SECOND LINE DRUG SENSITIVITY TEST OF MULTIDRUG RESISTANT TUBERCULOSIS (MDR-TB) ISOLATES USING PROPORTIONAL METHOD.

1Aminu, A.I. and 2Tukur, A.D.

1Department of Microbiology, Bayero University, Kano, Nigeria
2North West Zonal TB Reference Laboratory, Aminu Kano Teaching Hospital, Kano, Nigeria.

Correspondence author:*aishatuaminuibrahim@gmail.com, 08054503326.

ABSTRACT
Drug resistant Tuberculosis (DR-TB) continue to present challenges to the TB control programmes with Multidrug resistant TB (MDR-TB) becoming a major public health threat predisposing individuals to develop Extensively drug resistant TB (XDR-TB) and Totally drug resistant TB (XXDR-TB). The World Health Organization reemphasizes the need for drug Susceptibility testing (DST) for appropriate treatment of individual cases in order to minimize rapid transmission of tuberculosis especially DR-TB. The study aimed at performing second line drug sensitivity test of MDR-TB isolates to some of the major second line anti-TB drugs. Samples for the study comprised of 22 purposely selected MDR-TB isolates obtained from sputum samples of new AFB smear positive (MDR-TB) patients that were brought to the North West Zonal Tuberculosis Reference Laboratory located at Aminu Kano Teaching Hospital, Kano. The isolates were re-confirmed as MDR-TB using Line probe assay (LPA), and subjected to second line drug sensitivity testing using Proportional methods to some of the second line anti-TB drugs (Ofloxacin, Capreomycin, Ethionamide, & Kanamycin). The results of the study revealed that all the 22 isolates were re-confirmed as MDR-TB using the LPA. The results further shows that all the 22 isolates were sensitive to Ofloxacin (100%) and Capreomycin (100%), followed by Kanamycin (90.9%) and Amincakin (86.36%) and none of them was identified as XDR-TB. Most of the isolates 16 (72.72%) were however resistant to Ethionamide with only 6 (27.27%) of them being sensitive. The study revealed absence of XDR-TB among the studied isolates as all of them were sensitive to Ofloxacin and Capreomycin, however majority of them were resistant to Ethionamide and very few to Amikasin and Kanamycin. The study identifies the need for conducting drug susceptibility tests for isolates from patients identified as new AFB smear positive for appropriate treatment so as to reduce transmission of DR-TB.

KEYWORDS: Second Line Drug Sensitivity Test, Proportional Method, MDR-TB.

INTRODUCTION
Drug resistant Tuberculosis (DR-TB) continues to present challenges to the TB control programmes. Most importantly the increase in Multidrug Resistant TB (MDR-TB) cases in some parts of the world including Nigeria is a source of concern to all. McBryde (2017) revealed that despite the TB control measures, since 1985, the world has seen a constant rise in the levels of MDR-TB - defined as TB with in-vitro resistance to at least isoniazid and rifampicin, the two most potent first-line anti-TB drugs. The evolution of Extensively Drug Resistant TB (XDR) and Totally Drug resistant TB (XXDR) further worsened the situation. Extensively Drug Resistant TB identified in 2006 refer to strains of TB that are resistant not only to rifampicin and isoniazid, but also resistant to fluoroquinolone and to at least one of the second line injectable TB drugs and XDR-TB identified in 2007, is TB which is resistant to all the first and second line TB drugs and this makes it almost but not totally impossible to treat (Migliori, 2007). Thus, it is worrisome to note that MDR-TB is becoming a major public health threat predisposing individuals to develop XDR-TB and XXDR-TB.

The world health organization (WHO, 2017) reveals that an estimated 10.4 million incident TB occurred worldwide in 2016 up from 9.6m in 2014 and Nigeria was documented as 1st in Africa 4th among 6 countries that accounted for 60% of worldwide TB burden with reported 586/100,000 incident cases (WHO, 2017) up from 322/100,000 (WHO, 2015). Also, 580, 000 MDR/RR-TB incident cases occurred globally in 2015 with an estimated 3.9% new cases and 21% previously treated cases. Nigeria records 4.3% and 25% MDR/RR-TB in New & previous TB cases (WHO, 2017 and 2015). However, Nigeria and four other countries accounted for 60% of notified MDR-TB in 2015 with only 15% of the total burden of the disease in the country being notified in 2015 (WHO, 2017).
This indicates that majority of the MDR-TB cases in Nigeria were not reported and remain untreated as such becoming a major source of transmitting MDR-TB in the population. Insufficient TB diagnostic centres as well as surveillance and reporting systems are some of the major reasons that might be connected to the under-reporting of TB cases in the region. Bearing the enormous burden of MDR-TB especially in Nigeria and the fact that the WHO (2015) reemphasizes the need for drug Susceptibility testing (DST) in order to develop efficient regimen for appropriate treatment of individual cases, the study aimed at performing second line drug sensitivity test of multidrug-resistant tuberculosis (MDR-TB) isolates to some of the major second line anti-TB drugs used for treatment of TB.

**MATERIAL AND METHODS**

**Study area**

The study was conducted at the North West Zonal Tuberculosis reference laboratory located at Aminu Kano Teaching Hospital, Kano, which serves as a referral center for the diagnosis of tuberculosis in North western region of Nigeria and beyond.

**Collection and processing of samples**

Samples for the study comprised of 22 purposely selected MDR-TB isolates obtained from sputum samples of confirmed new AFB smear positive MDR-TB patients that were brought to the North West Zonal Tuberculosis Reference Laboratory located at Aminu Kano Teaching Hospital, Kano. All isolates that were identified by the center as MDR-TB were included in the study while those that were not were excluded. Ethical clearance for the study was obtained from the Ethical Review Committee of Aminu Kano Teaching Hospital. The Mycobacterial isolates collected were re-confirmed as MDR-TB using GenoType® MTBDR plus molecular line probe assay (LPA) that involves three major steps; DNA extraction, DNA amplification and Hybridization, and finally DNA detection. Accordingly, the DNA was extracted directly from the samples and the resistance-determining region of the gene was amplified using biotinylated primers with polymerase chain reaction (PCR). Labeled PCR products were hybridized with specific oligonucleotide probes immobilized on a strip and captured labeled hybrids were detected by colorimetric development, enabling detection of the presence of \textit{M. tuberculosis} complex as well as presence of wild-type and mutation probes for resistance (Barnard et al., 2012 and 2008; NTBLCP, 2011). The confirmed MDR-TB isolates were then inoculated into prepared Lowenstein Jensen (LJ) medium in duplicates for each sample and then incubated aerobically at 37°C for 8 weeks (NTBLCP; 2011; WHO, 2011). For the susceptibility testing, the inoculum was prepared by directly suspending colonies of MTBC isolates grown for approximately three weeks on Lowenstein Jensen drug free slopes to a turbidity equivalent to 1.0 MacFarland standard. The standardized suspension was further diluted to 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴. The control strain was obtained from National Tuberculosis and Leprosy Training Centre (NTBLTC) Saye, Zaria Nigeria Drug susceptibility testing of the MTBC isolates against some of the second line anti-TB drugs was carried out by proportional methods as described by NTBLCP, SOP (2011). For each type of anti-TB drug used in the DST, two (2) types of slopes (-a drug free slope and a drug containing slope) were prepared. The drug containing slopes were prepared by adding critical concentrations of Ofloxacin (2.0µg/ml), Capreomycin (2.0µg/ml), Ethionamide (40µg/ml) and Kanamycin (30µg/ml) to 200mls of LJ medium contained in tubes. Subsequently, for each sample the 10⁻² standardized MTBC suspension from above was inoculated on the drug-containing slopes. Three drug-free LJ slopes were inoculated with 10⁻², 10⁻³ 10⁻⁴ MTBC suspension. Furthermore, the drug-susceptible MTB reference strain ATCC 27294 (H37Rv) was used as a control. The slopes were incubated at 37°C and read after 4 and 6 weeks. After 28 days incubation, inoculated slopes were observed for growth. The average number of colonies obtained from drug-containing slopes indicates the number of resistant bacilli contained in the inoculum. Dividing the number of colonies in the drug containing slopes by that in the drug free slopes gives the proportion of resistant bacilli existing in the strain. An isolate was considered resistant if the proportion of bacilli resistant to the critical concentration of the drug exceeded 1%.

**RESULTS**

The results of the study revealed that all the 22 isolates were re-confirmed as MDR-TB using LPA. Table 1 revealed the drug susceptibility tests of the 22 MDR-TB isolates to second line anti-TB drugs using proportional methods and revealed that all the 22 isolates were sensitive to Ofloxacin (100%) and Capreomycin (100%) followed by Kanamycin (90.9%) and Aminkacin (86.36). Most of the isolates 16 (72.72%) were however resistant to Ethionamide with only six (27.28%) of them being sensitive to it.
DISCUSSION
The findings of this study demonstrated that all the 22 MDR-TB isolates were susceptible to Ofloxacin and Capreomycin and none of them was identified as XDR-TB. However, the isolates exhibited high level resistance (72.6%) to Ethionamide followed by Kanamycin and Amikacin. This indicates that the patients (who were documented as new MDR-TB cases) from whom this isolates were obtained have been infected primarily with MDR-TB isolates that were already resistance to some of the second line anti-TB drugs. This type of primary drug resistance to the reserved anti-TB drugs has a serious implication indicating that administration of second line drugs without initial performance of DST might not be beneficial to the patient and may even further lead to increase in the rate of resistance to the second line drugs which could lead to increase in the TB burden as well increase in acquisition of XDR-TB. This therefore reemphasizes the need to perform drug susceptibility test prior to administration of the drugs. According to the (WHO 2014) and da Silva and Palomino (2011) undiagnosed, untreated, or poorly treated drug-resistant TB contributes to sustained high drug-resistant TB prevalence, as well as high proportions of infectious drug-resistant TB cases among the community.

The high resistance to Ethionamide exhibited by the isolates of the study being them MDR-TB is not surprising since Ethionamide which a derivative of isonicotinic acid has been identified as a structural analogue of isoniazid. Resistance to Ethionamide is mediated by mutations not only in the etaA/ethA, ethR but also inhA genes and it has been established that mutations in the inhA gene mediate co-resistance to both isoniazid and ethionamide (Dookie et al., 2018). In comparison to the findings of this study other studies by Bakula et al. (2016) and Iqbal (2012) reported lower rates of resistance of 8.7% and 13.4% to Ethionamide respectively.

This study also reports that some of the isolates were identified as pre-XDR-TB as they were resistant to Kanamycin and Amikasin and did not exhibit resistance to Ofloxacin. Similar study by Osei-Wusu (2018) in Ghana revealed that 10.3% of their isolates were resistant to Amikasin. However, higher rates of resistance of 17.1% to Amikasin/Kanamycin were reported by Chen et al. (2016) compared to the findings of this study.

CONCLUSION
The study reports absence of XDR-TB among the studied isolates however majority of them were resistant to Ethionamide and very few to Amikasin and Kanamycin. The study identifies the need for conducting drug susceptibility test prior to administration of drugs to prevent transmission of MDR-TB and development of XDR-TB which is more difficult to treat as well XXDR-TB which does respond to any of the anti-TB drugs.

Acknowledgement
The authors acknowledge the Management of Aminu Kano Teaching Hospital and the TB Reference Laboratory for the use of their facility.

Conflict of Interest
None

REFERENCES


GenoType® MTBDRplus Assay (Hain Lifescience GmbH, Nehren, Germany).


