



# **Determination of Rifampicin Resistance Tuberculosis on Gene Xpert among Patients Attending Federal Medical Centre Yenagoa, Bayelsa State Niger Delta Region of Nigeria**

**Oluwayemisi A. Olorode<sup>1\*</sup>, Ofonime M. Ogba<sup>2</sup> and Joshua J. Tarilade<sup>1</sup>**

<sup>1</sup>*Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Nigeria.*

<sup>2</sup>*Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, University of Calabar, Nigeria.*

## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors OAO and JJT designed and conducted the experiment. Author OMO analyzed the data. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aim:** Department of Medical Laboratory Science (Medical Microbiology Section), Niger Delta University. Tuberculosis, being an oldest known human disease, is a major cause of mortality. Does gender determine Rifampicin Resistance *Mycobacterium tuberculosis* among the patients with tuberculosis in Bayelsa State? This work was carried out between February, 2019 and September 2019 to determine the prevalence of *Mycobacterium tuberculosis* and its resistance to Rifampicin anti-mycobacterium therapy among patients attending Federal Medical Centre, Yenagoa, Bayelsa State.

**Methodology:** A total of 250 sputum samples were collected from both HIV and non-HIV patients attending Federal Medical Centre, Mycobacteriology Laboratory (a referral laboratory in Bayelsa state); (age range 21-71 years) and immediately taken to the laboratory for analysis using GeneXpert machine, an automated cartridge based nucleic acid amplification diagnosis and

\*Corresponding author: E-mail: [yemisiolorode@yahoo.co.uk](mailto:yemisiolorode@yahoo.co.uk);

traditional Ziehl-Neelsen test for the detection of *Mycobacterium tuberculosis* and Rifampicin resistance gene.

**Results:** Findings showed that 50(100%) sputum samples (of which 24(48%) were from males and 26(52%) from females) out of the 250 samples examined were positive to *Mycobacterium tuberculosis*. Based on the GeneXpert analysis, the total of 8(16%); {4(8%) females and 4(8%) males}, samples were resistant to Rifampicin. Out of 50(100%) positive samples, the highest percentage prevalence of *Mycobacterium tuberculosis* was recorded within the age range of 21 and 30 years (52%); followed by 31-40 years (44%) and the least was 71 years (04%). The percentage prevalence of Rifampicin resistance *Mycobacterium tuberculosis* (RMB) was 16.0% while non-resistance was 84.0%; resistance was higher among the study subjects within the age range of between 21 and 30 years (6%) followed by 31-40 years (4%); 51-60 years (4.0%) and the least was 10-20 years and 70 years above(2%). There is no significant difference between the tuberculosis infected male and female resistance to rifampicin at P value= .88, t=14, df=4. However, out of 50 (100%) positive samples, 24 (48%) patients had HIV co-infection with tuberculosis while 26(52%) had tuberculosis only. There was no statistical significant difference between these two groups at P=.29, t=1.2 and df=4.

**Conclusion:** There is a progressive increase in the frequency of Rifampicin resistant *Mycobacterium tuberculosis* (RrMTB) in this research compared to the percentage prevalence (4%) reported by World Health Organization (WHO) in 2018. There is therefore a need to establish more medical diagnostic laboratories for rapid detection of tuberculosis infection in all the eight (8) Local Government Areas of Bayelsa state.

**Keywords:** *Mycobacterium tuberculosis*; rifampicin resistance; GeneXpert analysis; prevalence.

## 1. INTRODUCTION

Tuberculosis (TB) is an ancient disease known for its high mortality rate globally if left untreated, because the disease is curable and preventable despite its spread from person to person through air. The discovery of similarly deformed bones in various Neolithic sites in Italy, Denmark, and countries in the Middle East also indicate that TB was found throughout the world up to 4000 years ago. The etiological agent of Tuberculosis, *Mycobacterium tuberculosis*, has been the subject of much recent investigation and it is believed that the bacteria in the genus *Mycobacterium* like other Actinomycetes were initially found in soil and some species evolved to live in primates. The domestication of cattle, thought to have occurred between 10,000 and 25,000 year ago would have allowed the passing of the *Mycobacterium* pathogen from domesticated livestock to humans and in this adaption to a new host, the bacterium would have evolved to the closely related *M. tuberculosis* [1]. Tuberculosis is caused by bacteria that is rod shape, aerobic and spore formers. It was documented by some researchers, Adams and Woelke [2]; Baptista et al. [3] that two million people die each year from the malady. Tuberculosis has many manifestations, affecting bone, the central nervous system, and many other organs and systems. But it is basically a disease that is

initiated by the deposition of *Mycobacterium tuberculosis* contained in aerosol droplets, into the lungs alveolar surfaces, from this point the progression of the disease can have several outcomes, determined largely by the response of the immune system of the host [1]. Tuberculosis occurs in every part of the world and the worldwide incidence of new TB cases has been in decline since 2005. In September 2015 World Health Organisation (WHO) announced that the TB target of millennium development goal halting and reversing spread of the disease had been achieved. In 2017 the largest number of new TB cases occurred in the South-East Asia and Western Pacific Regions with 62% and 25% respectively; in African; 87% in the 30 high TB burden countries of which eight countries (India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa) accounted for two thirds of the new TB cases [4]. TB affects all age groups, but more severe in adults; over 95% of cases and deaths are in developing countries. People living with HIV stand 20 to 30 times risk of developing active TB. One million children 0 -14 years of age fell ill with TB and died from the disease in 2016 [5]. Ford et al. [6] stated that about one quarter of the world's population has latent TB; people who harbour the disease, but are not manifesting the illness and cannot transmit the disease. However, persons with compromised immune system such as people living with HIV, malnutrition or diabetes or

tobacco users are at higher risk of falling ill. However sustain progress is threatened by the emergence of antimicrobial resistance to first line therapy. Multidrug resistance (MDR) TB is cause by *Mycobacterium tuberculosis* organism that is resistant to Rifampicin and Isoniazid, and the estimated number of new MDR-TB cases rose from 250,000 in 2009 to 480,000 in 2013. Extensive drug resistance TB (XDR-TB) is defined as MDR-TB with addition resistance to any Flouroquinolone and any of the three second-line administered drugs (amikacin, capreomycin, and kanamycin); 9% of MDR-TB cases fulfil these criteria, and XDR-TB has been identified in over 100 countries [7].

Although the development of multi-drug resistance is a natural phenomenon, the inappropriate use of antimicrobial drugs, inadequate sanitary conditions, inappropriate food-handling and poor infection prevention and control practices contribute to emergence of multi-drug resistant Tuberculosis and encourage the further spread. The aim of this study is to determine the prevalence of Rifampicin Resistance Tuberculosis (RRTB). The objective is the collection of sputum samples from apparently healthy and immunocompromised Human Immunodeficiency Virus (HIV) Patients visiting Federal Medical Centre Yenagoa, Bayelsa State and subjection to analysis using traditional Ziehl Neelsen test and GeneXpert machine, an automated cartridge based nucleic acid amplification diagnosis for the detection of *Mycobacterium tuberculosis* and Rifampicin resistance gene. New target includes ending the TB epidemic by 2030 [3].

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out in Federal Medical Centre Yenagoa, Bayelsa State. The State is located within Latitude 4°15' North and Latitude 5°23' South. It is also within Longitude 5°22' West and 6°45' East. The State is bound by Delta State on the North, Rivers State by the East and the Atlantic Ocean on the Western and Southern parts.

### 2.2 Sample Size

Sample size was calculated using the Cochran formula (1999):

$$N = \frac{Z^2 P Q}{d^2}$$

Sample size calculation was done using the 95% confidence interval and 0.05 precision rate.

The prevalence rate of MDR-TB in Nigeria is 4% [8].

N=minimum sample size  
Z= confidence interval (95%) whose equivalent coefficient is 1.96  
P=prevalence rate (4%)  
Q=alternate proportion (1-P)  
D=degree of precision (5%)

$$N = \frac{(1.96)^2 \times (0.04) \times (0.96)}{(0.05)^2} = 59$$

Total sample size = 250

### 2.3 Sample Collection

The individual was provided with transparent, wide-mouthed, leak-proof, screw-capped containers and asked to produce sputum samples.

A total of 250 samples were collected from the study subjects.

The samples were analyzed in Federal Medical Centre, Yenagoa.

### 2.4 Sampling Technique

A Probability sampling technique was used (Stratified Random Sampling method).

The subjects were grouped into different strata.

### 2.5 Inclusion Criteria

- Patients living with HIV (PLHIV) with symptoms of TB.
- Patients (both HIV and Non-HIV) with failed 6 months regimen.
- Health workers (HIV and Non- HIV) with TB symptoms.
- Individuals without symptoms were used as control.

### 2.6 Sample Processing

There are various methods and samples used in the laboratory diagnosis of TB. But for the purpose of this work, it was specific on pulmonary TB.

For the detection of pulmonary TB and MDT the following methods are used.

**2.7 Microscopic Method; Sputum Smearing and Staining**

**2.7.1 Acid Fast Baccili (AFB) microscopy**

Sputum was spread evenly over the central area of a clean grease-free slide with its frosted ends appropriately labeled. The slides was placed on a dryer to air dry for about 30 minutes. The dried smear was heat fixed and taken to the staining rack. The smear was covered with strong carbol fuschin stain and the smear was heated, for 5 minutes intermittently, until vapour begins to rise. The stain was washed off with clean water and was covered with 3% v/v acid alcohol for 2-5 minutes for decolorization. The smear was rinsed with water and then was covered with Methylene Blue stain for 1-2 minutes and was rinsed with water. The slide was placed on draining rack to air dry and examined using the oil immersion objective.

**2.8 Smear Examination (According to [9])**

**2.8.1 ZN smears**

One length of the smear (2 cm) was examined with bright field microscope, using 1000x magnification. If less than 10 AFB are found in 100 fields, the number of AFB should be counted. For high positives, examination of only 20-30 fields is sufficient.

**2.9 Genexpert MTB/RIF**

The GeneXpert MTB/RIF is a cartridge based nucleic acid amplification test for rapid tuberculosis diagnosis and rifampicin resistance

test in a simultaneous condition. It is an automated diagnosis test that can identify *Mycobacterium tuberculosis* (MTB) DNA and resistance to Rifampicin (RIF).

**2.9.1 Mechanism**

The Xpert MTB/RIF can detect DNA sequences specific for *Mycobacterium tuberculosis* and rifampicin resistance by polymerase chain reaction. It is based on the Cepheid Genxpert system (nucleic acid amplification test NAAT). The Xpert does the work of purification and concentration of *Mycobacterium tuberculosis* bacilli from sputum samples, as well as isolation of genomic material from captured bacteria through the process of sonication and amplification of the genomic DNA by PCR. The process identifies most of the clinically mutations in the RNA polymerase beta (rpoB) gene in the *Mycobacterium tuberculosis* genome in a real time format using fluorescent probes called molecular beacons results are obtained from unprocessed sputum sample in 90 minutes [10].

Nucleic Acid Amplification Test Using Gene Xpert MTB/RIF (Cepheid, Sunnyvale, CA) a new molecular diagnostic test for TB, in addition to being highly sensitive for MTB, provides rapid detection of rifampicin resistance which is considered a surrogate for MDR-TB [11].

**2.10 Genexpert Procedure**

The Sputum sample collected from study subjects was mixed with the Genexpert reagent and mixed vigorously for 10 minutes to form a homogenous mixture using a sterile pipette, 2 ml of the solution was aspirated into the cartridge and the cartridge was loaded onto the

**Table 1. Grading of the smears is presented in the following table (HPF= high power field, or the view field size at 1000x magnification)**

| IUATLD/WHO scale | ZN 1000 x magnification, 100 HPF per length of the smear | Field Magnification 200-250x magnification, 30 fields= 300 HPF per length of the smear | Field Magnification 400x magnification, 40 fields= 200 HPF per length of the smear |
|------------------|--|--|--|
| Negative         | Zero AFB/1 length  | Zero AFB/1 length  | Zero AFB/1 length  |
| Scanty           | 1-9 AFB/1 length   | 1-29 AFB/1 length  | 1-19 AFB/1 length  |
| 1+               | 10-99 AFB/1 length                                       | 30-299 AFB/1 length  | 20-199 AFB/1 length  |
| 2+               | 1-10 AFB/1 HPF on average                                | 10-100 AFB/1 field on average  | 5-50 AFB/1 field on average  |
| 3+               | >10 AFB/1 HPF on average                                 | >100 AFB/1 field on average  | >50 AFB/1 field on average   |

GeneXpert machine. Following the principle of PCR, the nucleic acid of the MTB was amplified and the result recorded after the test running time of 1hour 55 minutes. The recorded results was obtained as follows: MTB not detected, MTB detected/RIF resistance not detected, MTB detected/Rif resistance detected or MTB detected/RIF resistance not detected.



**Fig. 1. Nucleic acid amplification test using Genexpert MTB/RIF system**

### 3. RESULTS AND DISCUSSION

Fig. 1. depicts Nucleic Acid Amplification Test Using Genexpert MTB/RIF System.

Table 2 Shows the prevalence of rifampicin resistance *Mycobacterium tuberberculosis* among presumptive subjects.

**Table 2. Prevalence of rifampicin resistance *Mycobacterium tuberculosis* among study subject using (gene-x pert)**

| Ages (years) | Number of cases examined | % TB infection | R. resistance | N.R. resistance |
|--------------|--------------------------|----------------|---------------|-----------------|
| 10-20        | 30(12.0)                 | 6(2.4)         | 0(0.0)        | 6(12.0)         |
| 21-30        | 53(21.2)                 | 13(5.2)        | 3(6.0)        | 10(20.0)        |
| 31-40        | 83(33.2)                 | 11(4.4)        | 2(4.0)        | 9(18.0)         |
| 41-50        | 41(16.4)                 | 10(4.0)        | 0(0.0)        | 10(20.0)        |
| 51-60        | 33(13.2)                 | 6(2.4)         | 2(4.0)        | 4(8.0)          |
| 61-70        | 74(29.6)                 | 4(1.6)         | 1(2.0)        | 3(6.0)          |
| ≥ 70         | 10(4.0)                  | 0(0.0)         | 0(0.0)        | 0(0.0)          |
| Total        | 250 (100.0)              | 50(20.0)       | 08(16.0)      | 42(84.0).       |

% = percentage, R = Rifampicin, NR = Non-Rifampicin

Table 3 Shows rifampicin resistance *Mycobacterium tuberculosis* based on gender n=50

Table 4 depicts Prevalence of rifampicin resistance *Mycobacterium tuberculosis* based on HIV CO-infections.

Table 5 shows relationship between HIV and MTB among the study subjects.

Table 2 shows the prevalence of rifampicin resistance *Mycobacterium tuberculosis* among study subjects attending federal medical Centre Yenagoa, Bayelsa using gene-xpert.

The prevalence of tuberculosis (MTB) in our study was 20% (50/250). The prevalence of *Mycobacterium tuberculosis* (MTB) was highest among subjects within 21-30 years (26%) (13/50), followed by 31-40 years (4.4%) and the least was in subjects above 71 years (2%). Furthermore, the prevalence of rifampicin resistance *Mycobacterium tuberculosis* (RMTB) in the present study was 16.0% while non-resistance was 84.0%. Resistance was more within the ages of 21-30 years (6.0%) followed by 31-40, 51-60 years 4.0% and least with ages of 10-20, and 70 above.

Table 3 shows Rifampicin Resistance *Mycobacterium tuberculosis* (RMTB) among the study subjects based on gender. A total of 48% infected with *Mycobacterium tuberculosis*, 8.0% were resistant to rifampicin in both male and female subjects. Non-resistance was 40% and 44% for male and female respectively.

The difference in rifampicin resistance between male and female subject was not statistically significant at P. value = 0.88, t =0.14 and df =4.0

**Table 3. Rifampicin Resistance *Mycobacterium tuberculosis* (RMTB) based on Gender n =50**

| Gender (%)     | Resistance (%) | Non-resistance (%) |
|----------------|----------------|--------------------|
| Male 24 (48)   | 04(8)          | 20(40.0)           |
| Female 26 (52) | 04(8)          | 22(44.0)           |
| Total 50 (100) | 08(16)         | 42(84.0)           |

*P. value 0.88, t=0.14, df =4.0*

**Table 4. Prevalence of rifampicin resistance *Mycobacterium tuberculosis* (RMTB) based on HIV co-infections**

| Variables | No examined | Positive cases (%) | Res.(%)  | N. Res(%) |
|-----------|-------------|--------------------|----------|-----------|
| HIV & MTB | 250         | 24(9.6)            | 03(12.5) | 21(87.5)  |
| MTB only  |             | 26 (10.4)          | 08(30.8) | 18(69.2)  |

*T = 1.20, df =4.0 P. value 0.29*

**Table 5. HIV and MTB statue among the study subjects**

| Variables           | Female    | Male      | Total      |
|---------------------|-----------|-----------|------------|
| <b>HIV status</b>   |           |           |            |
| % positive          | 53(21.2)  | 45(18.0)  | 98(39.2)   |
| % negative          | 93(37.2)  | 59(23.6)  | 152(60.8)  |
| Total               | 146(58.2) | 104(41.6) | 250(100.0) |
| <b>TB infection</b> |           |           |            |
| % positive          | 26(10.4)  | 24(9.6)   | 50(20.0)   |
| % negative          | 119(47.6) | 81(32.4)  | 200(80.0)  |
| Total               | 105(42.0) | 145(58.0) | 250(100.0) |

*HIV = Human Immunodeficiency Virus, TB = Mycobacterium Tuberculosis, %= Percentage*

Table 4 shows resistance of rifampicin among subjects studied based on Co-infection. Out of 250 presumptive subjects screen for HIV, 24 (9.6%) were tuberculosis (MTB) while 26 (10.4%) were positive for MTB only. HIV and TB co-infection showed 12.5% resistance while only TB infection showed increased resistance of 30.8% (26/250). The different in resistance between the two groups was not statistically significant, at *P. value = 0.29, t=1.20 and df = 4.0*.

Table 5 shows the HIV and *Mycobacterium tuberculosis* status among 250 presumptive patient students data shows 98(39.2%) HIV positive cases 21.2% were female (53/250) and 18.0% were male (45/250). While 152(60.8%) were negative.

Also a total of 20% positive for TB 10.4 (26/250) were females and 9.6 % ( 24/250) were males.

### 3.1 Discussion

Rifampicin is a major anti tuberculosis drug used for both new cases and previously treated *Mycobacterium tuberculosis*. Advancement in technology has made detection of the baccilli susceptibility to rifampicin drug easy. The

prevalence of rifampicin resistance *Mycobacterium tuberculosis* (RRMTB) in this present study was 16.0%; this finding is low compared to what was reported in a study from Lagos Nigeria by Keshavjee and Farmer (2017) [12] and in India by Jai, et al. [13] with a prevalence of 23.4% and 52.7% respectively. Findings from systemic review on the prevalence of drug resistance Tuberculosis in Nigeria and Ethiopia have shown that current estimate from new and previously treated tuberculosis patients for both countries were low, which is a proof that the burden of Drug rifampicin Tuberculosis in Nigeria (Bayelsa State) and other high tuberculosis burden countries in Africa is probably bigger than we can anticipate [14]. Furthermore, the current prevalence is equally higher than the 14.7% reported at the tuberculosis referral hospital and laboratory Igbogene Bayelsa State in 2018 by [15] while Otu et al. [16] in Calabar, Cross Rivers state reported a prevalence of 42%. Also, there was no gender difference in the prevalence of RRMTB in this present study similar to the reports from other parts of Nigeria [17]. However, studies from Southern parts of Nigeria and South Africa showed that the prevalence of drugs resistance tuberculosis was significantly higher

among males reported by McKay [18] who further proposed that the difference is attributed to poor health setting behaviour among men, social stigma and cultural behaviour.

Age specific prevalence in resistance in this study showed higher percentage at 31-40 years as against Edlin-Brain's [19] reports of increase prevalence at ages above 45 years.

#### 4. CONCLUSION

There is a progressive increase in the frequency of Rifampicin Resistant *Mycobacterium tuberculosis* (RRMTB) in our study area compare to the prevalence (4%) reported by World Health Organization (WHO) in 2018. There is therefore need to replicate laboratories and facilities for rapid detection of this infection in all the 8 Local Government Area of the state, so that the patients living around these areas can receive immediate medical laboratory attention instead of coming to the main city (Yenagoa) where FMC is located.

Obviously, reduction of DR-TB is a daunting task and these proactive measures are needed to urgently address the challenges of prompt diagnosis and early initiation of the treatment.

Finally, State Ministry of Health must put mechanism in place to ensure adequate treatment of drug sensitive tuberculosis cases.

#### CONSENT AND ETHICAL APPROVAL

All authors hereby declare that the individual patients' consent was sought and approved. The ethical committee of the Federal Medical Centre, Yenagoa approved the collection of the sputum samples.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist. This research was funded by personal efforts of the authors.

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