TB is one of the oldest diseases known to affect humans. It is a major cause of death worldwide and has been a major health problem for developing countries. If properly treated tuberculosis caused by drug susceptible strain is curable virtually in all cases. Both INH and RIF are potent bactericidal antituberculosis drugs used in tuberculosis control programs. So increasing drug resistance to commonly used drugs RIF and INH in isolates of *M. tuberculosis* is a major cause of concern.

Drug resistance in *M. tuberculosis* is caused by random genetic mutation in relatively restricted region of the genome. MDR-TB results from either primary infection or may develop in the course of patient treatment. The outcome of treatment of patients harboring MDR *M. tuberculosis* strains has been poor with high mortality rate. Their chance of being cured is very low and they require significant expenditure of healthcare resources. Moreover, these patients remain infectious for a prolonged period and may therefore be more likely to infect others. The level of drug resistance provides an epidemiological indicator to assess the amount of resistant bacteria transmission in the community as well as the success of tuberculosis control programs. Further, this influences the therapeutic regimens and policy decision also..Delay in recognition of drug resistance can result in increase in resistance rate of MDR, its transmission and high cost. Thus rapid detection of drug resistance will have obvious patient as well as public health benefits, including better prognosis, increased survival, prevention of acquisition of further resistance & reduce spread of drug resistant strains to vulnerable populations.

The pattern of drug resistant varies from place to place and at different period of time. The drug susceptibility pattern of *M. tuberculosis* is essential for
proper control of MDR-TB in every health care settings, hence the study was initiated with the aim of studying the prevalence of MDR-TB in our area.

Conventional culture & DST is a slow process, requiring isolation of mycobacteria from clinical specimens, identification of M. tuberculosis complex & testing of susceptibility pattern of strain in presence of anti-tuberculosis drugs. Depending on the methodology, culture & DST can take at least four months to be completed. During this time patient may be inappropriately treated drug resistant strains may continue to spread & amplification of resistance may occur. Drug resistance can now be assessed rapidly within one day. With this background, the present study was carried out to know the resistance rate to the first line drug in primary as well as acquired cases which can allow initiation of modified treatment. Also accuracy of LPA was detected. So in this study we evaluated the concordance between line probe assay and conventional method which was considered as gold standard was found.

In our study out of 392 clinical samples, Mycobacteria was grown in 206 samples thus the detection rate was 52.5%. A study conducted by Jain et al (2008) in Lucknow, Uttarpradesh showed 62.3% frequency.200

Gender distribution in the present study showed maximum males (73.1%) whereas females (26.9%) were lesser. The difference in percentage was found to be statistically significant (p<0.01). In our study male: female ratio is 2.7 similar to that reported in WHO SEAR Annual 2013.201

The patient profile of our study with respect to gender was quite similar to study conducted by Urassa et al (2008) (68% males and 32% females).202
The overall percentages of resistance to different antituberculosis drugs obtained from different surveys done by WHO\textsuperscript{203} using standardized guidelines showed that the levels of primary resistance to INH as single agent ranged from 0 - 16.9 per cent and for STR 0.1 - 23.5 per cent. Initial resistance to RIF as single agent was unusual with a rate ranging from 0 - 3 per cent. The rate of initial resistance to EMB was low ranging from 0 - 4.2 per cent globally. High rates of primary resistance to INH have been reported from Kenya, India, and Haiti while it is reported to be low in southeastern England, Melbourne and Argentina. High rates of resistance to streptomycin were reported in Zaire, Pakistan and Brazil while low levels of resistance were reported from China, Ethiopia, Bosnia and Herzegovina. There are fewer surveys of acquired drug resistance and the rates are usually higher than those of primary resistance. The rate of acquired resistance to INH ranged from 4 - 53.7 per cent, to STR from 0 - 19.4 per cent, to RIF from 0 - 14.5 per cent and to EMB from 0 - 13.7 per cent. Initial drug resistance reported from India is 18 - 20 per cent for INH, 4.8-14 per cent for STR. Prevalence of initial MDR-TB reported from India varied between 0-5 percent.\textsuperscript{204,205} Data from India on acquired resistance are limited and concluded that any resistance to INH was in between 47.7\textsuperscript{206} to 87.1 per cent.\textsuperscript{207} For RIF, it was 28.3 - 80.6 per cent and for MDR it was between 9.6 to 80.6 per cent.\textsuperscript{22} It is evident that prevalence of drug resistant tuberculosis varied considerably throughout the world and particularly in India. The reasons for this variation in different studies were the criteria of selection of patients studied, the extent of misuse of drugs, the quality of questionnaire used for eliciting history of previous treatment, inadequate laboratory support and reporting systems.
Primary drug resistance was found in 37.7% in our study which is lower than 43.5% study conducted in 1997-1999 by Malhotra et al (2002). But higher than 27.7% reported in North Arcot in 1999 & 21.9% in Raichur district.

Primary drug resistance to INH in present study was 20%, which is similar to several other centers in India, such as 17.4% at Bangalore and 20.1% reported at Lucknow in 2008 but lower than 32.8% reported at Kolar in 1987-89. INH, when taken irregularly and inadequately, is known to give rise to bacterial resistance very quickly and this could be a reason for the increase in prevalence as seen in our study.

Monoresistance in INH in Primary cases was 11.6% in our study; comparable to the range of 10.7% to 12.8%, as reported by other Indian Studies (10.7% in Wardha District, Maharashtra state and 12.2% and 12.8% in Raichur district, Karnataka and North Arcot District, Tamil Nadu state respectively).

On the other hand, primary drug resistance to RIF in these isolates was observed to be 4.4% which is comparable to several reports elsewhere in India, varying from 2% in North Arcot to 6.8% in Jaipur. This was low as compared to the resistance documented in 2010 from other parts of world Kazakhstan (28.4%) and Karakalpakstan (23.6%). A high rate of RIF resistance was observed in United Emirate (32.5%) in which drug susceptibility testing was done by disk method. Jain et al (2008) found 12.5% RIF resistance in Lucknow, Uttarpradesh.

In our study, the rate of primary resistance of M. tuberculosis to STR was 11.6% which comes in the range from 0.1% to 23.5% as reported worldwide. This is much higher than other reports worldwide. Primary resistance was found to be much higher than 3.9% at Mayurbhanj District, Orrisa in 2001 and 6.8% at TamilNadu state.
In 1997, in our study, the rate of any resistance of \textit{M. tuberculosis} to STR was 24.6%.

In our study, primary resistance to EMB was 8.7% which is lower than 12.2% that reported at Lucknow in 2008, whereas in India, initial EMB resistance (0-14.3%) has been reported by various investigators.

The rate of MDR-TB was 2.9% which is similar to the proportion of MDR-TB among new TB cases reported globally ranges from 0% to 28.3%. It is similar or marginally higher than 0.7% at North Arcot in 1985-89; 1.2% in Bangalore in 1985-86 and 3% at Jaipur in 1993. Levels of MDR-TB remain worryingly high in some parts of the world, notably countries in eastern Europe and Central Asia. Our MDR-TB resistance rate is higher than 13.2% that reported by Jain et al (2008) in Lucknow.

Acquired drug resistance to INH in our study was 47.2% which is similar to that reported from Jaipur 39.7%, though North Arcot reported a high incidence of INH resistance of 67%.

Acquired drug resistance to RIF in our study was 34.9% which is comparable to several reports elsewhere in India, varying from 12% in North Arcot to 66.8% in Bombay.

The reported prevalence of acquired resistance for EMB was 22.5% which is similar to 22.5% at Lucknow in 2000-2002 by Jain et al (2008). Our results showed higher proportion of EMB resistance as compared with the result of Cohn et al (1997) who have reported acquired EMB resistance of 1-13.7%. Higher
prevalence of EMB resistance in present study may be partly due the inclusion of relatively more isolates from treatment failure and relapse cases.

Acquired prevalence of STR was 33% in our study which is similar to 34.2% reported at Lucknow by Jain et al (2008) while lower than 14.2% reported at Delhi in 2009. The rate of MDR-TB in our study was high. 32.1% of our strains had acquired multi-drug resistance which is similar to 33.8% at Gujarat (1980-86) and 33.3% at New Delhi (1990-91). Our MDR acquired resistance is lower than 48.2% reported at Islamic Republic of Iran. 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.

In a study conducted by Jain et al (2014), out of total 2496 isolates of *M. tuberculosis* of previously treated cases tested, 1139 isolates (45.6%) were pan-sensitive and 370 (14.8%) were pan-resistant. Total 695 isolates (27.8%) were MDR. Maximum resistance was INH 42.8% followed by STR 33.7%, RIF 29.7% & EMB 24.6% which is similar to our study.

In this study, the line probe assay correctly identified 81.3%, 97.5% and 97.2% of INH, RIF and combined resistance (MDR), respectively and 100% specificity of each.

The sensitivity of detection of RIF resistance was similar to that reported from Vietnam, Germany, Italy, Finland, France, Denmark, Turkey and Taiwan (92-100%, p>0.05). With respect to culture isolates, the sensitivities of the
MTBDR assay for detection of RIF resistance were recently reported to be in the range of 95% to 99%.\textsuperscript{29-32}

The LPA failed to detect one of RIF resistant strain, which was caused by rare mutation which may not be present on strip or by probable mutation in other genomic region of rpoB gene. But the number is very low which compensates highly to the time taken in detection and DST by conventional method which would otherwise may lead to MDR transmission and also patient taking ineffective treatment.

RIF is one of the most important chemotherapeutic agents used to combat infections by \textit{M. tuberculosis} and can be assumed to be a surrogate marker for MDR-TB.\textsuperscript{231} Moreover, more than 90% of RIF-resistant isolates are also resistant to INH; therefore, detection of RIF resistance could also identify MDR strains.\textsuperscript{232} The occurrence of RIF monoresistance is rare and more than 90% of RIF resistant isolates are also resistant to INH.\textsuperscript{233} Therefore RIF is a good marker of MDR-TB, and thus can be used as a predictor of MDR-TB.\textsuperscript{44,234,235}

Sensitivity of detection of INH resistance i.e. 81.3% was similar to that reported in 2010 by Albert et al (2010)\textsuperscript{236} but lower than reports from Vietnam, Germany, Finland, Denmark, Russia & Taiwan (84-100%, p>0.05) but higher than reported from Turkey, Italy, France (35-73% p<0.05).\textsuperscript{29-33,156,229,230,237} The low sensitivity may be due to mutation being more complex involving mutations in at least four gene complexes and not all mutations result in an alteration of the wild type phenotype as compared to RIF which is associated mainly with single point mutations in one region of the RNA polymerase gene (rpoB), in contrast INH drug resistance which involves mutation in multiple genes.
Overall sensitivity of the Genotype® MTBDRplus assay for detection of RIF resistance, and MDR was high at 97.5%, and 97.2% respectively which is similar to previously reported results in studies from South Africa, Germany, and Italy\textsuperscript{156,238,239} supporting the use of this assay for MDR-TB screening.

Most molecular techniques have focused on the rapid detection of RMP resistance, the most potent drug, and a good marker for MDR-TB in high prevalence settings.\textsuperscript{91,92} So in our study LPA was taken to prove its utility as a rapid technique in this area also.

The sensitivity of line probe assay for detection of MDR resistance varies from region to region. In our study, sensitivity was found to be 97.2% which is in accordance with previous studies 97.1% by Yadav et al (2013)\textsuperscript{240} and Nikolayevskyy et al (2009).\textsuperscript{237} Sensitivity was found to be higher than 88.9% and 69.8% reported by Huyen et al (2010)\textsuperscript{28} and Miotto et al (2006).\textsuperscript{31}

With 100% specificity in detecting RIF, INH and MDR indicating that no patient would be given wrong treatment thus decreases the chance of emergence of drug resistance and also conferring good personal health and in avoiding false positive health.

The results of the present study have shown that the MTBDR\textit{plus} assay is easy to perform and has the capability for the rapid detection of RIF and INH-resistant \textit{M. tuberculosis}. As previously shown for other strip assays\textsuperscript{23,24,31,32} the MTBDR\textit{plus} assay has been proven to be suitable for application both with culture isolates and directly with smear-positive specimens.
LPA are not complete replacement for conventional culture & DST, as mycobacteriological culture is still required for smear-negative specimens while conventional DST is still necessary to confirm XDR, nevertheless, the implementation of LPA in MDR-TB screening algorithms may significantly reduce demand on conventional culture & DST laboratory capacity. Because only some of mutations are target so molecular test must be confirmed by phenotypic test.

In our study MDR rate is high so additional cost of test could be identified in all new cases or retreatment in which MDR-TB is suspected & results of screening could allow appropriate rapid management of patient.

In general, molecular methods offer several advantages over conventional techniques for the rapid detection and identification of *M. tuberculosis*, such as the turnaround time for results, reliability, reproducibility and possibility to improve patient management. However, due to the requirement of additional equipment and trained personnel, most of these methods have not yet gained easy access to the routine procedures performed in the clinical mycobacteriology laboratory especially in low-income countries where TB is a more important health problem.

The obvious advantages of molecular techniques are their ability to provide results within hours, with increased sensitivities and specificities compared to microscopy, increased sensitivity compared to microscopy, increased sensitivity compared to culture in some cases of extrapulmonary infections. Also conventional culture on solid media is labor-intensive and it may take several weeks for colonies to become detectable; even then, the process may require further subculture for definitive identification.
The high degree of accuracy, the substantial reduction in reporting time, and the possibility for high throughput with substantial cost savings suggest that molecular testing has the potential to revolutionize the diagnosis of MDR-TB.\textsuperscript{238}

Thus transmission of MDR-TB can be stopped by improving laboratory capacity for rapid diagnosis of MDR-TB, initiating and rapid scale up of MDR-TB services, effective treatment of MDR-TB patients and evaluating the extent of second-line anti-TB drug resistance and management strategies.\textsuperscript{242}

The LPA is a reliable test when used on clinical isolates, but culture growth requires 2 to 4 weeks, and sensitivity results are not available at the beginning of therapy.\textsuperscript{231} Performing the test on selected clinical specimens may give critical information needed for appropriate treatment in a very short time and can be crucial for avoiding the transmission of drug-resistant \textit{M. tuberculosis} strains. Indeed, the results are available in the same day.

Thus LPA is found to be effective tool for MDR-TB screening in a high MDR-TB prevalent area & also good concordance of LPA & phenotypic DST results was found in our study. We know that RIF is potent anti-TB drug as well as treatment of MDR and RIF resistant patient is very difficult so detection of RIF is very important which is fulfilling by our test in which sensitivity for RIF and MDR resistance is high and also specificity being 100% which enhances that no patient would be given wrong treatment so concluding that LPA can be used a rapid test in the area in which MDR rate found is high as has been documented in our study.