

Diagnosis of Multidrug Resistant Tuberculosis by MGIT 960 at Tertiary Care Hospital

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ABSTRACT

Introduction: MDR-TB is emerging as life threatening condition and lack of proper and accurate diagnostic tools can have very severe consequences. A study is needed to find the prevalence of MDR-TB using MGIT 960 in this particular area.

Aims and Objectives: To find the prevalence of MDR-TB cases in suspected tuberculosis patients.

Materials And Methods: 107 samples were collected from patients with clinically suspected tuberculosis visiting microbiology department, RIMS, Ranchi, between the period of January 2017 to November 2017. After sample processing was done, it was inoculated in MGIT 960 system for culture. Growth positive samples were tested to differentiate Mycobacterium Tuberculosis Complex from Mycobacterium other than tuberculosis (MOTT) by BD ID strip test. Antimicrobial susceptibility testing was done by SIRE and PZA susceptibility testing.

Results: Among 107 cases, 49 cases were growth positive, out of which 41 were Mycobacterium Tuberculosis Complex (MTbC) and 08 were MOTT. Among the MTbC cases 68.29% were male and 31.70% were female. Among 41 MTbC positive cases 31.71 % were both Isoniazid and Rifampicin resistant (MDR-TB). Maximum resistance was found against Isoniazid, 41.46%. MDR-TB was more associated with previously treated patients (treated for > 01 month), 15.71%.

Conclusion: Multidrug resistant TB is emerging in India at an alarming rate. Hence, it is important to use diagnostic methods using MGIT 960 as it has higher sensitivity. The appropriate diagnosis of tuberculosis and resistance pattern can aid in usage of suitable drugs and treatment of MDR-TB cases and thereby reduce the overall morbidity and mortality due to tuberculosis.


Keywords: Multidrug Resistant Tuberculosis, Mycobacterium Tuberculosis Complex, Mycobacterium Growth Indicator Tube (MGIT), Mycobacterium Other Than Tuberculosis.

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INTRODUCTION

Tuberculosis (TB) is a specific infectious disease caused by Mycobacterium Tuberculosis, primarily affecting lungs and causing Pulmonary tuberculosis but can also affect intestine, meninges, bones and joints, lymph glands, skin and other tissues of body. Transmission usually takes place through airborne spread of droplet nuclei produced by patients with infectious pulmonary TB. If properly treated, TB caused by drug-susceptible strains is curable in virtually all cases. If untreated, the disease may be fatal within 5 years in 50.65% of cases.¹ Latent TB do not have symptoms. 10% of latent infections progress to active disease with classic symptoms of chronic cough with blood containing sputum, fever, night sweats and weight loss.² Primary tuberculosis is the initial infection commonly located in lower lobe or lower part of upper lobe (Ghon focus). The Ghon focus together with enlarged hilar lymph node constitutes primary complex. Post pulmonary tuberculosis is due to reactivation of latent infection. It

mainly affects the upper lobe of the lungs, the lesions undergoing necrosis and tissue destruction, leading to cavitation.³

Multidrug resistant tuberculosis is defined as a form of TB infection caused by bacteria that are resistant to at least isoniazid (INH) and Rifampicin (RMP). XDR strains are extensively resistant strains. It is defined as MDR plus resistance to fluoroquinolones and one of the second-line injectables: Amikacin, Capreomycin, Kanamycin.³ India is one of the worst affected countries. More than 40 percent of population is infected and some 15 million suffer from tuberculosis.⁴ Unfortunately, even after years of advent of newer techniques for diagnosis of tuberculosis and detection of resistance to ATT in positive culture isolates, we don't have much data on MDR-TB cases in this part of the country in general, and in RIMS, in particular. Hence, it becomes important to study the resistance pattern to ATT and detect the MDR-TB cases so that these cases can be properly treated.

AIMS AND OBJECTIVES

1. The aim of the present study is to detect the MDR-TB cases (Resistance to isoniazid and Rifampicin) in suspected tuberculosis patients.
2. To evaluate the factors responsible for the development of MDR-TB in patients, and finding its prevalence, problems and constraints in diagnosis of MDR-TB in microbiology laboratory.
3. To assist in the programme run by Govt. of India – Revised National Tuberculosis Control Programme (RNTCP).
4. To help clinicians as well as patients who have developed MDR TB to detect, treat and cure the patients.

MATERIALS AND METHODS

Sputum sample was collected in a disposable, wide mouthed bottle, screw capped, plastic container about 100ml capacity. Proper aseptic measures and precautions were taken during collection of specimen and early morning sample was preferred. Specimen processing was done by NALC-NaOH solution. The collected specimen was taken in 50 ml plastic centrifuge tube in an amount not exceeding 10ml. NALC- NaOH solution was added

to the specimen in equal volume. The tube was filled upto 50ml with sterile phosphate buffer solution at pH6.8 and swirled by hand to mix. The specimen was concentrated in a centrifuge at a speed of 3000g for 20mins. The supernatant was carefully decanted. The sediment was resuspended with phosphate buffer using Pasteur pipette to achieve a final volume of 1-2 ml. Decontaminated and concentrated specimen was used for culture in BACTEC MGIT 960, 500µL of the specimen was inoculated into MGIT tube containing 7ml modified middlebrook-7H9 broth. The MGIT 960 tube was enriched with supplement and antibiotics (PANTA) prior to inoculation. Cultures were incubated at 37°C for upto 42 days. The direct microscopy by ZiehlNeelsen for detecting AFB bacilli. A test was done to differentiate M.TB from atypical mycobacterium(MOTT) by TBC-kit.

Antimicrobial susceptibility testing was done by SIRE supplement (0.8ml). 0.1ml of reconstituted drug was added. No drug was added to control. 0.5ml of well mixed culture suspension was added to each drug containing tube. Similarly, PZA (Pyrazinamide) susceptibility testing with growth control was done. The inoculum for control was diluted 1:10 and not 1:100 as in SIRE AST.

Table 01: Sex Wise Distribution of Cases

Sex	Suspected TB cases		Growth Positive MTbC cases	
	Number	Percentage	Number	Percentage
Males	74	69.16	28	68.29
Females	33	30.84	13	31.70
Total	107	100	41	100

Table 02: Age Wise Distribution of Cases

Sl. No.	Age in years	No. of cases	Percentage
1	0 – 10	03	02.80
2	11 – 20	23	21.49
3	21 – 30	24	22.43
4	31 – 40	21	19.63
5	41 – 50	14	13.08
6	51 – 60	10	09.35
7	61 – 70	12	11.21

Table 03: Drug Resistance Pattern of Isoniazid, Rifampicin, Ethambutol, Streptomycin, Pyrazinamide

Drug	Resistance		Sensitive	
	No.	%	No.	%
Isoniazid	17	41.46%	24	58.54%
Rifampicin	13	31.71%	28	68.29%
Ethambutol	11	26.83%	30	73.17%
Streptomycin	15	36.59%	26	63.41%
Pyrazinamide	14	34.15%	27	65.85%

Table 4: Multi-Drug Resistance Pattern (Isoniazid and Rifampicin)

Drug	Resistance pattern		
	No.	%	Prevalence in total suspected cases
Isoniazid and Rifampicin	13	31.71%	12.15%

Table 5: Comparison between Prevalence of MDR-TB Cases In New (Untreated or <01 Month) Patients and in Previously Treated Patients (Treated For >01 Month)

Resistance pattern	New patients (untreated or <01 month) {n=37}		Previously treated patients (treated for >01 month) {n=70}	
	No.	%	No.	%
Isoniazid and Rifampicin	2	5.41%	11	15.71%

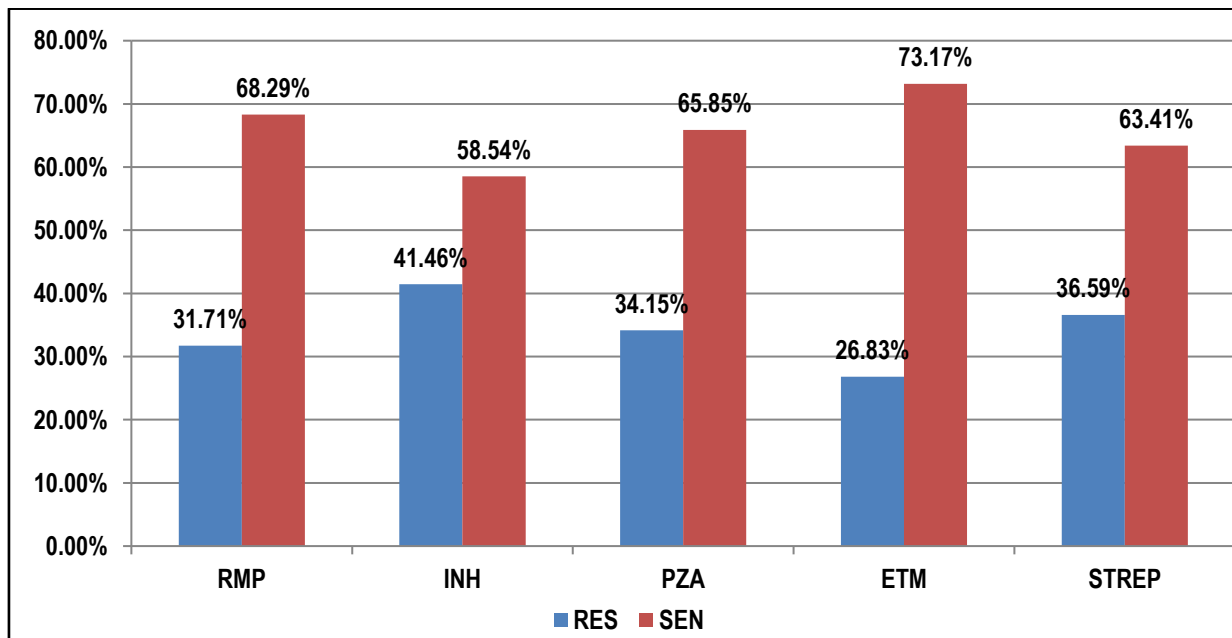


Fig 1: Drug Resistance Pattern of Rifampicin, Isoniazid, Pyrazinamide, Ethambutol, Streptomycin.

RESULTS

In the present study, 107 patients clinically suspected for either pulmonary or in some cases extra pulmonary tuberculosis were considered from November 2016 to September 2017. Among 107 cases, total 49 cases were growth positive, out of which 41 were Mycobacterium Tuberculosis complex (MTbC) and 08 were MOTT (Mycobacterium other than tuberculosis) differentiated with the help of BD MGIT 960 TbC ID strip tests and smear was also prepared from the positive samples for the Ziehl-Neelsen staining to examine the Acid-Fast Bacilli. The Mycobacterium Tuberculosis Complex (MTbC) positive on the test strip was subjected to the Drug sensitivity test (DST) for the first line anti-tubercular drugs (ATT) by BACTEC MGIT 960 system.

Out of 107 suspected cases 74 were males, comprising 69.16% of the total sample and 33 were females, comprising 30.84% of the total sample size. Among the 41 growth positive; Mycobacterium Tuberculosis complex cases, 28 (68.29%) were males and 13 (31.70%) were females. Males were more commonly affected than females in the ratio of 2.15:1 as shown in table 1.

In the present study the age of patient ranged from 3 years to 68 years, the youngest patient being 3 years and the oldest was of 68 years. Maximum no. of cases was found to be in the third decade of life (21-30) years, comprising 22.43% of the study population. However, the least number of patients were seen in the first decade of life (0-10) years, comprising only 2.8% of the study population as depicted in table 2.

In the present study, out of 41 positive MTbC samples subjected to drug susceptibility testing for first line drugs by liquid based media (MGIT 960 system), 17 samples were resistant to Isoniazid, comprising 41.46% of total MTbC positive samples and 24 samples were sensitive (58.54%) to Isoniazid.

Rifampicin resistance is always associated with resistance to one or more first line drugs. In present study all the Rifampicin resistant cases were also resistant to Isoniazid.

Among 41 MTbC positive cases 13 samples were Rifampicin resistant, comprising 31.71% of total MTbC positive samples and other 28 were sensitive to Rifampicin comparing 68.29% of MTbC positive samples. In 41 MTbC positive cases, 11 samples were

resistant to Ethambutol, comprising 26.83% of total MTbC positive cases. And remaining 30 samples were sensitive to Ethambutol, comprising 73.17% of total MTbC positive cases.

In 41 MTbC positive cases, 15 samples were resistant to Streptomycin, comprising 36.59% of total MTbC positive cases. And remaining 26 samples were sensitive to Streptomycin, comprising 63.41% of total MTbC positive cases.

In 41 MTbC positive cases, 14 samples were resistant to Pyrazinamide, comprising 34.15% of total MTbC positive cases. And the other 27 samples of 41 MTbC positive cases were sensitive to Pyrazinamide (65.85%). Resistance to Isoniazid and Rifampicin, the two mainstay drugs of first line anti tubercular treatment is known as multidrug resistance tuberculosis (MDR-TB). Out 107 suspected tuberculosis patients 13 were diagnosed with MDR-TB by MGIT 960 system. Thus, the prevalence of MDR-TB cases in the present study was 12.15%. In new patients, 02 cases out of 37 suspected cases were multi drug resistant (MDR) i.e. Isoniazid and Rifampicin resistant, comprising 5.41%.

In previously treated patients, 11 cases out of total 70 suspected cases were surfing from MDR-TB, comprising 15.71%.

DISCUSSION

When comparing various studies, authors have reported similar findings for sex wise incidence of suspected tuberculosis cases. In the present study, males accounted for 69.16% and females accounted for 30.84% of the total suspected tuberculosis cases. This correlated with the studies done by M.A.M. Siddiquiet al⁵.

Maximum number of patients expected to be suffering from tuberculosis were of the age group 21-30 yrs. (22.43%), followed by 11-20 (21.49%), 31-40 (19.63%), least prevalence was in the age group 0-10(2.8%). The present study correlated well with other studies. For example Mohammed A. I. Almaziniet al⁶ found maximum patients belonged to age group 25-34 years (28.99%) and M.A.M. Siddiqui et al⁵ found maximum number of patients of the age group 30-39 (37%).

Drug resistance pattern of Isoniazid: In the present study resistance to Isoniazid among positive MTbC cases detected by MGIT 960, SIRE Susceptibility testing was seen in 17 (41.46%)

cases and 24 cases (58.54%) were sensitive to isoniazid. The study result was similar to that stated by C. Scarparo et al⁷ with 49% cases resistant to Isoniazid.

Drug resistance to Rifampicin: Among 41 positive MTbC, 13 (31.7%) samples were resistant to rifampicin and 8 were susceptible. This finding was consistent with studies on resistance pattern done by C. Scarparo et al (29%), Tsi-Shu-Huang et al⁸ (34.07%).

Drug resistance to Ethambutol: In the present study, 26.83% of the MTbC samples were resistant to Ethambutol which correlates with the studies of Tsi- Shu Huang et al⁸ (27.65%), as well as with C. Scarparo et al⁷ (19%).

Drug resistance to Streptomycin: In the present study, 36.59% of the MTbC positive sample were resistant to Streptomycin which was similar to studies done by C. Scarparo et al⁷ 34 %, Tsi – Shu-Huang et al⁸ (34.07%) and P. Bemer et al⁹ (30.9%).

Drug resistance to Pyrazinamide: In the present study, 34.17% of the MTbC positive samples were resistant to Pyrazinamide which was comparable to C. Scarparo et al⁷ (30%) and Gaby E. Pfyffer et al¹⁰ (24.13%).

In the present study, the prevalence of MDR TB Isoniazid + Rifampicin was 12.15%. The present study was in agreement with study done by Sarder T. Hossain et al¹¹ (19.26%) in suspected TB cases. According to RadhaMunje et al¹² the prevalence of MDR-TB in India has been estimated to be 1-3% in new TB (untreated or treated for less than 1 month) cases and around 12-14% in previously treated TB cases. Globally according to WHO¹³, Global burden (2016) 3.6% new TB cases and 20.2% of previously treated cases are evaluated to be MDR-TB. In the present study similar findings were observed. Among new (untreated or treated for <1 month) cases, 5.41% of MDR was detected, and among previously treated cases (treated for > than 1 month) 15.71% of cases were MDR (i.e. INH + RMP).

CONCLUSION

Isoniazid was the drug against which most resistance appeared among MTbC positive cases. MDR-TB, resistance to Isoniazid and Rifampicin has appeared in an alarming rate (12.15%) Previous interrupted, inadequate, poorly administered drugs and poor adherence to treatment are the risk factors for the development of drug resistant TB.

It is worthwhile to mention the emergence of multi-drug resistant TB in India at an alarming rate. Hence, it is necessary to use diagnostic test which have higher sensitivity. MGIT 960 liquid culture based test has high accuracy and sensitivity as they do not detect dead bacilli or DNA. This appropriate diagnosis of tuberculosis can aid in the usage of appropriate drugs and prevent the emergence of MDR-TB and hence reduce the overall morbidity and mortality due to tuberculosis.

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