Original Article

Multidrug-Resistant Tuberculosis in Imo State, Southeast, Nigeria

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ABSTRACT

Background: Multidrug-resistant tuberculosis (MDR-TB) is a global health challenge. The emergence of MDR TB has contributed remarkably to the spread of tuberculosis and also poses a threat, which if not effectively addressed may wipe out the achievements of previous efforts in controlling tuberculosis. **Objective:** This study was aimed at detecting MDR-TB among patients in a setting prevalent with tuberculosis and HIV in Southeast, Nigeria. **Method:** Sputum specimens collected from 740 suspected tuberculosis (TB) patients were screened for acid-fast bacilli (AFB). All the 111 AFB positive samples were subjected to culture on Lowenstein-Jensen (LJ) medium and Mycobacterium Growth Indicator Tube (MGIT) 960 TB system. The isolates were then confirmed as *Mycobacterium tuberculosis* using SD Bioline Rapid Diagnostic Tests before being subjected to drug susceptibility testing to first-line anti-TB drugs. MDR-TB was determined by isolates being resistant to both isoniazid and rifampicin. HIV testing was performed for participants included in the study using standard rapid diagnostic tests. **Result:** Out of the 111 AFB-positive sputum samples, 65 (58.6%) were culture-positive for *Mycobacterium tuberculosis*. MDR-TB was found in 2 ([3.1%] 95% CI = 0.0–7.3) of the culture-positive samples. The rate of TB and HIV coinfection was 7.7%. Maximum single-drug resistance was seen in ethambutol 12 ([18.5%] 95% CI = 9.0–27.9). **Conclusion:** The MDR-TB rate of 3.1% found in this study was relatively low and efforts should be intensified to keep it low.

Keywords: *Imo State; Nigeria, Mycobacterium tuberculosis, multidrug-resistant tuberculosis, new and previously treated TB cases, tuberculosis*

Introduction

Multidrug-resistant tuberculosis (MDR-TB) is defined as tuberculosis that is resistant to at least isoniazid and rifampicin, the two most effective first-line anti-TB drugs.[1] MDR-TB patients require prolonged and expensive treatment using second-line medications that are less effective and more toxic.[1] Several mechanisms have been suggested to cause MDR-TB including poor adherence to anti-TB drug or previous TB treatment,[2] direct transmission of MDR-TB from person to person,[3,4] previous exposure to quinolones, use of inferior regimens, and high human immunodeficiency virus (HIV) coinfection.[1-4] The conventional methods of multidrug-resistance testing involve the culture of *Mycobacterium tuberculosis* on liquid or solid culture medium in the presence of anti-TB drugs to detect growth (indicating drug resistance) or inhibition of growth (indicating drug susceptibility).[5] Culture-based methods are the reference standard for the diagnosis of tuberculosis and MDR-TB.[6]

Molecular testing of MDR-TB involves the use of line probe assays and the Xpert MTB/RIF assay for rapid and simultaneous detection of *Mycobacterium tuberculosis* (MTB) and TB drug resistance.[7,8] These molecular technologies do not, however, eliminate the need for conventional microscopy, culture, and drug susceptibility test (DST) which are required to monitor treatment progress and to detect resistance to drugs other

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than rifampicin.[7,8] A rapid immunochromatographic identification test for the *M. tuberculosis* complex, which helps to differentiate it from mycobacteria other than *M. tuberculosis* is the SD BIOLINE TB Ag MPT64 Rapid.[9]

The pandemic of HIV/AIDS has been attributed to the upsurge of MDR-TB globally. MDR-TB in HIV-infected individuals leads to higher mortality compared to mortality in non-HIV-infected patients or HIV infected individuals with susceptible tuberculosis.[10]

MDR-TB is a growing global health challenge that currently accounts for 3.4% of all newly acquired tuberculosis cases worldwide.[11] The World Health Organization (WHO) reports that Nigeria currently ranks 7th in the world and 2nd in Africa, among the 30 countries with the highest burden of TB and MDR-TB.[12] Hence, early detection and treatment of these drug-resistant forms of TB have become paramount in the fight to curb the menace posed by the infection. Data on the MDR-TB rate in Imo State was not found in our literature search.

Materials and Methods

Study area and study population

This study was carried out in patients attending the chest clinics of three hospitals in Imo State, South East-Nigeria, namely: Imo State University Teaching Hospital 30th September, 2013, Umuna-Orlu; Holy Rosary Hospital, Emekuku; and Saint Damian's Hospital, Okporo. These hospitals represent major healthcare providers for TB patients in the state. Laboratory investigation was carried out at Dr. Lawrence Henshaw Memorial Hospital TB Reference Laboratory Calabar, Cross River State.

Ethical considerations

The samples were obtained from the three hospitals involved in the study with approval from the research and ethics committees of these institutions. All patients signed an informed consent form to participate in the main study and allow further testing on the samples and isolates.

Study design and sampling

This was a multicenter cross-sectional study wherein a total of 740 patients were enrolled using a consecutive sampling technique. Sputum samples from suspected, newly diagnosed on treatment, and previously treated tuberculosis patients who presented with signs and symptoms of pulmonary tuberculosis and were attending the chest clinics of these institutions were collected consecutively and examined for acid-fast bacilli (AFB). This was done until the desired sample size of 111 AFB positive sputa was achieved. The patients were also screened for HIV. All sputum samples with positive AFB results on Ziehl-Neelsen (ZN) staining were subjected to further testing. A standard semi-structured and interviewer-administered questionnaire was completed for each recruited patient to collect demographic, laboratory data, and management history.

Eligibility criteria

We included patients attending the chest clinics who were within the age range of 18 and 65 years who reported a cough of more than 2 weeks and whose sputum samples yielded a positive result for AFB on ZN staining. However, we excluded patients who developed cough due to diseases other than tuberculosis.

HIV testing

Determine (Abbott Laboratories, United Kingdom) and Unigold (Trinity Biotech, Ireland) rapid diagnostic test kits were used to detect the presence of HIV in the blood samples of participants using the serial algorithm method.[13]

Pretreatment of sputum

The sputum samples were digested, decontaminated, and concentrated before testing using N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH). The sediment of each sample was resuspended with 2 mL of sterile phosphate buffer.

Sputum microscopy using Ziehl-Neelson (ZN) staining technique

Dry sputum smear made on a clean and grease-free slide was stained using the ZN technique to detect AFB. This involved the use of strong carbol-fuchsin as the primary stain, 3% acid-alcohol for decolorization, and methylene blue as counterstain.

Culture of MTB in Lowenstein-Jensen medium

In a biosafety cabinet level II and using a sterile plastic pipette, two drops of the sediment of the sputum sample of each smear-positive patient were inoculated onto Lowenstein-Jensen (LJ) medium slope and incubated at 37°C for up to 8 weeks. A standard strain H37RV *M. tuberculosis* strain was used as positive control while the sterile LJ medium was used as a negative control. The growth and morphology of the colonies were noted, and the colonies were identified as *M. tuberculosis* using ZN smear microscopy and SD BIOLINE Rapid Diagnostic Test for MTB.[13]

Culture of MTB using BACTEC MGIT 960 automated TB culture system

About 0.8 mL of OADC-PANTA (oleic acid, albumin, dextrose, and catalase - polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) mixture was aseptically dispensed into each labeled Mycobacterium Growth Indicator Tube (MGIT) media using a sterile

pipette. Later 0.5 mL of the specimen concentrate was aseptically transferred to the corresponding MGIT tube using a sterile 1 mL pipette. Positive and negative controls similarly were set-up. The inoculated tubes were loaded into the MGIT 960 instrument and incubated for 42 days at 37°C. Positive cultures were flagged by a red light in front of the MGIT 960 drawer where the tubes were located while negative cultures at the end of 42 days incubation were flagged by a green light as negative. Positive tubes were scanned out of the machine first thing in the morning and the growth pattern in the media was observed. To check for contamination, positive cultures were grown on blood agar. ZN smear microscopy and SD BIOLINE tests were used to confirm *M. tuberculosis*.

Drug susceptibility testing using the agar proportion method

The cultures that yielded growth and confirmed to be *M. tuberculosis* by SD BIOLINE test, were subjected to drug susceptibility tests using the agar proportion method.[14,15] Each of the drug concentrations of streptomycin (S) 4 µg/mL, isoniazid (I) 0.2 µg/mL, rifampicin (R) 40 µg/mL, and ethambutol (E) 2 µg/mL were incorporated into LJ medium slope .Two dilutions of the bacilli, 10^{-2} and 10^{-4} dilutions (undiluted = 10^{6}–10^{8} CFU/mL) were inoculated on two sets of media (drug-containing and drug-free media), and incubated for 4–6 weeks. The critical proportion was taken at 1% for all drugs. When the bacterial growth on the medium with the specific drug was >1% compared to the control, the strain was identified as resistant to the specific drug. Besides, organisms were identified as sensitive to the drug when the growth rate was <1% compared to the control. The H37RV standard *M. tuberculosis* strain was used as positive control while inoculated slope without drug was used as a negative control.

Statistical analysis

Data were analyzed using the statistical analysis system (SAS) version 9.2. We calculated frequencies and percentages. We also conducted a Chi-square test of association between categorical variables, and Z-test for proportions. The probability value of less than or equal to 0.05 ($P \leq 0.05$) was considered statistically significant.

Result

Out of the 704 sputum samples collected, 111 (15.8%) were AFB positive. Of these AFB positive samples, 65 (58.6%) yielded culture-positive organisms using a combination of two different (LJ and MGIT) culture methods. Fifty-six (86.15%) of the 65 isolates grew on the LJ culture medium, while 64 (98.46%) of the isolates grew on the MGIT culture medium. The percentage of AFB positive sputum samples which yielded growth on the LJ medium was 50.5%, while that on MGIT was 57.7%. The 65 isolates were confirmed to be *M. tuberculosis* using the SD BIOLINE Rapid Diagnostic test for MTB complex.

Resistance to rifampicin was observed in 7 (10.8%) of the 65 *M. tuberculosis* isolates, with resistance to isoniazid, streptomycin, and ethambutol also observed in 6 (9.2%), 6 (9.2%), and 12 (18.5%) of the 65 MTB isolates, respectively. The prevalence of *M. tuberculosis* resistant to rifampicin and isoniazid (MDR-TB) was 3.1% [Table 1]. Maximum single-drug resistance was seen in ethambutol 12 ([18.5%] 95% CI = 9.0–27.9).

Only 5 (7.7%) of the culture and SD BIOLINE positive cases tested positive for HIV. There was no significant degree of association between the HIV status of patients and MDR-TB ($P = 0.678$) [Table 2].

There was a higher male-to-female ratio (2.4: 1) for subjects with culture-positive *M. tuberculosis,* with males comprising of 46 (70.8%) of the cases and females 19 (29.2%). There was no significant relationship between gender and the development of MDR-TB ($P = 0.356$) [Table 3].

Table 1: Test of proportions for drug resistance in *Mycobacterium tuberculosis*

Anti-Tb Drugs	Number Resistant *n* (%)	(CI)
Rifampicin	7 (10.8)	(3.2-18.3)
Isoniazid	6 (9.2)	(2.2-16.3)
Streptomycin	6 (9.2)	(2.2-16.3)
Ethanbutol	12 (18.5)	(9.0-27.9)
Rifampicin+ Isoniazid	2 (3.1)	(0.0-7.3)

n=Frequency; %=Percent; CI=Confidence Interval

Table 2: Cross-tabulation of HIV status and MDR-TB

HIV Status	MDR-TB		*P*
	No *n* (%)	Yes *n* (%)	
Negative	58 (96.7)	2 (3.3)	0.678
Positive	5 (100.0)	0 (0.0)	
Total	63 (96.9)	2 (3.1)	

n=Frequency; %=Percentage; *P*-value=Probability

Table 3: Cross-tabulation of gender and MDR-TB

Gender	MDR-TB		*P*
	No *n* (%)	Yes *n* (%)	
Female	19 (100.0)	0 (0.0)	0.356
Male	44 (95.7)	2 (4.4)	
Total	63 (96.9)	2 (3.1)	

n=Frequency; %=Percentage; *P*-value= Probability

Out of the 111 patients with AFB positive sputum, 108 (97.3%) never discontinued their medications for any reason while 100 (90.1%) never interrupted their anti-TB drugs.

Discussion

The study showed that there were more cases of tuberculosis among the males 46 (70.8%) than females 19 (29.2%) with a male-to-female ratio of 2.4: 1, which agrees with the findings by Surkova *et al.,* in 2012 that 70.7% of tuberculosis patients in a 2007 study were males with a male-to-female ratio of 2.4: 1.[16] This is was also consistent with reports which stated that in most countries, the majority of TB patients were males,[17,18] and also with findings by WHO that the male-to-female (M: F) tuberculosis ratio in 2013–2016 was greater than 1.7: 1. This suggests a potential role of gender in the epidemiology of tuberculosis.

The majority of patients with tuberculosis infection 60 (92.3%) were HIV-negative, with a TB and HIV coinfection rate of 5 (7.7%). This was lower than the 26% rate of TB and HIV coinfection reported in Nigeria.[19] Because of the established observation that most TB and HIV coinfected patients usually present with the smear-negative disease,[20,21] the low TB and HIV coinfection rate observed may then be attributed to the fact that samples included in this study were all ZN smear-positive.

Culture on LJ medium and MGIT culture system yielded less positive results than sputum AFB, which may probably be due to the sputum decontamination procedure before culture, which is used to prevent overgrowth by other microorganisms. It was also observed from this study that of the total AFB positive sputum samples, the percentage yield of *M. tuberculosis* on the MGIT culture system (57.7%) was more than that on the LJ medium (50.5%). The findings agree with the fact that all decontamination methods are to some extent harmful to *Mycobacteria* and culture is therefore not 100% sensitive.[22] Findings also agree with studies by Giovanni *et al.,* in 2008 that liquid culture media system is more sensitive than solid culture media and increases the case yield by 10% over solid media.[23]

The rates of single-drug resistance obtained for each of the drugs were higher than those reported in another Nigeria study.[24] This may be due to the observation that the pattern of drug resistance varies from place to place and at different periods.[25] The finding that rifampicin resistance of 10.8% was slightly higher than the lowest value of single-drug resistance observed in isoniazid (9.2%), has an important implication for national and global MDR-TB diagnosis because rifampicin resistance serves as a proxy for molecular detection of MDR-TB and single drug resistance often precedes and predicts the development of MDR-TB.[4]

A low MDR-TB prevalence of 3.1% was observed [Table 1]. This was lower than the 3.4% prevalence observed by WHO in a 2007–2010 study[11] as well as the 3.3% prevalence of global MDR-TB in new TB cases observed in 2014.[26] This justifies the use of these drugs as first-line drugs in the treatment of tuberculosis. The prevalence of MDR-TB (3.1%) obtained was also lower than the reported 3.9–5.0% prevalence of MDR-TB in newly diagnosed cases in Africa[27] and the 4.8% prevalence in Nigeria. Low rates in this study could be attributed to the fact that participants in this study showed good compliance with their first-line anti-TB medications wherein 108 (97.3%) of the participants never discontinued their medications for any reason, while 100 (90.1%) never interrupted their anti-TB drugs.

HIV infection is a known risk factor for the increased incidence of tuberculosis disease.[18] In this study, however, HIV infection was not found to have a statistically significant association with MDR-TB [Table 2]. This was consistent with the report by Berhan *et al*., 2013[28] as well as with the discovery that several reports from different parts of the world have shown that HIV infection has no statistically significant association with MDR-TB.[29,30]

Conclusion

The prevalence of MDR-TB was low in the study. Since the hospitals where patient recruitment took place are the major healthcare centers for TB patients in the state, this observed low prevalence may be representative of the MDR TB picture in Imo State, Nigeria. We recommend that a wider set of surveillance sites are investigated for MDR-TB, to obtain a more realistic view of the MDR-TB in Nigeria. Emphasis on regular monitoring and control of drug-resistant TB through prompt case detection, drug susceptibility testing, and systematic treatment observation will go a long way to curb the menace of MDR-TB in Nigeria.

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Prior presentations/submissions

This article has not been submitted or presented to any other organization before this time.

Authors' contributions/criteria for inclusion as author's

Akujobi CN, Ahiarakwem IIE, and Ekejindu IM conceived the idea. Ahiarakwem IIE, Ekejindu IM, and Akujobi CN designed the experiments, defined the intellectual content, and reviewed the manuscript. Ahiarakwem IIE performed the literature search and the laboratory experiments, as well as data acquisition. Ahiarakwem IIE and Aghanya IN analyzed the data, wrote, edited, and reviewed the manuscript. All authors approved the final version of the manuscript. Ahiarakwem IIE was the project leader, while Ekejindu IM and Akujobi CN were project supervisors. All authors made conceptual contributions.

Ahiarakwem IIE, Ekejindu IM serve as the guarantors.

Declaration statements

This is to certify that the manuscript has been read and approved by all the authors and that the requirement for authorship as stated in the NJCP authors' instructions, has been met. We believe that the manuscript represents honest work.

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Conflicts of interest

There are no conflicts of interest.

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