

Short Report

Pilot study on multidrug resistant tuberculosis in Nigeria

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Abstract

Background: Drug resistant tuberculosis (TB) has lately emerged and it represents a serious public health problem. We set out to determine drug resistance among TB patients.

Methods: Using automated BACTEC cultures, multidrug resistant-tuberculosis (MDR-TB) was investigated in 117 diagnosed cases in Abuja, Nigeria.

Results: Ten (31%) of 32 culture-positive patients were resistant to at least one and four (13%) to all of the four drugs tested. No association between drug resistance and human immunodeficiency virus (HIV) infection was found.

Conclusions: MDR-TB is present in Nigeria and larger studies are urgently required. TB clinical management and control efforts should be improved.

Keywords: Drug resistance, human immunodeficiency virus, prevalence, Nigeria, tuberculosis

Résumé

Arrière-plan: Résistant aux médicaments de la tuberculose (TB) est apparu récemment et il représente un problème sérieux pour la santé publique. Nous exposons à afin de déterminer la résistance aux médicaments parmi les tuberculeux.

Méthodes: Utilisation automatisée BACTEC cultures Multi-drug résistant-la tuberculose (TB-MR) a été étudiée dans 117 cas diagnostiqués à Abuja, Nigéria.

Résultats: 10 (31%) des patients de la culture-positif 32 étaient résistants au moins et 4 (13%) à l'ensemble des quatre médicaments testés. Aucune association entre l'infection par le virus d'immunodéficience humaines (VIH) et de résistance aux médicaments a été trouvée.

Conclusions: TB-MR est présent au Nigeria et grandes études sont nécessaires de toute urgence. Efforts de gestion et de contrôle cliniques TB devraient être améliorées.

Mots clés: Résistance au virus de l'immunodéficience humaine, prévalence, Nigéria, la tuberculose, la drogue

Introduction

Nigeria has the fourth highest burden of tuberculosis (TB) in the world, with an annual incidence of 311 cases per 100,000 population and a mortality rate of 81 per 100,000 population in 2006.^[1] While the directly observed treatment short-course (DOTS)

program was initiated by the National Tuberculosis and Leprosy Control Program (NTBLCP) in 1993 to streamline treatment, detection of new smear-positive cases still remained low at about 20% by 2006.^[1] The NTBLCP currently recommends treatment of newly diagnosed TB patients, with rifampicin, isoniazid, pyrazinamide, and ethambutol

or streptomycin for 2 months, followed by isoniazid and rifampicin for 4 months or isoniazid and ethambutol for 6 months to reduce rifampicin interaction with the drugs used for the treatment of the human immunodeficiency virus (HIV) infection. Nigeria has an HIV sero-prevalence rate of 4.4%, the third highest infection burden in the world, with an estimated 3 million individuals infected.^[1-3] In Nigeria, about 21% of all TB patients are dually infected with TB and HIV.^[1-3]

In vitro resistance to anti-TB drugs was first reported in Nigeria over three decades ago^[4] and local health practitioners have the perception that drug resistance has increased in the recent years.

As there has been no systematic study on drug sensitivity, we conducted a survey using automated BACTEC cultures and drug susceptibility testing (DST) of patients with features of pulmonary TB in Nigeria to generate preliminary data to support larger studies on the extent of drug resistant TB.

Materials and Methods

In this survey, patients, older than 15 years with cough for more than 3 weeks duration, who attended Wuse General Hospital, in Abuja, Nigeria, between May 2007 and August 2007, were studied. Patients, unclassified as both new and re-treatment patients were studied. Three sputum samples were collected as on-the-spot, morning and second on-the-spot specimens from consecutive patients. All sputum specimens were examined at the Research Laboratory of Zankli Medical Centre (ZMC) in Abuja, through a Private Public Mix Partnership (PPP). ZMC is the first center to have an automated BACTEC TB culture and susceptibility in Nigeria although two more have now become operational. As part of the PPP, it provides referral services in TB diagnosis and management in the Abuja area.^[3,5] Sputum smears were prepared using Ziehl-Neelsen technique as previously described^[5] and the specimen considered of best quality out of the three collected was cultured on an automated BACTEC 960 *Mycobacterium* growth indicator tube (MGIT; Beckton Dickinson, Erembodegem, Belgium).^[5] DST was conducted using four anti-TB drugs – streptomycin, isoniazid, rifampicin and ethambutol as described by Ardito *et al.*, using a BACTEC MGIT 960 automated system for DST of *Mycobacterium tuberculosis*.^[6] Briefly, the BACTEC 960 system was used for mycobacterial culture after specimens had been decontaminated using Petroff's method. Cultures were incubated at 37°C for up to 42 days. Facilities were not available for definitive confirmation of isolates as members of the *M. tuberculosis* complex (MtbC), therefore the term

“positive culture” here refers to the growth of Acid Fast Bacilli (AFB) in culture.

Drug solutions

For DST using BACTEC MGIT 960, 4 ml of sterile distilled water was added to a lyophilized vial containing low concentration of each drug (Becton Dickinson). Part of this solution (0.1 ml) was aseptically pipetted into an MGIT tube to obtain the following final drug concentrations in the medium (low or critical concentrations): 1.0 µg/ml for streptomycin (SM), 0.1 µg/ml for isoniazid (INH), 1.0 µg/ml for rifampicin (RMP), and 5.0 µg/ml for ethambutol (EMB). In addition, for SM, INH, and EMB, stock solutions at higher concentrations were prepared by dissolving each high concentration of lyophilized drug (Becton Dickinson) in 2 ml of sterile distilled water. Part of this antibiotic solution (0.1 ml) was transferred into the MGIT tube, yielding the final drug concentrations (high) of 4.0 µg/ml for SM, 0.4 µg/ml for INH, and 7.5 µg/ml for EMB.

BACTEC Mycobacterium growth indicator tube 960 drug susceptibility testing

To each 7-ml MGIT tube, 0.8 ml of MGIT 960 growth supplement and 0.1 ml of the drug stock solution were aseptically added, and finally 0.5 ml of the test inoculum was added. For each isolate, a growth control (GC) tube with growth supplement and without drug was included. For this GC, the inoculum was prepared by pipetting 0.1 ml of the test inoculum with 10 ml of sterile saline to make a 1:100 dilution; 0.5 ml of GC inoculum was added to a drug-free MGIT tube. All the inoculated tubes (seven drug-containing tubes and one drug-free tube for each isolate) were placed into the BACTEC MGIT 960 instrument on the same day of inoculation. The relative growth ratio between the drug-containing tube and drug-free GC tube was determined by the system's software algorithm. If the relative growth in the drug-containing tube was equal to or exceeded that of the GC tube, the isolate was considered drug susceptible; if the relative growth was less than that in the GC tube, the isolate was considered drug resistant. The instrument did the final interpretation and reported the susceptibility results automatically.^[3,5,6] All patients with smear-positive TB were offered HIV testing after counseling at Wuse General Hospital.

Quarterly External Quality Control (EQC) for smear microscopy is conducted in ZMC by the NTBLCP. Although there is no EQC system for culture, Internal Quality Control is routinely performed using blind controls with known AFB-positive and AFB-negative specimens for every batch stained and for culture and DST. Data are

described as frequencies and proportions (%). Relationship is explored using Fisher's exact test. All probabilities are two-tailed and a *P* value of less than 0.05 was considered to indicate statistical significance. Analysis was done using STATA (version 7.0) (College station, TX, USA).

Results

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One hundred and seventeen (males 59; females 58) patients with chronic cough were investigated during the study period. Their ages ranged from 18 to 86 years (mean age \pm standard deviation: overall – 35.4 ± 10.5 years; for males – 37.1 ± 10.8 years; and for females – 33.8 ± 10.0 years). Of these, 31 had smear-positive TB. Seventy-two patients had positive sputum culture, although *M. tuberculosis* complex isolates were only confirmed in 39 of these and 4 were deemed to be contaminants, leaving 35 specimens for analysis of DST. DST was performed in 32 of the 35 isolates; 10 (31%) were resistant to at least one of the four drugs tested and 4 (13%) were resistant to the four drugs tested and were classified as multidrug resistant-TB (MDR-TB). The mean age of the MDR-TB patients (two males; two females) was 39.0 years. Six (19%) isolates were resistant to rifampicin and 7 (22%) to isoniazid. Thirty-one of the 32 patients with DST data had also been tested for HIV and 19 (61%) were HIV positive. Seven of the 19 (37%) HIV-positive and 3 of the 12 (25%) HIV-negative patients had resistant isolates to any of the four drugs (Fisher's exact, *P* = 0.7) [Table 1].

Discussion

This study confirms that there is a high prevalence of anti-TB drug resistance in Nigeria and describes for the first time the presence of MDR-TB among new patients by using an automated culture system. Individual isoniazid and streptomycin resistance had been reported in Nigeria in 7 and 2% of new

patients with TB in the 1970s,^[4] and subsequent unpublished studies using Lowenstein-Jensen media and proportion DST methods suggested that there was a rising trend in drug resistance. For example, Kolo reported in 1991 that 19, 13 and 29% of newly diagnosed patients with TB in Zaria had isoniazid, streptomycin and pyrazinamide resistance^[7] and resistance to these drugs was reported to have increased to 29, 14 and 42% by 2006,^[8] suggesting that although poorly documented, MDR-TB is likely to have been present among newly diagnosed patients in Nigeria for some time. But the World Health Organization (WHO), without an actual survey, estimates a much lower MDR-TB burden of 1.9 and 9.3% for new and previously treated patients, respectively (1); the results reported here are among unclassified patients and could have been from re-treatment patients. However, it is probable that WHO probably underestimates Nigeria's MDR-TB burden. Taken together, this re-echoes the need for good clinical management practices and a prospective countrywide DST survey. It is likely that MDR-TB emerged in the 1990s as Idigbe *et al* reported that 56% of the strains recovered from 96 patients not responding to anti-TB treatment were resistant to one or more of the drugs used, with 38% being resistant to isoniazid, although only 2% were resistant to rifampicin at that time^[9] and did not find an association with HIV infection.^[10] The high HIV co-infection observed in Abuja highlights the need to strengthen the linkage between TB and HIV services in Nigeria.

The study strongly argues for more concerted TB control measures. Although DOTS treatment centers increased from 1605 in 2002 to 2015 by the end of 2006,^[11] the continued expansion and implementation of the strategy throughout the country should be pursued vigorously to prevent further increases in drug resistance. Additionally, operational interventions such as contact tracing, screening and early diagnosis, especially of first degree relatives of patients with MDR-TB, should be instituted. Measures to provide second-line drugs, supervision of drug distribution and compliance, enforcement of DOTS protocols, and sustained training of all personnel in TB management are crucial to prevent further development of drug resistance, as these alternative drugs are less effective, but more expensive and toxic and usually require parenteral administration.

The prevalence of MDR-TB in this study among unclassified cases is much higher than results in larger studies in the region among newly diagnosed cases^[12] and further studies are required to obtain more precise estimates of resistance in defined groups. In addition, there are very few studies published in

Table 1: Resistance to first-line anti TB drugs in Nigeria (N = 31)*

	Isolates	
	HIV-positive	HIV-negative
Susceptible to all drugs	12	9
Any resistance	7	3
Resistance to:		
Rifampicin alone	1	-
Ethambutol alone	-	1
Streptomycin and isoniazid	2	1
Rifampicin and ethambutol	1	-
Resistance to all drugs tested	3	1

*Result of HIV test result was not available in 1 of 32 patients with DST; Fisher's exact, *P* = 0.7

the literature, evaluating the comparability of drug sensitivities established using solid and liquid media, and our findings might not be directly comparable to previous studies in the country. Laboratory capacity in Nigeria should be strengthened to include drug resistance surveillance. This could be done through the provision of faster, automated culture and DST facilities or automated polymerase chain reaction (PCR) technologies that are emerging elsewhere. Future studies should investigate if extensively drug resistant TB (XDR-TB) strains are also affecting newly diagnosed patients with TB in Nigeria and if strains are still sensitive to fluoroquinolones and other alternative agents. In the short term, there is a need to establish one or more national reference laboratories and linkage with existing supra-national reference laboratories (SRL) overseas for technical support, quality assurance and surveillance.

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