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Research Article

AN INTEGRATED APPROACH OF RAPID PHENOTYPIC METHOD AND MOLECULAR DIAGNOSTIC TECHNIQUE FOR DIAGNOSIS OF DRUG RESISTANT TUBERCULOSIS

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Abstract- Drug Resistant tuberculosis(DR-TB) is a worldwide problem and to speed up diagnosis, to standardised testing procedures, scaling up management and surveillance of DR-TB in high throughput laboratories, genotypic or molecular methods have considerable advantages. Optimum utilization of rapid phenotypic method and liquid culture system MIGIT 960 (mycobacterial growth indicator tube) for culture and drug susceptibility testing (DST) of second line drugs for tuberculosis(TB), could help provide fast, reliable and accurate results for patient care, since our laboratory have all these facilities simultaneously. Passive case finding along can lead to missed cases or delayed diagnosis. Enhanced outstretched activities to detect more TB cases are critical to universal assess. We analysed percentage of MDR, pre-XDR and XDR cases of tuberculosis for presumptive tuberculosis, presumptive MDR and presumptive XDR patients, according to recent changes in RNTCP guidelines for case finding and diagnostic strategy to optimise treatment regime. To conclude, drug-resistant tuberculosis (DR TB) poses a significant threat to human health. MTBDR assay, which fits easily in the workflow of a routine laboratory, with effective planning and logistics, simultaneous use of combination of molecular based technologies and rapid phenotypic method can be successfully introduced into a reference laboratory setting with high throughput laboratories and high incidence country. Consequently, use of both molecular and phenotypic methods, will not only reduce the heavy work load of reference laboratories but also improves the quality of work done by the staff and thereby assuring the quality of reports released.

Keywords: Drug resistance, RNTCP, Tuberculosis, Line probe assay, Rifampicin

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Introduction

The diagnosis and management of drug resistant strains of mycobacterium tuberculosis is turning out to be an appalling challenge to tuberculosis control. The startling rises in multi drug resistant (MDR) cases have been registered globally, compounded by the emergence of extensive drug resistant (XDR) tuberculosis which is virtually untreatable in many settings [1]. Actualization of MDR and XDR cases calls for an necessary evaluation and wide scale implementation of molecular methods to screen the patients, at risk of MDR TB in combination with conventional but rapid phenotypic technique like liquid culture and culture based second line DST in order to diagnose pre XDR & XDR TB specially in well-equipped, high workflow, reference laboratories, not only initiate prompt and appropriate treatment to the patients but also decreases morbidity, mortality, and interrupt transmission.

MTB DR*plus* line probe assay have been endorsed by World Health Organisation for the rapid diagnosis of drug resistant tuberculosis and has implemented in revised national tuberculosis control program in India for screening of MDR TB cases. LPA technology is based on reverse hybridization of DNA on the strip, and the assay has shown good performance (98% sensitivity) RIF resistance detection compared to gold standard phenotypic DST [2]. The standard turnaround time (TAT) for reporting the LPA result is 48 to 72 hrs, as per WHO guidelines. RIF inhibits the RNA polymerase at the level of the beta subunit encoded by the rpoB gene. This molecular method is developed to target the rpoB gene, which consists of an 81-bp hot-spot region from codons 507 to 533, called the rifampin resistance-determining region (RRDR) [3]. Resistance to RIF in mycobacteria results from point mutations predominantly located in the 511 to 533 regions of the RpoB polypeptide [4,5]. So far more than 50 mutations have been characterized within this region by DNA sequencing but only point mutations at codons 526 or 531 are known to cause high levels of RIF resistance [6]. While, low-level resistance to RIF is caused by mutations in codons 511, 516, 518, 522, and 533. Mutations are rarely seen in other regions of the rpoB gene, for RIF resistance [7]. INH inhibits InhA, the enoyl-ACP reductase, which is a key enzyme for the biosynthesis of mycolic acids found in the cell wall [8]. As INH is a prodrug, this antibiotic need to be converted to an active form by the catalase-peroxidase KatG encoded by the katG gene [9]. Resistance to INH can therefore arise from a wide variety of mutations affecting either the binding of INH to the target InhA (such as Ile21Thr or Val, Ser94Ala, and Ile194Thr), the activation of INH by KatG (the most frequent mutation being Ser315Thr), or finally, the level of expression of the target InhA (by the C-to-T nucleotide substitution at 15 affecting the promoter region of the mabA-inhA operon [10-14]. We have high throughput, well equipped, ISO 15189 and NABL (National Accreditation Board for testing and calibrating laboratories) 112 Accredited, reference laboratory where we are using combination of genotypic (LPA for screening MDR and reporting results of DST for first line drugs) and phenotypic (liquid Culture and culture based DST for second line drugs) methodologies to screen and diagnose MDR, Pre-XDR and XDR cases of DR-TB.

Material and methods

Clinical samples. According to recent changes in RNTCP Technical and Operational Guidelines for Tuberculosis Control Programme in India – 2016 [15,

16]. From 496 patients, a total of 992 sputum specimens (two samples each patients) were collected from presumptive TB and presumptive MDR patients (diagnostic cases), and 417 single specimens were collected from presumptive XDR (follow up) patients [17].

Sample processing. All sputum samples were received through courier delivery in a cold chain in sterile, leak proof, wide mouth, transparent, and stopper plastic containers. and were processed using the N-acetyl-L cysteine-sodium citrate-NaOH (NALC-NaOH) method [18]. Samples were decanted following centrifugation, and the sediments were resuspended in 3 ml of phosphate buffer solution. Several aliquots were prepared from the processed sample, as per the quantity of the original sample. Processed samples were used to perform Ziehl-Neelsen (ZN) staining, LPA, and MGIT960 culture. Remaining sample aliquots were stored at -80°C for further use and quality control.

Line probe assay. LPA testing was done under the programmatic management of drug-resistant tuberculosis (PMDT) plan of the revised national tuberculosis control program (RNTCP) [19]. The LPA was performed according to the manufacturer's protocol [20]. The test is based on DNA strip technology and has three steps: DNA extraction, multiplex PCR amplification, and reverse hybridization. All three steps were performed as per the WHO recommendations [21].

MGIT960 culture and DST: Only DR TB confirmed cases from LPA were processed for liquid culture and liquid culture based DST. A 500-µl of decontaminated specimen was taken out from an aliquot cryovial and inoculated in Bactec-MGIT960. After the culture flashed positive, as indicated by visual signals from machine, presence of AFB bacilli appears as, granular serpentine growth in the medium, smear was made from positive flashed MIGIT tube and ZN staining was performed, the same growth was also inoculated on BHI agar to rule out bacterial contamination in the tube and the growth of MTBC was confirmed by performing rapid immunochromatography based (SD Bioline) MPT 64 Ag detection test. Drug Sensitivity Testing (DST) of Mycobacterium tuberculosis cultures on MGIT 960 for Second Line TB Drugs was performed on MDR TB confirmed cases. Critical Concentration of second line drugs was calculated as per WHO recommendations, kanamycin (K) 2.5 µg/ml, capreomycin (C) 2.5 µg/ml, levofloxacin (L) 1.5 µg/ml, pyrazinamide (PZA) 100 µg/ml, linezolid(LZD) 1 µg/ml, clofazimine (CLF) 1 µg/ml, moxifloxacin (M) was used in two concentrations 0.5 µg/ml for diagnostic cases and 2 µg/ml for follow up (XDR) cases. MGIT-DST was performed per the manufacturer's protocol Reading was interpreted as growth unit <100 is Susceptible and > 100 is Resistant as per manufacturer's instructions (MGIT 960 manual by BD). The standard strain of M. Tuberculosis, H37Rv, was used as a positive control.

Inclusion criteria: Pulmonary specimens positive for AFB by ZN smears

Exclusion criteria: Pulmonary specimens negative for AFB and extrapulmonary specimens.

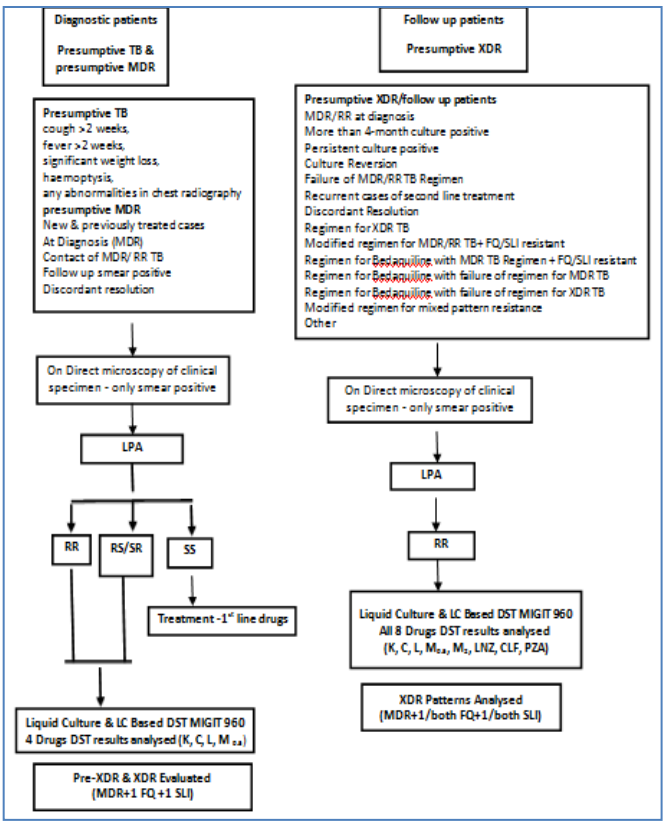
Flowchart:

Flowchart shows work flow processing of samples received according to recent changes in RNTCP Technical and Operational Guidelines for Tuberculosis Control Programme (2016). only smear positive specimens were taken in to consideration for LPA, and further work up, smear negative samples were processed for gene expert MTB/RIF assay which were not included in analysis of present data. (K-kanamycin, C- capreomycin, L levofloxacin, M-moxicycline, LZD- linezolid, CLF-clofazimine, PZA- pyrazinamide, FQ- fluoroquinolones, SLI- second line injectable, MDR- multi drug resistance, XDR- extensively drug resistance, RR- Isoniazid (INH) & rifampicin (RIF) resistance, RS/SR- Mono INH resistance, Mono RIF resistance, SS- Isoniazid (INH) & rifampicin (RIF) sensitive.

Results and Discussion

Total 913 samples were processed for identification of Mycobacterium tuberculosis from July 2016 to December 2016. According to changes in technical and operational Guidelines for rapid diagnosis of drug resistant tuberculosis, 496 patients were enrolled as diagnostic cases which were of presumptive TB and presumptive MDR, and 417 single specimens were received as follow up and as presumptive XDR cases. Four internal controls were run with every batch each time for LPA, (one negative control, one positive control, one extraction negative control and one swipe negative control) and for LC and DST H37RV was put as an internal quality control with every batch daily. Being an ISO 15189 (2012) NABL accredited laboratory the quality control and assurance of line probe assay and liquid culture and LC based DST technique is assured by having an effective external quality assurance programme (EQA) with National institute of Tuberculosis (NTI) Bangalore.

Flowchart



Line probe Assay: A better knowledge of the mechanisms of action of anti-TB drugs and the development of drug resistance will allow identifying new drug targets and better ways to detect drug resistance. More than 90% of mutations in the rpo B gene that codes for the β-subunit of the RNA polymerase is located at 81 base pairs region (codons 507–533), Because of this, conformational changes occur that decrease the affinity for the drug and results in the development of resistance. Isoniazid acts by inhibiting the synthesis of mycolic acids through the NADH-dependent enoyl-acyl carrier protein (ACP)-reductase, encoded by inhA [22]. Although simple in its structure, resistance to this drug has been associated with mutations in several genes, such as katG, inhA, ahpC, kasA and NDH.

We analysed 496 smear positive diagnostic cases out of 913 total samples in 6-month duration which showed, 266 (53.62%) strains were sensitive for isoniazid and rifampicin and 230(46.37%) were resistant. 417/913 patients enrolled as presumptive XDR and follow up cases, were all resistant for first line drugs (INH & RIP). On LPA 581 (88.18%) were rpoB resistant, 18 (2.82%) were rpoB indeterminate, 575 (88.91%) Kat G resistant and 210 (32.57) were inhA resistant. [Chart-1] 647 (230+417) out of 913 were the total resistant strains of MTB on LPA. 583/647 (90.26%) were screened as MDR were as 49/649 (7.42%) were mono resistant to INH and 15/647 (2.32%) were mono resistant to RIF. Another 29 (4.48%) strains did not develop TUB band on LPA, which were said to be negative for MTB. 18 (2.82%) were indeterminate for RIF and 5 cases were indeterminate

for INH in assay. Interpretations were done as per manufacturer's guidelines. And all in determinates on LPA turn out to be resistant on rapid phenotypic LC DST method.

Frequency of mutation: 581 (88.18%) strains were resistant to rpo B gene. The wild type (WT) rpo B probe hybridization band pattern showed WT 8 band was missing in 78.21% (506/647) cases. Other WT probe band for rpo B gene also gave negative hybridisation results with much less frequency eg; 32 (4.95%) strains of MTB were missing from 526-529 rpo B 'hot-spot' region followed by 29 (4.48%) from 513-517 codon, other WT strains were less frequently missing as shown in [Table-1]. 503 (77.74%) samples yielded positive hybridization result with mutation specific probe for S531L which was most frequently observed followed by H526Y, 9 (1.39%) samples, H526D, 5 (0.77%) samples, D516V, 3 (0.46%) samples respectively.

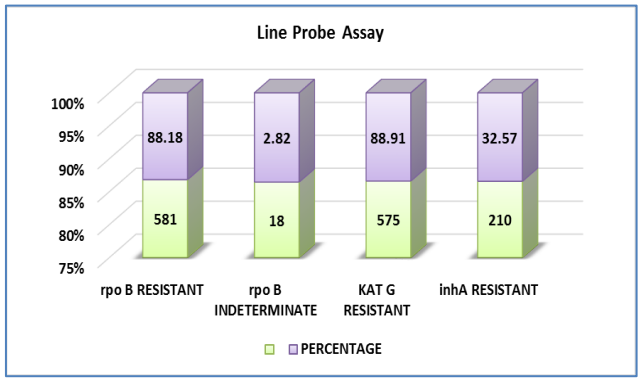


Chart-1

Table-1

FREQUENCE OF MUTATION ANALYSED FOR rpob, Kat G, & inh A					
Gene	Band	Gene Region or Mutation	OTAL RESISTAN STRAIN (647)	Inh Mono Resistance (49)	Rif Mono Resistance (15)
rpo B		WT	WT ABSENT	WT ABSENT	WT ABSENT
	WT 1	506-509	3	0	0
	WT2	510-513	24	0	5
	WT3	513-517	29	0	2
	WT4	516-519	13	0	1
	WT5	518-522	1	0	0
	WT6	521-525	1	0	1
	WT7	526-529	32	0	1
	WT8	530-533	506	0	7
		MUT	MUT PRESENT	MUT PRESENT	MUT PRESENT
	MUT1	D516V	3	0	0
	MUT2A	H526Y	9	0	0
	MUT2B	H526D	5	0	0
	MUT3	S531L	503	0	7
Kat G		WT	WT ABSENT	WT ABSENT	WT ABSENT
	WT	315	575	25	0
		MUT	MUT PRESENT	MUT PRESENT	MUT PRESENT
	MUT1	S315T1	554	30	0
	MUT2	S315T2	10	0	0
inh A		WT	WT ABSENT	WT ABSENT	WT ABSENT
	WT1	nus 15/16 Mir	174	16	0
	WT2	Minus 8	39	3	0
		MUT	MUT PRESENT	MUT PRESENT	MUT PRESENT
	MUT1	C15T	177	19	0
	MUT2	A16G	1	0	0
	MUT3A	T8C	20	2	0
	MUT3B	T8A	8	3	0

Similarly, INH-resistant strains were characterized by a mutation at codon 315 which results in decrease or total loss of catalase peroxidase activity which were revealed by a negative hybridization signal at the level of the corresponding wild-type specific probe. In 575 (88.87%) cases, WT 315 was missing, were as in 554/647 (85.63%) cases evaluated, serine was substituted for threonine at 315 (S315T1) while only 10 cases showed mutation at S315T2 region of Kat G. Mutation in Kat G showed high level of resistance (MIC > 1 µg/mL) while mutation in inh A region shows low level resistance (MIC < 1 µg/mL) to INH. In addition to being associated with katG gene mutations, resistance to isoniazid has also been associated with mutations in the inhA gene and its promoter found upstream from the *mabA-inhA* locus, similar findings has also been documented with other studies [23-25]. We found negative probe hybridisation results for WT -15/-16 in 174/647 (26.89%) and WT -8 in 39/647 (6.03%) cases respectively. 177/647 (27.36%) of them showed a 15 C-to-T substitution in the *inhA* promoter region, 20 (3.10%) and 8(1.24%) strains showed mutation in codon T8C and T8A respectively. There have been only one clinical isolates describing A16G amino acid substitution in present analysis. Frequency of mutation is also analysed for mono resistance in INH & RIF. [Table-1]

Patterns of mutations: Most common pattern of mutation found in 'hot-spot' region of 81 bp of rpoB is WT 8 absent and mut 3 (S531L) present seen in 490/647 (75.73%). Other than this there were 25 different patterns noted for rpo B gene. For kat G gene WT1 absent and mut 1 (S315T1) present (83.15%), and for inh A gene WT1 absent and mut 1(C15T) present (25.35%), was the most frequent patterns seen on line probe assay. Similarly, four different patterns were noted for Kat G gene and 11 different patterns were observed for inh A gene. Similarly, patterns for mono resistance in INH and RIF have also been evaluated. [Table-2A, 2B, and Table-3].

Table-2 A

PATTERNS OF MUTATION OR RESISTANCE ANALYSED				
MDR + MONO RIF + MONO INH				
SR NO	LPA BANDING PATTTERN (647 TOTAL SPECIMENS)			
rpo B	WT ABSENT	MUT PRESENT	NO OF SAMPLES	PERCENT(%)
1	8	3 (S531L)	490	75.73
2	2,3	NO	9	1.39
3	3	NO	3	0.46
4	2	3 (S531L)	1	0.15
5	2,8	3 (S531L)	1	0.15
6	7	2B (H526D)	4	0.62
7	7	NO	12	1.85
8	8	NO	12	1.85
9	6	NO	1	0.15
10	2	NO	6	0.93
11	2,3,7	2B (H526D)	1	0.15
12	7	2A (H526Y)	7	1.08
13	NO	3 (S531L)	10	1.55
14	7,8	NO	2	0.31
15	1,7	2A (H526Y)	1	0.15
16	2,7	NO	1	0.15
17	3,4	1 (D516V)	3	0.46
18	3,4	NO	6	0.93
19	1,7	NO	2	0.31
20	1	NO	1	0.15
21	2,3,7	NO	2	0.31
22	NO	2A (H526Y)	1	0.15
23	3,4,5	NO	1	0.15
24	2,3,4	NO	3	0.46
25	3,8	3 (S531L)	1	0.15

Table-2B

PATTERNS OF MUTATION OR RESISTANCE ANALYSED				
MDR + MONO RIF + MONO INH				
SR NO	LPA BANDING PATTTERN (647 TOTAL SPECIMENS)			
KAT G	WT ABSENT	MUT PRESENT	NO OF SAMPLES	PERCENT(%)
1	1	1 (S315T1)	538	83.15
2	1	2 (S315T2)	9	1.39
4	1	NO	11	1.7
PATTERNS OF MUTATION OR RESISTANCE ANALYSED				
MDR + MONO RIF + MONO INH				
SR NO	LPA BANDING PATTTERN (647 TOTAL SPECIMENS)			
inh A	WT ABSENT	MUT PRESENT	NO OF SAMPLES	PERCENT(%)
1	1,2	1 (C15T)	5	0.77
2	1	1 (C15T)	164	25.35
3	NO	1 (C15T)	8	1.24
4	2	3A (T8C)	17	2.63
5	NO	3A (T8C)	2	0.31
6	NO	3B (T8A)	2	0.31
7	1,2	NO	3	0.46
8	2	3B (T8A)	6	0.93
9	2	NO	1	0.15
10	1,2	2 (A16G)	1	0.15
11	1	3A (T8C)	1	0.15

Table-3

PATTERNS OF MUTATION				
PATTERN OF RESISTANCE				
MONO RIF RESISTANCE				
SR NO	LPA BANDING PATTTERN (15 rpoB SPECIMENS)			
rpo B	WT ABSENT	MUT PRESENT	NO OF SAMPLES	PERCENT(%)
1	8	3 (S531L)	6	40
2	2	3 (S531L)	1	6.66
3	6	NO	1	6.66
4	2	NO	3	20
5	7	NO	1	6.66
6	3,4	NO	1	6.66
7	2,3	NO	1	6.66
8	8	NO	1	6.66
PATTERNS OF MUTATION				
PATTERN OF RESISTANCE				
MONO INH RESISTANCE				
SR NO	LPA BANDING PATTTERN 32 (KAT G) RESISTANCT SPECIMENS)			
KAT G	WT ABSENT	MUT PRESENT	NO OF SAMPLES	PERCENT(%)
1	1	1 (S315T1)	23	71.88
2	NO	1 (S315T1)	7	21.88
4	1	NO	2	6.25
PATTERNS OF MUTATION				
PATTERN OF RESISTANCE				
MONO INH RESISTANCE				
SR NO	LPA BANDING PATTTERN 24 (inh A) RESISTANT SPECIMENS)			
inh A	WT ABSENT	MUT PRESENT	NO OF SAMPLES	PERCENT(%)
1	1	1	16	66.66
2	2	3B	3	12.5
3	NO	1	3	12.5
4	NO	3A	2	8.33

Hetero-resistance Analysed: It is presence of a mixed population of resistant and susceptible sub-populations in a clinical Isolate. It was analysed for both isoniazid and rifampicin. Ten patients showed all rpo B WT bands present along with presence of point mutation for S531L, while in one strain of MTB, mutation was noted at gene region H526Y, along with positive probe hybridization result for all WT bands, signifying the presence of both sensitive and resistant population of MTB for RIF. For isoniazid, hetero-resistance was not observed in high level resistance producing gene (Kat G) but was observed for inh A gene, eight strains of MTB showed presence of all WT bands and mutation in C15T, while in two other strain, mutation in T8C and T8A one each, was noted along with presence of corresponding WT band. They were resistant to rifampicin and isoniazid by liquid culture DST method.

Hetero-resistance can occur due to infection from two different strains, usually seen in new patients or in a single strain segregating into sensitive and resistant, as seen in previously treated patients, due to the positive selection pressure of drugs. LPA applied directly to clinical samples enhances the chance of detection of hetero-resistance, which can serve as an indicator of the quality of anti-TB programs [26]. Hetero-resistant samples on LPA eventually showed drug resistance by phenotypic drug susceptibility testing, that is, they corresponded to the mutated organism [27]. Therefore, if a clinical sample is detected to be hetero-resistant in the early screening by LPA, it can be inferred that the patient is drug-resistant and must give a regimen for drug-resistant TB.

Liquid culture and Liquid culture based DST: Drug-resistant tuberculosis poses a significant threat to human health. LC and LC based DST for 2nd line drugs were performed for strains resistant by LPA. All resistant strains 647 were inoculated in Bactec-MIGIT 960. For presumptive TB and presumptive MDR 131/230 (56.96%) and follow up and presumptive XDR 378/417 (90.65%)cultures were flagged positive and confirmed as MTBC by MPT 64 Ag detection test. 27 (11.74) cultures from diagnostic cases and 39 (9.35%) from follow up did not grew on culture as these may be dead bacilli but gave resistance pattern on LPA because it requires DNA and even dead bacilli can be amplified and resistance can be detected. 131 diagnostic cases were evaluated for second line DST for four drugs kanamycin, capreomycin, levofloxacin and moxifloxacin 0.5 ($KCLM_{0.5}$) pre-XDR, resistance to any one of the four drugs and XDR [MDR+1 FQ+1 SLI] were evaluated based on drug resistance interpreted as per manufacturer's instructions. For pre-XDR, maximum resistance was seen to levofloxacin 91/131 (69.47%), followed by moxifloxacin 0.5 66/131 (50.38%), kanamycin 27/131 (20.61%), and least resistance is seen to capreomycin 19/131 (14.5%). [Chart-1] In case of XDR, maximum resistance is seen in drug combination with (1 SLI+1 FQ) K+L 118 (90.07%), followed by C+L 110 (83.96%), K+M 93 (70.99%) and least resistance is seen in drug combination with C+M 85 (64.88). [Table-4]

Similarly, 378 presumptive XDR and follow up samples were analysed for second line DST for eight drugs kanamycin, capreomycin, levofloxacin, moxifloxacin 0.5, moxifloxacin 2, linezolid, clofazimine, pyrazinamide [K, C, L, $M_{0.5}$, M_2, LNZ, CLF, PZA] [Chart-2] and pre-XDR and XDR [MDR+1/bothFQ+1/both SLI] patterns was also evaluated for drug resistance. For pre-XDR, in follow up patients' maximum resistance was seen to PZA 325/378 (77.39%), followed by levofloxacin 273/378 (72.22%), moxifloxacin $_{0.5}$ 201/378 (53.17%), kanamycin 80/378 (21.16%), moxifloxacin $_2$ 68/378 (16.31), capreomycin 57/378 (15.08) LZN 30/378 (7.19%) and least resistance is seen to CLF 15/378 (3.6%). The pattern of resistance for XDR analysed is shown in [Table-5]. [MDR+1/bothFQ+1/both SLI] For follow up cases 30 patients showed resistance to all four drugs K+C+L+$M_{0.5}$ in combination which accounted for 7.93% of XDR cases.

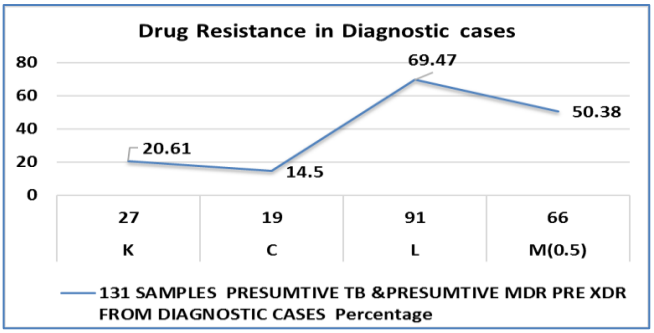


Chart-2 LIQUID DST FOR CULTURE POSITIVE SAMPLES ONLY. (131 positive LC & DST from diagnostic cases.)

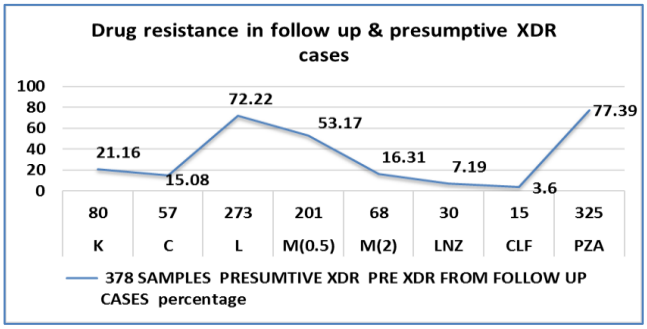


Chart-3 LIQUID DST FOR CULTURE POSITIVE SAMPLES ONLY. (378 positive LC & DST from follow up & presumptive XDR cases.)

Table-4

PRESUMTIVE MDR SAMPLES (131) DIAGNOSTIC CASES			PERCENTAGE
XDR COMBINATIONS MDR/RR-TB + 1FQ+ 1 SLI	K+M (27+66)	93	70.99
	C+L (19+91)	110	83.96
	K+L (27+91)	118	90.07
	C+M (19+66)	85	64.88

Table-5

PRESUMTIVE MDR SAMPLES (378) FOLLOW UP CASES				EXTENDED DST FOR SAME COMBINATIONS IN FOLLOW UP PATIENTS			
XDR COMBINATIONS			PERCENTAGE	M(2)	LNZ	CLF	PZA
MDR/RR-TB + 1 OR BOTH FQ + 1 OR BOTH SLI RESISTANT	RRRR	30	7.93	12	07	04	27
	RSRS	11	2.91	0	0	0	08
	SRSR	1	0.26	0	0	0	01

Identification for development in mechanism of drug resistance will help reduce circulation of drug resistant strain in population. Mutations in the gene pncA remain as the most common finding in pyrazinamide resistant strains. These mutations, however, are scattered throughout the gene but most occur in a 561-bp region in the open reading frame or in an 82-bp region of its putative promoter. Resistance to fluoroquinolone is not only due to amino acid substitutions in the putative fluoroquinolone binding region in gyrA or gyrB but also by active efflux mechanisms and involvement of fluoroquinolone-resistance protein from MTB; MfpA protein. In case of SLI, aminoglycoside drug resistance is associated with an A1401G mutation in the rrs gene coding for 16S rRNA. [high-level resistance to kanamycin] and mutations in the promoter region of the eis gene in M. tuberculosis. (low-level resistance to kanamycin), whereas mutations in the gene tlyA have been implicated in resistance to cyclical peptides (capreomycin). Cross-resistance between kanamycin and amikacin or kanamycin, capreomycin and viomycin to variable degrees have been reported. For LZN and CLF apart from mutation at gene region, involvement of efflux pumps or other non-ribosomal alterations are also suggested, and the same goes for bedaquiline (BDQ) also [28].

Conclusion

Early detection of all forms of drug resistance in TB is a key factor to reduce and contain the spread of these resistant strains. To effectively address the threats of drug resistant tuberculosis, global initiatives are required to scale-up culture and drug susceptibility testing capacities. In parallel efforts are needed to expand the use of novel and emerging molecular technologies for rapid diagnosis of drug resistance. Simultaneous use of both molecular and phenotypic methods, will not only reduce the heavy work load of reference laboratories but also improves the quality of work done by the staff and thereby assuring the quality of reports released.

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Ethical approval: This is a retrospective study, and permission was taken from Head of the Department. This article does not contain any studies with human participants or animals performed by any of the authors. This approval was obtained by Head of department, Microbiology.

Abbreviations

DR-TB = Drug Resistant tuberculosis
MDR TB = Multi drug resistance tuberculosis
XDR TB = Xtreme drug resistance tuberculosis
MTBDR assay = Mycobacterium tuberculosis drug resistance assay
RIF = Rifampicin
INH = Isoniazid
MIGIT 960 = mycobacterial growth indicator tube 960
PMDT = programmatic management of drug-resistant tuberculosis
RNTCP = Revised national tuberculosis control program

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