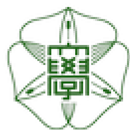
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Molecular analysis of streptomycin-resistance associating genes in *Mycobacterium tuberculosis* isolates from Nepal

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Abstract

Mutation in *rpsL* (encoding ribosomal protein S12), *rrs* (encoding 16S ribosomal RNA) and *gidB* (encoding 7-methylguanosine methyltransferase) are associated with resistance to streptomycin (STR), which is used for the treatment of multi-drug resistant tuberculosis (MDR-TB) in Nepal. The aim of our study is to analyze the correlation between mutations in the target genes and STR-resistance in 197 *Mycobacterium tuberculosis* (MTB) isolates from Nepal. Mutations in *rpsL* was harbored by 65.9% of isolates, in which the most common mutation in *rpsL* is caused by K43R (58.8%) and were significantly associated with Beijing genotype ($P<0.001$). About 13.2% of isolates harbored mutations in two highly mutable regions of *rrs*, the 530 loop and the 912 region. About 13.2% of *gidB* mutants do not show any mutation in *rpsL* and *rrs*, which might suggest the role of *gidB* mutations in STR-resistance in MTB. In addition, 5.6% of isolates do not show any mutations in three genes examined, suggesting the involvement of other mechanism in STR-resistance in MTB. Our findings can be implemented for the establishment of molecular STR-susceptibility testing, in which tuberculosis can be treated with appropriate drugs and can improve control strategies for DR-TB.

Keywords: *Mycobacterium tuberculosis*, streptomycin, drug-resistance, *rpsL*, *rrs*, *gidB*

42 **1. Introduction**

43 Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), remains a major global health
44 problem. According to the Global Tuberculosis Report (2019), World Health Organization (WHO)
45 estimated 10.0 million cases and 1.5 million deaths in 2018 [1]. Drug-resistant TB (DR-TB) tends to
46 increase worldwide, which causes difficulties in TB treatment. Multi-drug resistant TB (MDR-TB),
47 which is defined as TB resistant to at least two potent anti-TB drugs, isoniazid (INH) and rifampicin
48 (RIF), is a serious threat to fight against TB [2]. The WHO estimated that, worldwide in 2018, there
49 were half a million new cases of rifampicin-resistant TB (RR-TB), and of this, 78% had MDR-TB
50 [1]. Almost half (50%) of the global TB cases were in India (27%), China (14%), and the Russian
51 Federation (9%).

52 National TB Program (NTP) Nepal registered 32,474 TB cases in 2018 and estimated annual case
53 notification rate of 112/100,000 [3]. However, WHO estimated incidence rate of 151/100,000 and
54 death rate of 19/100,000 for Nepal, meaning NTP Nepal is yet to identify the missing cases in the
55 community and bring them to treatment [1].

56 Streptomycin (STR) has been an important drug for the treatment of pulmonary TB in human [4].
57 For the retreatment TB patients in Nepal, STR has been included in intensive phase for two months
58 with other first line anti-TB drugs [5]. However, NTP no longer recommends STR to be used in
59 retreatment regimen in Nepal [3]. According to the WHO recommended regimen as published in
60 "WHO treatment guidelines for MDR and RR-TB, 2018 Pre-final test"[6] and updated guidelines in
61 "WHO consolidated guidelines on drug-resistant tuberculosis treatment, 2019" [7], STR is
62 categorized into Group C agents in which it may be included in the treatment of MDR/RR-TB patients
63 aged 18 years or more on longer regimens when susceptibility has been demonstrated and adequate
64 measures to monitor for adverse reaction can be ensured, if amikacin is not available [3]. To move
65 into the new WHO recommendation, NTP will develop a comprehensive transition plan [3]. With an
66 increment of MDR-TB cases, and in addition of MDR-TB isolates resistant to second-line drugs and
67 newly discovered drugs, STR should be used in combination with other drugs for MDR-TB treatment
68 regimen. Despite its high rate of side effects and risk of developing drug resistance in TB of its single
69 use in a regimen [4], it is crucial to understand the molecular mechanism of resistance to STR in MTB
70 from Nepal.

71 STR impairs translational proofreading, leading to the blockade of protein synthesis. Resistance to
72 STR is associated with mutations in *rpsL* and *rrs* genes, encoding for ribosomal protein S12 and 16S
73 ribosomal RNA, respectively. The most frequent mutations localize around two regions of *rrs*, namely
74 the 530 loop and the 912 region, or at codons 43 or 88 within *rpsL* [8],[9]. Mutations within the *gidB*
75 gene, encoding a conserved 7-methylguanosine methyltransferase specific for the 16S rRNA, have

76 been reported to confer low-level STR-resistance in MTB [10]. Since, STR-resistance associating
77 genes vary geographically, it is important to perform the molecular studies which will detect the
78 common mutations in target genes conferring STR-resistance among MTB strains [10],[11].

79 The time consuming conventional proportional method using Löwenstein-Jensen medium is the
80 only available drug susceptibility testing (DST) for STR in Nepal. Recently, NTP have adopted the
81 latest diagnostic tools such as Xpert MTB/RIF, Line probe assay (LPA), and Mycobacterium Growth
82 Indicator Tube (MGIT) [3]. Despite the high number of MDR-TB cases with STR-resistant MTB
83 strains, no genetic information regarding the molecular mechanism of STR-resistant MTB in Nepal
84 is currently available.

85 The main aim of this study is to characterize the molecular mechanism of STR-resistance of MTB
86 in Nepal; to analyze the correlation between mutations in the *rpsL*, *rrs* and *gidB* and STR-resistant
87 MTB isolates from Nepal; and to study the association of those mutations with different MTB
88 genotypes. To elucidate mutations responsible for the resistance to STR, we performed sequence
89 analysis of STR-resistance associating genes (*rpsL*, *rrs* and *gidB*) in MDR-MTB isolates from Nepal.

90

91 ## 2. Materials and Methods

92 ### 2.1. Studied sample

93 MDR-TB isolates with resistant to all four first line drugs INH, RIF, STR and ethambutol (EMB)
94 has been preserved at the German Nepal Tuberculosis Project (GENETUP), Nepal. A total of 197
95 representative STR-resistant MTB isolates from April 2009 to March 2012 were selected in this study.
96 These samples were subjected to genotyping and mutation analysis.

97

98 ### 2.2. Drug susceptibility test (DST)

99 DST for INH, RIF, EMB (FatolArzneimittel GmbH, Schiffweiler, Germany) and STR (Sigma-
100 Aldrich, St. Louis, MO) were performed by the indirect proportional method on Löwenstein-Jensen
101 medium with the critical concentrations of 0.2, 40, 2 and 4 μg/ml, respectively [12],[13].

102

103 ### 2.3. DNA extraction

104 Extraction of DNA was done for PCR following the manufacturers protocol using the Genotype
105 MTBDR*plus*Ver2 (HainLifescienceNehren, Germany) [14].

106

107 ### 2.4. Spoligotyping

108 The genotypes of the MTB isolates were identified using spoligotyping which was performed as
109 described previously [15],[16].

110

111 **2.5. PCR amplification and sequencing**

112 Amplification of the 437 bp, 590 bp and 870 bp fragments of *rpsL*, *rrs* and *gidB*, respectively, were
113 performed using primers shown in Table 1. PCRs were carried out in a thermal cycler (Bio-Rad
114 Laboratories, Inc., Hercules, CA) under the condition previously described [17]. The presence of
115 PCR products was confirmed by agarose gel electrophoresis. Purified PCR products were
116 sequenced using DNA sequencer, 3500 Genetic Analyzer (Thermo Fisher Scientific, Inc., Waltham,
117 MA) using both forward and reverse primers same as for amplification. The obtained sequences
118 were aligned with the corresponding sequences from the wild-type *M. tuberculosis* H37Rv using
119 Bio-Edit software (version 7.0.9) [18].
120

121 **2.6. Statistical analysis**

122 All data were analyzed using R version 3.6. Pearson's Chi-square test was used to compare
123 different variances and to determine the association between MTB lineages, drug-resistance profile
124 and mutations in the genes examined. A p-value less than 0.05 was considered statistically significant.
125

126 **2.7. Ethical approval**

127 This study proposal was submitted to Nepal Health Research Council (NHRC) with register no
128 136/2013 on 20th Sep 2013 and approved by Ethical Review Board on 29th Nov 2013.
129

130 **3. Results**

131 **3.1. *Mycobacterium tuberculosis* genotypes**

132 Through spoligotyping, the genotypes of 197 STR-resistant MDR-MTB isolates were detected as
133 11 lineage 1, 116 lineage 2, 47 lineage 3 and 23 lineage 4 (Table 2).
134

135 **3.2. Mutation in *rpsL*, *rrs* and *gidB***

136 Mutations in only *rpsL* were harbored by 65.9% (130/197) of isolates. Mutations conferring
137 amino acid substitution at *rpsL* codon 43 and 88 were found in 58.8% (116/197) and 7.1% (14/197)
138 of isolates, respectively (Table 3).
139 There were seven different substitutions found at seven different positions in the *rrs* gene.
140 Mutations in only *rrs* was harbored by 13.2% (26/197) of isolates (Table 3). Mutations at positions

141 A514C and C517T of *rrs* were found in 5.6% (11/197) and 2.5% (5/197) of isolates, respectively.
142 Mutations around 912 loop (A906G, A907C, A908C or A912C) of *rrs* were found in 5.1% (10/197)
143 of isolates. A co-mutation in *rpsL* and *rrs* (*rpsL* K43R, *rrs* A907C; *rpsL* K88R, *rrs* A644G; and *rpsL*
144 G118D, *rrs* A514C) was found in 1.5% (3/197) of isolates.
145 Mutations in *gidB* was harbored by 25.8% (51/197) of isolates (Table 5). The *gidB* mutants
146 showing mutations in *rpsL* and/or *rrs* were found in 12.7% (25/197) of STR-resistant isolates
147 (Supplementary Table). About 13.2% (26/197) of *gidB* mutants do not show any mutation in *rpsL* and
148 *rrs* (Supplementary Table). No mutations in three genes examined in this study was found in 5.6%
149 (11/197) of isolates (Supplementary Table).
150
151 **4. Discussion**
152 According to our knowledge, this is the first study on the molecular characterization conferring
153 STR-resistance associating genes in MTB isolates from Nepal. It has been determined that mutations
154 in *rpsL* and *rrs* genes are associated with STR-resistance in MTB [20]. In MDR-TB isolates, about
155 80.7% (159/197) of STR-resistant MTB isolates harbored mutations in either *rpsL* or *rrs*. This is
156 within the range, reported from Asian countries, 40% to 98% [19],[20],[21],[22],[23]. Mutations in
157 *rpsL* was harbored by 65.9% (130/197) of isolates (Table 3), which suggest that these mutations
158 strongly correlate to STR-resistance in MTB. The most common mutation in *rpsL* codon K43R was
159 harbored by 58.8% of *rpsL* mutants in this study. The detection rate of mutations in *rpsL* codon K43R
160 varies considerably between geographical areas (Table 4). The high detection rate of mutation in *rpsL*
161 K43R in Nepal might be due to its non-restrictive mutation leading to no fitness cost in MTB [24]
162 which can be widely transmitted; and the bad practice of STR mono-therapy usage for the treatment
163 of TB and other bacterial diseases in the past in Nepal [25]. The predominant mutation *rpsL* K43R
164 was found in 80% (92/117) of Beijing isolates. There was a significant association between Beijing
165 strains and mutation K43R compared with the group of STR-resistant non-Beijing isolates ($P<0.001$).
166 Sun et al reported that mutation in *rpsL* causing K43R showed strong association with Beijing
167 genotype in STR-resistance isolates [22]. However, in India and Mexico, where Beijing genotype was
168 not frequent, mutations in these two loci had no significant contribution to Beijing genotype [19],[21].
169 The Beijing genotype has been reported as more virulent than other MTB genotypes, and has a
170 propensity to acquire the *rpsL* K43R mutation [22]. The high mutation rate in *rpsL* K43R represents
171 the geographical areas predominate by Beijing genotype family. In contrast, mutation *rpsL* K88R was
172 not significantly associated with the Beijing family ($P=0.34$). Mutations in *rpsL* K43R and G71D
173 were found in one STR-resistant non-Beijing isolate. As mutation K43R of *rpsL* strongly contributes
174 to STR-resistance in MTB, it is doubtful that whether mutation G71D of *rpsL* contribute to STR-

175 resistance in MTB or not. Mutation conferring G71D found in this study, was reported by study [26]
176 with an isolate with mutation in *rpsL* G71S (GGC→AGC), compounded with mutation in *rpsL* K43R;
177 and might be of only minor importance.
178 About 13.2% (26/197) of isolates harbored *rrs* mutations (except co-mutation in *rpsL*) in our study,
179 which showed a frequency range of 0 to 12.5% (Table 4) in the Mideast and Southeast Asian countries
180 [27],[28],[29],[30],[31]. Mutations in *rrs* were found in two highly mutable regions of *rrs*, the 530
181 loop and the 912 region, which suggest these mutations contributed to STR-resistance in MTB as
182 reported [8],[10],[19],[20],[21],[27],[32],[33],[34],[35],[36].
183 Three strains showed co-mutations in both *rpsL* and *rrs*. Among them, one strain showed
184 mutations in *rpsL* causing K43R and *rrs* 912 region A907C. Meier et al had reported the similar
185 findings of co-mutations in *rpsL* and *rrs*, which described that the *rrs* mutation (A→G at position
186 904) presumably conferred as yet ill-defined partial resistance to STR in vivo, thereby increasing
187 the number of cells which could acquire a second mutation in the *rpsL* (alteration of K88),
188 conferring resistance to STR-induced misreading [33]. Similar co-mutations *rpsL* K43R/*rrs* C789T
189 [37]; and *rpsL* K88Q/*rrs* A905G and *rpsL* K43R/*rrs* A513C [38] have been reported. In contrast,
190 Jagielski et al., had reported that mutations in *rrs* and *rpsL* were mutually exclusive, that is isolates
191 with *rrs* mutations had no *rpsL* mutations and vice versa [39]. The discrepancy in our results might
192 be due to the presence of mixed population of *rpsL* K43R mutants and *rrs* A907C mutants. This was
193 confirmed to be 1:1 mixture of A and G at nucleotide position 128 in *rpsL* by the electropherogram
194 obtained. One strain harbored mutations in *rpsL* causing K88R and *rrs* A644G. One studies had
195 reported an isolate with mutation in *rpsL* K43R and a second mutation in *rrs* nucleotide 645 A
196 deletion [26]. A mutation in *rrs* A644G is a novel mutation and this mutation is located far away
197 from hot spot mutation regions of *rrs*, the 530 loop and 912 region, which is associated with the
198 STR-resistance in MTB. As mutation in *rpsL* causing K88R is enough to cause STR-resistance in
199 MTB, a mutation in *rrs* A644G might not be associated with the STR-resistance in MTB but be a
200 natural polymorphism. Other strain harbored mutations in *rpsL* causing G118D and *rrs* A514C. As
201 MTB has a single rRNA operon [40], a single mutation in *rrs* A514C is enough to cause STR-
202 resistance in MTB. Mutation G118D have not been previously reported and this mutation is located
203 far away from frequent mutations in *rpsL*, K43R and K88R, which cause STR-resistance in MTB. It
204 is unclear that the mutation in *rrs* A644G and mutation in *rpsL* causing G118D may affect the
205 mechanism of STR-resistance in MTB. So, further studies should be carried out to find out the exact
206 role of these mutations in causing STR-resistance in MTB.
207 We observed that 13.7% (27/197) of STR-resistant isolates showed many mis-sense mutations in
208 *gidB* that were close to active or part of the interacting amino acid residues between the GidB protein

209 and S-adenosylmethionine (SAM) which are critical for methylation at the position G517 of 16S
210 rRNA [27]. These alterations may have an impact on the function of GidB which leads to low affinity
211 of STR to 16S rRNA and hence contribute to STR-resistance in MTB. The missense mutations in
212 *gidB* codons causing amino acid substitutions A19G, R20P, A27P, A27V, V36L, A82P, L101E,
213 V112G, V124G, A133V, M218V.

214 The *gidB* mutants harboring co-mutations in *rpsL*/*rrs* genes were found in 12.7% (25/197) of
215 isolates. These co-mutations of in *rpsL*/*rrs* of *gidB* mutants might suggest the evolution of *rpsL*/*rrs*
216 mutations for its existence as a compensation for losing methylation function of GidB caused by
217 distortion of its structure and consequently results in high level resistance to STR [10],[36].

218 It was found that 13.2% (26/197) of isolates showed mutations in *gidB* without those in *rpsL* and
219 *rrs* genes. All 13 mis-sense mutations had an exclusive *gidB* mutation without mutations in *rpsL* and
220 *rrs* conferring these *gidB* mutations contribute to STR-resistance in MTB. Deletion mutations in *gidB*
221 nucleotide without those in *rpsL* and *rrs* (112delC, 254delA, 347delG and 372delG) and insertion
222 mutations in *gidB* nucleotide (243insGC) causing frameshift in *gidB* in STR-resistant isolates found
223 in this study which alter the GidB protein and consequently leading to low-level STR-resistance in
224 MTB. Deletions with variable sizes at different positions in *gidB* were also reported [10],[8],[30].
225 Frameshift mutations (112delC, 254delA, 347delG, 372delG, 384delG and 243insGC) were
226 previously reported. The deletion mutation in *gidB* delG at nucleotide 98 have also been reported in
227 both STR-susceptible and STR-resistant isolates [39],[41]. Mutations in *gidB* found exclusively in
228 STR-resistant strains play a role in STR-resistance in MTB. These 13 mis-sense mutations and 5
229 frameshift mutations in *gidB* were highly associated with STR-resistance in MTB.

230 There were four *gidB* polymorphisms phylogenetically specific to MTB lineages which were found
231 in 75.6% (149/197) of isolates. The missense mutation E92D conferred by 100% (116/116) of lineage
232 2 isolates which was significantly associated with the Beijing family of lineage 2 ($P<0.001$). Similar
233 indication of high specificity to Beijing strains have been reported in previous studies
234 [34],[35],[36],[42]. The silent mutation in *gidB* codon at V110 was significantly associated with EAI
235 strains ($P<0.001$), which is also supported by previous findings [34],[42]. Another silent mutation in
236 *gidB* at codon A205 was present in 100% (23/23) of the Euro-American strains of lineage 4 which
237 was significant ($P<0.001$). In contrast, polymorphisms in *gidB* codon at A205 was absent from almost
238 all of the Euro-American strains in which this allele may be used as a specific marker for
239 differentiating Euro-American strains from non-Euro-American strains [34],[42]. Finally, all LAM
240 strains conferred the allele mutation in *gidB* codon at L16 was significant ($P<0.01$) which is also
241 confirmed by the previous findings [36],[43],[44].

242 In total, 94.4% (186/197) of STR-resistant isolates harbored mutations in either *rpsL* or *rrs* or *gidB*

243 alone or in combinations. This suggests that mutations in *rpsL*, *rrs* and *gidB* play a significant role in
244 the mechanism of STR-resistance in MTB. In this study, we found 11 isolates (one lineage 1 strains,
245 two lineage 2 strains, seven lineage 3 strains and one lineage 4 strains) do not harbor mutation in any
246 of the genes studied, accounting for 5.6% of the STR-resistant strains. This suggests that there might
247 be an involvement of other genes in the mechanism of STR-resistance such as the efflux system, cell
248 membrane permeability barrier or aminoglycosides converting enzymes.

249 In conclusion, the mutations in *rpsL* and *rrs*; and some exclusive mutations in *gidB* identified in
250 this study are responsible for STR-resistance in MTB. The sequence analysis of mutations in *rpsL*,
251 *rrs* and *gidB* could be used for the establishment of rapid and effective molecular diagnostic tools to
252 detect STR-resistant MTB strains in Nepal. This study can be further implicated for designing rapid
253 diagnostic tools considering geographic specificity in which MDR-TB transmission can be interrupt
254 worldwide.

255

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402

403 **Table 1 Primers used for PCR amplification and sequencing of streptomycin-resistance**
404 **associated genes in *M. tuberculosis***

Locus	Primers	Nucleotide sequence (5'-3')	Product size (bp)
rpsL	Forward (ON-1505)	GGCCGACAAACAGAACGT	437
	Reverse (ON-1506)	CTTCACCAACTGGGTGAC	
rrs	Forward (ON-1507)	GATGACGGCCTTCGGGTTGT	590
	Reverse (ON-1508)	AGGCCACAAGGGAACGCCTA	
gidB	Forward (ON-1509)	CGCCGAGTCGTTGTGCT	870
	Reverse (ON-1510)	AGCCTGGCCCGACCGTTA	

405 bp represents base pair

406 **Table 2 Distribution of 197 streptomycin-resistant *Mycobacterium tuberculosis* isolates into**
407 **different lineages**

Lineages	**Number of isolates**
1	11
2 (Beijing)	116
3	47
4	23
Total	**197**

408

409 **Table 3 Distribution of mutations in *rpsL* and *rrs* genes among different genotypes of 197**
410 **streptomycin-resistant *Mycobacterium tuberculosis* isolates**

***rpsL* nucleotide (amino acid substitution)**	***rrs* nucleotide**	**Lineage 1**	**Lineage 2**	**Lineage 3**	**Lineage 4**	**No. of isolates**
A128G (K43R)	-	2	91	13	9	115
A128G (K43R)	A907C		1			1
A128G (K43R), G212A (G71D)*	-			1		1
A263G (K88R)	-		12	2		14
A263G (K88R)	A644G*		1			1
G353A (G118D)*	A514C				1	1
-	A514C	1	5	2	3	11
-	A517T	1	1	2	1	5
-	A906C			1		1
-	A907C		3			3
-	A908C			3	2	5
-	A912G			1		1
-	-	7	2	22	7	38
Total		**11**	**116**	**47**	**23**	**197**

411 Fs denotes frameshift mutation
412 No. represents number
413 * denotes mutation previously not reported
414 - denotes no mutation.

415 **Table 4 Detection rate of mutations in *rpsL*, *rrs* and *gidB* between countries**

Countries / **Mutations**	**This study (N=197)**	**India (N=52)**	**Pakistan (N=32)**	**China (N=227)**	**Myanmar (N=141)**	**Thailand (N=46)**
***rpsL* codon K43R**	58.8%	32.6%	34.3%	47.5%	64.0%	69.5%
***rpsL* codon K88R**	7.1%	19.2%	3.1%	12.3%	6.0%	2.1%
rrs	13.2%	5.7%	12.5%	5.7%	3.5%	0%
gidB	13.2%	25.0%	21.8%	12.7%	2.7%	2.1%
Reference	This study	[27]	[28]	[29]	[30]	[31]

416

417 **Table 5 Distribution of mutations in *gidB* among different genoytpes of 197 streptomycin-**
418 **resistant *Mycobacterium tuberculosis* isolates**

***gidB* nucleotide (amino acid substitution)**	**Lineage 1**	**Lineage 2**	**Lineage 3**	**Lineage 4**	**No. of isolates**
G28C (A10P)			2		2
G28C (A10P), A119G (E40G)	1				1
C56G (A19G)*, γ, θ				1	1
G59C (A20P)*, β	1				1
G79C (A27P)*			1		1
C80T (A27V)*, α		1			1
G106C (V36L)*			1		1
C142A (H48N), γ				2	2
T236G (L79Y)			1		1
G244C (A82P)*			1		1
T302G (L101E)*			1		1
T335G (V112G)*			1		1
T371G (V124G)*			1		1
C398T (A133V)*, α		1			1
C413T (A138V), γ				1	1
G471A (G157G)*, α		2			2
A487G (K163E)			1		1
C492T (G164G)*			1		1
T583C (Y195H), α		7			7
G630A (G210G)*, α		1			1
A652G (M218V)*, γ				2	2
243insGC (Fs)*, γ				1	1
98delG (R33Fs)			6		6
98delG (R33Fs), β	3				3
98delG (R33Fs), γ				3	3
112delC (P38Fs)*, β	1				1
112delC (P38Fs)*, γ				1	1
254delA (D85Fs)*, γ				1	1
347delG (R116Fs)			1		1
372delG (V124Fs)*			1		1
384delG (L128Fs)			2		2
Α	1	104			105
Β	4				4
Γ				8	8
γ, θ				3	3
-			26		26
Total	**11**	**116**	**47**	**23**	**197**

419 A276C (E92D)=α, G330T (V110V)=β, G615A (A205A)=γ and T47G (L16R)=θ
420 Fs denotes frameshift mutation
421 No. represents number
422 * denotes mutation previously not reported
423 - denotes no mutation
424 del denote deletion mutation
425

Supplementary Table: Distribution of mutations in *rpsL*, *rrs* and *gidB* genes among different genotypes of 197 streptomycin (STR)-resistant *Mycobacterium tuberculosis* isolates

***rpsL* nucleotide (codon)**	***rrs***	***gidB* nucleotide (codon)**	**Beijing**	**EAI**	**Euro-American**	**CAS**	**Other**	**No. of isolates**
A128C(K43R)	-	α	77	2	1			80
A128C(K43R)	-	α, C398T(A133V)*	1					1
A128C(K43R)	-	α, T583C(Y195H)	7			1		8
A128C(K43R)	-	A445C(S149R), γ	1					1
A128C(K43R)	-	α, G630A(G210G)*	1					1
A128C(K43R)	-	112delC (P38Fs)*, β		1				1
A128C(K43R)	-	C286T (R96C)*	1					1
A128C(K43R)	-	G106C (V36L)*				1		1
A128C(K43R)	-	G28C (A10P)				2		2
A128C(K43R)	-	β	1					1
A128C(K43R)	-	γ	1		6			7
A128C(K43R)	-	A652G (M218V)*, γ			1			1
A128C(K43R)	-	γ, θ			1			1
A128C(K43R)	-	-	1			9		10
A128C(K43R)	A907C	α	1					1
A128C(K43R), G212A(G71D)*	-	-				1		1
A263G(K88R)	-	α	10					10
A263G(K88R)	-	G471A (G157G)*, α	2					2
A263G(K88R)	-	C492T (G164G)*				1		1
A263G(K88R)	-	-				1		1
A263G(K88R)	A644G*	α	1					1
G353A(G118D)*	A514C	98delG (R33Fs), γ			1			1
-	A514C	α	4					4
-	A514C	C80T (A27V)*, α	1					1
-	A514C	98delG (R33Fs), γ			2			2
-	A514C	β		1				1
-	A514C	γ, θ			1			1
-	A514C	-		1				1
-	A517T	α	1					1
-	A517T	β		1				1
-	A517T	γ, θ			1			1
-	A517T	-		1		1		2
-	A906G	-				1		1
-	A907C	α	3					3
-	A908C	C142A (H48N), γ			2			2
-	A908C	384delG (L128Fs)		1				1
-	A908C	-		1		1		2
-	A912G	384delG (L128Fs)				1		1
-	-	243insGC (Fs)*, γ					1	1
-	-	254delA (D85Fs)*, γ			1			1
-	-	α	2					2
-	-	A487G (K163E)				1		1
-	-	C413T (A138V), γ			1			1
-	-	112delC (P38Fs)*, γ					1	1
-	-	G244C (A82P)*				1		1
-	-	347delG (R116Fs)				1		1
-	-	372delG (V124Fs)*				1		1
-	-	G59C (A20P)*, β		1				1
-	-	γ			1			1
-	-	A652G (M218V)*, γ			1			1
-	-	G79C (A27P)*				1		1
-	-	98delG (R33Fs)		1		5		6
-	-	98delG (R33Fs), β		3				3
-	-	G28C(A10P), A119G(E40G)					1	1
-	-	β		1				1
-	-	T236G (L79Y)				1		1
-	-	T302G (L101E)*				1		1
-	-	T335G (V112G)*				1		1
-	-	T371G (V124G)*				1		1
-	-	C56G (A19G)*, γ, θ			1			1
-	-	-	1	1		6		8
Total			**117**	**16**	**21**	**40**	**3**	**197**

A276C (E92D)=α, G330T (V110V)=β, G615A (A205A)=γ and T47G (L16R)=θ

EAI, East-African Indian; CAS, Central Asian strain; and other, orphan and X2.

Fs denotes frameshift mutation.

* denotes frameshift mutation.

- denotes no mutation.

del denote deletion mutation.

Ins denote insertion mutation.

α, β, γ and θ represent A276C (E92D), G330T (V110V), G615A (A205A) and T47G (L16R), respectively.