ABSTRACT

BACKGROUND

Tuberculosis is one of the most important infectious disease with high mortality rates especially in developing countries. Mycobacterium tuberculosis is the most frequent cause of bacterial disease in humans. In 2012, there were an estimated 8.6 million incident cases (range, 8.3 million-9.0 million) of TB globally (equivalent to 122 per 100000 population). In 2012, 6.1 million cases of TB were notified to national TB programmes (NTP). Of these 5.7 million people were newly diagnosed in 2012 and 0.4 million were previously diagnosed TB patients whose treatment regimen was changed. Among TB patient notified in 2012, an estimated 300 000 (range 220 000-380 000) had multidrug-resistant TB (MDR-TB), (WHO Global TB Report 2013).

Tuberculosis is emerging as a global problem especially MDR-TB and associated HIV/AIDS. MDR-TB is defined as TB that is resistant to both isoniazid (INH) and rifampicin (RIF). LJ proportion method, though considered the gold standard earlier is time consuming and diagnostic delays are likely to contribute to the transmission of resistant isolates. The recent emergence of extensive drug resistance has greatly increased the significance of rapid susceptibility testing significance of rapid susceptibility testing of M. tuberculosis strains.

It is crucial to detect drug resistance timeously in clinical isolates of M. tuberculosis for administering of appropriate treatment to avert the development of further resistance and the spread of resistant strains. Line probe assay is one such molecular method which allows a safe and fast detection of the M. tuberculosis complex and its resistance to RIF and/or INH in a single procedure.
AIMS & OBJECTIVES

Aim of this study is Evaluation of line probe assay to assess drug susceptibility of *Mycobacterium tuberculosis*.

Objectives of the study are:

1. Isolation & Identification of *Mycobacterium tuberculosis* in clinically suspected tuberculosis patients.
2. To compare the performance of line probe assay with solid culture method for an early diagnosis of MDR-TB.

METHODS

In this study samples were collected from clinically suspected cases of pulmonary tuberculosis in Department of Microbiology, Subharti Medical College, Meerut. A total of 392 samples were processed and cultured on Lowenstein-Jensen medium and out of 206 culture positive samples 175 isolates of *M. tuberculosis* were grown and identified. The isolates were further tested for drug sensitivity by both conventional and molecular methods. Drug Susceptibility of isolated *M. tuberculosis* were performed by conventional 1 % proportional method against isoniazid, rifampicin, streptomycin and Ethambutol and Line Probe Assay (LPA) to detect mutations. Results of line probe assay were compared with that of conventional drug susceptibility testing (DST) on solid media [Lowenstein Jensen (LJ) method].

RESULTS

Out of 392 enrolled patients, 178(45.4%) were smear positive by ZN staining while 206 cultures were mycobacteria positive, of which 175(84.9%) were *M. tuberculosis* and 31(15.1%) were MOTT. DST results by LPA and LJ methods were compared in 175 isolates. Out of 175 *M. tuberculosis* culture positive patients, the percentage of
new patients was 69/175 (39.4%) and previously treated was 106/175 (60.6%). Prevalence of primary drug resistance was 20%, 4.4%, 8.7% and 11.6% for isoniazid, rifampicin, ethambutol and streptomycin respectively. 2.9% were multidrug resistant i.e. resistant to both rifampicin and isoniazid. Of the 106 isolates from cases with previous history of treatment of varying duration (acquired drug resistance), 66 (62.3%) were resistant to one or more anti-tuberculosis drugs. Resistance to isoniazid was estimated to be 47.2% followed by resistance to rifampicin in 37 (34.9%), streptomycin in 35 (33%) and ethambutol in 20 (22.5%). 32.1% strains of these drug resistant isolates were multidrug resistant. The LPA detected INH resistance in 52 of 64 cases, RIF resistance in 39 of 40 cases, and MDR-TB in 35 of 36 cases as compared to the conventional method. Overall (RIF, INH and MDR-TB) concordance of the LPA with the conventional DST was 93.1%. Compared to conventional drug susceptibility testing, the sensitivity and specificity were 81.3% and 100% respectively for INH resistance; 97.5% and 100% respectively for detection of RIF resistance; 97.2% and 100% respectively for detection of MDR-TB.

DISCUSSION

The pattern of drug resistance varies from place to place and at different period of time. Prevalence of resistance to any drug, MDR and individual drug resistance to isoniazid, rifampicin, ethambutol and streptomycin was significantly higher in patients who were treated in the past. The rate of MDR-TB was 2.9% in primary cases which is similar to the proportion of MDR-TB among new cases reported globally ranges from 0% to 28.3%. The reported prevalence of MDR-TB among acquired TB cases has varied from 8% to 67% in previous studies from different parts of India and our findings are in concordance with such observations.
Sensitivity of LPA for detection of RIF resistance, and MDR was similar to previously reported results in studies from South Africa, Germany and Italy. We observed that the LPA test results had a good concordance with the conventional DST with a additional advantage of a shorter turnaround time.

**CONCLUSION**

The Line probe assay provides an early diagnosis of monoresistance to isoniazid and rifampicin and is highly sensitive and specific for an early diagnosis of MDR-TB. The specificity of line probe assay is 100%, thus when compared with gold standard. Also LPA is failed to detect only 1 case of rifampicin resistant strains.

Based on these findings, it is concluded that LPA test is a rapid and easy-to-perform test for the detection of MDR *M. tuberculosis* strains that can readily be used in a routine laboratory work flow.

High sensitivity, short turnaround times and the potential for screening large numbers of specimens rapidly, make the GenoType® MTBDRplus assay suitable as a first-line screening assay for drug resistant TB.