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Original Article

Role of line probe assay in diagnosis and detection of drug resistance in *Mycobacterium tuberculosis*

Phalguni Malhotra [1], Deepinder Chhina*[1], Veenu Gupta [1], Akashdeep Singh [2] and Diljot Sandhu [1]

Departments of Microbiology [1] & Pulmonary Medicine [2], Dayanand Medical College and Hospital, Ludhiana, India

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ABSTRACT: Tuberculosis caused by Mycobacterium tuberculosis has remained a major global health problem worldwide. TB requires prolonged period of time for isolation by conventional culture methods. The emergence and spread of multi drug resistant (MDR-TB) poses great threats and challenges in controlling the infection. MDR-TB is resistant to both first line drugs rifampicin and isoniazid. PCR tests are based on targeting the mutation in rpoB, katG and inhA genes which can detect resistance to these drugs. To compare microscopy, conventional culture and Line probe assay for the detection of M. tuberculosis & detect rifampicin and isoniazid resistance using Lineprobe assay in various clinical samples. A total of 347 suspected patients of tuberculosis were included in the study. Demographic details & clinical presentation was noted. Various samples were received & processed for ZN staining, culture on LJ media and Line probe assay. Out of 347 cases, majority of cases were in the age group of 51-60 years (18.4%). Majority of the population was males (65.1%). Among suspected tuberculosis patients, cough with expectoration (55.9%) was the commonest complaint. Microscopy was positive in 17.3%, conventional culture was positive in 16.1% and line probe assay was positive in 26.2%. Out of 347, 91 were diagnosed with MTB, out of which 85.7% were sensitive to both rifampicin and isoniazid whereas 14.3% showed resistance to either rifampicin / isoniazid or both. LPA & direct microscopy are a good screening method for early diagnosis and detection of drug resistance but are not a complete replacement of conventional culture which is still a gold standard.

⇑ Corresponding author at:
Corresponding Author:
Dr. Deepinder Chhina, Professor & Head, Department of Microbiology, Dayanand Medical College & Hospital, Ludhiana, India
E-mail: deepinder.chhina@rediffmail.com

INTRODUCTION

Tuberculosis (TB) is a major public health disease caused by *Mycobacterium tuberculosis* (MTB). In 2016, there were an estimated 10.4 million new TB cases worldwide [1]. The estimated incidence of TB in India was 2.1 million cases in 2013, 16 % of which were new EPTB cases, equating to 336,000 people with EPTB [2]. The inability to rapidly confirm TB diagnosis and determine subsequent drug susceptibility remains one of the greatest hindrances to successful TB control [3]. Multidrug-resistant and extensively drug-resistant TB disproportionately affect HIV patients and result in increased morbidity and mortality [4].

The increasing demand for more rapid and reliable methods for the diagnosis of TB has led to the widespread introduction of molecular diagnostic procedures into the clinical microbiology laboratory. A rapid molecular test known as Geno Type MTBDR plus (Hain, Life Science) is a Line Probe Assay (LPA), which has been approved by WHO in 2008 for the diagnosis of MDR-TB [5]. Line probe assays use PCR and reverse hybridization methods for the rapid detection of mutations associated with drug resistance. They are designed to identify MTBC and simultaneously detect mutations associated with drug resistance [6]. This study was planned to evaluate the use of LPA for detection of MTBC directly from pulmonary as well as extrapulmonary samples & detect the resistance to INH and RIF

and simultaneously, compare the results with conventional culture.

MATERIALS AND METHODS: This prospective study approved by Institutional Ethical Committee was conducted over a period of one year (February 2017 to January 2018) in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana. Various samples like sputum, endotracheal secretion, BAL, pleural fluid, gastric aspirate, CSF, pus & tissue (except blood and urine) received from clinically suspected cases of TB from all age groups, admitted in various wards, ICUs and outdoor patients during the study period were included. Concentration and decontamination of all the specimens except CSF were done using the N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) standard method. Tissue biopsy samples were homogenized before decontamination. All the samples were subjected to microscopy, conventional culture (LJ) and LPA.

Direct Microscopy: The smears were prepared directly from the sample and after concentration procedures and subjected to Ziehl-Neelsen (ZN). The smears stained by ZN method were examined under oil immersion of light microscope for Acid Fast Bacilli (AFB) [7].

Conventional culture: Concentration and decontamination of specimens was carried out using NALC/NaOH method. 0.1 to 0.25ml of processed specimen was inoculated on 2 slopes of Lowenstein Jensen (LJ) medium and was incubated for 8 weeks [8].

Line Probe Assay: The test was performed as per manufacturer's guidelines. This assay is based on DNA STRIP technology. There are 3 Steps of LPA: DNA extraction from decontaminated samples, amplification by PCR and reverse hybridization.

RESULTS: A total of 347 clinically suspected patients of tuberculosis were enrolled in the study. Majority of cases were in the age group of 51-60 years (18.4%) followed by cases in age group ≤10 years (17.9%).Out of 347 cases 226(65.1%) were males and 121(34.9%) were females with male :female ratio of 1.9:1. Among suspected tuberculosis patients, cough with expectoration (55.9%) was the commonest complaint, followed by evening rise of temperature (49 %), anorexia (19.6%), and weight loss (14.4%).

A total of 369 samples were received from 347 clinically suspected patients of tuberculosis. Double requisitions were received for 22 suspected cases of tuberculosis. Various samples included sputum samples 117 (31.7%), pleural fluid 63 (17.1%), gastric aspirate 42 (11.4%), CSF 30 (8.1%) etc. (Figure 1).

On comparison of direct microscopy with line probe assay, microscopy was positive in 60 cases, out of which LPA was positive in 55 cases whereas negative in 5 cases. Out of 287 smear negative cases, LPA was positive in 36 cases. Accordingly, the positive & negative predictive value of LPA was 91.67% and 87.05% respectively (Table 1).

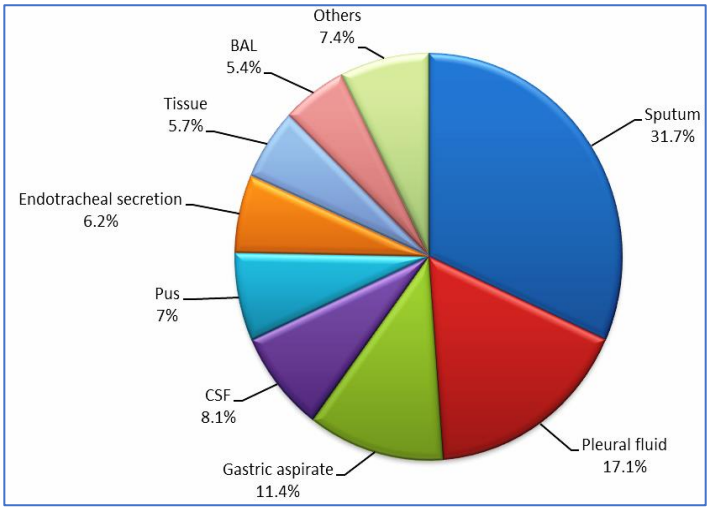

Figure 1: Distribution of various samples (n=369)

In our study, among 347 cases, positivity by Line probe assay was 91(26.2%) followed by 60(17.3%) by direct microscopy and 56(16.1%) by conventional culture (L-J medium) (Figure 2).

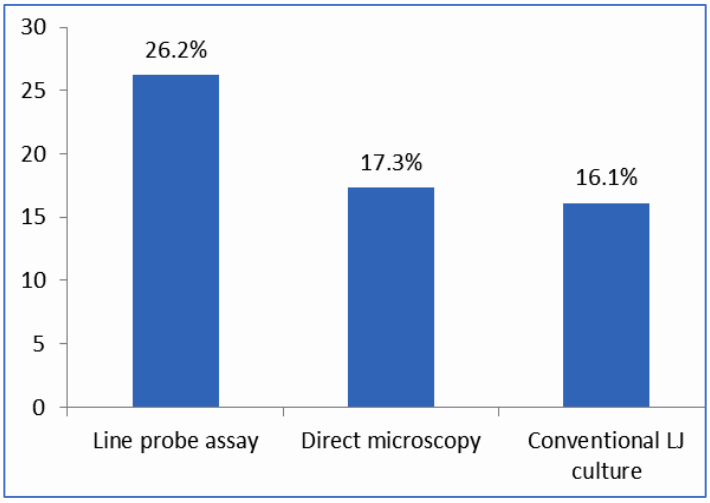

Figure 2: Comparison of positivity by different methods

Table 1: Comparison of Direct microscopy with Line probe assay (n=347)

		Line probe assay		Total	p-value
		Negative	Positive		
Direct microscopy	Negative	251	36	287	0.000
	Positive	5	55	60	
Total		256	91	347	

On comparison of conventional culture with line probe assay, culture was positive in 56 cases out of which line probe assay was positive in 48 cases & negative in 8 cases. Among 291 culture negative cases, LPA was positive in 43 cases. Accordingly, the positive & negative predictive value of LPA was 52.75% and 97.17% respectively **(Table 2).**

Table 2: Comparison of Conventional culture (LJ) with Line probe assay (n=347)

		Line probe assay		Total	p-value
		Negative	Positive		
Conventional culture (LJ)	Negative	248	43	291	
	Positive	8	48	56	0.000
Total		256	91	347	

In our study, out of 347 suspected cases, 91 were diagnosed MTB-positive, out of which, 78(85.7%) were sensitive to both RIF and INH and 13(14.3%) showed resistance to either or both rifampicin and isoniazid. Out of these, MDR were 2(2.2%), mono-resistant RIF were 3(3.3%) and mono-resistant INH were 8(8.8%).

DISCUSSION: The present study showed comparative analysis of conventional methods for diagnosis of tuberculosis with molecular methods. In our study majority of suspected TB patients were males 226 (65.1%) whereas females were 121 (34.9%). The average age at presentation ranged from 51-60 years. In a study conducted in the Netherlands on diagnosed cases of tuberculosis, the majority of the patients were males (65%) and the average age at presentation was 42 years [9,10]. In our study, male: female ratio was 1.9:1 whereas Baboolal *et al* reported male: female ratio of 4:1 in their study [11].

In our study direct microscopy, conventional culure & LPA positivity 17.3%, 16.1% and 26.2% respectively. Similar findings were observed in a study conducted by Arslan *et al* [12] in which direct microscopy and culture positivity was 11.33% and 15.47% respectively, whereas, study conducted by Jaishankar Sharma and his colleagues [13] showed higher positivity of 82%, 80% & 95% by ZN staining, culture & LPA respectively.

In our study, among 91 MTB-positive cases, on LPA, 85.7% were sensitive to both RIF and INH and 14.3% showed resistance to either or both rifampicin and Isoniazid whereas Yadav *et al* reported higher resistance (40%) [14].

Only 2(2.2%) isolates were MDR in the present study whereas in literature higher MDR (28%[14], 16.9%[15], 14.3%[16]) was reported by various authors.

In present study, monoresistance to RIF 3(3.3%) and INH 8(8.8%) was observed. Various other studies also depicted comparable results in which monoresistance to RIF (1%, 7.1%, 3.6%) & to INH (10%,7.1%, 19.6%) was reported [14,15,16].

CONCLUSION: Laboratory diagnosis by microscopy and culture is still the gold standard for the detection of *Mycobacterium tuberculosis*. Conventional culture using Lowenstein Jensen media takes prolonged time for identification that leads to the delay in treatment and spread of the disease. LPA performed directly on the samples gives results within 6-8 hours where Conventional culture and drug susceptibility test takes 8-12 weeks.

Increase in MDR cases has resulted in potential threat to community. Advent of molecular tests can reduce the risk for delay in treatment. LPA performed directly on samples is a excellent tool for fast detection of MTBC along with INH and RIF resistance. In this study we can conclude that line probe assay gives results within 6 -8 hours thus assisting in early diagnosis, preventing the delay in treatment and spread of multidrug resistant strains thus helping in halting the disease

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