Second-line drug susceptibility testing of *Mycobacterium tuberculosis* isolates from tuberculosis patients in Bayelsa State, Nigeria.

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ABSTRACT

Aim: To determine second-line drug susceptibility testing of *Mycobacterium tuberculosis* isolates from tuberculosis patients in Bayelsa State, Nigeria.

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Study design: A cross sectional study was carried out in this research.

Place and Duration of Study: Directly Observed Treatment Short (DOTS) Couse Centers across Bayelsa State, between March 2020 and November 2021.

Methodology: Ethical approval was obtained, Information source as age, sex and residential address was obtained with the help of a questionnaire. A total of 100 sputum sample was collected from 100 patients across all the Local Government Areas. Sputum sample decontamination and homogenization was done using the Sodium Hydroxide/N- Acetyl -L-Cysteine Citrate Solution. Sputum samples were cultured on solid Lowenstein Jensen Media. All growth were confirmed with Zeihl Neelsen staining and Standard Bioline antigen test. Drug susceptibility test was carried out after bacteria DNA was extracted, amplified using Line Probe Assay (MTBDRplus assay ver 2).

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Results: Out of 100 patients, 15 had confirmed growth of *Mycobacterium tuberculosis*. All isolate had no form of mutation on gyr gene, meaning 100% of isolates were susceptibility to flouroquinolones. There were also no mutation detected on rrs gene therefore all strains are also susceptible to Kanamycin, Amikasin and capreomycin. Out of the 15 isolates 14 had no mutation on the eis gene while 1% had mutation of WT2 and MUT1.

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Conclusion: A good percentage of the isolates are susceptible to second line drugs, therefore cases of extensive drug resistance is not common in Bayelsa State Nigeria

Keywords: tuberculosis, second-line, drugs, mutation, susceptibility and gene

1. INTRODUCTION

Mycobacterium tuberculosis (MTB) is responsible for tuberculosis (TB) disease condition globally. Nigeria is among the five countries in the world with high TB burden (1). The economy of Nigeria has depreciated over the years with high increase in poverty rate occasioned by corruption and bad governance. High rate of insecurity occasioned by

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insurgence in the North, agitations for self-determination in the South East, illegal oil refinery in the south and political tensions in the western part of Nigeria had worsen the life's of Nigerians. Therefore making more person susceptible to public health disease such as tuberculosis. According to Global Metrics Nigeria is currently among the poorest country in the world with poverty rate of 92.0% percentage under U.S 5.50 dollar per day. This is followed by Angola which is at 88.50%, Pakistan 76.20% and Lao PDR 70.40%. There are scarce published work on MTB molecular diversity and drug susceptibility test In Bayelsa State. This is owed to the fact that there are no centers for MTB culture and molecular characterization in Bayelsa State. This study will detect drug susceptibility test for second line drug using Line Probe Assay method (2)

Tuberculosis causing bacteria spread mostly from one person to the other through the inhalation of infected aerosol from a cough or sneeze (3). Though not all individual who are infected with MTB shows active TB disease condition. There are individuals with latent TB disease conditions. The strength of the immunity of an infected person determines the expression of the disease condition (4). Common symptoms of TB infections include; chest pain, persistent coughing, weight loss, fever and night sweats.

The laboratory diagnosis of TB includes; Ziehl Neelson (ZN) staining technique, GeneXpert and Culture. (5).

ZN staining technique cannot be used for molecular characterization of MTB. The preferred method is MTB culture using Lowenstein Jenson media. Though there are various drug susceptibility testing methods but the most preferred with faster turnaround time were Line Probe Assay technique using MDRTB plus version 2 genotypic technique.

Since the discovery of MTB by Koch in 1882 (6).The diagnosis and management of TB has improved over the years. The causative agent which is MTB has also evolved to have survived for this long. Hence the need for susceptibility test to second line drugs, such as Kanamycin, Amikacin, Caproemycin and Flouroquinolones.

Cases of multiple drug resistance has been reported in Bayelsa State (7), which is a case where MTB strain is resistant to more than one of the TB drugs. Extensive drug resistance is a situation where MTB is resistance to more than two anti TB agent including at list one of the second line drugs.

Genetic survey of TB susceptibility of antibiotic has being on for many years but improved molecular method had facilitated the process of TB drug susceptibility testing.

2. MATERIAL AND METHODS

2.1 Ethical approval was obtained from Bayelsa State Ministry of Health, in Yenagoa. Informed consent was also gotten from voluntary participants before proceeding with the administration of questionnaire in other to collect basic demographic data.

2.2 This research was carried out in all the eight Local Government Area of Bayelsa State, Nigeria. Each 100 participant produced above 2ml of sputum into a 50 ml falcon tube container. TB patients who are already diagnoses of TB but had not started ingesting any anti TB drug and those who are already on drugs are included in this research. Extra pulmonary TB cases and children below the age of 16 years as at the time of sample collection are excluded in this study. The handling of all sputum sample during analysis were carried out of bio safety grade level 3, all recommendations and standard Operational Procedure for the handling of Sputum sample by the Global Laboratory Initiative 2014 and Federal Ministry of Health, Nigeria 2008 was strictly adhere.

2.3 Sodium Hydroxide N-Acetyl-L-Cysteine citrate solution method were applied in the decontamination and homogenization of the sputum sample before culturing on solid egg based Lowenstein Jensen media. This were incubated at 37°C for 8 weeks, observation for growth was done every week. Culture media that had growth was subjected to further confirmatory test. The confirmatory test that were carried out were Zeihl Nelseen Staining technique and a MTB antigen based test called Standard Diagnosis Bioline TB AgMPT64 Rapid test. This was carried out in order to exclude all non MTB growth and contaminants. MTB drug susceptibility testing were carried out on the isolates using a Line Probe Assay method. The MDRTBplus Assay version 2 kit was used and the manufacturer's instruction was followed. DNA extraction was carried out using the Genolyse ver1 DNA extraction kit. The extracted DNA were subjected to a polymerase chain reaction technique, the amplified DNA were then subjected to hybridization before testing for Wide Type and MUT mutation.

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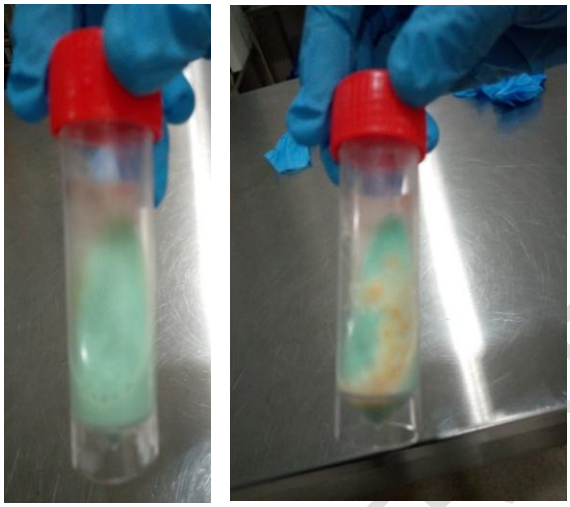
3. RESULTS AND DISCUSSION

Figure 1. shows tube A which displaces only greenish coloration of the LJ solid medium occasioned by the presence of malachite green. There were no growth indications on the slope of tube A which is a control tube incubated alongside with a homogenized decontaminated sputum sample. Tube B shows the visible growth of mycobacterium specie with buffy colored dry colonies on the slope surface of the medium.



A B

Figure 1. Positive and Negative Lowenstein Jensen solid culture Media

Out of 100 patients that were tested 15(15%) were confirmed to be *Mycobacterium tuberculosis* (MTB), while 2(2.0) were non tuberculosis species. Therefore the prevalence of MTB among the tested subjects were 15% (8). similar study in South west Nigeria isolated 73 MTB complex from 63 TB patients indicating a situation of mix strain infection. This was achieved by genetic sequencing of isolated species, meaning some patients were infected with more than one strain of MTB. Our study Bayelsa do not carry out gene sequencing of strains therefore situation of multiple strain infection were not among specific objectives. Our study focused on just Bayelsa State therefore had lesser number of isolate mean while south west of Nigeria had more than 3 States.

In this study 100% of the MTB complex isolated do not have any form of amino acid changes on the gyr gene. No WT (wild type) or MT (mutation) on the gyr gene of the 15 isolates Table 1. The gyr gene is responsible for activation of the flouroquinolones. Therefore it inferred that all strains that are isolated are susceptible to flouroquinolones. This do not agree with similar research conducted by (9) in Abuja, Ibadan and Nnewi which suggest that above 50% isolated had mutation /WT on their gyr gene. This result shows that there is a reduced chance of extensive drug resistance in Bayelsa State Niger

Table 1. Showing gyr Gene Mutation type.

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B- visible growth of mycobacterium specie with buffy colored dry colonies on the slope surface of the medium.

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MTB Isolates	MTBDRplus assay mutation (gyr gene)	Result
1	No Mutation	FLQ^S
2	No Mutation	FLQ^S
3	No Mutation	FLQ^S
4	No Mutation	FLQ^S
5	No Mutation	FLQ^S
6	No Mutation	FLQ^S
7	No Mutation	FLQ^S
8	No Mutation	FLQ^S
9	No Mutation	FLQ^S
10	No Mutation	FLQ^S
11	No Mutation	FLQ^S
12	No Mutation	FLQ^S
13	No Mutation	FLQ^S
14	No Mutation	FLQ^S
15	No Mutation	FLQ^S

FLQ = Flouroquinolones

s = susceptibility

MTB = *Mycobacterium tuberculosis*

The mutation type of rrs gene is sthown on table 2. This reveals that all the isolates are susceptible to kanamycin, Amikacin and Capreomycin, mine while a reseach carried out by (10) reveal 44.1% mutation on rrs gene of all the isolates. This study was carried out in a different location.

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Table 2. Showing rrs Gene Mutation type.

MTB Isolates	MTBDRplus assay	Result

	mutation (gyr gene)	
1	No Mutation	KAN^S,AMK^S,CAP^S
2	No Mutation	KAN^S,AMK^S,CAP^S
3	No Mutation	KAN^S,AMK^S,CAP^S
4	No Mutation	KAN^S,AMK^S,CAP^S
5	No Mutation	KAN^S,AMK^S,CAP^S
6	No Mutation	KAN^S,AMK^S,CAP^S
7	No Mutation	KAN^S,AMK^S,CAP^S
8	No Mutation	KAN^S,AMK^S,CAP^S
9	No Mutation	KAN^S,AMK^S,CAP^S
10	No Mutation	KAN^S,AMK^S,CAP^S
11	No Mutation	KAN^S,AMK^S,CAP^S
12	No Mutation	KAN^S,AMK^S,CAP^S
13	No Mutation	KAN^S,AMK^S,CAP^S
14	No Mutation	KAN^S,AMK^S,CAP^S
15	No Mutation	KAN^S,AMK^S,CAP^S

MTB = *Mycobacterium tuberculosis*
KAN = Kanamycin
AMK = Amikacin
CAP = Capreomycin
S = susceptibility

Among all the second line drug tested only isolate 6 had mutation of the WT2 and MUT1. It means that only 1% of all the isolate is resistance to all the second line drugs such as Kanamycin and Amikacin. This further prove the need for combine therapeutic administration during tuberculosis treatment. Single drug administration is seriously discouraged in tuberculosis treatment. A study carried out in India indicated that there are more frequent mutation on the C-14T region of the eis gene of their isolates (11)

Table 3 **Showing eis Gene Mutation type.**

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eis

MTB Isolates	MTBDRplus assay mutation (gyr gene)	Result
1	No Mutation	KAN^S,AMK^S
2	No Mutation	KAN^S,AMK^S
3	No Mutation	KAN^S,AMK^S
4	No Mutation	KAN^S,AMK^S
5	No Mutation	KAN^S,AMK^S
6	WT2,MUT1	KAN^R,AMK^R
7	No Mutation	KAN^S,AMK^S
8	No Mutation	KAN^S,AMK^S
9	No Mutation	KAN^S,AMK^S
10	No Mutation	KAN^S,AMK^S
11	No Mutation	KAN^S,AMK^S
12	No Mutation	KAN^S,AMK^S
13	No Mutation	KAN^S,AMK^S
14	No Mutation	KAN^S,AMK^S
15	No Mutation	KAN^S,AMK^S

MTB = *Mycobacterium tuberculosis*
KAN = Kanamycin
AMK = Amikacin
S = susceptible
R = Resistance

4. CONCLUSION

Cases of extensive drug resistance is not common in Bayelsa State currently, most of the isolated strains are still susceptible to the second-line drugs. This could be as a result of good management of cases of multiple drug resistance. Cease of multiple drug resistance are given preferential attention by the National Tuberculosis and Leprosy Control Program in Bayelsa State.

CONSENT

Informed consent was gotten from all welling participants

ETHICAL APPROVAL

Ethical approval was gotten from Bayelsa State Ministry of Health

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 not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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