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Prevalence of rifampicin-resistant *Mycobacterium tuberculosis* in Kebbi state, Nigeria

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ABSTRACT: Tuberculosis remains a global public health burden in low and middle-income countries. The emergence and spread of drug-resistant microbial strains in high-burden countries like Nigeria pose a threat to achieving the One health approach. This study aimed at determining the prevalence of rifampicin resistance in sputum specimens of patients in Kebbi State, Nigeria using the GeneXpert Assay. It was a retrospective cross-sectional study and was carried out in Kebbi, North-Western Nigeria among patients who were confirmed positive for tuberculosis infection and visited the designated health zones, for various local government areas within the state. Sputum samples were analyzed using the GeneXpert technique. Data entry was made using Microsoft Excel and analyzed with SPSS version 20. A p-value less than 0.05 was taken as significant. The overall prevalence of rifampicin-resistant Mycobacterium tuberculosis (RR-MTB) was 5.8% (14/240). The majority of the study participants were within the age grade 31-40 years (8.77%) and male participants (7.2%) were preponderant in comparison to female participants (2.7%). There was a significant association between settlement and rifampicin resistance in the study (p=0.05). The results showed that drug-resistant tuberculosis is prevalent in Kebbi State with a higher incidence observed in the Zuru Local Government Area of the state as compared to previous findings. This shows that improving the prevention and control efforts of tuberculosis in the state with relation to adequate regulatory strategies and policy formulation is of paramount importance.

Keywords: Tuberculosis; Rifampicin resistant; GeneXpert; Sputum; Public health; Nigeria.

1. INTRODUCTION

The early diagnosis and treatment of diseases in low and middle-income resource settings are decreasing simultaneously [1]. Tuberculosis (TB) is one of the major public health burden in medically and economically deprived settings with increasing mortality and morbidity rates globally [2,3]. It is estimated that 1.7 billion people are infected worldwide, with 8 to 10 million new cases and 3 million deaths per year [2]. About one-third of the World's population is suffering from latent TB cases with active forms in 10% of the general population [4].

Tuberculosis is a communicable chronic infectious granulomatous disease caused by *Mycobacterium tuberculosis* (MTB) complex [5]. It usually involves the lungs but may affect any organ or tissue in the body. Tuberculosis flourishes under conditions of poverty, crowding, and chronic debilitating illness particularly in elderly persons with weakened immune defenses [2]. The advent of antibiotics in the treatment of communicable and non-communicable diseases brought advancement in clinical decision-making, but this is currently threatened by the emergence of pathogenic resistant microbial strains.

Bacteriological examination of sputum samples serves as the gold standard in the detection of tuberculosis. This is based on the microscopic examination of sputum samples through smear microscopy and culture followed by drug susceptibility testing (DST) [6]. The detection of MTB strains involves the growth of *Mycobacterium tuberculosis* on a liquid or solid medium for a period of 8 weeks [7,8]. However, increased cost of the culture medium, prolonged turnaround time and empirical treatment of diseases without drug susceptibility testing, a common practice in many developing countries is believed to increase the risk of transmission of drug-resistant strains and timely treatment and diagnosis of TB [9,10].

Anti-tuberculosis drug (Anti-TB) resistance is a major health problem that poses a threat in the advancement of tuberculosis control globally [3]. It is a result of recurrent mutations in different genetic loci [11], which arises due to inappropriate and illogical use of anti-tubercular drugs in the treatment of susceptible TB individuals mostly, especially during the administration of improper treatment plans and negligence in ensuring that patients complete the whole course of treatment [3].

Rifampicin-resistant tuberculosis (RR-TB) is caused by tubercle bacilli that exhibit in vitro resistance to rifampicin, one of the active first-line drugs in tuberculosis treatment thereby initiating prolonged treatment regimens, alternate medications (2nd line), reduced compliance and higher occurrence of adverse effects [3]. Rifampicin, an active first-line drug acts by binding to RNA polymerase, the β-subunit of MTB thereby preventing messenger RNA elongation (mtchion). The majority of the resistance is attributed to one or more chromosomal mutations at the 81 base pairs spanning codons 507-533 of the rpoB gene [12]. One of these mutations is also a gene for mycolic acid synthesis, and another is in a gene for catalase-peroxidase, an enzyme required to activate INH within the bacterium [13]. Resistance to Anti-TB drugs by MTB can occur either through primary or acquired means. Patients without prior exposure to Anti-TB drugs usually showcase primary resistance while, patients who have previously been treated for TB infection exhibit an acquired form of MTB resistance [14,15].

The evolution and spread of drug-resistant MTB is one of the crucial challenges in the African region including Nigeria due to poor health infrastructure, health illiteracy, and insufficient drug surveillance [16]. Its control has been hindered by slow, insensitive methods most especially in the detection of microbial strains [1]. Unless infected individuals with TB infections are treated properly, the continuous spread of the disease in low and middle-income settings will spontaneously increase and accelerate the epidemics [17].

Early detection is vital in reducing the mortality and morbidity rate of TB. In addressing these issues of rifampicin drug-resistant strains, a method that can diagnose TB and identify TB is now been used for effective patient management [18]. Recently, a new nucleic acid amplification technology has been adapted for the detection of TB and resistance to rifampicin due to its sensitivity and specificity. The GeneXpert MTB/RIF is an assay that uses heminested real-time polymerase chain reaction (PCR), identifies a clinically important Rifampicin resistance-inducing mutation and amplifies a specific sequence of the RNA polymerase beta (rpoB gene). The determining region of the rpoB gene is probed with fluorescent probes named molecular beacons. This process enables the detection of rifampicin-resistant TB within 2 hours [19,3].

This study was initiated to determine the prevalence of rifampicin-resistant Mycobacterium tuberculosis among confirmed TB cases in Kebbi State using an automated GeneXpert system assay.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in Kebbi State, Nigeria. The research was conducted in some designated health zones, serving as reference laboratories for various local governments within the State, namely: Birnin-Kebbi, Kamba, Argungu, Zuru and Yauri. It is bordered by Niger Republic, Zamfara State and Sokoto State. It is mostly populated by Hausas and Fulanis. According to the National Population Commission, population figures stand at 3,256,541 persons spread over an area of 36,800 square kilometers of land [20].

2.2. Study design

The study design is a multi-staged sampling technique from patients suspected of having tuberculosis within Kebbi State. It entails two or more stages of random sampling based on the hierarchical structure of natural clusters within the population. The final stage of sampling involves choosing a random sample of people in the clusters selected at the penultimate stage [21].

2.3. Study population

The study population includes all patients that have been suspected of having TB who visited the designated health zones, serving as reference laboratories for various local governments within the State, namely: Birnin-Kebbi, Kamba, Argungu, Zuru and Yauri.

2.4. Eligibility criteria

All patient samples that are positive for tuberculosis within Kebbi State designated health centers were included in this research while patients that are negative for tuberculosis were excluded from this research.

2.5. Sample size determination and sampling technique

The sample size used was obtained using the formula $n = \frac{Z^2PQ}{d^2}$ [22], where:

n = Minimum sample size

d = desired level of significance (0.05)

z = Confidence interval (1.96)

p = Prevalence rate (29.2%) [23]

q = 1-P = (1-0.292) = 0.71

Using this formula, the minimum number of samples was:

$n=1.96^2 \times 0.17 \times 0.83 / 0.05^2 = 318.5 \approx 319$

A 10% attrition rate (31.9) will be added to the sample size: 319+31.9= 350.9 ≈ 351 samples

This was approximated to 351 samples for easy calculation in the study. The population (according to the 2006 census) and the number of Local governments present in each catchment area of health zones were put into consideration in determining sample size for different health zone within the state viz:

A total number of local government areas in Kebbi State = 21. A total number of health zones in Kebbi State are Birnin Kebbi (10 LGAs), Argungu (3 LGAs), Yauri (3 LGAs), Zuru (4 LGAs) and Kamba (1 LGA) respectively.

2.6. Data collection

After written informed consent was obtained from all study participants, a structured questionnaire was used to obtain the socio-demographic characteristics of the study participants.

2.6.1. Sample collection and processing

Sputum samples were collected in a wide-mouth, dry, clean, leak-proof container [24]. Trained National Tuberculosis and Leprosy Control Programme (NTBLCP) staff assisted in the collection of the 240 confirmed sputum samples used during the research. The sputum sample was collected as early morning on the spot specimen.

1 ml of collected sputum (particularly that which contains any yellow caseous material) sample was mixed with an equal volume of concentrated sodium hypochlorite (bleach) solution. It was left at room temperature for 10-15 minutes, shaking at intervals to break down the mucus in the sputum. About 8 ml of distilled water was added and then centrifuged at 3000 g for 15 minutes. Using a glass Pasteur pipette, the supernatant was discarded. A drop of the well-mixed sediment was transferred to a clean glass slide and spread to make a smear. It was allowed to air-dry. It was heat-fixed and stained using the Ziehl-Neelsen technique and examined microscopically [25]. A positive TB sample was then transferred for GeneXpert analysis.

Xpert MTB/RIF cartridges were labeled with the corresponding specimen identification number (ID). 1 mL of expectorated sputum was transferred to a conical, screw-capped tube using a sterile transfer pipette. 2 mL of Xpert MTB/RIF Sample Reagent (2:1) was added to the expectorated sputum using a sterile transfer pipette. The lid was replaced, and the tube was shaken vigorously for 10-20 times. The tube was allowed to stand upright for 5 minutes at room temperature and again mixed for another 10-20 times [26]. The tube was allowed to stand upright for another 10 minutes at room temperature to liquefy the sputum. Using a sterile transfer pipette, the liquefied specimen was aspirated and the sample was transferred into the open port of the Xpert MTB/RIF cartridge. The cartridge lid was closed and the test was started as per GeneXpert System manufacturer instruction [26]. Results were displayed on the computer screen after 2 hours of processing.

2.6.2. Data management and quality control

The smears of processed sputum samples were stained using the Ziehl-Neelsen staining technique and examined microscopically [25], before the GeneXpert procedure was performed. All laboratory procedures were carried out according to standard operating procedure (SOP).

2.7. Data analysis

Data were entered into Microsoft Excel and analyzed using SPSS (Statistical Package for Social Sciences) version 20. Data were summarized using descriptive measures and presented in tables and graph. Chi-square (X2) test was used to ascertain the association between the variables. Percentages were calculated and a p-value < 0.05 was considered statistically significant.

3. RESULTS

Table 1 shows a breakdown of the strategy behind the stratified sample collection employed in the five (5) health zones of detection, monitoring and treatment of tuberculosis in Kebbi State, Nigeria. The zones include: Birnin-Kebbi, Argungu, Zuru, Yauri and Kamba, representing the twenty-one (21) Local Government Areas (LGAs) in Kebbi state. The percentage of the sample size obtained from Birnin-Kebbi, Argungu, Zuru, Yauri and Kamba, are 45% (10 LGAs), 15% (3 LGAs), 20% (4 LGAs), 15% (3 LGAs) and 5% (1 LGA)

respectively. These accounted for the 157, 53, 70, 53 and 18 sputum samples in each health zones. The result of preliminary ZN staining confirmed the total number of 240 sputum samples to be positive for TB infection out of 351 samples collected accounting for an overall prevalence of 64.8% positive for tuberculosis (Table 1).

Table 1. Distribution of TB infection according to Health Zones in Kebbi State.

Number of local government areas	Health zones	Number of samples collected	Number of samples positive for TB	Percentage (%)
10	Birnin-Kebbi	157	108	30.77
3	Argungu	53	36	10.26
4	Zuru	70	54	15.38
3	Yauri	53	30	8.55
1	Kamba	18	12	3.42
21		351	240	68.38

Table 2 shows the socio-demographic characteristics of TB-positive patients in Kebbi State. Males had a percentage of 69.17% compared to females with a percentage of 30.83%. The majority of the study participants were within the age group of 21-30 years (27.1%) followed by 31-40, 41-50 and age group 71-80 having the lowest count (1.25%). Most of the positive TB cases dwelled in the rural settlement (57.5%) as compared to those in the Urban settlement (12.5%) and sub-urban (30%).

Table 2. Socio-demographic distribution of TB-positive cases in Kebbi State.

Variables	Frequency	n (%)
Gender		
Male	166	69.17
Female	74	30.83
Age group (years)		
1-10	12	5.0
11-20	30	12.5
21-30	65	27.1
31-40	57	23.8
41-50	43	17.92
51-60	17	7.10
61-70	13	5.42
71-80	3	1.25
Settlement		
Rural	138	57.5
Sub-Urban	72	30.0
Urban	30	12.5

Table 3 shows the prevalence of rifampicin-resistant TB (RR/MTB) across all Kebbi Health Zones. Out of 240 tuberculosis-positive samples collected from different health-registered zones, 14 samples were reactive for rifampicin-resistant strains, accounting for an overall prevalence of 5.8%. Birnin kebbi health zone had the highest prevalence of 2.5%. Argungu had a prevalence of 1.67%. It was followed by Zuru and Yauri health zones with a similar prevalence of 0.83% respectively. The remaining health zone showed a prevalence of 0% for Kamba. (p-value= 0.94).

Table 3. Prevalence of rifampicin-resistant TB across Kebbi Health Zones.

Health Zones	Number of TB-positive samples	Rifampicin-resistant	Percentage (%)
Birnin-Kebbi	108	6	2.5
Argungu	36	4	1.67
Zuru	54	2	0.83
Yauri	30	2	0.83
Kamba	12	0	0.0
Total	240	14	5.8

Table 4 shows rifampicin-resistant MTB in relation to other study variables in Kebbi State. Males were higher (7.2%) compared to females (2.7%). The majority of the study participants were within the age group of 31-40 years (8.77%) followed by (7.69%) in the age group 21-30 and 61-70. Other age grades had the lowest count. Most of the rifampicin-resistant TB cases dwelled in the Urban settlement (10%) as compared to those in the Sub-Urban settlement (2.90%) and Rural (9.72%). p-value was statistically significant in relation to settlement ($p = 0.05$)

Table 4. Rifampicin-resistant MTB in relation to other study variables.

Variables	Rifampicin resistant (%)	Chi-square (p-value)
Gender		
Male	12 (7.2)	1.909 (0.17)
Female	2 (2.7)	
Age group (years)		
1-10	0 (0.0)	4.2834 (0.75)
11-20	0 (0.0)	
21-30	5 (7.69)	
31-40	5 (8.77)	
41-50	2 (4.65)	
51-60	1 (5.88)	
61-70	1 (7.69)	
71-80	0 (0.0)	
Settlement		
Rural	4 (2.90)	5.696 (0.05)
Sub-Urban	7 (9.72)	
Urban	3 (10.0)	

4. DISCUSSION

Tuberculosis is one of the primary infectious diseases exhibiting resistance to chemotherapy. While it mainly affects the lungs, it can disseminate to other parts of the body. Drug-resistant TB develops due to inadequate treatment regimes, use of fake or counterfeited drugs, reduced or non-compliance, insufficient health literacy and education in active TB cases. In this study, the prevalence of rifampicin-resistant TB was analyzed in Kebbi State, Nigeria. Sample collection was carried out in the five (5) designated health zones in descending order as follows: Birnin-Kebbi, Zuru, Argungu, Yauri, and Kamba.

In this study, 240 positive TB cases were recorded among the study participants in all the health zones in the state with a prevalence of 68.4%. 14 positive TB cases were rifampicin-resistant thereby accounting for an overall prevalence of 5.8% for rifampicin-resistant tuberculosis in Kebbi State. Birnin-Kebbi, the state

capital, had the most samples collected, as well as the highest rifampicin-resistant TB cases (2.5%), Argungu (1.67%), Zuru and Yauri (0.83%), while Kamba had no rifampicin resistant TB cases (0%). This study differs significantly from the result of a similar study carried out in Zuru Local Government Area in Kebbi State where there was no rifampicin resistance (0%) among all tuberculosis-positive samples collected for the study [27]. It seems like efforts to reduce the spread of drug-resistant tuberculosis have reduced significantly in the state. It could be because more efforts and attention has been focused on battling the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) pandemic recently and variation in access to healthcare facilities.

Our findings were higher than the globally reported prevalence of RR-TB which was less than 1% [28]. However, the 5.8% reported in this study is lower than the figures such as 7.3% for Delta, Nigeria [5], 6.9% for Nnewi, Nigeria [29], 23% for Lagos [30], Nigeria and 18.8% for the general Nigerian populace [31]. The findings of this study in relation to RR-MTB in other African regions were higher compared to Ghana with a recorded prevalence of 4.52% [32], and lower compared to the prevalence of 7.7% reported in Swaziland [33], and 7.3% for South Africa [6]. This variation in data could be implicated by access to healthcare service delivery and differences in the study population. However, a key element of some of the aforementioned research is that the subjects used for sample collection were HIV-positive. We can therefore infer that HIV infection promotes rifampicin resistance in TB-positive patients. There is no significant association between the number of tuberculosis-positive samples and rifampicin resistance in this study (p-value=0.96).

Considering the socio-demographic data of this study, gender-based prevalence in relation to RR/MTB were high in the male gender in comparison to females. This is in accordance with different reports from Nigeria, other African regions and Asia [5,6,28,34]. Thus, this difference could be explained by increased drug abuse and misuse among males in comparison to females, sample distribution, degree of exposure to infection and behavioral factors. There was no significant association between gender and rifampicin resistance in this study (p-value=0.167).

The highest rate of RR/MTB was found to be within the age group 31-40 years compared to other age groups. This is in consonance with findings of other Nigeria studies that recorded similar incidence in age grades 20-40 years [26,35]. They stated that the high prevalence of TB among this age group could be influenced by the increase in reproductive and outdoor activities, overcrowding in most settlements and poor personal hygiene. There was a significant association between settlement and rifampicin resistance in this study (p-value=0.05). Thus, this agrees that different geographical locations exhibit varied prevalences of drug-resistant pathogens

Rifampicin resistance assay is crucial in the effective control of TB as well as the emergence and transmission of drug-resistant microbial strains. Epidemiological studies such as this are vital in providing useful information that could be effective in further studies. Looking into the prevalence of Non-Tubercular Mycobacteria (NTM) and testing their resistance pattern with all the first-line drugs is important in African regions for futuristic purposes.

5. CONCLUSION

Tuberculosis has been a significant public health burden in developing countries including Nigeria but increasing antimicrobial resistance has necessitated the need for more research. In a study of the prevalence of drug-resistant pathogens from Kebbi state, Nigeria, the prevalence of RR/MTB among TB confirmed cases was 5.8%. This study can help in the control of TB at the state and national level through policy formulation and assist in mapping drug-resistant TB cases. It will help in strengthening the AMR evidence-based data through

cost-effective global surveillance aimed at achieving the 2050 goal of eliminating tuberculosis as a public health burden.

This study adds:

- The prevalence of RR-MTB in our setting using GeneXpert rather than the conventional method.
- A higher incidence was recorded in one of the Local Government Area that had no resistance cases previously.

Authors' contributions: VOO conceived the main idea, performed the data collection, practical part, writing and submission, SBM and YKD supervised and revised the work, AIA analyzed the data, wrote the manuscript and final editing, ACU helped in sample collection, practical's and analyzed the data. All authors read and approved the final version of the manuscript.

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Disclaimer: All information that is presented in this article is presented after giving due credit to the original authors or sources. Furthermore, we cannot guarantee that the data available in the referenced articles is error-free.

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