

Rifampicin Resistant Tuberculosis in a Secondary Health Institution in Nigeria, West Africa

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Abstract

Background: Rifampicin-dependent tuberculosis is an unrecognized and potentially serious treatment issue. Rifampicin resistance is a risk factor for poor outcome in tuberculosis. It is prevalent in Nigeria. Therefore, we sought to examine the pattern of rifampicin resistance tuberculosis in Nigeria, West Africa.

Method: One hundred and forty tuberculosis cases were referred to the chest clinic of Sobi Specialist Hospital from January to December 2013. Sputum samples were obtained from them, smeared on glass slides, stained using Ziehl Neelsen Stain and later observed under light microscopy. The GeneXpert MTB/RIF assay was used to simultaneously detect TB and rifampicin resistance.

Result: The minimum age of the patients was 18years, while the maximum was 83. The mean age was 38.39±13.75. There was male preponderance 84(60%), compared to 56(40%) female. The secondary health institution made the highest referral. Forty eight (34.3%) had smear-positive TB, while 92(65.7%) were sputum negative. Thirty two (38.1%) male out of 84 and 12(21.4%) female out of 56 were sensitive to Rifampicin, while 6(7.1) male out of 84 and 4 (7.1%) female out of 56 were resistant to it. Forty four (31.4%) were MD-TB positive with a prevalence of 31.4%. Ten (7.2%) were Rifampicin resistant; this included 6 males and females. This was statically significant.

Conclusion: Our study highlights that physicians should have high index of suspicion for rifampicin resistant tuberculosis in patients refractory to anti-TB treatment. The MTB/RIF test is a useful method for rapid diagnosis of TB and detection of RIF-resistance strains. There is need for increasing effort to interrupt the transmission of RIF-TB.

Keywords: Rifampicin resistant tuberculosis; Secondary health institution; Nigeria; West Africa

Introduction

According to World Health Organization [1], nearly 2 billion people, one-third of the world's population have T.B. Almost 95% of T.B cases are found in the developing world. Nigeria currently ranks 10th among the 22 tuberculosis high burden countries in the world.

According to the World Health Organization, 650,000 people are infected by worldwide [2]. In Africa, 1.9% of new cases and 9.4% of diagnosed and treated patients are infected by a MDR strain [2]. The WHO estimates a Multi-Drug Resistant (MDR)-TB prevalence rate of 3.1% and 1% among new and retreatment TB patients respectively in Nigeria [3]. Multi-drug resistance (MDR) TB is defined as tuberculosis disease caused by a strain of *M. tuberculosis* that was resistant to at least isoniazid and rifampicin [4]. Emerging Multidrug-Resistant Tuberculosis-TB (MDR) is one of the major concerns of the Health policy [5]. Few studies had documented the presence of disease in Nigeria, with prevalent rates ranging from 4%-76.3% [6-9].

In a case study conducted in infectious Diseases Hospital, Kano, out of 80 patients sampled, 52 were diagnosed to be Acid Fast Bacilli (AFB) positive and 28 were Acid Fast Bacilli negative; 61.5% and 38.5% prevalence were found in male and female patients respectively, with age group 31-40years having the highest prevalence of 28.8% [10]. Sputum smear microscopy remains the most common way to diagnose pulmonary TB. Depending on the report and method used, smear microscopy can accurately detect TB in 20% to 80% (using fluorescence microscopy methods) of TB cases [11].

The acronym means: Multi-drug resistant TB, that is, TB in patients

whose infecting isolates of *M. tuberculosis* are confirmed to be resistant in vitro to both isoniazid (INH) and RIF. The categories of suspects are: failure to category 1 treatment, failure to convert at the end of intensive phase of CAT 2 or failure to CAT 2 treatment, Old cases (RAD, relapse, other etc), known symptomatic contacts of DR-TB, TB/HIV co-infected.

The GeneXpert MTB/RIF assay is a molecular-based rapid test with potential to revolutionize TB diagnosis. The regions of the target gene associated with RIF resistance (*rpoB* gene) is PCR amplified Table 4. 5 wild type (A-E) sites of the *rpoB* gene are present. Five molecular beacons are present and they bind only to the wild type *rpoB* gene. It can simultaneously detect *Mycobacterium tuberculosis* (MTB) complex DNA and mutations associated with rifampicin (RIF) resistance (a reliable proxy for) directly from sputum specimens in less than 2 hours, and it minimizes staff manipulation and bio safety risk [12]. Moreover, its ability to detect smear-negative TB provides a significant advantage, especially for PLHIV. In December 2010, the World Health Organization (WHO) endorsed Xpert for the rapid

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and accurate detection of TB, particularly among PLHIV and people suspected of having [12].

There is currently no country wide drug resistance survey hence, the exact data on the prevalence and pattern of rifampicin resistance tuberculosis is unknown. Moreover, there is paucity of data on the prevalence of in Nigeria in general and North-Central Nigeria in particular, hence the need for this study.

Materials and Methods

This study was conducted at the Chest Clinic of Kwara State Specialist Hospital, Ilorin, Nigeria. The target populations were tuberculosis suspects referred from the primary, secondary and tertiary health care facilities in Kwara State in particular and Nigeria in general. The chest clinic project of Sobi Specialist Hospital Ilorin, Kwara State was commissioned on Thursday 7th of June, 2012. It was donated by star deep water petroleum limited. The aim was to assist in the diagnosis of Tuberculosis and Drug Resistance Tuberculosis and to serve as referral center for tuberculosis cases. The hospital caters for infectious diseases such as HIV and tuberculosis.

Ethical approval was obtained from the Ethical Review Committee of the Kwara State Ministry of Health before commencement of the study. The respondents were adequately informed about the nature of the study and its benefits. An interviewer administered questionnaire was used. However, for subjects who do not understand English, a local dialect version of the instrument was used.

The inclusion criteria are tuberculosis (TB) patient who previously received Category 1 (new case) treatment and was declared cured or completed a full course of treatment and has once again development sputum smear-positive tuberculosis, or a TB patient who while on treatment remain, or becomes again smear positive five months or later after commencement of treatment, or a TB patient who completed at least one month of treatment and returns smear positive after at least 8 weeks of interruption of treatment, or a TB patient already registered for treatment in one local government area (LGA) who is transferred to another LGA where(s) he continues treatment, or a TB patient who

does not easily fit into one of the above case definitions. The exclusion criteria is a TB patient who has never had treatment for TB or who has taken anti-TB drugs for less than 4 weeks. A total of 140 Drug Resistance Tuberculosis Suspects cutting across age groups and of both sex were referred between January and December, 2013.

Respondent were grouped into different occupational classes using Oyedeji's classification [13] viz;

- I. Senior public servants, professionals, managers, large scale traders, business-men and contractor, senior military officers.
- II. Intermediate grade public servants, and senior school teachers, non-academic professionals e.g. Nurses, owners of medium sized business, secretaries.
- III. Non-manual skilled workers including clerks, typists, telephone operators, junior school teachers, drivers, artisans.
- IV. Petty traders, labourers, messengers, lower cadre civil servants.
- V. Unemployed, full-time house wives, students, subsistence farmers.

The predictive value of a positive test is calculated as follow:

$A/(A+B) \times 100$, where A represents those with oral lesions, B represents those without oral lesions and A + B, the total sample respondents.

Three sputum specimens were collected from each TB individual. The first sample was taken on the spot, the second sample was also an early morning sample and the third was taken on the spot when the client returns to the consulting room with the second sample.

The sputum was collected into a dry, clean, transparent, wide-mouthed leak proof container. The sides of the specimen containers were labeled correctly with the patient's name and referral Centre. The smear was heat-fixed with flame after complete air drying. Carbol fuchsin was applied onto the slides and heated gently for 5 minutes, then washed and drained. Later decolorized for three minutes, washed thoroughly, and covered with methylene blue for one minute. The slides were then washed, drained and ready for microscopy after drying [14]. The stained and dried smear was viewed under oil as a fine red rod against blue background. Interpretation of results was done using WHO guidelines [14]. Presence of pinkish red rods indicated presence of acid fast bacilli while absence of pinkish red rods meant absence of acid fast bacilli. AFB counts, recording and reporting were as follows: Table 1

No AFB seen in at least 100 fields	0
1-9 AFB in 100 fields	Report the exact number
10-99 AFB in 100 fields	+
1-10 AFB per field in at least 50 fields	++
Greater than 10 AFB per field in at least 20 fields	+++

Table 1: Acid Fast Bacilli report.

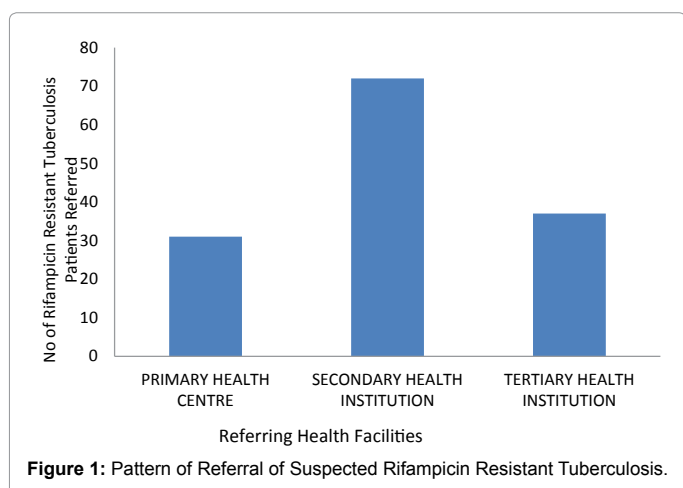
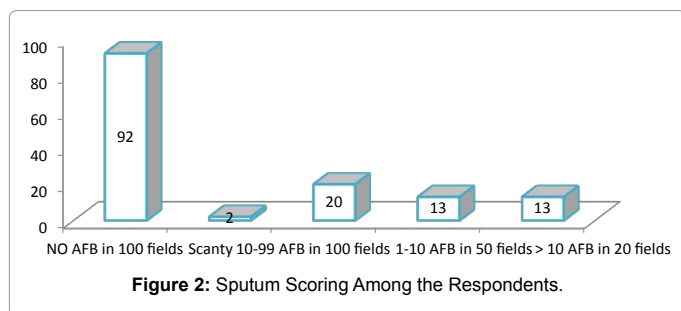


Figure 1: Pattern of Referral of Suspected Rifampicin Resistant Tuberculosis.

GeneXpert was used to diagnose TB and to test for rifampicin (RIF) resistance which was used as proxy for MDR-TB. The specimen for Gene Xpert was collected in an open field fresh. The patient was instructed to inhale deeply 2-3 times, breathe out each time, cough deeply from the chest, place the open container close the mouth to collect the specimen. Appropriate specimen used for Xpert was sputum, minimum of 1ml without food particles and stored 4°C max 10 days. Report results were as follows: MTB detected, MTB not detected, RIF resistance detected, RIF resistance not detected, RIF resistance indeterminate and Error/invalid. In December 2010, the World Health Organization (WHO) endorsed Xpert for the rapid and accurate detection of TB, particularly among PLHIV and people suspected of having [15].

Completed questionnaire and measurements were entered into a computer data base. The data were analyzed using the epidemiological information (Epi-info) 2005 software package of Center for Disease Control and Prevention (CDC). The 2 by 2 contingency tables were used to carry out Chi-square test and to find out the level of significance and values less than 0.05 were regarded as statistically significant.



Results

One hundred and forty patients consisting of 84 males; and 56 females with cough more than two weeks were investigated during the studied period. The minimum age of respondent was 18years, while the maximum was 83. The mean age was 38.39 ± 13.75 .

Figure 1 shows the pattern of referral of the TB suspects with the secondary health institution making the highest referral, while the primary health centre made the least.

Discussion

Demographic characteristic analysis showed that age group 31-40 had the highest drug resistance TB. This was comparable to the previous study in Nigeria [16], but differed from Imam and Oyeyi [17], Nigeria, where 15.29 age group had the highest percent distribution.

There was male preponderance, 84(60%) as against 56(40%) female; this was also in agreement with the previous studies [16-18]. and in concord with the work of Taura et al. [19], where male subjects had prevalence of 61.5% as against 38.5% of females. This disparity could be due to the fact that male subjects were more exposed to risk factors of TB infection such as smoking, which could make them more susceptible.

Forty eight (34.3%) had smear-positive TB. Comparable to 31(18.2%) obtained in the study by Lawson et al. [20] but lower than (8%) smear-positivity reported by Boyer et al. [21]. For TB diagnosis in sputum smear-positive samples, studies showed sensitivities ranging from 93% to 98% and specificities of 83% to 99% [22,23]. Sputum smear microscopy has some limitations because it can only be used to diagnose TB when sputum has sufficient bacillary load, and it cannot detect drug resistance. Thus, HIV-associated TB often goes undetected because people living with HIV (PLHIV), especially those with severe immune suppression generally have very low numbers of bacilli [24]. A more sensitive approach to diagnosis is to culture sputum samples, which can include testing for drug resistance. However, such techniques require expensive and sophisticated laboratory equipment and staff, and it take weeks or months to obtain results. Realistically, most people who need culture the tests to diagnose their TB will not have access to the test results on time, in other to commence medication on time there by preventing complication and spread to others. In this regard, GeneXpert assay is more sensitive than sputum smear microscopy in detecting TB, and it has similar accuracy as culture (Figure 2).

Eighteen (12.9%) were HIV positive and commoner among males than females. Age group 31-40 (33.57%) was more pronounced. This could be attributed to the social life style in the study area. Many of them lives a polygamous way of life, as well as wife inheritance as influenced by their religion. Alcohol use and misused was another contributing factor. Social class-V constituting 8(12.9%) of the respondents were unemployed. Majority of them were full time house wives, and some

of them were subsistence farmers. They were found to statistically significant (Table 2).

In this study HIV co-infection was not found to be statistically significant with anti-TB drug resistant, as found in other studies [25,26]. The current meta-analysis provides strong evidence to affirm that HIV co-infection may not have an influence on the development of anti-TB drug resistance. Additionally, with the exception of a report from Latvia and Ukraine, several reports from different parts of the world had shown that HIV infection had no statistically significant association with [27]. This was however in contrast to studies done about two decades ago, which showed that 90% of cases of occurred in HIV-co-infected individual [28]. Similarly, in the United State, it was

Variable	Frequency	(%)
Age		
< 21	9	-6.4
21-30	38	-27.1
31-40	47	-33.6
41-50	27	-19.3
51-60	8	-5.7
> 61	11	-7.9
Sex		
Male	84	-60
Female	56	-40
Marital Status	9	-6.4
Single	85	-60.8
Married	8	-5.8
Divorced	11	-7.8
Separated	17	-12.1
Widow	10	-7.1
Widower		
Level of Education		
Non-formal	84	-60
Primary	40	-28.6
Secondary	11	-7.8
Tertiary	5	-3.6
Religion		
Christianity	48	-34.2
Islam	85	-60.8
Traditional	5	-3.5
Others	2	-1.5
Occupation	58	-41.4
Business person	7	-5
Civil servant	11	-7.8
Retired workers	12	-8.6
Farmers	17	-12.2
House wives	29	-20.7
Unemployed	6	-4.3
Student		
Social Class		
I	0	0
II	5	-3.8
III	19	-13.5
IV	40	-28.5
V	76	-54.2
HIV Status		
Non-reactive	122	-87.1
Reactive	18	-12.9

Table 2, shows that age group 31-40 (33.57%) was more positive than others. Social class V was the most positive among the social classes. This was statistically significant.

Table 2: Socio-Demographic Characteristics of The Respondents.

Variables	RIFAMPICIN			Total	p-value
	Not on Rifampicin	Rifampicin Resistant	Rifampicin Sensitive		
Sex					
Male	46 (54.8)	6 (7.1)	32 (38.1)	84 (100)	0.076
Female	40 (71.5)	4 (7.1)	12 (21.4)	56 (100)	
Total	86 (61.4)	10 (7.2)	44 (31.4)	140 (100)	

Table 3 shows that 32 (38.1%) male out of 84 and 12 (21.4%) female out of 56 were sensitive to rifampicin, while 6 (7.1%) male out of 84 and 4 (7.1%) female out of 56 were resistant to it.

Table 3: Rifampicin Sensitivity Pattern.

Sex	MDR-TB				Total	p-value
	Negative	Positive	Positive RIF resistance	Invalid Results		
Male	40 (47.6)	32 (38.2)	6 (7.1)	6 (7.1)	84 (100.)	0.031
Female	39 (69.7)	12 (21.4)	4 (7.1)	1 (1.8)	56 (100)	
Total	79 (56.4)	44 (31.4)	10 (7.2)	7 (5.1)	140 (100)	

Table 4 shows the prevalence of MDR-TB and RIF resistance. Forty four (31.4%) were MDR-TB positive with a prevalence of 31.4%. Ten (7.2%) were Rifampicin resistant; this included 6 males and 4 females. This is statistically significant.

Table 4: Prevalence Of Multi Resistant Tuberculosis (Mdr-Tb) And Rifampicin Resistant (RIF)

reported that HIV infection was associated with [29]. Anastasis et al. expressed concern regarding the development of drug-resistant TB as the HIV epidemic progressed [30].

In our study, 44(31.4%) were rifampicin sensitive, while 10(7.2%) were rifampicin resistant (Table 3). This agreed with the findings of Olusoji et al. [16] where (8.6%) were resistant to rifampicin, but lower to the study of Lawson et al. [20] where (19%) isolates were resistance to rifampicin. This level of resistance was in keeping with the findings of Idigbe et al. [31] who reported 2% resistance to rifampicin in Lagos, Nigeria, but contrary to the study of Akaninyene and co-workers¹⁸ where no strain of rifampicin resistant was reported. Mycobacterium TB is more liable to undergo mutation when it is exposed to rifampicin than to many of the second line anti-TB drugs [32].

Forty four (31.4%) were positive and this was comparable to rates published in previous studies from India [33,34], but much lower than 77.4% reported by Olusoji et al. [16]. Few studies had documented the presence of in Nigeria, with prevalent rates ranging from 4%-76.3% [6-9]. According to the World Health Organization 650,000 people are infected by Worldwide and 12 million suffer from tuberculosis. In Africa, 1.9% of new cases and 9.4% of diagnosed and treated patients are infected by MDR strain [2]. The 2002-2007 global survey conducted by WHO also showed that the highest prevalence of among newly diagnosed and previously treated TB cases were 22% and 60% respectively [35]. The World Health Organization (WHO), without an actual survey, estimated a much lower burden of 1.9 and 9.3% for new and previously treated patients, respectively [1]. This could be attributed to lack of record keeping. It is probable that WHO probably underestimates Nigeria's burden. This re-echoes the dire need for good clinical management practices and a prospective countrywide DST survey and adequate record keeping. Idigbe et al. [31] reported that 56% of the strains recovered from 96 patients not responding to anti-TB treatment were resistant to one or more of the drugs used, with 38% being resistant to isoniazid, although only 2% were resistant to rifampicin at that time [9] and did not find an association with HIV infection [10]. There is a need to establish more National Reference Laboratories (SRL) in Nigeria for technical support, quality assurance and surveillance. Several factors might be responsible to the development of MDR-TB. These include patient factors such as poor adherence of patients to

first line anti-TB drugs, inappropriate treatment regimen, dosage and duration for treatment and non-compliance to national guidelines of TB treatment protocol by clinicians.

Since drug-resistance is a dynamic phenomenon, it is pertinent to monitor the trend of drug-resistance periodically among patients presenting with TB symptoms. Adequate enlightenment should be given to patients for proper drug compliance, because inadequate dosage intake on non-compliance on the part of the patients and clinician as well should be encourage to abide with the National Tuberculosis Protocol (NTP) in other to reduce the TB surge.

Conclusion

Rifampicin resistant TB is factor to be considered in refractory cases, hence the need for continuous monitoring of drug resistance trends, in order to assess the efficacy of current interventions and their impact on the TB epidemic.

Strengths

GeneXpert has the potential to significantly increase TB case detection where traditional diagnostics were woefully inadequate – people with suspected HIV-associated TB and MDR-TB.

Limitations

First of all, this is a hospital-based study, hence there could have been significant referral bias involved in patients selection. GeneXpert machine is recommended to test for RIF resistance, which is used as proxy for. Every RIF-resistant case should have also culture/DST to determine resistance against other first and second line drugs. However, GeneXpert is not a panacea, its implementation presents major challenges, particularly related to cost and infrastructure, which call for a thoughtfully phased and careful introduction.

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Conflict of Interest Statement

We declare that we have no conflict of interest

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