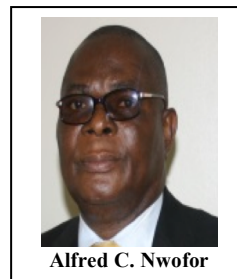


Performance of Genotype MTBDR_{plus} in the Detection of Resistance to Rifampicin and Isoniazid Among Clinical Mycobacteria Isolates in Ilorin, Nigeria

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Abstract: *Background:* Performance of Genotype MTBDR_{plus} assay against Lowenstein Jensen (LJ) proportion method of Drug Susceptibility Testing (DST) in detection of resistance among clinical mycobacteria isolates to rifampicin (RMP) and isoniazid (INH) was evaluated in Ilorin, Nigeria.

Methods: This retrospective study characterized one hundred mycobacteria isolates from pulmonary TB patients, stored on LJ medium and subcultured unto fresh LJ slants before being genotyped using MTBDR_{plus} assay. DST was performed on the isolates against RMP, INH, Ethambutol and Streptomycin.

Results: Genotype MTBDR_{plus} identified 97% and 3% of the 100 isolates as *Mycobacterium tuberculosis* Complex (MTBC) and Non-Tuberculous Mycobacteria (NTM) respectively. Fourteen of the isolates, (14%) were resistant to RMP while 86% were sensitive by the genotypic method. Three of these 14 RMP-resistant isolates were NTMs while 11 were MTBC. Twelve (12%) of the 100 isolates were resistant to INH. Three INH-resistant isolates were NTMs, and 9 were MTBC. Phenotypically and genotypically, the 3 NTMs were resistant to RMP and INH and ten of the 97 MTBC strains were RMP-resistant. One RMP-phenotypically-sensitive strain was genotypically resistant to RMP. Six of the MTBC isolates were resistant to both RMP and INH by both methods. Most mutations occurred in the S-531L and S315T1 codons of *rpoB* and *KatG* genes of RMP and INH, respectively.

Conclusion: The high specificity and positive predictive values recorded by MTBDR_{plus} in our study make it suitable for use in the programmatic management of drug-resistant TB in resource-limited settings.

Keywords: Codons, first-line anti-TB drugs, genotypic, MDR-TB, Nigeria, phenotypic.

INTRODUCTION

With the emergence of multidrug resistant-TB (MDR-TB), which is defined as resistance to at least rifampicin (RMP) and isoniazid (INH) coupled with Human immunodeficiency virus (HIV) pandemic, the early and rapid detection of drug resistance in TB becomes critical for effective patients care and Management [1]. Resistance to the two very important first-line anti-TB drugs of RMP and INH in most cases ends up in treatment failures with associated poor prognosis [2, 3]. MDR-TB is associated with high death rates among HIV-infected persons and treatment of this type of TB requires the use of drugs that are more toxic and less effective and are given at least three times longer and could cost up to 100 times more when compared to treatment of cases of susceptible strains of TB [4]. Cure rates in persons with MDR-strains range from as low as 6% to a high of 59% [5]. According to WHO, an estimated 3.5%

of new TB cases and 20.5% of previously treated cases have MDR-TB [6] Nigeria ranks 10th among the TB high burden countries of the world and 4th in Africa [7]. With the recent TB prevalence survey in Nigeria, the rate of MDR-TB among new TB and previously treated cases now stands at 2.9% and 14.3% respectively [8]

Under optimum program conditions, cure rates for drug-susceptible TB exceed 90%; and hardly exceed 70% for MDR-TB [9]. In most countries where the burden of TB is high, the diagnosis of MDR-TB is often made after prolonged treatment with the “first-line” anti-TB drugs and when it becomes obvious (clinically) that the treatment has failed. Treatment of drug-resistant TB with basic or standard “first line drugs of RMP, INH, streptomycin (S), ethambutol (EMB) and pyrazinamide - (TB drugs that generally have the greatest activity against TB organisms and form the core to any TB treatment regimen and are particularly used for someone with active TB disease that has not had TB drug treatment before) - which is generally inadequate, has some adverse implications which can be avoided if treatment is initiated with a regimen determined by the drug susceptibility patterns of the TB organism. When patients remain on inadequate treatment for long periods of time, the risk of treatment failure or death increases.

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Anti-TB drug combinations that are not adequate may not be able to block or inhibit the growth of *M. tuberculosis* and could result in resistance to drugs that the isolates were originally susceptible to, making the patient to remain infectious with the attendant transmission to close contacts of the patient [10]. In realization of this potential treat, the WHO has recommended the need for countries to start expanding their culture-based DST and to explore the need to introduce new molecular diagnostic methods that detects drug resistance in TB [11, 12]. Rapid detection of MDR-TB will allow for early initiation of treatment of patients with second-line anti-TB drugs, reduce the risk of treatment failure and relapse, decrease possible increase in drug resistance and transmission of MDR-TB.

Over 90% of mutations in the 81-base pair (bp) polymerase have been known to be associated with RMP resistance [13, 14], though with variations in the geographical distribution of certain types of gene mutations.

Monoresistance to RMP is rare but may, most often occur in strains of MTBC that are also resistant to INH. Resistance to RMP therefore can be used as an indicator for MDR-TB and this has led to the development of a variety of diagnostic tests aimed at improving the sensitivity of detecting mutations [13, 14].

Most mutations that confer resistance on INH have been found to occur in several genes and gene regions (15). Depending on variations, which is determined by geographical areas, between 50 to 100% of INH-resistant strains of MTBC harbour mutations in codon 315 *katG* (promoter gene) and/or in the promoter region of the *inhA* gene [16, 17].

The new molecular line probe assay has been shown to have high accuracy when used as screening tool for MDR-TB, achieving a significant reduction in diagnostic time. With superior performance characteristics, ahead of the conventional culture and DST techniques, a high throughput and moderate cost savings, this test has all it takes to revolutionize the diagnosis of MDR-TB [18]. The MTBDRplus assay is sensitive and has been shown to be easy to perform and has the capability for the rapid detection of resistance in *M. tuberculosis* against RMP and INH when applied to both culture isolates and clinical specimens [20]. The introduction of automated liquid culture systems has shortened the turnaround times when compared with solid media culture. However, the liquid systems still requires that mycobacteria cultures be isolated before DST. Because of infrastructural deficiencies, lack of resources and trained personnel, the implementation of these systems in resource-limited countries where the burden of TB and drug resistance are high may be difficult [20].

GenoType MTBDRplus is a commercially available molecular gene line probe assay developed by Hain Lifescience, Nehren, Germany for the differentiation of *Mycobacterium tuberculosis* complex (MTBC) and has capacity for the detection of resistance to RMP and INH by targeting the 81-bp 'hotspot' region of the *rpoB* gene of RMP, codon 315 of *katG* and *inhA* promoter regions of INH. The sensitivity and specificity of MTBDRplus for the detection of resistance to RMP and INH were also found to be high when the assay is applied directly to AFB smear-positive sputum specimens [16].

In this study, we evaluated the performance of MTBDRplus Line Probe Assay (LPA) against the conventional Lowenstein Jensen (LJ) proportion method of DST in the detection of resistance among clinical mycobacteria isolates to RMP and INH mycobacteria isolates in Ilorin, Nigeria.

MATERIALS AND METHODS

Setting

A total of 100 Mycobacteria stored isolates from pulmonary TB patients, grown on LJ were sub-cultured in fresh LJ slants and characterized using the Genotype MTBDRplus assay at the National TB reference Laboratory in Zaria, Nigeria. DST of the isolates to the first-line anti-TB drugs of RMP, INH, EMB and S were determined using the conventional proportion method on LJ media (21) and was carried out in the TB laboratory of Medical Microbiology and Parasitology Department of University of Ilorin Teaching Hospital, Ilorin, Nigeria. The LJ media which were prepared for the different drugs had final concentration of RMP 40µg, INH 0.2µg, EMB 2µg and S 4µg respectively. Serial 10-fold dilutions up to $1/10^4$ were prepared with sterile distilled water. Dilutions ($1/10^2$) were then used to inoculate drug containing media in duplicate while both the $1/10^2$ and $1/10^4$ were used to inoculate drug-free controls. *Mycobacterium tuberculosis* standard avirulent strain (H37Ra) was used as control strain [21]. An isolate is considered sensitive or resistant if the proportion of bacteria in the drug-containing medium to that of drug-free medium is less than or above 1. (The critical concentration of resistance = 1)

DNA Isolation from Stored Isolates

DNA for Genotype MTBDRplus assay were extracted from the mycobacteria isolates according to manufacturer's instructions, by making a bacterial suspension in 300 µL of sterile molecular grade water, heat-killed at 95°C for 20 min to inactivate the mycobacteria, sonicated in an ultrasonic for 15 minutes at room temperature and concentrated by centrifugation at 13,000 rpm for 5 min. The resultant ultrafiltrate contains the DNA which is used for the assay.

Genotype® MTBDRplus Assay

The MTBDRplus assay which is based on DNA strip technology was carried out according to manufacturer's recommendations <http://www.hain-lifescience.com> and as done by others [22, 19]. This assay consists of the steps of amplification [23], hybridization and interpretation [24]. By the process of multiplex polymerase chain reaction (PCR), the *rpoB*, *katG* and *inhA* genes were amplified. The resultant biotin-labeled amplicons were hybridized to the DNA probes bound to membrane strips. The hybridization was detected by addition of a streptavidin/alkaline phosphatase (AP) conjugate and an AP-mediated staining reaction. After hybridization and stringent washing, the strips were removed, air-dried and fixed on clean white paper. Each strip consists of 27 reaction zones or bands which include six controls (conjugate, amplification, *M. tuberculosis* complex - TUB, *rpoB*, *katG* and *inhA* controls), 8 *rpoB* wild-type (WT) and four mutant (MUT) probes, one *katG* WT and two MUT

probes, two inhA WT and four MUT probes. The results were interpreted according to the manufacturer's instructions. Susceptibility to RMP and INH is defined as hybridization to all the WT probes without any hybridization to any MUT probe. Hybridization to both a MUT and to the corresponding WT probe could represent a heterogeneous population of bacteria or a mixed infection made up of sensitive and resistant strain.

Capilia TB test is a simple immunochromatographic assay for the rapid differentiation of MTBC from NTM in liquid cultures and solid media [25, 26] was also performed on the isolates on LJ medium for the purpose of discriminating MTBC from NTMs among the mycobacteria isolates. Most MTBC strains have been correctly identified by Capilia TB tests with sensitivity and specificity of 99.2% and 100% respectively [27]. The test is based on the reaction of monoclonal antibodies against MPB64, one of the predominant proteins excreted by MTBC strains, with the exception of some sub-strains of *M. bovis* bacilli Calmette-Guérin (BCG) [28, 29]. The tests consist of a nitrocellulose membrane on a test plate with immobilized anti-MPB64 mouse monoclonal antibodies conjugated with gold colloid for the detection of MPB64 proteins. In case of an antigen-antibody reaction, a red-purple coloured band becomes visible within 15 min.

1µl of growth on LJ, confirmed to be Acid-fast bacilli (AFB) by Ziehl Neelsen staining was scrapped into 0.2ml of extraction buffer in a screw-capped bijoux bottle and vortexed to suspend the bacterial suspension used as specimen. 80-100µl of specimen was dropped into the specimen placing area of the test plate and allowed to stand at room temperature

Data Analysis

Pearson's Chi-square "Goodness of fit" statistic was used to compare the sensitivity, specificity, positive and negative predictive values of the tests at 95% confidence level.

RESULTS

This retrospective study revealed that the Genotype MTBDR_{plus} LPA identified 97 (97%) of the 100 isolates as MTBC and the remaining 3 (3%) as Non-Tuberculous Mycobacteria (NTM). Of the 97 MTBC isolates, 11 (11.3%) were resistant to RMP while 86 (88.7%) were sensitive by the genotypic method. Out of these 11 MTBC isolates that were resistant to RMP, 5 were mono-resistant to RMP. Nine (9.3%) of the 97 MTBC isolates were resistant to INH while 88 (90.7%) were sensitive, whereas 4 were mono-resistant to INH. The 3 NTMs were consistently resistant to both RMP and INH.

Considering the phenotypic DST as the 'Gold standard' [23], the sensitivity and specificity of the genotypic MTBDR_{plus} for detecting RMP and INH resistance were 86.67% 95% CI [59.51%-97.95%], 100% : 95% CI [95.56%-100%] and 100% 95 CI [62.91%-100%], 100% : 95% CI [62.91%-100%] while the positive and negative predictive values for RMP and INH were 100% : 95% CI [75.12%-100%], 100% : 95% CI [62.91%-100%] and 97.62% 95% CI

[91.64%-99.64%], 100% 95% CI [95.90%-100%] respectively.

Table 3 shows the results of MTBDR_{plus} for Isolates with Resistance to RMP and INH against the phenotypic proportion method on LJ. Of the 10/100 (10%) isolates (ID: IL-002, IL-004, IL-010, IL-042, IL-044, IL-057, IL-084, IL-090, IL-093 and IL-095) that were resistant to RMP by the phenotypic method, all (10) were also resistant by the genotypic method. However, the one sensitive to RMP (isolate ID—IL-003, Table 3) by the phenotypic method was resistant by the genotypic method. Six of the MTBC isolates were resistant to both RMP and INH (MDR-TB).

The mutation patterns produced by Genotype MTBDR_{plus} assay in this study which describes the frequency of mutation in the rpoB, katG and inhA genes are shown in Fig. (1).

Of the 10 RMP resistant MTBC strains detected by this assay, the most mutated codon on the rpoB gene in rifampicin resistance is rpoBMUT3[S-531L] (50%). This is followed by codon rpoBMUT2B [H-526Y] and codon rpoBMUT1 [D516V] with 30.0% and 20.0% frequencies respectively. The three mutations observed for the 7 INH resistances for katG genes were on codon katGMUT1[S-315T] (42.9%). No mutations were detected on the inhA genes.

DISCUSSION

This retrospective study evaluated the performance of Genotype MTBDR_{plus} assay against the conventional LJ proportion method for DST in the detection of resistance among clinical mycobacteria isolates to RMP and INH in Ilorin, Nigeria. The findings suggest that pulmonary TB patients in Nigeria from whom MDR-TB were detected by Genotype MTBDR_{plus} assay should be introduced to treatment with second-line anti-TB drug regimens without delay. The sensitivity of the genotypic MTBDR_{plus} for detecting RMP and INH resistance recorded in our study was 86.67% 95% CI [59.51%-97.95%], 100%: 95% CI [95.56%-100%] and this was similar to what was reported by independent research teams [30, 31, 32, 33, and 34] for RMP and consistent with the findings of [30, 31, 33], who reported 84-100% for INH. The data show substantial difference with previous findings that showed 73% and 34.6% respectively [34, 35]. Our study recorded specificities of 100% 95 CI [62.91%-100%], 100%: 95% CI [62.91%-100%] resistance to RMP and INH respectively. This is similar to the findings of [18, 30, 31]. The Positive predictive values for RMP and INH in this study were 100%, 95% CI [75.12%-100%] and 100%, 95% CI [62.91%-100%] respectively; findings that were in agreement with [18, 36]. Our finding in this study which showed a low frequency generation of INH resistance than RMP is different from others reported in Nigeria [5,37], probably because the persons from whom the isolates were recovered were all supposed to be TB treatment naïve and the uptake of IPT when the organisms were isolated was suboptimal.

There have been previous reports suggesting that the frequency of particular mutations and the correlation of RMP resistance to the MDR phenotype may vary [23, 24].

Table 1. Drug concentrations (added to LJ media) and critical proportion for determination of resistance.

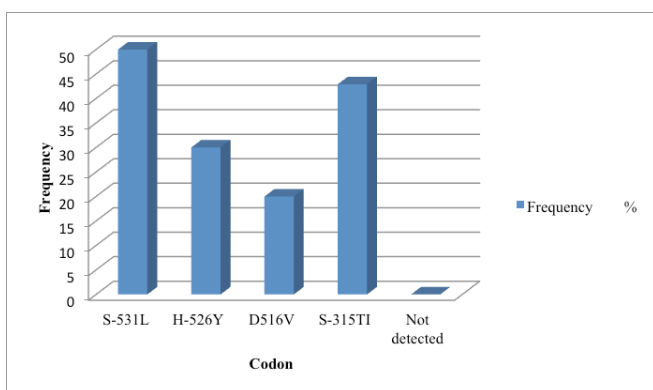
Drug	Media Concentration of Anti-TB Drugs	Critical proportion of growth of Mycobacteria to Determine Resistance
Streptomycin(dihydro-streptomycin sulfate)	4 µg/ml	1%
Isoniazid (INH)	0.2 µg/ml	1%
Rifampicin (RMP)	40 µg/ml	1%
Ethambutol (EMB)	2 µg/ml	1%

Table 2. The sensitivity, specificity, positive and negative predictive values (PPV, NPV) of genotype MTBDRplus assay on 97 Mycobacteria isolates.

	Sensitivity	Specificity	PPV	NPV
RMP	86.67%; 95%CI: [59.51-97.95%]	100%; 95%CI: [95.56%-100%]	100%; 95%CI: [75.12%-100%]	97.62%; 95%CI: [91.64% -99.64%]
INH	100%; 95%CI [62.91% -100%]	100%; 95%CI (95.90%-100%)	100%; 95%CI: (62.91%-100%)	100%; 95%CI (95.90%-100%)

Table 3. Results of MTBDRplus for Isolates with Resistance to RMP and INH using the LJ.

Strain	LJ Proportion Method	MTBDRplus Line Probe Assay					
		Rifampicin (RMP)		Isoniazid (INH)			
		<i>rpoB</i> gene		<i>KatG</i> gene		<i>inhA</i> gene	
		Missing WT	Mutation	Missing WT	Mutation	Missing WT	Mutation
IL - 002	R-R	WT8	<i>rpoB</i> MUT3 [S531L]	WT	<i>katG</i> MUT 1 [S315T]		
IL – 003	S-R	WT2, WT3, WT4	<i>rpoB</i> MUT1 [D516V]	WT	-		-
IL -004	R-R	WT 8	<i>rpoB</i> MUT3 [S531L]	-	<i>katG</i> MUT 1 ([315T]	-	-
IL – 010	R-S	WT 7	<i>rpoB</i> MUT2B [H526D]	-	-	-	-
IL -042	R-S	WT1, WT4	-	-	-	-	-
IL – 044	R-S	WT 7,	<i>rpoB</i> MUT 2B [H526D]	-	-	-	-
IL – 057	R-S	WT 8	<i>rpoB</i> MUT3 [S531L]		-	-	-
IL – 084	R-R	WT 8	<i>rpoB</i> MUT3 [S531L]	WT		-	-
IL – 090	R-R	WT 7	<i>rpoB</i> MUT2B [H526D]	WT	-	-	-
IL – 093	R-R	WT 2, W3, W4	<i>rpoB</i> MUT1 [D516V]	WT	-	-	-
IL – 095	R-R	WT3, WT4	<i>rpoB</i> MUT3 [S531L]	WT	<i>katG</i> MUT 1 [S315T]	-	-

**Fig. (1).** Results of MTBDRplus for isolates with resistance to RMP and INH showing the most mutated codons.

This study revealed that the most mutated codon in the resistance of the isolates to RMP (50%) was the *rpoB* MUT3[S-531L] of the *rpoB* gene. This finding is comparable to the frequencies reported by Huyen NT *et al.* [30]. Similar results have also shown that *rpoB*MUT3[S-531L] mutations in the *rpoB* gene occurred even more frequently i.e. 70.5% and 60.4% respectively [18, 23]. This was followed by Codon *rpoB*MUT2B [H-526Y] with 30% mutations. This figure is high and not in agreement with lower frequencies reported in other studies, i.e. 14.1%, 15% and 8,6% [18, 34], although there may be variations in the distribution in certain environments [38]. One factor, limiting the generalizability of this study is it's small sample size. The frequency of mutation of Codon *rpoB*MUT1 [D-516V] was 20%. This was in contrast to the findings of [18, 23] which detected similar frequency of 9.2%. As for INH resistances, out of the

7 resistant isolates detected by the MTBDR*plus* in this study, 3(42.9%) mutations occurred in the *katGMUT1*[S-315T1] codon of the *katG* gene and this is similar to the findings of [18]. The findings of this study are however in contrast to the frequency reported by [30, 39].

Certain mutations that occur on some genes or gene regions as well as some mutation mechanisms are not detected by MTBDR*plus*. Resistances occasioned by such mutation mechanisms in some isolates could be missed out and cannot be detected (MTBDR*plus* kit insert). Such mutations outside the *rpoB* gene region could lead to a missing wild type probe, which could confer resistance on the isolate. This could probably explain the reason why one of the isolates in this study was sensitive to RMP by the conventional LJ proportion (phenotypic) method but resistant by the MTBDR*plus* (genotypic) method. Although more than 90% of mutations associated with RMP resistance occur in the 81-bp core region of the *rpoB* gene [13, 14], there are certain mutations (though rare) that are also associated with RMP resistance and have been reported in the amino-terminal of *rpoB* [40]. Majority of laboratories that use molecular methods do not have the capacity to screen beyond the 81-bp region of *rpoB* gene and so could miss out such amino-terminal mutations. Further studies in this regard will be required since such was outside the scope of this study.

One limitation of this study was that we tested only a single concentration of INH (0.2 µg/ml) and this could have accounted for the non-detection of low level INH resistance in the isolates tested.

Rifabutin (a semisynthetic derivative of rifamycin S), together with RMP belong to the family of rifamycin. It is as effective as RMP in the treatment of TB but has lesser effect on antiretroviral metabolism. It is safe, effective and may save cost when used in combination with Protease Inhibitor (PI)-based regimens. This may explain why RFB is recommended for TB treatment in HIV co-infected patients [41]. Both RMP and RFB share some common mutation codons - *rpoBMUT2B* [H-526D] and *rpoBMUT3*[S-531L] which are reported to result in high-level resistance to both drugs.

The most mutated codons in the *rpoB* region of RMP as detected in this study were *rpoBMUT3* [S-531L], *rpoBMUT2B* [H-526D] and *rpoBMUT1* [D-516V] respectively. Whereas the isolates that showed alteration in the *rpoBMUT1* [D-516V] codon may be sensitive to RFB, those that have alteration in the *rpoBMUT3* [S-531L] and *rpoBMUT2B* [H-526D] codons will expectedly be resistant to both drugs. Rifabutin is widely available in the Nigeria TB program and is highly utilized. In Nigeria, rifabutin is preferred and only used for persons living with HIV (PLHIV) who require PI-based ART and anti-TB drugs [42].

The 6% MDR-TB cases found among these supposedly treatment naïve patients in this study have grave public health implications in Nigeria as it poses a great challenge to the TB control programs. With the current prevalence rate of MDR-TB (2.9% among new case and 14.3% among previously treated TB patients in Nigeria respectively), there are implications that there could be widespread transmission of MDR-TB among the communities.

Three out of the 100 mycobacterial isolates in this study (3%) came out as NTMs. There has been a rise in the diseases attributable to NTMs, with increasing frequency of the infections, especially pulmonary disease, with studies showing that NTMs are responsible for an increasing proportion of mycobacterial disease in developed and developing countries [43, 44]. Quite a significant proportion of mycobacterial diseases in both the developed and developing countries of the world are caused by NTM [43, 44]. Recent studies in Nigeria reported 4.1% and 15% NTM infection among patients seeking treatment for pulmonary tuberculosis [45, 46]. The frequency at which NTMs are being isolated from TB positive HIV-infected and non-infected persons within Sub-Saharan Africa is increasing [47] and apart from HIV disease, exposure to dust as an occupational hazard has been shown to be a risk factor of infection with NTMs [48].

NTMs are found virtually everywhere and could easily be isolated from such environmental sources as surface water, tap water and from the soil [48]. The isolation of an NTM therefore, from any respiratory specimen from a patient, without any clinical signs and symptoms of pulmonary disease could be spurious and does not in any way indicate NTM lung disease [48]. Even though there is an existing revised diagnostic criteria for NTM lung disease [49], there is need to rule out to a large extent, other possible causal agents of pulmonary disease, coupled with the presence of respiratory symptoms, a radiograph, showing compatible abnormal features as well as confirmed bacteriologic evidence of NTM. Only the confirmation of the clinical importance of the species should inform the need for DST [50] as the performance of DST on non-significant isolates will amount to a waste of time and resources both on the part of the patient and the laboratory. The results of such DST could be confusing and harmful for the care of the patient [51].

It is important to identify the species and clinical significance of NTMs because; the drugs used for the treatment are expensive, prolonged and often toxic. Treatment regimens may also differ by species of NTMs.

Treatment of these infections is time-consuming, complicated and often ineffective. The comparable ineffectiveness of standard-of-service smear microscopy and point-of care GeneXpert technology in discriminating between NTM and MTBC, underscores the urgent need for the introduction of molecular assays that could rapidly detect NTM pulmonary disease in high burden resource limited settings as a priority for strengthening public health response [46]. This will necessitate timely Drug Susceptibility Testing of the NTMs against other drugs; macrolides (clarithromycin) and aminoglycoside to which they may be sensitive so the patients can be treated and get cured.

CONCLUSION

The Genotype MTBDR*plus* assay has proved in this study to be reliable and easy to perform and to simultaneously detect resistance to both RMP and INH among MTBC isolates. With the high sensitivity for detecting RMP resistance, the high specificity and positive predictive values recorded for MDR-TB by MTBDR*plus* in

this study, we recommend that this test is suitable for use in the programmatic management of MDR-TB in Nigeria. No low level of INH resistance was detected among the isolates, suggesting that the technology can be used as a rapid method to detect RMP resistance and the high-level INH resistance among strains of *M. Tuberculosis* in a single assay. With the finding of 6% MDR-TB in this study, the establishment of a treatment center in Ilorin should be explored. There is however, an urgent need to explore and put in place mechanisms that will allow enhanced detection and diagnosis of NTM pulmonary disease, especially in countries with increased frequency of isolation, especially resource-limited countries where laboratories lack the diagnostic capacity. This will allow for timely initiation of treatment with the right regimen and better patient management [51]. The need for increased communication between clinicians and laboratory personnel for better treatment outcome is highlighted. Prompt consultation with one of the specialized centres of expertise is warranted with a need also to consider formulating national guidelines for diagnosis and treatment of MTN infections.

CONFLICT OF INTEREST

We declare that we have no conflicts of interest and no competing financial interest.

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