

Performance of Line Probe Assay (MTBDRplus) in the Detection of Drug Resistance Tuberculosis

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ABSTRACT

Objective/purpose: The development of multidrug resistant tuberculosis and the emergence of extensively and totally drug resistant TB worsen the overall TB control programs. The World Health Organization validates the use of Line Probe Assay (LPA) for rapid detection of MDR-TB. The study evaluates the performance of LPA (MTBDRplus) compared to proportional method (PM).

Methods: The study was conducted at TB reference Laboratory, Aminu Kano Teaching Hospital. Using cross-sectional study design 80 Rifampicin Resistant-TB sputum samples were purposely selected and processed according to standard mycobacteriological procedures. Their drug resistance status was detected using PM and MTBDRplus and the performance of LPA were evaluated using MedCalc Software.

Results: The study indicated that 88.5% of the samples provided an interpretable result with 46.5% and 30.99% of them detected by LPA and PM as Rifampicin mono resistant. Also, 32.9% and 45.07% of them were MDR-TB by the LPA and PM respectively. Compared to PM, the sensitivity, specificity, PPV and NPV for detection of Rifampicin resistance by the LPA was 98.15%, 82.35%, 94.64% and 93.33% respectively, for detection of Isoniazid resistance was 57.14%, 94.44%, 90.91% and 69.39% respectively, and for detection of MDR-TB resistance was 75.61%, 85.71%, 93.94% and 54.55% respectively.

Conclusion: The study demonstrated that the LPA (MTBDRplus) compared to PM performed very high in the detection of Rifampicin resistance more than Isoniazid resistance and MDR-TB. The study identifies the need for improving the MTBDRplus to contain other resistant gene regions to increase the detection rate of not only mono resistant TB but MDR-TB.

Key words:

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Author's contributions:

This work was carried out and approved in collaboration between all the authors. AAI, TAD designed the study; TAD, AAI, MAB, OJO, and AUM sourced for funding; TAD, AAI, MAB wrote the protocol; TAD, AAI, MUA contributed in literature search; TAD, AUM, OJO, UBR, RM did the experiments; AAI, TAD, did statistical analysis; AAI, TAD, MAB drafted the manuscript; AAI, TAD, MAB supervised the study; AAI, TAD Wrote the final manuscript; KAS proof read the manuscript.

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INTRODUCTION

Drug resistant tuberculosis continues to pose challenges not only to the individuals infected with *Mycobacterium tuberculosis* (*M. tuberculosis*) but the overall TB control programmes worldwide. The development of multidrug resistant TB (MDR-TB) and the emergence of extensively drug resistant TB (XDR-TB) further worsen the situation with some of them becoming totally drug resistant TB (XXDR) thereby defying all forms of TB treatment. Current reports by the World Health Organization (WHO) reveals that an estimated 10 million people (range: 9.0–11.1 million) are infected with TB and 1.3 million deaths occurs worldwide in 2017. Further reports indicated that, globally, an estimated 600,000 MDR/Rifampicin resistant-TB or RR-TB (i.e. 490,000-MDR-TB and 110,000-RR-TB) cases were reported with 3.5% of new TB cases and 18% of previously treated cases identified as MDR/RR-TB. Among cases of MDR-TB in 2017, 8.5% were estimated to have extensively drug-resistant TB (XDR-TB) (1, 2). Nigeria records 4.3% and 25% MDR-TB/RR-TB among new and old TB cases (1).

Early detection and prompt treatment of drug resistant TB especially rifampicin (RIF) and isoniazid (INH) drug resistant *M. Tuberculosis* still remains the key to a successful TB control programme. Previous reports by WHO revealed that the conventional methods for mycobacteriological culture and drug susceptibility testing (DST) are slow and cumbersome, and during this time patients may be inappropriately treated, drug resistant strains may continue to spread, and amplification of resistance may occur (3). In this regard the WHO identifies the need for the development of rapid diagnostic tests

and molecular assays to detect gene mutations that signal drug resistance are widely recognized as most suited. As such, novel technologies for rapid detection of anti-TB drug resistance becomes a priority in TB research and development, with molecular line probe assays (LPAs) on the fore front which focused on rapid detection of rifampicin resistance alone or in combination with Wilsonian. Consequently, the World Health Organization (WHO), Geneva and Foundation for Innovative New Diagnostics (FIND) validated the use of LPA test (GenoType MTBDR*plus*, Nehren, Germany) for rapid detection of MDR-TB directly from smear positive sputum specimens and *M. tuberculosis* cultures. The WHO noted that apart from the impact on morbidity, mortality and transmission of MDR-TB, introduction of LPA assays in screening and diagnostic algorithms could significantly reduce the need for sophisticated and costly conventional laboratory infrastructure, still vastly inadequate in most high-burden countries. The WHO, however, revealed that the Line probe assays are not a complete replacement for conventional culture and DST, as mycobacteriological culture is still required for smear-negative specimens while conventional DST is still necessary to confirm XDR-TB (3).

Earlier studies by WHO based on systematic reviews and meta-analyses revealed that the LPA were found to be highly sensitive and specific for the detection of rifampicin resistance, alone or in combination with isoniazid on isolates of *M. tuberculosis* and on smear-positive sputum specimens (3). Recently, Bai *et al.* (5) performed a meta-analysis to comprehensively evaluate the overall diagnostic accuracy of the GenoType MTBDR*plus* assay compared with

conventional DST and revealed that the results showed excellent pooled sensitivity and specificity for detection of resistance to RIF (96%, 98%), INH (91%, 99%) and MDR (91%, 99%), with lower and more inconsistent sensitivity than specificity. However, according to studies by Yadav *et al.* (6) the clinical utility of the LPA test varies with the prevalence of particular mutations (incorporated in the test) in different geographical regions and further noted that there are limited data on the performance of the test from high TB burden countries including India. Nigeria which is also among the high burden countries with TB had low reports of the performance of LPA in the region despite the fact that Nigeria was documented as 1st in Africa and 4th among 6 countries that accounted for 60% of worldwide TB burden and together with four other countries accounted for 60% of notified MDR-TB (1). The study therefore evaluate the performance of MTBDR*plus* by comparing it with conventional drug susceptibility test (proportional method-PM) among RR-TB isolates (suspected MDR-TB) obtained from TB centre located in Northern, Nigeria.

MATERIALS AND METHODS

The study was conducted at the TB reference laboratory located at Aminu Kano Teaching Hospital (AKTH) which serve as the North-West Zonal TB Reference Laboratory. A cross-sectional study design was used and 80 sputum samples were selected using purposive sampling from RR-TB samples (confirmed by Genexpert) obtained from various TB centers located across the zone and submitted to the TB Reference Laboratory (AKTH). Ethical clearance for the study was obtained from the Ethical Review Committee of Aminu Kano Teaching Hospital, Kano.

The samples were processed according to standard mycobacteriological procedures involving digestion, homogenization, decontamination and concentration in Biosafety Cabinet Level II (BSL II) using N-acetyl-L-cysteine (NALC)-sodium hydroxide (NaOH) (NALC-NaOH) solution, 0.067M phosphate buffer (pH 6.8) and buffered Saline (PBS) according to WHO, National Tuberculosis and Leprosy Programme Control (NTBLCP) SOP Manual, and National Committee for Clinical Laboratory Standards (NCCLS (3, 7, 8).

Drug susceptibility test of the MTBC isolates using the Line Probe Assay

The Line Probe Assay (Genotype® MTBDR*plus* assay) is a DNA strip-based tests that employs PCR amplification and reverse hybridization assay for detecting RIF and INH resistance of MTBC isolates (3). The molecular identification of RMP resistance is accomplished by detecting the most significant mutations in the 81-bp (base pair) region of the *rpoB* gene (which encodes the β -subunit of RNA polymerase, the essential enzyme that is inactivated by RMP). High-level resistance to INH is detected by screening for the most common mutations in the *katG* gene (which encodes catalase, the enzyme that activates INH). Low-level resistance to INH is detected by screening for mutations in the promoter region of the *inhA* gene (which encodes the NADH enoyl ACP reductase, involved in cell wall biosynthesis). Mutations are detected by: (i) the binding of amplicons to probes targeting the most commonly occurring mutations (MUT probes) or (ii) inferred by the lack of hybridization (i.e. lack of binding) of the amplicons to the corresponding WT probes. The post-hybridization reaction leads to the development of coloured bands on the test

strip detecting probe binding (3). Resistance to both INH and RIF by the MTBC isolates is described as Multidrug resistance TB (MDR-TB).

The samples in this study were further processed to detect their drug resistant status using the Line Probe Assay (LPA) which was carried out on each of the processed samples using the GenoType MTBDR*plus* molecular line probe assay according to the manufacturer's specification (HainLifescienceGmbH, Nehren, Germany, 2015) and WHO recommendations in three stages as described below (3, 9). The DNA extraction was performed in the BSL III laboratory, master mix preparation in a second room, and amplification and hybridization were performed in a third room (3, 9).

1- DNA EXTRACTION PROCEDURE WITH GENOLYSE

DNA extraction procedure was carried it out with genolyse. The genolyse contains the lysis buffer (A-LYS) and the neutralization buffer (A-NB). Exactly 0.5ml (500µl) of the decontaminated sputum sample was transferred into microcentrifuge tube. This was done for all the samples, after which the tubes were centrifuged for 15 minutes at 10000xg. The supernatant was discarded and 100ml A-LYS was added and re-suspended by vortexing gently for 30 sec. The tubes were arranged in a floater inside the BSC II and incubated for 5minutes in a water bath at 95°C. Then, 100ml A-NB was added and vortexed for 30 seconds and the tubes were centrifuged at 20,000xg. The heavier debris formed the pellet and the lighter DNA (free from impurities) was suspended in the supernatant which was transferred into clean labeled micro-centrifuge tubes for further use (3, 4).

2- PCR amplification of the extracted DNA.

The master mix was constituted by adding 10µl of the Amplification mix A AM-A that contain 10X buffer, nucleotides and DNA polymerase and 35µl of Amplification mix A AM-B reagent that contains the MgCl₂, the biotinylated primers and dye. The constituted master mix was then placed in a PCR tube labeled with sample number and mixed very well. This was prepared inside dead air box in a clean DNA free room. Then 5µl of each sample (containing the extracted DNA from above) was added to the corresponding tube containing the master mix and then mixed gently by pipetting up and down a few times in BSC II at the DNA addition room. The PCR tubes were then placed in a 30 cycle (10 + 20) thermal cycler program for amplification for the DNA amplification. After amplification the DNA contained in the amplicons were denatured in the TwinCubator which was pre-warmed to 45°C and 20µl of denaturation solution (NaOH) was added to each labeled well of the TwinCubator 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.

3- Hybridization and detection

Following denaturation of the amplicons, 1ml of the pre-warmed hybridization buffer (HYB) was carefully added to the wells using a pipette and thoroughly mixed. The tray was placed on the TwinCubator and labeled strips were added to each well ensuring that the strips were completely covered by the liquid and incubated at 45°C for 20mins. After incubation, the HYB buffer was aspirated completely from each well and 1ml of the pre-warmed red stringent wash buffer (STR) was then

dispensed into the tray. After 10 minutes incubation at 45°C in the TwinCubator, STR buffer was aspirated and was washed off with 1 ml of Rinse solution (RIN) for 1 minute. Then 0.9mL of the Conjugate (CON) solution was dispensed into each well and incubated for 20 minute on the TwinCubator. The strips were washed twice with 1ml of Rinse solution (RIN) for 1 minute in the TwinCubator. Then sterile distilled water was added and a 1 minute wash performed on the TwinCubator to wash off the RIN solution after which the distilled water was completely decanted. Substrate solution of 0.9mL was then dispensed into each well and incubated for 15 minutes on the TwinCubator 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 TwinCubator tray and placed onto absorbent paper. The developed strips were partially dried and transferred to the GenoType MTBD *plus* score sheet for interpretation.

Interpretation of LPA (MTBDR*plus*)

The LPA (MTBDR*plus*) screens for the absence and/or presence of “wild-type” (WT) and/or presence of “mutant” (MUT) DNA sequences within specific regions of three genes with the *rpoB* gene for RIF resistance, mutations in *katG* at codon 315 confer high-level resistance to INH, *inhA* confer low-level resistance to INH. Resistance was detected by the binding of amplicons to probes targeting the most commonly occurring mutations (MUT probes) or inferred by the lack of binding of the amplicons to the corresponding WT probes *katG* genes for high level INH resistance, and the *inhA* regulatory region gene for low-level INH resistance.

Standardization of inoculum for Drug susceptibility test of the MTBC isolates using the Proportional Method

For the susceptibility testing, the inoculum was prepared by directly suspending colonies of MTBC isolates grown for approximately three weeks on Lowenstein Jensen drug free slopes to a turbidity equivalent to 1.0 MacFarland standard. The standardized suspension was further diluted to 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} . The control strain was obtained from National Tuberculosis and Leprosy Training Centre (NTBLTC) Saye, Zaria Nigeria.

Drug susceptibility test of the MTBC isolates using the Proportional Method

Drug susceptibility testing of the MTBC isolates against RIF and INH or against both drugs (i.e. MDR-TB) was carried out by proportional methods (PM) as according to NTBLCP SOP manual (7). For both RIF and INH drug used in the DST, two (2) types of slopes (-a drug free slope and a drug containing slope) were prepared. The drug containing slopes were prepared by adding critical concentrations of 40µg/mL for RIF and 0.2µg/mL for INH to 200ml of LJ medium contained in tubes. Subsequently, for each sample the 10^{-2} standardized MTBC suspension from above was inoculated on the drug-containing slopes. Three drug-free LJ slopes were inoculated with 10^{-2} , 10^{-3} , 10^{-4} MTBC suspension. Furthermore, the drug-susceptible MTB reference strain ATCC 27294 (H37Rv) was used as a control. The slopes were incubated at 37°C and read after 4 and 6 weeks.

After 28 days incubation, inoculated slopes were observed for growth. The average number of colonies obtained from drug-containing slopes indicates the number of resistant bacilli contained in the inoculum. Dividing the number of colonies in the drug containing slopes by that in the drug free slopes gives the proportion of resistant bacilli existing in the strain. An isolate was considered resistant if the proportion of bacilli resistant to the critical concentration of the drug exceeded or equal to 1%.

Data analysis

Data generated from the study was presented using descriptive statistics inform of percentages

and the performance of LPA compared to PM was evaluated using MedCalc Software (2019) based on the sensitivity, specificity, PPV, NPV of LPA to detect RIF, INH and MDR resistance. The MedCalc Software (2019) utilizes the information generated from the data obtained from the study inform of true positives, true negatives, false positives and false negatives to provide the sensitivity, specificity, PPV and NPV.

RESULTS

The results of the study indicated that 71 (88.5%) out of the 80 samples collected for the study provided an interpretable result for both

LPA and the PM. The remaining 9 (11.25%) samples were excluded from the study as five of them were contaminated and the remaining four gave a negative result by the LPA (Table 1). The result further shows that 33 (46.5%) of the samples were detected by LPA as RIF resistant only while the PM detects 22 (30.99%) as RIF resistant only. None of the sample was detected as INH resistant only by the LPA but 4 (5.63%) were detected as INH resistant by the PM. Additionally, the result revealed that 23 (32.9%) and 32 (45.07%) of the samples were identified as MDR-TB by the LPA and PM method respectively (Table 1).

Table 1: Drug Resistant Status of 80 Tuberculosis Samples using the Line Probe Assay (LPA) (MTDBplus VER 2.0) and the Proportional Method (PM)

Drug Resistance Status	LPA (MTDBplus VER 2.0) (n=71)		PM (LJ Culture) (n=71)	
	No	%	No	%
Rifampicin Resistant only	33	46.5	22	30.99
Isoniazid Resistant only	0	0	04	5.63
Multidrug Resistant TB	23	32.39	32	45.07
Susceptible TB	15	21.13	13	18.31
Contaminated*	-	-	04*	-
Negative Result*	05*	-	-	-

Key: *Result not included in analysis.

Table 2: Performance of Line Probe Assay Compared to Proportional Method (LJ Culture)

LPA (MTBDRplus ver 2.0)	PM (LJ CULTURE) (n=71)				PERFORMANCE % (95%CI)			
	RIF-R	RIF-S	INH-R	INH-S	Sensitivity	Specificity	PPV	NPV
RIF-R	53	03	-	-	98.15	82.35	94.64	93.33
RIF-S	01	14	-	-	(90.11-99.95)	(56.57-96.20)	(86.34-98.01)	(66.48-99.00)
INH-R	-	-	21	02	57.14	94.44	90.91	69.39
INH-S	-	-	15	33	(39.35-73.68)	(81.34-99.32)	(71.62-97.54)	(60.53-77.01)

Note: RIF=Rifampicin; INH=Isoniazid; R=Resistant; S=Susceptible; CI=Confidence interval; PPV=Positive predictive value; NPV=Negative predictive value

In evaluating the performance of LPA, the results revealed that compared to PM, the sensitivity and specificity for detection of RIF resistance by the LPA was 98.15% (CI: 90.11%-99.95%) and 82.35% (CI: 56.57%-96.20%) respectively, and PPV and NPV of 94.64% (CI: 86.34%-98.01%) and 93.33% (CI: 66.48%-99.00%) respectively (Table 2). Also, Table 2 revealed that compared to PM, the sensitivity and specificity for detection of INH resistance by the LPA was 57.14% (CI: 39.35%-73.68%) and 94.44% (CI:

81.34%-99.32%) respectively, and PPV and NPV of 90.91% (CI: 71.62%-97.54%) and 69.39% (CI: 60.53%-77.01%) respectively.

Table 3 revealed that compared to PM, the sensitivity and specificity for detection of MDR-TB resistance by the LPA was 75.61% (CI: 59.70%-87.64%) and 85.71% (CI: 57.19%-98.22%) respectively, and PPV and NPV of 93.94% (CI: 80.94%-98.26%) and 54.55% (CI: 40.19%-68.18.00%) respectively (Table 3).

Table 3: Performance of Line Probe Assay (LPA) Compared to Proportional Method (PM) (LJ Culture) in the Detection MDR-TB.

LPA (MTDB ^{pl} <i>us</i> VER 2.0)	PM (LJ CULTURE) (n=56)		PERFORMANCE % (95%CI)			
	*RIF ^R IN ^H	RIF ^R IN ^S	Sensitivity	Specificity	PPV	NPV
*RIF ^R IN ^H ^R	21	02	75.61 59.70- 87.64	85.71 57.19- 98.22	93.94 80.94- 98.26	54.55 40.19- 68.18
RIF ^R IN ^H ^S	11	22				

Note: RIF=Rifampicin; INH=Isoniazid; R=Resistant; S=Susceptible; *RIF^RIN^H^R=MDR-TB; CI=Confidence interval; PPV=Positive predictive value; NPV=Negative predictive value

DISCUSSION

The findings of this study indicated that, independently the LPA detected 46.5% of the samples as RIF resistance only and the PM detected 30.99% of them as RIF resistance only. For INH resistance, the LPA detected none of the samples as resistant but the PM detected 5.63% of the samples as INH resistant. The varied observations between the two methods especially with regards to INH resistance indicated that there might be in existence other resistant gene regions that might not necessarily be in cooperated in the MTBDR^{plus} probe as such the assay cannot possibly identify these resistant regions. In a similar study, Yadav *et al.* (6) also revealed that the LPA test

failed to detect INH resistant strains in 7 specimens, suggesting presence of some unidentified mutations in other genomic regions (like *ahpC*, *kasA*, *furA*) which were not targeted by the assay. Earlier studies by Hazbo'n *et al.* (10) revealed that documented reports indicated that approximately 10% to 25% of INH-resistant strains do not contain mutations in any known gene targets for INH resistance. They further indicated that based on their study almost 56% of the mono-INH resistant isolates did not have *katG* mutations, and 34% of the mono-INH resistant isolates did not have any previously identified mutation that was likely to have caused INH resistance (10). They evidently discover that

the *katG* G316S, *kasA* D66N, *ahpC*_9 G-to-A, *inhA*_17 G-to-T, *inhA* I47T, and *inhA* I194T mutations, previously seen only in INH resistant *M. tuberculosis* isolates, could also be found in INH susceptible isolates and that *kasA269*, *kasA312*, *aphC*_46, *ahpC* D73H, and *ndh* V18A mutations could be detected in INH susceptible isolates. Hazbo'n *et al.* therefore suggests that many of the genetic causes of primary INH resistance remain to be discovered (10). Bai *et al.* (5) further indicated that the inclusion of testing mutations that cause INH resistance is highly desirable, especially in settings with relatively low MDR-TB prevalence.

Interestingly, in this study 4 of the contaminated results by culture gave an interpretable result by the LPA. Similarly, the 5 negative results by the LPA also gave an interpretable result with the culture methods. This highlights the need to repeat such test whenever the results turn out to be in disagreement. Luetkemeyer *et al.* (11) in their study revealed that 10 (1.6%) of their samples had no available result due to either due to test failure or site/lab error. Moreover, in this study 2 out of the 4 of the contaminated cases by culture were detected as MDR-TB by LPA. Similar observations were made by Aurin *et al.* (12) who revealed that 2 of the MDR-TB cases identified by LPA method in their study turn out to be contaminated with the conventional DST. They explained that such discrepancy might focus on the possibility of the onset of the false positive results by the molecular diagnostic methods although the frequency of such ambiguous cases was very negligible.

Numerous studies conducted on the diagnostic accuracy of GenoType MTBDR*plus* in different settings provided inconsistent results (5). The findings of this study were not exceptional as the outcome

gave results that in some instances provided comparable results to what was documented in the previous literature and in others provided a varied result. Overall, in this study, the performance of LPA compared to PM correlated very high in the detection of RIF resistance more than INH resistance and MDR-TB.

Evaluating the performance of LPA in the detection of RIF resistance compared to PM, this study observed similar results with the studies of Chen *et al.* (13) who revealed the sensitivities and specificities for MTBDR*plus* in detecting RIF and INH resistance as 85.94% and 93.13% and 76.47% and 95.44% respectively. This study however, provided lower results compared to studies by Albert *et al.* (14) who reported sensitivity, specificity, positive and negative predictive values of 100.0%, 96.1%, 83.3% and 100.0% for RIF detection respectively. For the detection of INH resistance this study reported a lower sensitivity but a similar specificity to the studies of Albert *et al.* who reported a sensitivity, specificity, PPV and NPV of 80.8%, 100.0%, 100.0% and NPV (14). Yadav *et al.* (6) also revealed higher values with sensitivity and specificity of 98% and 99% respectively for detection of RIF resistance and 92% and 99% respectively for detection of INH resistance. Ninan *et al.* (15) also showed that in their studies RIF resistance, and INH resistance gave a sensitivity of 100% and 89.3%, and specificity of 93.8% and 100%. In a meta-analysis performed to comprehensively evaluate the overall diagnostic accuracy of the GenoType MTBDR*plus* assay compared with conventional DST, Bai *et al.* (5) revealed that their results showed excellent pooled sensitivity and specificity for detection of resistance to RIF (96%, 98%), INH (91%, 99%) and MDR (91%, 99%), with lower and more inconsistent sensitivity than specificity. A recent systematic review

and meta-analysis by Nathavitharana *et al.* revealed that the pooled sensitivity and specificity were found to be 96.7% and 98.8% for RIF resistance and 90.2% and 99.2% for INH resistance respectively and concluded that the LPAs demonstrated high accuracy overall for the detection of RIF resistance and high specificity for INH resistance detection with good sensitivity (16). However, the various discrepancies observed regarding performance of LPA in this study compared to other studies mentioned above may not be unconnected with the observations made by Yadav *et al.* (6) who reiterated that the clinical utility of the test varies with the prevalence of particular mutations (incorporated in the test) in different geographical regions. This study therefore, support earlier documented studies that the performance of LPA (MTBDR*plus*) varies in different regions of the world.

The findings of this study also indicated that detection of MDR-TB by the LPA compared to PM provided varied results compared to documented studies. For example, compared to this study, Chen *et al.* (13) reported similar values of sensitivity (69.6%), specificity (96.7%), while studies by Yadav *et al.* (6) reported higher values of sensitivity (97%) and specificity (100%). A meta-analysis study by Bai *et al.* (5) revealed that the pooled sensitivity and specificity for detection of MDR-TB were 91.0% (95% CI = 86.0%–94.0%) and 99.0% (95% CI = 99.0%–100%), respectively. It is noteworthy to further mention that, apart from the 21 MDR-TB cases, the LPA compared to the PM also accurately detects 22 of the cases as potential MDR-TB cases being them RIF resistant and INH susceptible (i.e. RIF^RINH^S) bearing the fact that RIF resistance alone is being used as a surrogate marker for the development of MDR-TB. This implies that there is the need

for the relevant authorities to closely follow and monitor patients in this category for a successful TB control.

CONCLUSION

The study demonstrated that the LPA (MTBDR*plus*) compared to PM performed very high in the detection of RIF resistance more than INH resistance and MDR-TB. The study identifies the need for improving the MTBDR*plus* to contain other resistant gene regions that might not necessarily be incorporated in the current MTBDR*plus* probe as this will go a long way in increasing the detection rate of not only mono resistant but MDR-TB and will further strengthen the TB control strategies to curtail the increasing emergence of XDR-TB and XXDR-TB.

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