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# A Study on Prevalence & Drug Susceptibility Pattern of Newly Diagnosed Pulmonary Tuberculosis Patients in A Tertiary Care Hospital

**Brindha.V, Kalyani. M, Prem Kumar. L, Aravind Swaminathan**

**Abstract**
**Purpose:** A lot of effort has been put to eradicate Tuberculosis for which diagnosing muti-drug resistant cases early and treating them are of utmost importance. The knowledge of the prevalence and pattern of drug susceptibility helps in formulating ideal protocol for disease eradication.
**Methods:** The present study is a prospective observational study conducted for a period of 8 months from February 2015 to September 2015 in a total of 887 samples collected from suspected cases of primary pulmonary tuberculosis in a tertiary care hospital.
**Results:** A total of 78 culture positive samples were obtained from 767 samples contributing 10.16%. Biochemical examination for confirmation of *Mycobacterium tuberculosis* species was done. Drug susceptibility tests by MGIT 960 method revealed 2 cases of multi-drug resistant tuberculosis.
**Conclusion:** The prevalence of multi-drug resistance was found to be 2.56%. Only resistance to Rifampicin and Isoniazid was noted in the study group.

**Keywords:** Drug susceptibility test, INH resistance, MDR-TB, Multi-drug resistant.

## 1. Introduction

Tuberculosis (TB) is a major global health problem. It causes ill-health among millions of people each year and ranks alongside the Human Immunodeficiency virus (HIV) infection/ acquired immunodeficiency syndrome (AIDS) as a leading cause of death worldwide.[1] In 2014, out of the estimated global annual incidence of 9.6 million TB cases, 2.2 million were estimated to have occurred in India. [2]

Overall, a relatively small proportion (5–15%) of the estimated 2–3 billion people infected with *M. tuberculosis* will develop TB disease during their lifetime.[2] Developments in TB diagnostics in the last few years mean that the use of rapid molecular tests to diagnose drug-sensitive and drug-resistant TB is increasing, and some countries are phasing out use of smear microscopy for diagnostic purposes. In countries with more developed laboratory capacity, cases of TB are diagnosed via culture methods (the gold standard) as against smear microscopy in developing countries.

It has been estimated that about 2.2% of new TB cases and 15% of re-treatment TB cases in India are multi-drug resistance tuberculosis (MDR-TB). However, given the lack of community based epidemiological data, especially from endemic locals (like Mumbai) and the ill-equipped public health laboratory infrastructure, these numbers could well be an underestimation of the burden of MDR-TB in India. This has been emphasized by studies like the one done in Mumbai.[3]

Out of the estimated 300,000 MDR-TB among notified pulmonary TB cases worldwide in 2014, India hosts 71,000. In India, only a meager 1.7% of new bacteriologically confirmed cases have Drug Susceptibility Testing (DST) results. According to the targets set in the 'Global Plan to Stop TB', 20% of all new bacteriologically confirmed TB cases (i.e. those considered to be at high risk for MDR-TB) as well as all previously treated cases to undergo DST to first-line TB drugs. [4] We are far behind this WHO's target of conducting DST.

Drug susceptibility testing (DST) in which the five first line drugs are incorporated in the LJ media so that the antibiotic susceptibility pattern of the TB bacilli could be identified helps in

improving the susceptibility of detecting MDR-TB, which is now a rising global problem.

Hence the study for identification and analyzing the drug susceptibility pattern of Mycobacterium tuberculosis in suspected tuberculosis cases plays a vital role in effective diagnosis and management of these patients.

## 2. Materials & Methods

**(As per RNTCP Guidelines)**

The present study is a prospective observational study conducted for a period of 8 months from February 2015 to September 2015 and a total of 887 samples collected from suspected cases of primary pulmonary tuberculosis attending in-patient and out-patient department of General medicine and Chest medicine in a tertiary care hospital at Thandalam, Chennai. All suspected cases of TB attending the clinic were subjected to sputum smear examination, mycobacterial culture, biochemical tests and drug susceptibility testing (DST).

The definition of a new sputum smear-positive pulmonary TB case is based on the presence of at least one acid fast bacillus (AFB+) in at least one sputum sample in countries with a well-functioning External Quality Assurance (EQA) system.[5] In our study we have included patients with smear and/or culture positive for tuberculosis and has never had treatment for tuberculosis or has taken anti-tuberculosis drugs for less than one month. Cultures were done on commercially available Lowenstein Jensen (LJ) slopes by modified Petroff's method. All the isolates were identified as M. tuberculosis by their slow growth rate, colony morphology, niacin test, nitrate reduction test and catalase test. DST was carried out by culture based MGIT 320 instrument which uses MGIT 960 principle. DST is done for all drugs except pyrazinamide. MDR-TB was defined as TB caused by bacilli showing resistance to at least isoniazid and rifampicin. Human immunodeficiency virus (HIV) testing was carried out routinely in all the patients. Written informed consent was obtained from all patients.

## 3. Observation & Results

The present study is a prospective observational study conducted for a period of 8 months from February 2015 to September 2015 and a total of 887 samples collected from suspected cases of primary pulmonary tuberculosis attending inpatient and outpatient department of General medicine and Chest medicine in a tertiary care hospital at Thandalam, Chennai. 767 samples were subjected to microscopy examination by Ziehl-Neelsen staining method to screen for the presence of Acid-fast bacilli (AFB). Two smears were read for each sample and of the 767 samples, AFB was detected in 76 samples (9.90%) in either of the smears (Figure 1).

All the 767 samples were processed by Petroff's concentration method and cultured in Lowenstein-Jensen media. These cultures were incubated at $37^0$C and observed for growth between 8 to 10 weeks (Figure 2). Out of the 76 smear positive specimens, growth was noted in 73 samples (96.05%). The 3 samples without growth had scanty bacilli in smears. Among the 691 smear negative specimens, growth was observed in 5 samples (0.72%). Therefore, a total of 78 culture positive samples were obtained from 767 samples contributing 10.16% (Figure 1).

In total, 81 patients were diagnosed to have tuberculosis with a prevalence rate of 10.56%, whereas smear-positive tuberculosis prevalence rate was 9.9%. There were 66 male patients and 15 female patients contributing 81.5% and 18.5% respectively of the total positive samples. Mean age was 47.9 years. The age group between 51-60 years had the highest number of cases and about 74% of the total cases were between age group 31-60 years in the middle age.

All the culture positive samples with typical growth were subjected to Biochemical examination for confirmation of Mycobacterium tuberculosis species. The atypical growth samples were not considered for further study. The samples with Mycobacterium tuberculosis were subjected for drug susceptibility tests by MGIT 960 method (Figure 3).

Out of 78 cases, two cases of MDRTB were detected (Figure 4). Both were male, HIV negative, aged 27 and 48 years respectively and resistance pattern for both was for Isoniazid and Rifampicin. Thus, the prevalence of MDRTB among new culture positive pulmonary TB patients was 2.56 per cent. The resistance rates (%) observed to various first line drugs were Isoniazid - 2.56, Rifampicin - 2.56, Ethambutol - 0 & Streptomycin – 0.

## 4. Discussion

The conventional identification of *Mycobacterium tuberculosis* was based on microscopy of Ziehl-Neelsen staining, evaluation of growth on Lowenstein-Jenson media based on their typical colony morphology of slow growth rate, absence of pigmentation and biochemicals like nitrate reduction, niacin accumulation & catalase activity at $68^0$C.

Our study analysis showed that distribution of pulmonary tuberculosis is more among males (81%) than females (19%) which were in concordance with many studies like Gupta A *et al*,[6] Makeshkumar *et al* [7] and Kalo D *et al*, [8] were males out number females.

The age group between 51-60 years recorded the highest number of cases in our study which was in line with Bhat J *et al*, [9] Makeshkumar *et al* [7] and Joseph M R *et al*.[10] However in Khunjeli R *et al*[11] the age group of 25-29 years recorded maximum cases followed by 30-34 years.

In our study, the prevalence rate of cultures positive for *Mycobacterium tuberculosis* was 10.16% and for smear positive cases it was 9.9%. Overall, the prevalence rate of 10.56%, is similar to the prevalence rate in Kanchipuram district, which is 10%.[12] Gupta A *et al* [6] evaluated 3,704 clinical samples and found 345 culture positive cases with prevalence rate of 9.3%. Makeshkumar *et al* [7] evaluated 1030 patients with clinical evidence of pulmonary tuberculosis in a teaching hospital in Kanchipuram and noted 12.13% smear positivity. However, they later discarded samples with atypical mycobacteria, which were also included in the smear positivity rate giving a false high rate.

The biochemical testing of all culture positive samples detected *Mycobacterium tuberculosis* species. This may be due to the selection of cultures based upon the characteristic colonies and growth character.

Anti-tuberculosis drugs have proved to be a boon in the treatment of Tuberculosis, but on the other side, they, like other antibiotics have to be taken regularly according to the regime. If the patient who is on anti-tuberculosis treatment discontinues the treatment haphazardly, and becomes a defaulter, the chances of the organism developing resistance to the powerful and safer first-line anti-tuberculosis drugs increases. The increase in number of defaulters and thereby the development of multi-drug

resistant tuberculosis organism is a hindrance in controlling the disease in the community. Not only are the 're-treatment' patients difficult to manage medically but they pose varied challenges to the physician, as late detection of multi-drug resistant cases can worsen the disease in the patient and also puts the community at risk. Therefore, knowledge of the prevalence of multi-drug resistant organism in the local community and earlier detection are the key to combat this infection.[8] Hence forth, drug resistance in tuberculosis has been the picture of growing interest in the last decade.

In this study, the prevalence rate of multi-drug resistant tuberculosis is 2.56% among newly diagnosed culture-positive pulmonary tuberculosis patients. The reported prevalence of multi-drug resistant cases among newly diagnosed cases varies from 1.1% to 4.2% in previous studies [7,10,13-16] conducted in different parts of India, and our findings were in concordance with those observations (Table 1).

There were two samples with resistance to Isoniazid and Rifampicin but no samples showed resistance to Ethambutol or Streptomycin. These were detected in male patients, one in his 20s and the other a middle-aged man, both were HIV non-reactive. These patients were initially on regular Anti-tuberculosis regime and were converted to MDR regime with second-line drugs after these results Joseph M R *et al* [10] evaluated 305 sputum samples in Ernakulam district of Kerala and noted 2% of MDR-TB and 27.9% resistance to any drug. 62 culture positive patients in Nepal were evaluated by Khunjeli R *et al* [11] and detected MDR among 4.8%, Rifampicin resistance in 3.2% and Isoniazid resistance in 4.8%. Makeshkumar V *et al* [7] in Kanchipuram district found a MDR resistance rate of 4.05% (3 patients) among 68 pulmonary isolates subjected for drug susceptibility. They also evaluated 6 extrapulmonary samples, but none of them showed MDR. Saugat R *et al* [13] evaluated 100 new sputum positive patients in Bikaner, Rajasthan and detected 1 case of MDR-TB and 12 cases with Isoniazid resistance. Ramachandran R *et al* [14] in the state of Gujarat studied 1571 isolates from new patients and detected 11% (173 cases) resistance to Isoniazid and 2.4% (37 cases) MDR. Sharma S K *et al* [15] carried out their study in New Delhi among 218 sputum-positive pulmonary tuberculosis during 2008-2009 and detected a prevalence of 1.1% for MDR. Swaminathan S *et al* [16] studied the prevalence of MDR among HIV infected Pulmonary tuberculosis patients in Chennai and found it to be marginally higher in 4.2%.

In studies by Kandi *et al* [17] and Lahiri *et al* [18] evaluating MDR in re-treatment cases, the rate was 28% in 100 samples and 80.8% in 917 MDR suspected cases respectively. In our study we have not evaluated any re-treatment cases.

Because of the initiative by the WHO and RNTCP by the Indian government, detection of multi-drug resistance is carried out in many reference centers but, still coverage of remote areas and evaluation of all the newly diagnosed cases is not yet achieved. The time taken for organisms to grow in culture and susceptibility test to be performed and the high cost of rapid molecular tests have been hindrances in achieving this goal.

However, screening of all the newly diagnosed patients for drug susceptibility and equipping all the centers is the only way of detecting all the multi-drug resistant tuberculosis and preventing them from spreading resistance further in the community.

## 5. Tables and Figures

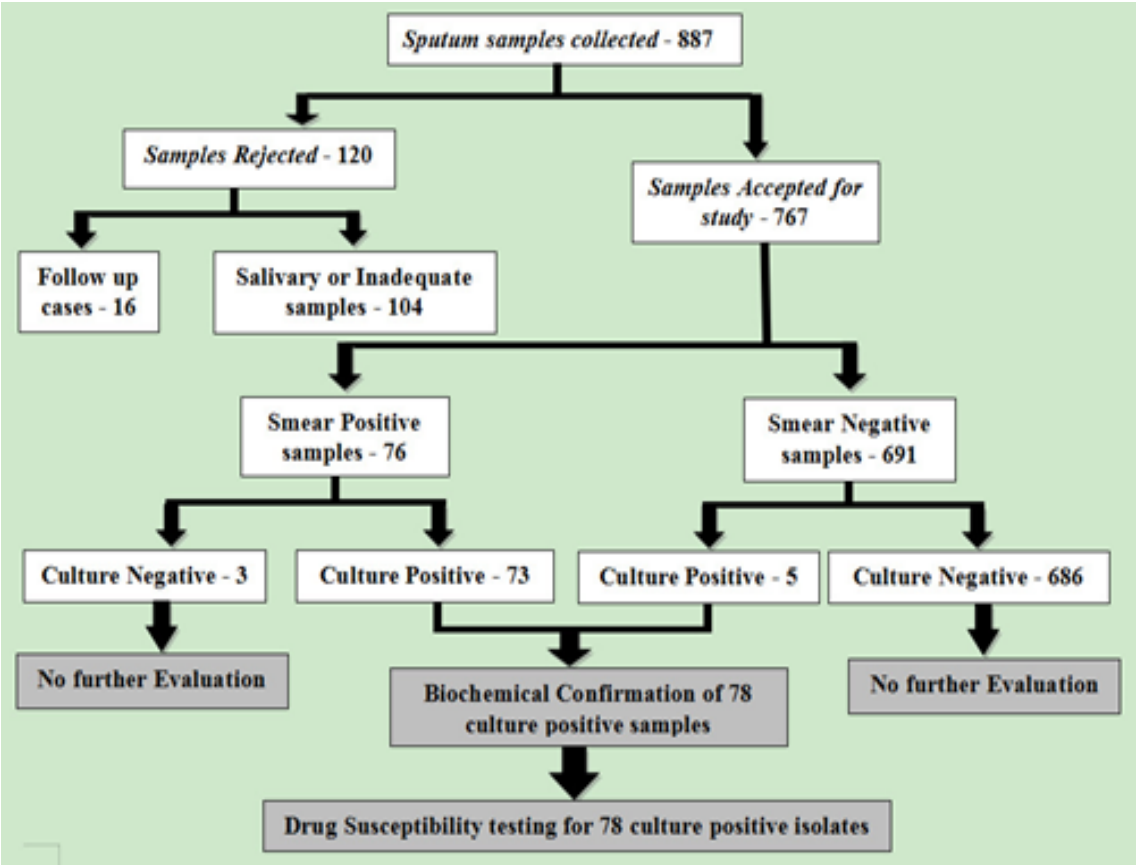


**Fig. 1:** Flowchart depicting method of study.

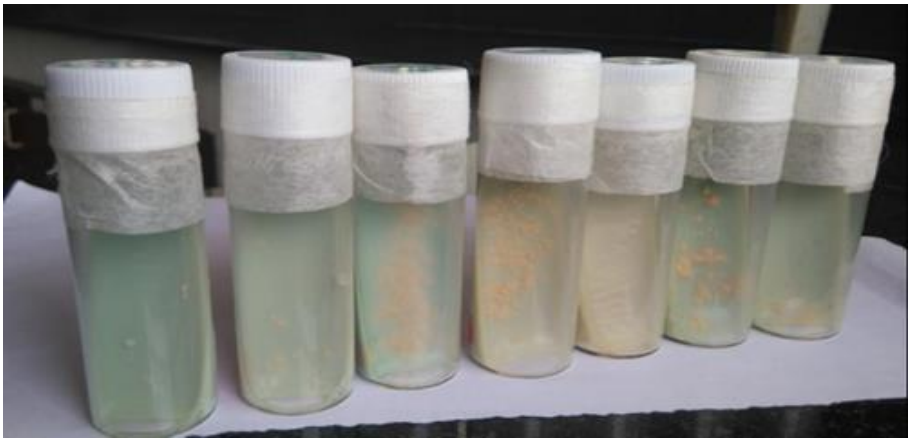

**Fig. 2:** Series of LJ medium bottles showing tuberculosis bacilli growth.

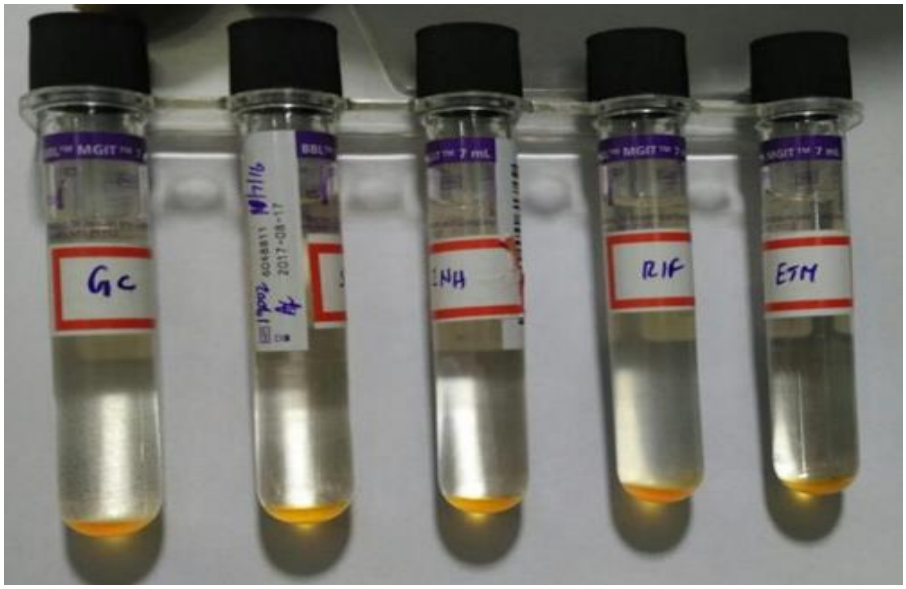


**Fig. 3:** MGIT tubes showing susceptibility to all first-line drugs
(GC - Growth control, STR - Streptomycin, INH – Isoniazid, RIF – Rifampicin, ETM – Ethambutol).

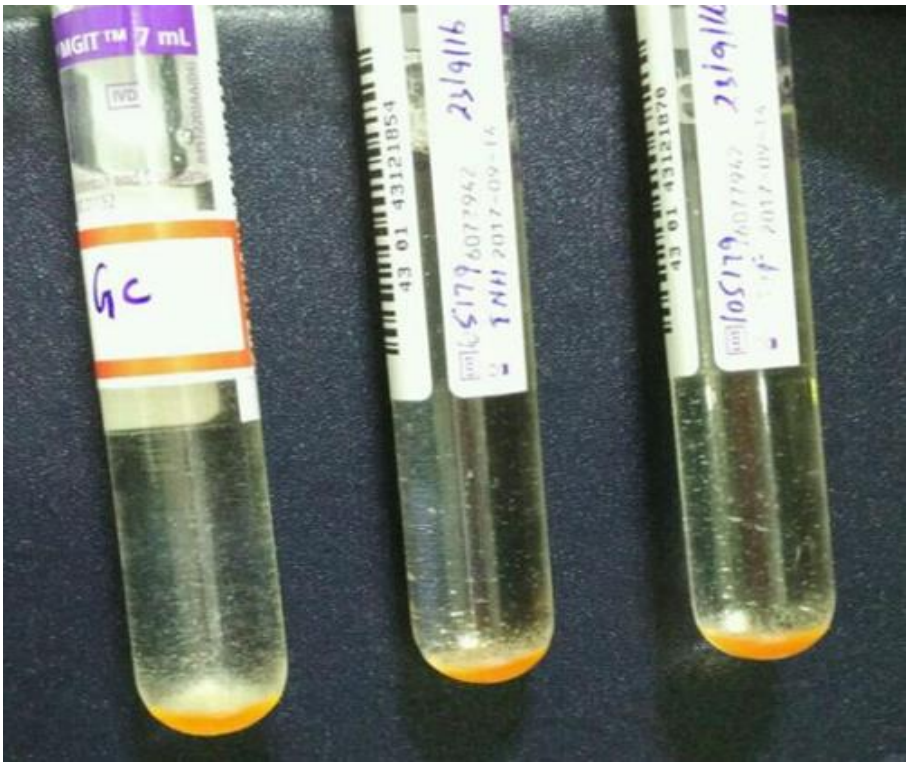


**Fig. 4:** MGIT tubes of INH and RIF showing growth of mycobacteria (MDR-TB case) similar to Growth control tube.

**Table 1**: Comparison of MDR and INH resistance in new cases among various studies.

| Studies | MDR in new cases | INH resistance in new cases |
|---|---|---|
| Makeshkumar V *et al* [7] (Kanchipuram dt) | 4.05% | 4.05% |
| Joseph M R *et al* [10] (Ernakulam Dt) | 2% | 8.8% |
| Khunjeli R *et al* [11] (Nepal) | 4.8% | 4.8% |
| Saugat R *et al* [13] (Bikaner, Rajasthan) | 1% | 12% |
| Ramachandran R *et al* [14] (Gujarat) | 2.4% | 11% |
| Sharma S K *et al* [15] (New Delhi) | 1.1% | 6.2% |
| SwaminathanS *et al* [16] (Chennai) | 4.2% (with HIV) | 13% (with HIV) |
| Our study | 2.56% | 2.56% |

## 6. Conclusion

The prevalence rate of *Mycobacterium tuberculosis* in the area covered by our tertiary care hospital was found to be 10.56%. The prevalence of MDR-TB in newly diagnosed pulmonary tuberculosis patients was found to be 2.56%. Only resistance to Rifampicin and Isoniazid was noted in the study group. Therefore, there is no alarming increase in multi-drug resistant tuberculosis in newly diagnosed cases in this part of the country. Accurate diagnosis and appropriate antibiogram is essential to combat MDR-TB and to prevent its progression to Extensively drug resistant (XDR-TB) strains, which in turn will improve the morbidity and reduce the mortality due to this dreadful disease. Limitations of this study are extra-pulmonary tuberculosis cases are not included in the study and the time taken for drug susceptibility testing of 3 weeks can delay initiation of treatment.

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