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## Gene mutation patterns of *Mycobacterium tuberculosis* complex and associated factors among suspected multidrug-resistant tuberculosis patients in Osun State, South-West, Nigeria

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### ABSTRACT

Tuberculosis (TB) drug resistance has become a substantial public health threat, posing a significant challenge to global TB control. Nigeria is one of the eight countries contributing two-thirds of the global TB cases in 2020, with a high rate of MDR/RR-TB. This study was designed to determine MDR-TB prevalence among suspected drug-resistant TB patients, MTBC mutation patterns, and the associated factors of MDR-TB in Osun State. We obtained fresh sputum samples from eligible participants who tested positive for rifampicin resistance on GeneXpert assay for analysis using the MTBDRplus LPA. SPSS version 23 was used for statistical analysis, significance was set at  $p < 0.05$ . We recorded a prevalence of 58.6 %, 37.1 %, and 2.9 % for MDR-TB, rifampicin and isoniazid mono-resistance. Majority of the MDR-TB cases were males (56.1 %), below 40 years (80.5 %), low-level education (75.6 %), unemployed (75 %), and low-income earners (64.1 %). 'Previous TB treatment' had a statistically significant effect on MDR-TB ( $P < 0.001$ , OR=65.46). Other factors that showed a strong odds ratio but were not statistically significant included: age (OR=2.52,  $P = 0.09$ ), diabetes (OR=1.99,  $P = 0.25$ ), smoking history (OR=1.51,  $P = 0.43$ ) and hypertension (OR=1.34,  $P = 0.63$ ). There were 67 and 46 reactions of the wild-type and mutant bands for *rpoB* gene, eight wild-type and five mutant bands for *katG* gene, and six wild-type and 24 mutant bands for *inhA* gene. WT8/MUT3 (64.2 %), WT/MUT1 (61.5 %), and MUT1 (80 %) were predominant for *rpoB*, *katG*, and *inhA* genes, respectively, with the occurrence of D516V *rpoB* gene mutation for the first time in Osun State. There is a need for the reinforcement of the current TB care system, empowerment and monitoring of TB-caregivers for adequate and effective follow-up of TB patients, and more citizens awareness, especially regarding TB/MDR-TB treatments. This is expected to improve the management of MDR-TB patients and mitigate the development and spread of MDR-TB in the community.

### Introduction

Tuberculosis (TB) is a contagious disease caused by *the Mycobacterium tuberculosis* complex (MTBC) [1] and predominantly by

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*Mycobacterium tuberculosis* (MTB) [2]. It has remained a significant cause of ill health and the leading cause of death from a single infectious disease outside COVID-19 [3,4]. MTBC comprises multiple clonal lineages and sub-lineages marked by various specific mutations, such as single nucleotide polymorphisms (SNPs) and genomic deletions [5].

The WHO classified Nigeria as one of the eight countries (first in Africa and sixth in the global ranking), contributing two-thirds of the global TB cases in 2020. Nigeria has an estimated TB-incidence rate of 219 per 100,000 population and 2100 laboratory-confirmed MDR/RR-TB cases in the same year, out of which 1600 (76.2 %) were started on second-line treatment with a treatment success rate averaging 77.5 % over four years covering 2015–2018 according to the WHO global report [3].

Sadly, drug resistance in TB has become a significant public health threat posing a great challenge to global TB control efforts due to the complex diagnostic and treatment requirements and high management cost [6,7]. Isoniazid (INH) and rifampicin (RIF) are the two most effective first-line drugs for the treatment of TB [8]. However, the resistance to both drugs (INH and RIF), which is known as multidrug-resistant TB (MDR-TB), is the most significant concern of the global TB community [3].

DNA sequencing studies have shown that RIF resistance within the MTBC has a mutation within the 81-bp region of the *rpoB* gene. At the same time, INH resistance is located in several regions, such as, in codon 315 of the *katG* gene, *inhA* promoter region and mutations in the *ahpC-oxvR* intergenic region. A number of molecular methods have been established to evaluate the *rpoB* and *katG/inhA* genes for determining resistance to RIF and INH, respectively [9,10].

Rapid and accurate TB/DR-TB disease diagnosis is a critical management strategy [2]. The WHO has endorsed the commercially available Xpert MTB/RIF and MTBDRplus [1] assays for the rapid detection of MTB and its drug resistance to the most important first-line drugs [11]. While the GeneXpert MTB/RIF can only detect resistance to RIF, MTBDRplus can detect resistance to RIF and INH.

The GeneXpert MTB/RIF (Cepheid, USA) assay is a nucleic acid amplification test that uses a disposable cartridge with the GeneXpert Instrument System. It detects MTBC and resistance to rifampicin via mutation of the *rpoB* gene (coding for the beta-subunit of the RNA polymerase) in less than 2 h [7]. The Xpert MTB/RIF assay primers amplify a portion of the *rpoB* gene containing the 81 base-pair "core" region. The probes can differentiate between the conserved wild-type sequence and mutations in the core region associated with rifampicin resistance [1].

The Genotype MTBDRplus LPA (Hain Lifescience GmbH, Nehren, Germany) is an innovative kit-based molecular method for detecting MTBC and its resistance to RIF and INH. This technology detects the most significant mutations of the *rpoB* gene for rifampicin resistance, and *katG* (associated with high-level INH resistance) and *inhA* (associated with low-level INH resistance) genes for isoniazid resistance. The kit has the advantage of accuracy and turnaround time [2,12].

Although culture is still the gold standard for detecting TB and determining drug susceptibility, it has the disadvantage of taking a long time to produce the result, which could be about 4–8 weeks [1]. Smear microscopy for acid-fast bacilli (AFB), on the other hand, is a rapid and inexpensive method of detecting TB but has the disadvantage of poor sensitivity and poor positive predictive value (PPV). Quick identification is essential to initiate early treatment, improve patient outcomes, and be more effective for public health interventions [7].

Studies conducted in various geographical regions of the world have shown that the burden of MDR-TB and the mutations responsible for drug resistance vary between regions [7,13]. This disparity may result from the wide variation in circulating MTBC strains across the world [12]. Several risk factors have been reported for TB disease, which include poverty, undernutrition, HIV infection, alcohol, smoking and diabetes [3,14]. However, Xi and colleagues [4] found lung cavity, previously diagnosed TB and history of anti-TB therapy as risk factors for MDR-TB.

The objectives of this study were two-fold: first, to determine the prevalence of MDR-TB among suspected MDR-TB patients and the mutation patterns of MTBC drug resistance using the Genotype MTBDRplus LPA. Secondly, to determine the associated factors of MDR-TB among study participants in Osun State, South-West, Nigeria, in order to improve the management of DR-TB cases.



Fig. 1. Map of Osun State, South-West, Nigeria.

## Materials and methods

### *Study design and location*

The institutional-based, cross-sectional study was conducted between July 2019 and December 2021. The study was conducted in Osun State, South-West Nigeria (Fig. 1), which is among the ten (10) states with the country's highest TB case notification rate. Samples were collected in the following locations in the state: Iwo, Ikire, Ife, Okebale and Ede centres. All centres are equipped with GeneXpert and DOTS treatment facilities.

### *Eligibility*

Patients whose samples tested positive for MTB with RIF resistance on a GeneXpert assay were recruited into the study. Others, such as MTB-detected RIF sensitive and MTB not detected, including invalid results, were excluded from the study, while those unwilling to participate were also excluded.

### *Ethical statement*

The study was conducted in accordance with the Declaration of Helsinki. Osun State Specialist Hospital Institutional Review Committee approved the study protocol (HREC/27/2015/SSHO/060). The researchers obtained written informed consent from the participants before their inclusion in the study, and their privacy and confidentiality were adequately guaranteed. All data collected from the participants, including test results, were de-identified, protected and secured in passworded personal computers and cloud storage.

### *Sample size and sampling technique*

All samples from the various study centres that met the inclusion criteria within the study period and willing to participate were included in the study. The number of eligible respondents was determined using the formula for cross-sectional studies [15], which gave a sample size of 70 at a 5 % level of significance and 95 % confidence interval, using a 4.8 % MDR-TB prevalence in line with the National DR-TB prevalence survey report in Nigeria. The study centres were selected by simple random technique from the various GeneXpert centres in the state.

### *Data collection*

Interviewer administered structured questionnaire was used for data collection from the study participants by trained research assistants. The research assistants were recruited among the health workers at the various study sites and trained in sputum sample handling and questionnaire administration. The data collection instrument was categorized into four (4) sections; section 1 included questions on the socio-demographics of participants (gender, age, marital status, occupation, income, and education), while sections 2, 3, and 4 comprised questions that border on TB, social and medical risk factors. Questions on TB risk factors included: previous TB treatment, treatment completion, treatment disruption, and contact with TB patients. Questions on social risk factors included history of alcohol use, history of drug use, and smoking history, while questions on medical risk factors included HIV status, diabetes, hypertension, and other health conditions (asthma, arthritis, hepatitis B, and fibroids). The questionnaire was administered to eligible participants before the commencement of antitubercular therapy.

### *GeneXpert*

GeneXpert (Cepheid, USA) analysis was done at the various sample collection sites according to the manufacturer's operational guidelines. The sputum samples submitted to the laboratory were liquefied and inactivated in a 2:1 dilution with the Xpert sample reagent at room temperature for 15mins, 2 ml of the preparation was transferred to the test cartridge and inserted into the test platform. The automated Xpert machine produced results within 2 hours.

### *Genotype MTBDRplus LPA*

Fresh sputum samples were collected from each eligible participant before the commencement of antitubercular therapy and transported to the African center of Excellence for Genomics of Infectious Diseases (ACEGID), Redeemer's University, Ede, Osun State, Nigeria, under a cold chain procedure. Digestion and decontamination of the specimens were done using equal volumes of NaOH/NALC—Na citrate solution. DNA extraction was done with Genolyse kit on the decontaminated sputum samples by centrifugation, lysis at 95 °C and neutralization. Multiplex amplification with biotinylated primers was done by adding 5ul of DNA sample to 10ul and 35ul of amplification mixes A and B (AM-A and AM-B) in PCR tubes. The tubes were transferred to Eppendorf thermocycler, using the amplification protocol provided by the manufacturer (15 min at 95 °C for 1 cycle, 30 s at 95 °C and 2 min at 65 °C for 20cycles, 25 s at 95 °C, 40 s at 50 °C and 40 s at 70 °C for 30cycles, and 8 min at 70 °C for 1cycle). Reverse hybridization was carried out using a shaking water bath at conditions and reagents provided by the manufacturer. Evaluation and interpretation of results were done in line with

the manufacturer's guidelines (Hain Lifescience, GmbH, Nehren, Germany).

### Quality control

We adhered to the manufacturers' quality control guidelines regarding sample analysis and the five (5) control zones on the test strip and ensured we added extraction and PCR negative controls to every batch of samples. At any point where the quality control failed, we repeated the test.

### Statistical analysis

Data generated from the fieldwork and results of laboratory analysis were entered in Excel spreadsheet (Excel 2013 version), exported, and analysed using the SPSS statistical package version 23 (SPSS Inc, Chicago, IL). The frequency of individual variables was done to determine data completeness and consistency. Numerical variables were presented in frequency tables and percentages, chi-square test and bivariate analysis were performed for categorical variables, while odds ratio was used to determine the association between potential risk factors and MDR-TB. The significance level was set at  $p < 0.05$  with a 95 % confidence interval.

## Results

### Socio-demographic characteristics of study participants

The study was carried out in five health facilities (Iwo, Ikire, Ife, Okebale and Ede) in Osun State, South-Western Nigeria, with a total of 70 participants comprising 38 (54.3 %) males and 32 (45.7 %) females. Most of the participants (72.8 %) were less than 40 years old, while other predominant socio-demographic characteristics included married people (71.4 %), artisans (48.6 %), low-income earners (55.7 %), and low-level education (75.7 %) which consist of 'No formal education' (11.4 %) and 'primary/secondary education' (64.3 %) categories. The socio-demographic characteristics of the study participants are presented in [Table 1](#).

**Table 1**

Socio-demographic characteristics of study participants with MDR-TB classification ( $n = 70$ ).

Variable	Occurrence (%)	MDR-TB		P-value
		Positive	Negative	
Location				0.081
Ikire	21 (30.0)	8(38.1)	13(61.9)	
Iwo	25 (35.7)	14(56)	11(44)	
Ife	8 (11.4)	7(87.5)	1(12.5)	
Okebale	7 (10.0)	5(71.4)	2(28.6)	
Ede	9 (12.9)	7(77.8)	2(22.2)	
Gender				0.717
Male	38 (54.3)	23(60.5)	15(39.5)	
Female	32 (45.7)	18(56.3)	14(43.7)	
Age (years)				0.315
20–29	24 (34.3)	16(66.7)	8(33.3)	
30–39	27 (38.6)	17(63)	10(37)	
40–49	10 (14.3)	3(30)	7(70)	
≥50	9 (12.8)	5(55.6)	4(44.4)	
Marital status				0.151
Single	10 (14.3)	9(90)	1(10)	
Married	50 (71.4)	27(54)	23(46)	
Divorced	3 (4.3)	1(33.3)	2(66.7)	
Widow/widower	7 (10.0)	4(57.1)	3(42.9)	
Occupation				0.758
Govt worker	14 (20)	9(64.3)	5(35.7)	
Artisan	34 (48.6)	20(58.8)	14(41.2)	
Private business	18 (25.7)	9(50)	9(50)	
Unemployed	4 (5.7)	3(75)	1(25)	
Income range				0.326
Low	39 (55.7)	25(64.1)	14(35.9)	
Medium	30 (42.9)	16(53.3)	14(46.7)	
High	1 (1.4)	0(0)	1(100)	
Education				0.589
No formal education	8 (11.4)	6(75)	2(25)	
Primary/Secondary	45 (64.3)	25(55.6)	20(44.4)	
Post-Secondary	17 (24.3)	10(58.8)	7(41.2)	

KEY: MDR-TB: Multidrug-resistant Tuberculosis; Govt: Government.

Associated risk factors of study participants

The associated risk factors of study participants (TB, social, and medical risk factors) are presented in Table 2. The survey showed that most participants (51/72.9 %) were previously treated for TB; however, 45 (88.2 %) of those previously treated completed their treatments, while 19 (42.2 %) of those who completed their treatments had treatment disruptions. Meanwhile, about three-fifths (28/54.9 %) of those previously treated were males, while 16 (22.9 %) of the study participants had previous contact with TB patients.

Regarding social risk factors, 32.9 % had a smoking history, while 27.1 % and 24.3 % had a history of alcohol and drug use, respectively. Interestingly, 87 % and 68.4 % of the participants with a history of smoking and alcohol use were males, while the majority (52.9 %) of those with a history of drug use were females. A significant statistical association was found between gender and the smoking history of participants ( $P < 0.001$ ).

In this study, associated medical risk factors included hypertension (20 %), diabetes (24.3 %), HIV (25.7 %), and other health conditions (30 %) comprising asthma, arthritis, hepatitis B, and fibroids. Most of the males were diabetic (64.7 %) and hypertensive (64.3 %), while the majority of the females had other health conditions (66.7 %). The association between gender and other health conditions ( $P = 0.021$ ) was statistically significant.

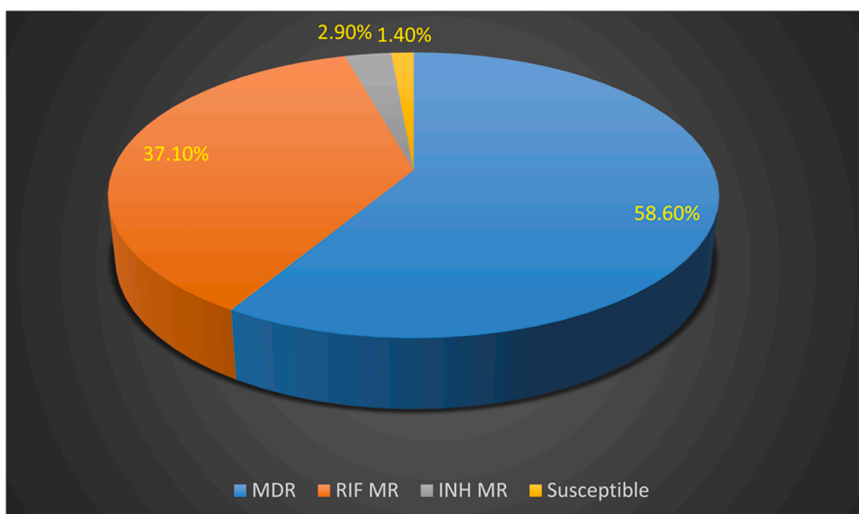
Distribution and prevalence of MDR-TB

In this study, MDR-TB prevalence by MTBDRplus LPA was 58.6 % (Fig. 2). The distribution of MDR based on location (Table 1) comprised Ikire (8/19.5 %), Iwo (14/34.1 %), Ife (7/17.1 %), Okebale (5/12.2 %), and Ede (7/17.1 %). In terms of prevalence, Ife center had the highest prevalence while Ikire had the lowest: (Ife{87.5 %} > Ede{77.8 %} > Okebale{71.4 %} > Iwo{56 %} > Ikire {38.1}). However, the relationship between MDR-TB prevalence and location was not statistically significant ( $P = 0.081$ ). The majority of the MDR-TB cases (23/56.1 %) occurred among males (Table 2), and the gender prevalence spread was 60.5 % (23/38) among males and 56.3 % (18/32) among females (Table 1).

**Table 2**  
Risk factors of study participants based on gender and MDR-TB classifications.

Variable	Gender			MDR-TB		
	Male	Female	P-value	Positive	Negative	P-value
Occurrence (%)						
Previous TB Treatment			0.865			<0.001
Yes	51 (72.9)	28 (54.9)	23 (45.1)	40 (78.4)	11 (21.6)	
No	19 (27.1)	10 (52.6)	9 (47.4)	1 (5.3)	18 (94.7)	
Treatment completion (N = 51)			0.538			0.756
Yes	45 (88.2)	24 (53.3)	21 (46.7)	35 (77.8)	10 (22.2)	
No	6 (11.8)	4 (66.7)	2 (33.3)	5 (83.3)	1 (16.7)	
Treatment disruption N = 45			0.685			0.925
Yes	19 (42.2)	11 (57.9)	8 (42.1)	15 (78.9)	4 (21.1)	
No	26 (57.8)	14 (53.8)	12 (46.2)	20 (76.9)	6 (23.1)	
Contact with TB patient			0.695			0.830
Yes	16 (22.9)	8 (50)	8 (50)	9 (56.3)	7 (43.7)	
No	54 (77.1)	30 (55.6)	24 (44.4)	32 (59.3)	22 (40.7)	
History of alcohol use			0.147			0.538
Yes	19 (27.1)	13 (68.4)	6 (31.6)	10 (52.6)	9 (47.4)	
No	51 (72.9)	25 (49)	26 (51)	31 (60.8)	20 (39.2)	
History of drug use			0.49			20.981
Yes	17 (24.3)	8 (47.1)	9 (52.9)	10 (58.8)	7 (41.2)	
No	53 (75.7)	30 (56.6)	23 (43.4)	31 (58.5)	22 (41.5)	
Smoking history			<0.001			0.430
Yes	23 (32.9)	20 (87)	3 (13)	15 (65.2)	8 (34.8)	
No	47 (67.1)	18 (38.3)	29 (61.7)	26 (55.3)	21 (44.7)	
HIV status			0.700			0.387
Positive	18 (25.7)	11 (61.1)		7 (38.9)	9 (50)	9 (50)
Negative	40 (57.2)	20 (50)		20 (50)	23 (57.5)	17 (42.5)
Unknown	12 (17.1)	7 (58.3)		5 (41.7)	9 (75)	3 (25)
Diabetes			0.332			0.248
Yes	17 (24.3)	11 (64.7)	6 (35.3)	12 (70.6)	5 (29.4)	
No	53 (75.7)	27 (50.9)	26 (49.1)	29 (54.7)	24 (45.3)	
Hypertension			0.401			0.627
Yes	14 (20)	9 (64.3)	5 (35.7)	9 (64.3)	5 (35.7)	
No	56 (80)	29 (51.8)	27 (48.2)	32 (57.1)	24 (42.9)	
Other health conditions			0.021			0.874
Yes	21 (30)	7 (33.3)	14 (66.7)	12 (57.1)	9 (42.9)	
No	49 (70)	31 (63.3)	18 (36.7)	29 (59.2)	20 (40.8)	
MDR-TB						0.717
Positive	41 (58.6)	23 (56.1)	18 (43.9)			
Negative	29 (41.4)	15 (51.7)	14 (48.3)			

KEY: MDR-TB: Multidrug-resistant Tuberculosis; Other health conditions (Asthma, arthritis, Hepatitis B and fibroids).



**Fig. 2.** Prevalence of Drug-Resistant Tuberculosis. MDR-TB: Multidrug-resistant Tuberculosis, RIF: Rifampicin, INH: Isoniazid, MR: Mono-resistance.

Regarding the age range of participants, MDR-TB cases were predominant (80.5 %) among young people under 40 years. The unemployed had the highest prevalence (75 %) among the occupations of the study participants, while low-income earners and low-level education had the highest prevalence of 64.1 % and 75.6 %, respectively (Table 1).

There was a significant statistical relationship ( $P < 0.001$ ) between MDR-TB occurrence and those previously treated for TB compared to treatment naïve participants, where previously treated participants had an MDR-TB prevalence of 78.4 % (Table 2).

Out of the 29 (41.4 %) non-MDR cases, 26 (37.1 %) were positive only for Rifampicin (Rifampicin mono-resistance), two (2.9 %) were positive only for Isoniazid (Isoniazid mono-resistance), while one (1.4 %) was susceptible to both Rifampicin and Isoniazid (Fig. 2).

**Table 3**  
Gene mutation patterns of study participants.

Locus/ band	Gene mutation	RIF MR (%)	INH MR (%)	MDR-TB (%)	Total (%)
<i>RpoB</i>					
WT3	513–517	5 (11.1)	12 (17.7)	17 (15.0)	
WT5	518–522	2 (4.4)	3 (4.4)	5 (4.4)	
WT7	526–529	0	2 (2.9)	2 (1.8)	
WT8	530–533	19 (42.2)	24 (35.3)	43 (38.1)	
MUT1	D516V	0	1 (1.5)	1 (0.9)	
MUT2B	H526D	0	2 (2.9)	2 (1.8)	
MUT3	S531L	19 (42.2)	24 (35.3)	43 (38.1)	
Total	45	68	113		
<i>katG</i>					
WT	315	1 (50)	7 (36.8)	8 (38.1)	
MUT1	S315T1	1 (50)	12 (63.2)	13 (61.9)	
<i>inhA</i>					
WT1	–15/–16	0	6 (16.7)	6 (16.7)	
MUT1	C15T	0	30 (83.3)	30 (83.3)	
Gene mutation combinations					
<i>rpoB</i>					
WT3	513–517	5 (19.2)	11 (26.8)	16 (23.9)	
WT3 & MUT1	D516V	0	1 (2.4)	1 (1.5)	
WT5	518–522	2 (7.7)	3 (7.3)	5 (7.4)	
WT7 & MUT2B H526D	0	2 (4.9)	2 (3.0)		
WT8 & MUT3 S531L	19 (73.1)	24 (58.5)	43 (64.2)		
<i>katG</i>					
WT & MUT1	S315T1	1 (50)	7 (63.7)	8 (61.2)	
MUT1	S315T1	1 (50)	4 (36.4)	5 (38.5)	
<i>InhA</i>					
WT1 & MUT1	C15T	0	6 (20)	6 (20)	
MUT1	C15T	0	24 (80)	24 (80)	

WT: Wild type band, MUT: Mutation band, MR: Mono-resistance, MDR-TB: Multidrug-resistant Tuberculosis, RIF: Rifampicin, INH: Isoniazid.

## Gene mutation patterns

A total of 41 MDR (58.6 %), 26 RIF mono-resistant (37.1 %) and 2 INH mono-resistant (2.9 %) cases, and 1 (1.4 %) susceptible to both RIF and INH (Fig. 2) were detected by MTBDRplus LPA using *rpoB* gene for RIF resistance while *katG* and *inhA* promoter regions coded for INH resistance. For the *rpoB* gene, 67 reactions to the wild-type and 46 reactions to the mutant bands were recorded, the 46 mutant bands reacted along with their corresponding wild-type bands, while 21 of the wild-type bands developed without any mutant band. The wild-type bands recorded in this study comprised WT8 (43/64.2 %), WT3 (17/25.4 %), WT5 (5/7.4 %) and WT7 (2/3.0 %) corresponding to the following gene mutation bands of the *rpoB* gene, 530–533, 513–517, 518–522 and 526–529 respectively, while the mutant bands comprised MUT3 (93.5 %), MUT2B (4.3 %) and MUT1 (2.2 %) corresponding to S531L, H526D and D516V respectively (Table 3).

For the *katG* gene, 13 reactions were observed comprising eight wild-type bands in combination with their corresponding mutant bands, while five mutant bands developed without their corresponding wild-type bands. All 13 mutant bands recorded for the *katG* gene belonged to the MUT1 band (S315T). There were 30 reaction bands for the *inhA* gene, six wild-type bands with the corresponding mutation band, while 24 mutant bands developed without any wild-type band. All the wild-type and mutant bands belonged to WT1

**Table 4**  
Risk factors of study participants on MDR-TB.

Variable	95 %CI	OR	P-value
Gender			
Male	0.46–3.10	1.19	0.72
Female			
Age (years)			
≤40	0.86–7.40	2.52	0.09
>40			
Occupation			
Poor	0.46–3.10	1.19	0.72
Good			
Income range			
Low/medium	0.31–0.54	0.41	0.23
High			
Education			
Poor	0.33–2.99	0.99	0.98
Good			
Previous TB Treatment			
Yes	7.85–546.05	65.46	<0.001
No			
Treatment completion (N = 51)			
Yes	0.73–6.70	0.70	0.76
No			
Treatment disruption N = 45			
Yes	0.26–4.47	1.07	0.93
No			
Contact with TB patient			
Yes	0.29–2.73	0.88	0.83
No			
History of alcohol use			
Yes	0.25–2.07	0.72	0.54
No			
History of drug use			
Yes	0.33–3.08	1.01	0.98
No			
Smoking history			
Yes	0.54–4.25	1.51	0.43
No			
HIV status			
Positive	0.21–1.84	0.63	0.39
Negative/Unknown			
Diabetes			
Yes	0.61–6.43	1.99	0.25
No			
Hypertension			
Yes	0.40–4.55	1.34	0.63
No			
Other health conditions			
Yes	0.33–2.59	0.92	0.87
No			

OR: odds ratio, MDR-TB: Multidrug-resistant Tuberculosis, CI: Confidence interval, Other health conditions (Asthma, arthritis, Hepatitis B and fibroids).

(-15/-16) and MUT1 (C15T), respectively (Table 3).

We observed diverse resistant patterns of wild-type and mutant bands (Table 3), 'WT8 & MUT3' (64.2 %) was the most predominant for the *rpoB* gene for RIF resistance, while 55.8 % of this combination coded for MDR-TB, 44.2 % coded for RIF mono-resistance. 'WT & MUT1' (61.5 %) and 'MUT1' (80 %) were most prevalent for *katG* and *inhA* genes for INH resistance. Interestingly, 100 % of the *inhA* gene coded for MDR-TB, 87.5 % of the 'WT & MUT1' *katG* combination coded for MDR, and 12.5 % for INH mono-resistance.

#### Effects of associated risk factors on MDR-TB

Among the factors tested (Table 4), only 'previous TB treatment' had a statistically significant effect on MDR-TB ( $P < 0.001$ , OR=65.46). Other factors that showed strong odds ratio, which were, however, not statistically significant, included: Age of participants (OR=2.52,  $P = 0.09$ ), diabetes (OR=1.99,  $P = 0.25$ ), smoking history (OR=1.51,  $P = 0.43$ ) and hypertension (OR=1.34,  $P = 0.63$ ).

## Discussion

This cross-sectional institutional-based study was conducted in five health facilities to determine the MDR-TB prevalence among GeneXpert-positive patients, the mutation patterns of MTB drug resistance using the MTBDR $plus$  LPA, and associated factors in Osun State, South-West, Nigeria. This study combined MDR-TB prevalence, mutation patterns, and associated risk factors (the risk factors covered TB-related, social, and medical risk factors) of MDR-TB. To the best of our knowledge, this is the first study in Osun State with this level of extensive description, and this is an advancement over similar previous studies in this region.

The higher percentage of males (54.3 %) recorded in this study is consistent with several other studies [1,6,16,17]. The majority of the MDR-TB cases were males (56.1 %), and males also had a higher prevalence of MDR-TB (60.5 %) with odds of 1.19 (OR=1.19;  $P = 0.72$ ; 95 %CI=0.46–3.10), these findings are in agreement with previous studies [18,19] which further support the fact that the male gender suffers more from MDR-TB compared to the females. The predominance of non-completion of treatment (66.7 %), treatment disruption (57.9), and HIV (61.1 %) among the males may have predisposed the male gender to the high prevalence of TB/MDR-TB.

The preponderance of MDR-TB among young people less than 40 years old (80.5 %) is consistent with previous studies [6,8,20] and differs from another study [21] that reported a higher prevalence among the elderly. Our study revealed that younger people, under 40, have a 2.52 likelihood (OR=2.52;  $P = 0.09$ ; 95 %CI=0.86–7.40) of developing MDR-TB compared to the elderly, which implied that MDR-TB upsets the younger generation in their productive age [22].

Most of the study participants (64.3 %) have a predominantly low-level education, which may signpost the role of poor education in the TB scourge [6]. However, the high rate of MDR-TB among the unemployed (75 %), low-income earners (64.1 %), and low-level education (75.6 %), which are all indicators of poverty [26] appear to support the findings of several researchers that reported poverty as a determinant of TB [21,23] and MDR-TB [24,25]. Consequently, the relevant stakeholders may need to focus on poverty eradication and education for all, especially among young people, as a means of improving TB management. When this is effectively implemented at the national and sub-national levels of government, it promises to move Nigeria towards achieving the first Aspiration of the Africa Union's Agenda 2063, specifically, Goals 1 (Priority Area 2), 2 (Priority Area 1), and 3 (Priority Area 1). In addition, Nigeria would also be able to move towards achieving the United Nations Sustainable Development Goals 1 (Targets 1.1 and 1.2) and 4 (Targets 4.1, 4.3 and 4.4).

We observed a large number of previously treated TB patients (72.9 %), comprising 57.2 % of MDR-TB and 15.7 % of non-MDR cases, and these findings are consistent with other studies [6,18]. The relationship between previous TB treatment and MDR-TB was statistically significant ( $P < 0.001$ ; OR=65.46; 95 %CI=7.85–546.05), which makes previous treatment the most potent risk factor associated with the occurrence of MDR-TB in this study. This outcome is similar to the findings of other researchers [18,27], which reinforces the role played by previous TB treatments in the development of MDR-TB. Therefore, the current system and practice of TB care in the country should be strengthened while treatment officers should be more empowered and monitored to ensure adequate and effective follow-up of TB patients on treatment to ensure treatment compliance. Effective and sustained treatment compliance among the infected populace would drive Nigeria towards TB reduction and eradication and contribute to a healthy life for the citizens, which would help Nigeria achieve SDG Target 3.3 and the first Aspiration (Goal 3, Priority Area 1) of the Africa Union's Agenda 2063.

The treatment completion (88.2 %) recorded in this study agrees with a similar survey in Lagos, Nigeria [28]. However, the 11.8 % non-completion of treatment and 42.2 % treatment disruption among those who claimed to have completed their treatments becomes remarkable when considered alongside previous reports that non-completion of therapy [17] and treatment disruption [18] contribute significantly to the development of TB drug resistance. Hence, the high rate of MDR-TB recorded in this part of the world may be attributable to cases of non-completion or disruption of treatment. Meanwhile, values as high as 23 % non-completion of therapy have been reported in a previous study in Nigeria [29]. Although there have been reports documenting non-completion of treatments, our study is thus adding a record of treatment disruption among the study participants to the body of TB knowledge in Osun State and Nigeria.

In terms of social risk factors, several researchers [18,21,30,31] have reported a history of smoking, alcohol, and drug use similar to our findings. Likewise, the predominance of alcohol use (68.4 %) and smoking (87 %) among the male gender in this study is also similar to the report of WHO and other researchers [3,31]. Previous studies have reported that smoking [21], alcohol consumption, and drug use [25,30] are connected with increased risk and severity of TB/MDR-TB due to the impact of these factors on the host immune system and damage to the liver [30–32].

We observed a higher percentage of associated medical factors (diabetes/70.6 %, hypertension/64.3 %, and other health

conditions/57.1 %) among the MDR-TB cases. Diabetes predominance in males (64.7 %) in this study was similar to the report of WHO [3], where a higher percentage of males with TB (6.3 %) had diabetes in contrast to the female gender (6 %). Diabetes has been reported to be associated with the risk of developing primary MDR-TB and an increased risk of poor TB treatment outcomes [33]. The current study observed that diabetic patients have 1.99 odds of developing MDR-TB compared to non-diabetic patients, though the relationship was not statistically significant ( $P = 0.25$ ,  $OR=1.99$ ,  $95\%CI=0.61-6.43$ ). However, the impact of diabetes on MDR-TB cannot be undermined, as a similar study [34] reported a significant association between diabetes and MDR-TB.

The prevalence of HIV-positive cases (25.7 %) in this study is comparable to the findings of a similar study in Nigeria [17]. The coverage of HIV testing among the participants in the current study was 82.9 % (25.7 % positive and 57.2 % negative); this is higher than the global coverage (73 %) and comparable to, though little less than the coverage (85 %) in the African region [3]. Although there was no significant statistical relationship ( $P = 0.39$ ,  $OR=0.63$ ,  $95\%CI=0.21-1.84$ ) between MDR-TB and HIV co-morbidity, the findings were consistent with the study report in the West African country of Mali [19].

Determining the drug-resistant profile of MTBC is critical for the effective management of TB disease, which may assist in standardizing treatment regimens for MDR-TB patients or promotes personalised management [26]. The high MDR-TB prevalence found in this study is comparable with the findings of similar studies [8,19,27,35] but differs from a study conducted in Ibadan, Nigeria [6]. The difference is likely attributable to the disparity in the study population, as it has been reported that DR.....TB prevalence is higher in hospital-based studies compared to community-based studies [16].

The prevalence of RIF mono-resistance (37.1 %) in this study was higher than INH mono-resistance (2.9 %), this is consistent with similar studies [8,12,35] and differs from other studies that reported a higher prevalence of INH mono-resistance [16,22]. The predominance of RIF mono-resistance in this study may be attributable to the choice of the study population that is essentially confirmed to be resistant to RIF by GeneXpert assay.

From the results of this study, 64.2 % of the RIF resistance had mutations in codon 531 and 3 % in codon 526 of the *rpoB* gene, which has been reported to confer high-level RIF resistance, while the remaining 32.8 % (23.9 % for codons 513–517; 7.4 % for codons 518–522 and 1.5 % for codon 516) belong to the codons that have been reported to confer low-level RIF resistance [11,13]. From our findings, more than three-fifths (67.2 %) of the study participants expressed mutations that confer high-level RIF resistance.

This study found WT8/MUT3 (S531L) as the predominant mutation pattern of the *rpoB* gene, which is in agreement with similar studies [1,36], while another study in Port-Harcourt, Nigeria [37] found MUT2B (H526D) as the predominant mutation pattern. However, the occurrence of D516V mutation in this study represents a paradigm shift in the *rpoB* gene mutation pattern in Osun State, southwest Nigeria. Mutations in the *inhA* gene, which usually confer a low-level INH resistance [38,39], were detected in 69.8 % of cases, while mutations in the *katG* gene identified to confer high-level INH resistance [39] were detected in 30.2 % of cases. However, this study did not record any combined mutation pattern between *inhA* and *katG* genes for INH resistance, which agrees with other studies [16,36,40]. The predominance of the *inhA* gene over the *katG* gene for INH resistance is consistent with a previous study in Nigeria [35], while other studies revealed the *katG* gene as the most predominant region of target for INH drug resistance [16,22,39].

The mutations causing resistance to the INH gene were detected at codons 15 and 315 of the *inhA* and *katG* genes, respectively. However, WT/MUT1 (61.2 %) and MUT1 (80 %) gene combinations were the predominant mutation patterns for *katG* and *inhA* genes, while 87.5 % and 100 % of the predominant *katG* and *inhA* genes coded for MDR, respectively.

### Limitations of the study

The test detects resistance associated with *rpoB*, *katG*, and *inhA* genes, hence could not detect resistance to second-line drugs. The test could also not differentiate between members of the MTBC. Thus, further investigation would be needed to establish a resistance to second-line drugs and determine the species specific MTBC.

### Conclusion

This study provided information on the MDR-TB prevalence, gene mutation patterns of MTBC, and associated factors among suspected multidrug-resistant tuberculosis patients, using the Genotype MTBDRplus LPA for the improvement of management and care for DR-TB patients. Therefore, rapid and accurate TB/DR-TB diagnosis is a critical management strategy that is often lacking in most resource-limited countries like Nigeria. However, our research provided a solution to the rapid determination of MDR-TB (RIF and INH resistance) against the current practice of using RIF resistance as a marker for MDR-TB. This is expected to impact policy formulation and implementation among the relevant government agencies at local and national levels.

Moreover, the current system and practice of TB care in the country should be reinforced while ensuring that TB caregivers are more empowered and monitored to provide adequate and effective follow-up of TB patients on treatment to improve treatment compliance. In addition, there is a need to enhance citizens' awareness, especially regarding TB/MDR-TB treatments, to improve treatment completion and reduce treatment disruption and associated risk factors. The relevant stakeholders are to ensure that this awareness drive gets to individuals in the community. The effectiveness of this campaign among caregivers and citizens will mitigate the development and spread of MDR-TB to others in the community.

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## Data availability

The data used for this study are available from the corresponding author on reasonable request.

## CRedit authorship contribution statement

**Onaiwu T. Ohiengbomwan:** Conceptualization, Formal analysis, Formal analysis, Writing – original draft, Writing – review & editing. **Judith Oguzie:** Formal analysis, Writing – original draft. **Philomena Eromon:** Formal analysis, Project administration, Writing – original draft. **Adeyemi T. Kayode:** Formal analysis, Project administration. **Taofeek Sola Afolabi:** Formal analysis, Project administration, Formal analysis, Writing – original draft. **Isaac O. Komolafe:** Project administration, Supervision, Writing – review & editing, Writing – original draft.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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