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Investigation of Rifampicin Resistance Outcome among Tuberculosis Patients Visiting Two Major Health Facilities in Port Harcourt in Niger Delta

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ABSTRACT

Background: *Tuberculosis is caused by Mycobacterium tuberculosis and is spread mainly through contact with air droplets and respiratory fluid from an infected person. Drug of choice for its treatment are rifampicin and isoniazid respectively. However, in recent times, resistance to these drugs as with other antibiotics has been observed across the globe. This study was thus aimed at determining the prevalence of rifampicin resistance strain among TB patients attending two major hospitals (Braithwaite Memorial Hospital and University of Port Harcourt Teaching Hospital) in Port Harcourt.* ***Method:*** *Sputum of patients was collected, tested for the presence of TB and rifampicin resistance of the isolates were determined using GeneXpert assay approach.* ***Result:*** *A total of 158 isolates from patients who were TB positive were tested for rifampicin resistance. 13.3% of these were resistant to rifampicin drug. 8.9% was from UPTH while 4.4% was from BMSH. 9.5% were less than age 45 while 3.8% were greater than or equal to age 45 (≥45). Males made up 8.2% of the total prevalence while females made up 5.1% of the total prevalence. Alternative drugs to rifampicin and isoniazid drug should however, be prescribed in confirmed cases of resistance outcome in our health facilities especially in the rural communities.* ***Conclusion:*** *Nonetheless, the use of antibiotics indiscriminately without the effective laboratory assay and physician's prescription should be discouraged at all levels and patients receiving treatment should be monitored to adhere strictly to the desired dosage as non-adhering to the gold standard option may lead to drug resistance outcome among vulnerable patients who are literally exposed to lack of health education and functional health facilities in the remote communities which are also hard to reach area due to the environmental terrine.*

Keywords: Rifampicin, Tuberculosis, Resistance strain, Public health, Port Harcourt, Niger Delta, Health facility

INTRODUCTION

Worldwide, tuberculosis has been seen and also regarded as a critical Public Health concern as the World Health Organization estimates that in 2012, there were 8.6 million cases of TB and 320, 000 deaths resulting from these infections which are also a communicable disease [1]. The Sub-Saharan region of Africa of which Nigeria belongs has a huge presence of this disease with Nigeria being ranked 13th in the global scale of tuberculosis [2]. The issue is however not in its presence but in getting a drug(s) which can combat it; this however has been slowed down by antibiotic resistance strain emergence.

Drug resistance has been on the increase in recent times, including that of antibiotic resistance. Rifampicin however, is an antibiotic used in the treatment against Mycobacterium tuberculosis but of recent, resistance of the bacterium to this drug has been observed. It is a rifamycin derivative which was first used in 1972 [3].

Multidrug-resistant tuberculosis has been observed as some TB are not only resistant to rifampicin but also to isoniazid [4]. 82.5% of rifampicin-resistant isolates were also found to be resistant to isoniazid in the United Kingdom [5]. Adequate treatment of such patients is aided by the quick identification of MDRTB and subsequently increased survival as well as minimizing its spread [6,7]. According to the Centres for Disease Control and Prevention, within

21 to 30 days of receiving a specimen, culture/identification as well as antibiotic susceptibility testing of the *M. tuberculosis* Complex (MTBC) should be completed respectively [8].

Line probe assay (LiPA) and GeneXpert are two molecular methods of determining rifampicin resistance. GeneXpert is however a molecular assay used not only in the detection of *M. tuberculosis* but also rifampicin resistance. GeneXpert makes use of real time Polymerase Chain Reaction in the detection of rifampicin resistance and *M. tuberculosis* specific sequence [9]. In rifampicin resistance, a missense mutation occurs in the rifampicin resistance-determining region (RRDR) of the rpoB gene; also, a mutation in 81 bp region of the rpoB gene is harbored by 95% of these resistant strains 5. According to evidence-based reports, the resistance to one or more anti-TB drugs has a prevalence of 3% to 37.3% in Africa [10-13]. A major challenge is the spread of multi-drug resistant tuberculosis (MDR-TB) in many third world countries [14].

Due to occurrences of drug resistance, the frequent prescription of rifampicin as the drug of choice and the fight to eradicate or reduce the incidences of TB has received a hugged setback. In Niger Delta region of Nigeria, there is paucity of data with respect to identification of rifampicin strain using the current trend of molecular technique as majority of studies conducted leveraged on the conventional evidence-based finding method with the application and use of AAFB slide staining mechanism, which has been linked to massive limitation especially in uncovering the rifampicin drug resistance strain in the sample in question. Nonetheless, this study was carried out to determine the prevalence of rifampicin resistance strain among TB patients visiting the University of Port Harcourt Teaching Hospital and the Braithwaite Memorial Specialist Hospital. It is strongly believed that the outcome of this study will not only provide epidemiological information of the current trend of the strain in our communities, but also, it will serve as a health survey approach that would underpin the need for more investments to be made in the area of robust health care facility that is well equipped with current state of the art infrastructure, that will potentially improve health care outcome through fast and accurate diagnostic technique.

METHODOLOGY

Study Area

Subjects for this study were drawn from the University of Port Harcourt Teaching Hospital and Braithwaite Memorial Specialist Hospital; both located within Port Harcourt City. Braithwaite Memorial Hospital (BMSH) is a state-owned hospital managed by the Rivers State Hospitals Management Board; named after Eldred Curwen Braithwaite, a British doctor and pioneer surgeon in Rivers State. Its location is the old Government Residential Area (GRA), 12.9416º N and 77.5669º E. Hospital was established in 1925 as Braithwaite Memorial Hospital and originally served as a medical facility for senior civil servants. It has about 375 licensed beds and 731 medical staff.

The University of Port Harcourt Teaching Hospital (UPTH) is located at East West Road, Port Harcourt, 4.8996N and 6.9286E. It is a major tertiary-care teaching and research facility in Rivers State and the Niger Delta Region that commenced operations in 1980 but officially commissioned in 1985. Currently, it has about 500 beds respectively.

Study Population

It is very critical to state that a total number of 285 subjects from UPTH and 271 subjects from BMSH were involved in this study respectively.

Both Hospitals are located within Port Harcourt, the capital city of Rivers State in the oil rich Niger Delta Region of Nigeria. According to the National Population Commission, the State had a population of 11 million as December 2016 (National Population Commission, 2016). Persons living in the State mainly engage in fishing, farming, trading and oil and oil exploration activities.

Subjects used in this study were those visiting both hospitals within the study period. The test population was those suspected with having pulmonary tuberculosis while non-TB subjects were the control subjects.

Ethical Clearance and Subject Consent

Ethical clearance was obtained from UPTH and Ministry of Health, Rivers State after due process. Informed consent was equally obtained from each participant after introducing the importance of the research and counseling. The confidentiality of the result was ensured using unique code number confidentiality. The results of laboratory findings

were provided to the study participants and those in need of medical attention were directed to respective physicians and laboratory personnel for further medical advice and support.

Inclusion Criteria

The study was conducted from January to September 2016 and all subjects regardless of their age and sex who were willing to participate were included in the study. Also, those who visited the Tb clinic for the first time who has been coughing for a period of one week and more that defiles other forms of antibiotic treatments, were included.

Exclusion Criteria

However, patients who were receiving TB treatment were excluded from the study. Also, those who refused to participate by not given their consent were excluded from the study. All collected sputum samples were analyzed for Mycobacterium tuberculosis and screened for its sensitivity to rifampicin using GeneXpert Assay.

Sample Collection

Expectorated sputum samples were collected on the same day of patients' enrollment. The minimum acceptable volume of sputum was 2 ml. Samples were assayed using the Xpert assay technique respectively.

Laboratory identification numbers, date, sex, were given and recorded. The sputa were collected in a sterile container (falcon tube) which was placed on an ice pack in a cool box, during sample transportation to the laboratory. All laboratory tests were performed by well-trained laboratory personnel. Standard operational procedures of the host laboratory were used to ensure the reliability and validity of test results.

Detection of *M. tuberculosis* and rifampicin resistance by GeneXpert Assay

The GeneXpert assay consists of a single use multi-chambered plastic cartridge preloaded with the liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction, and heminested real-time PCR. Sputum samples were treated with NaOH and isopropanol-containing sample reagent (SR). The SR was added at a 2:1 ratio to the sputum sample and incubated for 15 minutes at room temperature. The treated sample was transferred into the cartridge, and the cartridge loaded into the GeneXpert instrument, and an automatic process completed the remaining assay steps. The assay cartridge also contains lyophilized *Bacillus globigii* spores which served as an internal sample processing and PCR control. The spores were automatically re-suspended and processed during the sample processing step, and the resulting *B. globigii* DNA was amplified during the PCR step. The standard user interface indicated the presence or absence of M. tuberculosis, the presence or absence of RIF's resistance, and a semi quantitative estimate of M. tuberculosis concentration (high, medium, low, and very low). Assays that were negative for *M. tuberculosis* were reported as invalid. The results also stated whether resistance to Rifampicin was detected, not detected or indeterminate.

Statistical Analysis

Data from the results obtained were entered and represented using charts on Microsoft Excel spreadsheet. The data were represented in bar charts as shown in the result section.

RESULTS

This study was carried out to determine the prevalence of rifampicin resistance strain among TB patients attending the Braithwaite Memorial Specialist Hospital (BMSH) and the University of Port Harcourt Teaching Hospital (UPTH) respectively. The study recorded a total prevalence of 13.3% out of the 158 TB patients. Among these, 75 isolates were from the UPTH while 83 were from the BMSH. UPTH recorded 18.7% prevalence while BMSH recorded an 8.4% prevalence. Of the total prevalence, UPTH and BMSH recorded 8.9% and 4.4% respectively. 9.5% were less than age 45 while 3.8% were greater than or equal to age 45 (≥45). Males made up 8.2% of the total prevalence while females made up 5.1% of the total prevalence. The results are represented with the following charts based on percentage of each series (Figures 1-3).

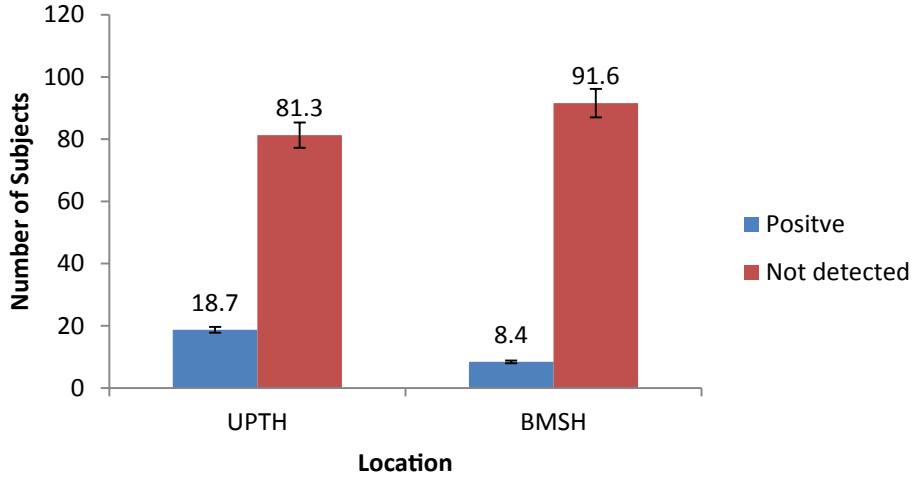

Figure 1 Rifampicin resistance based on location

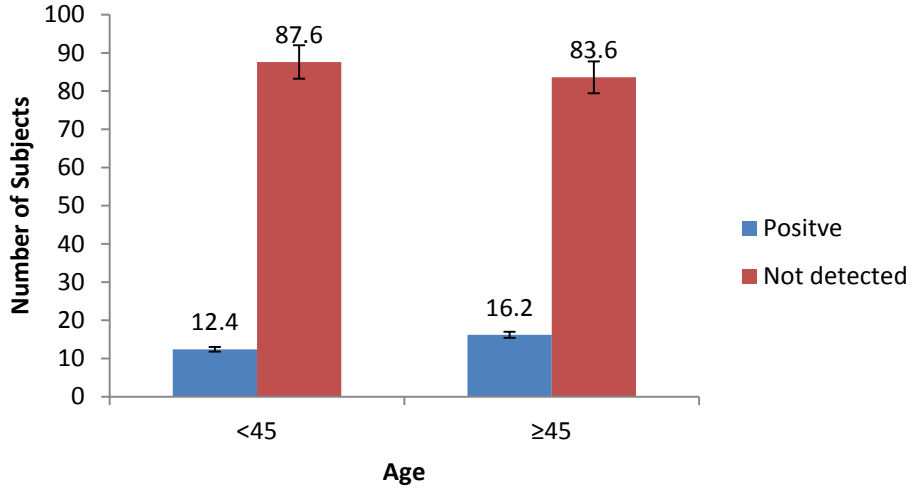

Figure 2 Rifampicin resistance based on age

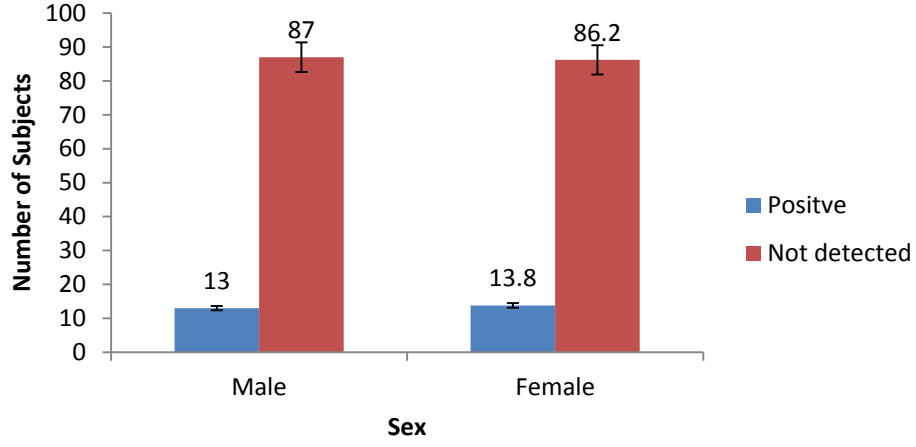

Figure 3 Rifampicin resistance based on sex

DISCUSSION

The use of GeneXpert in the detection of rifampicin resistance strain among TB patients has proved useful over time. In recent times, molecular biology has been very effective and fast in the detection of microorganisms as well as drug resistance outcome. Nevertheless, this is not to say that the conventional AFB slide method is useless. However, the ease with which molecular genetics acts, makes it a preferable choice as its sensitivity and specificity is always strongly underpinned through the application of molecular approach. This study recorded 13.3% rifampicin resistance out of the 158 subjects who were TB positive. This is higher than that recorded by Masenga, et al. [15] in Zambia where they recorded a prevalence of 5.9% among the 152 cases of TB as well as another conducted in Ghana [9].

Based on this study, females had a slightly higher occurrence of rifampicin resistance (13.8%) than males (13%). This similarity in percentage occurrence in the two genders was also recorded by Masenga, et al. [15] and in a study carried out in India [16]. This is also in consonance with the study carried out by Azuonwu, et al. [17] who also recorded a slightly higher prevalence in females compared with males. This obviously indicates that the risk of developing rifampicin resistant between the two genders is the same. As noted by Masenga, et al. [15], this may be due to the assumption that both sexes are equally exposed to the same predisposing factors that could lead to rifampicin resistance which include HIV and adherence. However, the larger number of males who were tested for rifampicin resistance (100) than females (58) should have invariably produced a corresponding higher prevalence among the males, but this was not so. This could be due to the notion that males are often more diagnosed of TB than females [15]. The reason for this is not very clear per se, but however, this could probably be linked to the fact that the males may have more and frequent chances of been in contact with massive risk factors, like sharing of a stick of cigarette among chain smokers, thus such risky practice may enhance the sporadic spread of the scourge among men.

In this study, the UPTH recorded a higher percentage occurrence (18.7%) than the BMSH (8.4%). It should also be known here that the number of subjects in the UPTH (75) was more than that of BMSH (83). Also, the UPTH is a bigger hospital compared with the BMSH and usually records a lot more patients attending than the latter. This increased population may have been exposed to a predisposing factor as one of the factors promoting the spread of TB is proximity to an infected person which he could easily be infected through the inhalation of aerosol from the infected subject. Nonetheless, the chances of developing more susceptibility could be seen among the Immune compromised group like in case of the HIV/AIDS patients.

According to this study, subjects whose ages were greater than or equal to 45 (≥45) recorded a lower percentage resistance (9.5%) than those who were less than 45 (3.8%). This may be attributed to the number of subjects who were less than 45 (121) being more than those who were less than 45 (37). This however, is in agreement with the study by Azuonwu, et al. [17] who recorded increased rifampicin resistance prevalence among TB patients who were less than 40 than those who were more 40 years of age in Bayelsa State of Nigeria.

The issue of drug resistance and especially multidrug resistance thrives due to non-compliance with treatment instruction, incorrect prescription, lack of adherence to physician's advice, inconsistent drug supply and lack of supervision [18]. Unhealthy behaviors such as substance abuse as well as alcoholism have been implicated in multidrug resistant TB [19]. Uzoewulu, et al. [20] has however suggested that multidrug resistant among TB patients may have resulted from previous treatment against TB. Other factors including poverty, lack of effective health care delivery system (especially in Nigeria), lack of or insufficient dedicated health care personnel and irregular and unimplemented government policies also promote multidrug resistance among TB patients [20,21]. These are further strongly underpinned by lack of health education amongst the already vulnerable subjects in our rural communities, even as they are massively attached to increasing passion of patronizing patent medicine shops and herbal native doctors to seek for cure of their cough against the option of medical treatable health conditions [22].

CONCLUSION AND RECOMMENDATIONS

This study carried out in a Federal Government owned (UPTH) and State Government owned (MBSH) hospital recorded a prevalence of 13.3% rifampicin resistance among TB patients visiting both hospitals. It also showed that persons whose ages were from 40 and above had a higher prevalence than those who were less than 40 years of age. Similarly, females recorded a slightly higher prevalence of rifampicin resistance than their male counterparts. This, however small it may seem, should not be neglected as these resistant strains can spread to other patients either within or outside the health facility.

Since antibiotic resistance can be transferred from one microorganism to another (possibly through plasmids) especially in hospital environments, it is important that patients adhere to their physicians' prescription by taking their medication at the right time, completing the dosage and taking the right dose at each point in time. In addition, awareness on the dangers of antibiotic resistance (not just rifampicin resistance) should be carried out by the relevant Government and Non-Government Agencies in the hospitals, churches, markets, and public gatherings.

DECLARATIONS

Conflict of Interest

The authors have disclosed no potential conflicts of interest, financial or otherwise.

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