VOLUME - 12, ISSUE - 04, APRIL - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Medical Microbiology

DETECTION AND CHARACTERIZATION OF MUTATIONS IN GENES RELATED TO SECOND-LINE ANTI-TUBERCULOSIS DRUGS RESISTANCE IN MDR TB

Diksha Zine Junior Resident, Department of Microbiology, GGMC and Sir JJH, Mumbai

Nilma Hirani Associate Professor, Department of Microbiology, GGMC and Sir JJH, Mumbai

Ameeta Joshi Professor and HOD, Department of Microbiology, Department of Microbiology, GGMC and Sir JJH, Mumbai

ABSTRACT

Introduction: Drug-resistant tuberculosis is a major global health issue. Multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) are particularly concerning. Conventional phenotypic methods being cumbersome and time consuming, molecular assays for the detection of mutations that are associated with resistance to anti-TB drugs have emerged as an important tool for rapid diagnosis. **Aims and Objectives:** To determine and characterize mutations associated with resistance to second line anti-TB drugs in MDR TB. **Methods:** First line LPA Confirmed MDR TB and/ or mono rifampicin or mono isoniazid resistant cases were subjected to second line LPA (MTBDRsl ver 2.0 kit)) and banding patterns of resistant cases were obtained. These were then subjected to extended phenotypic DST (Gold Standard) using MGIT 960 for Moxifloxacin – 1.0µg/ml, Kanamycin -2.5µg/ml, Capreomycin -2.5µg/ml. **Results:** The study analysed 1580 samples subjected to SL LPA and found that 37.65% were sensitive to both FQ and SLID. FQ resistance accounted for 60.56% and SLID resistance for 17.02%. Resistance to both seen in 15.15% samples. The most common mutation associated with FQ resistance was gyr A gene at codon D94G, for SLID resistance it was in rrs gene (A1401G) and eis gene (C-12T,G-10A). **Conclusion:** It was observed that there is a high prevalence (62.34%) of second-line anti-TB drug resistance and mutations in gyrA, gyrB (for FQ), rrs (for SLID) and eis promoter regions (for low level kanamycin) were significantly associated with second-line anti-TB drug resistance.

KEYWORDS : MDR – Multidrug resistant, XDR- Extensively drug resistant, LPA- Line probe assay, SLID- Second line injectable drug

INTRODUCTION

Tuberculosis (TB) remains one of the most infectious diseases and the leading cause of high mortality and morbidity worldwide [1]. India is one of the highest TB burden countries in the world, has an estimated incidence of 19.33 lakh cases in 2021[2]. At the start of 2020, the central government of India renamed the RNTCP as the National Tuberculosis Elimination Programme (NTEP) which aims to eliminate TB from the country by the year 2025[3].

Despite the historical challenges, technology and health care for treating TB have improved, but it yet continues to be the biggest health problem in India. The emergence of Multidrug resistant tuberculosis, which is defined as 'resistance to at least isoniazid and rifampicin' and more recently, the emergence of Extensively drug-resistant (XDR) TB, defined as 'MDR with resistance to fluoroquinolones (FQs) (levofloxacin or moxifloxacin) and at least one additional Group A drug (presently to either bedaquiline or linezolid [or both])' has been widely considered to be a serious threat to global health[4].

Since conventional phenotypic methods are cumbersome and take weeks to months to obtain drug resistance profiles, molecular assays for detection of mutations that confer the resistance to anti-TB drugs have emerged as an alternative tool for rapid, sensitive, faster and accurate diagnosis of TB and evaluation of resistance status of bacteria [5].

Hence, this study has been undertaken to characterize mutations associated with second line anti-TB drugs in MDR-TB patients.

Aim And Objectives:

To characterize mutations associated with fluoroquinolone and or / second line injectable drugs.

MATERIALS AND METHODS

This was a prospective and descriptive type of study conducted in TB culture and drug sensitivity laboratory in the Department of Microbiology at Tertiary care hospital, Mumbai from January 2021 to June 2022. Ethical committee approval was taken.

Inclusion criteria:

LPA confirmed MDR TB and or/ mono RIF mono INH cases

Exclusion criteria:

All cases of non-tuberculous mycobacterial infections. All pulmonary TB cases sensitive to first line anti-TB drugs

Sample collection:

All smear positive sputum specimens and culture isolates from MDR-TB and/ or mono RIF or mono INH resistant cases were included in the study

Methodology

Total number of 1580 First line LPA confirmed MDR TB and/ or mono rifampicin or mono isoniazid resistant cases were subjected to Second line LPA (MTBDRsl ver 2.0 kit)): The genotype MTBDR-sl (Hain Lifescience, Nephren, Germany) is the WHO approved qualitative in-vitro test for rapid detection of mycobacterium tuberculosis complex and its resistance to FQs and second line injectable anti-TB drugs from smear positive sputum specimen and cultivated samples[6].

FQ and or/ SLID resistant cases were obtained and banding patterns of resistant cases were studied.

These were then subjected to extended phenotypic DST (Gold Standard) using MGIT 960) for Moxifloxacin – 1.0µg/ml, Kanamycin -2.5µg/ml, Capreomycin -2.5µg/ml

In the BACTEC MGIT 960, the increase in the fluorescence in the sensor is measured automatically and designated as growth unit(GU).

If a drug is added to the medium which is bacteriostatic or bacteriocidal to the test mycobacteria, it inhibits growth and thus, there is little or no oxygen consumption, therefore little or no fluorescence of the sensor [7].

RESULTS

Table No. 1 shows that of the 1580 FL LPA confirmed MDR TB and/ or mono rifampicin or mono isoniazid resistant cases that were subjected to SL LPA, 37.65% (595/1580) were SL LPA sensitive (sensitive to by both FQ and SLID), whereas 60.56% (957/1580) were resistant to FQ & 17.02% (269/1580) were resistant to SLID and 15.15% (241/1580) were resistance to both FQ & SLID

Table 1: Second-line LPA results

Category	Number of cases	Percentage
SL LPA Sensitive	595	37.65%
SL LPA Resistant	985	62.35%
FQ and SLID Resistant	241	15.25%
Total FQ Resistant	957	60.56%
Total SLID Resistant	269	17.02%
Total	1580	100%

In table no 2, of the total 957 Second line LPA FQ resistant cases, 97.91% (937/957) had mutations in *gyrA* gene and 1.35% (13/957) had mutations in *gyrB* gene, while 0.74% (7/957) showed mutations in both *gyrA* and *gyrB* gene. For FQ resistance, most common banding pattern observed in 438 (45.76%) cases was *gyrA* gene WT 3 absent MUT 3C present indicating levofloxacin resistance and high level moxifloxacin resistance. Second most common banding pattern observed was WT 2 absent, MUT1 present indicating levofloxacin resistance, low level moxifloxacin resistance. In gyrB gene banding pattern observed in 22 (92.31%) cases was WT absent indicating levofloxacin resistance, low-level moxifloxacin resistance and high level moxifloxacin sensitive. Of the total 269 Second line LPA SLID resistant cases 49.81% (134/269) had mutations in *rrs* gene and similarly 49.81% (134/269) had mutations in *eis* gene, while one case showed mutations in both *rrs* and *eis* gene. In rrs gene most common banding pattern observed in 123 (91.12%) was WT1 absent MUT1 present indicating resistance to kanamycin, capreomycin and amikacin.

The second most common banding pattern (4 cases) observed was WT2 Absent, MUT2 present indicating resistance to kanamycin, capreomycin, amikacin and viomycin. In eis gene, the most common banding pattern observed in 120 (44.60%) cases was WT2 absent with no presence of MUT band indicating low-level kanamycin inferred resistance.

Table 2: Mutations associated with FQ and SLID resistance obtained from SL LPA

DRUGS	GENE	BANDING PATTERN OBSERVED	CODON	NUMBER OF CASES
FQ (957)	gyrA Mutations 937/957 (97.91%)	WT 3 Absent, MUT 3C Present	D94G	438/937 (46.74%)
		WT 2 Absent, MUT 1 Present	A90V	234/937 (24.97%)
		WT 3 Absent*	94	(7.68%)
		WT 3Absent, MUT 3A Present	D94A	(6.19%)
		WT 2 Absent, MUT 2 Present	S91P	(5.87%)
		WT 3 Absent, MUT 3B Present	D94N D94Y	(4.69%)
		WT 2 Absent*	S91P A90V	(2.03%)
		WT 1 Absent, MUT 1Present	G88A G88C	(0.64%)
		WT 1 Absent*	G88A G88C	(0.43%)
		WT 3 Absent, MUT 3D Present	D94H	(0.21%)
		WT 3 Absent, MUT 1Present	-	(0.21%)
		WT 3 Absent, MUT 1,3B,3C Present	-	(0.11%)
		MUT 2 Present#	S91P	(0.11%)
		MUT 3A present#	D94A	(0.11%)
	gyrB Mutations 13/957 (1.35%)	WT Absent*		
		WT Absent, MUT 2 Present	E540V	(7.69%)
	Both gyrA and gyrB 07/957 (0.74%)			
SLID 269	rrs gene Mutations 134/269 (49.81%)	WT1 Absent, MUT 1 Present	A1401G	(91.12%)
		WT2 Absent, MUT 2 Present	G1484T	(2.96%)
		WT 1 Absent*	C1402T	(2.96%)
		MUT 1 Present#	C14T	(2.96%)
	eis gene Mutations 134/269 (49.81%)	WT 2 Absent*	C-12T G-10A	(91.12%)
		WT 2 Absent, MUT 1 Present	C-14T	(4.44%)
		WT 1 Absent*	G-37T	(2.22%)
		MUT 1 Present#	C-14T	(2.22)
	Both rrs and eis gene mutation 01/269			

In Table number 3, among 957 total FQ resistant cases which were detected by SL LPA, 892 were subjected to phenotypic liquid DST using MGIT 960 system for moxifloxacin at a critical concentration of 1μg/ml, of which 36.65% (327/892) showed resistance, while 63.35% (565/892) were sensitive.

Among 269 total SLID resistant cases which were detected by SL LPA, 80 were subjected to phenotypic DST using MGIT 960 system for kanamycin and capreomycin at a critical concentration of 2.5μg/ml for both drugs, of which 82.5% (66/80) and 40% (32/80) showed resistance to kanamycin and capreomycin respectively, while 17.5% (14/80) and 60% (68/80) were sensitive to kanamycin and capreomycin, respectively.

Table no 3: Phenotypic DST using Bactec MGIT 960 system

Drugs	RESISTANT	SENSITIVE
Moxifloxacin (1.0μg/ml) N=892	327	565
	36.65%	63.35%
Kanamycin (2.5μg/ml)) N=80	66	14
	82.50%	17.50%
Capreomycin (2.5μg/ml)) N=80	32	48
	40%	60%

DISCUSSION

The overall second line anti TB drug resistance in MDR and/ mono isoniazid or mono rifampicin resistant cases in our study was **62.34%** which is extremely high as compared to a study conducted by Qianlin Li et al in China (48.2%)[8]. The overall FQ resistance was 60.56% which is much higher than the findings in the study conducted by Qianlin Li et al study (37.7%) [8] and a study by Sunil Sethi et al (34.2%) conducted in India in 2018 [9]. The overall SLID resistance was 17.02% while in the Qianlin Li et al study was 24.5%[8] and Sunil Sethi et al study was 8.1%[9]. FQ and SLID resistance was detected in 15.25% isolates, while in Yi Hu et al study, it was at 9.3% [10], Qianlin Li et al [8] study was at 10.9% and Sunil Sethi et al study at 8.6%[9].

A total 97.91% of FQ-resistant isolates had a mutation in the quinolone resistance-determining region of *gyrA* gene among which the commonest banding pattern seen was Wild type 3 absent MUT 3C present, signifying mutation at codon D94G (46.74%) which is comparable to the studies from South Africa (44.2%), China and India (47.3, 40 %) [10,11,9,12]. The second most common (24.97%) banding pattern observed in FQ-resistant isolates was Wild type 2 absent MUT1 present indicating mutation at codon A90V which is in concordance with studies conducted by Sunil Sethi et al [9] and (27%) Raj Narayan Yadav et. al[12]at New Delhi, India.

The *gyrB* gene mutation was found in 1.35% of cases which were also found in Qianlin Li (7 cases) et al [8] and Sunil Sethi et al (3.1%) [9] study.

Among the 17.02% SLID resistant cases, equal numbers of (135/269) *rrs* and *eis* gene mutation cases were observed. Among the *rrs* mutations, the most common (45.72%) banding pattern was *rrs* WT 1 absent MUT 1 signifying mutation at codon A1401G indicating resistance to SLID (AMK, KAN, CAP) which is comparable with studies from India (43.8%)[9] and South Africa (77.5%) [13,11]. In the *rrs* gene, the banding pattern Wild type 2 absent MUT2 was present in 4 cases similar findings (4 cases) were seen in Sunil Sethi et al study[9].

In the *eis* mutations, the most common banding pattern observed was WT 2 absent, no MUT band present which is reported as low-level kanamycin inferred resistance.

Of the 80 SLLPA SLID resistant cases, 66 were resistant to kanamycin phenotypically while 14 were sensitive. On studying the banding pattern of these 14 cases on SLLPA, it was found 13 cases had *eis* gene mutation which indicates low-level kanamycin inferred resistance, so these were sensitive phenotypically at a critical concentration of 2.5 μg/ml and requires MIC-based evaluation to confirm resistance. Similarly, 32/80 were resistant to capreomycin(2.5 μg/ml) phenotypically while 48 were sensitive. These 34/48 cases had *eis* gene mutations indicating low-level kanamycin resistance. Hence, these were phenotypically sensitive to capreomycin; but 14/48 cases had *rrs* gene mutation. Hence, to evaluate this discordance MIC based evaluation is needed.

CONCLUSION

This study provides data on genetic mutations associated with second-line anti-TB drug resistance.

The study concluded that a high prevalence (62.34%) of second-line anti-TB drug resistance, highlighting the need for early detection of TB-resistant strains and appropriate drug regimens for effective TB treatment.

Mutations in gyrA, gyrB, rrs, and eis promoter regions were commonly associated with second-line anti-TB drug resistance. The most common mutation associated with FQ resistance was in the gyrA gene, specifically at codon D94G, signifying levofloxacin resistance and high-level moxifloxacin resistance. Mutations in *rrs* and *eis* genes were associated with second-line injectable drug resistance. In the *rrs* gene, the most common mutation was A1401G while, in the *eis* gene they were C-12T and G-10A mutations. The study emphasizes the need to follow diagnostic algorithm proposed by National Tuberculosis Elimination Programme under Ministry of Health and Family Welfare to identify drug-resistant TB cases early and initiate effective anti-TB treatment.

REFERENCES

1. World Health Organization. World health statistics data. Geneva; 2009. http://www.who.int/whosis/whostat/2009/en/. Accessed 14th Apr 2017.
2. India TB report 2022 chapter 2
3. https://medicaldialogues.in/rntcp-gets-a-name-change-now-called-national-tuberculosis-elimination-program-ntepwhich
4. World Health Organization.2016. Global Terculosis Report. Genova; 2016. WHO/TB/2016.13.World Health Organization, Geneva, Switzerland.
5. Oudghiri, A., Karimi, H., Chetioui, F., Zakham, F., Bourkadi, J. E., Elmessaoudi, M. D., ... & El Mzibri, M. (2018). Molecular characterization of mutations associated with resistance to second-line tuberculosis drug among multidrug-resistant tuberculosis patients from high prevalence tuberculosis city in Morocco. *BMC infectious diseases*, *18*, 1-8.
6. SOP TB/19 SLLPA version 02
7. SOP TB/12 Drug sensitivity testing by MGIT
8. Li, Q., Gao, H., Zhang, Z., Tian, Y., Liu, T., Wang, Y., ... & Dai, E. (2019). Mutation and transmission profiles of second-line drug resistance in clinical isolates of drug-resistant Mycobacterium tuberculosis from Hebei Province, China. *Frontiers in microbiology*, *10*, 1838.9.Sunil Sethi, Priyanka Agarwal, Rajiv Khaneja et al Second-line Drug Resistance Characterization in Mycobacterium tuberculosis by Genotype MTBDRsl Assay, Journal of Epidemiology and Global Health Volume 10, Issue 1, March 2020, Pages 42–4
10. Hu, Y., Hoffner, S., Wu, L., Zhao, Q., Jiang, W., & Xu, B. (2013). Prevalence and genetic characterization of second-line drug-resistant and extensively drug-resistant Mycobacterium tuberculosis in Rural China. *Antimicrobial agents and chemotherapy*, *57*(8), 3857-3863.
11. Yadav, R., Saini, A., Kaur, P., Behera, D., & Sethi, S. (2018). Diagnostic accuracy of GenoType® MTBDRsl VER 2.0 in detecting second-line drug resistance to M. tuberculosis. *The International Journal of Tuberculosis and Lung Disease*, *22*(4), 419-424.
12. Yadav, R. N., Bhalla, M., Kumar, G., Sah, G. C., Dewan, R. K., & Singhal, R. (2022). Diagnostic utility of GenoType MTBDRsl assay for the detection of moxifloxacin-resistant mycobacterium tuberculosis, as compared to phenotypic method and whole-genome sequencing. *International Journal of Mycobacteriology*, *11*(2), 183.
13. Gardee, Y., Dreyer, A. W., Koornhof, H. J., Omar, S. V., Da Silva, P., Bhyat, Z., & Ismail, N. A. (2017). Evaluation of the GenoType MTBDR sl version 2.0 assay for second-line drug resistance detection of Mycobacterium tuberculosis isolates in South Africa. *Journal of clinical microbiology*, *55*(3), 791-800..