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Second-Line Drug Susceptibility Testing of *Mycobacterium tuberculosis* Isolates from Patients in Bayelsa State, Nigeria

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

Aim: To determine the second-line drug susceptibility testing of *Mycobacterium tuberculosis* isolates from patients in Bayelsa State, Nigeria.
Study Design: A cross sectional study was carried out in this research.
Place and Duration of Study: Directly Observed Treatment Short-course (DOTS) Centers across Bayelsa State, between March 2020 and November 2021.
Methodology: Ethical approval was obtained from Bayelsa State Ministry of Health, Yenagoa, Nigeria. Informations such 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 (LJ) Media. All growths on LJ media were confirmed with Ziehl Neelsen staining and Standard Bioline antigen test. Drug susceptibility test was carried out after bacterial DNA was extracted and amplified using Line Probe Assay (MTBDRplus assay ver 2).
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.
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.

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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 elicited by 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 lives of Nigerians, making more person susceptible to 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].

Mycobacterium tuberculosis 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 also 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 Neelsen (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 have 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 resistant to more than two anti TB agent including at list one of the second line drugs.

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

2. MATERIALS AND METHODS

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.

This research was carried out in all the eight Local Government Area of Bayelsa State, Nigeria. Above 2ml of sputum was collected from each participant, all participants were tuberculosis patients. Extra pulmonary TB cases and children below the age of 16 years as at the time of sample collection were excluded in this study. The handling of all sputum samples during analysis were carried out within bio safety grade level 3 cabinet, 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 adhered.

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 was incubated at 37°C for 8 weeks, observation for growth was done every week. Culture media that had growth was subjected to further confirmatory tests such as Ziehl Neelsen Staining technique and Standard Diagnosis Bioline TB AgMPT64 Rapid test. This was carried out in order to exclude all non MTB growth and contaminants.

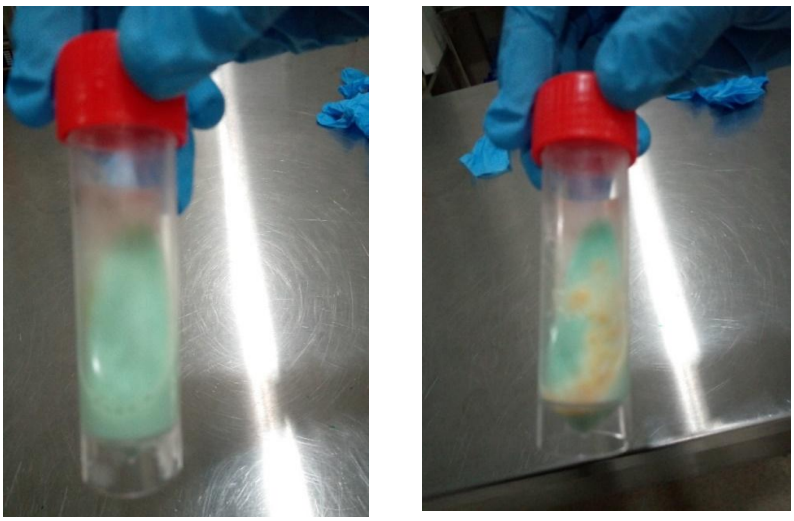
MTB drug susceptibility testing were carried out on the isolates using a Line Probe Assay method. The MDRTBplus Assay version 2 kit was used and the manufacturer's instruction was followed. DNA extraction was carried out using the Genolyse ver1 DNA extraction kit. The extracted DNA was subjected to a polymerase chain reaction technique, the amplified DNA were then subjected to hybridization before testing for Wide Type and MUT mutation.

3. RESULTS AND DISCUSSION

Out of 100 patients that were tested 15% were confirmed to be *Mycobacterium tuberculosis* (MTB), while 2% were non tuberculosis species. Therefore the prevalence of MTB among the tested subjects were 15%. A similar study in South west Nigeria [8] 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 did not carry out gene sequencing and it focused only one sate with lesser number of isolates.

In this study 100% of the MTB complex isolated had no form of amino acid changes on the *gyr* gene. No WT (wild type) or MT (mutation) on the

Fig. 1. A -Control - The green colour of the malachite green is visible without any growth. B - Visible growth of mycobacterium specie with buffy colored dry colonies on the slope surface of the medium.



A **B**

Fig. 1. Negative and positive Lowenstein Jensen solid culture media

Table 1. *gyr* Gene Mutation type

MTB Isolates	MTBDR plus 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

Table 2. *rrs* Gene Mutation type

MTB Isolates	MTBDRplus assay mutation (gyr gene)	Result
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

Table 3. *eis* Gene Mutation type

MTB Isolates	MTBDRplus assay mutation (*eis* 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

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 were susceptible to flouroquinolones. This do not agree with similar research conducted in three different cities in Nigeria (Abuja, Ibadan and Nnewi) [9] which suggest that above 50% isolated had WT mutation on their *gyr* gene. This result shows that there is a reduced chance of extensive drug resistance in Bayelsa State.

The mutation type of *rrs* gene is shown on (Table 2). This reveals that all the isolates are susceptible to kanamycin, Amikacin and Capreomycin, mine while a similar research carried out in Thailand reveal 44.1% mutation on *rrs* gene of all the isolates of MTB [10].

Among all the second line drug tested, only isolate number 6 had mutation of the WT2 and MUT1. It means that only 1% of all the isolate is resistant to some of the second line drugs such as Kanamycin and Amikacin that are activated by the *eis* gene. 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].

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. The positive effect of contact tracing program initiated by the National Tuberculosis and leprosy control program in

Bayelsa State may have led to a reduced cases of extensive drug resistant tuberculosis.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval was gotten from Bayelsa State Ministry of Health

DISCLAIMER

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.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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