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Molecular detection of multidrug resistant Mycobacterium Tuberculosis Complex (MTBC) in extra-pulmonary and pulmonary infections in Bauchi LGA, Bauchi State

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Abstract
Multidrug Resistant Tuberculosis has lately become a major public health problem that threatens the current achievement in Tuberculosis care and control worldwide, Multidrug Resistant Tuberculosis is a form of tuberculosis that is resistant to two primary drugs (Rifampicin and Isoniazid). This study was designed to identify TB and detect resistance to rifampicin using Genexpert system. MDR-TB was investigated in 250 suspects within the age bracket of 1-90years from 5 clinics in Bauchi. The overall prevalence of tuberculosis/RIF resistant detected was 3.2%, males had the highest prevalence with (2.0%) those at the age bracket of 16-30yrs and 31-45yrs having the high percentages. MDR-TB is not prevalent in Bauchi LGA. Because overall prevalence rate was low. There is need to do more on clinical management to totally eradicate the infection from the area, also, health care providers to encourage early and correct diagnosis, detection and treatments to all TB patients.

Keywords: Green computing, eco-friendly technology, carbon emissions, carbon foot print, e-waste, degradation

Introduction
Tuberculosis is caused by pathogenic bacteria of the *Mycobacterium tuberculosis* complex (MTBC) that consists of strains of nine human-adapted lineages and several animal-adapted lineages [1]. It affects the lungs (Pulmonary TB) and other parts of the body (extra pulmonary TB). Tuberculosis pose significant challenges to developing countries as it affects people during their most productive years. Tuberculosis (TB) had been declared as a worldwide global health emergency earlier in 1993 by WHO and remains one of the world's major causes of illness and death [2].

The growing threats of drug resistance, coupled with the devastating effects of TB-HIV coinfection threaten to undermine tuberculosis control efforts worldwide and put global tuberculosis control targets at risk. Drug resistant bacteria pass this resistance to their progeny and can spread from one person to another [3]. The average patient with multidrug resistant tuberculosis can infect additional 15-20 people. Today multidrug resistant (MDR) tuberculosis strains show resistance to at least isoniazid and rifampicin, the most effective first line drugs for treatment of tuberculosis and has become a global public health menace [4]. Development of new molecular biology methods based on mobile genetic elements (MGE) such as the Insertion Sequence *IS6110*, and applied it to tuberculosis patient population studies to identify potential genetic similarities between diverse MTB patients clinical isolates through fingerprint comparisons (or molecular clustering analysis) in order to infer tuberculosis case transmission. The molecular basis of resistance is due to mutation at one of the two main site in the either KatG or InhA genes; resistance to rifampicin is nearly always due to point mutations in the RPO gene on the beta subunit of DNA-Dependant RNA-polymerase [4]. Tuberculosis (TB) continue to remain one of today's global public health challenges, ranking as the leading infectious cause of death and one of the most burden-inflicting diseases in the world. The 2019 WHO Global Tuberculosis Report estimated a worldwide incidence of 10.4 million new cases and 1.5 million deaths in 2019 [5]. State of the art tools are needed to control TB worldwide.

Understanding genome-wide TB infection and persistence of infection during and after treatment is of the utmost importance. The discovery of new markers especially in recently discovered lineage [6] within Africa have shown limited attention on *Maf* and their consequential circulation in West African regions, the development of TB and adequacy of treatment. One would allow global determination of how-to-treat, what-to-treat, and how long to treat both for prevention and cure [7]. In this case, specialized and clear knowledge of *Maf* markers are of great importance to address TB in West Africa [8].

Of recent, there has been a marked period of significant progress in the TB research arena supporting the global effort to successfully achieve an end to tuberculosis [9]. Advances in the development of tuberculosis vaccines, therapeutics, and diagnostics have resulted from an intensified research effort characterized by increased investment in research resources and collaborations stimulated by both the declaration of the United Nations General Assembly high-level meeting on TB and the recent Lancet Commission on Tuberculosis report [10]. In tandem, the National Institute of Allergy and Infectious Diseases (NIAID), which supports 38.9% of the global research effort on tuberculosis, issued in late 2018 its strategic plan for TB research, which has laser-focused the efforts of the scientific community on the highest research priorities needed to develop new tuberculosis treatment and prevention tools that are essential to end tuberculosis within a generation [11]. Most of this aid is restricted to Major health centers in West Africa whereas the majority of patients are from rural areas of these regions. These facts and lack of dedicated resources have also resulted into less TB research studies in most West African urban and rural regions [12].

Infection with strain progresses slowly to TB disease, and is associated with impaired immunity in some settings but not all (e.g. HIV infection).

The members of the MTBC evolved from an environmental organism to an obligate pathogen through genome reduction and acquisition of new genes. In addition, it is known that some differences in gene content exist between strains of different lineages [13].

Study design

This is a prospective study in multiple sites with no intervention. Local Ethical Committees were sought in all sample collection centers selected to participate in this research protocol. Local reference laboratory committees adapted the protocol locally according to their own regulatory requirements. Immunocompetent individuals and immunocompromised Patients will be invited to participate in the study after receiving oral and written information explaining the nature and aim of the study. Informed consent may be translated into local useful languages for protocol acceptance and understanding.

The immunocompetent and immuno-compromised control group with Tb risk will also have an eligibility for routine screening. Apart from qualitative analysis, all result will be recorded in a qualitative manner. When recruiting patients, contributors will attempt to recruit similar number of patients with different level of clinical information to exclude any bias resulting from unequal patient selection.

Sample size: The convenience non-probability sampling techniques [14] was used in sample collection which is based on the samples available at the time of this research from MDR-TB suspected patients. Two Hundred and Fifty (250) samples were collected and used for this purpose. Patients were recruited with the aim to cover a wide spectrum of ages and sex, and existing treatment groups.

Control were recruited from the facility to ensure that controls and patients are comparable with respect to their place of origin and therefore share comparable TB-epidemiology characteristics.

Inclusion criteria for patients

- Individuals specified for TB Positive culture
- Pulmonary and Extra pulmonary TB patient
- Immunocompromised and immunosuppressed TB patients
- Written informed consent signed

Discomfort for participants

No discomfort imposed by participation in the study and no adverse events relating to these procedures are anticipated.

Sample collection

2mls of spot sputum sample free from food particles were collected in a clean sterile, wide mouthed, screw capped, leak proof sputum containers from 250 suspected MDR-TB patients from the five health facilities used for this study. Samples from all the collection points were well labelled and transported in a cold box following specific instructions for transportation of infectious agents [15].

Preparing the GeneXpert Machine

GeneXpert machine was switched on ensuring the barcode scanner beeped, windows were logged into and passwords input, the programs and the models were checked and ensured they were in place.

Sample processing

Appropriate personal protective (gloves, lab coats and respirator were used. Bench surface was disinfected and cartridges removed from pouch and labelled. Lid of the sputum container was carefully unscrewed and 2 volume of sputum sample added and shaken vigorously for 10-20 times and then incubated for 5mins at room temperature. Shaken again 10-20 times for 10 mins to ensure sample liquefaction with no visible clumps of sputum. Using the sterile transfer pipette, 2mls was aspirated of the liquefied sputum sample and gently transferred into an open pot of the cartridge, the lid closed making sure it snaps firmly into place. Sample identification number was entered on the machine and the barcode scanned, the cartridge containing the diluted sputum sample loaded into the module showing green lighten machine automatically run the test for a period of 2hrs and stopped. The result of the test run were read and recorded, used cartridges were removed, disinfected and disposed in a biohazard bag [16].

Statistical Analysis

Data analysis was performed using SPSS version 15.0. Chi-square was used to determine the association between *M. Tuberculosis* and sex of the infected patients. Two-way Analysis of Variance (ANOVA) was used to determine significant differences between sexes and age group. The association between TB and HIV was determined using the correlation analysis. All statistical tests were considered

significant at $p>0.05$.

Table 1: Prevalence of MDR-Tb detected in the study area

Total suspects	Frequency	Prevalence rate (%)
MTB Negative	187	74.8
MTB Detected/MDR Not detected	55	22.0
MTB Detected/MDR Detected	8	3.2
Total	250	100

Table 2: Distributions of patients with MDR-TB and sex

Test	MTB Negative	MTB/MDR Not Detected	MTB/RIF Detected
Male	120 (48.0%)	23 (9.2%)	5 (2.0%)
Female	87 (34.8%)	12 (4.8%)	3 (1.2%)
Total	207	35	8

Table 3: Distributions of patients with MDR TB detected and age

Age Group	MTB Negative	MTB/MDR Not Detected	MDR/TB Detected	Percentage (%)
01-15	21	4	3	0.0
16-30	70	14	2	1.2
31-45	66	8	1	0.8
46-60	29	4	1	0.4
61-75	16	9	1	0.4
76-90	9	16	1	0.4

Table 4: Distributions of patients with MDR TB detected and site

Clinic	MTB Negative	MTB/MDR Not Detected	MDR/TB Detected	Percentage (%)
CHCTB	26	18	0	0.0
DHCY	36	20	2	2.5
LKCHC	24	16	1	5.0
IDHB	68	37	4	10.0
LPHCC	21	9	1	2.5

Table 5: Prevalence of human immune deficiency virus among MDR-TB positive patients (N=8)

Test Result	Frequency	Percentage (%)
Positive	5	62.5
Negative	2	25.0
Unknown	1	12.5

Discussions

The overall prevalence of MDR-TB from the present study was found to be 3.2%. This study revealed a low prevalence of MDR-TB compared to a study by (17) who reported 7.33% rate in North East Nigeria with 4.18%nresistance coming from Gombe state. It also revealed a slight drop in the prevalence rate compared to report of 3, 5% prevalence rate globally in 2008-2013 by WHO, 2014. The low prevalence of resistance across the study area may also be attributed to the proximity of treatment centers and better follow up system.

MDR-TB shows higher prevalence among males who were found to have 2.0% while females recorded 1,2% prevalence which is in line with some previous studies by (18) that shows higher prevalence of MDR-TB in males than in females. This could be attributed to the fact that males stand a chance to be more expose to the infection due to the kind of activities they engage in than females in this part of the country who are mostly restricted at home. Prevalence of MDR-TB based on age groups revealed that age bracket of 16-30 had the highest prevalence of 1.2% followed by age group of 31-45 with prevalence of 0.8% of MDR-TB resistance to rifampicin, while group ranges of 46-60, 61-75,76-90 recorded the lowest prevalence of 0.4%. This may be due to the facts that individuals in the age range with high prevalence are in their most active stage in life and hence find it difficult to adhere to their schedule while regulated mobility played a part in the other age groups with less resistance [19].

High prevalence of HIV was observed among MDR-TB positive in this study, HIV seropositive patients as compared to seronegative patients showing a prevalence of 5(62.5%) where the HIV seronegative had prevalence of 2(25.0%). While those with unknown status recorded the prevalence rate of 1(0.4%). The higher prevalence of HIV in MDR-TB patients agrees with a report by WHO, 2014 showing high prevalence of HIV in seropositive patients. This is also in line with the work of (20) where there was a high prevalence of HIV among MDR-TB patients. This condition brings about complications leading to difficulties in treating of the disease. The patient also remains infectious longer increasing the risk to the public and to healthcare workers. MDR-TB confections further compromise the health and immune system of these patients. HIV itself does not increase the chance of drug resistance but if not carefully handled it does accelerate the progression of TB infection into MDR-TB which can cause death.

Conclusion

Overall findings emphasise the importance of continuing the systematic surveillance of Mycobacterium tuberculosis isolates to monitor the trends of drug resistance in different patient categories as well as its association with HIV across the country to timely modify and strengthen the national programs in order to prevent the emergence of MDR-TB strains and avert the threat of XDR-TB.

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Conflict of Interest

Not available

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Not available

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