EMERGENCE OF DRUG RESISTANCE TO FIRST AND SECOND LINE ANTI-TUBERCULAR DRUGS DURING TREATMENT OF PULMONARY TUBERCULOSIS

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ABSTRACT

The present study was planned to study the frequency of emergence of drug resistance to first and second line anti tubercular drugs during treatment.Culture positive pulmonary tuberculosis cases, with history of anti-tubercular treatment in past, were enrolled, put on anti-tubercular drugs & followed 3 monthly till the final outcome was established. Treatment details of all the patients were recorded. Sputum samples from all the patients at the time of enrollment and each follow up were subjected to mycobacterial culture and drug susceptibility testing for total six anti tubercular drugs. DST pattern of serial isolates from each patient was compared. Serial isolates from same patient showing changes in the DST pattern were genotyped to rule out new infection. Of 361 isolates from same number of patients; 130 (36%) were MDR and 231 were non-MDR. Of 130 MDR patients; 43 were cured and 87 had a poor outcome (48 patients expired, 31 were lost to follow up and 8 were treatment failures) at the end of follow up. Of 231 non-MDR patients; 132 cured and 99 had a poor outcome (45 patients expired, 43 were lost to follow up and 11 were treatment failures) at the end of follow up. The death rate was significantly higher in MDR-TB cases (P<0.0001) and cure rate was significantly higher (P=.005) in non-MDR–TB cases.Serial isolates from 11 MDR and 15 non-MDR patients showed change in resistance pattern to first line drugs. Serial isolates from 9 MDR and 12 non- MDR patients showed change in resistance pattern to second line drugs. Genotype change was recorded in serial isolates from only four MDR and only two non-MDR patients. Emergence of drug resistance to first line as well as second line during treatment needs attention.

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Introduction

India has the world's highest burden of TB (approximately 3.4 million cases), accounting for one fifth of global incident cases, and ranks first among the twenty two TB high-burden countries[1]. More than 4, 00,000 cases of Multidrug resistant tuberculosis (MDR-TB) emerge every year[2]. The rate of MDR-TB mortality is estimated to range from 40 to 60 percent [3]. It is increasingly common for MDR strains to acquire additional resistance and reaches the extensively drug-resistant (XDR) status. The development of resistance to first line as well as second line anti-tubercular drugs is a serious threat to management of tuberculosis. Resistance usually develops due to inadequate TB management, including improper use of medications, improper treatment regimens, and failure to complete the treatment course. Poor or sub optimal tuberculosis control programmes can also lead to rapid emergence of drug resistance especially where the prevalence of TB is high[4].Traditionally; it has been assumed that TB is caused by an infection with a single strain. The development of change in phenotypic characters during treatment and recurrences are the result of either mutations or reactivation of the strain causing the first episode[5]. The emergence of drug resistance may be due to mutation in infecting strain or a new infection[6, 7]. If the serial isolates have the same genotype, the episode is defined as mutation; otherwise, it is defined as new infection. Several molecular diagnostic tools are available to address whether the changes in phenotype are due to mutations in the infecting strain or due to new infection. Though unfortunate, but the drug resistant tuberculosis is a multi-factorial man made problem. Improper characterization of cases as multi drug resistant tuberculosis, and treating them with inadequate second line regimens remain one of them. There are studies which report the frequency of prescription errors seen in the prescriptions of TB patients written by qualified physicians[8].

The present study reports the changes in drug resistance pattern of serial mycobacterial isolates from pulmonary tuberculosis patients on anti-tubercular treatment and establishing whether these changes are due to mutations or new infection.

MATERIALS AND METHODS

Study protocol

Patients of pulmonary tuberculosis, fulfilling the inclusion and exclusion criteria, were enrolled from the Department of Pulmonary Medicine, Chhatrapati Shahuji Maharaj Medical University and Lucknow during December 2008 to December 2012. Patients of all age groups, and both sexes were included. A patient was included if she/ he was; (1) a case of pulmonary tuberculosis, (2) was giving history of treatment in past with anti-tubercular drugs for more than four weeks (3) sputum culture positive for *M. tuberculosis*, (4) currently taking the antitubercular treatment prescribed by his physician, and was (5)

consenting to participate. A patient was excluded if; (1) sputum culture was negative for *M. tuberculosis* (2) Sputum culture grew MOTT (3) was not consenting to participate (4) having known intolerance to anti tubercular drugs (5) having coexisting terminal illness and (6) residing outside study area.Treatment details of all these cases were recorded from their prescriptions. No interference/ intervention in the treatment protocol were done by investigators. The study was approved by Institutional ethical committee. All patients (or their parents in case of children) provided written consent (on a predesigned consent form approved by Institutional ethical committee to participate in the study. Patients or physicians were not identified by name.

After enrollment, all patients were subjected to sputum examination for presence of *M. tuberculosis* by Acid Fast Bacilli (AFB) smear examination & culture. The *M. tuberculosis* isolate from every patient was subjected to DST for four first line antitubercular drugs; isoniazid (H), rifampicin (R), streptomycin (S) and ethambutol (E) and two second line antitubercular drugs; ofloxacin (OFX) and kanamycin (KM). Based on the DST pattern patients were grouped as MDR or non-MDR TB patients.

Follow-up and outcome analysis

All MDR-TB patients were followed up for a period of two years or till the outcome was final; at an interval of three months for a maximum of 6 times. All non-MDR patients were followed up for a period of one year or till the outcome was final, at every three months for a maximum of 4 times. At the time of every follow up patients were asked to submit sputum samples which were processed like those collected at the time of enrollment. The patients were interviewed on every visit to ensure the treatment adherence. Drug resistance pattern of serial isolates from same patient was matched. Serial isolates from the same patient showing changed drug resistance pattern were genotyped by MIRU-VNTR.

Treatment outcomes were categorized in to two groups: cure and poor outcome (treatment failure, lost to follow up and expired).

Cure: Patients who have received treatment for 18-24 months, the proportion who remain smear and culture negative.

Expired: Of the patients initiated on treatment, the proportion who die during treatment.

Treatment failure: Of the patients who have received treatment for 18-24 months, the proportions that remain smear or culture positive.

Lost to follow up: Patients recruited into the study and were lost to follow up while on treatment.

Specimen collection, processing and culture & drug susceptibility testing

Three sputum samples (one on spot and two early morning samples) were collected in sterile container from each patient (minimum five ml of sputum) for sputum microscopy and mycobacterial culture. All sputum samples were examined for presence of AFB by Ziehl- Neelsen method[9]. Single sputum sample from each patient, showing maximum numbers of AFB, was

decontaminated by Petroff's method[10], and inoculated on to two Lowenstein-Jensen (L-J) tubes, and one L-J tube containing Paranitrobenzoic acid (PNB)[11]. The culture bottles were incubated at 37^{0}C for twelve weeks. These bottles were read after 48 hours of incubation to rule out contamination. There after bottles were read weekly; no growth in the LJ tube after twelve weeks of incubation was treated as negative. If growth was present in the presence of PNB, isolate was treated as Mycobacterium other than tuberculosis (MOTT) and patient was excluded from the study. If growth was present in the LJ tube but not in the PNB tube isolate was treated as *M. tuberculosis*.

Drug susceptibility testing (DST) was performed on LJ medium according to the conventional 1% proportion method [10] and [11]. The concentration of anti-TB drugs used was; 0·2µg/ml for H, 40µg/ml for R, 4 µg/ml for S, 2µg/ml for E, 2.0µg/ml for OFX and 30µg/ml for KM. All the drugs and chemicals were procured from Sigma, USA. External quality control for first line drugs DST was provided by the National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra (India). A standard H37Rv strain was used as the quality control strain for each new batch of medium throughout the study.

Genotyping

Serial isolates from same patient which were showing change in DST pattern were genotyped by PCR amplification of the original 12 MIRU VNTR loci (MIRU-02, -04, -10, -16, -20, -23, -24, -26, -27, -31, -39, -40) as per method described earlier[14].

Statistical analysis

The proportion of death in MDR/ non-MDR group was compared using Fisher Exact Test. STATA 11.1 software was used for all calculation. P value<0.05 was calculated to be significant. Patients lost to follow up during study were excluded from the statistical analysis.

Results

During study period 361 patients fulfilled the inclusion criteria and were enrolled. Among total 361 isolates, resistance to H, R, S, E, OFX and KM were 213 (59%), 163 (45.2%), 148 (40.9%), 176 (48.7%), 95 (26.3%) and 49 (13.5%) respectively. Multidrug resistance was detected in 130 (36%) isolates.

Anti-tubercular drugs prescribed to MDR and Non-MDR cases are summarized in table 1. As seen in table 1, patients are prescribed various combinations, many inclusive of second line drugs like fluroquinolones quite often.

During follow up, treatment outcomes revealed that 43 (33%) MDR and 132 (57.1%) Non-MDR patients were cured, 48 (36.9%) MDR and 45 (19.4%) Non-MDR patients expired, 31 (23.8%) MDR and 43 Non-MDR (18.6%) were lost to follow up, 8 (6%) MDR and 11 (4.7%) Non-MDR patients were treatment failure. Follow up details of MDR and Non-MDR patients are summarized in Figure1. Final outcome was not available in patients lost to follow up hence they were excluded from the statistical analysis and further analysis was done only on 99 MDR and 183 non-MDR

cases. Details of outcome and anti tubercular drugs prescribed to patients are summarized in table 1. As expected the death rate was significantly higher in MDR-TB cases (P<0.0001) and cure rate was significantly higher (P=.005) in non-MDR–TB cases (table 2).

Total 27 patients showed emergence of resistance to either first line or second line drugs and 6 patients showed genotype

Table 1. Anti tubercular drugs prescribed to patients of pulmonary tuberculosis, their outcome & emergence of drug resistance

Combinations of Drugs prescribed	Number of patients (361)	Cure	Expired	Lost to follow up	Treatment failure	Emergence of drug resistance	
						New infection	Mutation
MDR –TB patients (n=130)							
S H R E Z Levo	9	2	3	1	3	-	-
S H R Levo	5	1	-	4	-	-	-
K H Z E PAS Clarithro	6	-	3	3	-	-	-
R E Ethio Levo Cyclo	4	-	-	4	-	-	-
K H E Ethio Cyclo Levo	9	5	-	4	-	-	-
K H Z Ethio Cyclo Levo	7	4	-	3	-	1	3
K Z Ethio Cyclo Levo	4	2	1	1	-	-	-
K H PAS Ethio Cyclo Levo	21	6	9	2	**4**	-	2
K Z PAS Ethio Cyclo Levo	5	1	1	3	-	2	2
K Ethio Levo Z	5	-	2	3	-	-	-
K PAS Ethio Cyclo Levo	22	9	13	-	-	-	-
SHRE Cyclo Oflox PAS	14	5	6	3	-	-	-
K PAS Ethio Cyclo	5	3	2	-	-	-	-
K H Ethio Cyclo Oflo	2	0	2	-	-	-	-
KZ Ethio Oflox	12	5	6	-	1	1	1
Non-MDR-TB patients (n=231)							
RHE	65	46	5	14	-	-	2
S H R E Z	37	29	8	-	-	2	-
S H R E Z Levo	63	35	11	8	9	1	7
K H Ethio Oflox Z	17	8	8	1	-	-	-
K H PAS Ethio Cyclo Levo	11	4	3	1	2	-	-
K Ethio Levo Z	11	3	3	5	-	-	-
K PAS Ethio Cyclo Levo Z	27	7	7	13	-	1	2

S=Streptomycin; H=Isoniazid; R=Rifampicin; E=Ethambutol; Z=Pyrazinamide; K=Kanamycin; Ethio=Ethionamide; Cyclo=Cycloserine; Levo=Levofloxacin; Oflox=Ofloxacin; PAS=Para amino salicyclic acid; Clarithro=Clarithromycin

Table 2. Final outcome of the Patients

Outcome at the end of treatment	MDR (n=99)	Non-MDR (n=183)	P value
Dead	48	45	P<0.0001
Cure	43	132	P=.005
Treatment failure*	8	11	
Emergence of drug resistance to FLDs*	11	15	
Emergence of drug resistance to SLDs*	9	12	
Genotype change	2	4	

*Difference among groups is insignifican

Table 3. Change in DST pattern and MIRU-VNTR genotypes in serial isolates of MDR *M. tuberculosis*

S. no.	DST						MIRU profile												ATT prescribed	Remarks (interpretation)
	S	H	R	E	KM	OFL	4	26	40	10	16	31	2	23	39	20	24	27		
1a	r	R	r	s	s	s	2	2	4	6	5	6	3	8	3	2	2	3	K H Z Ethio	Two changes noted
1b	r	R	r	r	s	r	2	2	4	4	5	6	3	8	1	2	2	3	Cyclo Levo	(new infection)
2a	s	R	r	s	s	s	2	2	2	-	3	6	3	8	3	2	2	3	K H PAS Ethio	Same genotype
2b	r	R	r	r	s	r	2	2	2	-	3	6	3	8	3	2	2	3	Cyclo Levo	(mutation)
3a	s	R	r	r	s	r	2	2	3	6	5	2	2	5	2	1	2	3	K PAS Ethio	Three changes noted
3b	r	R	r	r	r	r	2	1	3	4	5	2	2	5	1	1	2	3	Cyclo Levo Z	(new infection)
4a	r	R	r	s	s	s	2	1	3	6	4	2	2	5	2	1	2	3	K H Z Ethio	Same genotype
4b	r	R	r	r	s	r	2	1	3	6	4	2	2	5	2	1	2	3	Cyclo Levo	(mutation)
5a	s	R	r	s	s	s	2	2	3	-	3	6	3	8	3	2	2	3	K Z Ethio Oflox	Same genotype
5b	r	R	r	r	s	r	2	1	3	-	3	6	3	8	2	2	2	3		(mutation)

6a 6b	s r	R R	r r	s r	s s	s r	2 2	2 1	3 2	2 2	3 3	2 2	5 5	2 2	5 3	3 3	5 5	3 3	K Z Ethio Oflox	Three changes noted (new infection)
7a 7b	r r	R R	r r	s r	-	-	3 3	2 2	3 3	4 4	5 5	2 2	8 8	4 4	2 2	3 3	6 6	3 3	K H Z Ethio Cyclo Levo	Same genotype (mutation)
8a 8b	s r	R R	r r	s r	s r	s s	2 2	2 2	3 3	3 3	3 3	2 2	5 5	2 2	5 5	3 3	5 5	3 3	K H PAS Ethio Cyclo Levo	Same genotype (mutation)
9a 9b	s r	R R	r r	r r	-	-	2 2	2 2	3 3	6 6	4 4	2 2	6 6	2 2	7 7	3 3	5 5	3 3	K PAS Ethio Cyclo Levo Z	Same genotype (mutation)
10a 10b	s r	R R	r r	s r	s r	S s	2 2	6 2	5 3	5 5	4 4	2 2	6 6	1 1	9 7	4 4	5 5	2 2	K PAS Ethio Cyclo Levo Z	Three changes noted (new infection)
11a 11b	r r	R R	r r	s r	s s	S r	2 2	2 2	3 3	6 6	4 4	2 2	5 5	3 3	7 7	4 4	5 5	2 2	K H Z Ethio Cyclo Levo	Same genotype (mutation)
12a 12b	r r	R R	r r	r r	s s	s r	2 2	2 2	3 3	3 3	3 3	2 2	6 6	2 2	7 7	3 3	5 5	3 3	K PAS Ethio Cyclo Levo Z	Same genotype (mutation)

DST= Drug susceptibility testing, MIRU= Mycobacterial Interspersed Repetitive Units, ATT=Antitubercular treatment, S=Streptomycin; H=Isoniazid; R=Rifampicin; E=Ethambutol; Z=Pyrazinamide; K=Kanamycin; Ethio=Ethionamide; Cyclo=Cycloserine; Levo=Levofloxacin; Oflox=Ofloxacin; PAS=Para amino salicyclic acid

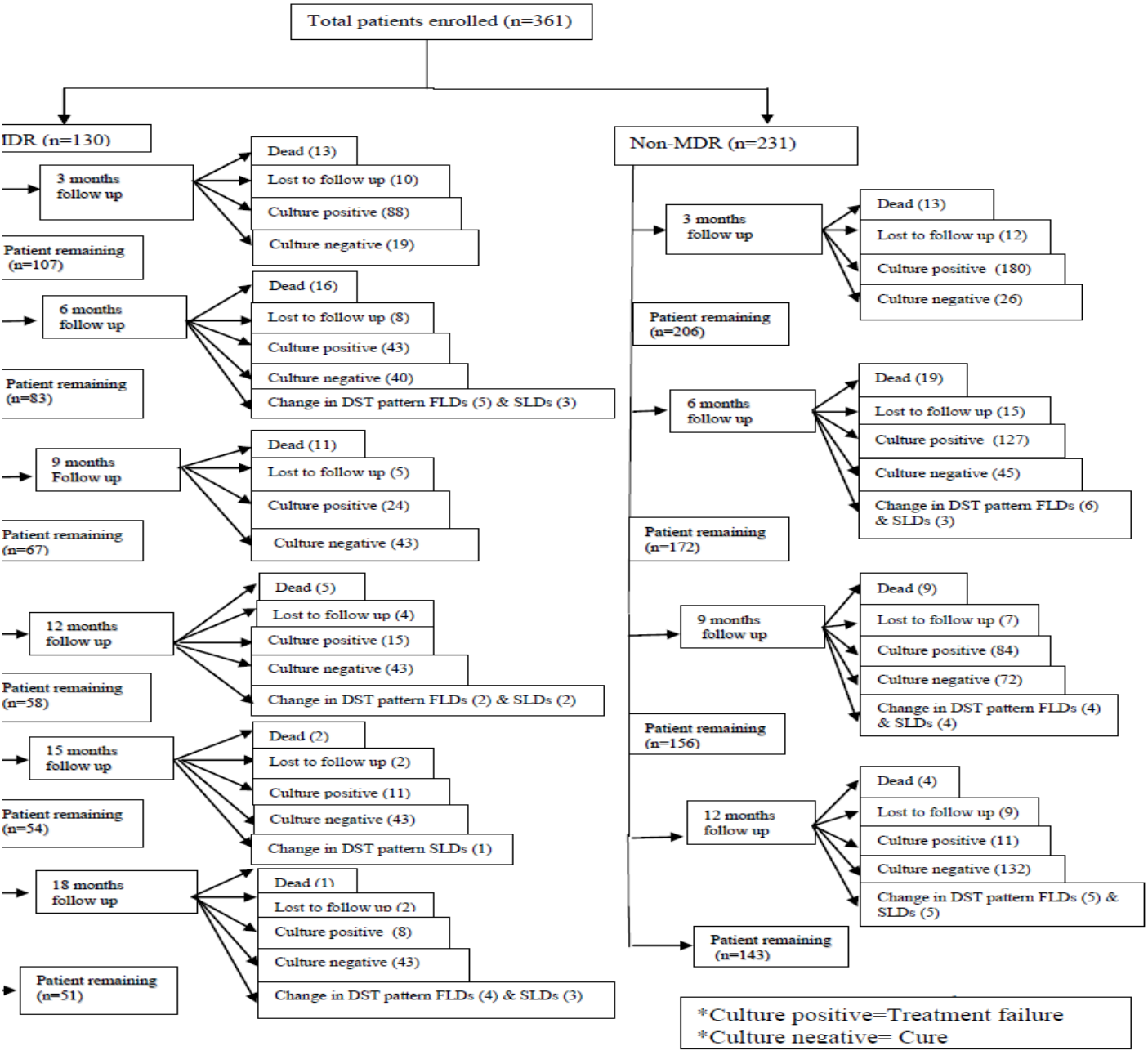
Table 4: Change in DST pattern and MIRU-VNTR genotypes in serial isolates of non-MDR *M. tuberculosis*

S. no.	DST						MIRU profile												ATT prescribed	Remarks (interpretation)
	S	H	R	E	KM	OFL	4	26	40	10	16	31	2	23	39	20	24	27		
1a	S	s	s	s	s	s	2	2	3	2	3	2	5	2	5	3	5	3	RHE	Same genotype
1b	R	r	r	r	s	r	2	2	3	2	3	2	5	2	5	3	5	3		(mutation)
2a	S	r	s	r	s	s	2	2	3	5	4	2	6	1	7	4	5	2	S H R E Z	Same genotype
2b	R	r	r	r	s	r	2	2	3	5	4	2	6	1	7	4	5	2		(mutation)
3a	S	s	s	s	s	s	2	5	4	6	4	2	7	2	1	4	6	4	S H R E Z Levo	Same genotype
3b	R	r	r	r	s	r	2	5	4	6	4	2	7	2	1	4	6	4		(mutation)
4a	S	s	s	s	s	s	2	2	3	5	4	2	6	1	7	4	5	2	K PAS Ethio Cyclo Levo	Four changes noted
4b	R	r	r	r	r	r	2	5	4	5	4	3	6	1	5	4	5	2	Z	(new infection)
5a	R	r	s	s	s	s	2	2	3	2	6	1	6	2	5	3	2	3	RHE	Same genotype
5b	r	r	r	r	s	r	2	2	3	2	6	1	6	2	5	3	2	3		(mutation)
6a	S	r	s	s	s	s	2	4	3	4	2	2	5	2	2	3	5	3	S H R E Z Levo	Same genotype
6b	s	r	r	s	s	r	2	4	3	4	2	2	5	2	2	3	5	3		(mutation)
7a	S	r	s	s	s	s	1	2	4	4	3	2	6	1	5	3	6	2	S H R E Z Levo	Same genotype
7b	S	r	r	s	r	s	1	2	4	4	3	2	6	1	5	3	6	2		(mutation)
8a	S	r	s	r	s	s	2	2	5	3	2	2	6	1	3	3	3	2	K PAS Ethio Cyclo Levo	Same genotype
8b	r	r	s	r	r	s	2	2	5	3	2	2	6	1	3	3	3	2	Z	(mutation)
9a	S	s	s	s	r	s	3	2	3	4	5	2	8	4	2	3	6	3	K PAS Ethio Cyclo Levo	Same genotype
9b	R	r	r	r	r	r	3	2	3	4	5	2	8	4	2	3	6	3	Z	(mutation)
10a	S	s	s	s	-	-	2	2	3	2	3	2	5	2	5	3	5	3	S H R E Z Levo	Same genotype
10b	R	r	r	r	-	-	2	2	3	2	3	2	5	2	5	3	5	3		(mutation)

11a 11b	S R	r r	s r	r r	s s	s r	2 2	2 2	3 3	6 6	3 3	2 2	5 5	2 2	5 5	3 3	5 5	3 3	S H R E Z	Same genotype (mutation)
12a 12b	R R	r r	s r	s r	s s	s r	1 1	2 3	3 4	6 5	4 4	2 2	6 6	2 2	5 5	4 4	6 6	3 3	S H R E Z Levo	Three changes noted (new infection)
13a 13b	S R	s r	s r	s r	- -	- -	2 2	2 2	3 3	6 6	3 3	6 6	3 3	8 8	3 3	2 2	2 2	3 3	S H R E Z Levo	Same genotype (mutation)
14a 14b	S R	s r	s r	s r	- -	- -	2 2	2 2	3 3	5 5	4 4	2 2	6 6	1 1	7 7	4 4	5 5	2 2	S H R E Z Levo	Same genotype (mutation)
15a 15b	S R	s r	s r	s r	s s	s r	2 2	2 2	3 3	5 5	3 3	2 2	5 5	2 2	5 5	2 2	5 5	3 3	S H R E Z Levo	Same genotype (mutation)

DST= Drug susceptibility testing, MIRU= Mycobacterial Interspersed Repetitive Units, ATT=Antitubercular treatment, S=Streptomycin; H=Isoniazid; R=Rifampicin; E=Ethambutol; Z=Pyrazinamide; K=Kanamycin; Ethio=Ethionamide; Cyclo=Cycloserine; Levo=Levofloxacin; Oflox=Ofloxacin; PAS=Para amino salicyclic acid

Figure 1: Follow up details of MDR and Non-MDR Pulmonary TB patients



change during follow up. Emergences of drug resistance, genotype change and anti-tubercular drugs prescription are detailed in table 1. Serial isolates from 12 (9.2%) MDR (table 3)_and 15 (6.4%) non-MDR patients (table 4)_showed emergence of resistance to first line drugs. Serial isolates from 9 (6.9%) MDR and 12 (5.1%) non-MDR patients showed emergence of resistance to second line drugs. Genotype change was seen in serial isolates from four MDR and two non-MDR patients. Serial isolates from rest of the patients were genetically similar (table 3&4). The treatment prescribed to each of these patients is listed in table 3&4. It is apparent that these patients were not prescribed standard regimens.

Discussion

The present study describes high prevalence of acquired MDR-TB. Most of instances of emerging first line and as well as second line drug resistance is due to acquisition of resistance and only few strains showed change in genotype. Instances of emergence of drug resistance are high and so are the inadequate prescriptions. Adverse outcomes are more often seen in MDR-TB cases than in non-MDR-TB cases.

Majority of cases of MDR or XDR TB develop as a result of acquired resistance[15]. Previous treatment for TB has been identified as an important risk factor for the acquisition of drug-resistant TB[16-17]. In India, several studies reported that the prevalence of MDR-TB in acquired cases is 25%- 57.73%[18-20].

MIRU-VNTR, is a useful method for studying genetic relatedness of *M.tuberculosis*[19, 20], for global epidemiological surveillance of TB because of its high resolution, simplicity, sensitivity, high reproducibility, and easy inter laboratory comparison[21]. Each isolate is typed by the number of copies of repeated units at 12 independent loci scattered throughout the genome. A number of studies have proven that MIRU-VNTR typing is a reliable and reproducible typing method[20]. This method has also been used to gain insight into the mechanisms resulting in changing drug-susceptibility patterns during the course of disease[22].

Contrary to our findings, a study conducted in South Africa, showed changes in the drug resistance patterns due to exogenous re-infection with drug-resistant strains[23]. The emergence of drug resistance during anti tuberculosis therapy is a result of several overlapping factors like, error in TB management such as the use of single drug to treat TB, the addition of a single drug to a failing regimen, the failure to identify preexisting resistance, the initiation of an inadequate primary regimen and variations in bioavailability of anti-TB drugs predispose the patient to the development of MDR-TB[24]. Many new cases of MDR-TB are created each year by physician's errors (drugs, dosing intervals, duration). Poor compliance with treatment is also an important factor in the development of acquired drug resistance[25, 26]. Shortage of drugs has been one of the most common reasons for the inadequacy of the initial anti-TB regimen[27].

Detection and treatment of MDR cases remains a major issue in developing countries mainly due to lack of accredited quality laboratories which are capable of performing DST and knowledge updates of practicing physicians. As a result there is often wrong categorization of patients treated in past with anti-tubercular treatment as MDR-TB cases. Treatment of these cases in unorganized private sector remains an issue where physicians often make prescription mistakes by not adhering to prescribed regimens and prefer designing a tailor made regimen for each patient. When regimens containing second-line drugs are selected or administered incorrectly, MDR-TB isolates acquire additional drug resistance and become even more difficult to cure[15]. In fact, the outbreaks of MDR-TB led to use and misuse of second-line drugs, and even concerns about extensively drug-resistant (XDR) TB have been raised. Irrational use of second-line drugs, like adding fluoroquinolones to a first-line regimen or a failing regimen, is a common practice in countries like India and should be avoided [16]. Due to lack of capacity to diagnose MDR-TB, the number of cases with correct diagnosis and treatment remains low. There is no standardized method of estimating laboratory error[28]. The conventional lab methods require a waiting period of minimum four–six weeks for DST results. It is urgently needed to improve the laboratory infrastructure and establish a nationwide network of quality-assured laboratories capable of carrying out first and second-line DST[29]. Limited availability of effective drugs, the reduced efficacy of second-line drugs, an increased number of adverse reactions to the drugs, the long duration of therapy, toxic and high cost of quality-assured second-line drugs etc. remain other major problems related to emergence of drug resistance[18]. The research is expensive, slow and difficult and requires specialized facilities for handling MTB.

In previous literature, failure rates of MDR-TB patients ranging from 4%–47% among new cases and 21%–50% among retreatment cases[26]. A recent study from Istanbul described that of 142 MDR-TB patients, failure was seen in 10 (7.0%) patients and 14 (9.9%) patients died during treatment[30]. One more study showed that out of 172 MDR-TB patients, 52 (30%) abandoned treatment, 22 (13%) died, three (2%) treatment failure, and 80 (47%) remained disease free without relapse during the follow-up period[31]. Goble et al reported in the United States, of 134 MDR-TB patients the cure rate was only 32%. The treatment outcomes of MDR -TB patients have been less favorable than the treatment outcomes of TB patients whose disease is caused by a pan-susceptible strain, a mono resistant, or a poly resistant strain of *M. tuberculosis*[32-33]. More intense TB-control programmes and periodic drug-resistance surveillance should be instituted for rapid diagnosis and aggressive treatment for favourable outcomes[34].

CONCLUSION

Emergence of drug resistance to first line as well as second line during treatment is an important finding. This mode of developing

TB drug resistance may be a major factor in the rapid rise in the number of MDR and XDR TB cases in India.

Contributors

AJ designed the study and provided the guidance for the data analysis and interpretation. PD did laboratory work, helped in the data analysis and wrote the manuscript. RP, RA, and RG clinically diagnosed the patients. AT enrolled the patients and did the data analysis and interpretation. AJ provided overall guidance for the laboratory work and manuscript preparation. All authors saw and approved the final manuscript.

Conflicts of interest

Nil

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