Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

rpoB Mutations are Associated with Variable Levels of Rifampin and Rifabutin Resistance in *Mycobacterium tuberculosis*

Ma-Chao Li[1,*], Xiao-Yue Wang[1,*], Tong-Yang Xiao[2,*], Shi-Qiang Lin[3], Hai-Can Liu[1], Cheng Qian[4], Da Xu[1], Gui-Lian Li[1], Xiu-Qin Zhao[1], Zhi-Guang Liu[1], Li-Li Zhao[1], Kang-Lin Wan[1]

[1]State Key Laboratory of Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China; [2]Department of Clinical Laboratory, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, Guangdong, People's Republic of China; [3]Department of Bioinformatics, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, Fujian Province, People's Republic of China; [4]Beijing Center for Disease Control and Prevention, Beijing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Li-Li Zhao; Kang-Lin Wan, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, P.O. Box 5, Changping, Beijing, 102206, People's Republic of China, Tel/Fax +86 10 58900779, Email zhaolili@icdc.cn; wankanglin@icdc.cn

Objective: To assess the relationship between the variant *rpoB* mutations and the degree of rifampin (RIF)/rifabutin (RFB) resistance in *Mycobacterium tuberculosis* (*M. tuberculosis*).

Methods: We analyzed the whole *rpoB* gene in 177 *M. tuberculosis* clinical isolates and quantified their minimum inhibitory concentrations (MICs) using microplate-based assays.

Results: The results revealed that of the 177 isolates, 116 were resistant to both RIF and RFB. There were 38 mutated patterns within the sequenced whole *rpoB* gene of the 120 isolates. Statistical analysis indicated that mutations, S450L, H445D, H445Y, and H445R, were associated with RIF and RFB resistance. Of these mutations, S450L, H445D, and H445Y were associated with high-level RIF and RFB MIC. H445R was associated with high-level RIF MIC, but not high-level RFB MIC. D435V and L452P were associated with only RIF, but not RFB resistance. Q432K and Q432L were associated with high-level RFB MIC. Several single mutations without statistical association with rifamycin resistance, such as V170F, occurred exclusively in low-level RIF but high-level RFB resistant isolates. Additionally, although cross-resistance to RIF and RFB is common, 21 RIF-resistant/RFB-susceptible isolates were identified.

Conclusion: This study highlighted the complexity of rifamycin resistance. Identification of the *rpoB* polymorphism will be helpful to diagnose the RIF-resistant tuberculosis that has the potential to benefit from a treatment regimen including RFB.

Keywords: *Mycobacterium tuberculosis*, rifampin resistance, rifabutin resistance, mutation

Introduction

Tuberculosis (TB) continues to be a major global public health threat causing 6.4 million new cases and 1.6 million deaths in 2021.[1] The alarming increase in drug-resistant, especially rifampicin-resistant TB (RR-TB), represents the major challenge for TB control. RR-TB requires prolonged use of a combination of drugs that are less efficient and more toxic. In the last year, there was a worldwide estimate of 450,000 incident cases of RR-TB.[1]

Rifampin (RIF) is one of the principal drugs in TB treatment and serves as a hallmark for the detection of multidrug-resistant TB (MDR-TB).[2] Rifabutin (RFB) is a semisynthetic derivative of rifamycin S, which together with RIF, belongs to the rifamycin family. These two drugs target the DNA-dependent RNA polymerase β-subunit, encoded by the *rpoB* gene. RFB is recommended for TB treatment in HIV-coinfected patients because it is less prone to drug–drug interactions than RIF in patients receiving antiretroviral therapy.[3,4] However, reports on the successful treatment of MDR-TB patients with RFB, even in patients with isolates susceptible in vitro to RFB, are very limited.[5,6] One possible reason is due to the

Received: 19 August 2022
Accepted: 17 November 2022
Published: 28 November 2022

fact that the clinical efficacy of RFB for the treatment of MDR-TB has not been well established because of general concerns about potential cross-resistance among the rifamycins.[7,8]

Certain mutations in the RIF resistance-determining region (RRDR) in *rpoB* gene, located between codons 426 and 452 in *M. tuberculosis*, are associated with cross-resistance to both RIF and RFB.[2,9–11] The mutations, S450L, H445Y, and H445D are most frequently observed in RIF- and RFB-resistant strains.[11–13] Although cross-resistance to RIF and RFB is common, some RIF-resistant strains carrying certain *rpoB* mutations may remain susceptibility in vitro to RFB.[14,15] Thus, RFB has the potential to be clinically effective against RIF-resistant strains.

Some reports have shown RFB susceptibility in RIF-resistant strains with specific *rpoB* mutations.[16–18] These studies also pointed out the possible uses for RFB in these cases with *rpoB* polymorphisms and their association with phenotypic drug susceptibility data.[16,17] However, most studies focused on the analysis of mutations in the RRDR region of *rpoB*.[11,17,18] Furthermore, several *rpoB* mutations were also detected in rifamycin-susceptible phenotypes. Thus, a detailed investigation of the different *rpoB* mutations and their association with different rifamycin resistance levels may be helpful to clinicians treating RIF-resistant TB, even multi-drug resistant TB (MDR-TB). To this end, we systematically performed quantitative RIF and RFP resistance phenotyping with MIC measurements for 177 *M. tuberculosis* clinical isolates from China and compared the effect of mutation within the whole *rpoB* gene on MIC changes for these two drugs.

Materials and Methods

M. tuberculosis Isolates

Overall 177 *M. tuberculosis* clinical strains, isolated from 177 epidemiologically unrelated patients with pulmonary tuberculosis (126 males; age range, 16–80 years; mean ± standard deviation, 42.2 ± 16.6), were collected in nine provinces of China, including Fujian (15 isolates), Guangxi (21 isolates), Guizhou (19 isolates), Hunan (25 isolates), Gansu (14 isolates), Jilin (26 isolates), Inner Mongolia (23 isolates), Xinjiang (16 isolates) and Tibet (18 isolates). All isolates were cultured on Lowenstein-Jensen (L-J) medium and freshly subcultured before being used for MIC testing.

MIC Testing

MICs were determined by in vitro 96-well microplate-based assay as described previously.[19] RIF concentrations prepared in Middlebrook 7H9 were 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 64, 128, and 256 μg/mL, and RFB concentrations tested were 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 μg/mL. The critical concentration was taken as 0.5 μg/mL for RIF and 0.5 μg/mL for RFB.[20,21]

Quality Control for MIC Testing

M. tuberculosis (ATCC 27294) was used as a quality control and was tested with each batch of MIC testing. This quality control strain is susceptible to both rifamycin drugs with MICs ≤0.125 μg/mL[21] in the present study.

DNA Extraction, Amplification, and Sequencing

All isolates on the L-J slants were collected and inactivated by heating at 95°C for 20 min. Supernatants containing genomic DNA were collected by centrifugation and stored at −20°C for further use.

The entire *rpoB* gene of each isolate was amplified using the primers and reaction conditions as previously described.[19] All amplified products were purified, dried, and loaded onto an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA). The sequences generated were compared with the H37Rv reference genome (GenBank accession number NC_000962) using BioEdit v7.05.3.

Data Analysis

The effects of *rpoB* mutations on rifamycin drugs were evaluated by the regression multivariate model and *P* value less than 0.05 was considered statistically significant. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, USA).

Results

MIC Results

Of the 177 tested isolates, 106 (59.9%) were resistant to RIF and RFB, 21 (11.9%) were RIF-resistant and RFB-susceptible, and 50 (28.2%) were susceptible to RIF and RFB. All the RIF-resistant isolates were categorized into the low-level group (MIC 1–64 μg/mL) and the high-level group (MIC ≥128 μg/mL). Among 127 RIF-resistant isolates, 38 (29.9%) and 89 (70.1%) had low and high levels of RIF MIC, respectively. Accordingly, MIC ranges for RFB resistance were categorized as high (MIC ≥8 μg/mL) and low (MIC 1–4 μg/mL) level groups, which contained 63 (59.4%) and isolates 43 (40.6%), respectively.

Mutations Within *rpoB*

DNA sequencing showed that 120 isolates harbored at least one non-synonymous mutation within the sequenced *rpoB* gene (Table 1), and 96 isolates (80.0%) had a single mutation, while 24 isolates (20.0%) had multiple mutations. It was notable that there were two isolates carrying both missense mutation and deletion mutation (Table 1). For all mutations, 38 genotype patterns distributed across 19 different sites were detected, including 28 polymorphisms in the RRDR and 10 polymorphisms outside the RRDR. The most frequent mutations were observed at codons 450, 445, and 435, which had mutated frequencies of 46.7% (56/120 isolates), 27.5% (33/120 isolates), and 17.5% (21/120 isolates), respectively. Other mutations were also observed at codons 45, 170, 400, 429, 430, 431, 432, 441, 446, 452, 460, 488, 491, 759, and 1056, which had a total mutated frequency of only 28.3% (34/120 isolates). Of these 34 isolates, 21 (61.8%, 21/34 isolates) were combined with additional mutations at 435, 445, or 450. It was notable that a single mutation V170F, located outside RRDR, occurred in one RIF and RFB-resistant isolate. Two novel deletions in *rpoB*, D435del (nucleotide 1303–1305 deletion) and F433-D435del (nucleotide 1296–1304 deletion) were also identified.

Correlation of *rpoB* Mutations with Drug Resistance

Most of the *rpoB* mutations (single or double) occurred in RIF- and RFB-resistant isolates. Only one RIF-susceptible isolate (MIC = 0.5 μg/mL) and 19 RFB-susceptible isolates (MIC ≤ 0.5 μg/mL) bearing the *rpoB* mutation were detected (Table 1).

As 20.0% of isolates with *rpoB* mutations harbored more than one mutation, the correlation between mutations and drug resistance was estimated through multivariate regression (Table 2). In the multivariate analysis, resistance to both RIF and RFB was predominantly correlated with S450L, H445D, H445Y, and H445R ($P<0.05$), with the OR values of 226.69, 45.99, 37.39, and 15.89 for RIF, and 425.87, 86.16, 70.06 and 17.98 for RFB, respectively (Table 2). Notably, mutations D435V (OR = 37.39) and L452P (OR = 11.59) were associated with resistance to only RIF ($P<0.05$), but not RFB, while mutations Q432K (OR = 13.75), Q432L (OR = 13.75), and L430P (OR = 15.99) were associated with resistance to only RFB ($P<0.05$), but not RIF. Furthermore, other mutations, except for those locating in the RRDR, were not associated with drug resistance according to multivariate analyses. In addition, there were still 8 RIF-resistant isolates and 5 RFB-resistant isolates that harbored no mutation in the whole *rpoB*.

Association Between *rpoB* Mutations and High-Level MIC

Isolates with higher MICs were more likely to harbor the S450L, H445D, and H445Y mutations (Table 1). Among the isolates harboring these three mutations, the proportions of high-level MIC isolates were 90.7% (49/54 isolates), 100.0% (11/11 isolates), and 88.9% (8/9 isolates) for RIF, and 64.8 (35/54 isolates), 54.5% (6/11 isolates) and 77.8% (7/9 isolates), and for RFB, respectively. The multivariate model also showed that these three mutations were most significantly correlated with high-level MICs ($P<0.01$), with the OR values of 50.39, 75.53, and 40.80 for RIF, and 17.35, 11.12, and 31.43 for RFB, respectively (Table 3). In addition, the H445R mutation (OR = 26.09) was significantly associated with high-level RIF MIC ($P<0.01$), but not high-level RFB MIC. However, mutations Q432K (OR = 20.46) and Q432L (OR = 20.46) were significantly associated with high-level RFB MIC ($P<0.05$), but not high-level RIF MIC.

There were 29 high-level RIF MIC isolates with *rpoB* mutations belonging to the low-level RFB MIC group. However, the number of high-level RFB isolates with low-level RIF MIC was only six, among which four isolates

Table 1 Distribution of *rpoB* Mutations and MICs of RIF and RFB

rpoB Mutations[a]		MIC (μg/mL)		No. of Mutated Isolates
M. tuberculosis	***E. coli***	**RIF**	**RFB**	
P45S/S450L	P25S/S531L	≥128	≥16	1
V170F	V146F	16	≥16	1
V170F/H445N	V146F/H526N	16	8	1
T400I/H445Y	T481I/H526Y	≥128	≥16	1
Q429H/H445Y	Q510H/H526Y	≥128	≥16	1
L430P	L511P	4	1	1
L430P/S431G	L511P/S512G	16	2	1
L430R/D435G	L511R/D516G	8	0.5	1
L430P/D435G	L511P/D516G	32	1	1
L430P/H445N	L511P/H526N	≥128	≥16	1
Q432H/F433-D435del*	Q513H/F514-D516del*	8	0.12	1
Q432K	Q513K	≥128	≥16	2
Q432L	Q513L	≥128	≥16	2
Q432P	Q513P	≥128	1	1
D435E/S441L	D516E/S522L	≥128	0.5	1
D435G/L452P	D516G/L533P	≥128	≥16	1
D435G/L452P	D516G/L533P	64	1	1
D435G/I491N	D516G/I572N	8	0.5	1
D435V	D516V	4	0.25	1
D435V	D516V	4	1	1
D435V	D516V	16	0.25	1
D435V	D516V	16	1	1
D435V	D516V	64	0.25	1
D435V	D516V	64	0.5	1
D435V	D516V	64	1	1
D435V	D516V	≥128	0.5	1
D435V	D516V	≥128	2	1
D435V/S450L	D516V/S531L	64	8	1
D435Y	D516Y	0.5	≤0.12	1
D435Y	D516Y	1	0.25	1
D435Y/S450G	D516Y/S531G	16	4	1
D435del*/E460G	D516del*/E541G	≥128	4	1
S441L	S522L	16	0.5	1
H445D	H526D	≥128	2	1
H445D	H526D	≥128	4	4
H445D	H526D	≥128	8	3
H445D	H526D	≥128	≥16	3
H445L	H526L	2	0. 5	1
H445L	H526L	4	0.25	1
H445N/L452P	H526N/L533P	≥128	4	1
H445N/L452P	H526N/L533P	≥128	8	1
H445P/K446Q	H526P/K527Q	≥128	≥16	1
H445Q/I491M	H526Q/I572M	8	0.25	1
H445R	H526R	≥128	0.5	1
H445R	H526R	≥128	1	1

(*Continued*)

Table 1 (Continued).

rpoB Mutations[a]		MIC (μg/mL)		No. of Mutated Isolates
M. tuberculosis	***E. coli***	**RIF**	**RFB**	
H445R	H526R	≥128	≥16	2
H445Y	H526Y	2.0	0.25	1
H445Y	H526Y	16	1	1
H445Y	H526Y	≥128	4	1
H445Y	H526Y	≥128	8	2
H445Y	H526Y	≥128	≥16	3
S450L	S531L	32	4	1
S450L	S531L	64	8	1
S450L	S531L	64	≥16	2
S450L	S531L	≥128	1	1
S450L	S531L	≥128	2	6
S450L	S531L	≥128	4	9
S450L	S531L	≥128	8	8
S450L	S531L	≥128	≥16	20
S450F	S531F	≥128	8	1
S450L/I488V	S531L/I569V	≥128	4	1
S450L/I491V	S531L/I572V	≥128	4	1
S450L/G759S	S531L/G846S	≥128	8	1
S450L/Q1056K	S531L/Q1264K	≥128	≥16	1
L452P	L533P	2	0.5	1
L452P	L533P	2	1	1
L452P	L533P	1	0.25	1

Notes: [a]Mutations are showed using either the *M. tuberculosis* or *E. coli* numbering system. *Mutation reported for the first time; del, deletion.

Table 2 Logistic Regression Multivariate Model Results Between *rpoB* Mutations and Drug Resistance

Mutation	Isolates	RIF			RFB		
		Median MIC (IQR)	OR	*P*	Median MIC (IQR)	OR	*P*
Q432K	2	128 (128, 128)	7.31	0.127	16 (16, 16)	13.75	0.047*
Q432L	2	128 (128, 128)	7.31	0.127	16 (16, 16)	13.75	0.047*
H445N	4	128 (72, 128)	/	/	8 (6, 12)	1.60	0.748
H445R	4	128 (128, 128)	15.89	<0.01**	8.5 (0.75, 16)	17.98	0.028*
H445L	2	3 (2, 4)	7.31	0.127	0.375 (0.25, 0.5)	2.77	1.000
H445D	11	128 (128, 128)	45.99	<0.000**	8 (4, 16)	86.16	<0.000**
H445Y	9	128 (128, 128)	37.39	<0.000**	16 (8, 16)	70.06	<0.000**
L430P	4	24 (10, 80)	7.31	0.127	1.5 (1, 9)	15.99	0.020*
L452P	7	2 (2, 128)	11.59	0.034*	1 (0.5, 8)	5.73	0.205
D435G	5	32 (8, 64)	7.31	0.127	1 (0.5, 1)	2.26	0.911
D435V	10	64 (16, 64)	37.39	<0.000**	0.75 (0.25, 1)	4.99	0.083
D435Y	3	1 (0.5 16)	6.31	0.312	0.25 (0.12, 4)	3.14	0.751
V170F	2	16 (16, 16)	3.06	0.492	12 (8, 16)	5.79	0.295
S441L	2	72 (16, 128)	7.31	0.127	0.5 (0.5, 0.5)	2.77	1.000
S450L	54	128 (128, 128)	226.69	<0.000**	8 (4, 16)	425.87	<0.000**

Note: **P*< 0.05 (significant); ***P* <0.01 (highly significant).

Table 3 Logistic Regression Multivariate Model Results Between *rpoB* Mutations and High-Level MIC

Drug and Mutation	OR Value	*P*
High-level RIF MIC		
H445R	26.09 (4.24, Infinity)	0.002**
H445D	75.53 (14.68, Infinity)	<0.000**
H445Y	40.80 (4.84, >999.99)	<0.000**
L452P	3.98 (0.52, 26.68)	0.205
D435V	0.77 (0.05, 5.95)	1.000
S450L	50.39 (16.39, 192.83)	<0.000**
High-level RFB MIC		
Q432K	20.46 (2.37, Infinity)	0.024*
Q432L	20.46 (2.37, Infinity)	0.024*
H445R	9.24 (0.60, 143.99)	0.120
H445D	11.12 (2.28, 56.64)	0.002**
H445Y	31.43 (4.95, 359.98)	<0.000**
L430P	3.17 (0.06, 45.44)	0.700
S450L	17.35 (6.68, 50.38)	<0.000**

Note: **P*< 0.05 (significant); ***P* <0.01 (highly significant).

had RIF MICs of 64 μg/mL, very close to the MIC definition of high-level RIF MIC (128 μg/mL). Interestingly, the V170F mutation was exclusively presented in two high-level RFB MIC but low-level RIF MIC isolates.

Discussion

Rapid and accurate determination of drug resistance in *M. tuberculosis* is essential for early initiation of appropriate anti-TB treatment. Although several molecular diagnostic techniques, such as GeneXpert MTB/RIF (Cepheid Inc., Sunnyvale, CA, USA) and GenoType MTBDRplus (HAIN Lifescience GmbH, Nehren, Germany), provide the rapid identification of *M. tuberculosis* drug resistance directly from specimens,[22–24] these molecular methods may miss isolates with mutations outside the target region, and cannot determine the level of resistance. Furthermore, previous reports indicated that some isolates with *rpoB* mutations are RIF-susceptible according to phenotypic drug susceptibility testing.[10,11,25] Similarly, RFB-susceptible isolates with *rpoB* mutations have also been reported,[11,14,15,18,26] highlighting the importance of the potential inclusion of RFB in the treatment regimen. For these reasons, it is crucial to understand the association of *rpoB* mutations with different RIF and RFB resistance levels.

Although cross-resistance to RIF and RFB is common, we still observed discordance between RIF and RFB in 21 isolates. Previous studies showed a proportion of RIF-resistant isolates with RFB-susceptibility ranging from 13% to 28%.[11,17] In our study, the proportion of RIF-resistant/RFB-susceptible isolates was 16.5% (21/127 isolates). The replacement of RIF by RFB might positively affect the treatment outcome in the cases carrying these isolates.

It is reported that the RNA polymerase β-subunit encoded by *rpoB* is a target for rifamycin drugs, and some amino acid changes in this protein can lead to rifamycin resistance.[27–30] Our study showed that 93.7% (119/127 isolates) of RIF-resistant isolates and 95.3% (101/106 isolates) of RFB-resistant isolates harbored at least one mutation in *rpoB*. Overall, 19 distinct codons in *rpoB* had mutations contributing to amino acid replacements, of which 11 codons are located in the RRDR and 8 are located outside the RRDR. Interestingly, almost all mutations outside the RRDR were accompanied by mutations in the RRDR. These results show the potential for using DNA sequencing of RRDR to rapidly predict RIF and RFB resistance.[2,10] However, a single mutation V170F outside the RRDR was also observed in one resistant isolate, similar to some reports.[20,31] This highlights the complexity of RIF and RFB resistance and emphasizes the requirement to investigate beyond the RRDR, especially when patient treatment effects are not as expected.

Our study also showed the mutations associated with RIF and RFB. Statistical analysis indicated that, of the 38 different *rpoB* polymorphisms, S450L, H445D, H445Y, and H445R were significantly correlated with RIF and RFB resistance. Miotto et al[32] and Whitfield et al[17] also classified these four mutations as RIF-resistant and RFB-resistant.

Mutations D435V and L452P were significantly associated with only RIF resistance, but not RFB resistance, consistent with the prior data.[17,32] Cases carrying the isolates with these two mutations may be RFB-susceptible and benefit from the RFB-containing treatment regimen. In addition, our results indicated that Q432K, Q432L and L430P were significant with only RFB-resistance. Although mutation L430P was classified as RFB-susceptible by Whitfield et al,[17] this mutation was observed in four RFB-resistant isolates. Of them, three isolates were accompanied by another *rpoB* mutation (Table 1). Interestingly, one isolate harboring double mutations (L430P/H445N) classified as RFB-susceptible by Whitfield,[17] had a MIC of ≥16 μg/mL. Thus, the actual effects of these double *rpoB* mutations on the RFB resistance deserve further investigation.

We also found that mutations 450L, H445D, and H445Y were strongly associated with high-level RIF/RFB MIC. This observation is similar to those of previous reports.[11–13,33] Moreover, mutation H445R was well correlated with high-level RIF MIC, while mutations Q432K and Q432L were associated with high-level RFB MIC. Our results, therefore, add important data on the association between mutations and rifamycin-resistant levels in TB. Notably, V170F mutation occurred exclusively in two high-level RFB MIC but low-level RIF MIC isolates. The function of this mutation on the interaction between RpoB and RFB is unknown and requires further exploration.

This study used the WHO current recommended clinical breakpoints (0.5 μg/mL) for susceptibility testing of RIF.[20] However, we still identified one RIF-susceptible isolate (MIC = 0.5 μg/mL) with the D435Y mutation, which has also been reported in some RIF-susceptible isolates.[14,15,31] It was notable that 19 RFB-susceptible isolates also harbored *rpoB* mutations, with the use of CLSI recommended clinical breakpoints (0.5 μg/mL) for susceptibility testing of RFB.[21] Of them, 9 had MICs of 0.5 μg/mL. Although most mutations presenting in these isolates were classified as RFB-susceptible, some reports also suggested that the critical testing concentration, currently recommended at 0.5 μg/mL, may be too high.[15,16] Moreover, some RIF/RFB resistant isolates lacking *rpoB* mutations were identified, indicating that resistance in these isolates may be attributed to other mechanisms, such as enhanced efflux pump activity or lowered cell wall permeability.[11,34]

This study also suffers some limitations. In total, large amounts of clinical isolates (n = 177) were recruited in this study. However, the number of isolates carrying mutations outside RRDR, such as T400I, G759S, and Q1056K, was very small, leading to insufficient data for further statistical analysis. Also, we only analyzed mutations in the whole *rpoB* gene, and changes in other genes or other mechanisms such as efflux pumps could not be identified in the present study.

In summary, we determined comprehensive profiles of *rpoB* mutations and their associations with RIF and RFB resistance levels in a variety of *M. tuberculosis* clinical isolates. Revealing the SNP-specific differential rifamycin phenotypic resistance will have the potential for using RFB to optimize the treatment of some RIF-resistant patients. However, clinical investigations are required to verify whether RFB can be applied effectively in these patients to promote outcomes.

Ethical Approval

This study was approved by the Ethics Committee of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. All patients participating in the study had written consent themselves.

Funding

This study was supported by the projects from the National Key Program of Mega Infectious Diseases (Grant No. 2018ZX10302302-001). The funder had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

Disclosure

All authors have no competing interests to report in this work.

References

1. World Health Organization. *Global Tuberculosis Report 2022*. Geneva: World Health Organization; 2022. licence: cc bY-Nc-sa 3.0 iGo.
2. Zaw MT, Emran NA, Lin Z. Mutations inside rifampicin-resistance determining region of rpoB gene associated with rifampicin-resistance in Mycobacterium tuberculosis. *J Infect Public Health*. 2018;11(5):605–610. doi:10.1016/j.jiph.2018.04.005
3. Lee SS, Meintjes G, Kamarulzaman A, Leung CC. Management of tuberculosis and latent tuberculosis infection in human immunodeficiency virus-infected persons. *Respirology*. 2013;18(6):912–922. doi:10.1111/resp.12120
4. Regazzi M, Carvalho AC, Villani P, Matteelli A. Treatment optimization in patients co-infected with HIV and Mycobacterium tuberculosis infections: focus on drug-drug interactions with rifamycins. *Clin Pharmacokinet*. 2014;53(6):489–507. doi:10.1007/s40262-014-0144-3
5. Jo KW, Ji W, Hong Y, et al. The efficacy of rifabutin for rifabutin-susceptible, multidrug-resistant tuberculosis. *Respir Med*. 2013;107:292–297. doi:10.1016/j.rmed.2012.10.021
6. Lee H, Ahn S, Hwang NY, et al. Treatment outcomes of rifabutin-containing regimens for rifabutin-sensitive multidrug-resistant pulmonary tuberculosis. *Int J Infect Dis*. 2017;65:135–141. doi:10.1016/j.ijid.2017.10.013
7. Aristoff PA, Garcia GA, Kirchhoff PD, Showalter HD. Rifamycins–obstacles and opportunities. *Tuberculosis*. 2010;90:94–118. doi:10.1016/j.tube.2010.02.001
8. Horne DJ, Spitters C, Narita M. Experience with rifabutin replacing rifampin in the treatment of tuberculosis. *Int J Tuberc Lung Dis*. 2011;15:1485–9, i. doi:10.5588/ijtld.11.0068
9. Jagielski T, Bakula Z, Brzostek A, et al. Characterization of mutations conferring resistance to rifampin in Mycobacterium tuberculosis clinical strains. *Antimicrob Agents Chemother*. 2018;62(10). doi:10.1128/AAC.01093-18
10. Campbell PJ, Morlock GP, Sikes RD, et al. Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2011;55(5):2032–2041. doi:10.1128/AAC.01550-10
11. Jamieson FB, Guthrie JL, Neemuchwala A, Lastovetska O, Melano RG, Mehaffy C. Profiling of rpoB mutations and MICs for rifampin and rifabutin in Mycobacterium tuberculosis. *J Clin Microbiol*. 2014;52(6):2157–2162. doi:10.1128/JCM.00691-14
12. Ohno H, Koga H, Kohno S, Tashiro T, Hara K. Relationship between rifampin MICs for and rpoB mutations of Mycobacterium tuberculosis strains isolated in Japan. *Antimicrob Agents Chemother*. 1996;40(4):1053–1056. doi:10.1128/AAC.40.4.1053
13. Pang Y, Lu J, Wang Y, Song Y, Wang S, Zhao Y. Study of the rifampin monoresistance mechanism in Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2013;57(2):893–900. doi:10.1128/AAC.01024-12
14. Farhat MR, Sixsmith J, Calderon R, Hicks ND, Fortune SM, Murray M. Rifampicin and rifabutin resistance in 1003 Mycobacterium tuberculosis clinical isolates. *J Antimicrob Chemother*. 2019;74(6):1477–1483. doi:10.1093/jac/dkz048
15. Schon T, Jureen P, Chryssanthou E, et al. Rifampicin-resistant and rifabutin-susceptible Mycobacterium tuberculosis strains: a breakpoint art fact? *J Antimicrob Chemother*. 2013;68(9):2074–2077. doi:10.1093/jac/dkt150
16. Rukasha I, Said HM, Omar SV, et al. Correlation of rpoB mutations with minimal inhibitory concentration of rifampin and rifabutin in Mycobacterium tuberculosis in an HIV/AIDS endemic setting, South Africa. *Front Microbiol*. 2016;7:1947. doi:10.3389/fmicb.2016.01947
17. Whitfield MG, Warren RM, Mathys V, et al. The potential use of rifabutin for treatment of patients diagnosed with rifampicin-resistant tuberculosis. *J Antimicrob Chemother*. 2018;73(10):2667–2674. doi:10.1093/jac/dky248
18. Berrada ZL, Lin SY, Rodwell TC, et al. Rifabutin and rifampin resistance levels and associated rpoB mutations in clinical isolates of Mycobacterium tuberculosis complex. *Diagn Microbiol Infect Dis*. 2016;85(2):177–181. doi:10.1016/j.diagmicrobio.2016.01.019
19. Li MC, Lu J, Lu Y, et al. rpoB mutations and effects on rifampin resistance in Mycobacterium tuberculosis. *Infect Drug Resist*. 2021;14:4119–4128. doi:10.2147/IDR.S333433
20. World Health Organization. *Technical Report on Critical Concentrations for Drug Susceptibility Testing of Isoniazid and the Rifamycins (Rifampicin, Rifabutin and Rifapentine)*. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
21. Wood GL. *CLSI Performance Standards for Susceptibility Testing of Mycobacteria, Nocardia Spp., and Other Aerobic Actinomy Cetes*. 1st ed. CLSI; 2018:M62.
22. Friedrich SO, Venter A, Kayigire XA, Dawson R, Donald PR, Diacon AH. Suitability of Xpert MTB/RIF and genotype MTBDR plus for patient selection for a Tuberculosis clinical trial. *J Clin Microbiol*. 2011;49(8):2827–2831. doi:10.1128/JCM.00138-11
23. Di Tanna GL, Khaki AR, Theron G, et al. Effect of Xpert MTB/RIF on clinical outcomes in routine care settings: individual patient data meta-analysis. *Lancet Glob Health*. 2019;7(2):e191–e199. doi:10.1016/S2214-109X(18)30458-3
24. Xie YL, Chakravorty S, Armstrong DT, et al. Evaluation of a rapid molecular drug-susceptibility test for Tuberculosis. *N Engl J Med*. 2017;377(11):1043–1054. doi:10.1056/NEJMoa1614915
25. Wi YM, Greenwood-Quaintance KE, Brinkman CL, Lee JYH, Howden BP, Patel R. Rifampicin resistance in Staphylococcus epidermidis: molecular characterisation and fitness cost of rpoB mutations. *Int J Antimicrob Agents*. 2018;51(5):670–677. doi:10.1016/j.ijantimicag.2017.12.019
26. Chen HY, Yu MC, Huang WL, et al. Molecular detection of rifabutin-susceptible Mycobacterium tuberculosis. *J Clin Microbiol*. 2012;50:2085–2088. doi:10.1128/JCM.00652-12
27. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update. *Tuber Lung Dis*. 1998;79:3–29. doi:10.1054/tuld.1998.0002
28. Rothman-Denes LB. Structure of Escherichia coli RNA polymerase holoenzyme at last. *Proc Natl Acad Sci USA*. 2013;110(49):19662–19663. doi:10.1073/pnas.1320604110
29. Goldstein BP. Resistance to rifampicin: a review. *J Antibiot*. 2014;67(9):625–630. doi:10.1038/ja.2014.107
30. 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. doi:10.1016/j.tube.2021.102083
31. WHO catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance; 2021. Available from: https://apps.who.int/iris/handle/10665/341981. Accessed November 21, 2022.
32. Miotto P, Tessema B, Tagliani E, et al. A standardised method for interpreting the association between mutations and phenotypic drug resistance in Mycobacterium tuberculosis. *Eur Respir J*. 2017;50(6):1701354. doi:10.1183/13993003.01354-2017

33. Shea J, Halse TA, Kohlerschmidt D, et al. Low-level rifampin resistance and rpoB mutations in Mycobacterium tuberculosis: an analysis of whole-genome sequencing and drug susceptibility test data in New York. *J Clin Microbiol*. 2021;59(4):e01885–e01920.
34. Louw GE, Warren RM, Gey van Pittius NC, McEvoy CR, Van Helden PD, Victor TC. A balancing act: efflux/influx in mycobacterial drug resistance. *Antimicrob Agents Chemother*. 2009;53(8):3181–3189. doi:10.1128/AAC.01577-08