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Drug Resistance Patterns of *Mycobacterium tuberculosis* – Isolates from Indore, India

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PS and RD designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author ISB managed the literature searches, analyses of the study performed for diagnosis by authors SN and VK to identify the species of infecting Mycobacterium. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Tuberculosis (TB) is an Infectious disease existing pandemically in our world.In parallel, the prevalence of multidrug-resistant tuberculosis (MDR-TB) is also increasing. The TB control programs were not successful due to the emergence of multidrug resistance in *M. tuberculosis* strains. Objective of the present study was to detect the rate of MDR-MTB in the central state of India.
Materials and Methods: The study included all new & old cases of pulmonary & extra pulmonary

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tuberculosis enrolled between January 2013 and December 2014 carried out in our Indore, lab, India. All the patients' samples found to be TB positive in our lab were tested by Mycobacterium culture and MTB isolated strains were put for first-line drug-susceptibility testing (DST). MDR-TB was defined as TB caused by bacilli showing resistance to at least isoniazid and rifampicin.

Results: A total of 60 MTB isolated strains; fourteen were MDRs (23.3%). Resistance to INH, RIF, PYRA, ETHAM,streptomycin (STREPTO) was found to be 41%, 36.6%, 23.3%, 30%, 25% respectively.

Conclusions: MDR-TB prevalence was found to be high among both new & old cases of pulmonary & extra pulmonary tuberculosis cases. Nation-wide and State-wide representative data on prevalence of MDR-TB are lacking in the state of Madhya Pradesh, in India. Efforts needs to be directed towards further continuous surveillance for MDR-TB among newly diagnosed TB cases and old diagnosed cases.

Keywords: MDR TB; pulmonary; extra pulmonary Madhya Pradesh India.

ABBREVIATIONS

TB: Tuberculosis; MDR: Multi Drug Resistance; INH: Isoniazide; RIF: Rifampicin; PYRA: Pyrazinamide; ETHAM: Ethambutol; STREPTO: Streptomycin; WHO: World Health Organization; MOTT: Mycobacteria Other Then Tuberculosis.

1. INTRODUCTION

Tuberculosis is a common, though preventable infectious disease of the world. It is known that India accounts for about 30 per cent of the total global TB burden [1] Currently, tuberculosis management and control is potentially devastating threat worldwide due to Did not profve to be successful though the therapy. Thus lead to formation of drug resistance bacteria spread in population there by entitling MTB Strains as manmade development of resistance as a consequence of suboptimal regimens and treatment interruptions by patients themselves [2].

Drug resistance in TB is classified in group of primary or that of acquired type when drug resistance is demonstrated in a patient, who has never received anti-TB treatment previously, it is termed primary resistance. Acquired resistance is that which occurs as a result of specific previous treatment [3].

Resistant to at least two major anti tuberculosis drugs; INH and RIF with or without resistance to other anti-TB drugs has been termed MDR-TB. MDR-TB is more difficult to treat than drug susceptible TB, requiring the use of less effective second line anti tubercular drugs which are often associated with major side effects [4].

Recently, WHO has estimated that around 3.7% of new TB cases are developed as MDRs. MDR-TB global average rate is 20%. About 9% of these cases also are resistant to at least one injectable second line antitubercular drugs. Extensively drug resistant (XDR) TB cases as reported earlier [5].

Major concern over drug resistance is a fear of spread of drug resistant organisms and ineffectiveness of chemotherapy in patients infected with them. The distribution and rate of MDR and XDR-TB are not uniform and vary in different places, regions, populations and countries. Limited data are available on the trends of prevalence of drug resistance in Indore region and therefore the find existence of Drug Resistant Mycobacterium. In our city in the Central State of India during the years 2013-2014.

2. MATERIAL AND METHODS

2.1 Study Setting

This study was done at Central Lab Onquest, Indore (M.P.), which is a private NABL accredited lab; the data presented are from January 2013 to December 2014.

We tested *a* total of 440 samples from all the age groups of clinical TB suspects of TB suspects from higher socioeconomic structure capable to efford private lab Indore & its surrounding region. Patient demographic data like age, gender, address were obtained. We received &processed all the pulmonary or extra

pulmonary samples. As our lab is private lab, some of the data some samples are submitted without prior history of patient. so we received direct samples in our lab and status of all patients whether new or old treated cases are not received in all cases. The patient samples were studied by viewing morphology using staining and under microscope, culture, along with culturing them identification and Drug Sensitivity testing (D.S.T). All tests were performed using patient's consentunder Medical supervision, according to guidelines for Good Clinical laboratoy practice (GCLP) in our NABL (National board for accreditation for testing and calibration for laboratory.

Samples obtained in the laboratory in a cold box and were processed on the same day or were kept at +4 degrees Celcius. in refrigerator, until their processing was done. The tissue samples were first decontaminated using N-Acetyl-L-Cysteine, 2% Sodium hydroxide and sodium citrate, PBS (pH 6.8). After they were decontaminated the smears were stained by Ziehl-Neelsen method and examined by trained technical staff for acid fast bacilli. and cultures were done on the Lowenstein -Jensen (LJ) egg based medium were carried out as per the standard methods.

All sample were processed according as instructed (Hi-Media) on the readymade Lowenstein-Jensen (LJ) medium (Hi-Media) and incubated for 8 weeks. All the isolates were identified as *M. tuberculosis* by their slow growth rate, colony morphology, inability to grow on L-J media containing p-nitrobenzoic acid (PNB), niacin and catalase test [6-8] DST was carried out on ready prepared Hi-media LJ medium first line drug kit according to kit information (Proportion method). First line drug include INH, RIF, PYRA, ETHAM and STREPTO. MDR-TB was defined as TB caused by bacilli showing resistance to at least INH and RIF. An un-inoculated tube of LJ medium was used as negative control and *M. tuberculosis* A.T.C.C. H37Rv was used as positive control.

3. RESULTS

We found Out that of small number of test samples of 440 samples, relatively small number, 60 showed growth of *Mycobacterium tuberculosis* isolates whereas 3 were identified as atypical mycobacteria (MOTT).

Fig. 1. The geographical representation of city Indore in Central state of India which shares borders with state Uttar Pradesh to the north-east state, the state Chhattisgarh, south-east, state Maharashtra and in south, with state Gujarat.

Fig. 1. Geographical representationof largest city Indore in of Central state (Madhya Pradesh) of India

Table 1 shows distribution of various samples types, from which growth of *Mycobacteium* was found.

Among 60 isolates in our lab, 33 were from male patients and 27 were from female patients. Maximum isolates were found in the age group 30-40 years followed by 40–50 years as shown in the Table 2.

Table 1. Sample wise distribution of the isolates

S. no	Type of sample	Sample	Total number (n=60)	Percentage positive (%)
1	Pulmonary	Sputum	30	50
2	Pulmonary	Broncho alveolar lavage	21	35
3	Extra pulmonary	Pus	6	10
4	Extra pulmonary	Peritoneal fluid	1	1.66
5	Extra pulmonary	Pleural fluid	1	1.66
6	Extra pulmonary	Lymph node	1	1.66

Table 3 shows percentage of resistance to various first line anti-tuberculosis drugs. Resistance to INH, RIF, PYRA, ETHAM, STREPTO was found to be 41.6%, 36.6%, 23.3%, 30%, 25% respectively.

Table 4 shows the resistance pattern of 60 drug resistant isolates not in any combinations of other drug. Single drug resistant was found in 18.33% of isolates, two drug resistant in 13.33%, three drug resistant in 10 %, and four drugs resistant in 6.66 % of isolates. MDR strains were found in 23.33% [9] of the isolates.

Among Mono resistance Isoniazid was found to be highest (41%) followed by RIF(36.66%) and in multiresistant strains. The highest proportion of drug resistance was found in *Mycobacteria* withusage of INH + RIF drug combination (5%).

Table 2. Age groups distribution of the isolates

Age groups (Years)	Number
1-20	3
21–30	10
31–40	30
41-50	15
51-60	8
>60	4
Total	60

Table 3. Sensitivity pattern of MTB to antitubercular (ATT) drugs

Drugs	Total number of resistant isolates
INH	25 (41.6%)
RIF	22 (36.66%)
PYRA	14 (23.3%)
ETHAM	18(30%)
STREPTO	15 (25%)

14 (23.3%) isolates were found to be MDR (i.e. Isoniazid and Rifampicin Resistant

4. DISCUSSION

Increasing drug resistance to commonly used drugs Rifampicin (RIF) and INH in isolates of *M. tuberculosis* is a major cause of concern. Because of these trends it is important to know the rate of primary and acquired drug resistance in an area at regular intervals.

Drug resistant tuberculosis is either acquired due to poor management of treatment or transmission from infectious drug resistant TB patients. As found in many other studies history of antitubercular treatment has been consistently associated with risk of MDR-TB [10].

Table 4. Resistance pattern of different patterns of 60 drug resistant strains of MTB to ATT drugs along with additional drug resistance in isolates

Number of drugs	Name of drugs	No. of resistant strains (%)
1 Drug	INH	04 (6.66%)
	RIF	04 (6.66%)
	ETHAM	01 (1.66%)
	PYRA	01 (1.66%)
	STREPTO	01 (1.66%)
2 Drug	INH + RIF	03 (5%)
	INH+PYRA	01 (1.66%)
	INH +STREPTO	02 (3.33%)
	RIF+EMB	01 (1.66%)
	RIF + STREPTO	01 (1.66%)
3 Drug	INH + RIF + ETHAM	01 (1.66%)
	INH + RIF + PYRA	01 (1.66%)
	RIF + ETHAM+ PYRA	01 (1.66%)
	RIF + EMB + STREPTO	01 (1.66%)
	RIF + PYRA + STREPTO	02 (3.33%)
4 Drug	INH + RIF + EMB + PYRA	03 (5%)
MDR TB		14

Overall MDR rate observed in this study is 14 (23.3%). Our findings are concordant with other studies reported from Chandigarh (27.6%) (10), of Tamil Nadu State studied by C. N. paramasivan et al. (25%) [11], D. Lina et al. Mumbai (25.25%) [12], and Ranganath R. et al. *study* in Mysore [13]. But higher rates were observed in in Study done in Dehradun (57.22%) by J. Rawat et al. [14] and DA. Khanna et al. in Delhi 53.6% [15] while the lowest rates were seen in Sewagram Wardha (9.2–9.6%), the study done by P. Narang et al. and N. K. Jain et al. [16,17]. High rate of MDRTB in our Setting may be because we received direct samples in our lab and status of all patients whether new or old treated cases were not known. Similar study was done earlier and reported byRanganath, R [13] MDR isolates.

The highest resistance is seen in Isoniazid (41%), which is the most popular drug for priscription to patients followed by RIF (36.6%),

ETHAM (30%), and STREPTO (25%). Similar resistance pattern was reported by Ranganath et al. from Mysore to INH (31.2%), RIF (28), EMB (17.6%) and STREPTO (21.6%)(13), by Sethi et al. from Chandigarh to INH (46.9%), RIF (27.6), STREPTO (22.22%), and EMB (10%) [9], and by Vijay et al. in Bangalore to INH (27.4%), RIF (15.5%), SM (23%), and EMB (6.6%) [18].

RIF resistance in our study is 36.6%. Resistance for RIF is considered as surrogate marker for detection of MDRTB. Similar findings were reported by Jain et al. from New Delhi (33.3%) [17], Paramasivan et al. from Tamil Nadu (25%) (3), Sethi et al [9]. from Chandigarh (27.6%), in cities of different states in India.

One most important limitation of this study is previous treatment histories, and information about second line drug susceptibility like quinolones were not available being a private lab for analysis, restricted our ability to derive concrete conclusions also including outdoor patients' tests done by our specialized clinicians in our lab. Present data also limits as representative of the whole community and are limited to few hospitals and outdoor registered patients for diagnosis. Our data can be well added to the community based multicenter study, from indore, with other studies including all parts of the country using the full spectrum of drugs, to describe the true prevalence of MDRTB in the state and country.

The pattern of drug resistance differs from place to place and at different periods of time. For a drug regimen to be effective with complete restriction of further growth or developing further resistant variations of *Mycobacterium*, it is important to know the drug resistance pattern of existing and spreading in that area, that needs to be conducted in periodic surveys of antibiotic drug resistance and to establish a continuous drug resistance surveillance program. As several of the studies cited in here including our present, study may have flaws with sampling, carefully planned long term studies with sufficient quality assurance are further required.

5. CONCLUSION

The study reported herewith lacks the detailed information about the previous treatment histories of medical treatment of patient and the information about second line drug susceptibility of *Mycobacterium* in the populations, which were not available for analysis, restricted us to derive concrete conclusions. Other limitations are of data as they are not representative of the whole community and are limited to few hospitals of Indore. A community based multicenter study, which includes all parts of the country and uses the full spectrum of drugs, is needed to further describe the true existence of MDRTB in Country. We herewith show the drug resistance pattern in both new & old cases of pulmonary & extra pulmonary tuberculosis cases in our City. Nation-wide and State-wide representative data on occurrence of MDR-TB are lacking I whole State. Efforts need to be directed towards further continuous surveillance for MDR-TB among newly diagnosed TB cases and old already diagnosed cases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory were followed, according to Indian National accridiation board for testing and Callibration for Testing laboratories (NABL), India with 15189:2007 protocol national laws for laboratories. All work have been examined and approved by the appropriate ethics committee

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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