DOI: 10.7860/JCDR/2015/14024.6793

Original Article

Community Medicine Section

Prevalence of Multidrug Resistant Pulmonary Tuberculosis in North Bihar

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ABSTRACT

Introduction: Multidrug resistant tuberculosis (MDR-TB) is caused by Infection with *Mycobacterium tuberculosis* which is resistant to both isoniazid (INH) and rifampicin (RIF), with or without any other anti tubercular drug. It is caused by resistant mutant strains due to inadequate treatment and poor compliance. Due to time taking conventional diagnostic methods, drug resistant strains continue to spread. Therefore rapid diagnosis and treatment of MDR-TB strains are prerequisites for the worldwide fight against TB.

Objective: To determine the prevalence of MDR TB in North Bihar by molecular diagnostic method and to facilitate early diagnosis and treatment. Also, to find out the number of those diagnosed cases who were successfully initiated the treatment in MDR TB Centre of DMCH.

Materials and Methods: This six month observational study was carried out in IRL Darbhanga, Damien TB research Centre of the Darbhanga Medical College and Hospital, Bihar, India. During the period of February-July 2014, 256 sputum samples were collected from suspected cases of multidrug resistant tuberculosis, from 6 districts of North Bihar around Darbhanga. These samples were subjected to routine microscopy and culture to detect *Mycobacterium tuberculosis*. Positive cases were subjected to drug sensitivity test by a molecular diagnostic method, Using Genotype MTBDR plus kit.

Result: Out of 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 Line Probe Assay (LPA), 39(15%) samples were found to have resistance to both INH and Rifampicin. Male female ratio was 4:1.

Conclusion: The Prevalence of Multi drug resistant pulmonary tuberculosis in North Bihar is 15%. It needs early diagnosis by molecular diagnostic method and prompt treatment to reduce the spread of MDR TB cases.

Keywords: DR TB (Drug Resistant Tuberculosis), INH (Isoniazide), IRL (Intermediate referral laboratory), LPA (Line Probe Assay), Programmatic management of Drug Resistant TB (PMDT)

INTRODUCTION

Multidrug resistant tuberculosis (MDR-TB) is caused by infection due to *Mycobacterium tuberculosis* resistant to Isoniazid and Rifampicin, with or without resistance to other antitubercular drugs [1]. Due to inadequate treatment and poor compliance of the patients, mutant resistant strains are developed which causes multidrug resistant tuberculosis [2]. The most important risk factor for the development of MDR-TB is previous anti-tuberculosis therapy [3].

In 2007, global burden of tuberculosis was reviewed and it was found that there were 500000 cases of MDR TB reported from high burden countries. Among these cases of MDR TB, number of cases reported from India were 131,000 cases from China were 112000, from Russia 43000, from South Africa 16000 and 15000 cases were from Bangladesh [4].

In India MDR TB cases were estimated in 3 different states. It was found that 35.7% cases of MDR TB cases were found from Varanasi, Uttar Pradesh, 66.6% from Sawai Madhopur, Rajasthan, and 43.8% from Buxar, Bihar [5]. Since 2006, PMDT (Programmatic management of drug resistant tuberculosis) is implemented in India in phased manner. Approximately 99,000 cases of MDR TB are estimated to occur per year [6].

The cause of drug resistant tuberculosis is loss of Sensitivity to drugs due to genetic mutation.

Spontaneous mutation causing resistance to INH occurs in about 1 in 10^6 replications and Rifampicin resistance occur in about 1 in 10^8 replications. Therefore spontaneous mutation causing resistance to both INH and Rifampicin may occur once in 10^{14} replications [7].

Detection of Mycobacteria and drug sensitivity by conventional method takes long time, about 12 weeks. Due to delay in diagnosis patients undergo inappropriate treatment in the beginning. During this period they develop drug resistant strains. So before starting anti-tubercular drugs, identification of drug resistant strains by rapid diagnostic method is needed to cure the patients and to prevent spread of MDR TB.

Now-a-days a rapid and sensitive molecular diagnostic method, known as LPA (Line Probe Assay) is available, which can be done by a diagnostic kit, known as Genotype MTBDR plus kit. It can detect mutated genes present in Mycobacteria causing resistance to INH or Rifampicin or both. This kit gives rapid result by using sputum or culture material. We can get the result within 5 hours in comparison to conventional method which takes 6-8 weeks. Thus it helps in early diagnosis and appropriate treatment within a short period. Thus transmission and spread of MDR TB can be reduced [8].

Considering all these things an IRL (Intermediate referral laboratory) has been established in TBDC Centre of Darbhanga Medical College with the help of Govt. and Non Govt. organization, where sputum samples are collected from all suspected cases of MDR TB of nearby district of north Bihar. Diagnosis of MDR TB is done by LPA using Genotype MTBDR plus kit. Diagnosed cases of MDR TB cases are admitted in MDR TB ward and are treated with appropriate medication under the supervision of the medical officer and other paramedical staffs appointed for that purpose.

OBJECTIVE

To determine the prevalence of MDR TB in North Bihar by molecular diagnostic method, to facilitate early diagnosis and treatment. Also to find out the number of those diagnosed cases who were successfully initiated the treatment in MDR TB Centre of DMCH.

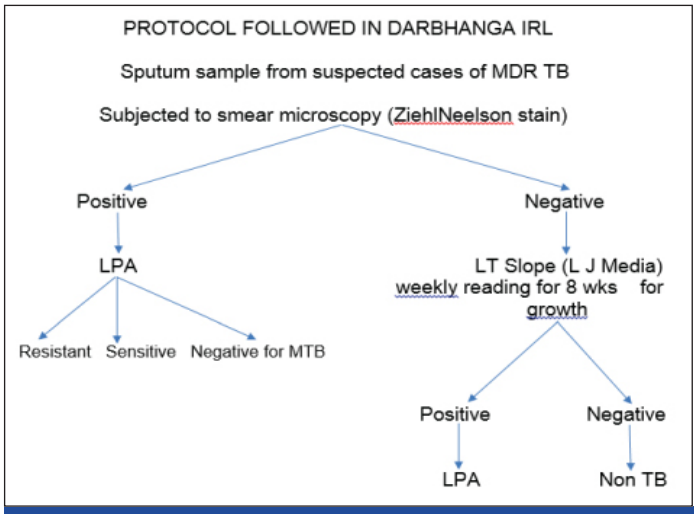
MATERIALS AND METHODS

This six month observational study (February-July, 2014) was carried out in IRL Darbhanga, Damien TB Research Centre of the Darbhanga Medical College and Hospital, Bihar. Approval was received from ethical committee of DMCH. Informed consent was taken.

Inclusion Criteria

All treatment failures of new cases smear positive treated cases, who continue to be smear positive after 4 months and all pulmonary TB cases who are contact of known MDR TB cases.

Total 256 sputum samples were received in IRL, Darbhanga from suspected cases of MDR TB according to the above criteria. Sputum samples were collected from patients of six different districts of North Bihar like-Darbhanga, Madhubani, Madhepura, Saharsa, Samastipur and Supoul.



[Table/Fig-1]: Flow chart showing the Protocol followed in our laboratory

Each sample was smeared, air-dried, fixed and stained with Zeihl Neelsen (Z-N) reagents. All sputum microscopy positive samples were subjected to Line Probe Assay (LPA) by Genotype MTBDR plus kit, where as microscopy negative samples were subjected to culture by LJ media. Culture was observed for 8 weeks and weekly reading was done. If culture became positive then they were subjected to LPA to know their drug sensitivity by Genotype MTBDR plus kit (HAIN Lifescience, GmbH, Germany) [9]. Then resistance to INH or Rifampicin or both could be determined by comparing the strip given along with the diagnostic kit. The whole protocol of diagnostic process which is followed in IRL Darbhanga, is shown through the flow chart in [Table/Fig-1].

The whole procedure was divided into three parts: 1) DNA extraction; 2) PCR amplification and; 3) Hybridization . We have used MTBDR plus kit for the present study and followed the instructions given by the manufacturer of the kit while performing each of the above mentioned procedures [9].

For diagnosis of MDR TB, 2 sputum samples, one early morning and another spot sputum sample, were collected in Falcon tube at the district level and were sent to TBDC Centre of Darbhanga by human carriers, from where samples were brought to IRL Darbhanga for detection and confirmation of MTB and for its drug sensitivity through the process shown above. Finally cases with drug resistance to Rifampicin or INH or resistance both were identified. Reports were sent to concerned district level. Then patients were sent from different district level to Darbhanga Medical College for their treatment in MDR TB Ward, under supervision of senior medical officer under DRTB centre.

RESULTS

In present study, 256 sputum samples were received in IRL Darbhanga from suspected cases of MDR from six districts of north Bihar, including Darbhanga district, over 6 months period from Feb-July 2014. Age of the patients was ranging from 7 to 68 years and male-female ratio was 4:1.

Out of 256 sputum samples, 39 (15%) samples were found to have MDR Tuberculosis i.e. resistance to both INH and Rifampicin. Among 39 cases of MDR TB majority of patients 33 (85%) belong to the age group of 16-45 years which is the most productive period of a human being. Out of these 33 cases, maximum patients 23 (59%) were in the age group of 16-30 years. Three patients (8%) were in 0-15 years age and only one (3%) patient was over the age of 60 years, shown in [Table/Fig-2].

While analysing sex distribution, there were 31(79%) males and 8 (21%) were females with MDR TB. Thus male- female ratio was approximately 4:1. Out of 31 male patients, majority, 22 (71%) were in the age group of 16-30 years. Among 8 females, majority i.e., 3 (38%) were in the age group of 31-45 years as shown in [Table/Fig-2].

In the present study, out of 256 sputum samples, 122 cases were microscopy positive for tuberculosis. Among these 122 cases, tuberculosis was confirmed by PCR in 114 cases. Thus the number and proportion of cases who undergone molecular diagnostic method for drug resistance were 114 & 45% respectively. Finally on drug sensitivity testing by Genotype MTBDR pluskit, 39 samples were found to have resistance to both INH and Rifampicin as shown In lab result [Table/Fig-3].

AGE RANGE	NUMBER	%	MALE	%	FEMALE	%
0-15	3	8	1	3	2	25
16-30	23	59	22	71	1	12
31-45	10	26	7	23	3	38
46-60	2	5	0	0	2	25
61-75	1	2	1	3	0	0
TOTAL	39	100	31	100	8	100

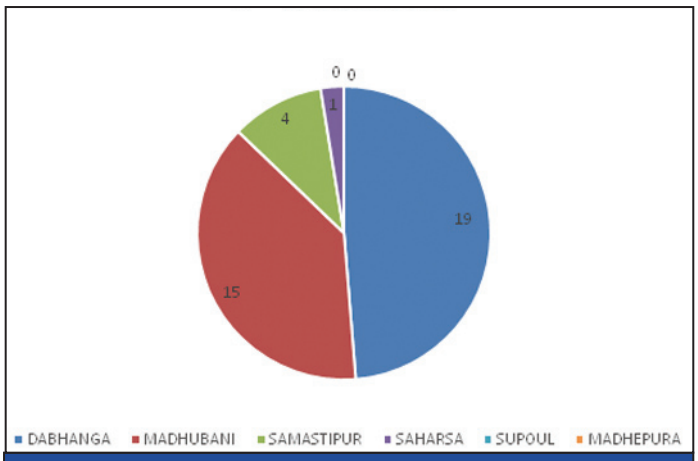
[Table/Fig-2]: Age and sex distribution of mdr tb cases

Month	No of Sample Received	Microscopy +ve	TB Detected by LPA	No of samples found resistant to INH & RIF both (By Genotype MTBDR plus Kit)
FEB	17	11	11	3
MAR	27	11	11	2
APR	42	18	18	3
MAY	24	6	6	4
JUNE	83	40	34	9
JULY	63	36	34	18
Total	256	122	114	39

[Table/Fig-3]: Lab result

MONTH	NO OF SAMPLE RECEIVED	NO AND PERCENTAGE OF MDR TB CASES	
		No	%
FEB	17	3	18
MAR	27	2	8
APR	42	3	7
MAY	24	4	17
JUNE	83	9	11
JULY	63	18	29

[Table/Fig-4]: Number and Percentage of cases diagnosed to be MDR PTB, Month (1) wise



[Table/Fig-5]: Pie Chart showing number of MDR TB CASES district wise

NAME OF DISTRICT	No of MDR TB CASES	% of MDR TB cases
DABHANGA	19	49
MADHUBANI	15	38
SAMASTIPUR	04	10
SAHARSA	01	3
SUPOUL	0	0
MADHEPURA	0	0
TOTAL	39	100

[Table/Fig-6]: Distribution of Number and Percentage of MDR TB cases district wise

Out of 256 sample, maximum number of sputum samples 83 (32%) were received in the month of June 2014, but maximum number, i.e., 18 (46%) of samples which were found to be of MDR TB, were received in July 2014 which can be seen from [Table/Fig-4], (month wise distribution)

While analysing distribution of patients district wise, we observed that maximum number 19 (49%) of MDRTB cases were from Darbhanga district and 15cases (38%) were from Madhubani, where as 4 cases (10%) were from samastipur and only 1 case (3%) was from saharsa as represented in pie chart [Table/Fig-5]. Multi drug resistant tuberculosis was not found in any sample received from Supoul and Madhepura. Percentage wise distribution of cases in different district is shown in [Table/Fig-6].

While analysing Mono-drug resistance, it was found that, out of 114 cases of tuberculosis, 27 (24%) were having resistance to single drug. Out of these 27 cases of mono resistance, there were 16 (59%) cases resistant to INH and 11 cases (41%) were resistant to Rifampicin only.

DISCUSSION

Tuberculosis (TB) is responsible for a large portion of morbidity and mortality worldwide. According to a WHO report, tuberculosis is responsible for at least 2 million deaths per year, 90% of these occurring in developing countries [10,11] Recently, it has been shown that multidrug-resistance (MDR) and extensively drug resistant tuberculosis (XDR-TB) are the most important factors resulting in death of patients with tuberculosis [12,13].

Over the counter availability of anti-tuberculosis drugs associated with indiscriminate use, inappropriate combinations by practitioners not well-versed in the proper use of drug therapy in tuberculosis, irregular supply of drug, poor case detection rate, and stoppage of drugs due to side effects of drugs also aggravated the problem of drug resistance [14].

Prevalence of MDR TB in North Bihar in and around Darbhanga, as determined by present study conducted in TBDC Centre of Darbhanga medical college and hospital, Bihar is 15%. Study conducted by Ramachandran, reported the prevalence of MDR TB in Gujarat was 17.4% in previously treated cases and only 2.4% in new cases. Thus our study is comparable to the study conducted by Ramachandran et al., [15]. In the study conducted by Vasanthakumari et al., in Tamilnadu out of 782 cases studied, 162 were found to be bacteriologically positive. Thirty three (20.3%) of these 162 cases were found to be resistant to both isoniazid and rifampicin [16].

When present study was compared with some international studies, as in Karachi, Pakistan, prevalence of MDR TB in treated cases of pulmonary tuberculosis was higher than us, i.e., 17.9% [17] (Ezaj et al.,), Where as our prevalence of MDR TB is 15.23%. In a report from Southeast of Iran, where the 16% of patients with PTB had MDR TB [18], this is comparable to the present study.

In North India, Delhi, prevalence of MDR TB is 33.7% and in South India it is 23.3% [19]. In both the places prevalence is higher than North Bihar, as found in present study. Difference in prevalence of MDR TB in urban/rural area has been reported by some authors. Almeida D et al., has reported that higher percentage (51%) of MDR TB was found in urban area (Mumbai) in comparison to a rural area, i.e., 2% in sakawar [20]. Similar result were noted in the present study, in Darbhanga (urban) MDR TB cases were higher (49%) in comparison to 3% in Saharsa (rural).

As per the result of present study 16 (59.25%) cases were resistant to INH and 11 cases (40.74%) were resistant to Rifampicin only. According to Ramachandran et al., (37%) cases had INH resistance. They have not reported any resistance to Rifampicin alone [14].

In present study almost 71% cases of MDR TB were young, belonging to 16-30 years of age. Dholakia et al., conducted a study in Mumbai where 67% of MDR TB cases were young (15-35 years) compared to Andhra Pradesh (Chadha SS et al.,) where 44% suspected cases were young patients [21,22].

Now-a-days prevalence of MDRTB is increasing because of increased awareness of the disease, availability of diagnostic procedures like culture and drug sensitivity testing and earlier suspicion of MDR tuberculosis in previously treated patients [23].

Present study also showed that number of sputum samples from suspected cases of MDR TB received in TBDC Centre increased from 17, in Feb-2014 to 63 in July 2014. Similarly number of cases diagnosed to have MDR TB was 3 in Feb 2014 which had increased to 18 in July 2014, probably due to increase in awareness between practitioners of these areas and paramedical staffs posted in DOTS Centers at different district level. Therefore they are sending more cases to Darbhanga medical college where early diagnosis could be done by molecular diagnostic method.

This study found that maximum number 19 (48.7%) case of MDRTB were from Darbhanga district, where early diagnostic facility is available and 38% were from Madhubani, whereas much less, i.e., 10% and 2% cases were from samastipur and saharsa respectively. The reason behind it may be, because this molecular diagnostic method available in IRL is situated in Darbhanga, which is within the reach of the patients and human carriers of darbhanga and madhubani, who bring the samples from various district levels, in comparison to those human carriers appointed in Saharsa and Samastipur. Therefore, it needs rapid diagnosis by molecular method in other regions of Bihar also, to facilitate early treatment and to prevent the spread of multi drug resistant pulmonary tuberculosis .

Previously MDR TB cases were sent to Delhi for their treatment, but now DRTB ward is established in Darbhanga medical college. Therefore all the cases (100%) of multidrug resistant TB diagnosed in DMCH were treated here only.

CONCLUSION

Prevalence of Multidrug resistant tuberculosis in North Bihar is 15%. All (100%) of diagnosed MDR TB cases were initiated treatment in DR TB ward of Darbhanga medical college and hospital. It is high

time to establish modern laboratories with molecular diagnostic facility in each district to diagnose MDR TB in early stage to prevent spread of MDRTB.

REFERENCES

[1] Sharma SK, Mohan A. Multidrug-resistant tuberculosis. *Indian J Med Res*. 2004;120:354-76.
[2] Andrews JR, Gandhi NR, Moodley P, Shah NS, Sturm AW, et al. Exogenous Reinfection as a Cause of Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis in Rural South Africa. *The Journal of Infectious Diseases*. 2008;198(11):1582-89.
[3] Sharma SK, Kumar S, Saha PK, George N, Arora SK, Gupta D, et al. Prevalence of multidrug-resistant tuberculosis among Category II pulmonary tuberculosis patients. *Indian J Med Res*. 2011;133:312-15.
[4] Donald PR, Van Helden PD. The Global Burden of Tuberculosis — Combating Drug Resistance in Difficult *Times N Engl J Med*. 2009;360:2393-95.
[5] Mathuria JP, Samaria JK, Srivastava GN, Mathuria BL, Ojha SK, Anupurba S, et al. Primary and acquired drug resistance patterns of Mycobacterium tuberculosis isolates in India: a multicenter study. *J Infect Public Health*. 2013;6:456-64.
[6] TB India 2011 Revised National TB Control Program Annual Status Report. From: http://www.tbcindia.nic.in/pdfs/RNTCP%20TB%20India%202011.
[7] David HL. Probability distribution of drug-resistant mutants in unselected populations of *Mycobacterium tuberculosis. Appl Microbiol*. 1970;20:810-14.
[8] Barnard M, Parsons L, Miotto P, Cirillo D, Feldmann K, Gutierrez C, et al. Molecular Detection of Drug-Resistant Tuberculosis by Line Probe Assay. *Foundation for New Diagnostics (FIND)*. 2012;1:6-10.
[9] HAIN Lifescience GmbH. Rapid diagnosis of tuberculosis, mycobacteriosis and Leprosy. www.hain-lifescience.de
[10] Pablos MA, Raviglione MC, Laszlo A, Binkin N, Rieder HL, Bustreo F, et al. Global surveillance for antituberculosis-drug resistance. *New Engl J Med*. 1998;338:1641-49.
[11] Metanat M, Mood BS, Shahreki S, Dawoudi SH. Prevalence of Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis in patients with Pulmonary Tuberculosis in Zahedan, Southeastern Iran. *Iran Red Crescent Med J*. 2012;14(1):1-3.
[12] Petrini B, Hoffner S. Drug-resistant and multidrug-resistant tubercle bacilli. *Int J Antimicrob Agents*. 1999;13:93-97.
[13] Pillay M, Sturm AW. Evolution of the extensively drug-resistant F15/LAM4/ KZN strain of Mycobacterium tuberculosis in KwaZulu-Natal, SouthAfrica. *Clin Infect Dis*. 2007;45:1409-14.
[14] Agarwal AK, Chugh IM, Shah A. Multidrug resistant tuberculosis: successful treatment with an unconventional regimen. *Ind J Tub*. 1998;45:227.
[15] Ramachandran R, Nalini S, Chandrasekar V, Dave PV, Sanghvi AS, Wares F, et al. Surveillance of drug-resistant tuberculosis in the state of Gujarat, India. *Int J Tuberc Lung Dis*. 2009;13(9):1154-60.
[16] Vasanthakumari R, Jagannath K. Multidrug Resistant Tuberculosis – A Tamilnadu Study. *LUNG INDIA*. 1997;15(4):178-80.
[17] Ejaz M, Siddiqui A R, Rafiq Y, Malik F, Channa A, Mangi R, et al . Prevalence of multi-drug resistant tuberculosis in Karachi, Pakistan: Identification of at risk groups. *Trans R Soc Trop Med Hyg*. 2010;104(8):511-17.
[18] Naserpour T, Naderi M, Mohagheghefard FA H, Sharifi-Moud B, Rezai E. Drug Resistance of Mycobacterium Tuberculosis Strains Isolated from Patients with Pulmonary Tuberculosis in South Eastern of Iran. *Journal of Medical Sciences*. 2006;6:321-24.
[19] Takkar J, Grover GS. Recent Advances in Multi-Drug-Resistant Tuberculosis and RNTCP. *Indian J Community Med*. 2008;33(4):219–23.
[20] Almeida D, Rodrigues C, Udwadia ZF, Lalvani A, Gothi GD, Mehta P, et al. Incidence of Multidrug-ResistantTuberculosis in Urban and Rural India and Implications for Prevention. *Clinical Infectious Diseases*. 2003;36:e152–54.
[21] Dholakia YN, Shah DP. Clinical profile and treatment outcomes of drug-resistant tuberculosis before directly observed treatment strategy plus: Lessons for the program. *Lung India*. 2013;30(4):316-20.
[22] Chadha SS, Sharath BN, Reddy K, Jaju J, Vishnu PH, Rao S, et al. Operational challenges in diagnosing multi-drug resistant TB and initiating treatment in Andhra Pradesh, India. *PLoS One*. 2011;6:e26659
[23] Sarkar S, Maity GN, Mukhopadhyay KK, Acharyya B, Ghoshal AG. Primary multidrug resistant tuberculosis. *Lung India*. 2007;24:97-99.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 16, 2015**
Date of Peer Review: **Jun 01, 2015**
Date of Acceptance: **Sep 14, 2015**
Date of Publishing: **Nov 01, 2015**