Detection of Multi-Drug Resistant Tuberculosis Using the Genotype MTBDR DNA Strip Assay and Lowenstein Jensen Proportion Method

Bosede Oyewumi Amuda[1], O. O. Oduyebo[2] and Philip Abutu[3*]

[1]*Microbiology Unit, Nigeria Institute of Medical Research, Lagos, Nigeria.*
[2]*College of Medicine, Lagos University Teaching Hospital, Lagos, Nigeria.*
[3]*Medical Laboratory, Paelon Memorial Hospital, Lagos, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author OOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BOA managed the analyses of the study. Author PA managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Pulmonary tuberculosis (TB) remains a significant public health issue in low-middle income countries. Accurate and timely diagnosis is key to effective management. Diagnosing Multi Drug Resistance Tuberculosis (MDR-TB) is mostly done with phenotypic Lowenstein Jenson (LJ) proportion method with long turn around which delays treatment. The genotypic MTBDR plus was introduced by World Health Organisation (W.H.O) for the same purpose hence, this study aims to detect MDR-TB using both two methods. Sputum samples were collected from cases of pulmonary TB diagnosed with Genexpert and Ziehl Neelsen stain. Positive samples were subjected to MTBDR plus and the LJ proportion method with the LJ method considered gold standard. Chi square analysis was used to evaluate the Sensitivity, Specificity, Positive Predictive value (PPV), Negative Predictive value (NPV), of the MTBDR plus method compared to the LJ Proportion method. Kappa values were also estimated as a measure of agreement between the two methods. In evaluating the performance of MTBDR plus compared to the LJ proportion method, the sensitivity, specificity,

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Corresponding author: E-mail: abutuphillip@gmail.com;

PPV and NPV for the detection of rifampicin and isoniazid was 93.7%, 90.2%, 92.5% ,91.7% and 88.5%, 95.5%,92.0% and 93.3% respectively, while evaluation MDR-TB was 74.5%, 94.4%, 88.4% and 86.6% with a Kappa value of 0.85,0.84 and 0.71 for Isoniazid, Rifampicin and MDR-TB which indicate almost perfect agreement for both rifampicin and isoniazid and substantial agreement for Multi Drug Resistance (MDR). Compared to LJ proportion method, MTBDRplus performed well in the detection of drug resistance to rifampicin, isoniazid and MDR-TB, hence, is a rapid and efficient tool for the diagnosis and initiation of treatment for MDR-TB.

1. INTRODUCTION

Tuberculosis (TB) is a communicable disease that is major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent ranking above human immunodeficiency virus and Acquired immune deficiency syndrome (HIV/AIDS). It is caused by the bacillus *Mycobacterium tuberculosis*. [1]. Although the global incidence reports diagnosis and successful treatment of people with TB, averts millions of deaths each year (an estimated 54 million over the period 2000–2017), there are still large and persistent gaps in detection and treatment. [2]. Tuberculosis is an infectious disease caused by the bacterium, *Mycobacterium tuberculosis* and occasionally *Mycobacterium bovis* (zoonotic tuberculosis). It primarily attacks the lungs (Pulmonary tuberculosis) in more than 80% of the cases, leading to primary tuberculosis. Extra-pulmonary tuberculosis occurs in less than 20% of cases and affects various organs of the body such as lymph nodes, the meninges, intestine, bone and joints among others [3]. Generally, pulmonary tuberculosis is characterized with malaise, weakness, weight loss, fever, night sweats, deep cough lasting more than 3 weeks with sputum production, chest pain and coughing-up blood (hemoptysis) [4]. Tuberculosis (TB), one of the oldest recorded human afflictions, is still one of the biggest killers amongst the infectious diseases, despite the worldwide use of live attenuated Bacillus-Calmette-Guerin (BCG) vaccine and several antibiotics [5].

Human tuberculosis is spread when patients with pulmonary TB expel the bacteria into the air by coughing, sneezing and singing. Overall, a relatively small proportion (5–15%) of the estimated 2–3 billion people infected with *M. tuberculosis* will develop TB disease during their lifetime. However, the probability of developing TB disease is much higher among people with HIV infection, diabetes mellitus, kidney disease and persons who have undergone transplant [6]. One of the most important factors influencing the current TB epidemic is resource-limited setting in poverty, which is closely related to malnutrition, overcrowding and lack of access to free or affordable health care services [7]. TB is both preventable and curable. The discovery of anti-tuberculosis drugs in the 1940s followed by combination chemotherapy made tuberculosis a curable disease. In the developed countries, effective treatment and surveillance reduced tuberculosis dramatically with high hopes of total eradication [8,9].

The management of MDR-TB cases starts with a reliable diagnosis, which is obtained by isolating *Mycobacterium tuberculosis* from clinical specimens and conducting drug-susceptibility tests. For years, conventional drug-susceptibility test (DST) on solid egg- or agar-based media was a standard technology and is still utilized in many countries worldwide including Nigeria; the methodologies are the proportion method, the absolute concentration method and the resistance ratio method for egg-based Lowenstein-Jensen medium. Data using the proportion methods on egg- and agar-based media have provided the most sufficient published evidence and thus these are reference methods in Nigeria and many other countries [10, 10]. The tuberculosis laboratory requires proper handling of *Mycobacterium tuberculosis* in order to ensure the safety of laboratory staff, patients and the community at large. The laboratory must be kept limited to trained TB personnel only, all Standard Operating Procedure must be strictly adhered to, appropriate Personal Protect Equipment should be worn, The use of biological safety cabinets and good laboratory and microbiological techniques [12].

1.1 Statement of Problem

Currently Genotypic DST is a major criterion for the commencement of patient for second-line TB regimen. This method being a molecular technique detects the presence of DNA with its

limitation on inability to detect viability of the organism. The full implementation of policies restricting the use of out of counter medicine(antibiotics) to treat symptoms of cough is still a major challenge in developing countries (Nigeria) before visiting the Directly Observed Treatment Short- course clinic which pose some challenges on the viability of the detected organism in respect to the technique use as against the acceptable regime for TB management as adopted by the National TB Control Program, hence the need to follow through with the phenotypic DST of the same specimen to evaluate the accuracy of Genotypic method prior to commencement of treatment.

1.2 Aim and Objectives

The aim of this study is to detect Multi-drug resistant *Mycobacterium tuberculosis* using GenoType MTBDR*plus* strip assay and Lowenstein Jensen proportion method The objective for this study is;

- To evaluate the sensitivity of Genotype MTBDR*plus* DNA strip assay in detecting MDR-TB.
- To determine any disparities between the two methods of drug susceptibility testing.
- To determine the strength and limitation of this method in relation to the gold standard, Lowenstein Jensen proportion method.

2. MATERIALS AND METHODS

2.1 Study Design

A prospective study among adult presumptive cases of pulmonary tuberculosis to detect Multidrug-resistant *Mycobacterium tuberculosis* by Genotype [R]MTBDR*plus* V2.0 assay and Lowenstein Jensen proportion method and to determine any disparities between the two methods. Sputum samples were collected from patients attending the Directly Observed Treatment Short course (DOTS) Center and all susceptibility testing done at the National Reference laboratory NIMR, Yaba Lagos.

2.2 Study Site

This study focused on Adult Presumptive TB patients that are attending the DOTS Center at the Nigerian Institute of Medical Research (NIMR), Lagos for diagnosis, treatment and care between November 2018 to July 2019. NIMR is a research institute with a mandate to conduct research into diseases and problems of public health importance in the country it. With an average of 10-15 patients seen on daily basis.

2.3 Study Population

Sputum samples were collected from participant with suspected pulmonary tuberculosis who presented themselves at the (DOTS) center NIMR.

2.4 Sample Size

Fischer's et al. (1998) exact test formula was used to determine the sample size.

$$no = \frac{z2pq}{e2}$$

Where,

n_o = the minimum samples size required for the study
Z = the critical value and in a two-tail test at 95% confidence level equals 1.96.
P = the estimated prevalence. The prevalence of MDR-TB in Nigeria is 11- 40%
(Gehre et al., 2008) P was taken to be 11%.
e = the absolute sampling error that can be tolerated (half width of the CI – measurement of precision). 0.05

q = 1 – p. q is 89%

n_o = $\frac{1.962(0.11 \times 0.89)}{(0.05)2}$
= 150 sputum samples

2.5 Inclusion Criteria

All patients who are of 15 years of age and above with clinical diagnosis of tuberculosis (coughing for more than two weeks, night sweat, weight loss, chest x-ray) who presented themselves at the DOTS clinic and are not yet on the Nigeria National TB Control Programme drug regime.

2.6 Exclusion Criteria

Patients who have taken the recommended anti-tuberculosis drugs.

2.7 Questionnaire

Interviewer administered questionnaire was employed to obtain information from consented

patients on demography, phone number, clinical signs and symptoms (such as deep productive cough lasting more than 2 weeks, chest pain, unexplainable weight loss, night sweat, fever, loss of appetite, hemoptysis and shortness of breath) present during the time of sample collection, HIV status, visited any chemist or pharmacy before visitation.

2.8 Sample Collection and Transport

Each consented patient was instructed to produce spot sputum sample at a designated area in a properly labeled, wide-mouth, sterile, leak-proof container with tight fitting lid. Thereafter, the samples were transported on ice pack to the DOTS laboratory, NIMR, YABA for processing. However, when processing was delayed, the samples were refrigerated at 2-8°C to limit the growth of oral normal flora (Moore et al., 2008).

2.9 Sample Processing

The samples were processed in a batch of 20 each in class II biosafety cabinet and level two biosafety practices were strictly followed. The two major stages of processing are decontamination of samples and culturing and drug susceptibility testing by Lowenstein Jensen Proportion Method.

2.10 Materials

Sodium hydroxide-sodium citrate (NaOH-Na-citrate) stock solution, N-acetyl-L-cysteine (NALC), weighing balance, foil paper, spatula, phosphate buffer and sputum sample.

2.11 Preparation of Decontamination Solution

The bench was decontaminated with absorbent soaked in 70% ethanol. Sterile NaOH-Na Citrate solution was prepared according to the manufacturer's instructions.1g of NALC crystals was dissolved in every 200 mL of decontamination solution required.

2.12 Procedure for Specimen Decontamination

The biosafety cabinet was first decontaminated with absorbent soaked in 70% ethanol and the tubes properly labeled with patients' identifiers and date. The sputum samples were poured into 50mL falcon tubes then equal volume of NaOH-NALC solution was added. The tube was cap

tightly and vortexed for 20 seconds, tube was inverted for NaOH-NALC solution to contact the entire interior surface of the tube and lid. The tube was allowed to stand for at least 15 minutes. The tube was filled to 45ML with phosphate buffer (pH 6.8) to neutralize alkali and terminate the decontamination process and the mixture was centrifuged using Eppendorf refrigerated centrifuge 5804R at 3000xg for 15minutes at 4°C, the supernatant was carefully poured out into a liquid waste container containing Lysol (disinfectant) and the sediment (pellet) re-suspended in 2 mL of phosphate buffer (Concepcion et al., 2001). From the decontaminated specimen two aliquots were made, for inoculating the Lowenstein Jensen (LJ) medium and for DNA extraction to analyze the presence of mutations conferring resistance to Rifampicin (RIF) and Isoniazid (INH) by Genotype MTBDR*plus*.

2.13 Concentrated Smear Microscopy

The sediment (pellet) was also used for smearing and stained using Ziehl- Neelson staining technique.

2.14 Isolation

The prepared Lowenstein Jensen (LJ) slopes were labeled with patient's names, date of inoculation and identification number. Excess water was removed on the LJ slope, using sterile transfer Pasteur pipette 200µL of the decontaminated sputum samples was inoculated on the LJ slopes. The caps were replaced not too tightly at least for the first 3days to allow for oxygen intake after which it was properly capped. The tubes were incubated aerobically at 37°C and observed daily for the first week of incubation and weekly thereafter until eight (8) weeks. Cultures showing evidence of growth at any time during this period were examined morphologically also making of smear and staining by the Ziehl-Neelsen procedure. All the positive cultures were further subjected to the rapid SD BIOLINE TB Ag MPT64 before Drug susceptibility testing (Martin et al., 1975; Concepcion et al., 2001; Srivastava et al., 2008; Gambo et al, 2013).

2.15 Characterization

SD BIOLINE TB Ag MPT64 Rapid is a rapid immune chromatographic identification test for the *M. tuberculosis* complex (MTBC) that uses mouse monoclonal anti-MPT64. The kit has sensitivity and specificity of 98.6% and 100% respectively. The test cassette consists of a

sample pad, a gold conjugate pad, a nitrocellulose membrane and an absorbent pad. Mouse monoclonal anti-MPT64 was immobilized on the nitrocellulose membrane as the capture material (test line). Another antibody which recognized another epitope of MPT64, conjugate with colloidal gold particles was used for antigen capture and detection in a sandwich type assay. One (1) µL of bacteria (equivalent to the amount of 1mm-diameter platinum micro-loop) growing on the solid medium slant, was suspended in 500 µL of the extraction buffer in a screw capped tube and vortex prior to the test. The cassettes were removed from the foil pouch and placed on a flat dry surface using a sterile pipette for each specimen, one hundred micro liters (100µL) of the suspended colonies in buffer was added into the sample well. As the test begins to work, a purple color moves across the result window in the center of the device. After 15minutes of sample application, the appearance of two colour bands ("T" test band and "C" control band) within the result window was considered a positive result, the appearance of color band on the "C" (control band) only indicate a negative result.

2.16 Drug Susceptibility Testing by Lowenstein Jensen Proportion Method

The proportion methods determine the percentage of growth (number of colonies) of a define inoculum on a drug-free control medium versus growth on culture media containing the critical concentration of the anti-TB drug. Susceptibility to isoniazid (INH) and rifampicin (RIF) was determined by the proportion method on Lowenstein Jensen egg-based slopes containing different critical concentrations of INH and RIF (0.2 µg/mL and 40µg/mL respectively) Standard antibiotic powders were obtained from Sigma-Aldrich.

The inoculum was prepared by directly suspending colonies grown for approximately 3-4 weeks on Lowenstein Jensen (LJ) drug free slope to a turbidity equivalent to MacFarland standard 1. The 1.0 MacFarland standardized suspension was further diluted to 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} The 10^{-2} suspension was subsequently inoculated on the drug-containing medium. Two drugs free LJ slopes were inoculated with 10^{-2} and 10^{-4} diluted suspension of 1.0 MacFarland standardized inoculum and this was done for each sample tested. The drug resistant ATCC

H37Rv was use as positive control and the LJ slopes was incubated at 37°C and read after 4 and 6 weeks. Susceptible wild-type strains are inhibited by this concentration and resistance is defined if over 1% of the bacterial population of a strain is able to grow (Canetti et al., 1963, Canetti et al., 1969).

2.17 Genotypic Assay

The GenoType®MTBDR*plus assays* were performed according to the instructions provided by the manufacturer (Hain Life science, Nehren, Germany). Firstly, Genolyse$^{(R)}$ Kit (Hain Life science, Germany) was used for the DNA Extraction.

2.18 DNA Extraction

Extraction of DNA was carried out on 1mL of decontaminated sputum sample. The decontaminated sputum was transferred into 1.5 mL screwed capped tubes which was centrifuged using the Mikro 200R micro-refrigerated centrifuge for 15minutes at 10,000 RPM, the supernatant was then discarded, and the pellet re-suspended in 100µL of lyses buffer(A-lyses). The re- suspended pellet was heat killed for 15min at 95^{0}C using the heating block.100µL of neutralization buffer (A-NB) was added, vortexed for 5 second, then centrifuge, at full speed 14,000 RPM for 5minutes to get the DNA to be used for amplification which is the next step after DNA extraction. The supernatants were transferred into clean labeled micro centrifuge tubes for amplification.

2.19 Pcr Amplification of the Extracted DNA

The master mix which comprises of 10µL Amplification Mix-A containing (buffer, nucleotide and DNA-polymerase) was mixed with 35µL Amplification Mix-B containing (MgCl$_2$, the biotinylated primers and dye) then 5µLof each sample's DNA was added to the mixture, making the final volume of the PCR mix to be 50µL. The tubes were transferred into a thermal cycler for amplification. according to the following protocol: 15 minutes of denaturation at 95°C, followed by 10 cycles comprising 30 seconds at 95°C and 120 seconds at 65°C; an additional 20 cycles comprising 25 seconds at 95°C, 40 seconds at 50°C and 40 seconds at 70°C and a final extension at 70°C for 8 minutes [13].

Table 1. Critical concentrations of isoniazid and rifampicin

Drug	INH	RMP
Critical concentration(µg/mL)	0.2	40

Table 2. Procedure for inoculation on lowenstein jensen slope

Suspension Dilution	Drug-free tubes (growth control G C)	INH 0.2 µg/mL	RMP 40 µg/mL
10^{-2}	X (GC)	X	X
10^{-4}	XX (GC)		

Table 3. Pcr reaction and the heating cycle used

Cycles	Temperature	Time	Sample type Clinical specimen
1 cycle	$95^{o}C$	15minutes	1 cycle
10 cycles	$95^{o}C$	30seconds	20 cycle
	$65^{o}C$	2minutes	
20 cycles	$95^{o}c$	25seconds	20 cycle
	$50^{o}C$	40seconds	
	$70^{o}C$	40seconds	
1 cycle	$70^{o}C$	8minutes	1 cycle

2.20 Denaturation of DNA

The Twin Cubator was pre-warmed to 45**°**C and 20µL of denaturation solution was added to each labeled well of the Twin Cubator tray followed by the addition of 20µL of the amplicons respectively. The mixture was mixed gently by pipetting up and down five times and then incubated at room temperature for 5mins.

2.21 Hybridization and Detection

One (1) mL of the pre-warmed hybridization buffer (HYB) was carefully added to the wells using a pipette. The tray was then carefully tilted back and forth so that the purple denaturation solution and green hybridization buffer were thoroughly mixed. The tray was placed on the Twin Cubator and the labeled strips added to each well ensuring that the strips were completely covered by the liquid. This was incubated at 45°C for 30mins. After incubation, the glass panel lid was opened and the condensate that formed during the incubation wiped off. The HYB buffer was aspirated completely from each well using a Pasteur pipette. One (1) mL of the pre-warmed red stringent wash buffer (STR) was dispensed into the tray using a multi-channel pipette avoiding the contact of the strips with the tips. After 15minute incubation at 45°C in the Twin Cubator, STR buffer was aspirated and disposed of with a Pasteur pipette. The STR buffer was washed off with 1 mL of Rinse solution (RIN) for 1 minute. One (1) mL of the Conjugate (CON) solution was dispensed into each well and the glass panel lid was closed and incubated for 30 minutes on the Twin Cubator. The strips were washed twice with 1mL of Rinse solution (RIN) for 1 minute in the Twin Cubator to wash off the excess CON solution. 1 ml of sterile distilled water was added to each strip-containing well, and a 1minute wash performed on the Twin Cubator to wash off the RIN solution after which the distilled water was completely decanted. One (1) mL of the Substrate solution was dispensed into each well and incubated for 15 minutes on the Twin Cubator after which the Substrate solution was aspirated, and the strips washed twice with sterile distilled water. A pair of clean tweezers was used to remove the strips from the Twin Cubator tray and placed onto absorbent paper. The developed strips were partially dried and transferred to the GenoType MTBDR*plus* score sheet.

2.22 Result Interpretation

The genotype MTBDRplus assay strip contain 27 reaction zone; 21 of them are probes for mutation and 6 are control probes verification of test procedure. The six (6) control probes include a conjugate control, amplification control, *Mycobacterium tuberculosis* complex specific control(TUB) an *rpo*B amplification control, a *kat*G amplification control and an *inh*A

amplification control for detection of rifampicin resistance the probes covers the *rpo*B genes while the isoniazid resistance specific probes covers position in *kat*G and *inh*A genes.

2.23 Quality Control

Quality control check was done on reagents and media; sterility check as well as performance checks. Control strain for drug-susceptible MTB reference strain ATCC 27294 (H37Rv) was used as a susceptible control and known resistant strains (ATCC 35825 H37Rv for INH, ATCC35838 H37Rv for RIF) was used as resistant controls.

2.24 Data Analysis

Data was analyzed by using soft statistical package for social science (SPSS) for data entry and appropriate diagrammatic presentation will be represented where necessary.

3. RESULTS

A total of 150 sputum samples were collected from the target participants recruited into the study. Out of these, 7 samples were contaminated, while 3 turned out to be Non-tuberculous mycobacteria (NTM) and thus were excluded from the study, leaving a total of 140 samples. Of these 140 samples, 95(67.9%) and 44(31.4%) were obtained from males and female respectively while the gender of one (0.7%) not stipulate. The participants had a mean age of 33.49 years (SD= 11.501) with a minimum age of 15 and a maximum age of 65. One hundred and ten participants were HIV negative, twenty were HIV positive, while ten did not disclose their status. The recruited participants were also screened for symptoms present at the time of sample collection, a total of 75(53.6%) were coughing, 30(21.4%) had fever, 48(34.3%) were experiencing night sweats, 66(47.1%) had weight loss, 35(25%) had chest pain, and 11(7.9%) had hemoptysis. Ninety (64.3%) participants had been to a chemist or pharmacy and were on self-medication. Table 4 shows the demographic and clinical characteristics of study population.

3.1 Results of Drug Susceptibility Testing

3.1.1 Result for Isoniazid (INH)

The drug susceptibility result for isoniazid (INH) is presented in Table 5, the table shows that 46 samples were resistant using the two methods,

while 4 were susceptible to the conventional method but resistant using the MTBDR*plus*, 84 samples were susceptible in both methods with 6 being susceptible to MTBDR*plus* and resistant to the conventional method. A total of 10 discordant results were recorded for isoniazid.

3.1.2 Result for Rifampicin (RIF)

The drug susceptibility result for Rifampicin (RIF) is presented in Table 6, the table shows that 74 samples were resistant using the two methods, while 6 was susceptible to the conventional method but resistant using the MTBDR*plus*, 55 samples were susceptible in both methods with 5 being susceptible to MTBDR*plus* and resistant to the conventional method. A total of 11 discordant results were recorded for Rifampicin.

3.1.3 Result for Multi-drug Resistance (MDR)

The drug susceptibility result for MDR are presented in Table 7, the table shows that 84 samples showed multiple resistance using the two methods, while 13 samples showed mono resistance or susceptibility to the conventional method, but multiple resistance using the MTBDR*plus*, 38 samples were susceptible in both methods with 5 samples showing monoresistance/susceptibility to MTBDR*plus* but multiple resistance to the conventional method. A total of 18 discordant results were recorded for MDR.

3.2 Performance of Genotype Mtbdrplus in Detecting Rifampicin and Isoniazid Resistance

In evaluating the performance of MTBDR*plus* the result revealed that compare to the Lowenstein Jensen proportion method, the sensitivity, specificity, positive and negative predictive values for the detection of RIF resistance by Genotype[R]MTBDR*plus* was found to be 93.7% (CI: 85.84% - 97.91%), 90.2% (CI: 79.81% - 96.30%), 92.5% (CI: 85.20% - 96.35%), and 91.7% (CI: 82.43% - 96.27%) respectively while for INH was 88.5% (CI: 76.56% - 95.65%), 95.5% (CI: 88.77% - 95.65%), 92.0% (CI: 81.46% - 96.99%) and 93.3% (CI: 86.82% - 96.75%) respectively. The Cohen coefficient for the agreement of the results for INH is Kappa =0.85 (95% CI, 0.604-0.887) with an observed accuracy percentage of 92.86%. The Cohen coefficient value of the results for RMP is Kappa =0.839 (95% confidence interval from 0.772 to 0.933). Percentage of observed accuracy is

92.14%. while the sensitivity and specificity for detection of MDR-TB resistance by MTBDR*plus* compared to Lowenstein Jenson proportion method was 94.4%(CI 95% 87.37%-98-15%) and 74.5%(CI95% 60.37-85.67%) respectively and PPV and NPV of 86.6%(CI95%80.12-91.20%) and 88.4%(CI95% 76.16%-94.76%). With a Kappa value of 0.71.

Table 4. Demographic and clinical characteristics of study population

Characteristics	Total (%)	P Value
Total (n)	140	
Sex		**0.50**
Female	44 (31.4)	
Male	95 (67.9)	
Age		
Mean Age ± SD	33.49 ± 11.50	
HIV status		**0.49**
Positive	20 (14.3)	
Negative	110 (78.6)	
Unknown	10 (7.1)	
TB Symptoms present at screening: Cough	75 (53.57)	0.50
Fever	30 (21.43)	
Night sweats	48 (34.34)	
Weight loss	66 (47.14)	
Chest pain	35 (25)	
Hemoptysis	11 (7.85)	
Visited a chemist or pharmacy	90 (64.28)	

Table 5. Isoniazid (inh) mtbdrplus*inh lj proportion dst cross tabulation

		LJ proportion DST		Total
		R	S	
MTBDR*plus*	R	46	4	50
	S	6	84	90
Total		52	88	140

Table 6. Rifampicin Mtbdr*plus* * rif lj proportion dst crosstabulation

		LJ proportion DST		Total
		R	S	
MTBDR*plus*	R	74	6	80
	S	5	55	60
Total		79	61	140

Table 7. Mdr Mtbdr*plus mdr lj proportion dst crosstabulation**

		Conventional		Total
		R	S	
MTBDR*plus*	R	84	13	97
	S	5	38	43
Total		89	51	140

Table 8. Performance of genotype mtbdr*plus* in detecting rifampicin and isoniazid resistance

	Rifampicin	Isoniazid	MDR
Sensitivity	93.7	88.5	94.4
Specificity	90.2	95.5	74.5
PPV	92.5	92.0	86.6
NPV	94.6	93.3	88.4
accuracy	91.7	92.9	87.1
Kappa value	0.84	0.85	0. 71

Fig. 1. Representative of DNA patterns obtained by the Mtbdr*plus* assa

4. DISCUSSION

The prevalence of drug-resistant TB is increasing in many parts of the world, and high rates of drug-resistant and MDR-TB has been reported in several countries. According to the WHO, an effective treatment regimen is dependent on optimal susceptibility testing of *Mycobacterium tuberculosis* to first-line drugs [14]. The accuracy of susceptibility testing results varies with the drug tested as well as with the method of drug susceptibility testing (DST) used [15]. Hence the aim of this current study was to detect MDR-TB using genotype MTBDR*plus* DNA strip assay and Lowenstein Jensen proportion method.

Considering the phenotypic DST as the 'Gold standard', data from this study showed the agreement for isoniazid (INH) was 92.1% using the MTBDR*plus* assay with a sensitivity value of 88.5%, specificity of 95 .5%, PPV 92.0%, NPV 93.3%, and a Kappa value of 0.85. According to McHugh, 1996 [16] kappa value of 0.01-0.20 indicates slight agreement, 0.21-0.40 indicating a fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 substantial agreement and 0.81-1.00

indicating that there is almost perfect or perfect agreement between the two tested methods. A kappa value of 0.85 as seen in the susceptibility testing of INH between MTBDR*plus* and Lowenstein Jensen proportion method showing that the strength of agreement is almost perfect (k= 0.81-1.00). This commensurate with some other studies that have found good sensitivity and specificity to INH. Chen, 2014 [17] reported sensitivity and specificity values of 76.5 and 95.4% respectively. However contrary, study by Huyen, 2010 [18] reported a sensitivity and specificity of 92.6 and 100% respectively. The varying level in sensitivity and specificity using both testing methods still remains unclear. These studies however, provided lower result compared to studies by Obionu, 2007 [3] who reported perfect values of 100% for both sensitivity and specificity for INH.

For this study, the INH resistance (4) of the discordant result was said susceptible by the proportional method and resistant by the MTBDR*plus* assay while CDC, 2013 [4] shows resistance by the proportional method and Susceptible by the MTBDR*plus*. the varied

observations between the two methods with regards to INH resistant to the conventional method but susceptible to the genotypic might be as a result of other resistant gene region that might not necessarily be in cooperated in the MTBDR*plus* probe as such cannot possible identify these resistance region.in a similar study, W.H.O, 2016 [19] also revealed that MDRDR*plus* test failed to detect INH resistant strains in 7 specimen suggesting presence of some unidentified mutations in other genomic region (like *ahp*C, *kas*A, *fur*A) which were not targeted by the assay. Earlier studies by Huyen *et al.* 2010 [18] revealed that approximately 10-25% of INH-resistant strains do not contain mutations in any known gene targets for INH resistance.

For discordant result for isoniazid susceptibility, only 10 yielded discordant results to both tests methods while 130 of the samples showed concordant results using both methods. In a similar study carried out by Atanda, 2015, [20], 97 isolates were subjected to testing by phenotypic DST method and genotypic MTBDR*plus* assay. Of the 97 isolates tested, 94 showed concordance with 3 discordant results for isoniazid. When subjected to both tests' methods, rifampicin showed an agreement of 92.1 % with a sensitivity value of 93.7.5%, specificity of 90.2%, PPV 92.5%, NPV 94.6%, and a Kappa value of 0.84. A kappa value of 0.84 as seen in the susceptibility testing of RIF between MTBDR*plus* Assay and Lowenstein Jensen proportion method shows that the strength of agreement to be almost perfect (k=0.81-1.00). This study corresponds with some other studies which showed high sensitivity to RIF, studies from South Africa, Germany and Italy, recorded a sensitivity of RIF to both methods reveals a sensitivity rate of 96.2% [21,22,23] which indicates the data is also in agreement with this current study. However, lower values for sensitivity of RIF by both test methods have also been reported. In a study by Schon et al. 2017 [24], it is reported that lower values of 86.6% for sensitivity of RIF by both methods which is contrary to data gotten from this study. The higher specificity of RIF (90.2%) detected in this study was similar to a study carried out in Cote d'Ivoire with a specificity of 88.2% by N'guessan et al. (2014) and 95% specificity by Schon et al. [24] For rifampicin susceptibility, 129 of the samples gave concordant results by both methods while 11 samples yielded discordant results. In previous studies carried out by Ahmad et al. (2016), 67 of the isolates gave concordant results by both

conventional DST and genotypic methods with only three discordant results. Also, similar studies carried out by Atanda 2015 had 97 isolates; Out of the 97 isolates, 87 showed concordance result for both DST with only ten discordant results for rifampicin, 6 of the discordant was said susceptible by the conventional method and resistant by the MTBDR*plus* assay while 5 showed resistance by the conventional method and susceptible by the MTBDR*plus* assay.

Data for Multi-Drug Resistant TB in this study showed an agreement of 87.1% with a sensitivity value of 74.5%, specificity of 94.4%, PPV 88.4%, NPV 86.6%, and a Kappa value of 0.71. A kappa value of 0.71 as seen in the susceptibility testing for MDR between MTBDR*plus* Assay and Lowenstein Jensen proportion method shows Substantial agreement. When compared to studies done by Tukur *et al.* (2019), it was in accordance with this current study where the sensitivity to MDR was reported as 75%. Although there was varied results of the specificity, PPV, NPV and agreement where it was reported as 85.7%, 93.4% and 54.5% respectively which contrasted with report from this study. Genotypic DSTs for anti-TB drugs based on the molecular method have the clinical advantages of rapid return of results and safety during the procedure. However, the discrepancy with the phenotypic DST in a real practice poses a clinical dilemma. Even though the incidence is low, as in our study, the potential clinical impact cannot be ignored. If the susceptibility shown by the genotypic DST is a false positive (false resistant), the patient would be prescribed an inappropriate drug unnecessarily long time, which increases the risk of side effects and lowers the efficacy. Similarly, if the susceptibility shown by the genotypic DST is a false negative (false susceptible), the patient would also be prescribed an incorrect drug, which would be ineffective and could induce resistance to that drug and, consequently, treatment failure. In particular, the former situation involving a false-positive result by genotypic DST is more confusing and challenging in choosing proper regimens for TB, to both the patient and clinician. Various explanations have been suggested for the genotypic–phenotypic discrepancy in INH or RIF susceptibility, these include rare gene mutations such as those in *kas*A or *msh*A for INH susceptibility, mutations at other regions of *kat*G and *inh*A or *rpo*B outside regions sequenced by the MTBDR*plus* assay, silent mutations, disputed mutations leading to increased minimal inhibitory

concentration below the critical concentration in some phenotypic DSTs, inadequate phenotypic results, hetero resistance, and random errors (9,15). A study that was done by Kang *et al.* (2019) showed genotypic susceptibility with phenotypic resistance to INH and RIF. DNA sequencing analysis detected new mutations of Ser315Asn (S315N) in *kat*G and Leu490Pro (L490P) for *rpo*B, both of which are not covered by the commercial line probe assay.

5. CONCLUSION

It can be concluded from this study that the detection of MDR-TB using MTBDR*plus* assay and the Lowenstein Jensen proportion method shows an almost perfect agreement (k=0.81-1.00) in the detection of mono-resistance of rifampicin and isoniazid (0.84 and 0.85) however substantial agreement (0.71) was observed for the detection of MDR-TB. The genotypic method presents a faster method for the diagnosis of MDR TB. However, the disparities seen in this study using the two methods can be clarified using Gene sequencing Hence, the MTBDR*plus* is a rapid, efficient and reliable tool for the diagnosis and initiation of treatment of MDR-TB.

6. RECOMMENDATION

It is recommended that gene sequencing can be adopted as a molecular technique for MDR-TB detection and to further clarify disparities whenever.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Lagos University Teaching Hospital Health Ethics Research Committee, Lagos; all institutional guidelines of ethics involving human experimentation in research were strictly complied with. Written informed consent and confidentiality was carried out.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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