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Original Article

Frequency and Distribution of Rifampicin Resistance in Mycobacterium Tuberculosis Clinical Isolates Using Gene Xpert MTB/RIF in Delta State, South-South Nigeria

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ABSTRACT

Introduction

The emergence and spread of multi-drug resistant-tuberculosis is a threat, which has complicated the diagnosis, management and control of tuberculosis. In addition to the simultaneous detection of *Mycobacterium tuberculosis* bacilli and rifampicin resistance, the Gene Xpert assay can also highlight the point of mutation if it occurs around the rifampicin resistance determination region (RRDR) of the rpoB gene, which is responsible for 95% of rifampicin resistance. This study seeks to estimate the prevalence of rifampicin resistance, determine the frequency and distribution of mutations along the rifampicin resistance determination region, and assay for the relationship that exists between these mutations and basic epidemiological variables.

Methods

Data of patients with presumptive tuberculosis screened for *Mycobacterium tuberculosis* and drug-resistant tuberculosis using Gene Xpert assay from January through December 2019 at Central Hospital Warri was considered, checked for correctness and analyzed using SPSS 22 statistical software.

Results

Of 1411 people that were screened, 252 (17.9%) had tuberculosis, with 16 out of the 252 had drug-resistant tuberculosis (6.4%). The active workforce age group of 16-50 years accounted for 75% of drug-resistant tuberculosis, while woman and men were equally distributed. Mutations at the region covered by probe E (codon 529-533, RRDR of the rpoB gene) had the highest frequency (37.5%) of mutations between the regions covered by the tested probes, while rifampicin resistances that were not associated with the considered probe regions had a frequency of 31.25%. There were varying frequencies and distributions of probe region-associated mutations among the study variables. However, all lacked statistical significance.

Conclusion

We reported a relatively high prevalence for drug-resistant tuberculosis (6.4%) and non-probe region-

associated mutations for drug-resistant tuberculosis (31.25%). Epidemiological variables (age, sex, retroviral status and nature of the specimen) had no statistical association with the pattern of probe region-associated mutation. Subsequently, studies with similar objectives with ours but with larger sample size and confirmatory methods (gene sequencing and drug susceptibility testing) are highly recommended.

Keywords: Frequency, distribution, rifampicin resistance, probe associated region mutation, rpo B gene, *Mycobacterium tuberculosis*, gene Xpert.

Abbreviations: multi-drug resistant-tuberculosis (MDR-TB); World Health Organization (WHO); the β subunit of the Mycobacterium DNA dependent RNA polymerase (rpoB); the rifampicin resistance determination region (RRDR).

INTRODUCTION

The emergence and spread of multi-drug resistant-tuberculosis (MDR-TB) (resistance of a strain of *Mycobacterium tuberculosis* to rifampicin and isoniazid) is a threat, which has complicated the diagnosis, management and control of the second greatest infectious killer globally[1]. In 2013, the World Health Organization (WHO) declared MDR-TB a public health crisis and reported a global estimate of 3.9% MDR-TB for new cases of tuberculosis and 21% for previously treated cases tuberculoses[2,3].

Nigeria is one of the countries included among the 30 high burden countries for TB, TB/HIV, and MDR-TB[4]. National figures for MDR-TB cases for new and previously treated cases stand at 4.3% and 25% respectively[5].

The introduction of Gene Xpert MTB/RIF assay (Cepheid, USA) has revolutionized the diagnosis, management, and control of tuberculosis via simultaneous detection of *Mycobacterium tuberculosis* and resistance to rifampicin[6]. Rifampicin is arguably the most important drug in the chemotherapy of tuberculosis[7]. It binds to the *β* subunit of the Mycobacterium DNA dependent RNA polymerase (rpoB) to inhibit transcription of MTB proteins. Rifampicin is usually well tolerated by patients, a key component of the first-line regime and its resistance accounts for about 90% of MDR-TB[8].

In addition to the simultaneous detection of MTB and rifampicin resistance, the Gene Xpert assay can also highlight the point of mutation if it occurs around the rifampicin resistance determination region (RRDR) of the rpoB gene. The Gene Xpert assay uses five overlapping molecular beacon probes (A-E) that target the RRDR of the rpoB gene. The probes are A (codon 507-511), B (codon 512-518), C (codon 518-523), D (codon 523-529) and E (codon 529-533)[9]. A strain is termed resistant when one or more of the probes fail to hybridize or when the difference between the first and last cycle threshold (ct) is more than 3.5[10].

The distribution and frequency of mutations along the rpoB gene vary for different geographical regions. There is limited data on these mutations for the study region. Thus, this study seeks to estimate the prevalence of rifampicin resistance, determine the frequency and distribution of mutations along the RRDR and assay for the relationship that exists between the mutations detected by used probes and basic epidemiological variables.

METHODS

Study design, region, and population

This was a hospital-based, retrospective, and a cross-sectional study conducted from January to December 2019 at Central Hospital Warri. Central Hospital Warri is a secondary healthcare and referral center located in Delta State, South-South Nigeria. Its Gene Xpert laboratory caterers for its patients, samples from other smaller facilities in Delta South and Central regions as well as most community outreach programs aimed at achieving TB control. All patients with presumptive TB that visited the study site during the study duration constituted the study population.

Laboratory analysis and data collection

Sputum or gastric aspirate sample (depending on age) was presented per participant for the diagnosis of tuberculosis. Samples were collected from the chest ward, other smaller facilities and from community outreach programs and were submitted at the Gene Xpert laboratory in a wide-open mouth sterile container.

These samples were diluted and decontaminated, and the Gene Xpert assay was performed according to the manufacturer's instruction. Samples for the diagnosis of extra-pulmonary TB and patients with

incomplete data (age, sex, HIV status and nature of the sputum) were excluded from the study.

Data analysis

Extracted data was checked for correctness, coded, entered and analyzed using Statistical Package for Social Sciences (SPSS) version 22. Basic epidemiological variables were characterized using descriptive statistics while Pearson's Chi-square was used to assay for significant association at p-values ≤ 0.05.

Ethical issues

Informed consent was not possible because of the nature of the study (retrospective). However, confidentiality was ensured as data was extracted

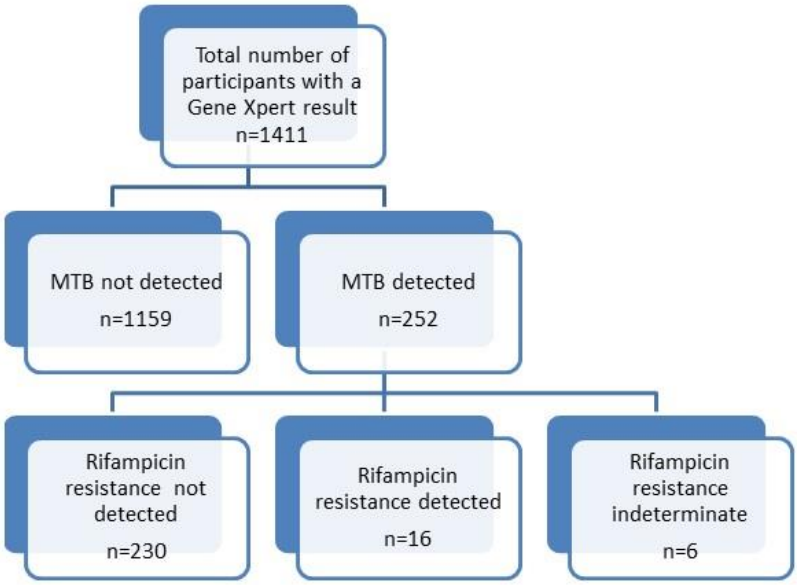

Figure 1. Flow chart of participants sampling

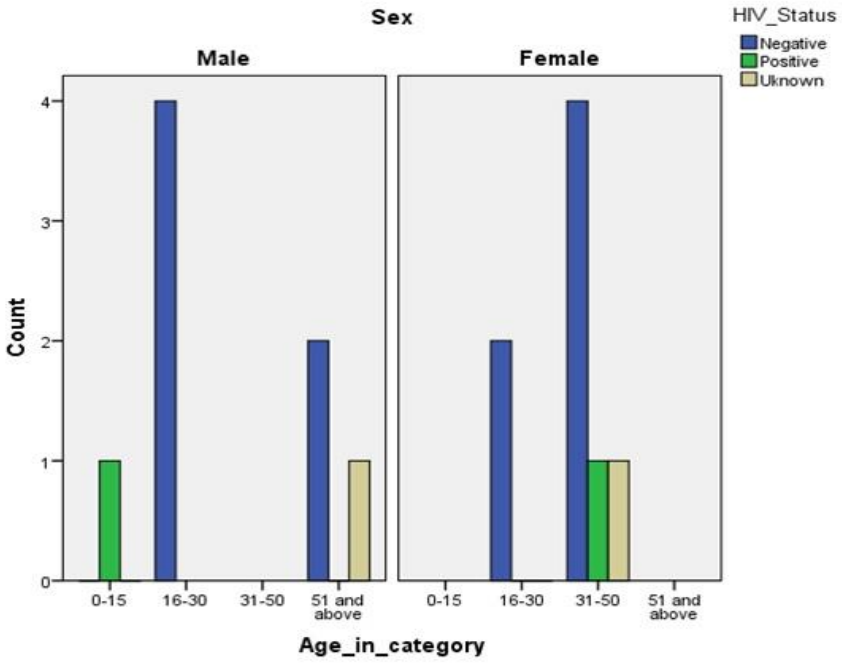

Figure 2. Characteristic of participants with Rifampicin resistance Pulmonary Tuberculosis

without identifiers, and access was strictly restricted to the research team. Permission was obtained from the unit head of the Gene Xpert laboratory after a detailed explanation of the study design and objectives.

RESULTS

A total of 1411 participants were eligible for the study. 252 (17.9%) of 1411 had pulmonary tuberculosis. 16 out of the 252 pulmonary TB cases (6.4%) had rifampicin resistance while 6 out of the 252 (2.4%) had rifampicin-resistant indeterminate results (Figure 1; Table 1).

A total of 16 participants with a mean age of 38.12 ± 18.25 (14-77 years) had rifampicin-resistant tuberculosis. The youngest participant was a 14-year old male with TB/HIV co-infection while all the elderly participants (age 51 and above) were also males. All female participants were within the active work force age group of 16-50 years (Figure 2).

The active work force age groups (16-30 and 31-

Table 1: Prevalence of pulmonary, rifampicin resistant, and rifampicin indeterminate tuberculosis

Variables	Positive	Negative	Percentage
Pulmonary tuberculosis	252	1159	17.9
Rifampicin resistance	16	236	6.4
Rifampicin indeterminate	6	246	2.4

Table 2: Characteristics of study population and missed probe distribution

Variables	Frequency	Percentage	Missed probe				
Age (years)			**B**	**C**	**D**	**E**	**None**
0-15	1	6.3 %	--	--	--	--	1
16-30	6	37.5 %	--	1	--	3	2
31-50	6	37.5 %	--	--	3	2	1
51 and above	3	18.7 %	1	--	--	1	1
Sex							
Male	8	50.0 %	1	1	--	2	4
Female	8	50.0 %	--	--	3	4	1
HIV status							
Negative	12	75.0 %	--	1	1	6	4
Positive	2	12.5 %	--	--	1	--	1
Unknown	2	12.5 %	1	--	1	--	--
Nature of sputum							
Salivary	3	18.7 %	--	--	--	2	1
Mucoid	6	37.5 %	1	--	2	2	1
Muco-purulent	5	31.3 %	--	1	--	1	3
Purulent	2	12.5 %	--	--	1	1	--

50) accounted for the bulk of the rifampicin-resistant TB cases (35.7%) each while pediatrics (0-15) accounted for about 6.3% (Table 2). It was an even distribution of rifampicin-resistant strains for both sexes, as they both accounted for 50% each (Table 2). HIV negative participants accounted for 75% of the rifampicin-resistant cases, while HIV positives and participants without knowledge of their HIV status both had 12.5% each (Table 2).

Mutation at probe E (codon 529-533) was responsible for the highest frequency of rifampicin resistance (6/16 (37.5%)). This was closely followed by rifampicin resistance without probe mutation (5/16 (31.25%)), while probe B and C were associated with just one mutation (6.25%) each (Table 3). Distribution of probe region-associated mutation varied among study variables. However, assay for significant relationship between study variables (age, sex, retroviral status and nature of the specimen) and probe region-associated mutation yielded p-values greater than 0.05 (Table 4).

DISCUSSION

In this study, we estimated the prevalence of rifampicin-resistant tuberculosis, measured the frequency and distribution of mutation along the RRDR, and assayed for the relationship between these mutations and basic epidemiological variables (age, sex, HIV status and nature of sputum). We recorded a prevalence of 6.4%, which was slightly lower than the 7.3% previously reported for the study site[7], but higher than the 3.2-5.4% prediction by WHO[11] for Nigeria. The 6.4% recorded is similar to the 6.0% reported by Ochang et al., for Cross River State, Nigeria[12], 5.9% for Abeokuta South-west, Nigeria[13], and 6.8% for Ethiopia[14]. However, studies from Yenagoa, Nigeria[7], Lagos Nigeria[15] and Ethiopia[1] gave 14.7%, 23.0% and 9.9% respectively, which are figures higher than ours. The variation in prevalence reported for the studies above may be largely attributed to the difference in study population, methods, and sample size.

Participants that constituted the active work force (age group 16-50 years) accounted for 75% of RR-TB cases. This finding is in consonance with the record of Ullah et al.[16], and Adejumo et al.[17], but in contrast to several other studies[7,18,19]. Sex-based distribution of RR-TB was even (50% for both sexes). Several reports have highlighted the male sex to be a risk factor for RR-TB[7,17]. However, reports form Gaifer et al.[2], and Lomtadze et al.[20], highlighted the female sex to account for higher cases of RR-TB. There have been inconsistencies in reports that assayed for the relationship between RR-TB and age/sex. While these inconsistencies are largely attributed to the socio-cultural and economic nature of the study population, the nature of TB transmission (household or community) may also be a defining factor. For populations where RR-TB is accounted for by the extremities of age (the youngest and the elderly), females tend to have the highest sex-based frequency. This pattern suggests household transmission while the reverse signifies community transmission.

For RR-TB cases associated with mutations in the regions covered by the probes, probe E had the highest frequency (6/16) of 37.5%. Probe D followed suit with a frequency of (3/16) of 18.75%, while probe B and C had the same prevalence of 6.25% each. Our findings are in agreement with the report of Alemu et al.,[21] who reported probe E, D, and B to be associated with rifampicin resistance in decreasing order. Several other reports[12,22,23] have highlighted probe E (codon 529-533) mutation to be responsible for most mutations along the RRDR. However, Diande et al.[24] and N'gessan et al.[25], reported probe B as the probe associated with most rifampicin resistance in Burkina Faso and Ghana respectively. While variation in geographical region may be responsible for the pattern reported for the studies above, the low fitness cost associated with mutations at around the probe E region has been speculated to be responsible for its high frequency.

RR-TB without probe associated mutation accounted for 31.25% of RR-TB cases recorded. This figure is higher than the 6.0% reported by Alemu et al.[21], but similar to the report of Diande et al.[24]. Uddin et al.[10], reported probe E as the probe associated with the highest frequency of RR-TB for all age and sex category. This finding is in contrast to our report. We reported probe E to have the highest frequency in age-grade 16-30 years and the female sex, probe D had the highest frequency for age-grade 31-50 years while non-probe associated mutation was highest for males and pediatric participants. The variation between both studies may be attributed to sample size variation. We reviewed 16 rifampicin-resistant cases while Uddin et al., worked with 205 samples. We reported no probe mutation associated with probe A and just one probe mutation associated with probe C. This report agrees with the findings of

Table 3: Prevalence of rifampicin resistance in relation to associated probe

Probe associated	Frequency	Percentage
A	0	0%
B	1	6.25 %
C	1	6.25 %
D	3	18.75 %
E	6	37.50 %
None	5	31.25 %

Table 4: Assay for significant association between mutant probe and study variables

Variables	Probe B n (%)	Probe C n (%)	Probe D n (%)	Probe E n (%)	Non n (%)	p-value
Age (years)						0.327
0-15	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	
16-30	0 (0.0)	1 (16.7)	0 (0.0)	3 (50.0)	2 (33.3)	
31-50	0 (0.0)	0 (0.0)	3 (50.0)	2 (33.3)	1 (16.7)	
51 and above	1 (33.3)	0 (0.0)	0 (0.0)	1 (33.3)	1 (33.3)	
Sex						0.113
Male	1 (12.5)	1 (12.5)	0 (0.0)	2 (33.3)	4 (50.0)	
Female	0 (0.0)	0 (0.0)	3 (37.5)	4 (50.0)	1 (12.5)	
HIV status						0.113
Negative	0 (0.0)	1 (8.3)	1 (8.3)	6 (50.0)	4 (33.3)	
Positive	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	
Unknown	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	
Sputum type						0.559
Salivary	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	
Mucoid	1 (16.7)	0 (0.0)	2 (33.3)	2 (33.3)	1 (16.7)	
Muco-purulent	0 (0.0)	1 (20.0)	0 (0.0)	1 (20.0)	3 (60.0)	
Purulent	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	

Uddin et al., who reported reduced frequency for mutations at both probes[10]. It is worthy to note that all probe D region associated mutation occurs for the female participants and those within the age grade of 31-50 years.

Limitation and strength

Results from Gene Xpert were not compared with phenotypic drug susceptibility testing and gene sequencing methods. Being a retrospective study, some relevant variables (contact history, previous TB status, and vaccination status) were lacking. Our sample size for rifampicin-resistant TB may be small but our study is among the very few to assay for the relationship between probe mutation variations and basic epidemiological variables.

CONCLUSION

We reported a prevalence of 6.4% for rifampicin resistant tuberculosis for the study region. Probe E (codon 529-533) and non-probe associated mutation had the highest frequency of 37.5% and 31.25% respectively. Probe A associated mutation was not recorded in the study, while probe B and C had just one mutation each. There were varying degrees of distribution of probe associated mutation among our study variables, but all lacked statistical significance. However, 31.25% of drug resistant tuberculosis cases in our study were not related to probe region-associated mutations: a finding which is rare. Epidemiological variables (age, sex, retroviral status and nature of specimen) had no statistical association with the pattern of the mutations in the region covered by the probes. Studies with increased sample sizes that will compare Gene Xpert results with gene sequencing and phenotypic drug susceptibility testing are recommended.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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