INTRODUCTION

Tuberculosis (TB) is still a major public health problem in most developing countries and its incidence is rising in many developed countries. TB is a re-emerging infectious disease caused by a bacterium called *Mycobacterium tuberculosis* (*M. tuberculosis*).\(^1\) TB is predominantly a disease of respiratory system but it can also affect bones and joints, vascular system, central nervous system, genitourinary systems and lymphatic system.\(^2\) In 1993 the World Health Organization declared tuberculosis a global public health emergency.

Of great concern for TB control is the emergence of drug resistance.\(^3\) The emergence of resistance to drugs used to treat TB, and particularly multi-drug-resistant TB (MDR-TB), has become a significant public health problem and an obstacle to effective TB control.\(^4\) Factors contributing to outbreak and continued spread of MDR-TB include upsurgence of human immunodeficiency virus (HIV) infection/acquired immune deficiency syndrome (AIDS), insufficient control procedures and laboratory delays in identification and susceptibility testing of *M. tuberculosis* isolates.\(^5\) The increase in incidence of drug resistance may be due to, easy accessibility of the drugs to the patients, indiscriminate use of rifampicin, inadequate treatment received, poor patient compliance because of nausea and vomiting and improper treatment regimens practiced in the past/ being practiced by medical personnel.\(^6\)

*M. tuberculosis* remains one of the leading causes of morbidity and mortality worldwide, but *Mycobacterium spp* other than tuberculosis (MOTT) are increasingly important pathogens. Speciation is particularly important when choosing antibiotic regimen for immunocompromised patients, in which the presence of any acid-fast bacilli may be considered significant.\(^7\) The rapid detection and identification of
clinically important *Mycobacterium* spp. is essential for patient management and infection control.

According to the World Health Organization (WHO), India is the highest TB-burden country, globally, accounting for one fifth of the global incidence.\(^8\) In several surveyed areas of India, 1.0% to 3.3% of new TB patients have MDR-TB.

India has about 1.9 million new cases of TB annually, accounting for a fifth of new cases in the world.\(^9\) WHO estimates that 9.27 million new cases of TB occurred in 2007 (139 per 100000 population), compared with 9.24 million new cases (140 per 100000 population) in 2006. In 2008, the annual incidence was 1.9 million cases, of which 0.8 million were infectious new smear positive pulmonary TB [PTB] cases.\(^10\) Prevalence of MDR-TB in new cases and previously treated cases is reported as 3% and 12-17% respectively.\(^11\) WHO has estimated that 99,000 MDR-TB cases emerging in India in 2008-the largest number for any country globally after China.\(^11\)

Almost 30,000 cases of MDR-TB were notified in 2008; this is 11% of the total number of cases of MDR-TB estimated to exist among cases notified in 2008.\(^12\)

TB kills more people in India and Southeast Asia than any other infectious disease. In India, with an estimated 19, 62,000 new cases and 331,000 deaths each year. Of these 19, 62,000 new TB cases, 873,000 were new smear positive and MDR TB is 2.8% among all new TB cases. One person dies every minute and 500,000 people die per year from TB.\(^12\) In the year 2010, 10,025 MDR-TB suspects have been examined for diagnosis; 2,967 MDR-TB cases have been confirmed and 2178 MDR TB have been initiated on Category IV treatment.\(^11\)
Pulmonary TB can be diagnosed by its symptoms, chest radiography, sputum smear microscopy and by cultivation of *M. tuberculosis*, which is considered as the gold standard.\textsuperscript{3,13}

Under the Revised National Tuberculosis Control programme (RNTCP), sputum microscopy forms the mainstay for diagnosis as well as for monitoring response to therapy. But it cannot be used for assessing drug susceptibility status.\textsuperscript{14,15} Under RNTCP, treatment failure is predicted based on continued or new smear positivity at the end of fifth month of Antituberculosis Treatment (ATT). Such patients are then advised culture and drug susceptibility testing (DST), the time lapse that compromises on success of therapy and results in spread of MDR-TB for longer periods.\textsuperscript{13}

Although microscopy is fairly rapid, it suffers from low sensitivity, especially in extrapulmonary tuberculosis and paucibacillary cases common among HIV-infected individuals, and is not species specific.\textsuperscript{16} The culture-dependent laboratory procedures may take 7 to 10 days on most recently developed liquid media and 4-6 weeks on solid media. Early detection and identification of *M. tuberculosis* is particularly important because it can be transmitted from one person to another, and because diseases due to NTM require adapted treatment regimens.\textsuperscript{17}

Recent advances in molecular biology and molecular epidemiology, and a better understanding of the molecular basis of drug resistance in TB, have proved new tools for rapid diagnosis\textsuperscript{6}, however, the high cost of most of these techniques, and their requirements for sophisticated equipment and skilled personnel have precluded their implementation on a routine basis, especially in low-income countries.\textsuperscript{18}
Drug resistance is a threat to TB control programs. It is a major public health problem because treatment is prolonged and complicated, cure rates are well below those for drug-susceptible TB, and patients may remain infectious for months or years, despite receiving the best available therapy. There is an urgent need to identify methods that would rapidly detect both the presence of *M. tuberculosis* and drug resistant status.

TB resistant to treatment with the two most important antitubercular drugs (isoniazid and rifampicin) are known as MDR-TB. MDR-TB is reported both from India and other parts of the world. The emergence and spread of MDR-TB is threatening to destabilize global tuberculosis control. The prevalence of MDR-TB is increasing throughout the world both among new tuberculosis cases as well as among previously-treated ones. Although previous treatment for TB is the strongest risk factor for development of MDR-TB, patients treated first time are also at risk due to either spontaneous mutations or transmission of resistant strains. The risk of transmission of resistant strains from close contacts is increasing day-by-day because of the growing burden of MDR-TB patients. Therefore, we sought to determine the prevalence of MDR-TB among new cases of sputum-positive pulmonary TB. Rapid detection of drug resistance would help not only to optimize treatment of MDR-TB but also in breaking chains of transmission and identification of MDR in the region for proper implementation of the TB control programs.

In case the tubercle bacillus is drug resistant the ATT started for the patient will be ineffective and only after the report is ready after 8-10 weeks, correct treatment can be started. The most rapid results could be achieved by direct testing of patient specimens by fast molecular methods. These methods are based on the
knowledge that resistance to RMP and INH in *M. tuberculosis* is most often attributed to mutations in the *rpoB*, *katG*, and *inhA* genes.

The treatment of tuberculosis is commonly through a multiple drug regimen that includes the antimycobacterial drugs streptomycin, isoniazid, rifampicin, and ethambutol (EMB). It is important that the antimycobacterial drugs prescribed show appropriate activity against *M. tuberculosis*; i.e., susceptibility of the isolate to the drug. This study reports to determine drug resistance and susceptibility pattern of *M. tuberculosis*.

Mortality is particularly high in cases infected with multi-drug-resistant strains- i.e., strains resistant to at least isoniazid (INH) and rifampicin (RIF). Hence early detection of *M. tuberculosis* cases and the drug sensitivity pattern improves survival by prompt and correct therapy. Also early detection of cases promotes contact tracing, implementation of institutional cross-infection procedures, and other public-health actions.²⁵

Early diagnosis of tuberculosis and initiating optimal treatment would not only enable a cure of an individual patients but will also curb the transmission of infection and diseases to others in the community. Due to slow growth of *M. tuberculosis*, rapid identification methods using molecular techniques have been developed and utilized in the clinical laboratory. Of the several distinct components of TB Control Programme, case finding remains the corner stone for effective control.²⁶

For a drug regimen to be effective it is important to know the drug resistance pattern in that area, therefore, there is need to conduct periodic surveys of antibiotic
drug resistance and also to establish a continuous drug resistance surveillance program.

The aim of this study is to compare the performance of Line probe assay (LPA) with solid culture method among MDR-TB suspects for an early diagnosis of MDR-TB.

In the present study, sensitivity and specificity of LPA in detecting RIF and INH resistant strains in samples from tertiary care hospital in Meerut were compared with the conventional susceptibility method.

The prevalence of drug-resistant tuberculosis continues to increase around the world. With current methods based on culture, it takes about two months after collection of sputum to obtain the result of drug resistance test, i.e after sample collection, the tuberculosis organisms needed to growth in culture, and then, the part of organisms grown on its culture are put on another drug sensitivity test culture. At the start of therapy, empirical therapy with several antituberculous drugs are needed, and if failed to cure due to drug resistance of TB, the duration of hospitalization would be quite long. In particular, the rapid detection of drug resistance would be expected to over-come this problem. Several new technologies have been developed for the rapid assessment of drug resistance using real time PCR and line probe assay. Genotype® MTBDRplus assay is a commercially available assay that combines detection of M. tuberculosis complex with predilection of resistance to rifampicin and INH. The assay combines detection of M. tuberculosis complex with detection of mutations in the 81-bp hotspot region of rpoB, at codon 315 of the KatG gene and in the inhA promoter region. It was found to have high sensitivity and high specificity for rifampicin and INH resistance and performs well when applied directly
to AFB smear-positive sputum specimens. A recent meta-analysis pooled all these studies and calculated pooled sensitivity and specificity rates of 99% (95% confidence interval (CI), 96%-100%) and 99% (95% CI, 98%-100%) respectively for rifampicin resistance, and of 96% (93-98%) and 100% (99-100%), respectively for isoniazid resistance. In this assay the time taken for detection is around 24-48 hrs.

Mutations of the inhA and KatG genes of *M. tuberculosis* are involved in about 80% of resistance to INH, mutations of the rpoB gene control about 95% of resistance to RIF.

The present study will provide rapid diagnosis and drug susceptibility pattern of *M. tuberculosis* in clinical specimens thus allowing the commencement of an appropriate TB treatment regimen earlier. This would also help prevent transmission of drug resistant TB bacilli.