HHS Public Access

Author manuscript

Int J Mycobacteriol. Author manuscript; available in PMC 2016 May 11.

Published in final edited form as:

Int J Mycobacteriol. 2016 March ; 5(1): 1–6. doi:10.1016/j.ijmyco.2015.09.001.

Correlating *rrs* and *eis* promoter mutations in clinical isolates of *Mycobacterium tuberculosis* with phenotypic susceptibility levels to the second line injectables

Priti Kambli[1], **Kanchan Ajbani**[1], **Chaitali Nikam**[1], **Meeta Sadani**[1], **Anjali Shetty**[1], **Zarir Udwadia**[2], **Sophia B Georghiou**[3], **Timothy C Rodwell**[3], **Antonino Catanzaro**[3], and **Camilla Rodrigues**[1]

[1]Microbiology Section, Department of Laboratory Medicine, P. D. Hinduja National Hospital and Medical Research Center, Mumbai, India

[2]Pulmonology Section, Department of Medicine, P. D. Hinduja National Hospital and Medical Research Center, Mumbai, India

[3]University of California, San Diego, Department of Medicine, San Diego, CA, USA

Abstract

Objective—To correlate *rrs* and *eis* promoter mutations, found in *Mycobacterium tuberculosis* (*MTB*) isolates,with corresponding Minimum Inhibitory Concentrations (MICs) of amikacin (AMK), kanamycin (KAN), and capreomycin (CAP).

Methods—Ninety *MTB* clinical isolates were analyzed in this study. MICs were determined by MGIT 960 for 59 isolates with resistance-associated mutations in the *rrs* and *eis* promoter gene regions and 31 isolates with wild-type sequences, as determined by the GenoTypeMTBDR*sl* (version 1) assay.

Results—The *rrs* A1401G mutation was identified in 48 isolates resistant to the second line injectables. The *eis* promoter mutations C-14T (n=3), G-10C (n=3), G-10A (n=3), and C-12T (n=2) were found within 11 isolates with various resistance profiles to the second line injectables. Thirty-one isolates had wild-type sequences for the *rrs* and *eis* promoter gene regions of interest, one of which was AMK, KAN and CAP-resistant. Isolates with the A1401G *rrs* mutation had AMK, KAN, and CAP MICs of >40, >20, and 5–15mg/L, respectively. Isolates with *eis* promoter mutations had AMK, KAN, and CAP MICs of 0. 25–1. 0, 0. 625–10, and 0. 625–2. 5mg/L, respectively.

Conclusions—This study provides a preliminary basis for the prediction of phenotypic resistance levels to the second line injectables based upon the presence of genetic mutations

Corresponding author: Camilla Rodrigues, Tel: 91-22-24447795, Fax: 91-22-24442318, dr_crodrigues@hindujahospital.com.

Competing interests

The authors have declared that no financial competing interests exist.

Authors' contributions

Study concepts: CR. Study design: CR, KA and PK. Data acquisition: PK, KA, MS, CN. Data analysis and interpretation: PK, SG and CR. Manuscript preparation: PK, SG and CR. Manuscript editing: Pk, SG and CR. Manuscript review: AS, ZU, SG, TR, AC and CR. All authors read and approved the final manuscript.

associated with AMK, KAN and CAP-resistance. Results suggest that isolates with *eis* promoter mutations have consistently lower resistance levels to AMK, KAN, and CAP than isolates with the *rrs* A1401G mutation.

Keywords

XDR-TB; Drug Susceptibility Testing; level of resistance; mutations

BACKGROUND

Drug-resistant tuberculosis poses a major threat to tuberculosis (TB) control efforts worldwide. There is an increasing global incidence of multi-drug resistant TB (MDR-TB) and extremely drug-resistant TB (XDR-TB), characterized by resistance to isoniazid, rifampin, a fluoroquinolone, and at least one of three second line injectable drugs (SLI): amikacin (AMK), kanamycin (KAN), and/or capreomycin (CAP). Effective treatment of MDR-TB, and the prevention of XDR-TB, is reliant upon the appropriate use of these SLIs. In order to establish appropriate treatment regimens, and to prevent the amplification of SLI resistance, it is critical that MDR-TB patients have full drug susceptibility profiles established for their particular *MTB* infections prior to treatment [1].

Conventional diagnosis of drug-resistant *MTB* relies upon the slow growth of mycobacterium cultures, and can take anywhere from several weeks to months to generate results [2]. While methods for the conventional diagnosis of MDR-TB are well established and generally effective, culture-based detection of SLI resistance is more complicated and results are less reliable [3]. As such, rapid molecular diagnostic tests, detecting genetic mutations associated with drug resistance, are essential to the future diagnosis and management of M/XDR-TB.

A high level of cross-resistance has been observed between the aminoglycosides AMK and KAN [4]. CAP, a cyclic polypeptide, can also be used as a substitute, though it is structurally dissimilar to the aminoglycosides [4, 6]. For patients with MDR-TB, if CAP resistance additionally occurs an increased incidence of treatment failure and patient mortality is observed [7]. Furthermore, studies conducted in clinical isolates and laboratory-generated *MTB* mutants have noted the additional occurrence of cross resistance between AMK/KAN and CAP, limiting treatment options for these infections [4, 5, 8]. AMK/KAN and CAP are known to effect protein synthesis in *MTB*, and resistance to these compounds is primarily conveyed by changes in the 16S rRNA (encoded by the *rrs* gene) [4, 5, 9]. The *rrs* mutation A1401G can cause high-level AMK/KAN resistance and lower-level CAP resistance [4]. Eis is an Aminoglycoside Acetyltransferase catalyzes the transfer of an acetyl group from acetyl-coenzyme A to an amine group of aminoglycoside. It has been reported that Eis of M. tuberculosis shows a multiacetylation capability at the 2′-, 3-or 6′ positions of aminoglycoside antibiotics [15]. Thus, it is critical to correlate specific genetic mutations with levels of SLI resistance in order to guide M/XDR-TB treatment regimens and improve patient outcomes. Herein, we establish MICs for90 clinical *MTB* isolates with specific *rrs* and *eis* promoter mutations.

MATERIALS AND METHODS

Setting and Ethical Approval

Written consent was waived for all participants as the study was carried out on 90 archived isolates for which pyrosequencing, GenoTypeMTBDR*sl* assay (version 1) and MGIT960 DST (utilizing WHO-approved critical concentrations) was previously performed. Sample collection, MGIT culture, GenoTypeMTBDR*sl* assay and Pyrosequencing (PSQ) were performed at the Mycobacteriology Laboratory of the P. D. Hinduja National Hospital and Medical Research Centre, a tertiary care hospital in Mumbai, India with a referral bias towards TB treatment non-responders. This study was approved by the Institutional Review Board of Hinduja Hospital.

Phenotypic MGIT Drug Susceptibility Testing

We performed standardized drug susceptibility testing (DST) to KAN using a BACTEC MGIT960 System (Becton Dickinson Diagnostic Systems, Sparks, MD) with Epicenter software. The manufacturer's protocol was strictly followed for preparation of the inoculums, inoculation and incubation. For KAN, a breakpoint concentration of 2. 5mg/L was utilized based upon previous findings [10]. Critical concentrations of AMK and CAP were 1. 0 and 2. 5mg/L, respectively. Stock solutions of AMK, KAN, and CAP (Sigma Aldrich) were prepared by dissolving the compounds in distilled water. The drugs were filtered, further diluted with distilled water, and stored at −80°C for up to 6 months. A genotypically-confirmed resistant strain and a pan susceptible strain, H37Rv, were tested weekly by MGIT960 DST as a quality control.

Quantitative DST

Six concentrations of KAN and seven concentrations of AMK and CAP were used to establish MICs for the *MTB* isolates included in this study. Two concentrations below the critical concentration (0. 25 and 0. 5mg/L) and three concentrations above the critical concentration (4. 0, 20. 0 and 40. 0mg/L) were used to establish AMK MICs; two concentrations below the critical concentration (0. 625 and 1. 25mg/L) and three concentrations above the critical concentration (5. 0, 10. 0 and 20. 0mg/L) were used for KAN; two concentrations below the critical concentration (0. 625 and 1. 25mg/L) and four concentrations above the critical concentration (5. 0, 10. 0, 15. 0 and 30. 0mg/L) were used for CAP.

GenoTypeMTBDR*sl* Assay

The procedure for the GenoTypeMTBDR*sl* assay was as follows

DNA extraction: DNA was extracted from all decontaminated patient samples using a GenoLyse kit (Hain Lifescience, Germany).

PCR: Multiplex polymerase chain reaction (PCR) amplification was conducted for the *rrs* gene of interest utilizing biotinylated primers. PCR was performed with the following cycling conditions: Initial Denaturation 95°C/15 min, [Denaturation 95°C/30 sec, Annealing

58°C/2 min, (10 cycles)], [Denaturation 95°C/25 sec, Annealing 53°C/40 sec, Extension 70°C/40 sec (30 cycles)], and Final Extension 70°C/8 min.

Hybridization: Reverse Hybridization/GenoTypeMTBDR*sl* assay was performed as per manufacturer instructions (HainLifescience, Germany) [11].

Analysis: The GenoTypeMTBDR*sl* assay evaluates the *rrs* gene for mutations at positions 1401/1402 and 1484. The absence of the test wild-type (WT) marker, WT1, and presence of the mutation (MUT) probe, MUT1, corresponds to the A1401G mutation, while the absence of the test WT2 marker and presence of the probe MUT2 corresponds to the G1484T mutation.

PSQ

A total of twenty *MTB* isolates were *rrs* sequence-confirmed and evaluated for the presence of *eis* promoter mutations by PSQ. Reagents from the Hot Start Taq kit, and deoxynucleotide triphosphate (dNTP) mixtures (Qiagen, Valencia, CA), were used in the PCR master mix. PSQ was performed as follows

DNA extraction: The PSQ assay utilized crude DNA, extracted by a simple heating procedure (95°C, 25min).

PCR solutions: Each PCR reaction contained 2. 5μL of extracted isolate DNA and 22. 5μL of PCR master mix (1×PCR buffer, 2. 5mM MgCl2, 0. 96mMdNTP mixture, 1× Q-solution, 0. 5μM *rrs* or *eis* promoter primers, and 1U of Hot Start Taq).

PCR steps: Initial activation of Hot Start Taq polymerase at 95°C for 15 min, 50 cycles of amplification (94°C for 15 s, 60°C for 30 s, and 72°C for 20 s), and a final extension at 72°C for 5 min.

PSQ reaction: PSQ was conducted for the -6 to -47 region of the *eis* promoter and the 1401/1402 region of the *rrs* gene. PSQ was performed with PyroMark Q96 reagents, utilizing the sequence analysis mode of the PyroMark Q96 ID system (Qiagen, Valencia, CA) [12].

RESULTS

Phenotypic DST and MIC Results

Sixty phenotypically XDR-TB strains were included in this study. Eleven (found to have *eis* promoter mutations) were resistant to KAN at the critical concentration, but sensitive to AMK and CAP. Forty-eight isolates (determined to have the *rrs* A1401G mutation) were found to be resistant to KAN, AMK, and CAP. One isolate (with wild-type sequences for both the *rrs* and *eis* promoter) was also resistant to KAN, AMK and CAP at the critical concentration. Thirty isolates were pan susceptible to all drugs tested. The MICs of AMK, KAN and CAP for all isolates are shown in Table 1.

Genotypic Results

The agreement between GenoTypeMTBDR*sl* assay results (based solely upon the presence of resistance-associated *rrs* mutations) and phenotypic DST was 97% for AMK, 96% for KAN and 86% for CAP. Results of 20 PSQ confirmed isolates are summarized in Table 2. One isolates, found to be phenotypically resistant to the three SLIs, and had *rrs* & *eis* promoter wild-type sequences by the GenoTypeMTBDR*sl* and PSQ assays.

Correlation of KAN MICs with Resistance-Associated Mutations

Isolates harboring the *rrs* A1401G mutation had AMK, KAN and CAP MICs of >40, >20, and 5-15mg/L, respectively, whereas isolates with *eis* promoter mutations were found to have AMK, KAN and CAP MICs ranging from 0. 25-1. 0, 5. 0-10, and 0. 625-2. 5mg/L, respectively (Table1). As such, the A1401G *rrs* mutation correlated with high-level AMK and KAN resistance and moderate-level CAP resistance. *eis* promoter mutations correlated with low-level AMK and CAP resistance and moderate-level KAN resistance.

DISCUSSION

This study correlates specific *rrs* and *eis* promoter mutations identified in *MTB* clinical isolates with AMK, KAN, and CAP phenotypic resistance levels. Overall, MIC ranges for *MTB* isolates with wild-type *rrs* and *eis* gene sequences were different from MIC ranges for isolates harboring resistance-associated mutations in these gene regions. *rrs* & *eis* promoter mutations associated with SLI-resistance were found in 59 of the 90 *MTB* isolates evaluated in this study. These two genes, alone, appeared to be highly sensitive markers for SLI-resistance, as only one of the 60 SLI-resistant isolates evaluated in the study was determined to have wild-type sequences for the gene regions evaluated. This isolate may have SLI resistance-associated genetic mutations in other genes, such as *tlyA* and *gidB* that were not assessed in this study. Whilst we are aware that it would have been ideal to perform PSQ for all isolates we feel that number of isolates selected were fairly representative. Overall, our results provide a preliminary basis for prediction of SLI phenotypic resistance levels based upon the presence or absence of specific resistance-associated mutations in both the *rrs* and *eis* promoter gene regions of *MTB* clinical isolates.

Peak serum concentrations for KAN, AMK and CAP have been previously reported at 22. 5mg/L, 20-40mg/L and 10-30mg/L, respectively [13,14]. Taking these concentrations into account, *MTB* resistance levels to each drug may be categorized as low, moderate or high. As noted in Table 1, the *rrs* A1401G mutation appears to consistently correspond to a high level of AMK and KAN resistance (MICs >40mg/L, >20mg/L). KAN MICs for isolates with the A1401G mutation have been previously documented to be as high as >80mg/L, and clinical studies have confirmed that this particular *rrs* mutation confers KAN-resistance even at these high concentrations, with additional cross-resistance to AMK with MICs of >64, [15] and, to a lesser extent, CAP with MICs of 5–10mg/L [16]. Conversely, isolates with *eis* promoter mutations have been documented to be resistant to KAN, but susceptible to the AMK and CAP at their respective breakpoints [4,16,18,19]. Our study identified 11 isolates with *eis* promoter mutations. These isolates had low to moderate-level KAN resistance and a low level of resistance to AMK and CAP. These *eis* promoter mutations, C-14T, G-10C,

G-10A, and C-12T, have been shown to result in a significant increase in *eis* transcript levels, as well as a corresponding increase in the expression of an enzyme that acetylates KAN, inactivating the drug [17,19].

Importantly, although the *eis* enzyme was found to have some cross-reactivity with AMK, *eis* mutants are largely susceptible to this other aminoglycoside, as reflected by current testing and treatment guidelines [18,19]. As Eis utilizes KAN as a substrate up to three times more efficiently than AMK, the noted lack of cross-resistance is unsurprising. This preferential substrate utilization by Eis is likely a result of aminoglycoside structural differences, as AMK contains an L-hydroxyaminobuteroyl amide group substitution in the N1 position of the deoxystreptamine ring [18, 19] which may sterically hinder acetylation. It is notable that even though *rrs* mutations confer cross-resistance to KAN and AMK, KAN-resistant isolates with *eis* promoter mutations may still be sensitive to AMK, and thus both KAN and AMK should be considered individually when determining phenotypic drug susceptibility profiles [19]. Indeed, *eis* promoter mutations were found only within AMK- and CAP-susceptible isolates in our study, suggesting that these drugs could potentially be used to treat strains housing *eis* promoter mutations.

Our observation that different *eis* promoter mutations correspond to different levels of phenotypic resistance to KAN, AMK and CAP is also notable. The *eis* promoter mutations C-14T and G-10A corresponded to low- or moderate-level resistance to KAN, with KAN MICs generally ranging from 2. 5–10μg/ml, and MICs corresponding with low-level resistance to AMK and CAP (0. 25–1. 0mg/L and 0. 625–2. 5mg/L), comparable to values reported by previous studies [16,19]. The association of the C-12T mutation with KAN-resistance, on the other hand, has been a point of contention. In one study, C-12T mutants were shown to have slightly increased KAN MICs though they retained sensitivity to KAN and AMK [19]. Another study found isolates with the C-12T mutation to have KAN resistance at an MIC of 5. 0mg/L, though CAP MICs were <2. 5mg/L [16]. Overall, all studies found aminoglycoside MICs to be lower for *eis* promoter mutants compared to *rrs* mutants [15, 16, 19]. This supports our characterization of *eis* promoter mutants as “low- to moderate-level” KAN-resistant, with MICs ranging from 0. 625-10mg/L, “low-level” AMK-resistant, with MICs from 0. 25–1. 0mg/L and “low-level” CAP-resistant, with MICs from 0. 625–2. 5mg/L. However, the overlap seen for KAN MIC ranges for the various mutants (i. e. MICs for -14T mutants were not very different than -10A or -12T mutants) suggests the need for further studies to clearly define associated phenotypic resistance levels.

The sequencing of additional gene regions not evaluated in this study, such as *gidB* and *tlyA*, might additionally contribute to our understanding of the genetic basis of observed SLI phenotypic resistance levels of *MTB* clinical isolates. These initial results, however, confirm that specific mutations in the *rrs* and *eis* promoter are promising markers for improved diagnostic assay sensitivity and specificity for the detection of injectable drug resistance. Furthermore, our findings suggest that each drug should be considered independently when considering the results of molecular diagnostic assays, in order to determine the most effective treatment regimens for M/XDR-TB cases.

CONCLUSION

These results suggest that the various mutations associated with SLI resistance may be correlated with specific SLI phenotypic resistance levels. The *rrs* canonical mutation, A1401G, was found to confer a high level of AMK and KAN resistance to *MTB* isolates, but a moderate level of CAP resistance. *eis* promoter mutations were generally determined to confer lower levels of AMK and CAP resistance and low- to moderate-levels of KAN resistance. Thus, together, the *rrs* and *eis* promoter mutations appear to have utility as molecular markers for aminoglycosides resistance levels, directing optimal treatment regimens for M/XDR-TB cases.

Acknowledgments

We are thankful to National Health and Education Society, Hinduja Hospital and Medical Research Centre.

Funding

The work was supported by the National Health and Education Society of Hinduja Hospital and Medical Research Centre. Antonino Catanzaro and Timothy C Rodwell were funded by National Institute of Allergy and Infectious Diseases (NIAID) grantU01-AI082229 and grant R01-AI111435-04.

References

1. Calver AD, Falmer AA, Murray M, Strauss OJ, Streicher EM, et al. Emergence of increased resistance and extensively drug-resistant tuberculosis despite treatment adherence, South Africa. Emerg Infect Dis. 2010; 16(2):264–271. [PubMed: 20113557]
2. Parsons LM, Somoskovi A, Gutierrez C, Lee E, Paramasivan CN, et al. Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. ClinMicrobiol Rev. 2011; 24(2):314–350.
3. Pfyffer GE, Bonato DA, Ebrahimzadeh A, Gross W, Hotaling J, et al. Multicenter laboratory validation of susceptibility testing of Mycobacterium tuberculosis against classical second-line and newer antimicrobial drugs by using the radiometric BACTEC 460 technique and the proportion method with solid media. J ClinMicrobiol. 1999; 37(10):3179–3186.
4. Sirgel FA, Tait M, Warren RM, Streicher EM, Bottger EC, Helden PV, Gey C, van Pittius NC, Coetzee G, Hoosain EY, Chabula-Nxiweni Mamisa, Hayes Cindy, Victor Thomas C, Trollip André. Mutations in the rrs A1401G Geneand Phenotypic Resistance to Amikacinand Capreomycin in Mycobacterium tuberculosis. MICROBIAL DRUG RESISTANCE. 2012; 18(2)
5. Maus CE, Plikaytis BB, Shinnick TM. Molecular analysis of cross-resistanceto capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2005; 49(8):3192–7. [PubMed: 16048924]
6. Capreomycin. Tuberculosis. 2008; 88:89–91. [PubMed: 18486038]
7. Migliori GB, Lange C, Centis R, Sotgiu G, Mütterlein R, Hoffmann H, Kliiman K, De Iaco G, Lauria FN, Richardson MD, Spanevello A, Cirillo DM. and TBNET Study Group. Resistance to second-line injectables and treatment outcomes in multidrug-resistant and extensively drugresistanttuberculosis cases. Eur Respir J. 2008; 31:1155–1159. [PubMed: 18515555]
8. Jugheli L, Bzekalava N, de Rijk P, Fisette K, Portaels F, Rigouts L. High level of cross-resistance betweenkanamycin, amikacin, and capreomycin among Mycobacteriumtuberculosis isolates from Georgia and a close relationwith mutations in the rrs gene. Antimicrob Agents Chemother. 2009; 53:5064–5068. [PubMed: 19752274]
9. Via LE, Cho S-N, Hwang S, Bang H, Park SK, Kang HS, et al. Polymorphisms associated with resistanceand cross-resistance to aminoglycosides and capreomycin inMycobacterium tuberculosis isolates from South Korean patientswith drug-resistant tuberculosis. J Clin Microbiol. 2010; 48:402–411. [PubMed: 20032248]

10. Rodrigues C, Jani J, Shenai S, Thakkar P, Siddiqi S, Mehta A. Drug susceptibility testing of *Mcobacteriumuberculosis*against second-line drugs using the Bactec MGIT 960 system. IntTuberc Lung Dis. 2008; 12(12):1449–55.
11. Ajbani K, Nikam C, Kazi M, Gray C, Boehme C, Balan K, Shetty A, Rodrigues C. Evaluation of Genotype *MTB*DRsl Assay to Detect Drug Resistance Associated with Fluoroquinolones,Aminoglycosides and Ethambutol on Clinical Sediments. PLoS One. 2012; 7(11):e49433. [PubMed: 23166667]
12. Lin SY, Rodwell TC, Rider TC, Rider L, Pham L, Catanzaro A, Desmond EP. Pyrosequencing for rapid detection of extensively resistant Mycobacterium tuberculosis in clinical isolates. J ClinMicrobiol. 2014; 52(2):475–482.
13. Bottger EC. The ins and outs of Mycobacterium tuberculosis drug susceptibility testing. ClinMicrobiol Infect. 2011; 17(8):1128–34.
14. Coyne KM, Pozniak AL, Lamorde M, Boffito M. Pharmacology of second line antituberculosis drugs and potential for interactions with antiretroviral agents. AIDS. 2009; 23(4):437–446. [PubMed: 19256042]
15. AngkanangSowajassatakul, TherdsakPrammananan, AngkanaChaiprasert andSaranyaPhunpruch*Molecular characterization of amikacin, kanamycin and capreomycin resistance in M/XDR-TB strains isolated in Thailand. BMC Microbiology. 2014; 14:165. [PubMed: 24953243]
16. Gikalo MB, Nosova EY, Lumila Y, Krylova LY, Moroz AM. The role of *eis* mutations in the development of kanamycin resistance in Mycobacterium tuberculosis isolates from the Moscow region. J Antimicrob Chemother. 2012; 67(9):2107–9. [PubMed: 22593564]
17. Magnet S, Blanchard JS. Molecular insights into aminoglycoside action and resistance. Chem Rev. 2005; 105(2):477–498. [PubMed: 15700953]
18. CLSI document M24-A2. 2. Wayne, PA: Clinical and Laboratory Standards Institute; 2011. Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard.
19. Zaunbrecher MA, Sikes RD Jr, Metchock B, et al. Overexpression of the chromosomally encoded aminoglycoside acetyl transferase eis confers kanamycin resistance in Mycobacterium tuberculosis. ProcNatlAcadSciUSA. 2009; 47:20004–9.

Int J Mycobacteriol. Author manuscript; available in PMC 2016 May 11.

Table 1

Mutations found within *Mycobacterium tuberculosis* clinical isolates and their associated ICs against the second line injectables

Mutation	LPA	Total No. of Isolates	No. of Isolates	MIC (mg/L)		
				AMK	KAN	CAP
rrs						
A1401G	MUT1	48	25	>40	>20	5. 0
			20	>40	>20	10
			3	>40	>20	15
eis promoter						
G-10C	Wild-type	3	1	0. 5	2. 5	0. 625
			1	0. 25	5. 0	0. 625
			1	0. 5	5. 0	0. 625
G-10A	Wild-type	3	1	1. 0	5. 0	2. 5
			1	1. 0	2. 5	0. 125
			1	0. 5	10	0. 625
C-12T	Wild-type	2	1	0. 5	5. 0	0. 625
			1	0. 25	5. 0	0. 625
C-14T	Wild-type	3	1	0. 25	10	0. 625
			1	1. 0	10	1. 25
			1	0. 25	0. 625	0. 625
Wild-type						
Wild-type[a]		1	1	4. 0	10	5. 0

Mutation	LPA	Total No. of Isolates	No. of Isolates	MIC (mg/L)		
				AMK	KAN	CAP
Wild-type		30	25	0. 25	0. 625	0. 625
			5	0. 5	0. 625	1. 25

[a]One isolate, characterized as wild-type but resistant to AMK, KAN and CAP at their respective breakpoints, could contain a mutation elsewhere that is not assessed by MTBDR*sl* or Pyrosequencing.

Table 2

Pyrosequencing results for 20 *Mycobacterium tuberculosis* clinical isolates with wild-type MTBDR*sl* assay results.

No. of isolates	Genotype MTBDR*sl assay*	DST critical concentration			PSQ
		AMK	KAN	CAP	
8	Wild-type	S	R	S	*eis* mutation
3	Wild-type	S	S	S	*eis* mutation
4	Wild-type	S	S	S	wild-type
1	Wild-type	R	R	R	Wild-type
4	*rrs*- MUT1	R	R	R	A1401G