Multidrug Resistance to *Mycobacterium* Tuberculosis in a Tertiary Hospital

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Objective: The magnitude of drug-resistant *Mycobacterium tuberculosis* infection (MDR-TB) in Nigeria, the most populous country in sub-Saharan Africa, is largely unknown. This information would assist policymakers to develop intervention strategies against tuberculosis (TB) in the country.

Materials and Methods: This is a one-year laboratory-based study. Specimens from suspected new TB patients sent to the TB laboratory of the Department of Medical Microbiology, University College Hospital Ibadan, Nigeria from May 1, 2005 to April 27, 2006 were processed and analyzed. The specimens were stained with Ziehl-Neelsen (Z-N) reagents and cultured on Lowenstein-Jensen medium, incubated at 37°C for 6–8 weeks. Isolates were confirmed as MDR-TB by Z-N reactions and biochemical methods. Drug susceptibility to streptomycin, ethambutol, rifampicin and isoniazid was done using Bactec 460 TB radiometric method.

Results: Of the 1,120 specimens processed, 80 (7.1%) were smear positive, while 56 (5.0%) were culture positive, even though the association was not statistically significant ($p>0.05$). Culture contamination rate was 8.8%. Thirty (53.6%) of the culture positive isolates were resistant to both isoniazid and rifampicin, while 26 (46.4%) were susceptible. About half—53.3%—of the resistant isolates were from the antiretroviral clinic, while 10 (33.4%) were from peripheral centers.

Conclusion: This study shows that MDR-TB is emerging in Nigeria. Further studies on MDR-TB are urgently needed in the country to ascertain the magnitude of the problem and to proffer solutions to it.

Key words: prevalence ■ tuberculosis ■ sub-Saharan Africa

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INTRODUCTION

Globally, tuberculosis (TB) constitutes a serious public health problem. TB has the largest impact among the working-age population and contributes significantly to loss of economic productivity. Multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) is a form of TB that develops in patients who do not complete the proper treatment for the disease. This can occur when a physician does not prescribe proper treatment regimen or when a patient is unable to adhere to therapy with first-line drugs. MDR-TB tends to occur in countries where there are inadequate trained medical personnel and erratic drug supply. Each year, approximately 400,000 new cases of MDR-TB occur worldwide, mostly in sub-Saharan Africa and Asia.[1] The World Health Organization (WHO) estimates that the average MDR-TB patient infects up to 20 other people in his/her lifetime.[2] Bacteriologically, resistance is caused by a genetic mutation which makes the drug ineffective against the mutant bacilli. MDR-TB is said to occur when the organism is resistant to ≥2 first-line drugs (isoniazid and rifampicin).[3] A recent global survey conducted by the WHO/IUATLD (International Union Against TB and Lung Diseases) in 2003 show that one-third of MDR-TB cases were resistant to all four of the first-line drugs tested.[4] Treatment of MDR-TB involves use of second-line drugs, which are costly, scarce, toxic and require longer durations of therapy.[5] MDR-TB is particularly threatening because without proper treatment, super-resistant strains can emerge for which there is no current cure.[1] This makes MDR-TB to be one of the greatest challenges facing public health. The main cause of MDR-TB is iatrogenic, although microbial, clinical and other issues are also contributory factors. The incidence of MDR-TB has increased since the first drug treatment in 1943, with subsequent widespread use of rifampicin since the 1970s.[4] The overall prevalence of MDR-TB is strongly associated with the burden of TB in a particular country.[6] Nigeria ranks fourth out of the 22 countries with the highest burden of the disease,[7] but in spite of this, information on the magnitude of MDR-TB in the country is largely unavailable.[3] Therefore, this study aimed to determine the prevalence of MDR-TB in

Ibadan, Nigeria. The information obtained will provide a baseline data that can be used to design intervention programs for prevention of MDR-TB not only in Nigeria but in other resource-poor countries with high burden of TB.

MATERIALS AND METHODS

Study Area

This prospective laboratory-based study was carried out in University College Hospital (UCH), Ibadan, southwestern Nigeria. Ibadan is the capital city of Oyo state of Nigeria and is believed to be the most populous indigenous city in west Africa. The TB laboratory at UCH, Ibadan, is a designated reference center for the isolation of *Mycobacterium tuberculosis*. It receives support from the Damien Foundation (Belgium) through the National TB Control Program. Specimens are received from medical outpatient clinics, including an antiretroviral clinic, neighboring hospitals and from distant peripheral centers.

Study Population

Specimens from suspected new TB patients from within and outside UCH that were sent to the TB laboratory between May 1, 2005 and April 27, 2006 were processed. Information such as age, sex, type of specimens and clinical diagnosis were extracted from the accompanying request forms.

Laboratory Investigations

Three specimens (labeled a, b and c) from each patient were collected into well-labeled wide-mouth containers covered with lids. They were then transported to the laboratory for processing. Specimens containing saliva instead of sputum were discarded. Each specimen was smeared, air dried, fixed and stained with Ziehl-Neelsen (Z-N) reagents using a known acid-fast bacilli (AFB)-stained slide as positive control and a stained slide made of egg albumin as negative control. Results were recorded according to the grading system of the IUATLD[8] as –, scanty, +, ++ or +++ AFB. Then, one of the specimens was cultured onto Lowenstein-Jensen (L-J) slope incubated at 37°C for 6–8 weeks. *Mycobacterium tuberculosis* strain H37RV and sterile L-J medium were used as positive and negative controls, respectively. Growth on L-J slope was restained with Z-N reagents at 2, 4, 6 and 8 weeks of incubation .Thereafter, visible growth was confirmed as *M. tuberculosis* by standard biochemical methods.[9] Confirmed isolates were collated and stored at 4°C in the refrigerator for subsequent drug susceptibility testing, while those cultures that were contaminated were noted and discarded. Revived *M. tuberculosis* isolates on L-J slope were tested for drug susceptibility against streptomycin, ethambutol, isoniazid and rifampicin using semiautomated radiometric method (Bactec 460^{TB})[10] at TB laboratory of the Nigerian Institute for Pharmaceutical Research and Development Abuja which receives support from the National Institute of Health (USA). Patients from whom MDR-TB were isolated were referred to the attending physician for further evaluation and treatment.

Table 1. Age and sex distribution of patients who submitted specimens

	Sex		
	Male	Female	Total
Age Group (Years)	No (%)	No (%)	No (%)
<10	13 (65.0%)	7 (35.0%)	20 (1.8%)
10–19	48 (60.7%)	31 (39.3%)	79 (7.1%)
20–29	99 (57.9%)	72 (42.1%)	171 (15.3%)
30–39	168 (60.2%)	111 (39.8%)	279 (24.9%)
40–49	206 (66.5%)	104 (33.5%)	310 (27.6%)
50–59	34 (33.3%)	68 (66.7%)	102 (9.1%)
60–69	36 (60.0%)	24 (40.0%)	60 (5.4%)
70–79	32 (60.4%)	21 (39.6%)	53 (4.7%)
≥80	26 (56.5%)	20 (43.5%)	46 (4.1%)
Total	662 (59.1%)	458 (41.9%)	1,120 (100.0%)

Table 2. Smear microscopy and culture results

	Smear Microscopy	Culture
Test Result	No (%)	No (%)
Positive	80 (7.1%)	56 (5.0%)
Negative	1040 (92.9%)	1064 (95.0%)
Total	1120 (100.0%)	1120 (100.0%)

Data Analysis

Data were entered into the computer using the Statistical Packages for Social Sciences® (Windows version 10.0, Chicago, IL). Data presentation was done with the aid of tables and charts. Demographic characteristics and laboratory variables were described in form of proportions and percentages.

Ethical Approval

Ethical approval was waived because the study was laboratory based.

RESULTS

Demographic Data

Specimens from 1,120 patients were processed during the study period. Six-hundred-sixty-two (59.1%) were from male patients, while 458 (41.9%) were from female giving a male:female ratio of 1.4:1. Only 20 (1.8%) of the specimens were from children, while a significant percentage came from age bracket 20–49 years. The age and sex distribution of patients who submitted specimens is shown in Table 1.

The majority of the specimens were sputum accounting for 82.6%, while the least frequent, comprising 25 cases (2.2%), were cerebrospinal fluid. Figure 1 shows the type of specimens processed during the period of study. A significant number 640 (57.1%) of the specimens were sent from the antiretroviral clinic located within the hospital, 320 (28.6%) from medical outpatient clinic, while 160 (14.3%) were from the peripheral health centers.

Laboratory Data

Eighty (7.1%) of the specimens were smear positive, while a lower percentage (5.0%) were culture positive even though the association was not statistically significant ($p>0.05$) (Table 2). All the culture-positive samples were from sputum specimens. Culture contamination rate was 8.8% (99 of the 1,120 specimens processed). Sixteen (1.4%) of them were smear negative but positive for culture, while eight (0.7%) were screened smear positive but culture negative. Only 40 (3.6%) were smear and culture positive, while the majority 1,056 (94.3%) were negative for the two tests.

Furthermore, 30 (53.6%) of the 56 culture-positive isolates were resistant to both isoniazid and rifampicin, 16 (28.9%) to all the four drugs tested (isoniazid, rifampicin, ethambutol and streptomycin), while 26 (46.4%) were susceptible. Of the 30 resistant isolates, 16 (53.3%) were from sputum sent from the antiretroviral clinic, four (13.3%) from medical outpatient clinic, while 10 (33.4%) were from distant health centers (Figure 2).

DISCUSSION

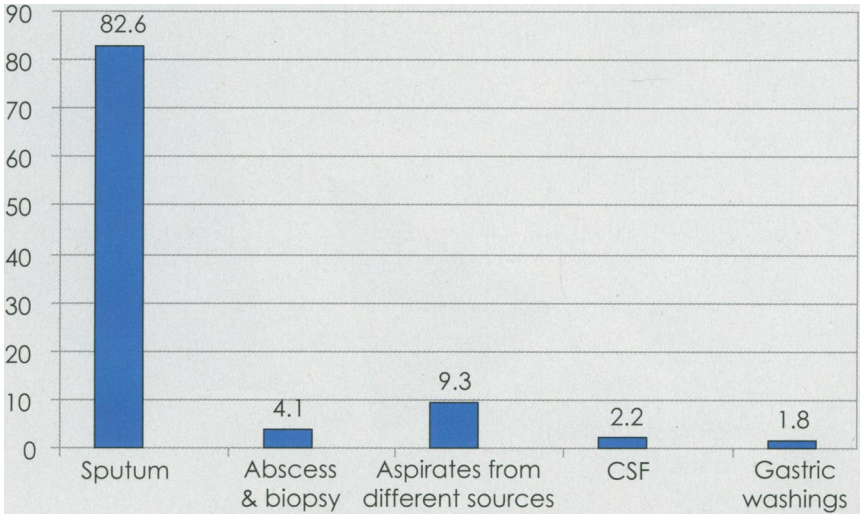
TB was considered on the brink of elimination in the developed world until the late 1980s, when new HIV-related TB cases and MDR-TB surfaced.[11] In developing countries, however, TB has remained an important public health problem, exacerbated in the last decade by poverty, demographic changes and the rapid spread of HIV.[12] The emergence of MDR-TB in resource-poor countries with high burden of the disease constitutes a big challenge to TB control program.

The majority of the specimens processed in this study were sputum, which supports the claim that pulmonary TB is the commonest presentation of TB. This finding is consistent with previous studies conducted in our environment and elsewhere.[13-15] Prevalence of extrapulmonary TB is low as evidenced by the low number of nonsputum specimens processed. This may be related to difficulty in obtaining such samples. A recent study carried out in the same centre by Ige et al. documented that cerebrospinal fluid accounted for 7.8% of the total specimens submitted for TB diagnosis, 4.1% for lymph node biopsy and 1.8% for abdominal aspirate.[15]

The study revealed that a significant percentage of the specimens processed were from the antiretroviral clinic located within the hospital. This is not surprising because TB is one of the commonest presentations of HIV/AIDS infection worldwide. The situation in sub-Saharan Africa is even more worrisome because the TB epidemic in the region is largely HIV driven.[4]

The majority (67.9%) of the specimens came from

Figure 1. Types of specimens



the age group 20–49 years (Table 1). This is in support of a previous study done in our environment which reported that more than half of infected TB cases came from people age <50 years[14] but in contrast to what obtained in developed countries where the elderly (age >60 years) were mostly affected.[16] The affected age bracket (20–49 years) corresponds to the productive workforce of the population, thus constituting a huge economic burden to the society.

A higher percentage of specimens were smear positive compared with those that were culture positive. This may be due to high contamination rate associated with our culture technique. There is a need to improve upon the standard of operation in our laboratory. At present, many of the TB laboratories in disease-endemic countries of sub-Saharan Africa are not affiliated to any supranational laboratory. This is necessary for laboratory proficiency and quality assessment. The inability of many of the low-income countries with a high burden of TB to accurately isolate *M. tuberculosis* on culture media has necessitated WHO to recommend smear microscopy as a case-defining tool. The method is rapid, specific and does not require highly skilled personnel. Furthermore, it is affordable to the generality of people mostly affected by the disease; however, its low sensitivity, especially in HIV coinfection, children and extra pulmonary diseases, is one of its major drawbacks.[17]

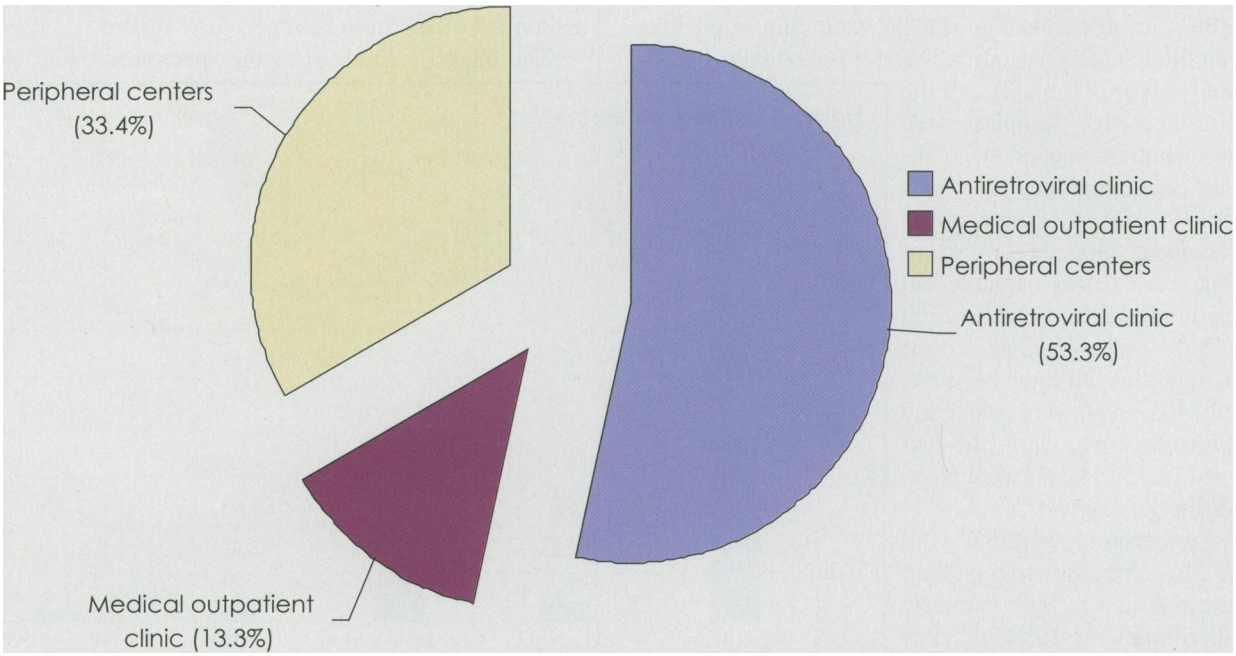
Sixteen (1.4%) of the sputum specimens were smear negative but positive for culture. Smear-positive cases are the focus of DOTS (Directly Observed Treatment Short Course–WHO global strategy for TB control) program. These undiagnosed cases are the reservoirs of disease transmission in the community. They tend to overload the laboratory and erode the predictive value of microscopy.

The definitive diagnosis of TB is by isolating the causative organism on selective medium. The required 6–8-week incubation period causes delay in initiating treatment and encourages the transmission of the disease in the community. This is one of the drawbacks of the culture method. There is an urgent need to introduce new diagnostic methods that would abbreviate diagnostic delay often associated with TB diagnosis in highly prevalent settings. This will allow prompt treatment of infected cases and ultimately a disruption in the cycle of disease transmission.

Of paramount public health importance were 30 (53.6%) of the 56 *M. tuberculosis* isolates that were resistant to both isoniazid and rifampicin. Drug resistance might have developed as a result of improper implementation of DOTS strategy. This may range from inadequate drug regimen prescribed by medical personnel to poor-quality/inadequate drug supply and poor drug compliance by the patient, hence the need to strengthen DOTS program in the country is urgent.

The results of this study cannot be used to ascertain the magnitude of MDR-TB in the community being hospital-based research. However, implementation of a sound infection control policy is needed to curtail the spread of the disease within the hospital setting.

Figure 2. MDR-TB isolates according to source



The treatment of MDR-TB requires the use of second-line drugs. The drugs are expensive, scarce, toxic and require long durations of therapy. The WHO and its partners through Green-Light Committee (GLC) advocate a strong foundation for DOTS program and a solid plan for management of MDR-TB for countries to benefit from GLC. To this end, the committee is saddled with the responsibility of providing technical assistance and second-line drugs at reduced price to countries with high burden of MDR-TB.[4]

More than half (53.3%) of the MDR-TB isolates came from the antiretroviral clinic, which is supported by the U.S.-funded President Emergency Fund for AIDS Relief (PEPFAR project). This is not surprising because TB is one of the first opportunistic infections to appear in HIV-infected patients. There is an urgent need to incorporate TB services into HIV treatment, care and a support program, especially in areas with high prevalence of HIV infection. This will require strengthening of DOTS strategy by interventions such as active case finding and treatment of latent TB infection in HIV infected individuals.[11,18] The strong association of both infections is a cause for concern worldwide, especially in sub-Saharan Africa, where up to 80% of TB patients are also HIV infected.[19]

One-third of the resistant isolates were from the community health centers (Figure 2). Active community participation needs to be incorporated into the DOTS strategy in order to bring TB services to the doorsteps of the rural dwellers who constitute more than half of the population in developing countries.

In conclusion, more studies are needed to ascertain the magnitude of MDR-TB in Nigeria. There is a need to strengthen DOTS strategy in Nigeria through expansion of laboratory capacity to carry out quality-assured microscopy, culture and drug susceptibility in order to reduce TB burden in the country.

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REFERENCES

1. The Lilly MDR-TB Partnership. www.lillymdr-tb.com Multi-drug resistant tuberculosis. Accessed 07/10/06.

2. World Health Organization. Global tuberculosis control: surveillance, planning and financing. WHO Report. WHO/HTM/TB/2006.362. Geneva, Switzerland; 2006:107-109.

3. World Health Organization. Anti-tuberculosis drug resistance in the world. Third Global Report. The WHO/IUATLD global project on anti-tuberculosis drug resistance surveillance (1999–2002). WHO/HTM/TB/2004. 343. Geneva, Switzerland; 2004:25-45.

4. World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis. WHO Report. WHO/HTM/TB/2006. 361. Geneva, Switzerland; 2006:32-56.

5. Carlevaro P. Multi-drug resistant tuberculosis: together we can fight it—the Lilly MDR-TB Partnership. *Ess Drugs Monitor WHO.* 2005;34:26-27.

6. Blower SM, Chon T. Modeling the emergence of the "Hot Zones": tuberculosis and the amplification dynamics of drug resistance. *Nat Med.* 2004;10(10):1111-1116.

7. World Health Organization. Global tuberculosis control: surveillance, planning and financing. WHO Report. WHO/HTM/TB/2005.349. Geneva, Switzerland; 2005:1-112.

8. Enarson DA. Laboratory diagnosis of pulmonary tuberculosis. In: Enarson DA, ed. Management of tuberculosis: a guide for low income countries. 5th ed, Paris, International Union Against Tuberculosis and Lung Diseases; 2000:1-50.

9. Cowan ST, Steel KJ. Acid-Fast Rods. In: Barrow GI, Feltham RKA, eds. Cowan and Steel's Manual for the identification of Medical Bacteria. Cambridge, UK: Cambridge University Press; 1995:91-93.

10. Koneman EW. Mycobacterial susceptibility testing. In: Koneman EW, Allan SD, Janda WM, eds. Color atlas and textbook of diagnostic microbiology. 5th ed. Philadelphia, PA: Lippincott-Raven Publishers; 1997:935- 937.

11. Family Health International. Tuberculosis control in high HIV prevalence areas. A strategic framework. FHI Report. Arlington, VA; 2001:1-110.

12. Abouya L, Coulibaly IM, Wiktor SZ, et al. The Cote-d'Ivoire national HIV counseling and testing program for tuberculosis patients: implementation and analysis of epidemiological data. *AIDS.* 1998;12:505-512.

13. Ankrah TC. The history of tuberculosis and its resurgence in the community. *WAJM.*1997;16:1-5.

14. Erhabor GE, Adebayo RA, Omodara JA, et al. Ten year review of patterns of presentation and outcome of pulmonary tuberculosis in Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria. *Nig J Health Sci.* 2003;3(1):34-37.

15. Ige OM, Sogaolu OM, Ogunlade OA. Pattern of presentation of tuberculosis and the hospital prevalence of tuberculosis and HIV co-infection in University College Hospital, Ibadan, Nigeria—a review of five years (1998–2002). *Afr J Med Med Sci.* 2005;34:329-333.

16. Tola E, Kochi E. Elimination of tuberculosis from Europe and the World. *Eur Res J.* 1991;4:1159-1164.

17. World Health Organization. Laboratory services in tuberculosis control part 1: organization and management. WHO Report. WHO/TB/98.258. Geneva; 1998:1-65.

18. Centers for Disease Control and Prevention. Prevention and treatment of tuberculosis among patients infected with human immunodeficiency virus: principle of therapy and revised recommendations. *MMWR.* 1998;47:20.

19. Reid A, Scano F, Getahun H, et al. Towards universal access to HIV prevention, treatment, care and support: the role of tuberculosis/HIV collaboration. *Lancet Infect Dis.* 2006;6:483-495. ■