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Molecular Epidemiology of *Mycobacterium tuberculosis* among Pulmonary Tuberculosis Patients in Ilorin, Nigeria

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Abstract

Background: Tuberculosis (TB) is an infectious disease and it is a public health concern due to the endemic nature of the disease. Nigeria's health systems still rely heavily on out-dated tuberculosis diagnostic tests; including sputum smear microscopy and chest radiography. This necessitated the urgent need to explore a more reliable, effective method that can rapidly enhance simultaneous detection of *Mycobacterium tuberculosis* (MTB) and rifampicin (RIF) resistance. **Aims**: We evaluated the prevalence of MTB and rifampicin resistance and the influence of socio-demographic factors. **Methods:** A longitudinal, descriptive study that employs the Xpert MTB/RIF automated molecular method for rapid detection of rifampicin resistance. Secondary data were collected from the medical record from March 2017 to December 2018. Primary data were collected by direct clinical observations and analysis with gene expert machine from January 2019-March 2019. **Results:** Study populations were 1893 subjects pooled as secondary data at the Gene Expert laboratory, with a mean age of 44.92 years and were categorized based on sex, 1125 (59.4%) males and 768 (40.6%) females respectively. The MTB detected were 117 (15.7%) for males while 76 (9.9%) were positive for females. 948 (84.3%) were negative for males and 692 (90.1%) negative for females. Also, the categories for age were grouped into three but the older age groups (29 - 54) and (55 - 80) were more vulnerable to MTB having a $p = 0.506$ which is statistically non-significant; only 14 (5.5%) have RIF while 239 (94.5%) do not have RIF resistance strains. Logistic regression analysis of MTB detected showed a significant association ($p < 0.05$) for sex and a non-significant association ($p > 0.05$) for age. The

prevalence rate for RIF Resistance is 5.53%, there is a statistical difference p = 0.001 between RIF Resistance and MTB in Ilorin. **Conclusion:** The utilization of validated molecular technique for MTB strain identification has shown stronger epidemiological impetus for an improved care management outcome specifically in resources limited areas.

Keywords

Mycobacterial tuberculosis, Gene Expert, Epidemiology, Care Outcomes, Pulmonary Tuberculosis

1. Introduction

Tuberculosis (TB) is one of the leading infectious diseases in the world and is responsible for more than 2 million deaths and 8 million new cases annually [1]. The disease is caused by a bacterium called *Mycobacterium tuberculosis*. Nigeria ranked fourth behind India, Indonesia and China as one of the six countries which accounted for 60 percent of the total TB burden. Some 1.8 million people died from TB in 2015, of whom 0.4 million were co-infected with HIV. The report urged the affected countries to "move much faster" to prevent, detect, and treat the disease if they are to meet global targets [2].

The bacteria usually attack the lungs, but can infect any part of the body such as the kidney, intestine, pleura, spine, and brain. If not treated properly, this infectious disease can be fatal. The most important control strategy for TB is the early detection and the appropriate treatment of infectious cases. However, globally the case detection rate (CDR) of TB has been estimated in only 64%, which means that about 36% of the incident TB cases remain undetected (WHO, 2015). For instance, America has about 79% of undetected and unreported of TB annually (WHO, 2015). In Nigeria, Lagos, Kano, and Oyo have the highest TB prevalence rate. Other states experienced a drop in cases notified, resulting in a 4% overall decline in 2010. Oyo increased by 46.5% from 2008-2010 [3]. Even since the discovery of *Mycobacteria tuberculosis* in 1882 by Sir Robert Koch, diagnosis of TB still remains elusive. For instance, diagnosis of TB based on observation of signs and symptoms like cough, fever, haemoptysis, weight loss, night sweat and abnormalities in lung particularly in chronic infection is not specific and may vary from total absence to gross abnormalities depending on the site of disease, severity of disease and associated complications. Similarly, other methods like Chest X-ray and other radiological imaging techniques may be suggestive of TB but are non-specific, and have unacceptably high inter and intrapersonal variation even among radiologist. Positive tuberculin test suggests infection not disease, and may be negative in active TB [4]. The laboratories play a central role in TB diagnosis and therefore the strengthening of the laboratory capacity and performance should be a priority for TB control as the present detection method largely depends on smear microscopy [4].

The best method of diagnosing an infectious disease is to demonstrate the causative organism in representative samples of tissue or fluid by either staining and or by culture. Sputum microscopy is accepted world-wide as the first line test as it is rapid, inexpensive and can be done in field condition. But it is less sensitive as it requires at least 104 bacilli per ml of sputum to be positive and it may be falsely positive in many conditions including environmental mycobacterial infection. Culture in solid media is the reference standard but it takes long time (4 - 6 weeks). Rapid culture in liquid media (BACTEC-MGIT) takes at least 10 - 14 days [5].

However, because of an urgent necessity for tests that can quickly diagnose TB, This study takes advantage of high sensitive and specific molecular method to demonstrate remnants of the TB bacilli in representative tissue/fluid samples in other to determine the true epidemiology of TB in Ilorin, Nigeria.

2. Materials and Methods

2.1. Ethical Consideration

Ethical consideration was obtained from the Kwara State University, Malete. Through the Head of Department of Public Health and General Hospital, Ilorin Kwara State ethical committee. Permission to conduct the study was sought from the Medical Laboratory Department. In addition, a written informed consent was obtained from the State Co-ordinator and focal person for National Tuberculosis and Leprosy Control Programme and all subjects of confidentiality was highly upheld throughout the study period till date.

2.2. Study Design

Cross-sectional descriptive study that employ quantitative laboratory based method of data collection. Primary data were collected in person by direct observation and analysis with gene expert machine from January 2019-March 2019 while Secondary data were collected from the medical record available since inception from March 2017 to December 2018.

2.3. Study Area

The study was conducted among patients presented for routine diagnosis at the General Hospital in Ilorin located in Ilorin West Local Government Area of Kwara State, Nigeria. Ilorin City is the traditional emirate and capital of Kwara State Western Nigeria. It also serves as a major trade centre between the Northern and Southern region of the country.

2.4. Samples and Subjects

A Convenience sampling technique was used in this study. In order to avoid bias, patients were chosen based on availability, proximity and presence of symptoms. A total of 1893 Clinical cases of suspected MTB patients (1477 secondary re-

spondents and 416 Primary respondents) and reported cases to General Hospital in Ilorin, Kwara State, Nigeria were included in this study.

2.5. Laboratory Investigation

Specimen Collection and Processing

Sputum samples were collected from patient in a clean and sterile specimen cup. Sputum liquefaction and inactivation with 2:1 sample reagent (buffer) 1.5 ml sample reagent to 0.5 ml deposit (sputum) was done, allowed to stand for 10 minutes. A repeated Shaking for 10 minutes was done, followed by additional shaking for 5 minutes. 2 ml of the sample was transferred to the cartridge aseptically. The cartridge was then placed inside the gene expert machine after the ban codes of each sample have been scanned together with entering of the necessary information into the Xpert machine. The Xpert machine was then allowed to run for 1 hour 45 minutes before the final reading of results.

3. Data Analysis

Data collection, collation and analysis were done using statistical package R. The descriptive statistics in the form of percentages and frequencies were computed to describe the data. Chi-square test was used to access the statistical significance of TB recovery rate between male and female with p-value of $p < 0.05$ were considered statistically significant.

4. Results

The overall prevalence of MTB in Ilorin is 13.4% out of the 1893 patients with a yearly incidence of 83 (15.8%) in 2017, 99 (10.4%) in 2018 and 71 (17.1%) in 2019 (Table 1).

The prevalence of MTB is higher in male 177 (15.7%) than in their female counterpart 76 (9.9%) with a statistical significant difference of $p = 0.001$ (Table 2).

The Age distribution of MTB in Ilorin shows a high prevalence of 14.3% (107) in age group 55 - 85 years with no statistical significance ($p = 0.346$) between age and MTB in the study (Table 3).

The prevalence of Rifampicin Resistant MTB positive patients in Ilorin is 5.5% (14) out of 253 with a statistical significant difference of $p = 0.001$ (Table 4) while the remaining 239 (94.5%) rifampicin resistant traits are not detected.

Table 1. Prevalence of MTB in ilorin.

Duration (Years)	MTB Detected	(%)	MTB Not Detected	(%)	Total	(%)
2017	83	15.8	443	84.2	526	(100%)
2018	99	10.4	852	89.6	951	(100%)
2019	71	17.1	345	82.9	416	(100%)
Total	**253**	**13.4**	**1640**	**86.4**	**1893**	

Source: Author (2020).

Table 2. Gender distribution of MTB in ilorin.

Gender	MTB Detected	(%)	MTB Not Detected	(%)	Total	p-value
Male	177	15.7	948	84.3	1125	0.001*
Female	76	9.9	692	90.1	768	
Total	**253**	**13.4**	**1640**	**86.6**	**1893**	

Source: Author (2020).

Table 3. Age distribution of MTB in ilorin.

Age Range	MTB Detected	(%)	MTB Not Detected	(%)	Total	p-value
3 - 28	54	12.8	367	87.2	421	0.346*
29 - 54	92	12.8	626	87.2	718	
55 - 85	107	14.3	647	85.8	754	
Total	**253**	**13.4**	**1640**	**86.4**	**1893**	

Source: Author (2020).

Table 4. Prevalence of rifampicin resistant detected among MTB positive patients in ilorin.

MTB Positive	MTB Rif. Resistant	(%)	MTB Negative	(%)	Total	p-value
Rif. Resistant Detected	14	5.5	948	84.3	1125	0.001*
Rif. Resistant Not Detected	239	94.5	692	90.1	768	
Total	**253**	**13.4**	**1640**	**86.6**	**1893**	

Source: Author (2020).

5. Discussion

This study reported cases of MTB in General Hospital Ilorin between March 2017 and March 2019 using molecular method (Table 1). We reported 253 (13.4%) cases of MTB in Ilorin between March 2017 and March 2019, this agrees with the work carried out by [6] on the review of the NTBLCP of Nigeria. The reason for the low percentage of positive patients to MTB despite presenting with two or more weeks of cough compare to the negative population suggest that two weeks of cough alone is not enough sign to suspect MTB, other signs and symptoms needed to be look into and taken serious too for example, haemoptysis, chest pain, and fever.

The MTB appeared to be more prevalent in Male in General Hospital Ilorin as its accounts for 1125 (59.4%) compared to the Females counterpart with prevalence rate of 768 (40.6%) (Table 2). This is consistent agrees with WHO report in 2012 that the prevalence of TB is more common among men than women.

There was statistical significant difference between gender and MTB from our findings This is substantiated by Kaulagekar that the inability of women in reaching health facilities because of their positions' in the household, economic dependence, and illiteracy thereby decrease the total number of TB cases notified [7].

Also, the prevalence of MTB appears to be highest within 55 - 80 years age bracket compared to age group 3 - 28 and 29 - 54 respectively, its accounts for 754 (39.8%) contrary to 421 (22.2%) and 718 (37.9%) recorded in age group 3 - 28 and 29 - 54 respectively (**Table 3**). This show that older age is most affected with MTB. A finding that is consistent with the study done by Schaaf [8] and similar report from 2010 by WHO [8] that 57% of all tuberculosis deaths globally occurred among people older than 50 years with more than half these deaths in those aged 65 years and above. However, there was no significant difference between age and MTB in Ilorin.

The prevalence of rifampicin resistance TB obtained in this present study was 14 (5.5%) (**Table 4**). This is close to 3.7% of new cases of multidrug resistant tuberculosis in Nigeria [9]. Our work found a statistical significant difference between Rifampicin resistance and the prevalence of MTB in Ilorin The slight difference in the prevalence is consistent with 2018 global TB report [10] that documents recent decrease in the prevalence of MDR-TB. In addition, studies from different geographic zones have showed varied prevalence of RIF resistance which all slightly differ from the prevalence obtained from this present study as reported by [11] [12]. Also, a study done in Nnewi, South East Nigeria shared a similar view with this study, although the general MDR-TB from their study was lesser than 7.7% which is close in range to that obtained in this study (5.53%). The low RIF resistance as reported by this study, and previous study mentioned above has a crucial implication for both national and global TB multidrug resistant diagnosis, because RIF incorporation in molecular technique is a vital indicator, for molecular detection of MDR-TB as seen in the Gene Xpert technology [13]. Multidrug resistant TB like the one reported in this study could be acquired as a result of wrong prescription, irregular supply of drugs, treatment noncompliance, lack of supervision and poor adherence to medical advice [14].

Similarly, the burden of MTB detection among gender between March 2017-March 2019 reveals high burden of TB among Male. Out of the 253 cases that were positive to MTB, Female account for 76 (30.04%) while Male account for 177 (69.96%). Globally, approximately 70% more males are notified of smear-positive TB tests than females [15]. According to World Health Organization, 2010, the prevalence of TB is more common among men than women [15]. The reason for this was stated in the work of [16] it was found that female patients are mostly illiterate and live in poor socio-economic conditions. Another study conducted in 2017 was also in agreement that females are often unable to reach health facilities because a woman's position in the household, economic dependence, and illiteracy would be restricting factors [6].

Furthermore, Age group 55 - 80 appears to have high burden of MTB; Out of 253 of different age groups that are positive to MTB. The reason for the prevalence of MTB signs and symptom in older population as revealed in a study that most of the TB patients seeking treatment belonged to 21 - 35 years of age group, compare to the older age group possibly due to the level of education and awareness on MTB in older people [17]. A similar result was found in Ibadan, Nigeria where a study conducted among pulmonary patients revealed more than 80% of the patients recognized coughing as major symptoms of TB and belong to the older age group [18].

6. Limitation of the Study

We were limited by inability to do a follow up for MTB and Rifampicin resistant patients. Also among their relatives in order to know if they are healthy carriers due to time constraint. Similarly, this study was unable to document other anti-tuberculosis resistance patterns due to lack of laboratory capacity and paucity of fund.

7. Conclusion

The study aimed at assessing the prevalence of MTB burden and its association with demographic factors and rifampicin resistance using the Gene Xpert. The prevalence of MTB, RIF resistance and MTB burden as obtained in this study have shown that more males in this population report at the health facility to be diagnosed for MTB compare to females, this may be due to the level of poverty and lack of awareness about the free gene expert diagnostic machine for MTB. Also, the older age group in this population of study shows high detection in MTB, they present with clinical symptoms and signs than the younger age in this study population which maybe as a result of old age diseases. The MDR-TB present in the study population is similar with the trend in other parts of Nigeria. This may be due to wrong prescriptions, irregular supply of drugs, treatment non-compliance, lack of supervision and poor adherence to medical advice. Therefore, there is need to scale up the much-needed technical support in this direction of study cannot be overemphasised, as the presence of the robust and more effective diagnostic tools are not readily available in our remote communities with large population of the vulnerable groups, who cannot potentially afford effective health care delivery services in the real sense of it.

Recommendations

Government needs to enhance TB (especially the drug resistant strain) diagnosis and management system in the country. Notably, other molecular diagnostic techniques which involve resistant to other drugs apart from RIF should be made available; as only few were resistant to RIF which was the only method used in this study, there could probably be other forms of resistance strains which could have been missed out, due to unavailability of other forms of diagnostic device.

The National Tuberculosis and Leprosy Control Programme (NTBLCP) in Nigeria should be well funded and empowered to reach to the rural arrears and women alike not minding the level of education or social status. Also to address low case detection of MTB, the government should ensure universal access to treatment and services, deployment and expansion of new technologies in diagnosing MTB, active case detection targeting high burden areas and high risk groups and to strengthen routine surveillance to include all points of contacts between patients and health care services.

There should be community mobilization for participation, ownership and sustainability; and advocacy at all levels of government and community for increased political commitment and resources toward the control and eventual eradication of TB.

Individual that has been seeing signs and symptoms of Tuberculosis (haven present with cough for two weeks or more, should quickly report at the nearest diagnostic centre for proper diagnosis and so that immediate treatment would begin.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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