Original Research Article

The significance of Age, Sex and Location in tuberculosis infection, using molecular technique to determine *Mycobacterium tuberculosis* infection among tuberculosis Patients in Bayelsa State, Nigeria.

ABSTRACT

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Introduction: Tuberculosis is a disease condition caused by *Mycobacterium tuberculosis*. Several predisposing factors such as Age, Sex and overcrowding may have affected the prevalence of tuberculosis in various locations including Bayelsa State and other parts of the world.

Aim: The aim of this study is to know if Age, Sex and Location determines tuberculosis rate of infection, using molecular technique to isolate *Mycobacterium tuberculosis* among tuberculosis patients in Bayelsa State, Nigeria.

Methodology: We analyzed a total of 102 sputum samples, which were collected from each of the participants. GeneXpert molecular method was used to identify *Mycobacterium tuberculosis* and Rifampicin resistant cases. The data and results obtained are statistically analyzed with Statistical Package for Social Science (SPSS) version 21.

Result: Out of the 102 sputum samples screened, 91(89.2%) were confirmed to have tuberculosis, Tuberculosis among male and female were 54(52.9%) and 37(36.3%) respectively, while rifampicin resistance among male and female were 3(2.9%) and 5(4.9%) respectively. Tuberculosis infection among male and female were not statistically significant ($p = .75$). The age intervals are 11-20, 21-31, 31-40, 41-50, 51-60, 61-70 and 71-80. 31-40 years had the highest tuberculosis prevalence of 30(29.4). The various locations were Brass, Ekeremor, Kolokuma/Opokuma, Nembe, Ogbia, Southern Ijaw and Sagbama Local Governmemt Areas. People residing in Yenagoa local Government had more positive case of both rifampicin resistant 5(4.9%) and non-rifampicin resistant tuberculosis infection.

Conclusion: Tuberculosis infection is more predominant among male in Bayelsa State than female but rifampicin resistance species are more predominant among females. The age group interval of 40-41 had the highest prevalence of tuberculosis infection while the highest location of tuberculosis infection is Yenagoa.

Keywords: Age, Sex, Location, Mycobacterium tuberculosis.

1. INTRODUCTION

Tuberculosis is a disease condition caused by *Mycobacterium tuberculosis*. Tuberculosis had been around for long, attracting global attention especially in developing countries. Globally tuberculosis is still among the leading cause of death especially in developing countries. Nigeria is among the 10 most tuberculosis bounding countries alongside India and Pakistan [1]. Pulmonary tuberculosis is commonly transmitted whenever an infected individual, sneezes, laughs, speaks or spits. The bacilli is expelled in aerosols and inhaled by other persons exposed in that environment. One third of the world's population have latent tuberculosis, this means they have tuberculosis but are not ill, and do not also have the ability to transmit the disease [2].

The overall global burden of TB remains alarmingly high, with about 10.4 million incident cases and 1.5 million deaths reported by the WHO in 2015 [3]. *Mycobacterium tuberculosis* (MTB) strains showed which display an in vitro resistance to isoniazid and rifampicin accounted for 480,000 incident cases and 250,000 deaths in 2015 according to WHO [3].

Multiple drug resistant tuberculosis (MDR-TB) strains display additional resistant to the fluoroquinolones and second-line injectable agents, and have been reported to cause disease in about 106 countries including Nigeria. Extensive Drug Resistant Tuberculosis (XDR-TB) poses a dire threat to public health, exacerbated by its deadly interaction with the HIV/AIDS epidemic [4].

Additional resistance beyond XDR has been described as totally drug-resistant TB, which displays further resistance to drugs used to treat XDR-TB, resulting in programmatically untreatable forms of TB. Published studies suggest that current treatment options for XDR-TB fail to cure 30%–75% of patients with XDR-TB. This situation is an emerging public health crisis. New drugs such as bedaquiline and delamanid, and repurposed drugs such as linezolid, have been introduced into drug-resistant TB (DR-TB) treatment regimens. Despite the availability of new drugs, limited access to them had contributed to ongoing poor outcomes in DR-TB, including treatment failure and mortality [5].

The management of DR-TB is further compounded by the high cost, long duration and debilitating toxicity of current available second-line drugs. Current treatment guidelines indicate standardized fixed dose 6 month regimens for new treatment and re-treatment of drug-susceptible TB (DS-TB). In the case of DR-TB, the conventional 18–24 month treatment regimen has been redesigned and now ranges between 9 and 24 months based on individual patient eligibility such as previous TB history and drug exposure. In addition to duration, complex multidrug regimens and optimal medication adherence are required for effective treatment of TB. Challenges of adherence are linked to complex dosing strategies, serious and often life-threatening drug side effects, and drug–drug interactions [6][7]. The aim of this study is discover if Age, Sex and Location affects the prevalence of tuberculosis infection in Bayelsa State, Nigeria.

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2. METHODOLOGY

Ethical approval was gotten from Bayelsa State ministry of health, Yenagoa. Questionnaire and consent form was issued to each of the participants. This research was carried out from

November 2020 to December 2021. A total of 102 Sputum samples were collected from patients across all the local Government Areas. Sputum samples collected from a far distance was transported in a cool chain at a temperature range of 2°c to 20°c. Samples were stored in the refrigerator when there was need for storage. GeneXpert machine system is the method used for the identification of MTB and Rifampicin Resistant strains. Each participant produced sputum sample in a wide mouth, screw cap, transparent sputum cup. The GeneXpert System is built with GX 2.1 software / computer, printer and barcode wand-reader and the GeneXpert machine. The machine are always available in a one, two, four or 16-module build up. The one used in this research has four module configuration, with serial number 805757.

The GeneXpert cartridge is a single-use Xpert MTB/RIF, where the genetic extraction, amplification and detection of MTB takes place. It also has a sample reagent of a maximum volume of 8ml which can be used for diluting just one sample. The GeneXpert System also comes with a sterile disposable pipette which is already marked for minimum value of diluted sample to be transferred to the cartridge, each sterile pipette is used for one sample.

The lid of the sputum container was unscrewed and the volume of the sputum was measured with the help of the already graduated screw cap sputum container given to the patient. Using a sterile disposable pipette, the sample reagent at 2:1 (v/v) ratio of the sputum sample was added. The lid of the sputum container was properly replaced tightly and mixed vigorously. The well mixed sample was incubated at room temperature for 10 minutes. It was mixed again and incubated for another 5 minutes.

Using a sterile disposable transfer pipette provided alongside with the GeneXpert kits, 2 ml of the liquefied sample was drawn and transferred into the open port of the GeneXpert Cartridge. The cartridge was then taken to the GeneXpert machine for further processing.

The cartridge were loaded inside the GeneXpert machine for a real time polymerase chain reaction. The automatically generated results were printed and recorded for further data analysis

Statistical Package for Social Science (SPSS) version 21 after collation with Microsoft excel spread sheet. Chi square was used to measure association between two variables. Mann Whitney test and kruskal Wallis were used to compare difference more than two variables respectively. All tests of significance in this analysis was at .05 alpha level.

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3. RESULTS AND DISCUSSION

The age group of 31- 40 years had the high MTB positive cases 30(29.4%) using the GeneXpert technique for MTB detection. Similar researches in various part of Nigeria had shown that subjects within 21-30 and 31-40 years age interval tend to have more positive TB cases than persons within other age groups [8][9][10]. The prevalence of tuberculosis within this age group may be attributed to their active live pattern, Adolescence are the most active and adventurous age group, therefore predisposing them to most air borne infections.

Statistical analysis within the various age interval showed p value =.45(not significant) table 1. The prevalence of TB in Bayelsa State among the tested subjects were 91(89.2%) while the prevalence of rifampicin resistance case were 8(7.8%).

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Table 1: Prevalence of TB in Bayelsa across various age intervals using GeneXpert

Age(yrs)	High	Medium	Low	Total

11 – 20	4(3.9)	2(2.0)	7(6.9)	13(12.8)
21 – 30	7(6.9)	8(7.8)	12(11.8)	27(26.5)
31 – 40	10(9.8)	5(4.9)	15(14.7)	30(29.4)
41 – 50	6(5.9)	2(2.0)	3(2.9)	11(10.8)
51 – 60	2(2.0)	0(0.0)	3(2.9)	5(4.9)
61 – 70	1(1.0)	0(0.0)	2(2.0)	3(2.9)
71 – 80	0(0.0)	1(1.0)	1(1.0)	2(2.0)

Number in parenthesis = percentages. P=.45 (not significant)
High= *Mycobacterium tuberculosis* detected in High quantity.
Medium= *Mycobacterium tuberculosis* detected in moderate quantity.
Low= *Mycobacterium tuberculosis* detected in small quantity.

Out of 102 samples that were examined 91(89.2%) were positive in the GeneXpert technique. Male 54(52.9%) had more positive cases of tuberculosis while females 37(36.3%) had lesser positive result. The high prevalence of MTB detection from this study could be attributed to the research methodology. Patient who were suspected of TB and those who have already been diagnosed of TB were the chosen population in this research. A similar research conducted in Northern part of Nigeria [9] also shows that male were more predisposed to TB infection than female. The distribution of MTB among the sex categories of the researched population is not statistically significant with a (p=.75) using SPSS.
The ability of the GeneXpert molecular method to identify mutation on the rpoB gene which is the receptor for rifampin were a major breakthrough in the fight against drug resistant tuberculosis. In this research it were observed that GeneXpert had 8 MTB strains that had mutation on the rpoB gene (Table 3). The overall prevalence of rifampicin resistance from GeneXpert technique were 8(7.8%). Though males had more TB cases in this research than females the prevalence of rifampicin resistant MTB were more predominant in females than males. Some female prefer the use of carbonated drinks which had been found to reduce drug absorption [11].Absorption of inadequate concentration of antibiotics can lead to drug resistance. The prevalence of rifampicin resistance in females was 5(4.9) while the males was 3(2.9%) had lesser prevalence of rifampicin as shown in table 3. This finding do not agree with a study carried out in another southern state in Nigeria [8] which reported higher prevalence of rifampicin resistance in males than females. Prolong use of drug is one of the reason why MTB were resistant to rifampicin, in most cases females tent to forget the daily intake of their drugs because of cultural responsibilities such as child care, domestic and work.

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Table 2: Prevalence of MTB among male and female in Bayelsa, Nigeria

GeneXpert positive Categories	Male	Female	Total
High	18(17.7)	12(11.8)	30(29.4)
Medium	11(10.8)	7(6.9)	18(17.7)

Low	25(24.5)	18(17.7)	43(42.2)
TOTAL	54(52.9)	37(36.3)	91(89.2)

Number in parenthesis = percentages. P= .75 (not significant)
High= *Mycobacterium tuberculosis* detected in High quantity.
Medium= *Mycobacterium tuberculosis* detected in moderate quantity.
Low= *Mycobacterium tuberculosis* detected in small quantity.

Table 3: Prevalence of drug resistant MTB among male and females

	No. Examined	**rifampicin resistant**
MALE	61(59.8)	3(2.9)
FEMALE	41(40.2)	5(4.9)
TOTAL	102(100)	8(7.8)

Number in parenthesis = percentages. P= .09 (not significant)

The prevalence of MTB infection using GeneXpert technique in all Local Government Areas as shown in table 4. Kolokuma\Opukuma (KOLGA) local government had the lowest TB prevalence cases of 1(1.0%). The highest TB prevalence was recorded in Yenagoa, with a prevalence rate of 48(47.1%). Yenagoa was identified as the local government Area with the highest TB prevalence rate in Bayelsa state, this also agrees with a study carried out by Amala and Goodluck in 2019 [12]. It also had the highest number of participants. The prevalence of rifampicin drug resistance is also recorded at its highest level in Yenagoa, so many cluster settlements like Amarata, swali waterside, and lack of social distancing may have also contributed to a high prevalence rate of DR-TB cases in Yenagoa. The dense populated nature of Yenagoa which is the State capital of Bayelsa State may had also contributed to the high prevalence rate of tuberculosis cases.

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Table 4: Prevalence of MTB by Local Government Area in Bayelsa.

	No. examined	Male	Female	Total
BRASS	4(3.9)	1(1.0)	2(2.0)	3(2.9)
EKEREMOR	8(7.8)	7(6.9)	0(0.0)	7(6.9)
KOLGA	1(1.0)	0(0.0)	1(1.0)	1(1,0)
NEMBE	6(5.9)	4(3.9)	1(1.0)	5(4.9)
OGBIA	9(8.8)	5(4.9)	3(2.9)	7(6.9)
S/IJAW	16(15.7)	7(6.9)	7(6.9)	14(13.7)
SAGBAMA	4(3.9)	3(2.9)	1(1,0)	4(3.9)
YENAGOA	54(52.9)	26(25.5)	22(21.6)	48(47.1)

Number in parenthesis = percentages. (P= .30) not significant

4. CONCLUSION

The prevalence of tuberculosis obtained from this research was high because samples were collected from tuberculosis patients attending chest clinic. The age group from 40-41 years had the highest prevalence. Overcrowding and age are risk factor associated with the spread of tuberculosis. The high prevalence of drug resistant *Mycobacterium tuberculosis* is of public health concern.

CONSENT

Informed consent were obtain from each of the patients before sample and data collection

ETHICAL APPROVAL

Ethical clearance was obtained from Bayelsa State Ministry of Health in Yenagoa Bayelsa State Nigeria.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO

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