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Drug resistance profile of *Mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients in Jos, Nigeria

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Summary The drug resistance profile of 100 *Mycobacterium tuberculosis* isolates from pulmonary tuberculosis (PTB) cases in Jos, Nigeria, was investigated between August 2006 and September 2007. Drug susceptibility testing for 50 new, 11 follow-up and 39 unclassified cases of PTB was performed on Löwenstein–Jensen medium by the proportion method, using isoniazid (0.2 μg/ml), rifampicin (40 μg/ml), ethambutol (2 μg/ml) and streptomycin (4 μg/ml). Susceptibility to all four drugs was found in 76, 62 and 55%, and multidrug resistance (combined resistance to isoniazid and rifampicin with or without resistance to any other drug) in 4, 31 and 18% of the new, unclassified and follow-up cases, respectively. Monoresistance was found in 15% of the cases. Nine of the 16 isolates (56%) showing multidrug resistance were resistant to all four drugs. These findings are critical and the risk to public health is high, particularly with an overall multidrug resistance of 16%. We suggest that TB management and control programs in Jos are revised to enhance patient's accessibility to treatment sites, promote patients' adherence to drugs, improve diagnostic practices, regularly assess drug resistance profiles, and undertake contact tracing for patients with multidrug-resistant TB.

1. Introduction

Tuberculosis (TB) came under significant control in the twentieth century following the discovery of effective drug combination therapy and interventions by global health programs. The cure rate with the use of anti-TB drugs was likewise remarkable.[1,2] The changing pattern of global health events over the last two decades, particularly the emergence and spread of HIV, with its characteristic immunosuppressive effects on the host, have aided the resurgence of infections caused by *Mycobacterium tuberculosis*, thus necessitating the advancement of alternative

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strategies for effective combat of the emerging global problem of drug-resistant *M. tuberculosis*.[3,4] Strains of *M. tuberculosis* resistant to anti-mycobacterial agents, including multidrug-resistant TB (MDR-TB) strains (MDR = combined resistance to isoniazid and rifampicin with or without resistance to any other drug), have been reported globally.[5–8] The recently described extensively drug-resistant TB (XDR-TB) has also been reported in South Africa and elsewhere[8] where studies have been carried out.

Nigeria, a nation in sub-Saharan Africa, with a population of about 150 million, is considered a high-burden zone for HIV and therefore TB, with about 50% of people living with HIV/AIDS dually infected with the TB bacillus. Jos city, located in the north central region of the country, with a population of about 3 million people, has a TB incidence of 293 per 100 000 (Dr L.A. Ibrahim, personal communication). Although the national TB control program recommends the use of directly observed treatment, short-course (DOTS),[9] its successful implementation has been difficult in Jos due to erratic drug supplies and poor organizational policies. It is also noteworthy that studies on drug susceptibility profiles of *M. tuberculosis* in Nigeria are inconsistent and scarcely reported due to deficiencies in diagnostic and research materials. A study in Ibadan city, southwestern Nigeria, reported a rather high MDR-TB prevalence of 53%.[10]

The aim of this preliminary study was to determine the drug susceptibility profile of *M. tuberculosis* in Jos, Plateau State, Nigeria, the first of its kind to be conducted here.

2. Materials and methods

2.1. Specimens

A total of 137 sputum samples positive for acid-fast bacilli (AFB) by Ziehl–Neelsen (ZN) method were picked up when available from the microbiology laboratories of four different TB units in Jos, Plateau State, Nigeria, during August 2006–September 2007. Centers A, B and D are located in Jos town, with Center D situated in the Jos University Teaching Hospital, which is a referral hospital for the entire north central zone of Nigeria, while Center C is in a suburban village area of Jos city. The specimens were categorized as new (new TB patients, which may include undisclosed previously treated cases, Centers A, B and C; $n = 86$) and unclassified (Center D; $n = 51$). All the samples were collected and transported in equal volume of 1% cetylpiridinum chloride (CPC)-sodium chloride for digestion and decontamination. Microscopy-positive or -negative purulent sputum specimens ($n = 94$), from patients 2 months after the start of intensive phase anti-TB treatment from Centers A, B and C were similarly treated.

Specimens were maintained in CPC at room temperature for 1–4 d and processed weekly for Löwenstein–Jensen (LJ) culture. Digested specimens were washed in sterile distilled water by centrifugation at 3500 *g* for 15 min to remove CPC. The centrifugation process was repeated for bacilli sedimentation after adding 10 ml sterile distilled water. Sediments were homogenized in 1 ml distilled water and 0.5 ml homogenate was inoculated on paired LJ egg-based slopes. All the cultures were incubated at 37 °C with weekly examination for growth of *Mycobacterium* spp. until 8 weeks.

Slow (2–8 weeks) growers showing evidence of *M. tuberculosis* colonies[11] were further examined for catalase production at room temperature and at 37 °C and in the nitrate reductase test. All isolates showing positive reaction for catalase and nitrate reductase assays were subcultured on LJ slopes to obtain pure and confluent growth required for drug susceptibility testing.

2.2. Drug susceptibility testing

Drug susceptibility testing for the 100 pulmonary isolates identified as *M. tuberculosis*, using isoniazid, rifampicin, ethambutol and streptomycin, was performed on LJ medium by the proportion method.[11,12] The isolates comprised 50 new and 11 follow-up cases from Centers A, B and C and 39 unclassified cases from Center D.

The patient information used was obtained from the laboratory requisition form. The laboratory aspect of this study was carried out in the microbiology research laboratory of the University of Jos, Nigeria.

2.3. Media preparation

LJ medium was prepared for each of the four drugs, with final concentrations of 0.2 μg isoniazid, 2 μg ethambutol, 40 μg rifampicin and 4 μg streptomycin. The drug-containing and drug-free LJ media were steamed at 85 °C in a slanting position for 50 min and prepared ready for use after storage at room temperature for 48 h to exclude contamination.

2.4. Bacterial suspension

Isolates of *M. tuberculosis* (up to 10 isolates at a time) were prepared for drug susceptibility testing by the proportion method. Using a 3 mm internal diameter (24 standard wire gauge) wire loop, about 4 mg fresh *M. tuberculosis* culture was scraped from LJ medium into 500 μl sterile distilled water in a bijou with five glass beads and vortexed for about 30 s to homogenize. The suspension was made up to 4 ml volume by adding 3.5 ml sterile distilled water and allowed to settle for about 30 min before gently aspirating the upper portion (1 mg/ml) into a fresh bijou bottle (S1 suspension). S1 was further diluted 10-fold to obtain S2–S4.

Bacterial suspensions were inoculated by concentrations (S1–S4) into drug-free and drug-containing LJ slopes and incubated at 37 °C. Growth was recorded at 28 and 42 days as: +++ for confluent growth, ++ for more than 100 colonies and 1–100 actual number of colonies. Susceptibility or resistance was recorded when the proportion of bacteria in drug-containing medium to that of drug-free medium was <1 or ≥ 1, respectively.

3. Results

In total, 115 isolates [57/86 (66%) new; 41/51 (80%) unclassified and 17/94 (18%) follow-up cases] of *Mycobacterium* spp.

Table 1 Drug resistance profile of *Mycobacterium tuberculosis* isolated from new and unclassified cases of pulmonary tuberculosis in Jos, Nigeria

Total no. cases examined				Resistance profile *n* (%)					
Center[a]	ZN (+)	Culture (+)	DST	PS	EMB	RIF	STR	MDR	MULT
A	29	21	21	15 (71)	0	2 (10)	4 (19)	0	
B	24	16	9	8 (89)	0	0	1 (11)	0	
C	33	20	20	15 (75)	0	0	2 (10)	2 (10)	1 (5)
Total	86	57	50	38 (76)	0	2 (4)	7 (14)	2 (4)	1 (2)
D	51	41	39	24 (62)	1 (3)	0	2 (5)	12 (31)	

DST: drug susceptibility test; EMB: ethambutol; MDR: multidrug resistant (combined resistance to isoniazid and rifampicin with or without resistance to any other drug); MULT: resistance to any drug combination other than isoniazid and rifampicin; PS: pan-susceptibile (susceptible to all four drugs); RIF: rifampicin; STR: streptomycin; ZN: Ziehl–Neelsen's acid fast bacilli.
[a] Specimens from Centers A, B and C are from new cases and those from Center D are unclassified cases.

Table 2 Drug resistance profile of *Mycobacterium tuberculosis* isolated from patients during intensive phase therapy in Jos, Nigeria

Total no. cases examined *n* (%)					Resistance profile *n* (%)			
Center	Total	ZN (+)	Culture (+)	DST	PS	MDR	STR	RIF
A	23	1 (4)	3 (13)	3 (13)	2 (67)	0	0	1 (33)
B	38	7 (18)	9 (24)	4 (11)	2 (50)	1 (25)	1 (25)	
C	33	4 (12)	5 (15)	4 (12)	2 (50)	1 (25)	1 (25)	
Total	94	12 (13)	17 (18)	11 (12)	6 (55)	2 (18)	2 (18)	1 (9)

DST: drug susceptibility test; MDR: multidrug resistant (combined resistance to isoniazid and rifampicin with or without resistance to any other drug); PS: pan-susceptibile (susceptible to all four drugs); RIF: rifampicin; STR: streptomycin; ZN: Ziehl–Neelsen's acid fast bacilli.

were obtained from 231 LJ cultures of sputum specimens. Phenotypic identification methods showed 50/86 (58%) new, 39/51 (76%) unclassified and 11/94 (12%) follow-up cases to be *M. tuberculosis* (Tables 1 and 2).

The drug susceptibility testing for the 100 isolates identified as *M. tuberculosis* showed 76, 62 and 55% susceptibility to all four first-line drugs and 4, 31 and 18% multidrug resistance for the new, unclassified and follow-up cases, respectively (Tables 1 and 2).

Monoresistance was observed in 11 streptomycin isolates (11%), 1 ethambutol isolate (1%) and three rifampicin isolates (3%). Monoresistance to isoniazid was not observed. Multiple resistance to rifampicin and streptomycin was observed in one isolate (1%) (Table 1).

The total MDR-TB prevalence of 31% in Center D, the source of the unclassified cases, was higher than that of the three other centers, in the new (4%) and follow-up cases (18%), combined (Tables 1 and 2). However, the difference was not significant using a two-tailed *t* test ($P = 0.339$).

4. Discussion

The level of resistance to anti-TB drugs observed in this study is high, particularly with an MDR-TB rate of 16% compared with a report from Ghana, a neighboring West African country, with an MDR-TB rate of 2%.[13] A study by Kehinde et al.[10] in Ibadan, Nigeria reported a multidrug resistance prevalence of 53% in new cases of TB. There could be selection bias in this reported study. Because the University Teaching Hospital Ibadan is a reference hospital that receives patients with chronic and life-threatening medical conditions from different parts of the country, some of the patients reported as new cases may therefore, have failed therapy during previous TB medications elsewhere.

The results of this study further showed that 12% of follow-up patients excreted *M. tuberculosis* in sputum 2 months after intensive phase treatment, 55% of the isolates were susceptible to all the four drugs tested and 18% were MDR. Although the sample sizes are small, the results suggest that multidrug resistance is likely to pose a significant risk to public health as well as impede the success of TB management in Jos, Nigeria if adequate control measures are not urgently implemented. The patients' adherence to administered drug dose and the compliance ethics of the health care staff in Jos may require evaluation and review for better results. The level of MDR-TB (31%) observed in Center D was higher than that for Centers A, B and C for new (4%) and follow-up cases (18%) combined and this level of multidrug resistance in supposedly new cases gives cause for concern. The scarcity of relevant information on the patients' laboratory investigation form in Center D was a major limitation, as patients' categories could not be ascertained from available information. TB cases that report to Center D are likely to include poorly treated cases referred from many secondary

and primary centers, given that Center D is a tertiary hospital and referral center for the north central zone of Nigeria, with a population of about 20 million people. It is therefore possible that a sizable number of the 39 cases from Center D represented a selection of referred difficult cases that may have failed or defaulted from previous treatment.

Jos University Teaching Hospital, site of Center D, also has the largest HIV treatment and care unit in the country, with over 12 000 patients enrolled for care; 40% have TB, with many cases of re-infection, relapse and treatment failure following poor TB diagnostic and monitoring approaches. There is therefore an urgent need to put in place strategies for effective early diagnosis and management of TB and MDR-TB. The high level of MDR-TB may also be an indication of XDR-TB. However, some default patients who conceal their history and readily get admitted to different treatment centers as new cases may constitute a significant source of the spread of MDR-TB in the community.

Center C, with an MDR-TB prevalence of 10% among new TB cases, is a suburban village area of Jos with scarcely standard socio-economic conditions. Poverty and a lack of knowledge about standard hygiene probably contribute to the high MDR-TB level.

The national guideline for management of TB uses DOTS and fixed-dose drug combinations. However, DOTS has been difficult to implement because of poor organization of facilities, stock-out of drugs, patient resource constraints, and the fact that patients are often unable to access treatment sites due to the long distances between DOTS centers and their homes. In addition, the program still relies on the use of ethambutol and isoniazid maintenance phase treatment instead of a 6 months' rifampicin regimen. However, Center D, using a non-DOTS approach, recommends the rifampicin-based regimen for treatment of TB. In the light of these shortcomings, it is necessary to consider the implementation of community-based DOTS programs in order to minimize some of the constraints encountered by patients, enhance patients' drug adherence and improve treatment outcome.

This study also indicated monoresistance to streptomycin (11%) and rifampicin (3%). Resistance to streptomycin may have been selected following previous and regular administration in the treatment of other bacterial diseases. Monoresistance to rifampicin, a major component of the anti-TB regimen, is said to be rare. The monoresistance to rifampicin observed cannot be explained within the scope of the study. However, rifampicin monoresistance can be used as a surrogate marker for MDR-TB.[14,15]

The limitation in the implementation of DOTS in Jos may have contributed significantly to the high level of MDR-TB observed in the study. There is rising prevalence of TB in Jos due to HIV/TB dual infection. The situation needs urgent attention, with special consideration to the high prevalence of MDR-TB observed in this study. Improved TB diagnosis, including screening for early diagnosis, and surveillance studies for MDR-TB and perhaps XDR-TB should be implemented.

The need for compliance with standard strategies at all levels of TB prevention and control in Jos, and in all of Nigeria, cannot be overemphasized.

Authors' contributions: AEA, JI and SLP conceived and designed the study; AEA and YBD carried out the laboratory investigations (ZN microscopy, culture and drug susceptibility tests for *M. tuberculosis*); AEA and JI analyzed and interpreted the data and drafted the manuscript; JI reviewed the manuscript critically for medical and intellectual content. All authors read and approved the final manuscript. AEA is guarantor of the paper.

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