MOLECULAR DIAGNOSIS OF DRUG RESISTANCE TUBERCULOSIS IN THE DISTRICTS OF TAMILNADU

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ABSTRACT

To diagnose the multi-drug resistant Mycobacterium tuberculosis by using molecular methods from pulmonary specimens of presumptive TB suspects belonging to the districts of Tamil Nadu. Two thousand three hundred and thirty five (2335) clinical specimens of presumptive MTB patients were collected from 23 districts of Tamil Nadu between the period of January and March 2015. Smear microscopy was performed by LED fluorescent microscopy. All smear positive samples were tested using Line Probe Assay (LPA) and smear negative samples were tested by Xpert MTB/RIF (Xpert) to detect the percentage of drug resistance pattern and to identify MTB complex. Among 1235 smear positives subjected to LPA method; 116 (9.4%) MTB was not detected and 3 (0.2%) showed invalid result; 1116 (90.4 %) strains showed MTB positive; 896 (80.3 %) were sensitive for both rifampicin (RIF) and isoniazid (INH) drugs; 128 (11.5 %) were resistance for INH; 18 (1.6 %) resistant for RIF and 58 (5.2 %) were resistance for RIF and INH and smear negative specimens were subjected to Xpert. Out of 1090 specimens; 647 (59.4 %) MTB was not detected; 30 (2.8 %) showed an invalid/error results; 413 (37.9 %) strains showed MTB positive of which 379 (91.8 %) were sensitive for RIF; 24 (5.8 %) showed resistant for RIF and 9 (2.2 %) showed MTB detection and RIF Indeterminate. LPA and Xpert molecular technology are the rapid, feasible and reliable methods for the detection of multi drug resistant (MDR) mutation.

Key Words: Multi Drug Tuberculosis, LPA, Gene Xpert, Rifampicin and Isoniazid.

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INTRODUCTION

World Health Organization (WHO) estimated, nine million people fell ill with TB and 4,80,000 people developed Multi Drug Resistant TB (MDR-TB) and 2,10,000 deaths were estimated in 2013. Isolation, identification and drug susceptibility testing (DST) of MTB take several weeks, because of its slow growth rate. Many methods have been developed which can potentially reduce the diagnostic time from days to hours. MDR-TB resistance to both RIF and INH is a global problem with an estimated death of 1,90,000 cases in 2014. Detection of tuberculosis and drug resistance pattern are slow by means of phenotypic method. Availability of molecular diagnosis has made possible the early detection of drug-resistant tuberculosis (DR-TB). Genotypic rapid molecular methods for the diagnosis of isoniazid and rifampicin resistance in MTB are based on the detection of genetic mutation in the rpoB gene, which encodes RNA polymerase enzyme of MTB and katG gene encoding the mycobacterial catalase peroxidase which is the only enzyme in MTB capable of activating the pro-drug INH to its active form. Mutation in the rpoB gene is responsible for majority of RIF’s resistance in MTB and the inhA shows low level isoniazid resistance. GenoType®MTBDRplus is one such commercially available assay which has been previously evaluated in Europe, South Africa, Far East and North America. INH and RIF are the key first-line anti-tuberculosis drugs and resistant to these drugs denotes MDR-TB. This multiplex Polymerase Chain Reaction (PCR) based reverse hybridization genotype MTBDR rapid line probe assay is to detect the most common mutations of katG and rpoB from smear positive sputum specimens and MTB growth positive cultures, either from liquid or solid, and it has the potential to shorten the overall turnaround time from specimen receipt to reporting of results of susceptibility testing. Xpert MTB/RIF is an automated molecular test for MTB and to identify the resistance to RIF, uses real-time PCR assay to amplify an MTB specific sequence of the rpoB gene. The Xpert assay utilizes molecular beacon technology to detect DNA sequences amplified in a hemi-nested RT-PCR assay. Five different nucleic acid hybridization probes are used in the same multiplex reaction. Each probe is complementary to a different target sequence within the rpoB gene of rifampicin susceptible MTB and is labelled with a differently coloured fluorophore. Together, these overlapping probes span the entire 81 bp core region of the rpoB gene. The assay utilizes single-use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing, the Xpert cartridge is then inserted into the Xpert device, DNA extraction and heminested RT-PCR, this provides results within 2h.

MATERIALS AND METHODS

CLINICAL SPECIMENS

A total of 2335 specimens of MDR suspects were received for culture and drug susceptibility testing for MTB from 23 District TB centre (DTC) of Tamil Nadu from January to March 2015. These specimens were collected, packed and transported as per standard operating procedure from DTC’s to Intermediate Reference Laboratory (IRL). Ten specimens were rejected due to leakage and for 2325 specimens, the smear microscopy was performed by fluorescent microscopy method (Auromine-O)-LED FM. The smear positive specimens were subjected to LPA genotypic method. Genotype MTBDR plus V2.0 (LPA method) according to the manufacturer’s instruction. Smear negative specimens were processed by Xpert automated molecular method.

SPECIMEN DIGESTION AND DECONTAMINATION

Collected specimens were processed by NALC-NAOH (4%) method (N –acetyl –L cystine – sodium hydroxide with sodium citrate 2.9 %) and neutralized with Phosphate buffer solution pH 6.8. Centrifuged at 3000g for 20 min and the supernatant was discarded and the pellet was reconstituted with 1 ml of PBS, after discarding the supernatant.

GENOTYPE MTBDR ASSAY

Rapid genotypic method Line Probe Assay (LPA) was used for the detection of MTB and their drug resistance pattern to RIF and INH, from various districts of Tamil Nadu smear positive specimens were processed by GenoType® MTBDR plus V2.0 (LPA method) according to the manufacturer’s instruction.

XPERT MTB/RIF TECHNOLOGY

Smear negative specimens were processed by Xpert MTB/RIF, an automated molecular test for MTB to identify the organism as well as resistance to rifampicin. Xpert uses real-time polymerase chain reaction (PCR) assay to amplify an MTB specific sequence of rpoB gene.

RESULTS

A total of 2335 specimens were received in the study period at the IRL,Chennai, Tamil Nadu. Of these,2325 specimens were processed by NALC-NAOH method. Based on smear microscopy, 1235 were observed as smear positive and 1090 as smear negative. Smear positive specimens were processed by LPA method and the results are shown in flow chart. Out of the 1235 smear positive subjected to LPA method; in 116 (9.4%) specimens MTB was not detected and 3 (0.2%) showed invalid results.1116 (90.4 %) specimens showed MTB positive of which 896 (80.3 %) were sensitive for both Rif and INH drugs. 128 (11.5 %) were resistant to INH, 18 (1.6 %) were resistant to RIF and 58 (5.2 %) were resistant to both Rif and INH. Smear negative specimens were subjected to Xpert. Out of 1090 specimens; MTB was not detected in 647 (59.4 %) and 30 (2.8 %) showed invalid/error results. 413 (37.9 %) specimens showed MTB positive of which 379 (91.8 %) were sensitive to RIF. 24 (5.8 %) showed resistance to RIF and 09 (2.2 %) showed RIF indeterminate results.
Figure 1
*Total TB suspects tested and the outcome of results by molecular technology*

![Diagram illustrating the testing process and results](image)

Table 1
*Age and gender wise distribution of presumptive TB cases with MTB and MDR detection*

<table>
<thead>
<tr>
<th>Age (Yrs)</th>
<th>Gender</th>
<th>Total suspects tested (A-B)</th>
<th>MTB detected (E+F)</th>
<th>MTB detected (%)</th>
<th>Mono INH Res (P)</th>
<th>Mono INH Res (%)</th>
<th>Mono RIF Res (N+O)</th>
<th>Mono RIF Res (%)</th>
<th>Both INH &amp; RIF res (M)</th>
<th>Both INH &amp; RIF res (%)</th>
<th>MTB Not detected (G+H)</th>
<th>MTB Not detected (%)</th>
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<td>0</td>
<td>0</td>
<td>6</td>
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<td>33.3</td>
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<td>72.5</td>
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DISCUSSION

There is an absolute necessity for application of new technology for rapid diagnosis of tuberculosis on day-to-day basis, which is the need of the technology in developing countries like India where the prevalence of TB is high. It is based on methods that permit recognition of mycobacterium products in clinical specimens. Genotypic testing is useful for rapid identification of M. tuberculosis in pulmonary samples, and results may be generated within a few hours. In addition, this technique has a higher sensitivity than sputum AFB smear microscopy. Genotypic approach based on amplification of MTB DNA by PCR in clinical specimens is a rapid and valuable screening method to overcome the limitations of the conventional techniques and it may have high impact on patient health and further will be in control of transmission of the disease. The current study is the first study to be carried out in the state of Tamil Nadu with such a large sample size (2335) for detection of Mycobacterium and INH and RIF resistance. In our study 2335 specimens were transported from 23 districts of Tamil Nadu of which 10 (0.42 %) samples were rejected due to inappropriate quality sample collection and packing. The smear positive specimens were processed by LPA method. Of the 1235 smear positive subjected to LPA method; 1116 (90.4 %) specimens were positive for MTB; 11.5 % were identified to be mono resistant to INH; 1.6 % were identified to be mono resistant to Rif and 5.2 % were identified as MDR (resistant to both Rif and INH). The resistance patterns of these MTB strains using LPA for detection for Rif and INH resistance were observed to be 6.2 % and 11.5 % respectively this percentage has been observed to be uniform for both the drugs across districts in Tamil Nadu and which is lesser than other published study, ie MDR 9 % and Mono Rif resistant11 %. Smear negative specimens were subjected to Xpert technology. Out of 1090 smear negative specimens; 37.9 % specimens were showed MTB positive; 5.8 % showed resistance to Rif as per study conducted across India the positive predictive value for detecting rifampicin resistance among smear negative using Xpert MTB/RIF is 5.1 %. Using XPERT technology MTB was detected in 413 (37.9 %). This percentage is low, since the samples from people living with HIV (PLHIV) patients and smear negative patients were tested even though our study shows higher percentage of MTB detection compared to Gidado et al study.28 Challenges in the amplification of mycobacterial DNA on extraction from biological specimens such as sputum are due to several inhibitors in these specimens, which may lead to several errors. LPA invalid results were very minimal (0.2 %) and Xpert error/invalid was observed in 2.8 % which is lesser than other study ie 4 %. In our study, it has been observed that the number of female suspects are very less compared to male suspects (Fig. 2), which is similar to the other study ie, only 28 % females were suspected themselves for testing, which may be due to several reasons such as ignorance, social stigma towards the disease, and reluctant attitude towards self-health and also the high prevalence of MTB (63.6%) between the age group of 30 and 60 yrs (Table 1), this is similar to that described by Holmes et al.29 This lower notification rates are reported among women, whereas men having high rates of notification are at older adolescence. The limitation of this study is related to the Socio-economic characteristics. Clinical information details were available in DTC and not documented in IRL; Only LPA and Xpert technique for 2325 samples have been performed and not compared with other techniques to detect INH and RIF resistance. Studies have shown that solid culture from smear negative specimens for MTB diagnosis and susceptibility testing, may take up to 84 days. The application of molecular methods in the study has enabled early diagnosis (2h) from smear negative cases using Xpert technology by 38 %. This technology is rapid for diagnosis of MTB/RIF resistance, which in-turn has reflected in early treatment and thus reduction in transmission of MDR in the society. Hence, these methods are simple and easy to use.

CONCLUSION

Based on the molecular methods, the diagnosis of MDR TB from presumptive TB suspects of 23 districts in TN, the following pattern of resistance have been observed in our study: among the smear positives 11.5 % have been observed to be resistant to INH, 6.2 % resistant to Rif and 5.2 % resistant to both INH and Rif using LPA facility. Among the smear negative 5.8 % were observed to be resistant to Rif using Xpert facility. It is also observed that the presumptive TB suspects were less in females 22.4 % than male patients 77.6 % and MTB infection is more common 73% in the male adults in the age group 30 to 60 Yrs. This study also clearly demonstrates the rapid diagnosis of MTB from smear negative patients, which in-turn would help in appropriate treatment and thus reduction in transmission of MDR-TB in the society.

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CONFLICT OF INTEREST

Conflict of interest declared None.
REFERENCES


