ORIGINAL RESEARCH PAPER | Medical Science | Volume : 6 | Issue : 8 | August 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

# Molecular Line Probe Assay for the Detection of Multi Drug Resistant Tuberculosis and Comparison of Results with Conventional Solid Culture and Drug Susceptibility Testing

KEYWORDS


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**ABSTRACT** ***Background:*** *Drug-resistant TB often goes undetected and untreated in many countries. The laboratory is an essential component in TB control programs to provide culture and drug susceptibility test services. In this study we evaluated the performance of line probe assay (LPA) in comparison with LJ culture DST.* ***Methods:*** *Samples from Three thousand and sixty two (N=3062) MDR-TB suspects were included in the study. Sputum smear microscopy was done for all the specimens and all smear positive specimens were processed for LPA and LJ culture and DST.* ***Results:*** *Overall concordance between conventional DST and LPA was observed among 652 specimens. In comparison to 238 culture isolates observed sensitive on LJ C-DST, 226 were found sensitive by LPA (concordance=95%). Further, In comparison to 426 culture isolates observed resistant on LJ C-DST, 12 more cases were found resistant by LPA (Concordance= 97.3%).* ***Conclusion:*** *Overall concordance of the LPA with conventional DST was 96%.*

**Introduction**

The global threat of multidrug-resistant tuberculosis (MDR-TB; strains of *Mycobacterium tuberculosis* resistant to at least rifampicin and isoniazid) to TB control, underscores the importance of prompt and rapid identification of such resistant *Mycobacterium tuberculosis* strains. Isoniazid (INH) and rifampicin (RIF) are the key first-line anti-tuberculosis drugs, and resistance to these drugs i.e. MDR-TB, is likely to result in treatment failure and poor clinical outcomes.[1] India has the largest number of estimated MDR-TB cases amongst notified TB patients of any country.

Smear microscopy has long been known as the primary method for screening of TB, with a case detection rate of not more than 68%.[2] Conventional culture-based methods, Lowenstein Jensen (LJ) culture and drug susceptibility testing (C&DST) remain the "gold standard" for TB diagnosis in developing countries as these techniques have been greatly improved and routinely used over the past decade. However, the time for a bacteriological culture-based diagnosis of TB may require several weeks to months.[3] To address such delay in TB diagnosis as well as to discretely improve the diagnosis accuracy, molecular diagnosis aspects need to be considered for the early detection of MTB which involves the detection of the mutation in specific genes imparting resistance against rifampicin (RIF) and/or isoniazid (INH), mostly used as the first line anti-tubercular drugs.

Newly developed molecular based methods have advantages over conventional phenotypic methods in terms of both accuracy and turnaround time. The GenoType MTBDRplus assay is a commercially available line probe assay (LPA) from Hain Lifescience, Nehren, Germany, and is designed to simultaneously detect the most important gene mutations conferring R (*rpoB* genes) and H (*inhA*, *katG*) resistance in MTB isolates in 8 hours.[4]

Although the GenoType MTBDRplus assay has been studied in several laboratories, there is a wide variation in circulating MTB strains across the globe, and false negative results can occur due to the presence of unique genetic mutations in the different settings. Hence validation in different settings is needed to ensure acceptable performance.

Therefore, in the present study we attempted to evaluate the performances and utility of the LPA assay for the rapid and detection of multi drug resistant tuberculosis. The results of the molecular diagnostic method was compared with that of the conventional phenotypic drug susceptibility test (DST) to demonstrate the sensitivity, specificity and accuracy of LPA with its capacity to shorten the time required for TB diagnosis compared to that of the conventional method.

**Materials and Methods**

**Selection of cases**

Samples from a total of Three thousand and sixty two (N=3062) MDR-TB suspects were included in the study

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from February 2014 to December 2015. All these cases were enrolled at different chest clinics of Delhi. Two sputum samples (spot or morning) were collected per patient in 50 ml sterile conical centrifuge tubes and were transported on same day to New Delhi Tuberculosis centre. In all these specimens, quality was examined by visual appearance. Sputum samples from 2431(79.4%) suspects were found Mucoid / Mucopurulent, 227(7.42%) were blood stained and 404(13.18%) were saliva.

**Sputum Microscopy**

Smears were prepared from all these specimens stained by Auramine staining and were read using LED flurescence microscope .[5] All smear positive sputum specimens were processed for molecular DST by Line probe assay (LPA) to screen resistance for two first line key drugs, isoniazid and rifampicin.

**Line probe assay (LPA)**

Sputum specimens were processed using N-acetyl-cysteine-sodium hydroxide (NALC-NaOH) decontamination (NaOH final concentration, 1.5%). Following centrifugation, the pellet in each tube was suspended in 2.5 ml of phosphate buffer pH 6.8. Processed sediments from the same patient were pooled and mixed thoroughly. One portion of the sediment was used for LPA and another portion was used for culture in LJ media.

Five hundred microlitres of processed sediment was used to perform the Genotype MTBDR*plus* (Hain Lifescience GmbH) assay, according to the manufacturer's instructions.[6]

**Conventional culture and DST**

Samples sediments were inoculated on to LJ media and were incubated at 37 C. The bacterial growth was observed on weekly basis. As soon as growth was observed in any particular tube, it was labelled as positive.

Primary culture isolates (N=664) were stored at -80°C. Before DST, frozen isolates were thawed and confirmed as *M. tuberculosis* complex using Nitrate reduction test and Catalase test and checked for contamination by growth on blood agar medium for 48 hours at 37°C prior to setting up DST for isoniazid and rifampicin according to manufacturer›s instructions (0.1 µg/ml isoniazid and 1 µg/ml rifampicin).

LPA testing was completed well in advance before conventional DST. Solid culture and DST was performed independently and without knowledge of LPA results. Results from both methods included in the data analysis after completion of all testing.

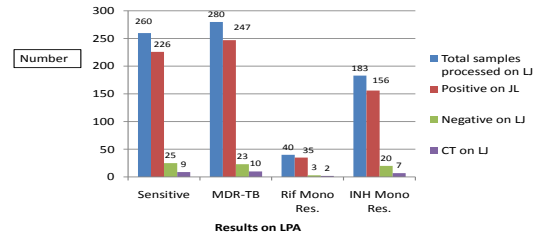
**Results**

Specimens from 2055 (67.1%) MDR-TB suspects were read as positive and specimens from1007 (32.9%) suspects were read as negative. All smear positive specimens were processed for LPA and total of 2019 specimen's generated valid LPA results and 36 specimens were found invalid / non-interpretable band pattern.

Of 2019 valid LPA tests, 1427 (69.4%) were found sensitive and 503 (24.9%) resistant to both or any of the two drugs. MDR pattern was observed among 280 (11.7) specimens, Mono rifampicin resistance among 40 (3.9%) and mono isoniazid resistance in 183 (8.9%) specimens.

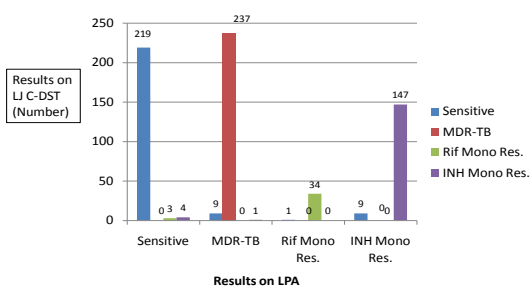
All MDR-TB (280), all Rif mono resistant (40), all INH mono resistant (183) and 260 randomly selected pan-sensitive cases were selected and specimen sediments of all these cases were processed for culture. Out of total 763 specimens processed for culture of LJ medium, 664 turned out to be culture positive, 71 culture negative and 28 became contaminated. All positive cultures were subjected to DST on LJ media (Fig 1).

**Figure 1** Culture outcome of smear positive specimens inoculated on LJ medium




Overall concordance observed between conventional phenotypic DST and LPA was observed among 652 specimens. In comparison to 238 culture isolates observed sensitive on LJ C-DST, 226 were found sensitive by LPA (concordance=95%). Further, In comparison to 426 culture isolates observed resistant on LJ C-DST, 12 more cases were found resistant by LPA (Concordance= 97.3%) (Fig 2).

**Figure 2:** Correlation of LPA and solid C&DST results




The sensitivity, specificity, PPV, NPV and overall accuracy of LPA results were compared to the conventional MGIT DST results for rifampicin, isoniazid, multidrug resistance and the ability of rifampicin resistance alone to predict MDR. An analysis of banding patterns associated with rifampicin and isoniazid resistance in MDR-TB and non MDR-TB strains was performed.

Overall concordance of the LPA with conventional DST was 96%. Sensitivity and specificity , PPV and NPV were 92%, 99%, 95% and 99% respectively for detection of RIF resistance; 97%,96%,94% and 96% respectively for detection of INH resistance 100% 96% , 96% and 100% respectively for detection of MDR TB. Table 1.

**Table 1: Performance of LPA test as compared to LJ C-DST in detecting resistance to rifampicin, isoniazid, and MDR-PTB in 664 smear positive sputum samples**

| | Rifampicin | Isoniazid | MDR |
|---|---|---|---|
| LPA | Resistant (N=35) | Resistant (N=156) | Resistant (N=247) |
| | Sensitive (N=629) | Sensitive (N=508) | Sensitive (N=417) |

Volume : 6 | Issue : 8 | August 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

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|---|---|---|---|
| Sensitivity, % | 92 (88-96) | 97 (94-99) | 100 (98-100) |
| Specific-ity, % | 99 (96-100) | 96 (94-98) | 96 (94-99) |
| PPV, % | 95 (90-99) | 94 (91-97) | 96 (90-99) |
| NPV,% | 99 (97-100) | 98 (95-109) | 100 (97-100) |

**Discussion**

The diagnosis of MDR-TB and XDR-TB is hampered by the absence of effective and affordable rapid diagnostic techniques for drug sensitivity. Several approaches, phenotypic and molecular, have been explored to develop rapid, reliable and accurate methods for the rapid detection of drug resistance in *M tuberculosis*.

The GenoType MTBDRplus enables the simultaneous molecular genetics identification of the *M. tuberculosis* complex and its resistance to rifampicin and isoniazid from clinical specimens or cultivated samples. The benefits of using Geno Type MTBDRplus are Efficient, Rapid, User-friendly, Flexible and Cost-efficient. Taking in to account of all above facts, the use of LPAs has been recommended by the WHO[7] and subsequently by RNTCP.

The findings of the present study indicated that the molecular method has been highly consistent with the conventional culture and DST method. Low frequency of discordant results and higher sensitivity in mono-drug and multi-drug resistance detection by the LPA method would be supportive of this fact. The method could almost successfully detect the mutation in *rpoB* gene (responsible for RIF resistance) as 100% sensitivity was estimated when compared to that of the conventional DST. The specificity (99%) and accuracy (98%) were also found to be higher than those from the conventional diagnostic methods.

Overall concordance of the LPA with conventional DST was 96%. Sensitivity and specificity , PPV and NPV were 92%, 99%, 95% and 99% respectively for detection of RIF resistance; 97%,96%,94% and 96% respectively for detection of INH resistance 100% 96% , 96% and 100% respectively for detection of MDR TB. Results of our study are in agreement with large scale demonstration studies conducted in South Africa in two laboratories for validation of Genotype MTBDR plus assay.[8] In Cape town laboratory the results obtained Sensitivity, specificity, PPV,NPV for detection of RIF were 98.9%, 99.4%, 97.9% and 99.7% respectively. Sensitivity, specificity, PPV, NPV for detection of INH was 92.2%, 99.7%, 99.1% and 97.9% respectively. Sensitivity, specificity, PPV, NPV for detection of MDR was 98.8%, 100%, 100% and 99.7% respectively. In Johannesburg, sensitivity, specificity, PPV, NPV for detection of RIF were 90.7%, 99.7%, 98.3% and 98.0% respectively. Sensitivity, specificity, PPV, NPV for detection of INH was 91.2%, 98.7%, 92.9% and 99.5% respectively.

In our study the target of completing the test within the turnaround time was achieved in 99% cases and the report was delivered to the concerned physicians within a day. Early detection of MDR or Rifampicin resistant by LPA led to early initiation of MDR treatment. Before the introduction of LPA in Delhi state, only 70% of diagnosed MDR TB cases by conventional LJ Culture and DST were put on treatment and the remaining cases were either died or defaulted. But after the introduction of LPA,, more than 90% of diagnosed cases were started on treatment.

It was reported earlier that over all the cost of performing the MTDB DR plus test was lower when the assay is performed directly on smear positive sputum specimens.[7, 9] In our study it was not possible to do costing analysis due to various factors like support by the government in the form of new equipment and supplies in addition to fulfilment of man power.

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