

1 Specific *gyrA* gene mutations correlate with high prevalence of 2 discordant levofloxacin resistance in *Mycobacterium tuberculosis* 3 isolates from Beijing, China

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17 ABSTRACT

18 Although molecular diagnostics are highly sensitive in the diagnosis of fluoroquinolone
19 (FQ)-resistant tuberculosis (TB), the discordant results with the sequential phenotypic
20 drug susceptibility testing (DST) invariably occur. To investigate the prevalence and
21 the cause of discordant results between molecular and phenotypic DST for
22 levofloxacin (LFX), the cases who were determined to be LFX-susceptible using the

phenotypic DST but LFX-resistant using the MeltPro assay were retrospectively reviewed in Beijing, China. We measured LFX minimal inhibitory concentrations (MICs) using Middlebrook 7H9 broth. Sanger sequencing was used to determine genotypic characteristics of discordant isolates. Between January and December 2018, 126 (22.1%) out of 571 smear-positive TB patients were identified as LFX-resistant TB by MeltPro assay. Among the 126 LFX-resistant TB, there were 34 isolates identified as LFX-susceptible TB by phenotypical DST. This result demonstrated a discordance prevalence of 27.0%. LFX MICs were majorly centered around the critical concentration, and 7 (21.2%) and 13 (39.4%) had MICs of 2.0 mg/l and 4.0 mg/l, respectively. The most prevalent mutations conferring discordant LFX resistance were the amino acid substitutions of Ser to Leu in 90 codon (13, 39.4%) and Asp to Ala in 94 codon of *gyrA* (11, 33.3mg/l belonged to Ala90Val and Asp94Ala group. In conclusion, our data demonstrate that more than one quarter of LFX-resistant isolates by molecular method are susceptible to LFX using the phenotypic DST. The high prevalence of discordant LFX resistance is majorly due to the isolates with specific mutations within *gyrA* gene conferring proximity of MICs to the critical concentration.

KEYWORDS: tuberculosis, levofloxacin, drug susceptibility testing, MeltPro assay, *gyrA*

INTRODUCTION

Despite the great achievements in tuberculosis (TB) control globally, the epidemic of

drug-resistant tuberculosis, especially multidrug-resistant tuberculosis (MDR-TB), poses a major barrier to eliminate TB (1, 2). In 2018, the World Health Organization (WHO) estimates that 48, 400 MDR/RR-TB cases have occurred worldwide, only 32% of which were successfully diagnosed and treated (2). Therefore, rapid and accurate laboratory diagnosis of drug susceptibility of MTB is essential to ensure early initiation of appropriate treatment for drug-resistant TB patients, thus preventing further transmission in the community (3, 4).

Conventional diagnosis of drug-resistant TB requires the phenotypical drug susceptibility testing (DST) using growth-based methods (5). Due to the slow growth rate of tubercle bacilli, it takes weeks to months to yield final results (5). Recently, several commercial molecular tests have been endorsed by WHO for the initial diagnosis of drug-resistant TB, such as GeneXpert MTB/RIF (Cepheid, USA), and GenoType MTBDRplus (Hain Lifescience, Germany) (6, 7). These tests detect drug resistance by identifying the core genetic regions responsible for drug resistance in a timely manner (3). Although a serial of clinical trials have demonstrated that molecular tests are highly sensitive in the diagnosis of drug-resistant TB, especially for rifampin, isoniazid and fluoroquinolone, the discordant results with the sequential phenotypic DST invariably occur (8). The situation is more complicated if the “false-positive” result is reported by molecular test considering that the patients have undergone the therapy for drug-resistant TB. Discordant results between molecular and phenotypic rifampicin susceptibility testing are reported, resulting in diagnostic

and clinical management dilemmas (9). Understanding the interpretation of these discordant results is thus important to ensure optimal diagnosis and treatment.

Fluroquinolones (FQs) are cornerstone drugs in the treatment of MDR-TB (10). The subunit A of gyrase (*gyrA*) is the primary target of current FQs in MTB, thus killing bacteria via inhibiting DNA supercoiling and disrupting DNA replication (11, 12). Thus, the mutations located in *gyrA* gene are considered as an alternative for predicting FQ resistance in MTB (12). In China, the MeltPro TB assay (MeltPro, Zenssen, China) is the only commercial molecular test approved for the diagnosis of FQ-resistant TB, and also widely used in clinical practice since 2018 (13). However, limited knowledge is available to examined the prevalence of false-positive results between molecular and phenotypic DST for FQs. To address this concern, we retrospectively reviewed the cases who were determined to be levofloxacin (LFX)-susceptible using the phenotypic DST (pDST) but LFX-resistant using the MeltPro assay.

MATERIALS AND METHODS

Study Design. This is a retrospective analysis of laboratory results and stored clinical isolates were collected for routine clinical practice. The study was conducted in the Beijing Chest Hospital, which is the National Clinical Centre on Tuberculosis in China. Consecutive individuals with LFX resistance by MeltPro were reviewed between January 2018 and December 2018. The cases without confirmatory LFX

resistance testing were excluded from further analysis. We collected the MTB isolates from the cases with discordant LFX resistance results between MeltPro and phenotypic DST. Discordance was defined as an initial MeltPro result with LFX resistance with only LFX-susceptible follow-up phenotypic DST tests. The sputum specimens from the smear-positive TB patients were collected for MeltPro TB assay. The MeltPro assay testing was performed as previously described (Fig. 1) (13). In addition, the commercial 96-well plate containing lyophilized antibiotics were used for determination of *in vitro* susceptibility of MTB isolates to first- and second-line anti-tuberculosis drugs according to the manufacturer's instructions (Encode Medical Engineering Co., Zhuhai, China). Susceptibility was judged on the basis of the following drugs and concentrations: isoniazid (0.2 mg/l), rifampicin(1.0 mg/l), levofloxacin(2.0 mg/l), ethambutol(2.5 mg/l), amikacin(1.0 mg/l) , capreomycin(2.5 mg/l).

Bacterial strains and culture condition. All MTB isolates were stored in the Middlebrook 7H9 medium containing 5% glycerol at the BioBank of Tuberculosis, Beijing Chest Hospital. Prior to experiments, the bacterial cells were recovered on Lowenstein-Jensen (L-J) medium for 4 weeks at 37°C.

Minimal inhibitory concentration (MIC). The MICs for LFX were determined in Middlebrook 7H9 broth containing oleic acid-albumin-dextrose-catalase (OADC) (14). Fresh colonies were harvested from the surface of L-J medium, and added to a

saline solution with glass beads. After vortex for 1 min, the suspension was adjusted to turbidity at a McFarland standard of 1.0. 200 µl bacterial diluent was transferred to 20 ml of 7H9 broth, and 100 µl of this dilution was inoculated into each well. The drug concentrations tested were doubling dilution, ranging from 0.063 to 64 mg/l. Plates were incubated at 37°C in 5% CO₂ for one week. Then 70 µl of AlamarBlue and 5% Tween80 2:5 mixed solution was added to each well, incubated for 24 h at 37°C, and assessed for color development. As a consequence of bacterial growth, the oxidation-reduction indicator AlamarBlue changed color from blue to pink (15). The MIC was defined as the lowest concentration of drug that prevented the color change from blue to pink. The reference MTB strain H37Rv (ATCC 27249) was tested in both rounds for quality control purpose.

DNA sequencing. The crude genomic DNA was extracted from freshly cultured bacteria using the simple boiling method as previously described (16). The genomic DNA was used as template to conduct PCR amplification. The partial fragment of *gyrA* gene, containing quinolone resistance-determining region, were amplified (17). The PCR mixture were prepared as follows: 25 µl 2×PCR Mixture, 2 µl of DNA template and 0.2 µM of each primer set. After completion of the thermal cycler program, the amplicons were sent to Tsingke Company (Beijing, China) for DNA sequencing service. DNA sequences were compared with the homologous sequences of MTB H37Rv strain to identify whether the sequences had genetic mutations within QRDR.

RESULTS

Between January and December 2018, 571 smear-positive TB patients were screened by MeltPro assay (Fig. 2). Of these, 126 (22.1%) were identified as LFX-resistant TB. Phenotypical DST showed that 92 isolates were LFX-resistant, while the remaining 34 were LFX-susceptible, demonstrating a discordance prevalence of 27.0%. After exclusion of one isolate due to subculture failure, a total of 33 discordant isolates from 33 patients were further analyzed. As showed in Table 1, the isolates displayed a wide range of MICs with the majority centered around the critical concentration of 2.0 mg/l. Out of isolates tested, 7 (21.2%) and 13 (39.4%) had MICs of 2.0 mg/l and 4.0 mg/l, respectively.

DNA sequencing data revealed that the most prevalent mutations conferring discordant FQ resistance were the amino acid substitutions of Ser to Leu in 90 codon (13, 39.4%) and Asp to Ala in 94 codon of *gyrA* (11, 33.3%), respectively. Mutations Thr91Pro ($n=1$), Asp94Gly ($n=1$), Asp94Val ($n=1$), Asp94Tyr ($n=1$) and Asp94Asn ($n=1$) were detected in five separate isolates. In addition, two heteroresistant isolates were identified by Sanger sequencing plots. In one isolate, the mutant G allele in 94 codon of *gyrA* (94Gly) was mixed with the wild-type A allele (94Asp) (Fig. 3A). Double point mutations were observed in the other heteroresistant isolate carrying both the Thr91Pro and Asp94Gly mutations (Fig. 3B). Notably, two FQ-resistant isolates reported by MeltPro assay had no mutation within *gyrA* gene by DNA

sequencing. By re-examination of MeltPro patterns, we found that both these isolates had two melting curve peaks (Fig. 4), representing the simultaneous presence of mutant and the wild type in the specimens.

Mutations in the *gyrA* gene and corresponding MICs of the 33 selected isolates are shown in Table 2. 18 of these isolates were confirmed to be resistant to LFX by using 2.0 mg/l as cut-off value, and the remaining 15 isolates were susceptible to LFX. The LFX MICs for MTB isolates with the mutation Ala90Val ranged from 2.0 to 8.0 mg/l. In addition, the lower LFX values were observed for Asp94Ala mutants, ranging from 1.0 to 4.0 mg/l; however, all five other *gyrA* mutants had MICs of ≥ 4.0 mg/L. At the same time, the 2 genetically heterogeneous populations harbored MIC values of 4.0 mg/L. Importantly, all 13 LFX-susceptible isolates with *gyrA* mutations belonged to Ala90Val and Asp94Ala group.

DISCUSSION

Fluroquinolones are widely used in the treatment of MDR-TB, and have also been contained in several regimen trials for the treatment of drug-susceptible and drug-resistant TB (10, 18), highlighting the urgent need for early diagnosis of *in vitro* FQ susceptibility. In the present study, our data demonstrated that more than one quarter of LFX-resistant TB cases identified by molecular diagnostics were phenotypically LFX-susceptible. The high rate found in this study may be majorly due to the fact we used broth-based assays for drug susceptibility testing instead of the

solid agar-based methods, which are more likely to identify the drug-resistant MTB isolates with low-level resistance (19). In addition, although phenotypical DST is considered as gold standard for detection of *in vitro* drug susceptibility, there is increasing evidence that the low-level resistance is associated with a marked decrease in reproducibility of this phenotypical method, even in the sophisticated laboratories (8, 20). As a consequence, the isolates with MICs that is very close or equal to the defined critical concentration for the tested drug are prone to be missed. These isolates are termed “challenging isolates”, as they are responsible for the majority of discordant results between phenotypical and genotypical method.

In addition, the heteroresistance is another important cause of the discordant LFX susceptibility results (21). On one hand, the coexistence of wide-type and mutant tubercle bacilli may result in a shift toward lower MIC value compared with that of mutant bacilli (16). Despite having the mutant population (Asp94Gly) with high-level resistance to LFX, the MIC values of two isolates located around the critical concentration. On the other hand, the Sanger sequencing failed to identify the presence of mutant tubercle bacilli from the two isolates recovered from clinical sputum. Numerous previous studies have demonstrated that the *gyrA* mutations do not only seem to have an impact on the FQ resistance but also on the fitness of the strains (22). Thus, the loss of relative fitness associated with specific mutations in *gyrA* gene may explain why the bacterial population structure changed significantly during the subculture period.

199

200 It is well known that various *gyrA* mutations are associated with different levels of
201 resistance to LFX (11). In consistent to previous findings, we found that the MICs of
202 isolates with either Ala90Val or Asp94Ala mutation are scattered around the critical
203 concentrations of LFX (11, 23). In a previous retrospective study, the treatment failure
204 with FQ-containing regimen was reported in patients infected with isolates harboring
205 these mutations, suggesting that they are probable resistant to FQ (24), but may be
206 missed by the conventional DST methods. Considering that Ala90Val and Asp94Ala
207 mutation are the most predominant mutation sites conferring FQ resistance in China
208 (11), the high prevalence of discordant levofloxacin resistance could be expected in
209 clinical practice.

210

211 Our findings provide important implications for the diagnosis and management of
212 drug-resistant tuberculosis. First, the low-level drug resistance is believed to be the
213 most important reason for the disputed LFX resistance. In other words, the molecular
214 diagnostic algorithm outperforms the phenotypical DST methods for detecting the
215 LFX-resistant isolates with mutations conferring proximity of MICs to the critical
216 concentration. Thus, the reliable LFX-resistance by molecular methods should be
217 adopted in individualization of multidrug regimens. Second, operational aspects of the
218 methods should be mentioned. Conventional DST methods remain technically
219 cumbersome, and the culture-based characteristic requires time to obtain positive
220 isolates (3). In contrast, the commercial molecular DST methods could be directly

used on sputum samples (preferably smear positive), which saves intervals over phenotypic methods requiring culture. A recent external quality assessment (EQA) study of TB laboratories in China demonstrated that approximate 40% of laboratories failed to provide certified conventional DST results; however, the EQA results for molecular diagnostics were satisfactory for these laboratories (25). Taken together, these results highlight the potential clinical application of molecular diagnostics in diagnosis of drug-resistant TB, especially for resource-limited settings. Third, previous molecular epidemiological studies on FQ resistance always included phenotypically resistant MTB isolates determined by phenotypical DST (11, 23). In view of their restricted capability to detect low-level resistant isolates, the prevalence of mutations within *gyrA* locus conferring low-level resistance may be underestimated.

We also acknowledge the limitations in the study. First, the major limitation is the small sample size in our analysis. The first commercial kit for detecting FQ susceptibility, MeltPro TB assay, was approved by Chinese FDA in 2018. Hence, the short-term application of this method become the major barrier to recruit more cases. Further study will be carried to validate our conclusion in larger samples. Second, the discordant LFX susceptibility was not assessed by another phenotypic DST method. It is difficult to compare the capability of different phenotypic methods to detect low-level LFX resistance. Third, the clinical outcomes of these cases with discordant LFX susceptibility were not evaluated due to the high proportion of loss to follow-up.

243

244 In conclusion, our data demonstrate that more than one quarter of LFX-resistant
245 isolates by molecular method are susceptible to LFX using the phenotypic DST. The
246 high prevalence of discordant LFX resistance is majorly due to the isolates with
247 specific mutations within *gyrA* gene conferring proximity of MICs to the critical
248 concentration. Our results will provide important implications into the clinical
249 interpretations of discordant LFX resistance between phenotypical and genotypical
250 DST methods.

251

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260 **REFERENCES**

- 261 1. Van Rie A, Warren RM. 2015. MDR tuberculosis control: time to change the dogma?
262 Lancet Respir Med 3:907-9.
- 263 2. World Health Organization. 2019. Global Tuberculosis Control 2019.
- 264 3. Weyer K, Mirzayev F, Migliori GB, Van Gemert W, D'Ambrosio L, Zignol M, Floyd K,
265 Centis R, Cirillo DM, Tortoli E, Gilpin C, de Dieu Iragena J, Falzon D, Raviglione M.
266 2013. Rapid molecular TB diagnosis: evidence, policy making and global implementation
267 of Xpert MTB/RIF. Eur Respir J 42:252-71.

- 268 4. Pang Y, Xia H, Zhang Z, Li J, Dong Y, Li Q, Ou X, Song Y, Wang Y, O'Brien R, Kam KM,
269 Chi J, Huan S, Chin DP, Zhao Y. 2013. Multicenter evaluation of genechip for detection
270 of multidrug-resistant *Mycobacterium tuberculosis*. *J Clin Microbiol* 51:1707-13.
- 271 5. Kim SJ. 2005. Drug-susceptibility testing in tuberculosis: methods and reliability of
272 results. *Eur Respir J* 25:564-9.
- 273 6. World Health Organization. 2013. Using the Xpert MTB/RIF assay to detect pulmonary
274 and extrapulmonary tuberculosis and rifampicin resistance in adults and children.
- 275 7. World Health Organization. 2019. The use of molecular line probe assays for the
276 detection of resistance to isoniazid and rifampicin.
- 277 8. Van Deun A, Barrera L, Bastian I, Fattorini L, Hoffmann H, Kam KM, Rigouts L,
278 Rusch-Gerdes S, Wright A. 2009. *Mycobacterium tuberculosis* strains with highly
279 discordant rifampin susceptibility test results. *J Clin Microbiol* 47:3501-6.
- 280 9. Mvelase NR, Pillay M, Sibanda W, Ngozo JN, Brust JCM, Mlisana KP. 2019. *rpoB*
281 Mutations Causing Discordant Rifampicin Susceptibility in *Mycobacterium tuberculosis*:
282 Retrospective Analysis of Prevalence, Phenotypic, Genotypic, and Treatment Outcomes.
283 *Open Forum Infect Dis* 6:ofz065.
- 284 10. World Health Organization. 2019. WHO consolidated guidelines on drug-resistant
285 tuberculosis treatment
- 286 11. Zhang Z, Lu J, Wang Y, Pang Y, Zhao Y. 2014. Prevalence and molecular characterization
287 of fluoroquinolone-resistant *Mycobacterium tuberculosis* isolates in China. *Antimicrob*
288 *Agents Chemother* 58:364-9.
- 289 12. Zhang Y, Yew WW. 2015. Mechanisms of drug resistance in *Mycobacterium tuberculosis*:
290 update 2015. *Int J Tuberc Lung Dis* 19:1276-89.
- 291 13. Pang Y, Dong H, Tan Y, Deng Y, Cai X, Jing H, Xia H, Li Q, Ou X, Su B, Li X, Zhang Z,
292 Li J, Zhang J, Huan S, Zhao Y. 2016. Rapid diagnosis of MDR and XDR tuberculosis
293 with the MeltPro TB assay in China. *Sci Rep* 6:25330.
- 294 14. Zhang T, Jiang G, Wen S, Huo F, Wang F, Huang H, Pang Y. 2019. Para-aminosalicylic
295 acid increases the susceptibility to isoniazid in clinical isolates of *Mycobacterium*
296 *tuberculosis*. *Infect Drug Resist* 12:825-829.
- 297 15. Yajko DM, Madej JJ, Lancaster MV, Sanders CA, Cawthon VL, Gee B, Babst A, Hadley
298 WK. 1995. Colorimetric method for determining MICs of antimicrobial agents for
299 *Mycobacterium tuberculosis*. *J Clin Microbiol* 33:2324-7.
- 300 16. Zhang Z, Wang Y, Pang Y, Liu C. 2014. Comparison of different drug susceptibility test
301 methods to detect rifampin heteroresistance in *Mycobacterium tuberculosis*. *Antimicrob*
302 *Agents Chemother* 58:5632-5.
- 303 17. Pang Y, Zong Z, Huo F, Jing W, Ma Y, Dong L, Li Y, Zhao L, Fu Y, Huang H. 2017. In
304 Vitro Drug Susceptibility of Bedaquiline, Delamanid, Linezolid, Clofazimine,
305 Moxifloxacin, and Gatifloxacin against Extensively Drug-Resistant Tuberculosis in
306 Beijing, China. *Antimicrob Agents Chemother* 61.
- 307 18. Conde MB, Lapa ESJR. 2011. New regimens for reducing the duration of the treatment of
308 drug-susceptible pulmonary tuberculosis. *Drug Dev Res* 72:501-508.
- 309 19. Rigouts L, Gumusboga M, de Rijk WB, Nduwamahoro E, Uwizeye C, de Jong B, Van
310 Deun A. 2013. Rifampin resistance missed in automated liquid culture system for
311 *Mycobacterium tuberculosis* isolates with specific *rpoB* mutations. *J Clin Microbiol*

- 312 51:2641-5.
- 313 20. Banu S, Rahman SM, Khan MS, Ferdous SS, Ahmed S, Gratz J, Stroup S, Pholwat S,
314 Heysell SK, Houpt ER. 2014. Discordance across several methods for drug susceptibility
315 testing of drug-resistant Mycobacterium tuberculosis isolates in a single laboratory. J Clin
316 Microbiol 52:156-63.
- 317 21. Ng KCS, Supply P, Cobelens FGJ, Gaudin C, Gonzalez-Martin J, de Jong BC, Rigouts L.
318 2019. How Well Do Routine Molecular Diagnostics Detect Rifampin Heteroresistance in
319 Mycobacterium tuberculosis? J Clin Microbiol 57.
- 320 22. Luo T, Yuan J, Peng X, Yang G, Mi Y, Sun C, Wang C, Zhang C, Bao L. 2017. Double
321 mutation in DNA gyrase confers moxifloxacin resistance and decreased fitness of
322 Mycobacterium smegmatis. J Antimicrob Chemother 72:1893-1900.
- 323 23. Kambli P, Ajbani K, Nikam C, Khillari A, Shetty A, Udwadia Z, Georgiou SB, Rodwell
324 TC, Catanzaro A, Rodrigues C. 2015. Determination of MICs of levofloxacin for
325 Mycobacterium tuberculosis with *gyrA* mutations. Int J Tuberc Lung Dis 19:1227-9.
- 326 24. Rigouts L, Coeck N, Gumusboga M, de Rijk WB, Aung KJ, Hossain MA, Fissette K,
327 Rieder HL, Meehan CJ, de Jong BC, Van Deun A. 2016. Specific *gyrA* gene mutations
328 predict poor treatment outcome in MDR-TB. J Antimicrob Chemother 71:314-23.
- 329 25. Du J, Shu W, Liu Y, Wang Y, Zhan Y, Yu K, Gao J, Li L, Pang Y. 2019. Multicenter
330 feasibility study to assess external quality panels for molecular diagnostics for
331 tuberculosis in China. Eur J Clin Microbiol Infect Dis DOI: 10.1007/s10096-019-03730-9

332

333 **Figure Legends**

334 **Figure 1 The region of *gyrA* targeted by the probe of MeltPro TB assay.**

335 A specific probe covers region of *gyrA* codons 88 to 94, consisting of the most
336 common mutation types conferring FQ resistance. The probe is perfectly matched
337 with the wild-type sequence of *gyrA* gene. The corresponding genotype was
338 interpreted according to the T_m of melting curve of double-stranded structures
339 between probe and targeted *gyrA* gene.

340

341 **Figure 2 Enrolment of MTB isolates in this study.**

342

343 **Figure 3 The *gyrA* gene sequencing chromatograms of two heteroresistant MTB**

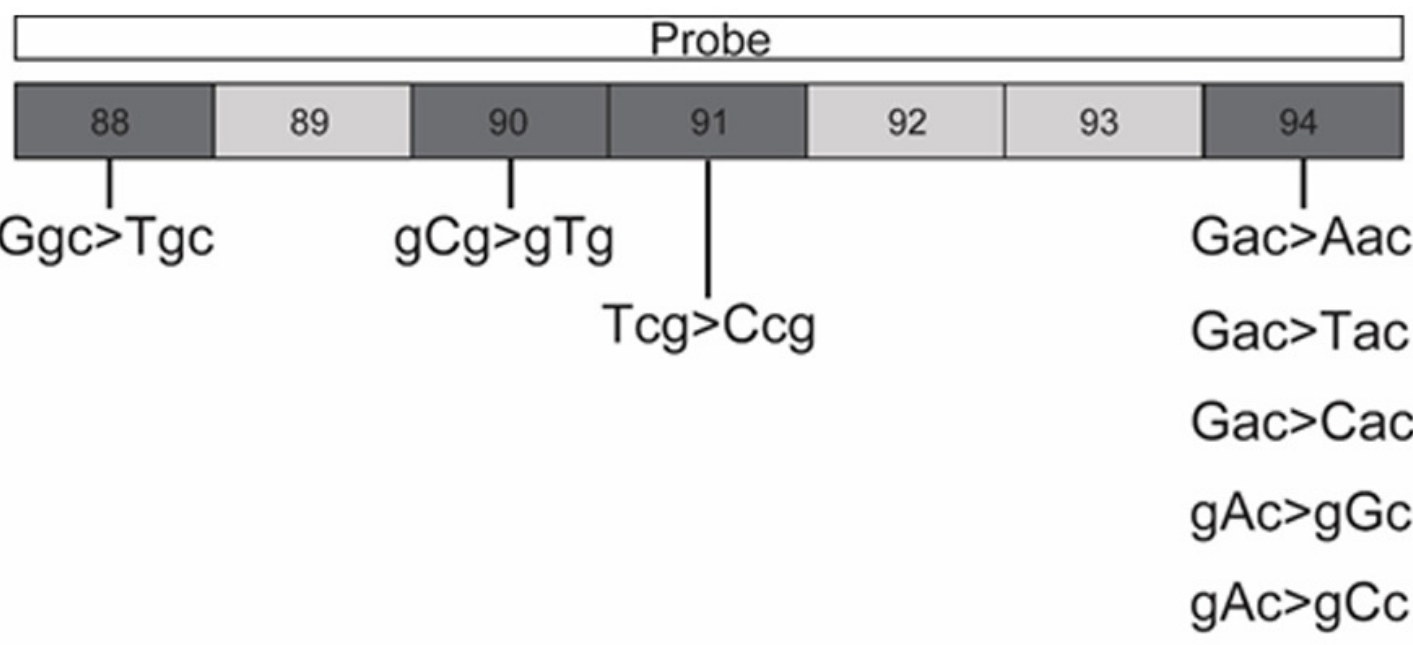
344 **isolates.**

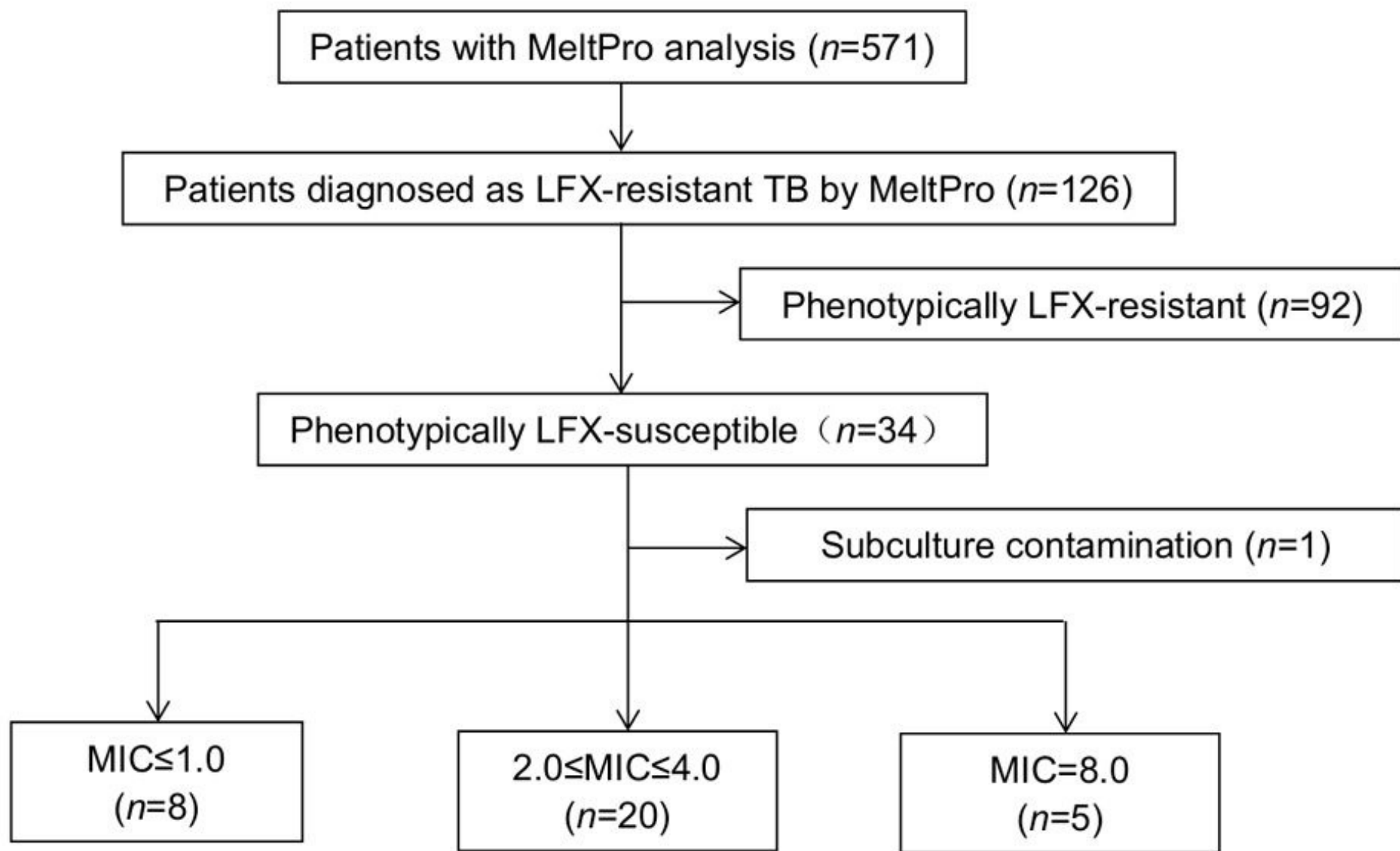
345 A. The mutant G allele in 94 codon of *gyrA* (94Gly) was mixed with the wild-type A
346 allele (94Asp). B. Double point mutations were observed in the heteroresistant isolate
347 carrying both the Thr91Pro and Asp94Gly mutations.

348

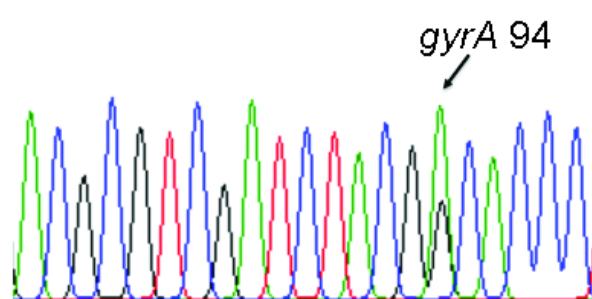
349 **Figure 4 The raw melting curve analysis by MeltPro TB assay of two MTB**
350 **isolates with wide-type *gyrA* locus**

351 The two isolates both had two melting curve peaks in the melting curve, indicating the
352 occurrence of wide-type and mutant *gyrA* gene in the initial specimens.

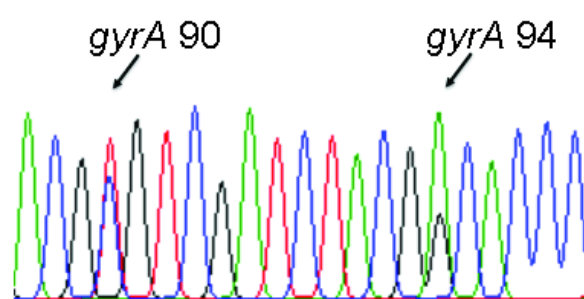




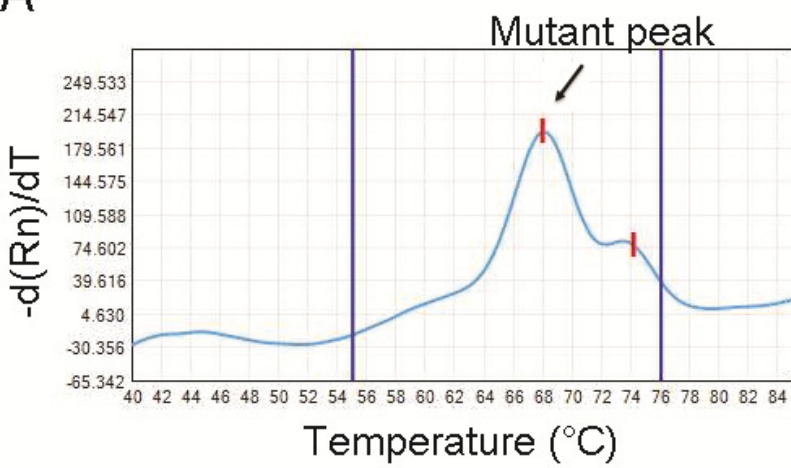
A



B



A



B

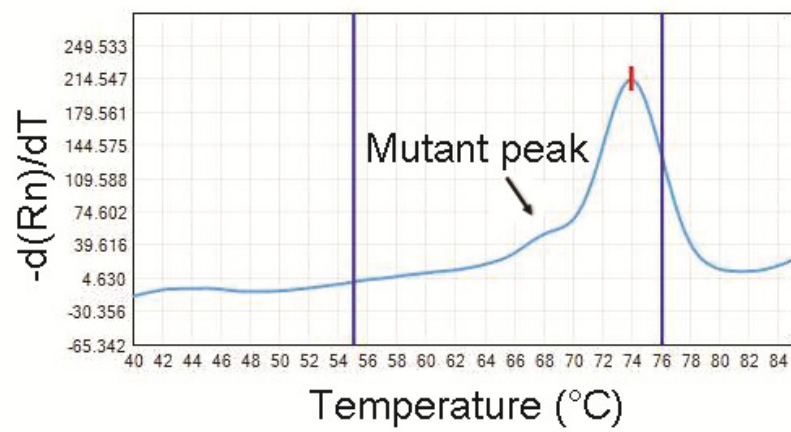


Table 1 Distribution of MTB isolates with different MICs stratified by *gyrA* mutations

Mutation type	No. of isolates with different MICs (mg/L)					Total (n=33)
	≤0.5 (n=2)	1 (n=6)	2 (n=7)	4 (n=13)	8 (n=5)	
Ala90Val	0(0.0)	0(0.0)	4(57.1)	7(53.8)	2(40.0)	13(39.4)
Thr91Pro	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	1(3.0)
Asp94Ala	0(0.0)	6(100.0)	3(42.9)	2(15.4)	0(0.0)	11(33.4)
Asp94Gly	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	1(3.0)
Asp94Val	0(0.0)	0(0.0)	0(0.0)	1(7.7)	0(0.0)	1(3.0)
Asp94Tyr	0(0.0)	0(0.0)	0(0.0)	1(7.7)	0(0.0)	1(3.0)
Asp94Asn	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	1(3.0)
Heteroresistance ^a	0(0.0)	0(0.0)	0(0.0)	2(15.4)	0(0.0)	2(6.1)
Wide type	2(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(6.1)

^aHeteroresistance was defined as a heterogeneous population of tubercle bacilli according to the sequencing chromatograms.