Tuberculosis continues to be a leading public health problem across the globe, particularly in the developing countries. India accounts for nearly one third of the global TB burden\(^1\) Further, the situation in India is worsened due to the emergence of multi-drug resistant (MDR) strains of Mycobacterium tuberculosis. Although, the phenomenon of drug resistance in Mycobacterium tuberculosis was observed as early as fifty years ago, the current threat is due to the emergence of strains resistant to the two most potent anti-TB drugs namely, isoniazid (H) and rifampicin (R) (multidrug resistant-tuberculosis, MDR-TB)\(^3\). The response of patients with MDR-TB to treatment is poor and the mortality rate is usually high. Since these patients need to be treated with expensive and toxic second line drugs, and may require hospitalization to manage their toxic reactions and other complications, they require a sizeable proportion of health care resources. Therefore, the detection of drug resistant TB (INH or RIF resistance) and other mycobacterial infections in the early stage of disease is utmost important to trim down the incidence. However, the available techniques such as BBL MGIT and molecular methods, which are widely used, in fact are not cost effective and affordable for most of the clinical microbiology laboratories in developing countries\(^91\). For this purpose, various rapid methods both colorimetric and non-colorimetric methods have been proposed, but these methods have not been evaluated in field conditions\(^5\)\(^-\)\(^24\). Hence the current study was designed to evaluate the performance of two rapid methods (direct TTC assay and direct MODS assay) for the detection of MDR TB with proportion method (using LJ media) on Ziehl-Neelsen smear positive sputum specimen. The results were analyzed in terms of sensitivity, specificity, positive predictive value, negative predictive value, turnaround time, contamination rates, and cost per sample.

In this study, clear descriptions of all enrolled patients were recorded for the reader to compare the TB suspects in our settings with other settings as well. Table IIa shows the demographic details of the study participants. The study was
conducted on direct AFB smear positive sputum specimens (N=1000) collected from 68.65% and 32.89% male and female patients, respectively confirming the usual sex distribution of PTB in the Southeast Asia region. However, the age distributions showed a peak case detection among the age range of 20-40 years were comparable with previous reports published by WHO\textsuperscript{137}. It is evident from the Table IIIa that 28.3% study participants having smoking habits, which was comparable earlier reports as well\textsuperscript{138-140}. However, a higher percentage of (26.94%) study participants were having the family history of TB. This was comparable with Bhargava et al\textsuperscript{140} wherein 23% patients where possessing family history of TB. Further, in the current study, 90.11%,80.21%,89.63%, 77.59%,100%,6.67%48.39%,50.3% and 51.85%, were having persistent low grade fever, night sweats, weight loss, loss of appetite, cough more than two months, hemoptysis, fatigue, chest pain, dysapnoea respectively. This data was comparable with previous reports as well\textsuperscript{39,43}

The results of the study, which included for the analysis, were categorized as valid results. However; the results which were not included for the analysis were classified as invalid results. In the present study, of the thousand sputum specimens processed, a total of 83.9% of sputum samples produced valid results (Table Ia). This data was comparable with previous results as well, wherein 94% of the samples showed valid results\textsuperscript{17,43}. In the current study, a total 16.1% of sputum samples showed invalid results. However, Bwanga et al demonstrated a total of 6% invalid results. This can be substantiated due to the fact that the current study protocols insist the collection of sputum specimens from patients receiving anti tuberculosis treatment as well. This may in turn affected the viability of mycobacteria in culture, resulting in the lack of growth in various culture techniques investigated in the study. Further, it is evident from Table IVa that the distribution of the sputum samples processed using Indirect LJ PM, Direct TTC assay, Direct MODS assays. Among these, Indirect LJ PM, Direct TTC
assay, Direct MODS assays could produce 85.2%, 84.9%, 84.3%, valid results respectively. This data was comparable with previous results as well, wherein 94 % and 95%, respectively (for LJ PM and Direct MODS assays of the samples) showed valid results\textsuperscript{17}. However, we could not compare the direct TTC assays valid results (84.9%) with other techniques, as no studies have been documented hitherto in literature. But it may be due to the similar basal media (Middlebrook 7H9 broth along with Bacto casitone, OADC enrichment and PANTA antibiotic mixture) used in both direct TTC assay and direct MODS assay, the difference (among the valid results) between both direct TTC assay and direct MODS assay were minimal.

Invalid results occur in the MTB drug susceptibility assays due to contamination of the assays, no growth of the MTB strains in the culture media, cross contamination of assays, growth of non tubercular mycobacteria\textsuperscript{92}. The invalid results were varied from 14.8%, 15.1% and 15.7% for LJ PM, Direct TTC assay, Direct MODS assays, respectively. These decreased invalid results in indirect LJ PM Method (14.8%) may be due to the low contamination rate\textsuperscript{93}. In the current study, Table V demonstrates the overall distribution of invalid results as obtained by indirect LJ PM, direct TTC assay, direct MODS assay. Among the invalid results (N=161), Indirect LJ PM, direct TTC assay and direct MODS assay could not demonstrate growth in 66.4%, 62.73%, and 63.98% case of AFB smear positive sputum specimens. This may be probably due to the death of MTB strains caused by exposure to alkali treatment (the current study protocol uses NALC – NaOH pretreatment method for decontamination of all the sputum specimens) or it may also be due to physiological or chemical changes of various sputum specimens after pretreatment with NALC-NaOH method\textsuperscript{94}. Alternatively, the Indirect LJ PM, Direct TTC assay, Direct MODS assay could detect growth of non tuberculous mycobacteria in 3.1% in each technique. This reduced occurrence of NTM may be due to the excellent laboratory sterilization techniques (proper
sterilization of bio safety cabinets after carrying out the smear and culture techniques) adopted in the current study, which must have in turn reduced the laboratory cross-contamination, it is known that NTMs are ubiquitous in environment and may cross contaminate the laboratory LJ cultures\textsuperscript{95}. In addition, among the invalid results (N=161), 21.12%, 24.84% and 26.09% sputum specimens were contaminated in indirect LJ PM, direct TTC assay, direct MODS assay, respectively. This low contamination rate may possibly be due to the use of low concentration NaOH (final concentration of NaOH was 1%) for decontaminating the sputum specimens\textsuperscript{94}. Interestingly, it was also noted that 1.24%, 3.1% and 4.35% of the sputum specimens were confirmed to be cross contaminated. This data was comparable with previous workers wherein the cross contamination rate was found to be 0.5-6\%\textsuperscript{95-99}

Most of the TB laboratories confront contamination of culture media particularly in the liquid culture media\textsuperscript{101}. In the present study, the rate of contamination (monthly variation) among various drug susceptibility tests were noted (Table VI). A total of 3.4%, 4% and 4.2% specimens were contaminated in indirect LJ PM, direct TTC assay and direct MODS assay, respectively. This contamination rate was comparable with earlier reports wherein the overall contamination rate was 3.3% and 3\%, respectively\textsuperscript{17,102}. However, the contamination rate of liquid media in the current study was low. This probably may be due to the addition of BBL PANTA mixture (Polymixin B 6000 units, Azlocillin 600µg, Nalidixic acid 2400 µg, TMP-600µg, Amphotericin B-600 µg) in the liquid media. Further, the maximum contamination rate (1.2\% 1.3 and 1.3 \% specimens for indirect LJ PM, direct TTC assay and direct MODS assay) were reported in between June and July. This relatively higher contamination rate in the June- July months may be possibly due to the hot and humid climate conditions prevailed during that time, which must have in turn favored the growth of contaminating organism. However, no contamination was
reported during December and January months, possibly due to the cold climate condition, which might have minimized the multiplication of contaminating organism in the sputum specimens.

The distribution of TB culture contaminating organisms varies between study settings\textsuperscript{97-99,101}. In the current study, Table VII demonstrates the distribution of contaminating organisms. In all the drug susceptibility tests, fungal contamination was prevailing over bacterial contamination (but among bacteria, gram positive bacilli contamination was predominant over gram positive cocci contamination). This predominance of fungal contamination of drug susceptibility results can be attributed to the fact that the respiratory system of the pulmonary tuberculosis patients are colonized with various fungal agents\textsuperscript{103}. These fungal agents must have overgrown in the culture media, giving rise to contaminated culture results or these fungal agents may also be acquired from the environment while transportation. However, the higher preponderance of Gram positive bacilli contamination in the study probably may be originated from exogenous source more specifically; acquired from the external environment while transportation. However the predominance of the Gram positive cocci contamination in the study may be resulted from the overgrowth of endogenous normal flora (sputum specimens contain gram positive cocci as normal flora.

In the current study, Table VIII demonstrates overall performances of the entire drug susceptibility assay investigated in the study. Among the valid results (n=839), Indirect LJ PM, direct TTC assay and direct MODS assay could detect 84.74%, 84.03%, and 83.31%, respectively as susceptible isolates (susceptible to either INH or RIF or both). This data was comparable with previous reports as well in which 65%, 62% and 66% were identified as susceptible (to both Isoniazid and rifampicin ) by direct nitrate reductase assay (a colorimetric assay), direct MODS assay (non colorimetric assay) and indirect proportion method, respectively\textsuperscript{17}. Mohammadzadeh et al studied the efficiency of indirect TTC assay
and proportion method for the detection of MDR TB strains on isolates obtained from patient samples. This data (52.17% and 47.83% isolates were detected as susceptible by direct PM and Indirect TTC assay, respectively) was comparable with the current study wherein 84.74% and 84.03% isolates were susceptible to Indirect LJ PM, direct TTC assay, respectively. In addition, the Indirect LJ PM, direct TTC assay and direct MODS assay could detect 15.26%, 15.97% and 16.69%, respectively as resistant isolates (resistance to either INH or RIF or both) from the AFB smear positive sputum specimens. This prevalence rate was comparable with previous studies from other parts of India and across the world as well. On the other hand, the detection rates of MDR strains (15.26% and 16.69% cases of MDR strains were detected by Indirect LJ PM and direct MODS assay) were comparable with previous report as well wherein 19% and 17% isolates were detected by Indirect LJ PM and direct MODS assay, respectively. However due to paucity of literatures, the detection rate of direct TTC assay was not compared with Indirect LJ PM. But Mohammadzadeh et al compared the proportion method and indirect TTC assay for the detection of MDR strains directly on MTB isolates obtained from clinical specimens wherein the proportion method and TTC assay could detect the 47.83% and 52.17% isolates as resistant to either INH or RIF or both. This detection rates where comparable with the current study in which, the Indirect LJ PM and direct TTC assay could detect 15.26% and 15.97%, respectively from clinical specimens. However, this disparity in the detection rates of MDR TB between the current study and previous study (Mohammadzadeh et al) may possibly due to the difference in distribution of resistance pattern among the isolates tested, more specifically; the current study was carried out on a direct AFB smear positive sputum samples (n=839). However, Mohammadzadeh et al carried out the study on twenty three MTB isolates (MDR TB isolates, n=11, susceptible TB isolates, n=12). In addition, the results of other colorimetric methods such as Nitrate reductase assay
the MTT test, and the resazurin microtitre assay were comparable with direct TTC assay (15.97%) of the current study. 

Rate of detection of drug resistance from clinical specimens varies between drug susceptibility testing techniques. In the present study, Table X and XI illustrates the comparison of rate of detection of drug resistance between Indirect LJ PM, direct TTC assay and direct MODS assay (for INH). Indirect LJ proportion method and Direct TTC assay could detect 29.68%, 32.85%, 31.43% isolates as resistant to INH, respectively. This data was comparable with a study carried out at Christian Medical College, Vellore, Tamil Nadu (2012), wherein the INH resistance detected by Indirect LJ proportion method and direct MODS assay was found to be 27.5% and 25.63%, respectively. Alternatively, Mohammadzadeh et al affirmed the complete agreement between indirect TTC assay and proportion method for the detection of INH resistance (directly on MTB isolates). However, in the current study, the direct TTC assay could detect 32.85% of isolates as resistant to INH when compared to indirect LJ PM (isoniazid resistance was found to be 29.68%). This disparity in the detection rates of these two assays possibly may due to the low specificity of direct TTC assay for the detection of INH resistance directly from sputum specimens. However, this difference was not statistically significant (Fishser’s exact test; P value 0.8996). But for the rate of detection of RIF resistance, we have compared Indirect LJ PM with direct TTC assay and direct MODS assay (Table XII and XIII). It is evident from the table XII and XIII that the indirect LJ proportion method, direct TTC assay, direct MODS assay could detect 10.94% and 10.45% and 10.45% of isolates (for each) as resistant to RIF, respectively. This data was comparable with a study carried out by Lazarus et al, in which Indirect LJ proportion method and direct MODS assay could detect 22.12% and 21.88%, respectively. However in the current study Indirect LJ proportion method and direct MODS assay could detect 10.94% and 10.45% isolates as resistant to RIF,
respectively. This disparity in the detection rates of these two assays possibly may due to the low specificity of direct MODS assay for the detection of RIF resistance directly from sputum specimens, although this difference was not statistically significant (Fisher’s exact test; P value 1.000). On the other hand, Mohammadzadeh et al affirmed the complete agreement between indirect TTC assay and Proportion method for the detection of RIF resistance (directly on MTB isolates)\textsuperscript{8} This finding was in accordance with the current study in which both indirect LJ PM and direct TTC assay could detect all the rifampicin resistance strains confirming the higher specificity for the detection of rifampicin resistant strains from the sputum specimens. In addition, resistance to both INH and RIF were also studied using indirect LJ PM, Direct TTC assay and direct MODS assay (\textbf{Table XIV and XV}). Indirect LJ proportion method, Direct TTC assay and Direct MODS assay could detect 59.38\%, 59.7\%, 59.29\% isolates as resistant to both INH and RIF, respectively. This four (in direct TTC assay) and seven false positive results (direct MODS assay) may be possibly due to the reduced specificity of direct TTC assay and direct MODS assay for the detection both INH and RIF resistant MTB strains directly from sputum specimens.

Drug resistance in TB may be broadly classified as primary and acquired. Drug resistance in a patient who has never received anti-TB treatment previously is termed as primary resistance. ‘Acquired resistance’ is that which occurs as a result of specific previous treatment. The term ‘initial resistance’ is used to indicate primary resistance and resistance among patients whose history of previous chemotherapy is not known. WHO and the IUATLD have now replaced the term ‘primary resistance’ with the term ‘drug resistance among new cases’ and ‘acquired resistance’ with the term ‘drug resistance among previously treated cases’\textsuperscript{109}. It is evident from the \textbf{Table XVI} that 18.59\% isolates were resistant to either INH or RIF or both. Among these (n=156) isolates, 13.83\% and 4.77\% samples were previously treated or newly diagnosed cases, respectively. This was
in accordance with a study conducted at Bangalore in which the multi-drug resistance in previously treated cases was found to be 12.8 percent. Further, NTI, Bangalore conducted drug resistance surveillance (DRS) in the four districts of Karnataka [Mysore (2001), Hoogly (2003), Mayurbhanj (2003) and Bangalore city (1999)] in which MDR-TB levels amongst patients with no history of previous treatment (drug resistance in new cases) were found to be 1.2 percent, 3.0 percent, 0.7 percent, and 2.2 percent, respectively. This data was comparable with current study as well wherein 4.77% of the patients were belongs to newly diagnosed TB cases.

In the current study, of the 41.13% of the resistant isolates detected among the previously treated TB patients, 11.7%, 5.32% and 24.11% of the MTB isolates were resistant to INH, RIF and both INH and RIF, respectively (Table XVII). This data was comparable with a study conducted at Bangalore wherein the multi-drug resistance among previously treated cases was found to be 12.8 percent and ranged from 8.4 to 17.2 percent. In addition, a single time-point cross sectional survey carried out by TRC, Chennai in a cohort of 3,357 smear-positive patients in North Arcot district found the frequency of acquired drug resistance to be 67% to Isoniazid, 12 % to Rifampicin and 11% to both Isoniazid and Rifampicin. Another study conducted, on 440 patients belongs to the model DOTS area in Tiruvallore district of Tamil Nadu (1999-2003) revealed the incidence of MDR TB to be 11.8 per cent. However, due to the paucity of literature and surveys regarding the prevalence drug resistance pattern from various parts of India particularly from Northern India; the comparison with other studies was not established. Further, the studies from India are deficient in several aspects, such as lack of standardized methodology, improper elicitation of previous treatment history, sample selection, non-uniformity in bacteriological procedures, sub-standard drug powders used for susceptibility testing and lack of quality assurance studies. In addition, the higher MDR prevalence rate among
previously TB treated cases may be attributed to many factors such as a) poor case holding, administration of sub-standard drugs, inadequate or irregular drug supply and lack of supervision ; b) improper prescription of regimens; c) interruption of chemotherapy due to side effects d) non-adherence of patients to the prescribed drug therapy; e) availability of anti-TB drugs across the counter, without prescription; f) massive bacillary load g) illiteracy and low socio-economic status of the patients the epidemic of HIV infection; (h) laboratory delays in identification and susceptibility testing of M. tuberculosis isolates; (i) use of non standardized laboratory techniques, poor quality drug powders and lack of quality control measures; . However, the data of current study was comparable with data across the globe as well. Among the previously treated cases, median prevalence of resistance to any drug was 33.4 per cent (range 0-93.8%). The median prevalence of MDR-TB among treated cases was 7.0 per cent, ranging from 0 per cent in eight geographical settings to a maximum of 58.3 per cent in Oman116

Newly diagnosed TB patients are at risk of developing MDR TB due to the transmission of resistant strains from MDR TB affected patients. Further, the risk is enhanced by spontaneous mutations of the strains117. Of the 7.18% of the resistant isolates, 3.05%, 0.89% and 3.24% of the MTB isolates were resistant to INH , RIF and both INH and RIF, respectively (Table XVIII). This data was comparable with a previous reports wherein the median prevalence of MDR-TB in new cases of tuberculosis was 1.1 per cent (range 0-14.2%)4. Further, the Tuberculosis Research Centre (TRC) carried out controlled clinical studies to estimate the prevalence of primary drug resistance among newly diagnosed TB patients. Among these Controlled clinical studies [TRC, Chennai] a total of 3500 patients were included over the last decades. The isoniazid, , rifampicin and both isoniazid and rifampicin resistance was found to be 10-16%, 05-1% and < 1%, respectively4. Paramsivan etal also reported an initial resistance to rifampicin to
range from 2.5-4.4% while the prevalence of MDR-TB was around 3 per cent.\textsuperscript{118-119} The results of another study in the Wardha district of Maharashtra revealed resistance to isoniazid, rifampicin and to both drugs to be 15.2, 0.5 and 0.5 per cent respectively.\textsuperscript{120} An additional short-term study carried out at Jabalpur district of Madhya Pradesh showed an initial resistance to isoniazid, rifampicin and to both drugs to be 16.1, 1.8 and 1.1 per cent, respectively (TRC, unpublished data).

Various culture based methods have been proposed to determine the drug susceptibility of MTB\textsuperscript{5-34}. In the current study, indirect LJ PM, direct MODS and direct TTC assays were evaluated to determine the distribution of INH, RIF and both INH and RIF resistance among previously treated and newly diagnosed TB cases (Table XIX -XXI). Among the both previously treated cases and newly diagnosed TB patients, the INH, RIF and both INH and RIF resistance (overall resistance by combining both previously treated cases and newly diagnosed TB cases) was found to be 24.36%,28.21% and 28.21% (for indirect LJ PM, direct TTC assay, direct MODS assay, respectively),8.97%,8.97% and 10.9% (for indirect LJ PM, direct TTC assay, direct MODS assay, respectively) and 48.72%,51.28% and 53.21% (for indirect LJ PM, direct TTC assay, direct MODS assay, respectively), respectively. This data was comparable with study carried out by Bwanga et al wherein indirect LJ PM, direct Nitrate reductase assays (a colorimetric assay) and direct MODS assay could detect resistance among 9%,11% and 11% MTB strains (INH resistance), 2%,3% and 2% MTB strains (RIF resistance), 17%,9% and 17% (both INH and RIF resistance), respectively. This relatively higher percentage of INH, RIF or both INH and RIF resistance among the MTB strains in the current study, probably may due to the inclusion of the resistance among previously treated TB cases and resistance among newly diagnosed cases altogether more specifically; the analysis of resistance is made from the total drug resistance cases [N=156 (116+40)]. However, Bwanga et al made the observation of drug resistance pattern of INH, RIF or both INH and RIF
from the whole study population, probably this would have been decreased the percentage of resistance. But the comparison between the percentage of resistance among previously treated TB cases and resistance among newly diagnosed TB cases were not performed independently due to the scarcity of literatures.

Grading of sputum smears has been emphasized in the WHO directly observed treatment short term course (DOTS) strategy\textsuperscript{121}. In the current study, a total of 20.26%, 38.25%, 22.29%, and 19.19% sputum specimens (percentage calculated by combining both susceptible and resistant sputum specimens obtained in Indirect LJ PM) were having the smear scores 3+, 2+, 1+, and scanty, respectively (Table XXII and XXIII). This distribution was in accordance with previous studies\textsuperscript{39-43}. It is apparent from Tables XXIV-XXXV that as the smear score increases the turnaround time for the detection of MTB also decrease, confirming an inverse relation between the turnaround time and smear scores of the sputum specimens. This inverse relation between the smear scores and the turnaround time possibly may be attributed to the number of tubercle bacilli in the clinical specimens, more specifically; as the smear scores increases, the number of parent bacilli (yielding progenies) also increases, thereby decreasing the duration of colony formation (each colony consist of millions of bacteria formed from a single parent cell). Further, it is also evident from the tables 22-33 that the TTD of indirect LJ PM, Direct TTC assay, and direct MODS assay varies from 38-120 days, 7-42 days, and 5-42 days, respectively. The low TTDs of direct TTC assay and direct MODS assay when compared to indirect LJ PM attributed to many factors; 1) the MTB multiplies well in liquid media than in solid media 2) the nutrients (for instance; Bacto casitone and OADC enrichments) supplemented in the direct TTC assay culture media enhances the growth of MTB strains 3) PANTA antibiotic mixture incorporated in the media inhibits the growth of contaminating organisms, making more selective for the growth of MTB 4) The TTC dye incorporated in the Middlebrook 7H9 broth changes to red colored
insoluble TTC formazan crystals (formed due to activity of mitochondrial dehydrogenase enzyme of living cells) formed as a red ring at the bottom of the tube and after one or two days the crystals undergo lysis producing reddish discoloration, confirming the growth of MTB or any other multiplying organism. In addition, the growth may be visible much faster than other colorimetric assays wherein addition of particular reagents at the particular interval is required to detect the growth of MTB. 5) Direct MODS assay utilizes the principle of direct microscopic observation of MTB chords (serpentine arrangement of MTB), which could be seen relatively few hours or days before the production of formazan crystals, producing relatively much faster results than the direct TTC assay. Further, it is also evident from the Tables XXVI, XXVIII, XXIX, XXXII, XXXIII, XXXIV, XXXV the TTD of MTB growth in indirect LJ PM, Direct TTC assay and direct MODS assay varies among newly diagnosed and previously treated cases. For instance, it is obvious from the table 26 (newly diagnosed TB cases) that the median TTD of indirect LJ PM method (46,63,86 and 94 days for 3+,2+,1+ and scanty, respectively), direct TTC assay (9,9,9,11 days for 3+,2+,1+ and scanty, respectively) and direct MODS assay (7,7,7,16 days for 3+,2+,1+ and scanty, respectively). it is also evident from the table 24 that (previously treated TB cases) the median TTD of indirect LJ PM method (76,46 69 and 89 days for 3+,2+,1+ and scanty, respectively), direct TTC assay (12,12,18,16 days for 3+,2+,1+ and scanty, respectively) and direct MODS assay (12,12,17,15 days for 3+,2+,1+ and scanty, respectively). This discrepancy of median TTD (an increased time to detection of MTB among previously treated TB patients than newly diagnosed TB cases) of all the DST techniques may be attributed to the fact that sputum specimens of patients treated with anti-tuberculosis drugs will reduce the number of AFBs in the sputum specimens, irrespective of the duration of treatment. In addition, the specimens with varying durations of treatment may
respond differently to sputum processing methods. This possibility might have increased the TTD of MTB growth among the previously treated patients.

In general, it is known that detection rate of MTB either in the direct AFB smear microscopy or in culture methods depends on gross appearance of sputum specimens as well. Therefore, various sputum-submission instructions have been reported to improve the detection rate of smear-positive TB, especially in females suspected of TB. In the present study, a total of 83.08%, 10.01%, 4.05% and 2.86% sputum specimens (percentage calculated by combining both susceptible and resistant sputum specimens obtained in Indirect LJ PM) were possessing the mucopurulent, mucoid, blood tinged and salivary consistency, respectively (Table XXXVI and XXXVII). This distribution was in accordance with earlier reports as well. In addition, it is evident from tables 36-47 that as the quality of sputum specimens increases, the turnaround time for the detection of MTB also decrease, confirming a contrary relation between the turnaround time and quality of the sputum specimens as well. This inverse relation between the quality of sputum specimens and the turnaround time possibly may be attributed to the quantity of tubercle bacilli in the clinical specimens, more specifically in the current study most of the sputum specimens with higher smear grades (3+, 2+) were possessing the mucopurulent consistency (as the smear scores increases, the number of parent bacilli (yielding progenies) also increases, thereby decreasing the duration of colony formation (each colony consist of millions of bacteria formed from a single parent cell). This must have resulted in decreased TTD of MTB from sputum specimens with mucopurulent consistency confirming that the mucopurulent sputum samples are ideal for isolation as well as relatively fast detection of MTB (Yoon et al. 2012). This higher percentage of rate of recovery and comparatively reduced median TTD of mucopurulent sputum samples [ for instance; table 37 shows a median detection time of mucopurulent sputum samples as 54, 12, 9 days for Indirect LJ PM, direct
TTC assay and direct MODS assay, respectively when compared to mucoid (86,14,7 days for Indirect LJ PM, direct TTC assay and direct MODS assay, respectively), blood tinged (86,9,9 days for Indirect LJ PM, direct TTC assay and direct MODS assay, respectively) and salivary (96,31,28 days for Indirect LJ PM, direct TTC assay and direct MODS assay, respectively) are attributed to the fact that the purulent sputum samples are formed in the lower respiratory tract when the severity of infection or tubercle bacillary load increases, more specifically as the severity of infection increases, the number of alveolar macrophage infiltration also increases in the lower respiratory tract. These macrophages engulf the tubercle bacilli and some of bacilli are killed while others survive or multiply or die inside the macrophages. Theses dead or persisting macrophages (containing large number of viable TB bacilli) are expectorated along with the lower respiratory tract secretions while spitting, resulting in sputum specimens with high bacillary load. In contrast, sputum samples with mucoid consistency, could detect the growth of MTB relatively longer median TTD than mucopurulent sputum specimens. This may be due to the low bacillary load in the mucoid sputum specimens more specifically; if the severity of infection is low, the number of macrophage infiltration also decreases, resulting in expectoration of mucoid or salivary sputum specimens with low bacillary load and macrophages. This probably could have in turn reduced the rate of detection of MTB from mucoid or salivary sputum specimens. In the current study, of the blood tinged sputum specimens could detect growth of MTB relatively faster than mucoid and salivary sputum specimens. This relatively rapid detection rate of MTB from blood tinged sputum specimens may be attributed to the severity of MTB infection. More specifically, the tubercle bacilli causes superficial mucosal inflammation and edema that further lead to the rupture of the superficial blood vessels causing bleeding in the respiratory tract. This blood is expectorated while patient is spitting indicating the apparent infection. 

Tables XXXX - XXXXIV,
XXXVI, XXXVII, XXXVIII XXXIX, demonstrate the TTD of MTB growth in indirect LJ PM, Direct TTC assay and direct MODS assay varies between newly diagnosed and previously treated TB cases. For instance, it is evident from the table 40 that (newly diagnosed TB cases) the median TTD of indirect LJ PM method (52, 86 86and 95 days for mucopurulent, mucoid, blood tinged and salivary, respectively.), direct TTC assay (9, 14,9 and 36 days for mucopurulent, mucoid, blood tinged and salivary, respectively.), and direct MODS assay (7, 7,9 and 28 days for mucopurulent, mucoid, blood tinged and salivary, respectively.). It is also evident from the Table XXXX (previously treated TB cases) that the median TTD of indirect LJ PM method (83, 76 76and 90.5 days for mucopurulent, mucoid, blood tinged and salivary, respectively.), direct TTC assay (14, 14,28 and 20 days for mucopurulent, mucoid, blood tinged and salivary, respectively.), and direct MODS assay (12, 12,7 and 13.5 days for mucopurulent, mucoid, blood tinged and salivary, respectively.). This discrepancy of median TTD (an increased time to detection of MTB among previously treated TB patients than newly diagnosed TB cases) of all the DST techniques may be attributed to the fact that sputum specimens of patients treated with anti tuberculosis drugs will reduce the number of AFBs in the sputum specimens, irrespective of the duration of treatment. In addition, the specimens with varying durations of treatment may respond differently to sputum processing methods. This possibility might have increased the TTD of MTB growth among the previously treated patients.

The sensitivity, specificity, positive predictive value and negative predictive value of the direct TTC assay and direct MODS assay were calculated and compared with indirect LJ PM. It is evident from Tables L-LXXIII that the diagnostic accuracy of direct 2,3,5-Tri Phenyl Tetrazolium Chloride (TTC) assay with indirect LJ PM among both newly diagnosed TB patients and previously treated TB patients. The sensitivity, specificity, PPV and NPV of direct 2,3,5-Tri Phenyl
Tetrazolium Chloride (TTC) assay for the detection of resistance to RIF, INH and their combination (MDR-TB) was excellent when compared to indirect LJ PM was excellent. The sensitivity, specificity, PPV and NPV of direct TTC assay was 

a) For newly diagnosed TB patients; 97.54%, 77.78%, 98.85%, 61.76%, respectively (for INH): 99.26%, 83.33%, 99.44%, 78.95%, respectively (for RIF) : 98.29%, 76.66%, 98.67%, 71.88%, respectively (for both INH and RIF).

b) For previously treated TB patients; 93.33%, 90.8%, 95.79%, 85.87%, respectively (for INH): 95.71%, 86.11%, 95.26%, 87.32%, respectively (for RIF): 91.3, 87.76%, 93.33%, 84.31%, respectively (for both INH and RIF).

However, this data was not compared with earlier reports due to lack of literatures. However, the overall sensitivity, specificity, PPV and NPV of Direct TTC (95.44%, 84.29%, 97.32% and 73.82%, respectively for INH; 97.48%, 84.72%, 97.35%, 83.13%, respectively for RIF; 94.8%, 82.21%, 96%, 78.1%, respectively for both INH and RIF) was comparable with other colorimetric assays (such as indirect TTC assay and direct nitrate reductase assays) as well. This excellent sensitivity, specificity, and ease of implementation show that the direct TTC assay to be technically suitable for rapid diagnosis of MDR-TB in low income high TB burden countries. Since most of the retreatment patients have non-MDR disease, this highly sensitive test should be used for rapid detection of MDR cases and to confidently exclude the majority without MDR disease. In addition, the relatively short time between specimen acquisition and susceptibility results, indicates that the direct TTC assay may be useful as a direct drug susceptibility screening tool in high burden TB countries. Further, the direct TTC assay isolates can be identified after sub culturing on solid media or if an additional MB7H9 broth containing para nitrobenzoic acid (PNB) tube included in the assay, may further reduce the time to detection.

The sensitivity, specificity, PPV and NPV of direct MODS assay for the detection of resistance to RIF, INH and their combination (MDR-TB) was excellent when compared to indirect LJ PM was excellent (Tables LXXIV-LXXXVII). The
sensitivity, specificity, PPV and NPV of direct TTC assay was ; a) For newly diagnosed TB patients: 98.49%, 85.19%, 99.24%, 74.19%, respectively (for INH); 99.07%, 94.44%, 99.81%, 77.27%, respectively (for RIF). 98.1%, 83.33%, 99.04%, 71.43%, respectively (for both INH and RIF). b) For previously treated TB patients: 92.33%, 90.8%, 95.79%, 84.95%, respectively (for INH); 94.76%, 87.5%, 95.67%, 85.14%, respectively (for RIF); 90.22%, 88.78%, 93.78%, 82.85%, respectively (for both INH and RIF). However, this data was not compared with other studies due to the lack of literatures. However, the overall sensitivity, specificity, PPV and NPV of Direct MODS assay (95.66%, 84.99%, 97.51% and 79.57%, respectively for INH; 96.92%, 90.97%, 97.74%, 81.23%, respectively for RIF; 94.16%, 86.06%, 96.41%, 77.14%, respectively for both INH and RIF) was comparable with other studies as well where the sensitivity and specificity ranged from 90%–100% 17,108,128,130. Additionally, more cases of false MDR-TB were detected with the MODS assay compared to the direct TTC assay. In the current study, MODS false resistant results could happen if artifacts are interpreted as cords since the identification test used was visual “cord formation”. It appears that failure to distinguish artifacts from cords and non-TB Mycobacterial growth from MTB cords can lead to a false resistant interpretation. Earlier reports also found false positive results with the MODS assay 131. These false positive results may also be due to the low specificity of direct MODS assay to detect MDR TB effectively from sputum specimens. Further, the specificity of MODS assay may be enhanced if molecular techniques [for instance; GenotypeH MTBDRplus assay (Hain Lifescience GmbH, Germany)] are incorporated in the study design. This assay detects mutations in the 81-bp hot spot region of the rpoB gene for RIF resistance and in the katG gene or inhA promoter region for INH resistance 132. In addition, a recent modification of MODS assay such as addition of a well with a Para-Nitrobenzoic Acid (PNB) – a reagent that prevents growth of MTB complex but not other mycobacteria would help to minimize false resistant
The MODS assay is however, potentially an economical test in laboratories with many samples but less incubator space since one plate is adequate for at least five samples. However, its lower technical performance compared with direct TTC assay in the study setting is a disadvantage. In addition, a disagreement between LJ proportion method and Direct MODS were also noted; a possible explanation for the disagreement between MODS test results and those of the comparator LJ proportion method lies in the qualitative nature of the MODS assay. In MODS testing, unlike solid-agar methods, there are no discrete colonies to count and, therefore, a resistance proportion cannot be calculated. In the MODS method, any growth in drug-containing medium indicates drug resistance, whereas in solid-agar testing, growth of less than 1% of that on drug-free medium is interpreted as susceptible. However, the relatively short time between specimen acquisition and susceptibility results, indicates that the MODS assay may be useful as a direct drug susceptibility screening tool. Further, liquid culture systems in diagnostic mycobacteriology are burdened with risk of aerosolisation when compared to conventional solid culture methods. Therefore, strict infection control measures need to be taken while processing the samples, right from inoculation to the reading of the plates.

Estimation of cost of assays are also important to implement the technique in the field conditions particularly in low income high burdened TB settings. In the current study, the cost estimates of the indirect LJ PM, direct TTC assay and direct MODS assay were calculated (Table LXXXVIII). For all the assays, the sputum processing charges (using NALC-NaOH method) was two rupees fifty paisa as the same processing method was used for all the techniques. However, for Indirect LJ PM; two LJ slants (one containing glycerol and the other one containing sodium pyruvate) for the isolation of MTB and eight LJ slants (for drug susceptibility testing) - four slants were containing INH and RIF separately, three plain LJ slants one for NEAT inoculation and other two for diluted MTB
culture ($10^{-2}$ and $10^{-4}$ dilutions) one slant with Para nitro benzoic acid and the other one act as growth control. The estimated cost for one indirect LJPM was fifteen rupees fifty paisa excluding the bottle charge, electricity, labor charges. The estimated cost for one indirect LJPM was eleven rupees fifty paisa this charge was excluded from the bottle charge, electricity, labor. For direct TTC assay; two test tubes with MB7H9 broth without antibiotic and two test tubes with INH and RIF separately, the estimated cost was nineteen rupees and eighty eight paisa. The direct TTC assay requires test tubes, growth supplements and PANTA mixture that may inevitably increased the cost per test 9 rupees more than Indirect LJ PM. For direct MODS assay; two wells with MB7H9 broth without antibiotic and two wells with INH and RIF separately and the estimated cost was thirty nine rupees one paisa. The MODS assay uses 24 well tissue culture plates; growth supplements and PANTA mixture have increased the cost per test (twenty seven rupees one paisa). The MODS assay also requires the use of an inverted microscope, which is not available in most TB laboratories. More recently, a less costly inverted microscope has been designed and in the future the MODS assay might cut the investments costs\textsuperscript{135}. However, it was difficult to estimate the costs of drug susceptibility assays studied as it depends on many parameters such as the variation the in the costs of reagents, labor charges, usage of electricity, usage of various instruments such as bio safety cabinets, inverted microscopes, incubators, micropipette tips and micropipettes, usage of gas. Therefore, the cost differences varies from place to place as reported in previous studies\textsuperscript{17, 136}.