Original Article

Drug susceptibility testing and line probe assay of first-line anti-tuberculosis drugs among presumptive tuberculosis patients attending a secondary care hospital in Bhubaneswar

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Abstract

Background: Pyrazinamide (PZA) is important for identification in multi-drug-resistant tuberculosis patients before starting therapy. PZA drug susceptibility testing (DST) is essential for the management of drug-resistant and susceptible TB patients. **Aims:** The degree of drug resistance among TB patients and discrepancy between DST results of the phenotype and genotype were assessed. **Materials and Methods:** Socio-demographic and clinical profiles of TB patients recruited in the study were documented. Sputum samples were processed for diagnosis using TrueNat Xpert MTB, TrueNat Xpert MTB Plus, and MGIT culture. **Results:** Rifampicin (RIF) line probe assay (LPA) showed the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 100%, whereas isoniazid (INH) LPA testing showed a sensitivity of 85.7%, a specificity and PPV of 100%, and NPV of 94.8%. The gene mutation for RIF resistance was between the codon, 530–533 of *rpo*B gene, and that for INH resistance was at the codon, 315 of *kat*G gene. **Conclusion:** Our findings demonstrated high prevalence of mono- and poly-drug resistance as well as pyrazinamide resistance.

Keywords: Bhubaneswar, drug susceptibility testing, line probe assay, TB patients

Introduction

Tuberculosis (TB), one of the major public health concerns in the 21st century, is a communicable disease caused by the *Mycobacterium tuberculosis* that normally causes pulmonary TB affecting the lungs and extra-pulmonary TB affecting other organs. WHO (World Health Organization) reports that India's TB pandemic is the worst in the world (WHO, 2006).[1,2] India was responsible for 26% of the new cases of TB in 2020, and the rate of incidence is 192 per one lakh people. The overall incidence of notified TB including new and relapse cases during 2021 showed a 19% rise (from 1,628,161 in 2020 to 1,933,381).[3,4]

Over the past few decades, concern over the development of drug-resistant (DR) TB (DR-TB) has grown considerably, which complicates TB treatment. WHO in 2022 reported that among 0.45 million cases of rifampicin-resistant TB worldwide, 78% of these cases are multi-drug-resistant (MDR) and India is at the top of the list with 133,000 prevalent MDR-TB patients.[5]

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A national assessment on DR-TB in India revealed that 21% of cases that had previously received treatment and 7.1% of newly diagnosed cases were MDR-TB. The projected prevalence of TB in 2022 was 28%, and an increasing rate of MDR-TB has been associated with treatment failure.[6] The inadequate utilization of the treatment of drug-sensitive (DS) TB (DS-TB) and DR-TB, extensive and ineffective use of antibiotics, the unavailability of drug susceptibility testing (DST), and the correlation of human immunodeficiency virus (HIV) has a great impact on DR-TB. Inadequate treatment outcomes, poor compliance, inferior quality medicines, and unsatisfactory infection control measures are among the main causes of developing drug resistance. With increasing drug resistance to anti-TB medications, MDR, pre-extensive drug resistant (Pre-XDR), and extensively drug resistant (XDR) strains pose a threat to the TB control strategy.[7,8] Basically, resistance to isoniazid (INH) and rifampicin (RIF) drugs is the major health concern today. The first-line drugs INH, RIF, PZA (pyrazinamide), and EMB (ethambutol) are used to treat drug-susceptible TB. PZA serves as a crucial foundation of this regimen due to its exceptional capacity to eliminate persisting bacilli, thus shortening the period of treatment from 9 to 6 months. PZA's unique manner of activity (interfering ATP generation) and regimens for treating drug-sensitive TB and MDR-TB will probably continue to include it as a key component. Despite the fact that PZA is an essential part of TB regimens, there is no known study regarding the PZA resistance, especially globally. Technically, DST for PZA is complex and is infrequently carried out as a part of the standard medical care procedure or standard drug monitoring in settings with bounded resources, but it is important for primary care physicians.[9,10]

Among two phenotypic PZA DSTs, BACTEC MGIT 960 and BACTEC 460TB, only BACTEC MGIT 960 is commercially available. The selection of a genetic mutant has an important role in the growth and development of the resistant strain of *Mycobacterium tuberculosis*. By choosing antibiotic resistance as a pre-dominant trait, *M.tb* population survive by taking advantage of the arising mutant under selective pressure. Mutation in the 561-nucleotide pncA gene is associated with PZA resistance, whereas mutation in katG, inhA linked with INH resistance, and rpoB gene can explain RIF resistance. It is crucial to detect PZA resistance among MDR-TB patients due to its distinctively bactericidal impact. The pro-drug PZA transforms to its active form by the pcnA-encoded non-essential pyrazinamidase enzyme (PZase) when it enters the bacteria through passive diffusion. In an acidic environment, PZA alters the energetics of bacterial membranes and prevents membrane transfer. Various mutations in genes of *Mycobacterium tuberculosis* are responsible for resistance to a range of drugs. Line probe assay (LPA), a polymerase chain reaction (PCR)-based test labeled with primers and complementary oligonucleotides probes that amplify DNA, may assess many mutations concurrently in a single reaction with a higher level of sensitivity than the conventional PCR methods.[11,12] In this study, we assessed the degree of drug resistance among TB patients and the discrepancy between DST results of the phenotype and genotype. High prevalence of both uni- and multi-drug resistance was observed among TB patients who were either newly diagnosed or previously treated, thus emphasizing the necessity for an in-depth research of this problem.

Materials and Methods

Ethics approval

The Human Ethics Committee of this Institute at Bhubaneswar, Odisha, has reviewed and authorized the elaborate procedure of the study. The results of TrueNAT MTB/RIF were not used in clinical decision making.

Study design

Participants attending the Dept. of TB and Chest Diseases at Capital Hospital, Bhubaneswar, were recruited in the study during November 2019 to December 2021. Socio-demographic and clinical profiles, risk factors, namely, previous treatment of TB, and recent exposure, if any, were documented using structured questionnaires. Written informed consent was obtained and two sputum samples were collected from the participants for clinical diagnosis using TrueNat Xpert MTB, TrueNat Xpert MTB Plus, and MGIT culture. All samples were processed at the BSL-III facility of National Reference Laboratory, RMRC, Bhubaneswar. *M. tuberculosis* clinical strains that were non-repetitive were extracted from AFB-positive sputum samples by Ziehl–Neelsen (ZN) staining.

Specimen collection and processing

The NALC/NAOH (N-acetyl-L-cysteine and sodium hydroxide) method was used to prepare the specimens.[13] The pre-treatment sputum was cultured for mycobacteria using the BACTEC MGIT (mycobacterial growth indicator tubes) 960 system (Becton Dickinson BACTECTM MGITTM 960 System, USA). Mycobacteria species were identified using the SD Bioline TB Ag MPT64 Rapid test. MTBc test validated the species of *M. tuberculosis* complex.[11] This was followed by inoculating *M. tuberculosis* strains onto BHI plates to establish the absence of contamination. The absence of growth/development on BHI plates indicated that the culture is free of contamination. SIRE and PZA DST were conducted using the automated BD BACTEC MGIT 960 system.[14] Simultaneously, LPA (Hain Lifescience, Nehren, Germany) was performed on 101 chosen samples.

MGIT sub-culture and DST

Sub-culturing and detection of growth were performed by using 200 µl of MGIT 960 TB culture in the MGIT vials. The positive cultures were stained with ZN stain in order to verify the presence of *M. tuberculosis*. In all, 374 strains were tested using phenotypic MGIT SIRE and PZA DST. 369 of them were satisfactorily evaluated throughout the process. The following medication concentrations were used for the drug susceptibility tests: RIF 1.0 g/ml, INH 0.1 g/ml, PZA 100.0 g/ml, EMB 5.0 g/ml, and SM 1.0 g/ml, as per the manufacturer's recommendations. Upon

reaching a value of 400 growth units (GU), the instrument displayed the completion of the DST set. At that time, the DST set report was printed out to recover the GU values of the tubes containing drugs.

DNA isolation and line probe assay

Due to limited resources, the LPA DST was performed on 101 different isolates for the first line of testing. As per the manufacturer's instructions, DNA was removed using the Genolyse (R) Kit (Hain Lifescience, Germany) from liquid cultures. With the MTBDRplus (version 2) assays, we were able to detect TB strains resistant to first-line drugs by following the kit's protocols. For amplification, 5 µl of DNA was used. The LPA system's primers were used for all amplifications, and the PCR conditions for the cultures were kept constant. The PCR mixture utilized for this experiment was 10 µl AM-A GT, 35 µl AM-B GT, and 5 µl DNA template from *M.tb* isolate. DNA templates derived from the *M.tb* H37Rv strain served as a positive control. Two negative controls, an extraction negative control (ENC) including lysis and neutralization buffer, and a master mix negative control were used (MNC). A thermal cycler (Applied Biosystems by life technologies) was programmed into alternate cycles of denaturation (95°C for 15 min, 30 s and 25 s), elongation (65°C for 2 min., 8 min), and annealing (50°C for 40 s). Amplicons were tagged with biotin and hybridized to DNA probes on a strip. The Twin Cubator was used for the hybridization process. The appearance or non-appearance of wild and mutated probes was assessed using an LPA card.[15]

Quality control

In this study, *M. tuberculosis* H37Rv (ATCC 25177) strain, sensitive to the first line drugs, was used for quality control (QC) in all methods.

Results

In all, 1059 study participants were enrolled, of which 384 (36.3%) were culture-positive and 8 (2.1%) were contaminated sputum samples. Two of 384 (0.5%) samples had non-tuberculous mycobacteria (NTM). Thus, 374 samples positive for *M. tuberculosis* were processed for further analysis. MGIT SIRE along with PZA DST was carried out on 374 strains. Of these, 369 (98.7%) were successfully isolated, while 5 (1.3%) could not be isolated because the growth control tube of three of them had over growth with a X400 error, that is, grew prior to 4 days of incubation. The control tube of other two isolates showed under-growth with X200 error, that is, no growth in the incubation period. Phenotypic DST was performed successfully on 369 sputum samples. Of these, 264 were from TB patients, 94 were from patients having both diabetes and TB, 10 were from HIV-TB co-infected patients, and one patient was co-infected with TB, HIV, and diabetes. The result of phenotypic DST showed 237 (64.2%) males and 132 (35.8%) females were suffering from TB. Among males, 210 (56.9%) were sensitive to all the five drugs (STR, INH, RIF, EMB, and PZA) and 27 (7.3%) were resistant to at least one drug. Among females, 117 (31.7%) were sensitive and 15 (4.1%) were resistant to at least one drug. With regard to age groups, among sensitive females resistant to one drug, 91 (24.7%) and 9 (2.4%) were in 16–30 years, 129 (35.0%) and 16 (4.3%) were in 31–45 years, and 107 (29.0%) and 17 (4.6%) were more than 46 years. As per the Asian criteria, body mass index (BMI) was categorized into underweight (<18.5), normal (18.5-22.9), and overweight (≥23). Among sensitive and resistant, 88 (23.8%) and 15 (4.0%) were underweight, 165 (44.7%) and 19 (5.1%) were normal, and 74 (20.1%) and 8 (2.2%) were overweight. Among urban, rural, and slum dwellers, 78 (21.1%), 106 (28.7%), and 143 (38.8%) were sensitive to all drugs and 9 (2.4%), 14 (3.8%), and 19 (5.1%) were resistant to at least one drug. Among diabetes and HIV patients, 64 (17.3%) and 6 (1.6%) were all sensitive and 31 (8.4%) and 5 (1.4%) were resistant. Out of 369 positive samples, 337 (91.3%) were from new cases and 32 (8.7%) were relapse cases. Among new and relapse cases, 303 (82.1%) and 24 (6.5%) were sensitive and 34 (9.2%) and 8 (2.2%) were resistant. Risk factors for TB were observed among 26 (7.0%) patients, of which 19 (5.1%) were sensitive and 7 (1.9%) were resistant. Of the successful 369 isolates, genotypic first-line probe assay was performed on selected 101 isolates [Table 1]. The result of genotypic DST (LPA) showed 63 (62.4%) males and 38 (37.6%) females were suffering from TB. Among males, 48 (47.5%) were sensitive to both the drugs (INH and RIF) and

Table 1: Results of drug susceptibility test and line probe assay

Variables	MGIT DST		MTB-DRplus (LPA)	
	Susceptible *n* (%)	Resistance *n* (%)	Susceptible *n* (%)	Resistance *n* (%)
Gender				
Male	210 (56.9)	27 (7.3)	48 (47.5)	15 (14.9)
Female	117 (31.7)	15 (4.1)	29 (28.7)	9 (8.9)
Age (in years)				
16-30	91 (24.7)	9 (2.4)	23 (22.8)	4 (4.0)
31-45	129 (35.0)	16 (4.3)	17 (16.8)	10 (9.9)
≥46	107 (29.0)	17 (4.6)	37 (36.6)	10 (9.9)
BMI				
<18.5	88 (23.8)	15 (4.0)	26 (25.7)	7 (6.9)
18.5-22.9	165 (44.7)	19 (5.1)	34 (33.7)	13 (12.9)
≥23	74 (20.1)	8 (2.2)	17 (16.8)	4 (4.0)
Location				
Urban	78 (21.1)	9 (2.4)	20 (19.8)	7 (6.9)
Rural	106 (28.7)	14 (3.8)	25 (24.8)	7 (6.9)
Slum	143 (38.8)	19 (5.1)	32 (31.7)	10 (9.9)
Diabetes Status				
Present	64 (17.3)	31 (8.4)	38 (37.6)	13 (12.9)
Absent	263 (71.2)	11 (3.0)	39 (38.6)	11 (10.9)
HIV Status				
Positive	6 (1.6)	5 (1.4)	9 (8.9)	2 (2)
Negative	321 (87.0)	37 (10.0)	68 (67.3)	22 (21.8)
TB RX history				
New	303 (82.1)	34 (9.2)	69 (68.3)	20 (19.8)
Relapse	24 (6.5)	8 (2.2)	8 (7.9)	4 (4.0)
Risk for TB				
Yes	19 (5.1)	7 (1.9)	7 (6.9)	1 (1.0)
No	308 (83.5)	35 (9.5)	70 (69.3)	23 (22.8)

15 (14.9%) were resistant to at least one drug. Among females, 29 (28.7%) were sensitive and 9 (8.9%) were resistant to one drug. Among sensitive and resistance to at least one drug, 23 (22.8%) and 4 (4.0%) were in 16-30 years, 17 (16.8%) and 10 (9.9%) were in 31-45 years, and 37 (36.6%) and 10 (9.9%) were above 46 years of age groups. Among sensitive and resistant, 26 (25.7%) and 7 (6.9%) were underweight, 34 (33.7%) and 13 (12.9%) were normal, and 17 (16.8%) and 4 (4.0%) were overweight. Among urban, rural, and slum dwellers, 20 (19.8%), 25 (24.8%), and 32 (31.7%) were sensitive to both the drugs and 7 (6.9%), 7 (6.9%), and 10 (9.9%) were resistant to at least one drug. Among diabetes and HIV patients, 38 (37.6%) and 9 (8.9%) were sensitive and 13 (12.9%) and 2 (2%) were resistant. Out of 101 positive samples, 89 (88.1%) were newly diagnosed cases and 12 (11.9%) were cases with previous treatment history. Among new and relapse cases, 69 (68.3%) and 8 (7.9%) were sensitive and 20 (19.8%) and 4 (4.0%) were resistant. Risk factors for TB were observed in 8 (7.9%) patients, out of which 7 (6.9%) were sensitive and 1 (1.0%) was resistant, as depicted in Table 1.

Out of the 369 TB patients tested for DST, 337 (91.3%) were newly diagnosed and 32 (8.7%) had previous history of treatment for TB. Among these isolates, 42 (11.4%) were resistant to not less than one drug, 25 (6.8%) for STR, 28 (7.6%) for INH, 20 (5.4%) for RIF, 25 (6.8%) for EMB, 31 (8.4%) for PZA, and 20 (5.4%) for MDR. Among new TB patients, 303 (89.9%) were sensitive, 12 (3.6) were mono-resistant, and 22 (6.5%) were multi-resistant. Among the TB patients who were previously treated, 24 (75.0%) were sensitive, 4 (12.5%) were mono-resistant, and 4 (12.5%) were multi-resistant [Table 2]. LPA and MGIT were performed on the 101 isolates, out of which 81 (80.2%) isolates were found to be sensitive to RIF by both the methods. Of these 101 isolates, 20 (19.8%) were resistant to RIF by both the methods. Conversely, for INH testing, there were 73 (72.3%) sensitive and 24 (23.8%) resistant concordant by both the methods, and 4 (3.9%) isolates were inconsistent showing sensitivity by LPA and resistance by MGIT. There were 81 sensitive isolates and 20 resistant isolates by both genotypic and phenotypic methods for MDR testing. Furthermore, RIF LPA compared to MGIT

Table 2: First-line Drug susceptibility Test Results of MTBC Isolates

TB Cases	Number of Isolates (%)	First-Line Anti-TB Drugs					Drug Resistance Type Number (%)
		STR	INH	RIF	EMB	PZA	
New (*n*=337)	303 (89.9)	S	S	S	S	S	Pan-susceptible 303 (89.9)
	2 (0.6)	S	R	S	S	S	Mono-resistance 12 (3.6)
	2 (0.6)	S	S	S	R	S	
	8 (2.4)	S	S	S	S	R	
	1 (0.3)	R	S	S	S	R	Poly-resistance 22 (6.5)
	1 (0.3)	S	R	S	S	R	
	2 (0.6)	R	R	S	R	S	
	2 (0.6)	R	R	R	R	S	
	16 (4.7)	R	R	R	R	R	
Re-treatment (*n*=32)	24 (75.0)	S	S	S	S	S	Pan-susceptible 24 (75.0)
	1 (3.1)	S	R	S	S	S	Mono-resistance 4 (12.5)
	3 (9.3)	S	S	S	S	R	
	1 (3.1)	R	R	S	S	S	Poly-resistance 4 (12.5)
	1 (3.1)	R	R	S	R	S	
	2 (6.3)	R	R	R	R	R	
Any resistance (*n*=369)	42 (11.4)	25 (6.8)	28 (7.6)	20 (5.4)	25 (6.8)	31 (8.4)	MDR 20 (5.4)

Table 3: Comparison of LPA and MGIT results

DST	Type of Drugs	Nature of Isolates	MGIT		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
			Susceptible	Resistance				
LPA	RIF	Susceptible	81	0	100	100	100	100
		Resistance	0	20				
	INH	Susceptible	73	4	85.7	100	100	94.8
		Resistance	0	24				

Table 4: Frequency of Gene Mutations by GenoType MTB-DRplus

Drug	Locus	Failing wild type band	Codons analyzed	Developing mutation band	Mutation	Nucleotide change	Frequency (no. of isolates)
RIF	*rpo*B	*rpo*B WT8	530-533	*rpo*B MUT3	S531 L	TCG TTG	20
INH	*kat*G	*kat*G WT	315	*kat*G MUT1	S315 T1	AGC ACC	24

Table 5: Studies from different regions

Authors	Place	Study period	No. screened	Results
Yigzaw *et al.*[30]	University of Gondar Hospital.	2018-2019	A total of 376 patients were enroled. Sputum samples from 126 patients suspected of active pulmonary TB	*Mtb* was isolated from 126 patients. 106/126 (84%) patients were newly diagnosed with TB and 20 patients with prior TB treatment. 70 (66.0%) were susceptible to all anti-TB drugs tested. 25 (19.8%) of the isolates were resistant to isoniazid, 12 (9.5%) to rifampicin and six (5%) were multi-drug-resistant. Among previously treated TB patients, 4 (20.0%) and 5 (25.0%) were mono-resistant and poly-resistant.
Singhal *et al.*[17]	Mumbai	January 2015 -December 2015	223 patients of tuberculosis who were culture-positive and *Mycobacterium tuberculosis* was resistant to rifampicin and isoniazid	The results showed that 43 patients (81.13%) had pulmonary and 10 patients (18.87%) had extra-pulmonary TB. Of the 43 pulmonary cases, 18 (41.86%) had drug-sensitive TB and 25 (58.13%) had drug-resistant TB. In drug-resistant pulmonary cases, pre-XDR TB was found in 15 (60%), MDR TB in 6 (24%), XXDR TB in 2 (8%), XDR TB in 1 (4%), and INH mono-resistance in 1 (4%). Overall, the incidence of pre-XDR TB was 61.9% (19 patients)
Tripathy *et al.*[27]	IRL Darbhanga, Damien TB research Centre of the Darbhanga Medical College and Hospital, Bihar, India	February-July 2014	256 samples	256 sputum samples from suspected cases of MDR TB, 122 cases were microscopy-positive for tuberculosis. Among these 122 cases, tuberculosis was confirmed by PCR in 114 cases. Finally, with the help of LPA, 39 (15%) samples were found to have resistance to both INH and rifampicin.
Joseph *et al.*[25]	Ernakulam District, Kerala, South India	Tuberculosis Research Centre, Chennai.	A total of 305 (88.7%) sputum samples were positive for culture.	Resistance to any drug was seen in 27.9%, and MDR-TB was observed in 2%. Mono-resistance to rifampicin and streptomycin was observed in, respectively, 1% and 17% of cases, and 27.1% resistance was observed to any drug in the younger age group. MDR-TB is within expected ranges in Ernakulam District.
Myneedu *et al.*[24]	Lala Ram Sarup (LRS) district, under RNTCP, New Delhi	July 2011 to June 2012	453 samples	Primary MDR was found to be 18/453; (4.0%). Extensively drug resistance (XDR) was found in one strain (0.2%), which was found to be resistant to other antibiotics
Shivekar *et al.*[28]	South India	2013-2018 retrospective analysis	MTBDRplus assay and MGIT liquid culture performed on 20,245 sputum specimens obtained from presumptive MDR-TB cases	MDR, rifampicin mono-resistant and isoniazid mono-resistant TB were found in 5.4%, 2.5%, and 11.4% cases of presumptive MDR-TB, respectively. Based on the *rpoB* gene, true resistance, hetero-resistance, and inferred resistance to rifampicin were found in 38%, 29.3%, and 32.7% of the 1582 MDR cases, respectively. S450L (MUT3) was the most common *rpoB* mutation present in 59.4% of the rifampicin-resistant cases. Of the 3390 isoniazid-resistant cases, 72.5% had mutations in the *katG* gene, and 27.5% had mutations in the *inhA* gene. True resistance, heteroresistance, and inferred resistance accounted for 42.9%, 22.2%, and 17.3% of the 2459 *katG* resistant cases, respectively. MDR-TB, i.e., resistance to both rifampicin and isoniazid, is strongly correlated with treatment failure, spread through contact, and not to treatment compliance.
Ahmed *et al.*[22]	Uttar Pradesh	2016-2018	A total of 1268 sputum samples of MDR-TB suspects were subjected to fluorescent microscopy.	MDR-TB was detected in 11.02%, 20.03% in new and previously treated cases. Among MDR-TB patients, S531 L was the most common mutation detected in rpoB gene, 71.43% in new, and 72.17% in previously treated cases. S315T1 was the most common mutation noted in katG gene; 100% in new and 81.74% in previously treated. While in hA gene, it was C15T (7.8%) among previously treated cases.

Contd...

Table 5: Contd...

Authors	Place	Study period	No. screened	Results
Rao *et al.*[16]	Udupi, Karnataka	September 2011–August 2014	990 cases of smear-positive PTB patients	A total resistance of 33.4% was observed that includes the mono-resistance of 22.5%, MDR of 6.3%, and XDR of 0.3%.
Raizada *et al.*[18]	RNTCP state level Intermediate Reference Laboratories (IRL) in Hyderabad (Andhra Pradesh State), Ahmadabad (Gujarat State), and the Mycobacteriology laboratory at SMS Medical College, Jaipur (Rajasthan State)	November 2008 and January 2009, National Reference Laboratory	Smear-positive sputum specimens from 320 patients were subjected to LPA and results compared against those from conventional Lowenstein Jensen (LJ) culture and drug susceptibility testing (C and DST).	A total of 248 patients had both LJ and LPA DST results available; 232 (93.5%) had concordant R DST results. Among the 16 discordant R DST results, 13 (81%) were resolved in agreement with LPA results. LPA proved highly accurate in the rapid detection of R resistance.
Sharma *et al.*[21]	Nepal	2013-2014	54 culture samples were analyzed for DST using both conventional proportion method and MTBDR*plus*, where conventional DST identified 43 isolates (79.6%) as drug-resistant.	Among the 43 drug-resistant isolates, 30 isolates (69.7%) were found to be MDR. Of all observed mutations using MTBDR*plus*, codon 531 of *rpoB* gene and codon 315 of *katG* gene were found to have highest mutational frequency for RIF resistance (64.7%) and INH resistance (96.8%), respectively. In the present study, MTBDR*plus* assay was shown to have excellent specificity (100%) for both RIF and INH resistance, while sensitivity of the assay was little lower with a value of 89.4% for RIF resistance and 91.4% for INH resistance.
Thakur *et al.*[15]	Himachal Pradesh	2013-2015	A total of 770 sputum samples of the MDR-TB suspects which were received at Intermediate Reference Laboratory, Government TB Sanatorium, Dharampur, Solan, Himachal Pradesh	Among the 521 MTBC strains, 19.76% were found to be MDR and mono-resistance to isoniazid and rifampicin was detected in 8.63% and 6.14% strains, respectively. About 74.81%, 76.35%, and 5.40% strains harbored known mutation in *rpoB*, *katG*, and *inhA* genes, respectively.
Yadav *et al.*[19]	All India Institute of Medical Sciences hospital, New Delhi	January to September 2012	269 previously treated sputum-smear acid-fast bacilli (AFB) positive MDR-TB suspects	DST results by LPA and LJ methods were compared in 242 MDR-TB suspects. The LPA detected rifampicin (RIF) resistance in 70 of 71 cases, isoniazid (INH) resistance in 86 of 93 cases, and MDR-TB in 66 of 68 cases as compared to the conventional method. Overall (rifampicin, isoniazid and MDR-TB) concordance of the LPA with the conventional DST was 96%.
Abdella *et al.*[29]	Jimma, Southwest Ethiopia	March, 2012 and April, 2013	79 re-treatment cases	The prevalence of MDR-TB was 31.4% (22/70). Place of residence, duration of illness, and frequency of prior TB therapy were significant factors for any drug resistance. Moreover, history of treatment failure was found to be associated with MDR-TB. The overall prevalence of MDR-TB among re-treatment cases around Jimma was high. The rate of MDR-TB was higher in patients with the history of anti-TB treatment failure.
Gupta *et al.*[23]	Institute of Medical Sciences, Banaras Hindu University (BHU). Sir Sundar Lal Hospital, a tertiary-care hospital of BHU	January 2008 to January 2010	3,704 clinical specimens, 345 (9.3%) were culture-positive, and drug-susceptibility testing was carried out for 301 MTB strains	A high level of primary and acquired drug resistance of MTB was observed in the region studied, with weighted mean of 10.5% and 28.08%, 12.81% and 29.72%, 17.12% and 29.94%, 11.97% and 27.84%, and 10.74% and 23.54% for rifampicin, isoniazid, streptomycin, ethambutol-resistant and MDR cases, respectively. Drug resistance was significantly higher in pulmonary and acquired drug-resistant TB cases. Any drug resistance and MDR TB were significantly associated with HIV-seropositive cases.
Sinha *et al.*[26]	All India Institute of Medical Sciences and National Institute of Tuberculosis and Respiratory Diseases, New Delhi	2013-2016	1103 patients	Of the total 1103 patients (median age 34 years, 59% and 41% males and females, respectively), there were 683 new PTB cases with liquid culture-positive. From these patients, 62 (9.1%) were resistant to rifampicin, 75 were resistant to isoniazid (11%), and 60 (8.7%) patients were resistant to both drugs

showed the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 100%. INH LPA testing showed a sensitivity of 85.7%, a specificity of 100%, PPV of 100%, and NPV of 94.8% [Table 3]. The gene mutation for RIF resistance[20] was between the codon, 530–533 of *rpo*B gene, and 315 of *kat*G gene for INH resistance,[24] as shown in Table 4.

Discussion

In this study, the prevalence of first-line drug resistance among pulmonary TB patients was demonstrated. In all, 1059 (264 TB patients, 94 patients having both diabetes and TB, 10 HIV-TB co-infected patients, and one patient was co-infected with TB, HIV, and diabetes) patients were enrolled. Phenotypic MGIT SIRE along with PZA DST tests revealed that 337 (91.3%) of TB patients were freshly detected, whereas 32 (8.7%) patients had history of previous treatment for TB. RIF LPA compared to MGIT showed the sensitivity, specificity, PPV, and NPV of 100%. INH LPA testing showed 85.7% sensitivity and 100% specificity. Mono-resistance against pyrazinamide (Z) was a significant finding in our study. We found high resistance patterns for pyrazinamide (8.4%) among the total TB isolates.

Drug resistance is a significant obstacle in treatment and prevention of TB in developing nations. The detection and treatment of MDR-TB is one of the biggest health issues.[16] First-line anti-TB regimens are insufficient for obtaining high cure rates because drug-resistant strains are spread throughout the community, replacing susceptible strains.[17,18]

Since *M. tuberculosis* is a slow-growing bacterium and DST is costly and time-consuming, all diagnosed patients are treated with the first-line anti-TB medications irrespective of the drug sensitivity status. In view of this, identifying the strain in each region related to drug resistance can be effective in determining the best approach for containing MDR strains.[19]

Rapid and reliable techniques like DST and LPA play a crucial role for faster diagnosis and treatment of TB since it benefits both the patients with high risk of drug-resistant TB and health care professionals treating them before phenotypic DST results become available.[20,21]

Although the DOTS-plus therapy is the most effective way to avert the development of MDR-TB by diagnosis and treatment, these patients undergo toxic and complicated treatments for a longer period of time, leading to the emergence of XDR-TB. Therapy for MDR-TB is difficult, time-consuming, and expensive. Therefore, use of DOTS and DOTS-plus methods, which are a pillar in the fight against the emergence and spread of MDR-TB and XDR-TB, needs to be strengthened.

PZA-DST is indispensable for the appropriate supervision of patients with DS, DR, and MDR-TB. Additionally, PZA will be crucial for shorter regimens for both DR and DS-TB in order to avoid the development of resistance to new medications.[21,22]

Several authors from different regions of India and the world have reported findings of drug-resistant TB as depicted in Table 5.[15-19,21-30] Together, these studies have emphasized that treatment prior to DST, previous record of treatment failure, tertiary level healthcare, place of residence, duration of illness, and HIV positivity were associated with resistance to first- and second-line drugs.

Strengths and limitations

We could enrol a number of patients with TB. The processing of samples in the NRL was an added advantage. DST was carried out using MGIT and LPA assays, and PZA resistance has been reported. Due to financial constraints, we could process a few samples for LPA.

Conclusion

The predominant gene mutations concurrent with RIF and INH resistance were noted in 315 and 531 codons of the kat G gene and rpoB gene, respectively. High prevalence of mono- and multi-drug resistance among freshly detected cases of TB and patients with history of previous treatment as well as pyrazinamide resistance was observed in our study, thus emphasizing the need for an in-depth research of this issue. In phenotypic DST, along with SHRE, PZA should also be initiated to diagnose resistance to complete range of first-line drugs. LPA showed exceptional concurrences of sensitivity and specificity for diagnosing single- and multi-drug-resistant TB. Thus, phenotypic and genotypic avenues are reciprocal for acquiring better sensitivity and specificity in diagnosis of drug resistance and sensitivity to precisely speculate cure for MDR-TB.

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Conflicts of interest

There are no conflicts of interest.

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