INCIDENCE OF RIFAMPICIN-RESISTANCE PRESUMPTIVE M. TUBERCULOSIS CASES AMONG OUTPATIENTS IN KEBBI STATE, NIGERIA

Mohammed Bashar Danlami1*; Basiru Aliyu2; and Grace Samuel3

1Department of Microbiology, Federal University Birnin Kebbi, PMB 1157 Kebbi State, Nigeria.
2Department of Microbiology, Faculty of Life Sciences, Kebbi State University of Science and Technology, Aliero, P.M.B.1144. Birnin Kebbi, Kebbi State, Nigeria.

*Corresponding author's e-mail: mohammed.bashar@fubk.edu.ng or mr.bash@gmail.com

Abstract

Background: The present study determined the incidence of rifampicin resistance M. tuberculosis among outpatients at the General Hospital Yauri, Kebbi State, Nigeria.

Materials and Methods: The study is a cross-sectional study conducted from February 2018 to October 2019. Sociodemographic data were collected from hospital registration books. Rifampicin resistance M. tuberculosis was detected using GeneXpert Model GX-IV following manufacturers' instruction. Descriptive statistics and logistic regression were computed using SPSS version 20. The results were presented as odds ratios associated 95% confidence intervals, and P-value at 0.05.

Result: Of the 837 samples, 65.8% (551/837) were males, and 34.2% (286/837) females, 11.4% (95/837) HIV-seropositive. M. tuberculosis was detected in 15.5% (130/837), of which 116/130 (89.23%) were males and 14/130 (10.77%) females. M. tuberculosis-HIV co-infection was detected in 9.47% (9/95) of HIV positive. Rifampicin resistance was observed in 1.3% (11/837), 7.7% (10/130) in M. tuberculosis patients and 1.05% (1/94) in HIV seropositive. In logistic regression, the odds ratio for having a rifampicin-resistant M. tuberculosis was 0.49 (0.15-1.54) for >30 years; taking <30 years as the reference value, 1.02 (1.00-1.03) for male; taking female as the reference value, and 0.78 (0.09-6.15) for HIV positive, taking negative as the reference value.

Conclusion: This study reported the current incidence rate of rifampicin-resistant M. tuberculosis at the General Hospital Yelwa Yauri, Kebbi State, Nigeria, among presumptive TB patients. Patients diagnosed with rifampicin-resistant M. tuberculosis were predominantly male adults. Thus, frequent screening is vital for surveillance and reduces the risk of transmission and spread of M. tuberculosis infections.

Keywords: Rifampicin resistance, Mycobacterium tuberculosis, GeneXpert, Kebbi, Nigeria

List of Abbreviations: HIV: Human immunodeficiency virus, MDR-TB: Multidrug-resistant tuberculosis, MTB: M. tuberculosis, RIF: Rifampicin-resistant, TB: tuberculosis, CI: Confidence interval, OR: Odd ratio, EQA: External quality assurance, WHO: World Health Organization, SPSS: Statistical package for social sciences.

Introduction

The spread of multidrug-resistant tuberculosis (MDR-TB) remains a public health problem in Nigeria (Audu et al., 2017). The country is among the high-TB-endemic in the world. An estimated 4.3% of tuberculosis patients in the country are MDR, out of which 32% are newly diagnosed (WHO, 2016; Ukwamedu et al., 2019). A recent national survey in Nigeria recorded a 2% increase in the prevalence of MDR-TB among pretreatment cases (Onyedum et al., 2017). In Kebbi State, comprehensive information about the patterns of multidrug-resistant tuberculosis is lacking, and only a few studies have assessed the extent of multidrug-resistant tuberculosis among likely TB patients in other states of the federation (Aminu and Tukur, 2017; Onyedum et al., 2017). Rifampicin and isoniazid are essential drugs in the treatment of tuberculosis. The two drugs are critical for short-term treatment with reduced toxicity because of long-term therapy (WHO, 2016; Ukwamedu et al., 2019). Studies have
shown that MDR-TB to rifampicin is accompanied mainly by resistance only to isoniazid (WHO, 2016; Ukwamedua et al., 2019).

In the last few decades, culture is the most common laboratory diagnostic technique for M. tuberculosis (Ma et al., 2006; Derseh et al., 2017). The technique requires sophisticated and expensive biosafety laboratory facilities (Arega et al., 2019). Of recent, Gene Xpert assay, an automated molecular assay detects mutations on the rpoB gene responsible for 90% cases of rifampicin-resistance in TB patients with less sophisticated facilities (Okonkwo et al., 2017; Onyedum et al., 2017). Thus, the treatment of M. tuberculosis without susceptibility testing increases the risk of transmission and spread of MDR-TB strains in a population (Xiao-li et al., 2014; Onyedum et al., 2017; WHO, 2018).

With the projected incidence of 219 new TB cases per 100,000 people per year in the country (Onyedum et al., 2017; Ukwamedua et al., 2019), screening for rifampicin-resistance tuberculosis within the population is vital for the treatment of MDR-TB. This study will provide up-to-date insight into the incidence and extent of rifampicin-resistance among likely TB patients in one of the referral hospitals in Kebbi State of Nigeria.

Materials and Methods

Study design, area and period

The study was conducted at General Hospital Yelwa Yauri, an Emirate Region situated in the southern part of Kebbi State, Nigeria, between February 2018 to October 2019. In Yelwa Yauri, 54% of the populations live in rural areas, and half of the communities are illiterates. The hospital is a secondary level health facility located in the heart of the town.

Study design and Data collection

Participants microbiological and clinical information were collected from the hospital records for all the presumptive patients. Patients' records with incomplete data, e.g., age, gender, Xpert MTB/RIF results, and HIV status and unsuccessful results were excluded from the study. Failed results were classified as invalid, error and undetermined. The inclusion criteria for this study included participants willing to provide their samples freely and those who presented themselves as patients with a long-term duration of cough and chest pain and patients that are booked for AFB screening.

Gene Xpert assay

Single sputum samples per patient were used for the diagnosis of Mycobacterium tuberculosis using Gene Xpert assay. Samples were processed using Model GX-IV of the Gene Xpert (Cepheid Sunnyvale, CA, USA) following the manufacturer's instruction. Briefly, 0.5 ml sputum sample and Xpert sample reagent were added in a ratio of 1:2, vortex twice for 15 min at room temperature. Consequently, 2 ml of the reaction mixture was transferred to the Xpert test cartridge; the cartridge was then loaded into the Xpert machine for 90 mins. The results were interpreted from measured fluorescent signals automatically as MTB detected, not detected and if present- rifampicin resistant.

Data processing and statistical analysis

The database was generated in Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Descriptive analysis was adopted using the Statistical Package for the Social Sciences (SPSS®, version 21; SPSS Inc., Chicago, IL, USA). Frequencies and percentages were calculated, and odds ratio and nominal 95% confidence intervals (CI) were presented to explain the association between variables - age, gender, marital status, RIF resistance and HIV status independently as against MTB detection. A two-sided p-value < 0.05 was considered significant.

Ethical issue

This study was approved by the ethical research and review committee of the Ministry of Health Birnin Kebbi, Kebbi State, Nigeria (KSUSTA1510204007), on 02/05/2019.

Results

Of the 896 patients that submitted samples for TB diagnosis, 82.2% (837/896) were included in the study. Five samples were external quality assurance (EQA) samples, 21 samples were invalid, 19 were error, and the remaining 14 samples were undetermined. Out of these 837, 65.8 % (551/837) were males, and 34.2 % (286/837) were females. The study population median age was 35 years, 27.7% (232/837) were in the age range of 21–30 years, while 2.4% (20/837) were in the age range under ten years. Among total study participants, 11.4% (95/837) were HIV-seropositive, and the remaining 88.6% (742/837) were HIV-seronegative (Table 1).
Table 1: Sociodemographic distribution and clinical characteristics of tuberculosis patients in (N = 837)

| Variables             | Total Patients | Percentage % |
|-----------------------|----------------|--------------|
| **Age**               |                |              |
| Under 10              | 20             | 2.4          |
| 11-20                 | 105            | 15.5         |
| 21-30                 | 232            | 27.7         |
| 31-40                 | 176            | 21.0         |
| 41-50                 | 143            | 17.1         |
| 51-60                 | 100            | 11.9         |
| Above 60              | 61             | 7.3          |
| **Gender**            |                |              |
| Male                  | 551            | 65.8         |
| Female                | 286            | 34.2         |
| **Marital Status**    |                |              |
| Single                | 233            | 27.8         |
| Married               | 604            | 72.2         |
| **MTB Status**        |                |              |
| Detected              | 130            | 15.5         |
| Not Detected          | 707            | 84.5         |
| **RIF Resistant**     |                |              |
| Detected              | 11             | 1.3          |
| Not Detected          | 826            | 98.7         |
| **HIV Status**        |                |              |
| Positive              | 95             | 11.4         |
| Negative              | 742            | 88.6         |

Among the enrolled presumptive TB patients, MTB was detected in 15.5% (130/837), of which 116/130 (89.23%) were males, and 14/130 (10.77%) were females. Of presumptive MTB patients, the age group 31–40 years with a median age of 35 years, found prone to MTB infection 40/130 (30.8%). MTB-HIV coinfection was detected in 9.47% (9/95) of HIV positive patients, table 2. Rifampicin-resistant was observed in 1.3% (11/837) in the presumptive TB patient, while the total RIF-MTB was 7.7% (10/130) in all MTB detected patients and 1.05% (1/94) in HIV seropositive patient (Table 2).

Table 2: Characterization of *M. tuberculosis* in presumptive TB patients based on age, gender, HIV status and marital status in Kebbi State, Nigeria (N = 837)

| Variables           | Detected | Not Detected | OR(95%CI)       | *P* Value |
|---------------------|----------|--------------|----------------|-----------|
| **Age* MTB**        |          |              |                |           |
| Under 10            | 3        | 17           |                |           |
| 11-20               | 6        | 99           | 0.35(0.08-1.50) |           |
| 21-30               | 33       | 199          | 0.94(0.26-3.39) |           |
| 31-40               | 40       | 136          | 1.67(0.47-6.0)  |           |
| 41-50               | 25       | 118          | 1.20(0.33-4.41) |           |
| 51-60               | 13       | 87           | 0.85(0.22-3.30) |           |
| Above 60            | 10       | 51           | 1.11(0.28-4.51) |           |
| **Gender* MTB**     |          |              |                |           |
| Male                | 116      | 435          | 0.19(0.11-0.34) | .000     |
| Female              | 14       | 272          |                |           |
| **Marital Status* MTB** |        |              |                |           |
| Single              | 18       | 215          | 00*            |           |
| Married             | 112      | 492          | 0.37(0.22-0.62) | .000     |
| **RIF Resistant* MTB** |        |              |                |           |
| Detected            | 10       | 120          | 0.02(0.002-0.13)|           |
| Not Detected        | 1        | 706          | 00*            |           |
| **HIV Status* MTB** |          |              |                |           |
| Positive            | 9        | 121          | 1.86(0.91-3.8)  | 0.09      |
| Negative            | 86       | 621          | 00*            |           |

00* = Reference category
MTB = *M. tuberculosis*
OR: Odd ratio
In a logistic regression model, taking <30 years as the reference value, the odds ratio for having a rifampicin-resistant MTB was 0.49 (95% CI, 0.15-1.54) for age groups > 30 years, 1.02 (95% CI, 1.00-1.03) for male taking female as the reference value. It is 1.75 (95% CI, 0.33-8.17) for married, single as a reference value, and 0.78 (95% CI, 0.09-6.15) for HIV positive individuals, taking negative as the reference value (Table 3).

**Table 3:** Characterization of rifampicin-resistant *M. tuberculosis* in presumptive TB patients based on age, gender, HIV status and Marital Status in Kebbi State, Nigeria *(N = 837).*

| Variables            | Detected | Not Detected | OR(95%CI) | P_Value |
|----------------------|----------|--------------|-----------|---------|
| Age* RIF             |          |              |           |         |
| <30 years            | 4        | 353          | 00*       |         |
| > 30 years           | 7        | 473          | 0.49(0.15-1.54) | 0.29 |
| Gender* RIF          |          |              |           |         |
| Male                 | 11       | 540          | 1.02(1.00-1.03) | 0.02 |
| Female               | 0        | 286          | 00*       |         |
| Marital Status* RIF  |          |              |           |         |
| Single               | 2        | 231          | 00*       |         |
| Married              | 9        | 395          | 1.75(0.33-8.17) | 0.74 |
| HIV Status* RIF      |          |              |           |         |
| Positive             | 1        | 94           | 0.78(0.09-6.15) | 1.00 |
| Negative             | 10       | 732          | 00*       |         |

00* = Reference category

**Discussion**

The spread of rifampicin resistant *M. tuberculosis* is a threat to treatment and control of tuberculosis (Onyedum *et al.*, 2017; WHO, 2018). Thus, early detection is essential for the management and prevention of transmission of the disease. In the present study, we combined sociodemographic data and Xpert MTB/RIF assay and assessed the incidence of rifampicin resistant *Mycobacterium tuberculosis* among outpatients referred to the General Hospital Yauri, Kebbi State, Nigeria. This study is first to describe the scale of *M. tuberculosis* cases in the study population.

The overall incidence rate of new *M. tuberculosis* cases in Yauri Emirate in Kebbi State was 15.5%. The result was slightly higher than the studies reported in Zaria (12%) in the North-west region of the country a decade ago (Ogboi *et al.*, 2010). Though, the incidence is significantly lower than reports from previous studies in a referral hospital in South-west Nigeria (37.7%) (Adejumo *et al.*, 2018) and among patients previously treated for pulmonary tuberculosis in North-west of Nigeria (29.2%). The incidence is equally lower than it has been reported in another study in Nasarawa State in the North-central (18.8 %) (Audu *et al.*, 2017). This result is considerably lower when compared with the results obtained using a line probe assay from a referral hospital in Kano State (54.5 %) (Aminu and Tukur, 2017). The low incidence rate reported in this study compared with other studies could be attributed to sampling techniques, size and methodology adopted.

Similarly, this study reported a 1.3% incidence rate of rifampicin resistance (11/ 837). The rate is significantly lower than the 7.2% reported in Kwara State, Nigeria, 6.9% recorded in Nnewi, Nigeria (Dim and Dim, 2013; Okonkwo *et al.*, 2017). Also, the incidence rate is relatively below the projected rate of 3.2–5.4% for the country (WHO, 2016; WHO, 2018). Although the resistance in this study is very low due to the sample size. The low incidence rate recorded may be due to (i) this study considered only presumptive TB cases from outpatients using gene Xpert assay, (ii) the rifampicin resistance positive cases were infected with a resistant *M. tuberculosis* strain. This study also found a high number of *M. tuberculosis* cases and rifampicin resistance among males compared to females. All the 10 rifampicin-resistant *M. tuberculosis* were detected in presumptive TB patients. Analysis of statistical significance reveals that gender is associated with rifampicin-resistant tuberculosis with p-values of 0.02. The result was in line with a high rate of multidrug resistance TB reported in men in North and southern regions of Nigeria (Iliyasu and Babashani, 2009; Dersch *et al.*, 2017) and many African countries (Cox *et al.*, 2010; Kirenga *et al.*, 2015). Also, the result was consistent with other studies that stated that the male gender is at risk of developing drug resistance due to behavioural factors such as poor health-seeking behaviour, not finishing an antibiotic course and cultural activities that expose male gender to infectious agents (Bello and Itiola, 2010).

The average age range with the highest rate of *M. tuberculosis* cases was 31-40 years, and rifampicin resistance was in > 30 years. This result correlates with other studies reported in Nigeria, where patients between 20-40
years had a higher prevalence of rifampicin resistance. However, the results disagree with studies that reported younger age had been associated with PTB and MDR-PTB (Onyedum et al., 2017; Ukwemdua et al., 2019). The distinction observed in this study was 48% of the total population in this study were adults between 20-40 years of age and 72.5% rural farmers (data not shown) associated with unpasteurized cow milk in local porridge as a staple food in the farm. The unpasteurized cow milk was the primary source of M. tuberculosis to the farmers (Araújo et al., 2014; Lorente-Leal et al., 2019).

The association between MTB-HIV coinfection has long been established (Oshi et al., 2014). Despite this, a low rate of MTB-HIV coinfection was reported in this study. However, the reduced rates of MTB-HIV coinfection recorded in this study may be because a significant number of the study participants had no contact with HIV because of increasing public enlightenment in the population (Keating et al., 2006; Gambo et al., 2013).

**Conclusion**

This study reported the current incidence rate of rifampicin-resistant M. tuberculosis at General Hospital Yelwa Yauri Kebbi State, Nigeria, among possible M. tuberculosis. The patients diagnosed with rifampicin-resistant M. tuberculosis were predominantly adult males. This result demonstrated that GeneXpert assay is a convenient tool for the early diagnosis of rifampicin-resistant M. tuberculosis. Accordingly, frequent screening and surveillance within the population are vital for the management and treatment of M. tuberculosis infections.

**Conflicts of interest:** The authors declare no conflict of interest.

**Acknowledgement**

The authors are very grateful to all laboratory personnel of General Hospital Yelwa Yauri in Kebbi State, Nigeria, for their support.

**References**

1. Adejumo OA, Oluwol-Faley B, Adepoju V, Abimbola B, Sunday A, Ayodeji F, Henry O, Kehinde O, Shaafaat O, and Oluwatosis A. (2018). Prevalence of rifampicin-resistant tuberculosis and associated factors among presumptive tuberculosis patients in a secondary referral hospital in Lagos, Nigeria. African Health Sciences. 18(3):472-478.

2. Aminu AI and Tukur AD. (2017). Detection of Multidrug-Resistant Tuberculosis (MDR-TB) among Rifampicin-resistant TB patients using Line Probe Assay (LPA) in Kano, Nigeria. Bayero Journal of Pure and Applied Sciences. 9(2): 1 – 8.

3. Arega B, Menbere F, and Getachew Y. (2019). Prevalence of rifampicin-resistant Mycobacterium tuberculosis among presumptive tuberculosis patients in selected governmental hospitals in Addis Ababa, Ethiopia. BMC Infectious Diseases. 19(307):1-5.

4. Audu E, Gambo M, and Yakubu A. (2017). Rifampicin resistant mycobacterium tuberculosis in Nasarawa State, Nigeria. Nigerian Journal of Basic and Clinical Sciences. 14(1), 21-25.

5. Bello SI and Itiola OA (2010). Drug adherence amongst tuberculosis patients in the university of Ilorin teaching hospital, Ilorin, Nigeria. African Journal of Pharmacy and Pharmacology. Vol. 4(3):109-114.

6. Cox HS, McDermid C, Azevedo V, Muller O, Coetzee D, John S, Marinus B, Gerrit C, Gilles van C, and Eric GI. (2010) Epidemic Levels of Drug-Resistant Tuberculosis (MDR and XDR-TB) in a High HIV Prevalence Setting in Khayelitsha, South Africa. PLoS ONE 5(11): 1-8.

7. Araújo CP, Ana Luiza AR., Klaudia S.G. Jorge1, Carlos A.N. Ramos, Antonio F. Souza F, Carlos EV, Aguera P.C. V, Eliana R, Adalgiza SR, Philip NS, Antônio A. Fonseca J, Marcio RS, José D. BN, Valíria DC, and Flávio RA. (2014). Direct detection of Mycobacterium tuberculosis complex in bovine and babaline tissues through nested-PCR. Brazilian Journal of Microbiology. 45(2): 633-640.

8. Derseh D, Moges F, and Tessema B. (2017). Smaer positive pulmonary tuberculosis and associated risk factors among tuberculosis suspects attending spiritual holy water sites in Northwest Ethiopia. BMC Infectious Diseases. 17:100:1-8.

9. Dim, C. and Dim, N. (2013). Trends of tuberculosis prevalence and treatment outcome in an under-resourced setting: The case of Enugu state, South East Nigeria. Nigerian Medical Journal. 54(6): 392-397.

10. Gambo A, Samer SE, Alash'le A, Nicholas E, Iwakun M, Laura H. Clayton B, Kathleen JT, Joshua O and William B. (2013). Mycobacterial Etiology of Pulmonary Tuberculosis and Association with HIV Infection and Multidrug Resistance in Northern Nigeria. Tuberculosis Research and Treatment. 2013, ID 65056:pp. 1-9.

11. Iliyasu Z, and Babashani, M. (2009). Prevalence and predictors of tuberculosis coinfection among HIV-seropositive patients attending the Aminu Kano Teaching Hospital, northern Nigeria. Journal of Epidemiology. 19(2):81-7.
12. Keating J, Meekers D, and Adewuyi A. (2006). Assessing the effects of a media campaign on HIV/AIDS awareness and prevention in Nigeria: Results from the VISION Project. BMC Public Health. 2006, 6:123.
13. Kirenga BJ, Sengooba W, Catherine M, Lydia N, Stephen K, Samuel K, Frank M, Martin B, Moses J, and Alphonse O. (2015). Tuberculosis risk factors among tuberculosis patients in Kampala, Uganda: Implications for tuberculosis control. BMC Public Health. 15:13:1-7.
14. Lorente-Leal V, Liandris E, Castellanos E, Bezos J, Domínguez L, de Juan L and Romero B (2019) Validation of a Real-Time PCR for the Detection of Mycobacterium tuberculosis Complex Members in Bovine Tissue Samples. Front. Vet. Sci. 6:61.
15. Ma X, Wang H, Deng Y, Yunfeng D, Zhimin L, Yong X, Xi P, James MM. and Edward AG. (2006). rpoB gene mutations and molecular characterization of rifampin-resistant Mycobacterium tuberculosis isolate from Shandong Province, China. Journal of Clinical Microbiology. 44(9):3409-3412.
16. Ogboi S. J., Idris S. H, Olayinka A. T. and Ilyas Junaid. (2010). Sociodemographic characteristics of patients presenting pulmonary tuberculosis in a primary health centre, Zaria, Nigeria. Journal of Medical Laboratory and Diagnosis. 1(2):11-14, ISSN 2141-2618.
17. Okonkwo RC, Onwunzo MC, Chukwuka CP, Ele PU, Anyabolu AE, Onwurah CA, Ifeanyichukwu MO, Akujobi CN, Enemuo E and Ochei KC. (2017). The Use of the Gene Xpert Mycobacterium tuberculosis/Rifampicin (MTB/Rif) Assay in Detection of Multi-Drug Resistant Tuberculosis (MDRTB) in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria. Journal of HIV and Retro Virus. 23(1):1-5
18. Onyedum CC, Aloub I, Ukwaja KN. (2017). Prevalence of drug-resistant tuberculosis in Nigeria: A systematic review and meta-analysis. PLoS ONE. 12(7): 1-17.
19. Oshi DC, Oshi SN, Aloub I, and Ukwaja KN. (2014). Profile, Outcomes, and Determinants of Unsuccessful Tuberculosis Treatment Outcomes among HIV-Infected Tuberculosis Patients in a Nigerian State. Tuberculosis Research and Treatment. 2014.ID 202983:1-8.
20. Ukwamedua H, Omote V, Etaghene J, Oseji ME, Agwai IC, and Agbroko H. (2019). Rifampicin resistance among notified pulmonary tuberculosis (PTB) cases in South-Southern, Nigeria. Heliyon. 58(6):161-166.
21. World Health Organisation (2018). Global Antimicrobial Resistance Surveillance System (GLASS) Report. Geneva, World Health Organization 2016-2017; 2018. ISBN 978-92-4-151344-9.
22. World Health Organization. (2016). WHO treatment guidelines for drug-resistant tuberculosis. World Health Organisation. 2016. ISBN 978 92 4 154963 9.
23. Xiao-li Yu, Zi-li W, Gao-zhan C, Rui L, Bing-bing D, Yu-feng Y, Yao Li, Hai Wu, Xiao-kui G, Hong-hai W, and Shu-lin Z. (2014). Molecular characterization of multidrug-resistant Mycobacterium tuberculosis isolated from South-central in China. Journal of Antibiotics. 67(4):291-7.