Research article

Estimation of genotype MTBDRPLUS line probe assay in detection of rifampicin and isoniazid resistance in comparison to liquid culture (BACTEC-960) drug susceptibility testing in a tertiary care hospital from Eastern India

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ABSTRACT

Introduction and Aim: India has the uppermost trouble of Multidrug resistant tuberculosis (MDR-TB) is a major challenge controlling resistance, reducing transmission and improving handling outcomes in MDR-TB patients is dependent on susceptibility testing. Isoniazid (INH) and rifampicin (Rif) are the key first-line antituberculosis drugs, and resistance to these drugs *i.e.*, MDR-TB, is likely to result in treatment failure and poor clinical outcomes. The present study was done to compare the performance of line probe assay test (GenoType® MTBDRplus) with liquid culture (MGIT 960) system for the detection of resistance to first-line drugs.

Materials and Methods: We estimate the performance of LPAs to BACTEC MGIT 960 system for susceptibility testing of bacterial resistance to first-line drugs: rifampicin (RIF), isoniazid (INH).

Results: We performing Drug susceptibility testing (DST), 219/258 MTB cultures were viable after subculture the results of DST using the MGIT 960 system were compared to those obtained by line probe assay. LPA detected a total 46/258 (17.81%) samples as drug resistant, of which 35/258 (13.70%) were resistant to both rifampicin and isoniazid (MDR), 6/258 (2.28%) were rifampicin mono-resistant samples and 11/258 (4.11%) were isoniazid mono-resistant. Out of the culture-positive samples (219), LPA detected 39/219 (17.83%) as drug-resistant, of which 31/219 (14.2%) were resistant to both rifampicin and isoniazid, 5/193 (2.08%) were rifampicin mono-resistant and 8/219 (3.7%) were isoniazid mono-resistant.

Conclusion: Drug resistant TB poses an enormous threat to TB control programs worldwide. Effective treatment of MDR-TB is very expensive, particularly in middle income countries such as India.

Keywords: GenoType MTBDRplus test; BACTEC MGIT 960; MDR-TB; Rifampicin (RIF); Isoniazid (INH).

INTRODUCTION

Tuberculosis (TB) residue a most important global health problem, an estimated 86 million individuals developed TB and 1.3 million died from the infection (1). With a population of around 1.24 billion, India is the largest country in the South East Asian region. It ranks first among the high TB burden countries which contributed 26% of the estimated global incident TB cases in 2012 and also among the 27 multidrug-resistant TB (MDR-TB) high-burden countries worldwide, contributing to 21% of all MDR-TB cases estimated among notified cases (2). Multi-Drug Resistant TB (MDR-TB) is distinct as resistance to, at slightest, isoniazid and rifampicin among first-line drugs, while Extensively Drug Resistant TB (XDR-TB) refers to MDR-TB with further resistance to, at slightest, any fluoroquinolone and a few one of second-line injectable drugs in calculation to isoniazid and rifampicin.

BACTEC MGIT 960, a rapid liquid phenotypic DST method, is used in frequent laboratories to test for resistance to first-line drugs. The MGIT preparation normally information drug susceptibilities according

to predefined algorithms 4 to 13 days after inoculation (3). In 2008, WHO approved the use of the molecular test GenoType® MTBDR plus (Hain Lifescience, Nehren, Germany) for rapid revealing of high-risk MDR-TB cases constantly preparatory specimens (4, 5). The GenoType MTBDR plus assay is a commercially available Line Probe Assay (LPA) that allow specific gene markers associated with RIF and INH resistance to be detected. The GenoType® MTBDRplus evaluation is a molecular PCR-based extension and reverse blotting evaluate that employs accurate probes hybridized to nitrocellulose strips to recognize tuberculosis and its resistance to rifampicin (RIF) and isoniazid (INH) drugs (6). The approximation detects mutations in the *rpoB* gene for RIF resistance, in the *katG* gene for high-level INH resistance and in the *inhA* regulatory region gene for low-level INH resistance (3). During this study was appearance of molecular line probe assay to a MGIT 960 system for the detection of resistance to Anti-TB drugs.

MATERIALS AND METHODS

Study Design

This was a prospective study in Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna and Associated Hospital of Bihar, India between September 2018 and October 2020 were integrated. All tuberculosis isolates from sputum samples of clinically suspected cases of tuberculosis were seen in the study. The study protocol was approved by the IGIMS ethics committee (IEC numbe-485/IEC/2018).

MGIT subculture and DST

A whole volume of 200 µl of MGIT 960 TB culture was being inoculated in the MGIT 960 vials for sub culturing and growth detection. Positive cultures will be stained using the Ziehl-Neelsen (ZN) staining method to confirm M. tuberculosis.

Inclusion criteria

Multidrug-resistant TB (MDR-TB) and pre-MDR isolates **ii)** Patient of 18 year to 75 year of age groups **iii)** both sexes (male and female).

Exclusion criteria

Other than Pulmonary Tuberculosis (PTB) and Extra Pulmonary Tuberculosis (EPTB).

DNA isolation and line probe assay

For LPA DST, isolate were all the samples subjected to LPA Mention the culture medias being used for the 1st line LPA.

Agar proportion method

We lined out discrepancies between LPA and MGIT liquid culture by behavior DST with the agar proportion technique on the Microplate (Lasec, SA) from Lowenstein-Jensen medium culture (7). A deferment of 5– 10 mg of drug resistant-TB culture was used to make a McFarland standard.

Specimen collection

Sputum sample was being collected from PTB suspected patients, who was being attended hospital OPD. The Standard RNTCP protocol [8] was being followed for sample collection. The patients were provided a clean, dry, sterile wide-neck, leak-proof plastic container. Patients were being educated to cough deeply to produce sputum specimen and how to collect sputum without contaminating the collection container (8).

Processing of samples

Microscopic observation: Direct smear was being prepared from specimen for ZN staining and it will be examined under binocular bright field microscope, as per the standard procedure (8).

Conventional drug susceptibility testing using BD BBL™ MGIT™ AST SIRE SYSTEM

Subsequent to the cultures become positive, they were experienced for DST according to the BD BBL™ MGIT™ AST SIRE system within 7 days. For every sample, 4 ml of Middlebrook 7H9 broth was deposit in a sterilized universal bottle with 8–10 glass beads. A 10 µl sterile inoculating loop was used to abrade colonies from the LJ medium. The colonies were hanging in the broth to a turbidity of 1.0 McFarland standard. The deferment was vortexed for 2–3 min to break the large colonies. The suspension was then permitted to sit for 20 min to let the sediment settle at the bottom of the tube. The supernatant was suspiciously transfer to another sterile universal bottle and allowed to sit for another 20 min. The supernatant was then transfer to a third sterile universal bottle and adjusted to 0.5 McFarland. The adjustment was done by comparing the turbidity of the suspension with the turbidity of the McFarland turbidity standard made by combining 1% sulfuric acid (H₂SO₄) and 1% anhydrous barium chloride (BaCl₂) in the following volumes: for 1.0 McFarland (9.95 ml H₂SO₄ and 0.05 ml BaCl₂) and for 0.5 McFarland (9.9 ml H₂SO₄ and 0.1 ml BaCl₂). For susceptibility testing, 1 ml of the 0.5 McFarland-adjusted deferment be diluted in 4 ml of sterile saline to make 1:5 dilution (9, 10).

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), based on conformity between the DST gold standard and the index assay and 95% confidence intervals (95% CIs), were considered for both the antibiotic class and individual drugs. The agreement, sensitivity, specificity, positive and negative predictive values of LPA compared to BACTEC MGIT 960 system were intentional for Isoniazid (INH), Rifampicin (RIF).

RESULTS

Two hundred and eighty-two sputum positive samples of patients suspected to have drug resistant TB were picked and subjected to LPA and Culture MGIT. MGIT was positive for M. tuberculosis in 258/282(91.48%) were AFB smear positive.

Socio demographic characteristics

Of the 258 samples, 90 (34.80%) were from female and 168 (65.20%) from male participants. Transmission of tuberculosis is influenced by gender. Tuberculosis is more common among males than females (CDC, 2016; WHO, 2016) [17]. The prevalence of tuberculosis among males 168 (65.20%) was higher than that of females 90 (34.80%) and male to female ratio (M: F) was 1.7 (168/90), this difference was highly significant (p<0.01).

Table 1: Distribution of TB patients according to gender

Gender	Culture Based Diagnosis on Liquid culture	
	+Ve	%
Male	168	65.20
Female	90	34.80
Total	258	100%
Chi-square- χ^2	-	21.6791
P-value	-	0.00001

To evaluate the performance of LPA on smear positive results, 258 samples were used by LPA detected 241/282 (85.46%) samples as positive. LPA reported sensitivity and specificity of 99.31 and 97.43% respectively with positive and negative predictive value of 99.31% and 98.70% respectively. Of 282 samples tested, Culture MGIT detected *M. tuberculosis* in 219 (77.66%) samples, LPA detected *M. tuberculosis* in 241/282 (85.46%). Using Culture MGIT as the gold standard, the LPA correctly recognized *M. tuberculosis* in 216 of 219 Culture MGIT positive samples.

Drug susceptibility testing (DST)

MGIT culture positive for *M. tuberculosis* in 219/258 (84.88%) were AFB smear positive. Of MGIT cultures, 6/258 (2.32%) were contaminated, and due

First line DST

Table 2: Results of drug susceptibility test in first line anti-Tb drugs

Category, Number and Percentage of Isolates		Drug	Frequency (%)
Susceptible to all drugs		INH+ STP + RIF +EMB	78 (35.62%)
Resistant 141 (64.38%)	Monodrug resistance 29 (13.24 %)	RIF	5 (2.28%)
		INH	6 (2.74%)
		STP	14 (6.39%)
		EMB	4 (1.83%)
	Multidrug resistance 96 (43.84 %)	INH + RIF	12 (5.48%)
		INH + RIF+EMB	27 (12.33%)
		INH + RIF +STP	
		INH+ STP + RIF +EMB	57 (26.11%)
	Polydrug resistance 16(7.30%)	RIF+EMB	16 (7.30%)
		RIF+STP	
		RIF+STP+EMB	
		INH+STP+EMB	
		EMB+STP	
INH+EMB			
	INH+STP		
Total		-----	219 (100%)
Any resistance to:		INH	113 (51.59%)
		STP	102 (46.58%)
		RIF	99 (45.21%)
		EMB	74 (33.79%)
Chi-square- χ^2		---	11.342
P-value		---	0.0003

INH= Isoniazid, STP = Streptomycin, RIF= Rifampicin, EMB= Ethambutal, Monodrug = Single drug resistance, Multidrug resistance = resistance at least INH and RIF, Poly drug =Resistance to two and more drugs without the combination of INH and RIF.

Results of Line Probe Assay (LPA) test

LPA detected a total 46/258 (17.81%) samples as drug resistant, of which 35/258 (13.70%) were

to site/lab error 8 had the X200 error, and 12 had the X400 error. The remaining Two hundred nineteen (219) samples were analysed.

Drug susceptibility testing against first line drugs which used for TB treatment was carried out on 219 sputum culture positive isolates. (Table 1). The results of the routine DST using the MGIT 960 system were compared to the results obtained by LPA. Drug-resistance isolates were distributed as follows: 29 (13.24 %) isolates were resistant to one drug (mono drug resistance) including 5 (2.28%) isolates resistant to RIF, 6(2.74%) to INH, 14 (6.39%) to STP and 4(1.83%) to EMB. 96 (43.84%) isolates were multidrug resistance (MDR) of which 12 (5.48%) isolates were resistant against both INH and RIF, 55 (26.11%) were drug resistant against all four drugs, while 27 (12.33%) isolates were resistant to different combinations of three drugs. Finding of MDR-TB isolates to combinations of 3 and 4 drugs may point to presence of XDR - TB. Poly drug resistance against two or three drugs was observed in 17(7.76%) isolates. The results also showed the highest prevalence of resistant to any drug was identified against INH (51.59%), followed by STP, RIF and EMB (46.58%, 45.21%, and 33.79%) respectively.

resistant to both rifampicin and isoniazid (MDR), 6/258 (2.28%) were rifampicin mono-resistant samples and 11/258 (4.11%) were isoniazid

mono-resistant. Out of the culture-positive samples (219), LPA detected 39/219 (17.83%) as drug-resistant, of which 31/219 (14.2%) were resistant to both rifampicin and isoniazid, 5/193 (2.08%) were rifampicin mono-resistant and 8/219 (3.7%) were isoniazid mono-resistant (Figure-1). When compared to MGIT liquid DST for finding of

drug resistance, LPA accurately detected all samples resistant on MGIT liquid DST but disastrous to detect two samples of isoniazid mono-resistant and showed them as sensitive. Sensitivity and specificity for DR-TB recognition was found to be 95.83% (95% CI: 80.8–99.3) and 99.53% (95% CI: 97.7–100), respectively.

Table 3: Hybridization patterns obtained with the GenoType MTBDRsl assay

DISCUSSION

Early diagnosis and treatment halt the spread of drug resistant bacilli in the community but the current convent Effective treatment of MDR-TB is very expensive, particularly in middle income countries such as India. Early diagnosis and successful treatment require a sensitive and specific diagnostic tool. According to the WHO, a successful handling regimen depends on optimal susceptibility testing of Mycobacterium tuberculosis to anti-TB drugs (11). The precision of susceptibility testing result varies with the drug tested as well as with the DST method.

The present study was finished to evaluate two rapid molecular diagnostic tests for the diagnosis of drug resistance with the conventional gold standard method i.e., MGIT liquid culture DST. In this study was comparing the routine of the MGIT 960 system to line probe assay for the detection of drug susceptibility to first line drugs.

Our LPA result compares to the sensitivity and specificity of LPA investigative platform in India two studies showed a sensitivity and specificity of 96 and 99% respectively in 248 smear positive patients (12) South African and South American was 81 and 100% correspondingly (13) and, sensitivity and specificity of 68.4 and 89.3% correspondingly from 213 sputum smear negative samples (14).

In learning in New Delhi agreement sensitivity and specificity of 97.6 and 94.4% when they use LPA in smear positive samples (15) respectively to the South Africa study description a sensitivity and specificity of 97.7 and 91.8% respectively (16). In previous studies done in African countries, the sensitivity and specificity of Rifampicin mono resistance uncovering was 96.4 and 100% among smear positive samples and 77.8 and 98.2% among smear negative samples in Ethiopia and 100 and 96.1% among smear positive population in Uganda (17).

The current learning information a high consistency comparable to what was report in India, of 100% between MGIT960 and LPA results (8, 19) equally LPA reporting the same with the conventional DST at 96% in New Delhi (20).

Limitations

LPAs are as composite to achieve as conventional culture and DST methods and entail skilled and well-trained laboratory employees, as well as adequate laboratory space and design to reduce the risk of false-positive results. dispensation of smear-positive specimens for unswerving testing should be performed in a BSL-2 level laboratory, whereas performing LPAs on positive cultures requires BSL-3 laboratory infrastructure.

CONCLUSION

TB control programmes are needed to ensure universal access to MDR-TB diagnosis and reduce the development and transmission of drug-resistant TB. We experiential enormous diagnostic presentation of the LPA, confirming its reliability and efficiency for the rapid diagnosis of MDR-TB. The current study performance sensitivity and specificity in detecting MDR-TB cases with a appreciably lesser turnaround time as compared to liquid culture DST method and instantaneous detection of Rif and INH resistance. This performance saves several days of time necessary for liquid culture and DST. Hence, a schedule use of LPA can considerably reduce the time to diagnose RIF and INH resistance and can potentially permit previous beginning of proper drug therapy, thereby facilitating prevention of further transmission of drug-resistant strains. In the background of rising drug resistance in MTB, the MTBDR plus assay may be a good tool in the diagnosis and management of MDR-TB.

CONFLICT OF INTEREST: Authors declare no conflict of interest.

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