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# Comparative evaluation of GenoType MTBDR*plus* line probe assay and solid culture for detection of drug-resistant tuberculosis in northcentral Nigeria

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## Abstract:

**Background**: Multi-drug resistant tuberculosis (MDR-TB) poses a great threat to the TB control programs worldwide. Early detection of rifampicin (RIF) and isoniazid (INH)-resistant *Mycobacterium tuberculosis* (MTB) is essential for effective treatment and control of MDR-TB. This study evaluated the performance of GenoType MTBDR*plus* Line Probe Assay (LPA) with the solid culture method for early diagnosis of MDR-TB.
**Methodology:** We enrolled a total of 112 consented and GeneXpert-confirmed MDR-TB participants from different health facilities in the States of northcentral Nigeria between January and December 2024 into the study. Sputum samples collected from the participants were transported using the triple sample packaging system in a cold chain to Zankli TB Reference Laboratory, Bingham University, Karu, and analysed by AFB microscopy, LPA, solid culture and drug susceptibility test (DST) methods. The diagnostic accuracy of LPA and solid culture for detection of MDR-TB was compared, with the DST as the 'gold standard'.
**Results**: Of the sputum samples from the 112 patients, 95.5% were positive for MTB on solid culture, 1.7% were negative, 1.7% were contaminated, and 1.1% were non-tuberculous mycobacteria (NTM). For LPA, 96.4% of the 112 patient samples were positive, 3.6% were negative, and 1.1% were NTM. The sensitivity for detection of mono-resistance to rifampicin and isoniazid, and resistance to both drugs (MDR) by LPA compared to the 'gold standard DST was respectively 95.7%, 72.9%, and 97.1%, while the specificity was respectively 88.4%, 88.7%, and 100.0%. The most frequently occurring mutation detected by LPA among the MDR strains was *rpoB* MUT2 and *katG* MUT2.
**Conclusion:** The LPA test was highly sensitive and specific for the detection of mono-resistance to rifampicin and isoniazid, and multi-drug resistance in sputum samples of TB patients. This makes it a valuable tool for early diagnosis of MDR-TB.

**Keywords**: MDR-TB, GenoType MTBDR*plus*, Drug Susceptibility Test, Line Probe Assay, Solid culture

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# Évaluation comparative du test GenoType MTBDR*plus* sur sonde lignée et de la culture solide pour la détection de la tuberculose pharmaco-résistante dans le centre-nord du Nigéria

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## Résumé:

**Contexte:** La tuberculose multirésistante (TB-MR) représente une menace majeure pour les programmes de lutte contre la tuberculose dans le monde entier. La détection précoce de *Mycobacterium tuberculosis* (MTB) résistant à la rifampicine (RIF) et à l'isoniazide (INH) est essentielle pour un traitement et un contrôle efficace de la TB-MR. Cette étude a évalué les performances du test GenoType MTBDR*plus* sur sonde lignée (LPA) avec la méthode de culture solide pour le diagnostic précoce de la TB-MR.
**Méthodologie:** Nous avons recruté un total de 112 participants atteints de TB-MR, consentants et confirmés par GeneXpert, provenant de différents établissements de santé des États du centre-nord du Nigéria entre janvier et décembre 2024. Français Les échantillons d'expectorations prélevés sur les participants ont été transportés à l'aide du système d'emballage triple échantillon dans une chaîne du froid jusqu'au laboratoire de référence Zankli TB, Université Bingham de Karu, et analysés par microscopie AFB, LPA, culture solide et tests de sensibilité aux médicaments (DST). La précision diagnostique du LPA et de la culture solide pour la détection de la TB-MR a été comparée, le DST étant la «référence absolue».
**Résultats:** Sur les échantillons d'expectorations des 112 patients, 95,5% étaient positifs pour MTB sur culture solide, 1,7% étaient négatifs, 1,7% étaient contaminés et 1,1% étaient des mycobactéries non tuberculeuses (NTM). Pour le LPA, 96,4% des 112 échantillons de patients étaient positifs, 3,6% étaient négatifs et 1,1% étaient des NTM. La sensibilité de détection de la mono-résistance à la rifampicine et à l'isoniazide, et de la résistance aux deux médicaments (MR) par le test LPA, comparée au test de sensibilité aux médicaments de référence, était respectivement de 95,7%, 72,9% et 97,1%, tandis que la spécificité était respectivement de 88,4%, 88,7% et 100,0%. Les mutations les plus fréquemment détectées par le test LPA parmi les souches MR étaient *rpoB* MUT2 et *katG* MUT2.
**Conclusion:** Le test LPA s'est révélé très sensible et spécifique pour la détection de la mono-résistance à la rifampicine et à l'isoniazide, et de la multirésistance dans les échantillons d'expectorations de patients tuberculeux. Cela en fait un outil précieux pour le diagnostic précoce de la MR.


**Mots-clés:** MR-TB, GenoType MTBDR*plus*, test de sensibilité aux médicaments, test de sonde en ligne, culture solide

## Introduction:

Tuberculosis (TB) is a serious infectious disease caused by members of the *Mycobacterium tuberculosis* complex (MTBC). It is one of the leading causes of death worldwide, with an estimated 1.5 million people dying from the disease in 2020 (1). Multidrug-resistant tuberculosis (MDR-TB) is a form of TB that is resistant to at least two of the most commonly used anti-TB drugs, rifampicin and isoniazid. MDR-TB is more difficult to treat and is associated with a higher mortality rate than drug-susceptible TB (1,2). MDR-TB poses a great threat to the TB control programs worldwide. Early diagnosis of rifampicin (RIF) and isoniazid (INH)-resistant MTB is essential for efficient treatment and control of MDR-TB (2).

MDR-TB develops when *M. tuberculosis* (MTB) mutates and become resistant to anti-TB drugs. This resistance often arises when TB patients do not complete their full course of treatment or when they receive sub-optimal doses of the drugs (3). The symptoms of MDR-TB are like those of drug-susceptible TB, and can include cough, fever, night sweats, weight loss, fatigue, and chest pain (4).

The diagnosis of MDR-TB can be challenging, as the symptoms are often like those of other respiratory infections. The most common methods for the diagnosis of MDR-TB include culture and molecular methods. Culture is the 'gold standard' method for the diagnosis of TB, but it is time-consuming, requires specialized equipment, and can take up to 8 weeks to produce results. Molecular tests are becoming increasingly available and are more accurate than sputum microscopy or culture for diagnosis of MDR-TB. Molecular tests can detect mutations in the TB bacteria that are associated with resistance to anti-TB (5,6).

The World Health Organization (WHO) has recommended the conventional drug susceptibility testing as the 'gold standard', but due to its long turnaround time, the treatment given to the patients is delayed, resulting in a higher risk of treatment failure and continuous transmission of MDR-TB (7). The choice of diagnostic method for MDR-TB will depend on the availability of resources and the clinical presentation of the patient. In resource-limited settings, sputum microscopy may be the only available option (7). In resource-rich settings, molecular tests are preferred, as they are more accurate. Early diagnosis of MDR-TB is important, as it allows for prompt treatment and reduces the risk of transmission to others. Although treatment for MDR-TB is long and complex, MDR-TB can be cured with the right combination of drugs. (5).

New methods such as the Line Probe Assay (LPA) have been developed to detect resistance faster and reliably using genotype rather than phenotype and have been endorsed by the WHO for fast and reliable detection of MTBC and its resistance to RMP and/or INH (8). Solid and liquid culture methods for drug susceptibility testing (DST) are time-consuming, requiring weeks to months for results, with culture and DST contamination challenges. This study aimed at evaluating the performance of GenoType MTBDR*plus* LPA with solid culture method for early diagnosis of MDR-TB in northcentral Nigeria and at detecting the mutation patterns associated with *rpo*B, *katG* and *inhA* genes.

## Materials and method:

**Study design**:

This is a cross-sectional study of pulmonary TB (PTB) patients that compared the accuracy of the GenoType MTBDR*plus* line probe assay (LPA) and solid culture for early diagnosis of MDR-PTB.

**Ethical considerations:**

Ethical approval was obtained from the Bingham University Clinical Research Ethics Committee (NHREC/21/05/2005/01500). Informed consent was obtained from all participants before sample collection, ensuring their right to confidentiality and voluntary participation. Participants' data were anonymized using study codes with limited access to study personnel only.

**Study participants:**

The study consisted of 112 confirmed MDR-TB participants who were enrolled from January to December 2024 at different health facilities in the States of northcentral Nigeria. Participants were selected based on their confirmed MDR-TB status on the GeneXpert MTB/RIF platform at each facility.

**Sample and data collection:**

Sputum samples were collected from each consenting participant and packaged using the triple sample packaging system in a cold chain and shipped to Zankli TB Reference Laboratory, Bingham University, Karu, Nigeria. The sputum samples were analyzed using different diagnostic methods (8). The demographic data of each participant were also collected at enrolment with a designed form.

**Decontamination of sputum and culture procedure:**

The standard N-acetyl L-cysteine (NA LC)/sodium hydroxide (NaOH) method was used to process the sputum samples in a BSL-2 laboratory. To prevent the loss of mycobacterial viability, NaOH was neutralized by adding phosphate buffer after 15 min. The mixture was centrifuged at 3000g for 15 min, and the supernatant was discarded. The sediment was used for concentrated acid-fast bacilli (AFB) smear microscopy and inoculation on solid Lowenstein-Jensen (LJ) media (9,10,11).

The Ziehl-Neelsen (ZN) AFB staining was carried out on the sputum smears, and the results graded as scanty (1-9 AFB/100 fields), 1+ (10-99/100 fields), 2+ (1-10 AFB/field), or 3+ (more than 10 AFB/field) (11). Approximately 0.5 ml of the processed sample was inoculated onto prepared L-J slants and incubated at 37°C. The media were checked for growth weekly for 8 weeks. ZN staining was performed on the isolate from positive cultures to detect AFB, and MTBC SD Bioline was used to confirm the presence of MTBC.

**Drug susceptibility test (DST):**

The DST was performed on all positive cultures using standard proportion method (12), to first-line anti-TB drugs (isoniazid, rifampicin, and ethambutol), which classifies them as MDR-TB or drug-susceptible TB (DS-TB) strains (12).

**Line Probe Assay (LPA):**

***DNA extraction and multiplex PCR amplification***

DNA extraction was carried out on the sediments from the decontaminated samples using a commercial GenoLyse extraction kit, following the manufacturer's instructions. The GenoType MTB CM LPA speciation was then performed according to the manufacturer instruction which include master mix preparation, addition of templates, amplification, reverse hybridization, and interpretation of results (13).

Briefly, 10µl of amplification mix A (AM-A) was combined with 35µl of amplification mix B (AM-B), which contained deoxynucleotides, biotinylated primers, and dye. Following this, 5µl of the extracted DNA sample was added to the master mix in a separate room to avoid contamination. PCR amplification was carried out in a thermocycler under the following conditions; initial denaturation of 1 cycle at 95°C for 15 minutes, followed by first round amplification of 10 cycles at 95°C for 30 seconds, 58°C for 2 minutes, and 70°C for 40 seconds; second round amplification of 20 cycles at 95°C for 25 seconds, 53°C for 40 seconds, and 70°C for 40 seconds; and final extension of 1 cycle at 70°C for 8 minutes.

***Reverse hybridization assay procedure:***

The hybridization assay was performed using the GT-Blot 48® automated hybridizer (Hain Life Science, Nehren, Germany) following the manufacturer's guidelines. Preheated hybridization and stringent buffers, as well as diluted conjugate and substrate solutions, along with rinsing solutions and sterile distilled water, were loaded into their respective color-coded slots in the GT-Blot 48®. Suction heads were positioned into their corresponding color-coded solutions to ensure proper dispensing. Equal volumes (20µl) of the denaturing reagent and PCR amplicons were mixed in wells of a tray placed in the GT-Blot 48® and allowed to stand for 5 minutes at 25°C for denaturation.

After this, DNA test strips [that targets specific MTBC, wild type (WT) and mutation (MUT) sequences], labeled with sample identification numbers, were placed into the appropriate wells. The automated hybridizer then executed pre-programmed sequence of steps, including hybridization, stringent wash, rinsing, conjugate application, rinsing with sterile distilled water, substrate application, and final

rinse with sterile distilled water. Once the steps were completed, the test strips were dried using the integrated heating system of the GT-Blot 48®. The colour bands produced on the test strips are read and interpreted as presence or absence of resistance to rifampicin, isoniazid or both drugs, in line with the WHO guideline (13).

**Data analysis:**

The results obtained from AFB microscopy, LPA, solid culture and DST were recorded and compared. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the LPA and solid culture for MDR-TB diagnosis were calculated using SPSS version 26.0. The accuracy of LPA for detecting drug resistance was evaluated by comparing its result with the 'gold standard' DST method.

## Results:

A total of 112 GeneXpert MTB/RIF confirmed positive pulmonary TB cases were enrolled in to the study; 68 (60.7%) were males, 13 (11.6%) were HIV positive, and 3 (2.7%) were age ≤ 14 years, with an average age of 44 years (Table 1). The AFB smear microscopy yielded a total of 87 (77.7%) positive cases with 34 (30.3%) $1^+$, 23 (20.5%) $2^+$, and 30 (26.7%) $3^+$. On solid culture, 107 (95.5%) of 112 patient samples were positive, 2 (1.7%) were negative, 2 (1.7%) were contaminated and 1 (1.1%) grew non-tuberculous mycobacterium (NTM), while on LPA, 108 (96.4%) were positive, 4 (3.6%) were negative, and 1 (1.1%) was NTM as shown in Table 1.

LPA correctly identified MTB in 104 (97.2%) of the 107 MTB culture-positive patient samples but wrongly identified MTB in 4 (80.0%) of 5 MTB culture-negative or contaminated patient samples on solid cultures. One patient sample positive for NTM on the solid culture was negative on LPA by the absence of MTBC TUB control bands on the LPA test (specificity of LPA assay of 100%, 95% CI) as shown in Table 1.

Table 1: *Mycobacterium tuberculosis* detection by solid culture from sputum samples of GeneXpert-confirmed pulmonary tuberculosis patients in relation to LPA, AFB, gender, age, and HIV status

| **Variables** | | **MTB solid culture results** | | | | |
|---|---|---|---|---|---|---|
| | | **MTB positive (%)** | **MTB negative (%)** | **NTM (%)** | **Contaminated (%)** | **Total (%)** |
| LPA | | | | | | |
| | Positive | 104 (92.8) | 2 (1.7) | 0 | 2 (1.7) | 108 (96.4) |
| | Negative | 3 (2.6) | 0 | 1 (1.1) | 0 | 4 (3.6) |
| | Total | 107 (95.5) | 2 (1.7) | 1 (1.1) | 2 (1.7) | 112 (100.0) |
| AFB | | | | | | |
| | Negative | 20 (17.8) | 1 (1.1) | 1 (1.1) | 0 | 22 (19.6) |
| | Positive | 87 (77.7) | 1 | 0 | 2 | 90 (80.4) |
| | 1+ | 34 (30.3) | 0 | 0 | 1 (1.1) | 35 |
| | 2+ | 23 (20.5) | 0 | 0 | 1 (1.1) | 24 |
| | 3+ | 30 (26.7) | 1 (1.1) | 0 | 0 | 31 |
| | Total | 107 (95.5) | 2 (1.7) | 1 (1.1) | 2 (1.7) | 112 (100.0) |
| Gender | | | | | | |
| | Male | 67 (59.8) | 1 (1.5) | 0 | 0 | 68 (60.7) |
| | Female | 40 (35.7) | 1 (2.3) | 1 (1.1) | 2 (1.7) | 44 (39.3) |
| | Total | 107 (95.5) | 2 (1.7) | 1 (1.1) | 2 (1.7) | 112 (100.0) |
| Age group (years) | | | | | | |
| | ≤14 | 3 (2.6) | 0 | 0 | 0 | 3 (2.7) |
| | ≥15 | 104 (92.8) | 2 (1.7) | 1 (1.1) | 2 (1.7) | 109 (97.3) |
| | Total | 107 (95.5) | 2 (1.7) | 1 (1.1) | 2 (1.7) | 112 (100.0) |
| HIV status | | | | | | |
| | Positive | 12 (10.7) | 0 | 0 | 1 (1.1) | 13 (11.6) |
| | Negative | 95 (84.8) | 2 (1.7) | 1 (1.1) | 1 (1.1) | 99 (88.4) |
| | Total | 107 (95.5) | 2 (1.7) | 1 (1.1) | 2 (1.7) | 112 (100.0) |

LPA=Line probe assay; AFB = Acid fast bacillus; HIV=Human immunodeficiency virus; MTB = *Mycobacterium tuberculosis*; NTM = Non-tuberculous mycobacteria

Table 2: Comparison of LPA and phenotypic DST results for rifampicin mono-resistance, isoniazid mono-resistance, and MDR-TB

| Test method | Total positive samples | RIF mono-resistant | INH mono-resistant | MDR-TB (RIF + INH resistant) |
|---|---|---|---|---|
| LPA | 108 | 29 | 19 | 38 |
| DST | 107 | 31 | 9 | 37 |

LPA=Line Probe Assay; DST= Drug Susceptibility Test; RIF=Rifampicin; MDR=Multi-drug resistant; INH: Isoniazid

For assessing the performance of the LPA test, all culture-negative and contaminated samples, LPA test reports with absent TUB control bands, or invalid LPA results were excluded. Only valid genotypic LPA and conventional phenotypic DST results were compared for the 112 samples. For the 108 LPA positive samples, 67 were rifampicin resistant (with 29 rifampicin mono-resistant), 57 were isoniazid resistant (with 19 isoniazid mono-resistant) and 38 were multi-drug resistant (rifampicin + isoniazid-resistant). Of the 107 positive solid cultures/DST, 31 were rifampicin mono-resistant, 9 were isoniazid mono-resistant and 37 were multi-drug resistant (Table 2).

From Table 3, the sensitivity for rifampicin and isoniazid monoresistance and that of MDR were 95.7%, 72.9%, and 97.1%, respectively, while their specificity was 88.4%, 88.7%, and 100%, respectively. Their PPV and NPV were 93.0%, 87.8%,98.5%, and 92.7%, 74.6%, 99.0%, respectively (Table 3).

All the 29 rifampicin-mono-resistant strains had mutation on the *rpoB* WT7, WT8, MUT1, MUT2, and MUT3 bands, while the 19 isoniazid mono-resistant strains had mutation on the *katG* WT, MUT1, MUT2, and the *inhA* WT1, WT2, MUT1, MUT2, and MUT3 bands. The most frequently occurring mutations among the MDR were *rpoB* MUT2 and *katG* MUT2 (Table 4).

Table 3: Diagnostic performance of LPA test in detecting resistance to rifampicin, isoniazid, and multi-drug resistance in comparison with the DST 'gold standard'

| Drug/Diagnostic parameter | Rifampicin | Isoniazid | Rifampicin + Isoniazid |
|---|---|---|---|
| | Resistance (n=67) Sensitive (n=41) | Resistance (n=57) Sensitive (n=51) | Multi-drug resistance (n=38) Non-MDR (n=74) |
| Sensitivity (%) | 95.7 | 72.9 | 97.1 |
| Specificity (%) | 88.4 | 88.7 | 100 |
| PPV (%) | 93.0 | 87.8 | 98.5 |
| NPV (%) | 92.7 | 74.6 | 99.0 |

MDR = multi-drug-resistance; NPV = negative predictive value; PPV = positive predictive value (values in parentheses are with 95% confidence intervals)

Table 4: Band patterns of drug-resistant *Mycobacterium tuberculosis* strains with the Line Probe Assay

| Gene | Band | Gene region/mutation | RIF mono-resistant strain (n=29) | INH mono-resistant strains (n=19) | MDR-PTB strains (n=38) |
|---|---|---|---|---|---|
| *rpoB* | | | | | |
| | WT1 | 506–509 | 0 | 0 | 0 |
| | WT2 | 510–513 | 1 | 0 | 1 |
| | WT3 | 513–517 | 0 | 0 | 0 |
| | WT4 | 516–519 | 0 | 0 | 0 |
| | WT5 | 518–522 | 0 | 0 | 8 |
| | WT6 | 521–525 | 0 | 0 | 10 |
| | WT7 | 521–525 | 3 | 0 | 35 |
| | WT8 | 53o- 533 | 28 | 0 | 35 |
| | MUT1 | D516V | 28 | 0 | 24 |
| | MUT2 | H526 Y | 29 | 0 | 38 |
| | MUT3 | S531 L | 29 | 0 | 2 |
| *katG* | | | | | |
| | WT | 315 | 0 | 11 | 4 |
| | MUT1 | S315 T1 | 0 | 3 | 10 |
| | MUT2 | S315T2 | 0 | 2 | 23 |
| *inhA* | | | | | |
| | WT1 | -15/-16 | 0 | 4 | 9 |
| | WT2 | -8 | 0 | 1 | 2 |
| | MUT1 | C15T | 0 | 18 | 10 |
| | MUT2 | A16G 0 | 0 | 19 | 1 |
| | MUT3 | T8C | 0 | 12 | 22 |

RIF = rifampicin; INH = isoniazid; MDR-PTB = multidrug resistant-pulmonary tuberculosis; WT=Wildtype; MUT=Mutation

## Discussion:

This study compared the accuracy of GenoType MTBDR*plus* LPA and solid culture for the early diagnosis of MDR-TB in north central Nigeria on a total of 112 GeneXpert-confirmed MDR-PTB patients. Our finding shows that LPA, compared to conventional solid culture, is reliable for the diagnosis of MDR-TB, with a sensitivity of 92.8%, meaning that it was able to correctly identify 92.8% of patients with MDR-TB as confirmed by solid culture. LPA also had a specificity of 88.4%, 88.7%, and 100,0% for rifampicin, isoniazid, and MDR-TB, respectively. The results of this study are consistent with a study conducted in India, which reported LPA sensitivity of 93.3% and specificity of 97.0% for the diagnosis of MDR-TB, while solid culture had a sensitivity of 85.7% and a specificity of 94.8% (14). Although our study showed that LPA correctly detected resistance to rifampicin and MDR-TB, it had a lower sensitivity (72.9%) for isoniazid resistance detection.

The specificity of LPA for detecting resistance to rifampicin, isoniazid, and MDR-PTB of 88.4%, 88.7%, and 100%, respectively, indicates that LPA had moderate specificity for rifampicin and isoniazid, but a high specificity for MDR-PTB. The India study (14) similarly reported that LPA had high sensitivity and specificity for detecting rifampicin resistance and MDR-TB, but lower sensitivity for detecting isoniazid resistance. The present study reports a lower sensitivity of 72.9% for isoniazid, while the India study (14), contrarily, reported a higher sensitivity of 92.0%. Additionally, the current study also reports moderate specificity for isoniazid resistance of 88.7%, while a meta-analytical study (15) reported high specificity of 99.0%. The differences in rates may be due to differences in sample sizes, study populations, or methodologies between studies, that could potentially contribute to the variations in these rates.

The positive predictive value (PPV) of a test indicates the probability that a positive test result accurately detects what is being tested for. In our study, the PPV of the LPA for detecting resistance to rifampicin, isoniazid, and MDR-TB was 93.0%, 87.8%, and 98.5%, respectively. This means that the LPA has a high probability of correctly identifying true resistant cases for rifampicin, isoniazid, and MDR-PTB. On the other hand, the negative predictive test (NPV) of a test indicates the probability that a negative test result correctly identifies absence of resistance. In this study, the NPVs of the LPA for detecting resistance to rifampicin, isoniazid, and MDR-PTB were 92.7%, 74.6%, and 99.0%, respectively. This means that LPA has a high probability of correctly identifying absence of rifampicin-resistant and MDR-TB cases, but a lower probability of such for isoniazid resistance. The results suggest that LPA, among molecular tests, offers a more comprehensive resistance profile within a short turnaround time compared to the culture method. Additionally, it has the added benefit of determining the appropriate drug regimen for patients with INH mono-resistance.

The findings from our study further revealed that most of the gene mutations were in the regions of *rpoB* H526 Y and 530-533 in rifampicin resistance, while those of INH resistance were in gene mutation regions *inhA* A16G0 and C15T. However, for the MDR strains, they were at the gene mutation regions *katG* S315T2, *inhA* T8C, and *rpoB* 530-533. A similar study (16) reported that the most common gene mutations were in codons 531 (34.0%), 526 (9.3%), and 516 (2.3%) in the rifampicin resistance-determining region (RR DR) of the *rpoB* gene, whereas the most associated mutations with rifampicin resistance was in codon 531, followed by 526 mutations. Another study (17) reported that up to 95%–98% of rifampicin-resistant strains exhibited mutations in the *rpoB* gene at codon 531. The evidence from our study did not show any relationship between specific mutation conferring rifampicin and isoniazid resistance, which is similar to other studies (14,18).

Our study is limited by the fact it was conducted in one specific region (northcentral) of Nigeria, with a relatively small sample size of 112 participants. Therefore, the findings may not be truly representative of the entire population or apply to other regions of the country. Our study also compared only LPA with solid culture and did not include other molecular tests for the diagnosis of MDR-TB. Therefore, the comparative performance of LPA with other molecular tests remains unknown. In addition, our study did not assess the cost-effectiveness of LPA compared to other diagnostic methods. Therefore, the financial implications of implementing LPA in resource-limited settings are not fully understood and need further investigation.

## Conclusion:

This study provides evidence that the GenoType MTBDR*plus* LPA is accurate and reliable for the early diagnosis of MDR-TB, with a higher sensitivity and specificity for detecting MDR-TB. Therefore, it is recommended that TB programs should consider implementing LPA as part of their diagnostic protocol for MDR-TB.

Further research and validation studies should be conducted to confirm the findings of this study and to evaluate the performance of LPA in different settings and populations. This will help to establish the generalizability and reliability of LPA as a diagnostic tool

for MDR-TB.

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## Contribution of authors:

TTE conceptualized the study, participated in conducting the laboratory work, analyzing data, and writing the first draft of the manuscript. ABA participated in the writing, proofreading, and editing of the manuscript. KJA analysed some data and participated in writing the manuscript. BE and IP participated in the laboratory work. BJS supervised the study and reviewed the manuscript. All authors approved the final copy of the manuscript.

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Authors declare no conflict of interest

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The dataset of this study is available from the corresponding author on reasonable request.

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