RESEARCH ARTICLE

Rifampicin and isoniazid resistance not promote fluoroquinolone resistance in *Mycobacterium smegmatis*

Qin Zhou[1,2], Na Pu[2], Ge Xu[2], Hangchi Liu[2], Xudong Jia[2], Xiaomin Wang[1], Peng Xu[1]*

1 National Clinical Research Center for Infectious Diseases, Shenzhen Clinical Research Center for Tuberculosis, Shenzhen Third People's Hospital, Shenzhen, Guangdong, China, **2** Zunyi Medical University, Zunyi, Guizhou, China

* WXM_ZMU@163.com

Abstract

Background

The emergence of drug-resistant Tuberculosis (TB) has made treatment challenging. Although fluoroquinolones (FQs) are used as key drugs in the treatment of multidrug-resistant tuberculosis (MDR-TB), the problem of FQs resistance is becoming increasingly serious. Rifampicin (RIF) resistance is considered a risk factor for FQs resistance. The objective of this study was to investigate the impact of RIF and isoniazid (INH) resistance on the FQs resistance in vitro experiment.

Methods

FQs resistant strains were selected in vitro from RIF and/or INH resistant *Mycobacterium smegmatis* (*M.sm*). The sequencing of the *gyrA* gene, and the minimum inhibitory concentration (MIC) of FQs (ciprofloxacin, levofloxacin, moxifloxacin and gatifloxacin) were performed for FQs-resistant strains.

Results

A total of 222 FQs-resistant *M.sm* strains were selected, all of which had the *gyrA* mutation. Seven *gyrA* mutations were detected, with mutations at loci 90 and 94 being the most common. There were no differences in FQs resistance developed from RIF and/or INH resistant *M.sm*. There was a significant difference in the MIC of the *gyrA* mutant types to FQs. The highest resistance to FQs was observed in the Gly88Cys mutant strains. *M.sm* with the identical *gyrA* mutation showed the highest resistance to ciprofloxacin and relatively low resistance to gatifloxacin and moxifloxacin.

Conclusions

In this study, we found no evidence that RIF and/or INH resistance directly affects FQs resistance in *M.sm* in vitro experiments. Resistance profiles of different *gryA* mutations to the

four FQs drugs were also presented. These findings provide a more comprehensive under-
standing of FQs resistance.

Introduction

Tuberculosis (TB) is one of the three major infectious diseases threatening global public health,
and is caused by infection with *Mycobacterium tuberculosis* (*M.tb*) [1]. The WHO reports that
10.6 million people worldwide had TB in 2022, an increase of 300,000 compared to 2021. Of
these, 7.5 million were newly diagnosed with TB, the highest peak in 28 years of global TB sur-
veillance[2, 3]. Approximately 500,000 multidrug-resistant/rifampicin-resistant TB (MDR/
RR-TB) cases are reported each year, and drug-resistant is a major cause of TB deaths [4]. The
cure rate for MDR/RR-TB patients is only 63%, posing a serious threat to public health [5–8].

Fluoroquinolones (FQs) are important drugs in the treatment of MDR/RR-TB [9]. FQs are
commonly used in the treatment of drug-resistant TB because of their efficacy, oral adminis-
tration, relatively few side effects and low cost compared with other second-line injectable
drugs [10]. FQs can inhibit bacterial DNA gyrase and disrupt genome replication to exert bac-
tericidal activity. Bacteria develop resistance to FQs through mutations in the *gyrA* gene,
which encodes a DNA gyrase [11, 12]. In recent years, FQs resistant TB has become increas-
ingly common in clinics, posing a challenge to treating the disease. Treatment success rates for
pre-XDR (resistant to one of the FQs while fulfil the definition of MDR/RR-TB) and XDR
(resistant to bedaquiline or linezolid while fulfil the definition of pre-XDR) TB are only
30~40% [5, 13–15].

Clinical data show that the drug resistance profile may influence the risk of other drug resis-
tance in TB. Compared to susceptible TB, rifampicin (RIF) monoresistant patients and MDR
patients had a 6.3-fold and 13.8-fold risk of FQs resistance, respectively [16, 17]. Therefore, to
guide the use of FQs in the clinic, it is essential to clarify whether the drug-resistant back-
ground influences the development of resistance to FQs. Due to the slow growth and biosafety
concerns of *M.tb*, *Mycobacterium smegmatis* (*M.sm*) is often used as a model organism to
study *M.tb* drug resistance [18–22]. Therefore, we used *M.sm* to investigate whether RIF and/
or isoniazid (INH) resistance influences the development of FQs resistance.

Materials and methods

RIF-R, INH-R and MDR strains

To investigate the effect of different drug-resistant *M.sm* on FQ resistance, the RIF-resistant
(RIF-R), INH-resistant (INH-R), MDR (both RIF and INH resistant), and drug-susceptible
(both RIF and INH susceptible) MC^2-155 strains were used, which were *rpoB* gene S531L
mutant (MS^{RIF-R}), *katG* gene G280D mutant (MS^{INH-R}), *rpoB* gene S531L and *katG* gene
G280D double mutant (MS^{MDR}), and wild type (MS^S), respectively.

In vitro selection of FQs-resistant mutants

We isolated spontaneous mutant colonies using 2× minimal inhibitory concentration (MIC)
[23] of levofloxacin (0.5 μg/mL) with the Luria-Delbrück fluctuation assay [24]. For the initial
preparation of the cell suspension, the optical density (OD) at 600 nm was used to measure the
CFU, as a bacterial solution with an OD600 equal to 1.0 corresponds to approximately $2×10^8$
CFU/ml [25]. For each strain, an approximately $2.3 × 10^5$ CFU were added to 90 ml of

Middlebrook 7H9 liquid medium containing 10% OADC (7H9-OADC), mixed well, and divided into 20 culture tubes. Each tube contains 4ml of cell suspension, approximately 104 CFU. These parallel cultures were then incubated at 37˚C until the OD_{600} reached 1.0 [26]. Among twenty parallel cultures for each strain, 15 were used for isolating spontaneous FQ-resistant mutant colonies and 5 for counting CFU. Spontaneous FQ-resistant mutant colonies were selected on Middlebrook 7H11 solid medium with 10% OADC (7H11-OADC) and 1.0 µg/mL levofloxacin (2× MIC). Serially diluted cell suspensions were added to drug-free 7H11-OADC for CFU counting. Mutation rates and 95% confidence intervals (CI) were calculated by the online tools of FluCalc, which was able to accurately estimate mutation rates using the fluctuation assay [27].

DNA extraction and *gyrA* gene sequencing

Bacterial genomic DNA was extracted by boiling method [28, 29]. The gyrA-1R (5′-CGAC GAACTGTTCTCCATCC–3′) and gyrA-1F (5′-CCGGTCTTGTACGTGTCCTC–3′) primers were used to amplify the 869 bp fragment of the *gyrA* gene [13]. The obtained PCR products were sent for Sanger sequencing to identify the FQs-resistant mutations.

Minimal inhibitory concentration (MIC) assay for FQs

MICs were determined in 7H9-OADC by the resazurin microtiter assay (REMA) using a two-fold FQ drugs dilution [30, 31]. Ciprofloxacin was tested at a range of 0.25 to 128 µg/mL, while levofloxacin at a range of 0.0156 to 8 µg/mL, moxifloxacin at a range of 0.0625 to 32 µg/mL and gatifloxacin at a range of 0.0625 to 32 µg/mL. The MIC is interpreted as the minimum drug concentration that prevents the color changing from blue to purple or pink.

Statistical analysis

The chi-squared test was used to compare the mutations between the groups, while the one-way ANOVA method and the *t*-test were used to compare the MIC. All analyses were performed with SPSS version 29.0 and results with a *p*-value less than 0.05 were considered statistically significant.

Results

Selection of FQs-resistant clones

A total of 609 FQs-resistant clones were selected by the Luria-Delbrück fluctuation assay. The number of FQs-resistant clones from MS[S], MS[INH-R], MS[RIF-R] and MS[MDR] were 96, 349, 88 and 76, respectively. The frequency of FQs resistance was $2.93×10^{-10}$ (95% CI: $1.67–4.42×10^{-10}$) for MS[S], $5.28×10^{-10}$ (95% CI: $3.17–7.77×10^{-10}$) for MS[INH-R], $8.11×10^{-10}$ (95% CI: $4.33–12.72×10^{-10}$) for MS[RIF-R] and $5.09×10^{-10}$ (95% CI: $2.56–8.23×10^{-10}$) for MS[MDR]. Of the 609 FQs-resistant clones, 222 were randomly selected for *gyrA* sequencing and FQs MICs testing, of which 66 clones were from MS[S], 66 clones from MS[INH-R], 41 clones from MS[RIF-R] and 49 clones from MS[MDR].

FQs-resistant mutations

Each sequenced clone harbored a mutation in the *gyrA* gene. There were seven types of mutation, located at loci 88, 90, 91 and 94 of the *gyrA* gene (Table 1). Mutations at loci 90 and 94 were the most common, accounting for 54.1% (120/222) and 30.6% (68/222) of all samples, respectively. Chi-squared test showed a significant difference in the mutation profile between MS[S] and MS[INH-R] (*p* = 0.004), and between MS[INH-R] and MS[RIF-R] (*p* = 0.002) (S1 Table). The

Table 1. *gyrA* mutation profile of *M.sm* strains.

Mutation	MS^S	MS^INH-R	MS^RIF-R	MS^MDR
	n = 66 (%)	n = 66 (%)	n = 41 (%)	n = 49 (%)
Gly88Cys	11 (16.7)	2 (3.0)	5 (12.2)	3 (6.1)
Ala90Val	36 (54.5)	40 (60.6)	14 (34.1)	30 (61.2)
Ser91Pro	3 (4.5)	1 (1.5)	9 (22.0)	0 (0.0)
Asp94His	0 (0.0)	1 (1.5)	1 (2.4)	0 (0.0)
Asp94Tyr	6 (9.1)	0 (0.0)	0 (0.0)	2 (4.1)
Asp94Gly	10 (15.2)	21 (31.8)	12 (29.3)	14 (28.6)
Asp94Ala	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)

Abbreviations: Gly, Glycine; Cys, cysteine; Ala, Alanine; Val, Valine; Ser, Serine; Pro, Proline; Asp, Aspartic acid; His, Histidine; Tyr, Threonine.

https://doi.org/10.1371/journal.pone.0315512.t001

mutation profile comparisons between MS^S and MS^INH-R, and between MS^INH-R and MS^RIF-R were performed independently. Compared to MS^INH-R, the MS^S strain had a significantly higher proportion of Gly88Cys and Asp94Tyr mutations, but a significantly lower proportion of Asp94Gly mutation. And MS^INH-R strain had a significantly higher proportion of Ala90Val mutation, but a significantly lower proportion of Ser921Pro mutation than MS^RIF-R strain.

FQs MIC of *M.sm* strains

In order to ascertain whether drug-resistance backgrounds influence FQs resistance, this study analyzed the MIC of FQs in MS^S, MS^INH-R, MS^RIF-R, and MS^MDR with identical *gyrA* mutations. The MICs of ciprofloxacin, levofloxacin, moxifloxacin and gatifloxacin for strains with the same mutation were found to be generally consistent, with MIC differences observed primarily within a range of one titer (Fig 1). The results indicated that resistance to RIF and/or INH did not influence the level of FQs resistance. Consequently, the MIC results of different strains with the identical mutation were combined and subjected to further analysis. (S2 and S3 Tables).

FQs MIC of *gyrA* mutations

The *gyrA* mutation increased the MIC of ciprofloxacin from 0.5μg/mL to 4.0~16.0 μg/mL, levofloxacin from 0.25μg/mL to 2.0~8.0 μg/mL, moxifloxacin from 0.125μg/mL to 1.0~4.0 μg/mL, and gatifloxacin from 0.25μg/mL to 1.0~2.0 μg/mL (Fig 1). An analysis of the FQs MICs for different mutations revealed that the MIC of the Gly88Cys mutation was significantly higher than that of the Ala90Val (p = 0.015) and Ser91Pro (p = 0.016) mutations (Fig 2A). This suggests that the Gly88Cys mutation may confer higher resistance to FQs, while Ala90Val and Ser91Pro confer lower. No significant difference was observed in the MICs between the remaining mutations.

Differences in MIC of four FQ drugs

In general, the mutant clones exhibited variability in their MICs for the four FQ drugs. The mutant clones had the highest resistance to ciprofloxacin (4.0~16.0 μg/mL), followed by levofloxacin (2.0~8.0μg/mL), and the lowest resistance to moxifloxacin (1.0~4.0 μg/mL) and gatifloxacin (1.0~2.0 μg/mL) (Fig 1). With the exception of no difference being found in MICs between moxifloxacin and gatifloxacin, significant differences were observed in the comparisons of MICs for all the drugs ($p<0.001$) (S4 Table)
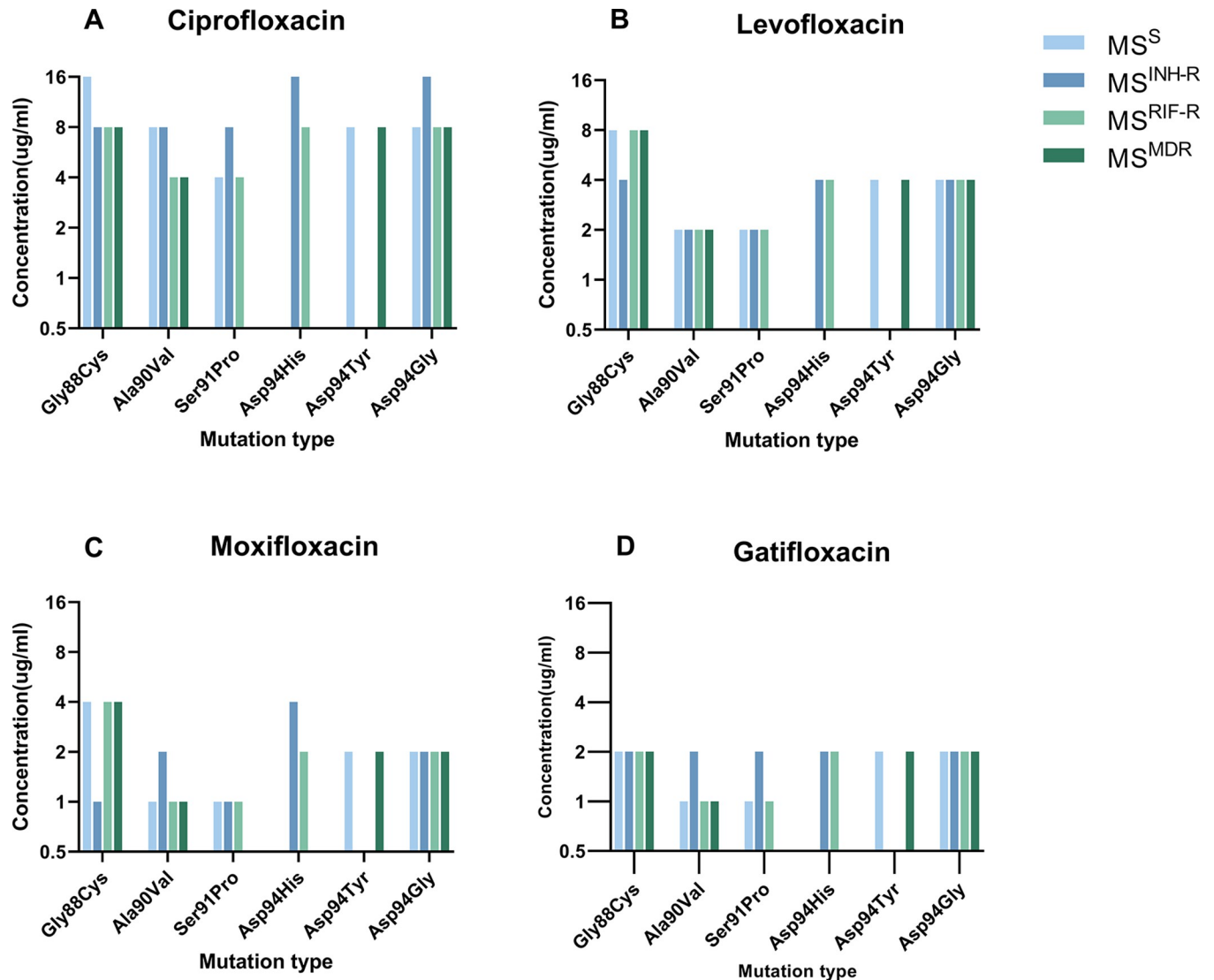
Fig 1. The FQs MIC among *M.sm* strains. (A) Ciprofloxacin, (B) Levofloxacin, (C) Moxifloxacin, (D) Gatifloxacin. No clone with *gyrA* Ser91Pro mutation was selected form MS^MDR, no clone with *gyrA* Asp94His mutation was selected form MS^S and MS^MDR, and no clone with *gyrA* Asp94Tyr mutation was selected form MS^INH-R and MS^RIF-R.

https://doi.org/10.1371/journal.pone.0315512.g001

The relative changes in the FQs MIC of the mutant strains relative to the wild-type strain were further analyzed. Resistance mutations resulted in the smallest mean increase in minimum inhibitory concentration (MIC) for gatifloxacin, with a mean increase of 6.8-fold (interquartile range: 4–8), whereas levofloxacin, ciprofloxacin, and moxifloxacin showed a larger mean increase in MIC, with a mean increase of 16-fold (IQR: 8–16) (Fig 2B). The relative changes in MIC of gatifloxacin were significantly lower than those of levofloxacin, ciprofloxacin, and moxifloxacin ($p<0.001$) (S5 Table). Among all the mutation types, Ala90Val and Ser91Pro showed lower relative changes than Gly88Cys, Asp94Tyr and Asp94Gly (Fig 2B).

Discussion

In this study, a total of 222 FQs-resistant *M.sm* strains were selected in vitro from RIF and/or INH resistant *M.sm*, all of which had the *gyrA* mutation. The MICs of FQs developed from *M.*
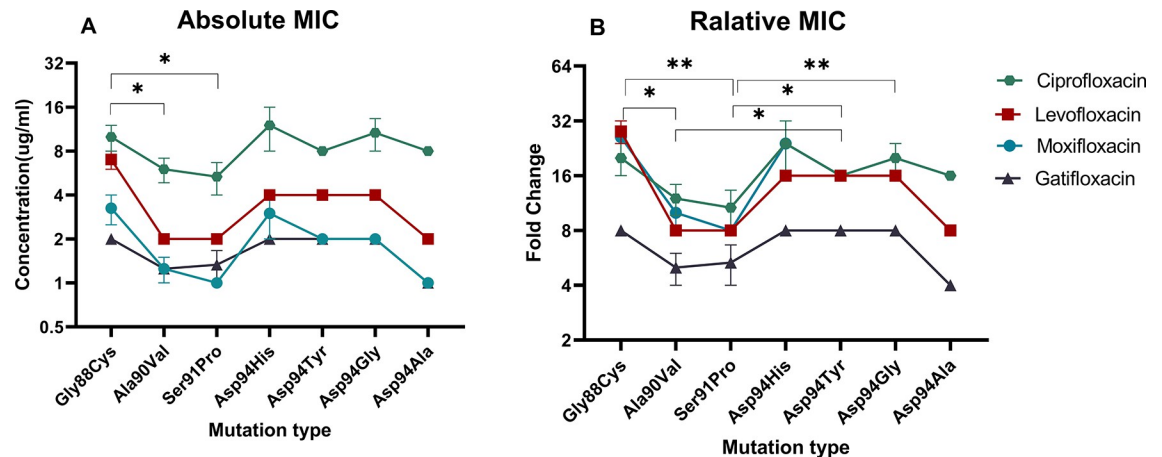
Fig 2. The FQs absolute MIC (A) and relative MIC (B) of *gryA* mutations. * indicates a *p*-value of less than 0.05, ** indicates a *p*-value of less than 0.01.

sm strains resistant to RIF and/or INH were found to be no significantly different. Among seven *gyrA* mutations were detected, the highest resistance to FQs was observed in the Gly88Cys mutant strains. *M.sm* with the identical *gyrA* mutation showed the highest resistance to ciprofloxacin and relatively low resistance to gatifloxacin and moxifloxacin. The findings will contribute to a more comprehensive understanding of drug resistance in FQs.

The *gyrA* gene encodes the α-subunit of bacterial DNA gyrase, which is the target of FQ drugs [32, 33]. FQ resistance is the consequence of the drug-resistant mutations in the *gyrA* gene [34, 35]. A total of seven FQ-resistant mutations at four codon sites have been observed in this study. All of these mutations have been reported in clinical FQ-resistant *M.tb* strains [36–38]. We also found more than one mutation type in codon 94 conferring FQ resistance, which is consistent with clinical *M.tb* strains [36, 39]. These results suggest that *M.sm* can be used as a model for the study of FQ resistance in *M.tb*.

Clinical studies have demonstrated that the prevalence of FQs resistance in MDR/RR-TB cases is considerably higher than in susceptible cases, indicating that INH/RIF resistance may contribute to the risk of FQs resistance [40, 41]. Nevertheless, our study did not identify a significant difference in the frequency of FQs resistance among RIF and/or INH-resistant *M.sm*. This suggests that it is not drug resistance profiles but other factors that increase the risk of FQ resistance. One potential explanation is that FQs are primarily used for the treatment of drug-resistant TB [9], whereas patients with susceptible TB have limited opportunity to use FQs. Consequently, the overall FQs resistance rate is higher in drug-resistant TB than in susceptible TB [41]. Studies have shown that subinhibitory concentrations of drugs induce bacteria to generate reactive oxygen species (ROS) that increase the mutation rate [42], thereby increasing the risk of drug resistance. Because the in vivo environment is complex and drug concentrations in tissues vary spatially and temporally [43, 44], it is possible that subinhibitory concentrations may exist in patients and increase the rate of drug resistance mutations. Compared to susceptible strains, drug-resistant strains have more opportunities to be in a subinhibitory environment, which could potentially favour the development of drug resistance.

Not only patient factors, but also bacterial factors other than drug resistance may promote resistance to FQs. Bacteria can prevent drugs from entering into cells to reduce its susceptibility, and can also excrete drugs out of bacteria through efflux pumps to increase the ability of drug resistance, which involving many genes, such as *pe11*, *mymA*, *virS*, *cpnT*, *mmpL3*, *bacA*, *efpA* [45]. These mechanisms that affect drug susceptibility are usually not specific to a single

drug, but affect multiple drugs [46]. Drug-resistant strain can also acquire compensatory mutation that partially or fully restore the deficiency caused by the drug resistance conferring mutations [47]. A secondary in the *rpoA*, *ropB* and *rpoC* genes was a common compensatory mutation to restore the deficiency caused by RIF resistance [48], may have a potential contribution to the development of FQs resistance. In addition, genetic background may have an effect on susceptibility to FQs, as the MIC of lineage 3 strains was lower than that of lineages 4 [49]. Whether these bacterial factors contribute to the promotion of FQs resistance requires further investigation.

FQs are frequently used to treat MDR-TB patients as an important component of the WHO-recommended short-course treatment regimen for TB [50, 51]. FQs were initially discovered as broad-spectrum antibiotics in 1962, and four generations of FQ drugs have been developed to date [52]. Our study involved three generations of FQs, including second-generation (ciprofloxacin), third-generation (levofloxacin), and fourth-generation (moxifloxacin and gatifloxacin) [53]. Strains with the same *gyrA* mutation differed in their resistance to different generations of FQs. In general, the more recent generation of FQs exhibited a lower MIC. The MIC of ciprofloxacin for the Gly88Cys mutant strain was 16 μg/mL, while that of gatifloxacin was 2 μg/mL, representing an 8-fold difference. This phenomenon makes it possible for patients to achieve the same therapeutic effect and reduce the risk of side effects by taking lower doses of new-generation FQs. It is also possible to treat low-level drug-resistant TB by using high-dose new-generation FQs.

This study had several limitations. Although, *M.sm* is often used as a model organism to study *M.tb* drug resistance [18–22], there are genetic and physiological differences between them. The results of this study require further validation using both *M.tb* reference and clinical strains with different drug resistance profiles. Secondly, the diversity and complexity of drug resistance mechanisms in clinical *M.tb* strains, which may have evolved under different selective pressures in vivo [54], were not considered in this study. Thirdly, this study did not assess the effect of other bacterial factors on the development of resistance to FQs, in particular compensatory mutations, which are common in clinical RIF-resistant strains. These factors need to be studied in order to draw more comprehensive conclusions.

In summary, this study assesses the impact of RIF and/or INH-resistant profile on the development of FQs resistance in *M.sm*. We found that there was no significant direct effect of RIF and/or INH resistance profile on FQs resistance in *M.sm* in vitro experiments. This indicates that there are other reasons for the higher FQs-resistant rate in drug-resistant TB. Furthermore, the distribution of MICs between generations of FQs and *gyrA* mutations was presented. These findings provide a more comprehensive understanding of FQs resistance.

Supporting information

S1 Table. The original data and *p*-value for between-group differences in mutation composition ratios for different drug-resistant strains.
(DOCX)

S2 Table. The original data and *p*-value for difference in absolute MIC between groups of different resistant strains.
(DOCX)

S3 Table. The original data and *p*-values of differences in relative MICs between different strains.
(DOCX)

S4 Table. The original data and *p*-value for difference in absolute MIC between groups of different FQs.
(DOCX)

S5 Table. The original data and *p*-value for difference in relative MIC between groups of different FQs.
(DOCX)

Author Contributions

Conceptualization: Xiaomin Wang, Peng Xu.

Investigation: Qin Zhou, Na Pu, Ge Xu, Hangchi Liu, Xudong Jia.

Supervision: Xiaomin Wang, Peng Xu.

Writing – original draft: Qin Zhou.

Writing – review & editing: Xiaomin Wang, Peng Xu.

References

1. Singh R, Dwivedi SP, Gaharwar US, Meena R, Rajamani P, Prasad T. Recent updates on drug resistance in Mycobacterium tuberculosis. J Appl Microbiol. 2020; 128(6):1547–67. Epub 20191029. https://doi.org/10.1111/jam.14478 PMID: 31595643.

2. Bagcchi S. WHO's Global Tuberculosis Report 2022. Lancet Microbe. 2023; 4(1):e20. Epub 20221212. https://doi.org/10.1016/S2666-5247(22)00359-7 PMID: 36521512.

3. Organization WH. Global tuberculosis report 2023 2023. Available from: https://www.who.int/zh.

4. Organization WH. WHO consolidated guidelines on tuberculosis. Module 1: prevention–tuberculosis preventive treatment 2020. Available from: https://www.who.int/zh.

5. Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respir Med. 2017. Epub 20170315. https://doi.org/10.1016/S2213-2600(17)30079-6 PMID: 28344011.

6. Lange C, Dheda K, Chesov D, Mandalakas AM, Udwadia Z, Horsburgh CR, Jr. Management of drug-resistant tuberculosis. Lancet. 2019; 394(10202):953–66. https://doi.org/10.1016/s0140-6736(19)31882-3 PMID: 31526739.

7. Lange C, Chesov D, Heyckendorf J, Leung CC, Udwadia Z, Dheda K. Drug-resistant tuberculosis: An update on disease burden, diagnosis and treatment. Respirology. 2018; 23(7):656–73. Epub 20180411. https://doi.org/10.1111/resp.13304 PMID: 29641838.

8. Garcia PK, Annamalai T, Wang W, Bell RS, Le D, Martin Pancorbo P, et al. Mechanism and resistance for antimycobacterial activity of a fluoroquinophenoxazine compound. PLOS ONE. 2019; 14(2): e0207733. https://doi.org/10.1371/journal.pone.0207733 PMID: 30794538

9. Vanino E, Granozzi B, Akkerman OW, Munoz-Torrico M, Palmieri F, Seaworth B, et al. Update of drug-resistant tuberculosis treatment guidelines: A turning point. Int J Infect Dis. 2023; 130 Suppl 1:S12–s5. Epub 20230312. https://doi.org/10.1016/j.ijid.2023.03.013 PMID: 36918080.

10. Lan Z, Ahmad N, Baghaei P, Barkane L, Benedetti A, Brode SK, et al. Drug-associated adverse events in the treatment of multidrug-resistant tuberculosis: an individual patient data meta-analysis. Lancet Respir Med. 2020; 8(4):383–94. Epub 20200317. https://doi.org/10.1016/S2213-2600(20)30047-3 PMID: 32192585; PubMed Central PMCID: PMC7384398.

11. Aldred KJ, Blower TR, Kerns RJ, Berger JM, Osheroff N. Fluoroquinolone interactions with Mycobacterium tuberculosis gyrase: Enhancing drug activity against wild-type and resistant gyrase. Proc Natl Acad Sci U S A. 2016; 113(7):E839–46. Epub 20160120. https://doi.org/10.1073/pnas.1525055113 PMID: 26792518; PubMed Central PMCID: PMC4763725.

12. Spencer AC, Panda SS. DNA Gyrase as a Target for Quinolones. Biomedicines. 2023; 11(2). Epub 20230127. https://doi.org/10.3390/biomedicines11020371 PMID: 36830908; PubMed Central PMCID: PMC9953508.

13. Luo T, Yuan J, Peng X, Yang G, Mi Y, Sun C, et al. Double mutation in DNA gyrase confers moxifloxacin resistance and decreased fitness of Mycobacterium smegmatis. J Antimicrob Chemother. 2017; 72 (7):1893–900. https://doi.org/10.1093/jac/dkx110 PMID: 28387828.

14. Kabir S, Tahir Z, Mukhtar N, Sohail M, Saqalein M, Rehman A. Fluoroquinolone resistance and mutational profile of gyrA in pulmonary MDR tuberculosis patients. BMC Pulm Med. 2020; 20(1):138. Epub 20200511. https://doi.org/10.1186/s12890-020-1172-4 PMID: 32393213; PubMed Central PMCID: PMC7216623.

15. Tritar F, Daghfous H, Ben Saad S, Slim-Saidi L. [Management of multidrug-resistant tuberculosis]. Rev Pneumol Clin. 2015; 71(2–3):130–9. Epub 20140819. https://doi.org/10.1016/j.pneumo.2014.05.001 PMID: 25153927.

16. Xu P, Li X, Zhao M, Gui X, DeRiemer K, Gagneux S, et al. Prevalence of fluoroquinolone resistance among tuberculosis patients in Shanghai, China. Antimicrob Agents Chemother. 2009; 53(7):3170–2. Epub 20090413. https://doi.org/10.1128/AAC.00177-09 PMID: 19364851; PubMed Central PMCID: PMC2704692.

17. Wang JY, Lee LN, Lai HC, Wang SK, Jan IS, Yu CJ, et al. Fluoroquinolone resistance in Mycobacterium tuberculosis isolates: associated genetic mutations and relationship to antimicrobial exposure. J Antimicrob Chemother. 2007; 59(5):860–5. Epub 20070405. https://doi.org/10.1093/jac/dkm061 PMID: 17412727.

18. Borrell S, Teo Y, Giardina F, Streicher EM, Klopper M, Feldmann J, et al. Epistasis between antibiotic resistance mutations drives the evolution of extensively drug-resistant tuberculosis. Evol Med Public Health. 2013; 2013(1):65–74. Epub 20130308. https://doi.org/10.1093/emph/eot003 PMID: 24481187; PubMed Central PMCID: PMC3868377.

19. Danilchanka O, Pavlenok M, Niederweis M. Role of porins for uptake of antibiotics by Mycobacterium smegmatis. Antimicrob Agents Chemother. 2008; 52(9):3127–34. Epub 20080616. https://doi.org/10.1128/AAC.00239-08 PMID: 18559650; PubMed Central PMCID: PMC2533485.

20. Yoshida M, Nakata N, Miyamoto Y, Fukano H, Ato M, Hoshino Y. A rapid and non-pathogenic assay for association of Mycobacterium tuberculosis gyrBA mutations and fluoroquinolone resistance using recombinant Mycobacterium smegmatis. FEMS Microbiol Lett. 2018; 365(23). https://doi.org/10.1093/femsle/fny266 PMID: 30418577.

21. Lelovic N, Mitachi K, Yang J, Lemieux MR, Ji Y, Kurosu M. Application of Mycobacterium smegmatis as a surrogate to evaluate drug leads against Mycobacterium tuberculosis. J Antibiot (Tokyo). 2020; 73 (11):780–9. Epub 20200529. https://doi.org/10.1038/s41429-020-0320-7 PMID: 32472054; PubMed Central PMCID: PMC7554168.

22. Kim KH, An DR, Yoon HJ, Yang JK, Suh SW. Structure of Mycobacterium smegmatis Eis in complex with paromomycin. Acta Crystallogr F Struct Biol Commun. 2014; 70(Pt 9):1173–9. Epub 20140829. https://doi.org/10.1107/S2053230X14017385 PMID: 25195887; PubMed Central PMCID: PMC4157414.

23. Gillespie SH, Basu S, Dickens AL, O'Sullivan DM, McHugh TD. Effect of subinhibitory concentrations of ciprofloxacin on Mycobacterium fortuitum mutation rates. J Antimicrob Chemother. 2005; 56(2):344–8. Epub 20050614. https://doi.org/10.1093/jac/dki191 PMID: 15956099.

24. Sebastian J, Swaminath S, Nair RR, Jakkala K, Pradhan A, Ajitkumar P. De Novo Emergence of Genetically Resistant Mutants of Mycobacterium tuberculosis from the Persistence Phase Cells Formed against Antituberculosis Drugs In Vitro. Antimicrob Agents Chemother. 2017; 61(2). Epub 20170124. https://doi.org/10.1128/AAC.01343-16 PMID: 27895008; PubMed Central PMCID: PMC5278719.

25. Fang H, Yu D, Hong Y, Zhou X, Li C, Sun B. The LuxR family regulator Rv0195 modulates Mycobacterium tuberculosis dormancy and virulence. Tuberculosis (Edinb). 2013; 93(4):425–31. Epub 20130511. https://doi.org/10.1016/j.tube.2013.04.005 PMID: 23673208.

26. Yan S, Xu M, Wang R, Li Q, Yu Z, Xie J. Overexpression of Rv2788 increases mycobacterium stresses survival. Microbiol Res. 2017; 195:51–9. Epub 20161117. https://doi.org/10.1016/j.micres.2016.11.007 PMID: 28024526.

27. Radchenko EA, McGinty RJ, Aksenova AY, Neil AJ, Mirkin SM. Quantitative Analysis of the Rates for Repeat-Mediated Genome Instability in a Yeast Experimental System. Methods Mol Biol. 2018; 1672:421–38. https://doi.org/10.1007/978-1-4939-7306-4_29 PMID: 29043640; PubMed Central PMCID: PMC5741187.

28. Afghani B, Stutman HR. Polymerase chain reaction for diagnosis of M. tuberculosis: comparison of simple boiling and a conventional method for DNA extraction. Biochem Mol Med. 1996; 57(1):14–8. https://doi.org/10.1006/bmme.1996.0003 PMID: 8812720.

29. Hosseinali Z, Mohammadshahi J, Teimourpour A, Habibzadeh S, Esmaelizad M, Arzanlou M, et al. Molecular identification of multiple drug resistance (MDR) strain of Mycobacterium tuberculosis. Mol

Biol Rep. 2023; 50(12):10271–5. Epub 20231116. https://doi.org/10.1007/s11033-023-08867-7 PMID: 37971566.

30. Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2002; 46(8):2720–2. https://doi.org/10.1128/AAC.46.8.2720-2722.2002 PMID: 12121966; PubMed Central PMCID: PMC127336.

31. Rivoire N, Ravololonandriana P, Rasolonavalona T, Martin A, Portaels F, Ramarokoto H, et al. Evaluation of the resazurin assay for the detection of multidrug-resistant Mycobacterium tuberculosis in Madagascar. Int J Tuberc Lung Dis. 2007; 11(6):683–8. PMID: 17519102.

32. Dighe SN, Collet TA. Recent advances in DNA gyrase-targeted antimicrobial agents. Eur J Med Chem. 2020; 199:112326. Epub 20200428. https://doi.org/10.1016/j.ejmech.2020.112326 PMID: 32460040.

33. Mayer C, Takiff H. The Molecular Genetics of Fluoroquinolone Resistance in Mycobacterium tuberculosis. Microbiol Spectr. 2014; 2(4):Mgm2-0009-2013. https://doi.org/10.1128/microbiolspec.MGM2-0009-2013 PMID: 26104201.

34. Chaiyachat P, Chaiprasert A, Nonghanphithak D, Smithtikarn S, Kamolwat P, Pungrassami P, et al. Whole-genome analysis of drug-resistant Mycobacterium tuberculosis reveals novel mutations associated with fluoroquinolone resistance. Int J Antimicrob Agents. 2021; 58(3):106385. Epub 20210620. https://doi.org/10.1016/j.ijantimicag.2021.106385 PMID: 34161790.

35. Maruri F, Guo Y, Blackman A, van der Heijden YF, Rebeiro PF, Sterling TR. Resistance-Conferring Mutations on Whole-Genome Sequencing of Fluoroquinolone-resistant and -Susceptible Mycobacterium tuberculosis Isolates: A Proposed Threshold for Identifying Resistance. Clin Infect Dis. 2021; 72 (11):1910–8. https://doi.org/10.1093/cid/ciaa496 PMID: 32348473; PubMed Central PMCID: PMC8315129.

36. Li J, Gao X, Luo T, Wu J, Sun G, Liu Q, et al. Association of gyrA/B mutations and resistance levels to fluoroquinolones in clinical isolates of Mycobacterium tuberculosis. Emerg Microbes Infect. 2014; 3(3): e19. Epub 20140312. https://doi.org/10.1038/emi.2014.21 PMID: 26038513; PubMed Central PMCID: PMC3974338.

37. Uddin MKM, Ather MF, Nasrin R, Rahman T, Islam A, Rahman SMM, et al. Correlation of gyr Mutations with the Minimum Inhibitory Concentrations of Fluoroquinolones among Multidrug-Resistant Mycobacterium tuberculosis Isolates in Bangladesh. Pathogens. 2021; 10(11). Epub 20211102. https://doi.org/10.3390/pathogens10111422 PMID: 34832578; PubMed Central PMCID: PMC8623510.

38. Chien JY, Chiu WY, Chien ST, Chiang CJ, Yu CJ, Hsueh PR. Mutations in gyrA and gyrB among Fluoroquinolone- and Multidrug-Resistant Mycobacterium tuberculosis Isolates. Antimicrob Agents Chemother. 2016; 60(4):2090–6. Epub 20160325. https://doi.org/10.1128/AAC.01049-15 PMID: 26787695; PubMed Central PMCID: PMC4808166.

39. Malik S, Willby M, Sikes D, Tsodikov OV, Posey JE. New insights into fluoroquinolone resistance in Mycobacterium tuberculosis: functional genetic analysis of gyrA and gyrB mutations. PLoS One. 2012; 7(6):e39754. Epub 20120628. https://doi.org/10.1371/journal.pone.0039754 PMID: 22761889; PubMed Central PMCID: PMC3386181.

40. Zaidi SMA, Haseeb A, Habib SS, Malik A, Khowaja S, SaifUllah N, et al. Emergence of fluoroquinolone resistance among drug resistant tuberculosis patients at a tertiary care facility in Karachi, Pakistan. BMC Res Notes. 2017; 10(1):313. Epub 20170725. https://doi.org/10.1186/s13104-017-2633-6 PMID: 28743312; PubMed Central PMCID: PMC5526320.

41. Ali S, Khan MT, Khan AS, Abbas Q, Irfan M. Fluoroquinolone Resistance Among Isolates of Mycobacterium tuberculosis in Khyber Pakhtunkhwa, Pakistan. Microb Drug Resist. 2021; 27(6):786–91. Epub 20201030. https://doi.org/10.1089/mdr.2020.0118 PMID: 33124944.

42. Bhattacharya G, Dey D, Das S, Banerjee A. Exposure to sub-inhibitory concentrations of gentamicin, ciprofloxacin and cefotaxime induces multidrug resistance and reactive oxygen species generation in meticillin-sensitive Staphylococcus aureus. J Med Microbiol. 2017; 66(6):762–9. Epub 20170609. https://doi.org/10.1099/jmm.0.000492 PMID: 28598307.

43. Turnidge J. Pharmacokinetics and pharmacodynamics of fluoroquinolones. Drugs. 1999; 58 Suppl 2:29–36. https://doi.org/10.2165/00003495-199958002-00006 PMID: 10553702.

44. Ji B, Lounis N, Maslo C, Truffot-Pernot C, Bonnafous P, Grosset J. In vitro and in vivo activities of moxifloxacin and clinafloxacin against Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1998; 42 (8):2066–9. https://doi.org/10.1128/AAC.42.8.2066 PMID: 9687408; PubMed Central PMCID: PMC105862.

45. Xu G, Liu H, Jia X, Wang X, Xu P. Mechanisms and detection methods of Mycobacterium tuberculosis rifampicin resistance: The phenomenon of drug resistance is complex. Tuberculosis. 2021; 128:102083. Epub 2021/05/12. https://doi.org/10.1016/j.tube.2021.102083 PMID: 33975262.

46. Gygli SM, Borrell S, Trauner A, Gagneux S. Antimicrobial resistance in Mycobacterium tuberculosis: mechanistic and evolutionary perspectives. FEMS Microbiol Rev. 2017; 41(3):354–73. https://doi.org/10.1093/femsre/fux011 PMID: 28369307.

47. Alame Emane AK, Guo X, Takiff HE, Liu S. Drug resistance, fitness and compensatory mutations in Mycobacterium tuberculosis. Tuberculosis (Edinb). 2021; 129:102091. Epub 20210521. https://doi.org/10.1016/j.tube.2021.102091 PMID: 34090078.

48. Brunner VM, Fowler PW. Compensatory mutations are associated with increased in vitro growth in resistant clinical samples of Mycobacterium tuberculosis. Microb Genom. 2024;10(2). https://doi.org/10.1099/mgen.0.001187 PMID: 38315172; PubMed Central PMCID: PMC10926696.

49. Consortium CR. Quantitative measurement of antibiotic resistance in Mycobacterium tuberculosis reveals genetic determinants of resistance and susceptibility in a target gene approach. Nature Communications. 2024; 15(1):488. Epub 20240112. https://doi.org/10.1038/s41467-023-44325-5 PMID: 38216576; PubMed Central PMCID: PMC10786857.

50. Falzon D, Schünemann HJ, Harausz E, González-Angulo L, Lienhardt C, Jaramillo E, et al. World Health Organization treatment guidelines for drug-resistant tuberculosis, 2016 update. Eur Respir J. 2017;49(3). Epub 20170322. https://doi.org/10.1183/13993003.02308-2016 PMID: 28331043; PubMed Central PMCID: PMC5399349.

51. Mirzayev F, Viney K, Linh NN, Gonzalez-Angulo L, Gegia M, Jaramillo E, et al. World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. Eur Respir J. 2021; 57(6). Epub 20210604. https://doi.org/10.1183/13993003.03300-2020 PMID: 33243847; PubMed Central PMCID: PMC8176349.

52. Takahashi H, Hayakawa I, Akimoto T. [The history of the development and changes of quinolone antibacterial agents]. Yakushigaku Zasshi. 2003; 38(2):161–79. PMID: 15143768.

53. King DE, Malone R, Lilley SH. New classification and update on the quinolone antibiotics. Am Fam Physician. 2000; 61(9):2741–8. PMID: 10821154.

54. Castro RAD, Borrell S, Gagneux S. The within-host evolution of antimicrobial resistance in Mycobacterium tuberculosis. FEMS Microbiol Rev. 2021; 45(4). https://doi.org/10.1093/femsre/fuaa071 PMID: 33320947; PubMed Central PMCID: PMC8371278.