

## Original Research

### Assessment of pattern of drug resistance in sputum smear positive cases of previously treated pulmonary tuberculosis using Line probe assay

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#### ABSTRACT:

**Background:** Tuberculosis (TB) is an ancient human disease caused by *Mycobacterium tuberculosis* which mainly affects the lungs, making pulmonary disease the most common presentation. Drug-resistant tuberculosis (MDR-TB) has emerged as an important global health concern and is on the rise throughout the world. Hence; the present study was assessment of pattern of drug resistance in sputum smear positive cases of previously treated pulmonary tuberculosis using Line probe assay. **Materials & methods:** A total of 100 patients were included in this study. The first 100 patients fulfilling the inclusion criteria, willing to participate and given consent were taken as subject for the study. Informed consent was taken for getting the detailed history including previous anti tuberculosis treatment, for performing systemic physical examination and for required investigations as per proforma attached. Two Sputum samples from each patient were collected in sterile containers and immediately sent for AFB smear microscopy. All sputum smear positive cases with previous history of treatment with first line antituberculosis drugs were instructed to collect sputum sample in a sterile, leak proof falcon tube. All sputum smear positive previously treated cases were subjected to Line Probe assay. Chest X ray was done before the start of treatment and thereafter during treatment if necessary. All the data were compiled in Microsoft excel sheet and were analysed by SPSS software. **Results:** H (Isoniazid): Sensitive, R (Rifampicin): Sensitive was found to be present in 51 percent of the patients, while R: Sensitive, H: Resistant was found to be present in 27 percent of the patients. H: Sensitive, R: Resistant was found to be present in 5 percent of the patients while H: Sensitive, R: Sensitive was found to be present in 17 percent of the patients. **Conclusion:** Line probe assay is a rapid and accurate tool for identification of drug resistance in pulmonary tuberculosis patients. Our findings carry significant importance because there have been scarce data on the prevalence of MDR-TB among pulmonary TB patients from the recent past.

**Key words:** Drug, Resistance, Tuberculosis

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#### INTRODUCTION

Tuberculosis (TB) is an ancient human disease caused by *Mycobacterium tuberculosis* which mainly affects the lungs, making pulmonary disease the most common presentation. However, TB is a multi-systemic disease with a protean presentation. The organ system most commonly affected include the respiratory system, the gastrointestinal (GI) system, the lymphoreticular system, the skin, the central nervous system, the musculoskeletal system, the reproductive system, and the liver.<sup>1-3</sup>

Successful treatment is as much about building rapport with the patient as about using appropriate chemotherapy. An empathic doctor or other health worker who can build a good relationship with patients is crucial to compliance with treatment and follow-up. National tuberculosis programmes that ensure, by appropriate selection and training, that health workers treat patients with respect and understanding are likely to achieve more than those that place less emphasis on good relationships with patients.<sup>4</sup> Drug-resistant tuberculosis (MDR-TB) has emerged as an important global health concern and is on the rise

throughout the world. According to the World Health Organization (WHO) report, 3.7% of new TB cases (patients who have never had treatment for TB, or had been on anti-TB treatment for less than four weeks) and about 20% of retreatment patients (TB patients who had been cured for one month or more) had MDR-TB in 2012. Nine percent of these patients in 2012 had extensive drug-resistant tuberculosis (XDR-TB).<sup>5-8</sup>

To be defined as MDR-TB, the patient shows resistance to at least isoniazid (INH) and rifampin (RMP). Furthermore, XDR-TB is defined as resistant to INH, RMP, any fluoroquinolone and any second-line injectable medications (i.e. amikacin, capreomycin and kanamycin). Treatment of MDR-TB is a costly and time-consuming (18 months) process which can be associated with numerous complications. The overall economic burden of an MDR-TB patient is high and if left untreated, MDR-TB has an estimated 80% fatality rate.<sup>7,8</sup> Hence; the present study was assessment of pattern of drug resistance in sputum positive smear cases of previously treated pulmonary tuberculosis using Line probe assay.

**MATERIALS & METHODS**

This study was conducted between March 2018 to March 2019 through inpatient department of T.B and Chest hospital, a dedicated T.B center at Guru Gobind Singh Medical College and Hospital in Faridkot (Punjab). Patients with potential symptoms suggestive of pulmonary tuberculosis and history of treatment with antituberculosis drugs reported to Chest and T.B department at Guru Gobind Singh Medical College and Hospital, Faridkot. So, study was conducted on sputum smear positive previously treated pulmonary TB patients and follow up sputum smear positive new pulmonary TB patients. A total of 100 patients were included in this study. The study was conducted for duration of one year from March 2018 to March 2019. The first 100 patients fulfilling the inclusion criteria, willing to participate and given consent were taken as subject for the study. Informed consent was taken for getting the detailed history including previous anti tuberculosis treatment, for performing systemic physical examination and for required investigations as per proforma attached.

Two Sputum samples from each patient were collected in sterile containers and immediately sent for AFB smear microscopy. All sputum smear positive cases with previous history of treatment with first line antituberculosis drugs were instructed to collect sputum sample in a sterile, leak proof falcon tube. Sputum examination was done under direct microscopy by Auramine Rhodamine staining before the start of treatment and then at 2, 6 months in category I patients and at 3, 8 months in category II patients. The intensive phase was prolonged for one month if the sputum was positive at the end of intensive phase and sputum was sent for DST. All sputum smear positive previously treated cases were subjected to Line Probe assay. Chest X ray was done before the start of treatment and thereafter during

treatment if necessary. All the data were compiled in Microsoft excel sheet and were analysed by SPSS software. Fischer’s exact t test was used for assessment of level of significance. P- value of less than 0.05 was taken as significant.

**RESULTS**

In the present study, a total of 100 patients of pulmonary tuberculosis were analyzed. Mean age of the patients of the present study was 38.47 years. 39 percent of the patients belonged to the age group of 31 to 50 years. 31 percent of the patients belonged to the age group of 16 to 30 years. 63 percent of the patients were males while the remaining 37 percent were females. Fever and cough was found to be present in 90 and 97 percent of the patients. Shortness of breath and weight loss was found to be present in 49 percent and 76 percent of the patients. Night sweats, hemoptysis and chest pain was found to be present in 42 percent, 6 percent and 12 percent of the patients. ADR was the most common cause of lost to follow-up found to be present in 22 percent of the patients. Feeling of wellbeing, history of prior default and migration were the other common causes of lost to follow-up found to be present in 16 percent, 9 percent and 2 percent of the patients. H (Isoniazid): Sensitive, R (Rifampicin): Sensitive was found to be present in 51 percent of the patients, while R: Sensitive, H: Resistant was found to be present in 27 percent of the patients. H: Sensitive, R: Resistant was found to be present in 5 percent of the patients while H: Sensitive, R: Sensitive was found to be present in 17 percent of the patients.

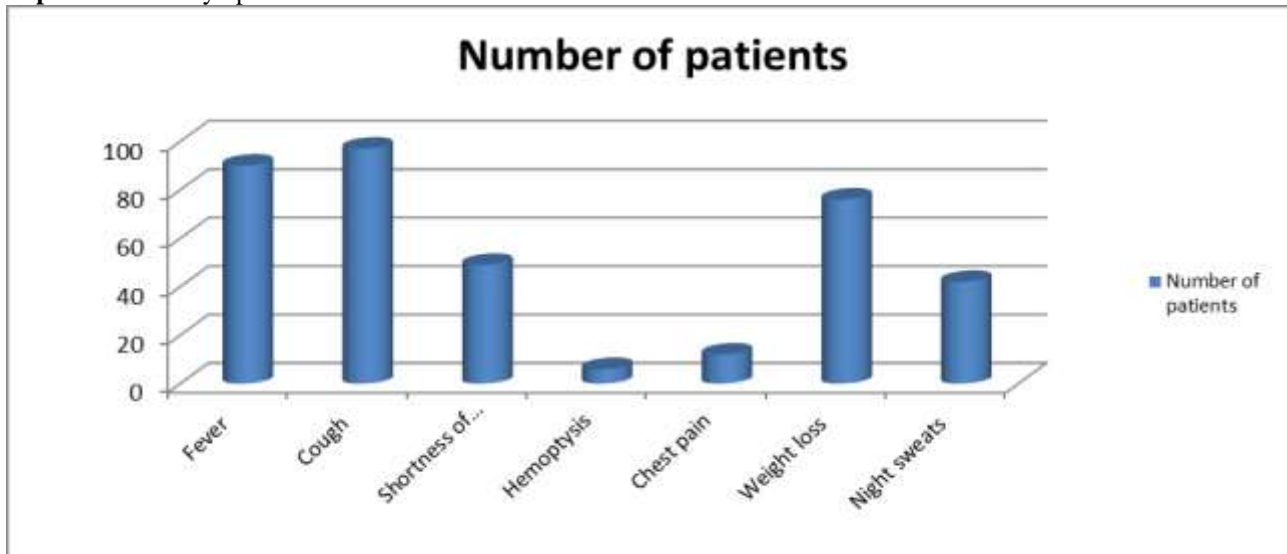
**Table 1:** Age-wise distribution of patients

Age group (years)	Number of patients	Percentage of patients
16 to 30	31	31
31 to 50	39	39
51 to 70	24	24
More than 70	6	6
<b>Total</b>	100	100
<b>Mean</b>		38.47
<b>SD</b>		15.93

**Table 2:** Pattern of H and R resistance

H and R pattern	Number of patients	Percentage	p-value
<b>H: Sensitive R: Sensitive</b>	51	51	
<b>H: Resistant R: Sensitive</b>	27	27	0.0001
<b>H: Sensitive R: Resistant</b>	5	5	
<b>H: Resistant R: Resistant</b>	17	17	
<b>Total</b>	100	100	

**Graph 1: Clinical symptoms**



**Table 3: Causes of lost to follow-up**

Causes	Number of patients	Percentage of patients
Feel of well being	16	16
ADR	22	22
Migration	2	2
History of prior default	9	9

**DISCUSSION**

Tuberculosis continues to be a major cause of morbidity and mortality worldwide. There are an estimated 8.8 million incident cases of tuberculosis globally. India has more new tuberculosis (TB) cases annually than any other country. According to WHO report 2019, total incidence of TB in Indian population is approximately 27 lakh. Also, incidence of MDR in TB has been reported to be 1.3 lakh.<sup>8</sup> It is estimated that an untreated smear-positive patient may infect >10 contacts annually. Delay in diagnosis and treatment leads to more advanced disease, more complications, higher mortality, and has resulted in community outbreaks.<sup>9</sup> Tuberculosis is caused by infection of lung with small aerobic non-motile bacillus *Mycobacterium tuberculosis* (MTB). It spreads through the air when people who have an active MTB infection cough, sneeze, or otherwise transmit their saliva through the air.<sup>10</sup>

In the present study, a total of 100 patients were enrolled. Mean age of the patients of the present study was 38.47 years. 70 percent of the patients belonged to the age group of less than 50 years. Our results were in concordance with the results obtained by previous authors who also reported similar findings in their respective studies. In a previous study conducted by Singhal R et al, authors reported that 76.7 percent of the patients belonged to the age group of 15 to 45 years.<sup>9</sup>

In the present study, Isoniazid and rifampicin mono-resistance was found to be present in 27 percent and 5 percent of the patients. Both isoniazid and rifampicin resistance was found to be present in 17 percent of the patients. Therefore; in 49 percent of the study population, patients were found to be resistance to at least one out of two anti-tubercular drugs included in the present study. Hence; it can be inferred that isoniazid mono-resistance is more prevalence than rifampicin mono-resistance. Variable results have been reported by various authors in the past literature. In a previous study conducted by Li Q et al, authors reported that only Rifampicin resistance was found to be present in 10.2 percent of the patients, while only isoniazid resistance was found to be present in 14.4 percent of the patients. Their study showed that 22.3% of TB patients were resistant to at least one first-line anti-TB drugs and 8.0% were MDR.<sup>10</sup>

In another study conducted by Pradhan N et al, Isoniazid and rifampicin mono-resistance was found to be present in 8 percent and 13 percent of the patients respectively. Both isoniazid and rifampicin resistance was found to be present in 12 percent of the patients.<sup>11</sup>

Siddiqui S, et al compared the diagnostic performance of the GenoType MRBDRplus assay with the gold standard phenotypic drug susceptibility testing in the detection of drug resistance among culture isolates obtained from patients in Karachi, Pakistan. *Mycobacterium tuberculosis* isolates were obtained from 96 consecutive tuberculosis patients found to have resistance to isoniazid from two health centers in Karachi. Isolates were tested for drug resistance against rifampin and isoniazid using the MTBDR plus assay. Results were compared with conventional drug-susceptibility testing and the frequency of specific mutations were reported. The MTBDR plus assay had a sensitivity for rifampin resistance of 98.8% (95% CI: 93.4–

100) and for isoniazid resistance of 90.6% (95% CI: 83.0–95.6). The MTBDRplus assay showed mutations in *rpoB* in 81 of the 96 (84.4%) isolates. Of the 87 isolates showing resistance to isoniazid via the MTBDRplus assay, 71 (74.0%) isolates had mutations in the *katG* gene only, 15 (15.6%) isolates had mutations in the *inhA* promoter region, and 1 (1.0%) showed mutations in both genes. The GenoType MTBDRplus assay in Pakistan can identify subgroups at high-risk of having isolates with mutations in the *katG* and/or *inhA* genes.<sup>12</sup>

## CONCLUSION

From the above results, the authors conclude that line probe assay is a rapid and accurate tool for identification of drug resistance in pulmonary tuberculosis patients. Our findings carry significant importance because there have been scarce data on the prevalence of MDRTB among pulmonary TB patients from the recent past.

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