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Drug Resistance and Mutation Patterns Among Tuberculosis Patients in National Tuberculosis and Leprosy Training Centre Zaria

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Abstract

Tuberculosis (TB) has afflicted humans for millennia and continues to pose a significant threat to public health worldwide. The World Health Organization classified TB as one of the top 10 causes of death globally, with approximately 10 million new cases and 1.4 million TB associated deaths in 2019 alone. Tuberculosis has high prevalence rates in certain regions, including Nigeria. Traditional diagnostic methods for TB and drug resistance diagnosis have limitations in accuracy and speed, highlighting the need for continuous studies on advanced molecular techniques. The study assessed the prevalence of tuberculosis and drug resistance among patients attending the National Tuberculosis and Leprosy Training Centre (NTBLTC), Saye Zaria using molecular techniques, with the aim of contributing to the improvement of diagnosis, treatment, and control strategies for tuberculosis. The cross-sectional study involved 342 suspected TB patients. Samples collected were analysed by microscopy, GeneXpert MTB/RIF and Line probe Assays. The results of this study showed an overall prevalence rate of 39.5% ($p<0.05$) of Mycobacterium Tuberculosis determined from GeneXPert MTB/RIF positive confirmed cases. further assay using Line Probe Assay (LPA) revealed an MDR-TB prevalence rate of 18(5.3%). Poly-resistance was also detected in 2 (0.58%). However, no Pre-Extensively Drug Resistant Tuberculosis (Pre-XDR-TB) and Extensively Drug Resistant-Tuberculosis (XDR-TB) were found. The overall results showed a slight but comparable rise in the prevalence of MTB against reviewed studies while the MDR-TB prevalence was lower. The study underscores the need for early diagnosis and treatment of TB to stall the occurrence of MDR-TB and other forms of severe TB infections.

Keywords: *Line Probe Assay (LPA), Multidrug Resistant TB (MDR-TB), Rifampicin Resistance, Tuberculosis.*

Introduction

Tuberculosis (TB) is an ongoing global health problem caused primarily by the bacterium Mycobacterium tuberculosis and occasionally by related species such as Mycobacterium bovis and Mycobacterium africanum. Nigeria is listed as one of the countries with the highest burdens for TB [1, 2, 3, 4, 5]. TB continues to be a major target in the global TB control effort that has plagued humans for many years and continues to pose a serious threat to public health worldwide. The World Health Organization (WHO) ranks tuberculosis among the top ten causes of death worldwide, with approximately 10 million new cases and 1.4 million people died from tuberculosis in 2019 [6].

In 2022, a total of 1.3 million people died of tuberculosis (TB), and a further 167,000 cases occurred in people infected with HIV.

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Globally, tuberculosis is the second most common infectious cause of death after Covid-19 and ahead of HIV and AIDS [7]. In the same year, around 10.6 million people worldwide became ill with tuberculosis. This number included 5.8 million men, 3.5 million women and 1.3 million children. Tuberculosis occurs in different countries and in different age groups and is a treatable and preventable disease [7].

The persistence of multidrug-resistant tuberculosis (MDR-TB) represents a major public health crisis and poses a threat to health security. As far back as 2016, there was a 4.1% surge in new cases of MDR-TB and up to 19% cases previously treated were reported to have potential of progressing into Pre-XDR-TB and XDR-TB [8]. Up to 240,000MDR-TB related deaths were reported from drug resistant surveillance which may include previously mentioned forms of MDR-TB [9, 10]. Worryingly, in 2022, only 40% of people with drug-resistant tuberculosis sought treatment. Since 2000, collaborative global efforts against tuberculosis have saved approximately 75 million lives. Achieving the goals set at the 2018 UN High-Level Meeting on Tuberculosis requires $13 billion in annual funding for the prevention, diagnosis, treatment and care of tuberculosis. The United Nations' health-related Sustainable Development Goals (SDGs) include ending the tuberculosis epidemic by 2030 [7].

Tuberculosis (TB) has a high prevalence rate in some regions, including Nigeria. The National Tuberculosis and Leprosy Training Center in Saye Zaria is an important health facility in northern Nigeria fighting tuberculosis. Traditional methods for diagnosing tuberculosis and drug resistance have limitations in accuracy and speed, highlighting the need for continued research into advanced molecular techniques [6].

The World Health Organization (WHO) emphasizes the importance of accurate and rapid diagnosis to control the spread of tuberculosis and curb the emergence of drug resistance. Traditional methods such as sputum smear microscopy lack the sensitivity required for early diagnosis, delaying the start of treatment. Molecular techniques, including polymerase chain reaction (PCR) and genotyping, enable more precise identification of TB strains and drug-resistant variants. These methods have the potential to revolutionize diagnosis by providing faster and more accurate results and allowing appropriate treatment to be initiated quickly [6]. This study is therefore aimed at researching the patterns of drug resistance among TB patients attending the NTBLTC using molecular techniques to contribute to knowledge and to the limited existing data in the study population for enhanced TB diagnosis and patient management.

Materials and Methods

The research was carried out at the National Tuberculosis and Leprosy Training Center (NTBLTC) located in Saye, Zaria on the old Kaduna-Zaria Road, Kaduna State - Nigeria. The center also houses the second National reference laboratory for Tuberculosis in the Northwest of Nigeria. The Center's responsibilities include training staff under the National TBL Control Program (NTBLCP), providing tuberculosis, HIV and leprosy services (diagnosis, chemotherapy, etc.) and operational research on tuberculosis, HIV and leprosy. The National Tuberculosis Reference Laboratories (NTRL) is recognized as the leading tuberculosis reference center in Northern Nigeria. The NTRL offers biosafety levels 2 and 3 (BSL 2 and 3) and is equipped with state-of-the-art facilities. Patients with suspected tuberculosis were included in the study and unsuspected subjects were excluded. Ethical approval was duly obtained from the health research ethics committee of the center. This study was conducted between August 2022 and November 2023 on three hundred and forty-two (342) sputum samples from

patients who attended the National Tuberculosis and Leprosy Training Center. The sample size was calculated based on a national prevalence rate of 590,000 inhabitants out of a current population estimated at 177,476,000 inhabitants [11].

Sample Collection

Two specimens of sputum were obtained from each of the 342 individuals suspected of having pulmonary tuberculosis who consented using sputum containers and labelled appropriately. All the 342 sputum samples were collected as on spot samples in line with recent TB guidelines for routine laboratory investigations for TB suspected patients. All samples collected were pre-treated (digested, homogenized, decontaminated and concentrated) in a Biosafety level II cabinet using reagents and methods as described in the NTBLTC manual. Detailed patient Biodata and laboratory-specific information was recorded on a standardized case record form and captured on excel using double data entry.

Sample Analysis

Smear Microscopy & GeneXpert MTB/RIF

Samples were processed with Ziehl Neelsen to detect Acid Fast Bacilli (AFB) under controlled microbiological conditions. All Acid-Fast Bacilli positive samples were further analysed using GeneXpert MTB/RIF to detect Rifampicin-positive samples [12].

Line Probe Assay

Upon detection of rifampicin-resistant tuberculosis, the sputum samples were further tested utilizing both the MTBDRplus and MTBDRsl line probe assays (Hain Life Science GmbH, Nehren, Germany). Reverse hybridization technology was used to detect TB resistance mutations to both first- and second-line anti-TB drugs. While the MTBDRplus was used for the identification of INH and RIF resistance by revealing mutations in the katG, inhA, and rpoB genes, the MTBDRsl detects resistance to Fluoroquinolones (Qs), ethambutol (EMB), aminoglycosides such as kanamycin (KAN), amikacin (AMK), viomycin (VIO), and cyclic peptide like capreomycin (CAP).This is achieved by finding mutations in three separate loci, that is; gyrA, embB, and rrs, respectively [13, 14, 15, 16]. The processes included DNA extraction procedure with genolyse, PCR amplification of the extracted DNA and hybridization followed by detection. GeneXpert MTB/RIF, Genotype MTBDRsl and MTBDRplus line probe assays strictly followed the guidelines provided by the manufacturers.

Data Analysis

Statistical analysis was carried out using both excel and statistical package for social science (SPSS) version 20. The test of significance was set at $p<0.05$.

Results

Results of this research showed that a total of 342 samples were collected and assayed (comprising 200 males and 142 females). Out of this figure, 135 GeneXpert confirmed rifampicin-resistant TB cases (Table 1), giving a prevalence rate of 135(39.5%). Distribution of rifampicin resistance Tuberculosis among the study subjects about sex showed that 93(27.2%) and 42(12.3%) were males and Females respectively (Table 2). The highest number of rifampicin resistance cases was found in the age range of 20 – 29 with 43(12.57%) of the study population being positive while the least was age range 01 – 09 with 3(0.88%) being positive. MDR-TB was detected using the GenoType®MTBDRPlus molecular line Probe Assay in which MDR-TB was found in 18 patients (Tables 3 & 4); out of which 13 were males while 5 were females. There was no significant relationship statistically between sex and the method of detection as $p>0.05$. In terms of the pattern of drug resistance, Rifampicin monoresistance

was the highest in prevalence (33.63%) while 18 MDR-TB-positive cases were found to be resistant to Rifampicin and Isoniazid, confirming the presence of MDR-TB. The overall prevalence of MDR-TB was determined to be 18 (5.26%) among the studied population (Table 4). The number of Resistance to Rifampicin and Amikacin was found to be 2(0.58%), p>0.05. However, no resistance to Fluoroquinolones was detected. In terms of mutation pattern, results from our study also showed that 80% of resistance due to rifampicin had mutations between codons 526 and 531 of the rpoB gene (Table 5). The other 20% had mutations between codons 513 and 517.KatG gene mutation was detected in 16 patients of the study population while InhA mutation was detected in 2 patients. Gene mutations were detected on codons 15 and 315 of the inhA and KatG genes respectively.

Table 1. GeneXpert MTB/RIF Detection Among Patients Attending NTBLTC Saye, Zaria

Genxpert MTB/Rif	**NO. (%)**
Resistance detected	135(39.5)
Undetected	207(60.5)
Total	342(100)

Table 2. Distribution of Age and Sex Among GeneXpert MTB/RIF Positive TB Patients Attending NTBLTC Saye, Zaria

AGE (yrs.)	**SEX**		**Total**
	Male No. (%)	**Female No. (%)**	
01 – 09	3	0	3
10 – 19	14	7	21
20 – 29	23	20	43
30 – 39	28	6	34
40 – 49	11	6	17
50 – 59	6	2	8
60 – 69	8	1	9
Total	**93(27.19)**	**42(12.28)**	**135(39.47)**

Table 3. TB Drug Resistance from Line Probe Assay Among Patients Attending NTBLTC Saye, Zaria

Drug	**Rifampicin**	**Isoniazid**	**Fluoroquinolones**	**Amikacin**
Resistance	135	18	0	2

detected				
Undetected	0	117	135	133

Table 4. Drug Resistance Pattern Among Patients Attending NTBLTC Saye, Zaria

Resistance Pattern	**Frequency**	**Percentage (%)**
Mono Resistant (Rif)	115	33.63
Poly Resistant TB	2	0.58
MDR-TB	18	5.26
Pre-XDR-TB	0	0.00
XDR-TB	0	0.00

Table 5. Genetic Mutation Pattern of MTBC Among Rifampicin-Resistant TB Patients Attending NTBLTC Saye, Zaria

Gene	**Band**	**Mutation Region**	**Rifampicin (n=135)**	**Isoniazid (n=18)**	**MDR (n=18)**
rpoB	WT1	506-509		0	18
	WT3	513-517	27	0	0
	WT7	526-529	98	0	0
	WT8	530-533	10	0	0
KatG	WT	315	0	16	0
	MUT1	S315T1	0	0	0
InhA	WT1	-15/-16	0	**2**	0

Discussion

There is no doubt that MDR-TB constitutes a major challenge across the globe [8]. MDR-TB and other forms of TB such as pre-extensively drug resistant TB and complicated treatment and management [8]. This cross-sectional study in which a total of 342 suspected TB patients were included was conducted between August 2022 and August 2023 to determine the drug resistance patterns among the study population.

The findings from this study showed that GeneXpert MTB/RIF positive cases were 135(39.5%) of the study population. Male positive cases were determined to be 93(27.19%) and Females were 42(12.28%) of the study population. The percentage of infected males recorded in this study was higher than that of females which is in agreement with findings of other studies [17,18]. The prevalence of Rifampicin resistance by GeneXpert MTB/RIF assay was

found to be 39.5%. This finding is in agreement with those reported by [2,19,20,21].

Our findings also showed the prevalence of INH resistance to be 5.3%, confirming the presence of MDR-TB. This finding corroborated a similar report by [2] indicating a low prevalence level of INH resistance in a similar setting. The Prevalence of Poly-resistance was found to be also low (0.58%). However, no Pre-Extensively Drug-Resistant Tuberculosis (Pre-XDR-TB) and Extensively Drug-Resistant-Tuberculosis (XDR-TB) were found in our study. The overall prevalence of MDR-TB detected among the study population was 18(5.3%). This figure fell below the 54.5% reported in Kano in a similar study [22,23], which was far higher than the findings at this centre. The low prevalence of MDR-TB at the NTBLTC may be due to early detection and treatment strategies in place. This may be an indication that the fight against MDR-TB and TB as a whole is gradually being won at the centre. Rifampicin mono resistance was higher than other forms of resistance 115(33.63%). This finding is consistent with other findings [18]. The reason for this may not be unconnected with the method used i.e. GeneXpert MTB/Rif used for confirmation of Rifampicin resistance as well as the NTBLTC being a treatment centre for the most difficult TB cases including MDR-TB. Findings from this study also showed that 119 (34.80%) of resistance due to rifampin had mutations between codons 526 – 531 of the rPoB gene which several studies have proven to contribute to high Rifampicin resistance, while 16(11.86%) had mutations in codons between 513 – 517 which are associated with low Rifampicin resistance level. These findings are in agreement with those reported by [18, 24, 25].

KatG gene mutation associated with high INH resistance level was detected in 16 patients of the study population while inhA mutation associated with low INH resistance level was detected in 2 patients. Gene mutations responsible for INH resistance were detected on codons 15 and 315 of the inhA and KatG genes respectively.

Conclusion

The findings of this study have contributed to the body of knowledge on the pattern of drug resistance prevalent in the researched population. It also gave a picture of the subsisting gene mutations that could cause drug resistance within the population all of which are important for the accuracy of TB diagnosis and Management, especially in the complicated forms of the disease.

Conflict of Interest

I declare that there is no conflict of interest regarding the research presented in this paper. I affirm that I have not received any financial support and benefit or engaged in any commercial, legal, or professional relationship with other organizations, or with the people I am working with, that could influence this study.

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