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Risk Factors and Treatment Outcome Analysis Associated with Second-Line Drug-Resistant Tuberculosis

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Abstract: The present study aimed at analyzing the treatment outcomes and risk factors associated with fluoroquinolone drug resistance having mutations in the *gyrA* and *gyrB* genes. A total of 258 pulmonary tuberculosis samples with first-line drug-resistant (H, R, or HR) were subjected to GenoType MTBDRsl assay for the molecular detection of mutations. Among the 258 samples, 251 were drug-resistant tuberculosis and seven were sensitive to all first-line TB drugs. Out of 251 DR-TB cases, 42 cases were MDR TB, 200 were INH mono-resistant and nine cases were RIF mono-resistant tuberculosis. Out of 251 DR-TB cases performed with a MTBDRsl assay, 14 had Pre-XDR-FQ, one patient had pre-XDR-SLID, one had extensively drug-resistant tuberculosis (XDR-TB) and 235 cases were sensitive to both FQ and SLID drugs. The study group had a mean average of 42.7 ± 16.4 years. The overall successful treatment outcomes among the MDR, INH mono-resistant, and pre-XDR patients were 70.6%, 82.0%, and 51%, respectively. The percentage of risk for the unfavorable outcomes in the pre-XDR, INH-mono-resistant, and XDR cases were 113.84% increased risk with RR 2.14; 95% CI 0.7821–5.8468. The independent risk factor associated with the unfavorable outcomes to failure was 77.78% increased risk with RR 1.78; 95% CI 0.3375–9.3655. Logistic regression analysis revealed that the percentage relative risk among MDR-TB patients for gender, male (RR: 1.85), age ≥ 61 years (RR: 1.96), and diabetics (RR: 1.05) were 84.62%, 95.83%, and 4.76%, respectively. The independent risk factors associated with INH mono-resistant cases of age 16–60 (RR: 1.86), ≥ 61 year (RR: 1.18), and treated cases (RR: 5.06). This study presaged the significant risk of INH mono-resistant, pre-XDR, and MDR among males, young adults, diabetics, and patients with previous treatment failure. Timely identification of high-risk patients will give pronounced advantages to control drug resistance tuberculosis diseases.

Keywords: *Mycobacterium tuberculosis*; MTBDRplus; MTBDRsl; fluoroquinolones; aminoglycosides



Citation: Aaina, M.; Venkatesh, K.; Usharani, B.; Anbazhagi, M.; Rakesh, G.; Muthuraj, M. Risk Factors and Treatment Outcome Analysis Associated with Second-Line Drug-Resistant Tuberculosis. *J. Respiration* **2022**, *2*, 1–12. <https://doi.org/10.3390/jor2010001>

Academic Editor: Cesar A. Moran

Received: 17 November 2021

Accepted: 22 December 2021

Published: 28 December 2021

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1. Introduction

The emergence of drug-resistant tuberculosis jeopardizes the TB control programme activities globally. The development of drug-resistant to any antimicrobial agent by *Mycobacterium tuberculosis* is due to the interplay of biological, clinical, and microbiological reasons such as intrinsic drug resistance, complexity of TB granulomas, phenotypic resistance, acquired resistance and also non-adherence of patients to their six months therapy [1]. Drug-resistant tuberculosis continues to be a major public health problem. In the year 2019, globally close to half a million people developed rifampicin-resistant TB (RR-TB), of

which 78% had multidrug-resistant TB (MDR-TB). MDR-TB is defined tuberculosis strains resistant to at least two first-line drugs, rifampicin (RIF) and isoniazid (INH). The countries accounting for 50% of the global burden of MDR/RR TB were India (27%), China (14%), and the Russian Federation (8%). The average proportion of MDR TB cases with XDR TB was 6.2% globally. The proportion of MDR/RR-TB cases with resistance to fluoroquinolone (FQ), including levofloxacin (LFX) and moxifloxacin (MFX), was 20.8% among twenty-four high TB or MDR-TB burden countries [2]. Globally, in 2020, 3.4% of new TB cases (patients never treated with anti-TB medicine or treated for <1 month) and 18% of previously treated cases (patients treated for \geq one month in the past) had MDR/RR-TB. The highest proportions (>50% in previously treated cases) were in countries of the former Soviet Union [3]. India alone accounts for about one-fourth of the global burden of MDR-TB. The Indian government survey conducted from 2014 to 2016 revealed that the estimated incidence of MDR-TB was 2.84% in new cases and 11.6% among previously treated patients [4]. Further, rifampicin mono-resistance was negligible, and INH resistance was invariably associated with rifampicin resistance. Worldwide, INH mono-resistance in new cases is 7.2% and 11.6% in previously treated TB cases. In India, INH mono-resistance was observed in 3.8% and 7.8% of new and previously treated cases, respectively [5].

Extensively drug-resistant TB (XDR-TB) is defined as MDR-TB strain that is resistant to any fluoroquinolone drug and one of the second-line injectable drugs (i.e., amikacin, kanamycin, or capreomycin). Pre XDR-TB is defined as the MDR-TB strain that is resistant to either fluoroquinolones (FQs) or second-line injectable drugs agent but not both [6,7]. Aminoglycosides (kanamycin, amikacin) act against mycobacterial species by binding to the 30S ribosomal subunit, affecting polypeptide synthesis, resulting in inhibition of translation. Resistance to aminoglycosides develops due to mutation of the ribosome target binding sites, although cross-resistance is observed between amikacin and kanamycin. Fluoroquinolones (levofloxacin, moxifloxacin) act by trapping gyrase and topoisomerase IV on DNA as ternary complexes, which block the movement of replication forks and the transcription process. Unlike other bacterial species, *Mycobacterium tuberculosis* lacks topoisomerase IV but contains the genes *gyrA* and *gyrB* encoding the A and B subunits, respectively, of DNA gyrase. Resistance to fluoroquinolones is associated with mutations in the conserved quinolone resistance-determining region (QRDR) of *gyrA* and *gyrB* involved in the interaction between the drug and DNA gyrase [8]. Mutations in *gyrA* and *gyrB* lead to acquired resistance to fluoroquinolones and these have been widely used as predictive markers for fluoroquinolones resistance in molecular diagnostics [9,10]. This study aims to assess the susceptibility of *M. tuberculosis* strains to fluoroquinolones and second-line injectable drugs and survey the putative mutations associated with quinolone resistance in the α -subunit of the *gyrA* and *gyrB* genes in the clinical strains of *M. tuberculosis* isolated from patients belonging to Puducherry state.

2. Materials and Methods

2.1. Patient Enrollment

A total of 258 smear-positive tuberculosis patients were registered at State TB Training and Demonstration Centre, Government Hospital for Chest Disease, between May 2018 and July 2019 from eight districts of Tamil Nadu and Puducherry state, India were enrolled in this study. The specimens were collected per the guidelines described in the National TB Elimination Programme for diagnostic workup. The grant for this study was supported by the Indian Council of Medical Research through short-term scholarship (Project Identification Code: 2019-04445). The study protocol was approved by the Institute Ethical Committee (SVMC/IEC/2019-22 dated 20 March 2019) and written informed consent was obtained from each study subject. All methods were applied in accordance with relevant guidelines and regulations. Demographic information about the patient was obtained by review of the medical records. The isolates were transferred to the Intermediate Reference Laboratory at Government Hospital for Chest Disease for further drug susceptibility testing. According to the drug treatment history, the MDR-TB suspected cases were divided

into seven groups: previously treated cases (patients treated for \geq one month in the past), new TB cases (patients never treated with anti-TB medicines or treated for <1 month), previous treatment failure cases, retreatment cases, contact of known MDR-TB, and HIV-TB cases, based on the guidance of the National Tuberculosis Elimination Programme, India. The previously treated cases represent the pulmonary tuberculosis patients, still sputum smear-positive at the end of three months of treatment. The previously treated failure cases represent pulmonary tuberculosis patients, still, sputum smear-positive after the completion of treatment (six months) or treated for five months. Retreatment cases are defined as pulmonary tuberculosis patients who were once cured but relapsed or were treated more than one month, but treatment interruption was longer than two months. The retreatment failure cases are defined as retreated pulmonary tuberculosis patients, still sputum smear-positive after the completion of treatment (nine months) or treated for five months.

2.2. Line Probe Assay

First-line drug susceptibility testing for rifampicin and isoniazid was done using GenoType MTBDR plus assay (Hain Life science, Nehren, Germany) and was performed as per the manufacturers' instructions. The sputum decontamination and DNA extraction were carried out in the Biosafety Cabinet (Class II A2 type, ESCO Singapore) at the Biosafety level III facility. Testing was conducted as per the recommendation of the manufacturer using trained laboratory staff. Master Mix amplification and hybridization involved in this assay were carried out in separate clean rooms with restricted access and unidirectional workflow. DNA strip-based tests determine the drug resistance profile of *M. tuberculosis* complex strain through the pattern of binding of amplified products to probes targeting the most common resistance-associated mutations to first and second-line TB drugs and to probes targeting the corresponding wild-type DNA sequence. The mutations are detected either by binding PCR amplified products to probes targeting the most commonly occurring MUT probes or (inferred) by the lack of binding of the amplified products to the corresponding wild-type probes. Reversed hybridization was performed with a fully automated GT Blot (Hain Life science) machine. The results were interpreted as per the instructions given in the insert by the manufacturer. Second-line drug susceptibility testing by methods similar to the ones described above was carried out using the GenoType version 2 MTBDRsl assay (Hain Life science, Nehren, Germany) to detect resistance to fluoroquinolones (FQLs), amikacin (AMK), capreomycin (CAM), and kanamycin (KAN) [9].

2.3. Treatment Outcome Analysis

The conformed MDR/RR-TB patients were treated with longer regimens containing all three Group A (fluoroquinolones (levofloxacin and moxifloxacin), bedaquiline and linezolid) agents with at least one Group B (clofazimine and cycloserine or terizidone) in the intensive phase and at least three in the continuation phase of treatment, with a total treatment duration of 18–20 months for most patients. The conformed isoniazid-resistant and rifampicin-sensitive tuberculosis patients were treated with rifampicin, ethambutol, pyrazinamide, and levofloxacin for six months as per the WHO's guidelines. Treatment outcomes were analyzed after the completion of treatment as per the WHO guidelines. The treatment outcomes were defined according to WHO recommendations as cured, treatment completed, treatment failed, death, and lost to follow-up. The sum total of cured (treatment completed and three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase) and treatment completed (treatment completed but no record that three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase) is commonly used as an indicator of favourable outcome, or treatment success. Treatment failure, death and loss to follow-up were considered as unfavorable treatment outcomes [10,11].

2.4. Statistical Analysis

Data were entered using Epi-Data version 3.1 and analyzed using SPSS version 20 (SPSS Inc., Chicago, IL, USA) software. Completeness of data and consistency was checked by running frequencies of each variable. Quantitative variables were intimated as mean \pm standard deviation; qualitative variables were denoted as number of observations with percentages. We conducted a logistic regression analysis to determine the factors associated with multidrug-resistant tuberculosis (MDR-TB), extremely drug-resistant tuberculosis (XDR-TB), and treatment outcomes. Cumulative incidence functions were used to estimate time to events (i.e., time to poor treatment outcomes, time to lost to follow-up, and time to unfavourable outcomes), and a competing-risks survival regression model was used to identify predictors of treatment outcomes.

3. Results

Out of 258 patients screened by Genotype MTBDRplus assay for the molecular detection of mutations, 251 cases were drug-resistant tuberculosis and seven cases were sensitive to all first-line TB drugs. Overall, 188 (72.9%) were male and 70 (27.1%) were female patients. Among the 251 DR-TB patients, 42 (16.3%) cases were multidrug-resistant tuberculosis, 200 (77.5%) and 9 (3.5%) cases were identified as isoniazid and rifampicin mono-resistant, respectively. The remaining seven cases were sensitive to both rifampicin and isoniazid drugs (PAN sensitive). Out of 251 DR-TB patient samples subjected to genotype MTBDRsl assay, 15 (15/258, 5.8%) cases were pre-XDR-TB and one (1/258, 0.39%) case was extensively drug-resistant tuberculosis (XDR-TB). Among the 15 pre-XDR-TB cases, 14 cases were fluoroquinolone-resistant, and one was resistant to second-line injectable drugs. Almost all age groups were prone to have pre-XDR except to the pediatric age group. Of the 16 s-line drug-resistant patients, one XDR (1/42, 2.4%) and four pre-XDR-TB (4/42, 9.5%) cases were from multidrug resistant tuberculosis cases. Among other nine pre-XDR-TB (9/200, 4.5%) patients, eight cases were fluoroquinolone-resistant, and one had resistance to the second-line injectable drug from isoniazid mono-resistant case, and two pre-XDR-TB (2/9, 22%) were from rifampicin mono resistant cases (Figure 1).

Out of 51 multidrug-resistant cases, six cases were pre-XDR (6/51, 11.8%-fluoroquinolone-resistant) and one case was XDR (1/51, 1.96%-Extremely Drug-Resistant). Among the seven fluoroquinolone-resistant, two cases were inferred resistant (Wild type band missing and no corresponding mutant band developed) belonging to the *gyrA* and *gyrB* gene (497–502 region), and five cases were true resistant (Wild type band missing and corresponding mutant band developed). One XDR was true resistant due to the development of multiple mutation bands in the *gyrA* gene. Among the five true resistant cases, there were two fluoroquinolone-resistant resistant to levofloxacin, Moxifloxacin (Asp94His GAC→CAC) at a high level, and three were at lower level resistant to Moxifloxacin (Ala90Val, GCG→GTG; Ser91Pro TCG→CCG) drugs (Table 1). Two inferred fluoroquinolone-resistant cases were resistant to levofloxacin and as well as resistant to Moxifloxacin (497–502 codon regions) at lower level.

Out of 200 isoniazid mono-resistant cases, nine were pre-XDR (9/200, 4.5%-fluoroquinolone-resistant). Among nine pre-XDR-TB cases, two cases were hetero-resistant, six cases were true resistant and one case was inferred. In seven cases, the wild-type band is missing without the development of the corresponding mutant band; hence, all seven cases were considered as inferred resistant and two cases were true resistant (wild-type band missing and corresponding mutant band develops). Among the two true resistants, one case was (Ser91Pro TCG→CCG), resistant to levofloxacin and also resistant Moxifloxacin at the lower level, and another one case was (second-line injectable true resistant g1484t) resistant to Amikacin, Kanamycin, and Capreomycin. Out of the seven inferred fluoroquinolone-resistant cases, one case belongs to the *gyrA* gene (85–97 codon regions), and six belonged to the *gyrB* gene (497–502 codon regions), resistant to levofloxacin, and resistant to Moxifloxacin at the lower level (Table 2).

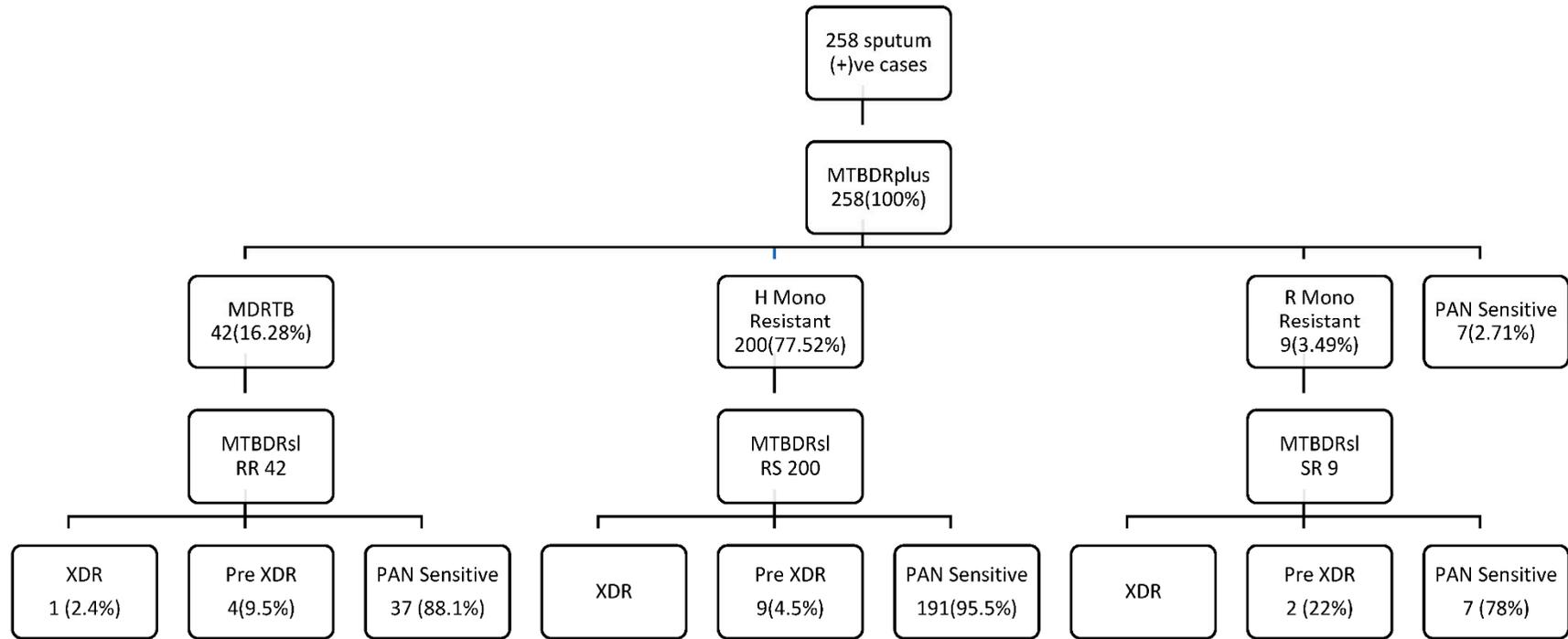


Figure 1. Flow diagram of drug case enrollment for MTBDRsl assay.

Table 1. Characteristics of *gyrA* and *gyrB* gene mutations in 51 MDR isolates.

Gene Target	Number of Isolates	MTBDRsl	Codon/Mutation or Region	Resistant	Number (%)
	with SR ^r /RR ^r Pattern	Probe			
<i>rpoB</i> gene					
	True resistant (5)				
	H445Y	MUT3D	Asp94His (GAC→CAC)	Lfx,Mox	1 (14.3%)
	S450L	<i>gyrB</i> WT1 (ND)	497–502	Lfx,Mox(LL)	1 (14.3%)
	S450L, S315T1	MUT2	Ser91Pro (TCG→CCG)	Lfx,Mox(LL)	1 (14.3%)
	H445D, S315T1	MUT3D	Asp94His (GAC→CAC)	Lfx,Mox	1 (14.3%)
	H445D,S315T1	MUT1	Ala90Val (GCG→GTG)	Lfx,Mox(LL)	1 (14.3%)
	Inferred (2)				
	432–438,315	<i>gyrB</i> WT1 (ND)	497–502	Lfx,Mox(LL)	1 (14.3%)
	445–448, c-15t	MUT1	Ala90Val (GCG→GTG)	Lfx,Mox(LL),Km,Am,Cm,	1 (14.3%)
		MUT2	Ser91Pro (TCG→CCG)		
		<i>rrs</i> MUT1	a1401g		

D-Developed; ND-Not Developed; Lfx-Levofloxacin; Mox-Moxifloxacin; LL-Low Level; Am-Amikacin; Km-Kanamycin; Cm-Capreomycin.

Table 2. Characteristics of *gyrA* and *gyrB* gene mutations in 200 isoniazid-resistant isolates.

Gene Target	Number of Isolates	MTBDRsl	Codon/Mutation or Region	Resistant	Number (%)
	With H ^r Pattern	Probe			
<i>KatG</i> gene					
	Heteroresistant (2)				
	WT1 (D) + S315T1	<i>gyrA</i> WT1 (ND)	85–90	Lfx,Mox(LL)	1 (11.1)
		<i>gyrB</i> WT1 (ND)	497–502	Lfx,Mox(LL)	1 (11.1)
	True resistant (4)				
	S315T1	<i>gyrB</i> WT1 (ND)	497–502	Lfx,Mox(LL)	3 (33.3)
	S315T2	<i>gyrB</i> WT1 (ND)	497–502	Lfx,Mox(LL)	1 (11.1)
<i>inhA</i> gene					
	True resistant (2)				
	c-15t	MUT2 (<i>gyrA</i>)	Ser91Pro (TCG→CCG)	Lfx,Mox(LL)	1 (11.1)
		MUT2 (<i>rrs</i>)	g1484t	Am,Km,Cm	1 (11.1)
	Inferred resistant (1)				
	WT1 ND	<i>gyrB</i> WT1 (ND)	497–502	Lfx,Mox(LL)	1 (11.1)

D-Developed; ND-Not Developed; Lfx-Levofloxacin; Mox-Moxifloxacin; LL-LowLevel; Am-Amikacin; Km-Kanamycin; Cm-Capreomycin.

3.1. Risk Factors Associated with RR-TB

Table 3 shows the results of the risk factors associated with RR-TB amongst diagnosed pulmonary tuberculosis cases. After the diagnosis of XDR and pre-XDR, 100% of patients received standardized treatment regions, and the study group had a mean average age of 42.7 ± 16.4 years. Out of the 51 multidrug-resistant tuberculosis patients, the overall successful treatment outcome was 36 (70.6%), and the poor unfavorable outcome was 15 (29.4%), as shown in Table 3. Among 15 poor outcome patients (29.4%), 2 (3.9%) patients died before completing treatment, 8 (15.7%) patients had treatment failure, and five (9.8%) patients were lost to follow-up. Patients who were lost to follow-up interrupted treatment after a median of 58 days. Most of these interruptions occurred during the intensive phase of the treatment. The independent relative risk associated with RR-TB cases were at the age ≥ 61 years, RR 1.96; 95% CI 0.3065–12.5127. The percentage relative risk of diabetics and new cases among multidrug-resistant tuberculosis was 4.76% increased risk with RR 1.05; 95% CI 0.1474–7.4453 and 30% increased risk with RR 1.3 95% CI 0.288–5.866 respectively. Among the eight failure cases, one was XDR-TB (Ala90Val GCG→GTG; Ser91Pro TCG→CCG and a1401g), another one was pre-XDR-TB (D94C), and six were non-XDR-TB but typical multidrug-resistant tuberculosis cases. The two deaths belonged to non-XDR cases.

Table 3. Multivariate analysis to evaluate the risk factors of pre-XDR among 51 MDR patients.

Characteristics	Number (%) of	Number (%) of Isolates		RR (95% CI)	Increased Risk (%)
	Isolates (n = 51)	Resistant	Susceptible		
Total	51 (100%)	7 (13.73%)	44 (86.27%)		
Sex					
Male	39 (76.47%)	6 (11.76%)	33 (64.71%)	1.85 (0.2459, 13.8578)	84.62%
Female	12 (23.53%)	1 (01.96%)	11 (21.57%)		
Age					
≤15	0	0	0		
16–60	47 (92.2%)	06 (11.76%)	41 (80.3%)	0.51 (0.0799, 3.2627)	
≥61	04 (07.84%)	01 (1.96%)	03 (05.88%)	1.96 (0.3065, 12.5127)	95.83%
District					
Urban	38 (74.5%)	5 (9.8%)	33 (64.7%)	0.86 (0.1882, 3.8875)	
Rural	13 (25.5%)	2 (3.9%)	11 (21.6%)		
Treatment history					
New	12 (23.5%)	2 (3.9%)	10 (19.6%)	1.3 (0.2881, 5.866)	30%
Treated cases	39 (76.5%)	5 (9.8%)	34 (66.7%)		
HIV					
Positive	0	0	0		
Negative	51 (100%)	7 (13.73%)	44 (86.27%)		
Diabetics					
Yes	7 (13.7%)	1 (1.9%)	06 (11.8%)	1.05 (0.1474, 7.4453)	4.76%
No	44 (86.3%)	6 (11.8%)	38 (74.5%)		
Treatment outcomes					
Successful treatment					
(Cure+ treatment completed)	36 (70.6%)	3 (5.9%)	33 (64.7%)	0.31 (0.0794, 1.2303)	
Poor treatment					
(Failure + death + Lost to follow-up)	15 (29.4%)	4 (7.84%)	11 (21.57%)	3.2 (0.8128, 12.5987)	220%

3.2. Risk Factors Associated with INH Mono-Resistant

Aiming to evaluate the risk factors independently associated with the development of isoniazid mono-resistant logistic regression analysis was performed. Out of 200 isoniazid mono-resistant patients, a successful treatment outcome was observed in 171 (85.5%) cases, and the unfavorable outcomes observed among isoniazid mono-resistant patients were 29 (14.5%). Out of the 29 (14.5%) poor outcomes, nine (4.5%) patients died before completing treatment, 4 (2.0%) patients had treatment failure and 16 (8.0%) patients were lost to follow-up. The percentage relative risk among the age group of 16–60 and ≥ 61 years was 86.08% increased risk with RR 1.86; 95% CI 0.2354–14.7096 and 17.95% increased risk with RR 1.18; 95% CI 0.2549–5.4584 respectively. The percentage relative risk of new cases among isoniazid mono-resistant was 405.56% increased risk with RR 5.06; 95% CI 1.3798–18.5237 as shown in Table 4. There were nine deaths and four treatment failure cases observed in non-XDR-TB cases, but nine had mutations in the *katG* gene, and four had mutations in the *inhA* gene. The treatment success rates in the INH mono-resistant and multidrug-resistant tuberculosis were 82% and 70.6%, respectively, significantly higher than the success rate (51.0%) in the pre-XDR-TB and XDR-TB cases. The unfavorable outcomes in the pre-XDR-TB and XDR-TB cases were 49%, which is higher than the poor outcome rate of INH mono-resistant (14.5%) and multidrug-resistant (29.4%) tuberculosis cases.

Table 4. Multivariate analysis to evaluate the risk factors of pre-XDR among 200 isoniazid mono-resistant patients.

Characteristics	Number (%) of	Number (%) of Isolates		RR (96% CI)	Increased Risk (%)
	Isolates (<i>n</i> = 200)	Resistant	Susceptible		
Total	200 (100%)	09 (04.5%)	191 (95.5%)		
Sex					
Male	146 (73.0%)	05 (2.5%)	141 (70.5%)	0.46 (0.1289, 1.6582)	
Female	054 (27.0%)	04 (2.0%)	050 (25.0%)		
Age					
≤ 15	03 (1.5%)	0	03 (1.5%)		
16–60	158 (79.0%)	07 (3.5%)	151 (75.5%)	1.86 (0.2354, 14.7096)	86.08%
≥ 61	39 (19.5%)	02 (1.0%)	37 (23.5%)	1.18 (0.2549, 5.4584)	17.95%
District					
Urban	189 (94.5%)	9 (4.5%)	180 (90.0%)		
Rural	11 (5.5%)	0	11 (5.5)		
Treatment history					
New	18 (9.0%)	3 (1.5%)	15 (7.5)	5.06 (1.3798, 18.5237)	405.56%
Treated cases	182 (91.0%)	6 (3.0%)	176 (88.0%)		
HIV					
Positive	7 (3.5%)	0	7 (3.5%)		
Negative	193 (96.5%)	9 (4.5%)	184 (82.0%)		
Diabetics					
Yes	36 (18.0%)	0	36 (18.0%)		
No	164 (82.0%)	9 (4.5%)	155 (77.5%)		
Treatment outcome					
Successful treatment (Cure + completed)	171 (85.5%)	8 (4.0%)	163 (81.5%)	1.36 (0.1762, 10.4475)	35.67%
Poor treatment (Failure + death + Lost to follow-up)	29 (14.5%)	1 (0.5%)	28 (14.0%)	0.57 (0.0735, 4.4117)	

3.3. Predictors of Poor Treatment Outcomes

Out of the 15 unfavorable outcomes from RR-TB cases, two (3.9%) patients died before completing treatment, eight (15.7%) patients had treatment failure, and five (9.8%) patients were lost to follow-up. In the multivariate logistic regression analysis, poor treatment outcomes associated with failure, death, and loss to follow-up were 220% increased risk with RR 3.2; 95% CI 0.8128–12.5987 and its percentage relative risk was 133.84 shown in Table 5. Out of the 29 unfavorable outcomes from INH mono-resistant tuberculosis cases, nine (4.5%) patients died before completing treatment four (2.0%) patients had treatment failure, and 16 (8.0%) patients were lost to follow-up. In the multivariate logistic regression analysis, poor treatment outcomes associated with failure, death, and lost follow-up were RR 0.57; 95% CI 0.0735–4.4117.

Table 5. Multivariate analysis of treatment outcomes pre-XDR-TB among multidrug-resistant/rifampicin mono-resistant and isoniazid mono-resistant tuberculosis patients ($n = 251$).

Characteristics	Number (%) of	Number (%) of Isolates		RR (95% CI)	Increased Risk (%)
	Isolates ($n = 251$)	Resistant	Susceptible		
Total	251 (100%)	16 (6.4%)	235 (93.6%)		
Treatment outcome	207 (82.5%)	11 (4.4%)	196 (78.1%)	0.47 (0.1710, 1.2786)	
Cure	156 (62.2%)	9 (3.6%)	147 (58.6%)	1.47 (0.3285, 6.5882)	47.12%
Treatment completed	51 (20.3%)	2 (0.8%)	49 (19.5%)	0.68 (0.1518, 3.0440)	
Poor unfavorable treatment	44 (17.5%)	5 (2.0%)	39 (15.5%)	2.14 (0.7821, 5.8468)	113.84%
Failure	12 (4.8%)	2 (0.8%)	10 (4.0%)	1.78 (0.3375, 9.3655)	77.78%
Death	11 (4.4%)	1 (0.4%)	10 (4.0%)	0.75 (0.0935, 6.0177)	
Lost to follow-up	21 (8.4%)	2 (0.8%)	19 (7.6%)	0.73 (0.1349, 3.9515)	

4. Discussion

Because of the diversity of drug resistance epidemiological situation, the actual status of second-line drugs and their rate of resistance would be distinctive in different regions. The emerging resistance of pre-XDR-TB and XDR-TB is the major hurdle for the tuberculosis control programme in developing countries like India. This study showed that the prevalence of fluoroquinolone resistance among MDR-TB was 13.73%, which was higher than the 6.3% in Amhara region of Ethiopia [12] and the prevalence of fluoroquinolone resistance among non-MDR-TB (isoniazid mono-resistance) was 4.5%. The rate of fluoroquinolone resistance prevalence among non-MDR-TB is higher than 0.8% of the global estimate, which may be a warning against the widespread use of fluoroquinolone in the community. The overall prevalence rate of fluoroquinolone resistance among tuberculosis patients is 6.2%, which was lower than 26.2% of the global estimate for fluoroquinolone resistance [13].

The common mutations in the *gyrA* gene that confers resistance in *M. tuberculosis* to fluoroquinolone are the Ala90Val (GCG→GTG), Ser91Pro (TCG→CCG), and Asp94His (GAC→CAC) [14]. In this study, among the seven fluoroquinolone-resistant from 51 RR-TB cases, two cases were inferred resistant (Wild type band missing and no corresponding mutant band developed) belonging to the *gyrA* and *gyrB* gene (497–502 region), and five cases were true resistant wild-type band missing and corresponding mutant band developed). One XDR was true resistant due to the development of multiple mutation bands in the *gyrA* gene. Among the five true resistant cases, there were two fluoroquinolone-resistant resistant to levofloxacin, Moxifloxacin (Asp94His GAC→CAC) at a high level, and three were at lower level resistant to Moxifloxacin (Ala90Val, GCG→GTG; Ser91Pro TCG→CCG) drug. Two inferred fluoroquinolone-resistant cases were resistant to levofloxacin and resistant to Moxifloxacin (497–502 codon regions) at a lower level. It was striking that isolates with the Ala90Val and Ser91Pro mutations for the two different drugs showed a lower level of moxifloxacin resistance.

In contrast, isolates carrying the Asp94His mutations showed a higher level of moxifloxacin resistance [15]. These targeted amino acids, alanine, serine, and asparagine, are nonessential amino acids, which are produced by our body. These nonessential amino acids are very helpful for the removal of toxins, promoting brain functioning, synthesis of red blood cells and white blood cells. The functional changes of these nonessential amino acids due to mutation may lead to hard-to-make-up proteins that are required for the repair, growth, and maintenance of cells. Even though the functional groups of alanine, serine, and asparagine differ, the elaborate study on this with the considerable volume of samples in the future might be giving the solutions as these are not reported yet.

The occurrence of fluoroquinolone-resistant in *M. tuberculosis* is due to mutations equally in both the *gyrA* gene (7/258; 2.7%) and *gyrB* (8/258; 3.1%) genes, which is contradicting previous studies of Takiff et al., Pitaksajakul et al., Wang et al., and Feuerriegel et al. [16–19]. All resistances associated with *gyrB* were inferred, whereas the resistance associated with the *gyrA* gene was truly resistant except for one inferred resistance. The most common mutation site in the *gyrB* gene that confers resistance to *M. tuberculosis* is the codons 497–502 regions. It is observed that the strongest risk factors independently associated with pre-XDR were on gender (RR; 84.62%), followed by the age group 16–25 (RR; 172.73%). The resistance in the younger generation might be due to recent transmission, poor adherence to the previous antituberculosis medication [16]. In the present study, the regression analysis has shown that multidrug-resistant tuberculosis with diabetics had four times more relative risk than non-diabetic cases. Our study has shown a strong association between the history of previously treated cases and drug-resistant TB cases as reported in previous literature [20,21].

The difference in treatment success rate for MDR-TB, pre-XDR, and INH mono-resistant patients is reported in the literature. In our study, the treatment success rates of H-mono-resistant (82.0%), MDR-TB (70.6%) were a little lower, and pre-XDR-TB (51.0%) were a little higher than the previous study [22]. The unfavorable poor outcome rates among the INH-mono-resistant, MDR, TB, and pre-XDR-TB were 29.4%, 14.5, and 49.0%, respectively. In our study, we found that the rate of loss to follow-up was 11.16%. The main reason for poor treatment outcomes might be the death and higher percentage of loss to follow-up cases, which is lower than the previous study [23,24]. Out of the 15 unfavorable outcomes from RR-TB cases, two (3.9%) patients died before completing treatment, eight (15.7%) patients had treatment failure, and five (9.8%) patients were lost to follow-up. In the multivariate logistic regression analysis, poor treatment outcomes associated with failure, death, and loss to follow-up were RR 3.2; 95% CI 0.8128–12.5987 and its percentage relative risk was 133.84, as shown in Table 5.

Out of the 29 unfavorable outcomes from INH mono-resistant tuberculosis cases, 9 (4.5%) patients died before completing treatment, 4 (2.0%) patients had treatment failure and 16 (8.0%) patients were lost to follow-up. In the multivariate logistic regression analysis, poor treatment outcomes associated with failure, death, and lost follow-up were RR 0.57; 95% CI 0.0735–4.4117. The treatment success rates in the INH mono-resistant and multidrug-resistant tuberculosis were 82% and 70.6%. This has been significantly higher than the success rate (57.0%) in the pre-XDR-TB and XDR-TB cases [25]. The unfavorable poor outcome rate in the pre-XDR-TB and XDR-TB cases was 49%, which is higher than the poor outcome rate of INH mono-resistant (14.5%) and multidrug-resistant (29.4%) tuberculosis cases.

5. Conclusions

In conclusion, the rate of at least one second-line drug resistance among MDR-TB patients is alarming in the region. Our study limitations are the small sample size and the higher proportion of loss to follow-up patients limited our conclusion. The correlation of the mutation region in the target gene with the patient's outcome might give better insight into the drug-resistant tuberculosis management for controlling tuberculosis effectively. Further study is warranted to explore the role of various ecological factors on the observed

high distribution of DR-TB and identify transmission networks in the community using molecular epidemiological methods to locate hotspots for targeted interventions.

Author Contributions: Conceptualization, M.A. (Muralidhar Aaina) and M.M.; methodology, M.A. (Muralidhar Aaina); software, K.V.; validation, G.R., M.A. (Muralidhar Aaina) and M.M.; formal analysis, M.A. (Muthukumar Anbazhagi); investigation, M.A. (Muralidhar Aaina); resources, M.M.; data curation, B.U.; writing—original draft preparation, K.V.; writing—review and editing, M.A. (Muthukumar Anbazhagi); visualization, B.U.; supervision, M.A. (Muralidhar Aaina); project administration, M.M.; funding acquisition, G.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Indian Council of Medical Research (ICMR), the Government of India, through Short Term Studentship (STS) Ref.No.2019-04445. The funders had no role in the study design, data collection and analysis, the decision to publish, or preparation of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

MDR	multidrug resistant
XDR	extremely drug resistant
H/INH	isoniazid
R/RIF	Rifampicin
SLID	second line injectable drug
RR	relative risk
FQ	fluoroquinolone
LFX	levofloxacin
MFX	moxifloxacin
RR-TB	rifampicin resistant tuberculosis
QRDR	quinolone resistance-determining region
AMK	amikacin
CAM	capreomycin
KAN	kanamycin
DR-TB	drug resistant tuberculosis
STS	short term studentship
CI	confidence interval

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