29/41	151/154	91/95	38/41	30/31
(%)	(83.3)	(88.4)	(87.6)	(96.2)	(71.4)	(70.7)	(98.1)	(95.8)	(92.7)	(96.8)
95% CI	77.1-88.5	80.2-94.1	79.0-93.7	87.0-99.5	56.7-83.4	54.5-83.9	94.4-99.6	89.6-98.8	80.1-98.5	83.3-99.9
Specificity										
no./total	122/123	201/208	200/212	210/250	245/249	256/257	149/149	208/208	256/257	267/267
(%)	(99.2)	(96.6)	(94.3)	(84.0)	(98.4)	(99.6)	(100)	(100)	(99.6)	(100)
95% CI	95.6-100	93.2-98.6	90.3-97.0	78.9-88.3	95.9-99.6	97.9-99.9	97.6-100	98.2-100	97.9-100	98.6-100

Abbreviations: DST, drug susceptibility testing; INH, isoniazid; OFL, ofloxacin; MXF, moxifloxacin; KAN, kanamycin; AMK, amikacin; CI, confidence interval

Table S9. Sensitivity and Specificity of the investigational assay, by enrollment site (DST main analysis population)

Site	Phenotypic DST						DNA Sequencing			
	INH	OFL	MXF 0.5 µg/mL	MXF 2.0 µg/mL	KAN	AMK	INH	MXF/ OFL	KAN	AMK
China										
Sensitivity no./total (%) 95% CI [%]	90/109 (82.6) 74.1-89.2	43/52 (82.7) 69.7-91.8	42/50 (84.0) 70.9-92.8	24/26 (92.3) 74.9-99.1	18/27 (66.7) 46.0-83.5	16/22 (72.7) 49.8-89.3	91/93 (97.8) 92.4-99.7	48/51 (94.1) 83.8-98.8	20/21 (95.2) 76.2-99.9	17/17 (100) 80.5-100
Specificity no./total (%) 95% CI [%]	101/102 (99.0) 94.7-100	155/160 (96.9) 92.9-99.0	155/160 (96.9) 92.9-99.0	162/186 (87.1) 81.4-91.6	178/181 (98.3) 95.2-99.7	185/186 (99.5) 97.0-100	118/118 (100) 96.9-100	161/161 (100) 97.7-100	186/187 (99.5) 97.1-100	191/191 (100) 98.1-100
South Korea										
Sensitivity no./total (%) 95% CI [%]	60/71 (84.5) 74.0-92.0	41/43 (95.4) 84.2-99.4	36/39 (92.3) 79.1-98.4	27/27 (100) 87.2-100	17/22 (77.3) 54.6-92.2	13/19 (68.4) 43.5-87.4	60/61 (98.4) 91.2-100	43/44 (97.7) 88.0-99.9	18/20 (90.0) 68.3-98.8	13/14 (92.9) 66.1-99.8
Specificity no./total (%) 95% CI [%]	21/21 (100) 83.9-100	46/48 (95.8) 85.8-99.5	45/52 (86.5) 74.2-94.4	48/64 (75.0) 62.6-85.0	67/68 (98.5) 92.1-100	71/71 (100) 94.9-100	31/31 (100) 88.8-100	47/47 (100) 92.5-100	70/70 (100) 94.9-100	76/76 (100) 95.3-100
Total										
Sensitivity no./total (%) 95% CI [%]	150/180 (83.3) 77.1-88.5	84/95 (88.4) 80.2-94.1	78/89 (87.6) 79.0-93.7	51/53 (96.2) 87.0-99.5	35/49 (71.4) 56.7-83.4	29/41 (70.7) 54.5-83.9	151/154 (98.1) 94.4-99.6	91/95 (95.8) 89.6-98.8	38/41 (92.7) 80.1-98.5	30/31 (96.8) 83.3-99.9
Specificity no./total (%) 95% CI [%]	122/123 (99.2) 95.6-100	201/208 (96.6) 93.2-98.6	200/212 (94.3) 90.3-97.0	210/250 (84.0) 78.9-88.3	245/249 (98.4) 95.9-99.6	256/257 (99.6) 97.9-99.9	149/149 (100) 97.6-100	208/208 (100) 98.2-100	256/257 (99.6) 97.9-99.9	267/267 (100) 98.6-100

Abbreviations: DST, drug susceptibility testing; INH, isoniazid; OFL, ofloxacin; MXF, moxifloxacin; KAN, kanamycin; AMK, amikacin; CI, confidence interval

Supplementary Results. Detail of DNA sequencing genotypes of isolates with discrepant investigational assay and phenotypic DST results

A. Sequencing results for specimens found resistant by phenotypic testing and susceptible by the investigational assay (Figure 2A):

isoniazid: *katG* S315T (n=1); *katG* S315/wild-type mix (n=1)

moxifloxacin (0.5 µg/mL): *gyrA* A90V/wild-type mix (n=1); *gyrA* A90V+D94G/wild-type mix(n=1); *gyrA* D94C/wild-type mix (n=1)

moxifloxacin (2.0 µg/mL): *gyrA* D94C/wild-type mix (n=1)

ofloxacin: *gyrA* A90V/ wild-type mix (n=1); *gyrA* A90V+D94G/wild-type mix (n=1); *gyrA* D94G/wild-type mix (n=1); *gyrA* D94C/wild-type mix (n=1)

kanamycin: *rrs* A1401G (n=1); *eis* G(-10)A (n=1); *eis* G(-37)T (n=1)

amikacin: *rrs* A1401G (n=1)

B. Sequencing results for specimens found susceptible by phenotypic testing and resistant by the investigational assay (Figure 2B):

isoniazid: *katG* S315T (n=1)

moxifloxacin (0.5 µg/mL): *gyrA* A90V (n=7); *gyrA* A90V/wild-type mix (n=1); *gyrA* S91P (n=3); *gyrB* D500H (n=1)

moxifloxacin (2.0 µg/mL): *gyrA* A90V (n=15); *gyrA* S91P (n=5); *gyrA* D94G (n=5); *gyrA* D94A (n=5), *gyrA* D94N (n=1); *gyrA* D94Y (n=2), *gyrA* D94N+D94Y (n=1); *gyrA* A90V/wild-type mix (n=1); *gyrA* A90V+D94N/wild-type mix (n=1); *gyrA* D94A/wild-type mix (n=1); *gyrB* D500N (n=2); gyrB E540D wild-type mix (n=1)

ofloxacin: *gyrA* A90V (n=5); *gyrA* A90V/wild-type mix (n=1); *gyrB* E540D/wild-type mix (n=1)

kanamycin: *eis* G(-10)A (n=1); *rrs* A1401G (n=1); *rrs* A1401G/wild-type mix (n=1)

amikacin: *rrs* A1401G/wild-type mix (n=1)