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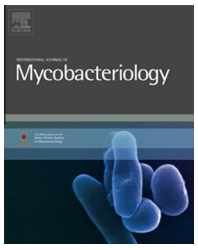

Multidrug resistance and demography of newly diagnosed tuberculosis patients in Cross River State, Nigeria

Benjamin Thumamo Pokam [a,*], *Anne E. Asuquo* [b], *Lydia N. Abia-Bassey* [b], *Mandor B. Idasa* [c], *Nse O. Umoh* [d,1], *Francis O. Eko* [e], *Nalin Rastogi* [f]

[a] *Department of Medical Laboratory Science, Faculty of Health Sciences, University of Buea, Cameroon*
[b] *Department of Medical Laboratory Science, College of Medical Sciences, University of Calabar, Nigeria*
[c] *University of Calabar Teaching Hospital, Calabar, Nigeria*
[d] *Ministry of Health, Uyo, Akwa Ibom State, Nigeria*
[e] *Morehouse School of Medicine, Atlanta, USA*
[f] *WHO Supranational TB Reference Laboratory, TB & Mycobacteria Unit, Institut Pasteur de la Guadeloupe, 97183 Abymes, Guadeloupe, France*

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ABSTRACT

Setting: Nigeria has the world's fourth largest tuberculosis burden, and multidrug resistant tuberculosis (MDR-TB) represents a serious public health problem.

Objectives: To describe the demography of TB patients and determine the susceptibility of *Mycobacterium tuberculosis* isolates to the major TB drugs.

Methods: One hundred and thirty-seven newly diagnosed TB patients (26 (19%) being HIV positive) from all age groups were recruited into the study. Each specimen was cultured using BACTEC MGIT960, followed by inoculation and growth on Lowenstein–Jensen (LJ) medium. Primary identification was carried out using an immunochromatographic technique (Capilia TB-Neo), and further confirmed by genotyping. Drug susceptibility testing (DST) was carried out by the agar proportion method.

Results: Of the 97 pure mycobacterial cultures on LJ medium, 81 (83.5%) isolates were identified as *M. tuberculosis* complex, while 16 (16.5%) were Capilia negative. DST was carried out on 58 isolates. The drug susceptibility pattern showed that resistance occurred in 16 (27.6%) for streptomycin, 11 (19%) for isoniazid, 9 (16%) for rifampicin, and 10 (17.2%) for ethambutol. Rifampicin monoresistance occurred in 2 (3.4%) cases. MDR (combined resistance to isoniazid and rifampicin), also involving resistance to streptomycin and ethambutol, occurred in 6/58 (10.3%) isolates; although laboratory cross-contamination could not be excluded in 4/6 MDR strains with identical MIRU patterns characterized by consecutive strain numbers. Considering that first out of these 4 isolates was not due to laboratory carryover, the results of this study still report a minimal MDR-TB rate of 3/58 (5.2%) among newly diagnosed TB patients in Cross River State, Nigeria.

Conclusions: An increase in drug resistance was observed in this study as compared with previous studies in the country. Hence, introduction of culture in routine diagnostic mycobacteriology laboratories will prevent the emergence and dissemination of MDR-TB, while

* Corresponding author. Address: Department of Medical Laboratory Science, Faculty of Health Sciences, University of Buea, P.O. Box 63, South West Region, Buea, Cameroon. Tel.: +237 77358743.
E-mail address: thumamo@yahoo.fr (B.T. Pokam).
[1] Directorate of Public Health

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improved quality control strategies would in parallel prevent laboratory cross-contamination, thereby reducing mislabeling, unnecessary treatment, and drug toxicity for patients.

Introduction

Globally, tuberculosis (TB) continues to pose major health challenges that result in disability and death. Nigeria has the world's fourth largest TB burden; with nearly 460,000 estimated new cases annually [1]. The country adopted the Directly Observed Treatment Scheme (DOTS) strategy for TB control since 2003, but both the case detection and treatment success rates are among the lowest of high-TB burden countries [2]. There were 3.3 million people living with HIV in the country by the end of 2009, an estimated 3.6% of the population [3,4], and about 21% of all TB patients were dually infected with TB and HIV [5–7]. Anti-TB drug resistance among TB patients in some countries has increased in the last decade, progressing from multiple drug-resistance (MDR), to extensive drug resistance (XDR), to extreme and total drug resistance (TDR) [8,9]. In 2008, there were an estimated 440,000 new multidrug-resistant TB (MDR-TB) cases emerging worldwide with 150,000 deaths, and it was estimated that in 2009, 3.3% of all new TB cases had MDR-TB [10]. However, precise information on drug-resistance patterns of *Mycobacterium tuberculosis* complex is lacking for Cross River State, Nigeria. Consequently, the present investigation was aimed to have a first idea of drug resistance and demographic characteristics of *M. tuberculosis* strains isolated from newly diagnosed TB patients in this area.

Materials and methods

The study was carried out in three senatorial districts (northern, central and southern) of the Cross River State, located in the South-South part of Nigeria between June 2008 and May 2009. A total of 137 sputum samples were collected from newly diagnosed smear-positive patients without any family or previous history of TB. Both genders (age range <15 to >64 years) recruited from major hospitals and TB care facilities were retained for the study. Ethical approval and informed medical consent were appropriately obtained for the study.

Sputum specimens obtained from patients were preserved using sodium carbonate (75 mg), and/or refrigerated until cultured. Specimens were decontaminated using modified Petroff's method, and cultured using BACTEC MGIT960 (Becton Dickinson, Franklin Lakes, NJ 07417, USA). Smears were made from isolates obtained from the BACTEC MGIT tubes, stained using the Ziehl Neelsen staining method, and examined for the presence of acid-fast bacilli (AFB). The growth on each AFB positive MGIT tube was further inoculated into two Lowenstein–Jensen (L–J) slants, one containing sodium pyruvate. The cultures were examined twice weekly, and their rate of growth and colonial morphologies were recorded. Contaminated slants were further re-decontaminated and re-cultured.

Primary identification of organisms was performed using Capilia TB-Neo (TAUNS Laboratories Inc., Japan) according to manufacturer's instructions (http://capilia.jp/english/capilia_tb_neo.html) and further confirmed by genotyping of *M. tuberculosis* complex isolates as reported [11]. *M. tuberculosis* complex isolates were tested for drug susceptibility against 0.2 μg/ml for isoniazid (INH), 40 μg/ml for rifampicin (RIF), 8 μg/ml for streptomycin (STR), and 2 μg/ml for ethambutol (ETH) using the proportion method on Lowenstein–Jensen medium.

Results

Gender, age and HIV status of TB patients

More males ($n = 86$, 62.8%) than females ($n = 51$, 37.2%) were encountered in this study. The highest frequency of disease occurred in the age range between 15 and 44 years with a total of 115 (83.9%) out of 137 cases. The age range of 25 to 34 years had the highest number of cases (58/137, 42.3%) with 35/86 (40.7%) males and 23/51 (45.1%) females (Fig. 1). Twenty-six (19%) of the 137 patients enrolled in this study were HIV positive, while 81 (59.1%) were HIV negative. Information on the HIV status of 30 (21.9%) patients was not available at the time of specimen collection.

Culture and primary identification

Following specimen workup, including microscopy by the Ziehl Neelsen method, primary isolation on MGIT, and subculture on LJ, a final working culture of 97 isolates was obtained after recording 19 (13.9%) negative cultures, 6 (4.4%) mislabeled vials, and discarding 15 (10.9%) heavily contaminated LJ slants. Using the immunochromatographic technique (Capilia TB-Neo) and genotyping, 81 (83.5%) were identified as members of the *M. tuberculosis* complex [11], and 16 (16.5%) isolates were Capilia negative.

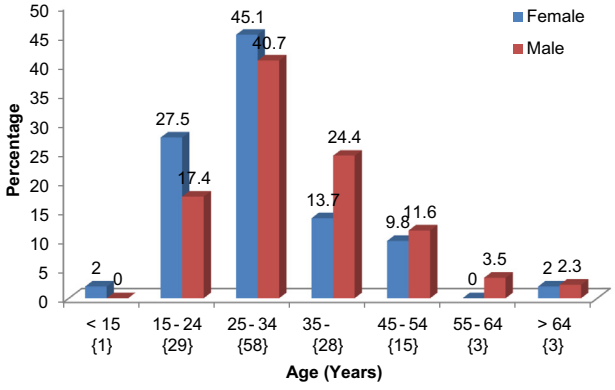

Fig. 1 – Sex and age distribution of smear-positive patients.

Drug susceptibility testing

A total of 58/81 of the *M. tuberculosis* isolates obtained were evaluated for drug sensitivity. Their susceptibility patterns and the HIV status of the patients are shown in Table 1. Thirty-five (60.3%) isolates were pansusceptible, and resistance to one or more of the standard drugs was detected in 23 (39.7%) patients. Irrespective of resistance combinations, 16 (27.6%) isolates were resistant to streptomycin, 11 (19%) to isoniazid, 9 (16%) to rifampicin, and 10 (17.2%) to ethambutol. Among the three HIV positive patients with drug resistance, two were monoresistant to streptomycin and rifampicin, and one was resistant to all four TB drugs ($p = 0.7$). Globally, MDR occurred in six isolates in this study; all the six strains were found to be resistant to all first-line anti-TB drugs (FLD). However, 4/6 of these MDR strains shared an identical MIRU (mycobacterial interspersed repetitive unit) pattern 223315153321 which corresponded to MIRU International Type MIT266. Since all these four strains were characterized by consecutive strain numbers, laboratory cross-contamination could not be excluded in these strains [11]. Nonetheless, considering that the first out of these four isolates was not due to laboratory carryover, a minimal MDR-TB rate of 3/58 (5.2%) is still reported among newly diagnosed TB patients in Cross River State, Nigeria.

Discussion

With 86 males and 51 females, globally there were 1.7 times more males than females in this study, an observation which is in agreement with the fact that male gender has been identified as a risk factor for TB [12–14]. Ninety-five percent of the TB patients in this study were aged between 15 and 54 years – in agreement with the observation that more than 75% of TB-related disease and deaths occur among people in this age range which is also the most economically active segment of the population [15]. Indeed, TB affects the most productive age group worldwide [10]; and interestingly, of the 51 female TB patients in this study, 44 (86.3%) were aged between 15 and 44 years. Lastly, 20% of the TB patients in this study were HIV positive (despite the fact that 22% did not know their HIV status). Currently in Nigeria, 79% of tested TB patients know their HIV status and 25% are HIV-positive (WHO TB data consulted on October 15, 2011; http://www.who.int/tb/country/en/index.html).

Only cases of primary drug resistance are assumed to be owing to transmission of drug-resistant strains. In this study, none of the patients had any history of prior anti-TB treatment. Drug resistance irrespective of reported prior drug intake in this study occurred in about 40% of cases. This is similar to reports in Chad, a neighboring country, where 61% of isolates were susceptible to all drugs, and 39% were resistant to at least one drug [16]. Other studies have shown that a steady increase in drug resistance in the country is a reality. Unpublished data carried in Zaria (Northern Nigeria) among newly diagnosed TB patients confirmed this fact (19% and 13% for isoniazid and streptomycin, respectively, in 1991, against 29% and 14% by 2006; [17,18]). In the present study, resistance to streptomycin was recorded at 27.6%; similar high levels of resistance to streptomycin have been reported in neighboring Cotonou (Benin Republic), as well as in several other African countries, which may be partially related to the widespread use of low-cost aminoglycosides to treat respiratory infections other than TB [19,20].

Similarly, 19% of primary resistance to isoniazid in this study is higher as compared with other African countries

Table 1 – Drug susceptibility patterns of isolates and HIV status of patients in Cross River State.

Drugs	*M. tuberculosis* ($n = 58$)	%	HIV[a] status		
			Pos[b]	Neg[c]	Unk[d]
Pansusceptible	35	60.3	6	20	9
Monoresistance					
STR[e]	7	12.1	1	6	–
INH[f]	2	3.4	–	2	–
RIF[g]	2	3.4	1	1	–
ETH[h]	2	3.4	–	1	1
Resistance to two drugs					
STR + INH	1	1.7	–	1	–
INH + ETH	1	1.7	–	–	1
STR + RIF	1	1.7	–	1	–
Resistance to three drugs					
STR + INH + ETH	1	1.7	–	1	–
Multidrug resistance					
STR + INH + RIF + ETH	6	10.3	1	2	3

[a] Human immunodeficiency virus.
[b] Positive.
[c] Negative.
[d] Unknown.
[e] Streptomycin.
[f] Isoniazid.
[g] Rifampicin.
[h] Ethambutol.

such as the neighboring Cameroon (12%; [21]), Equatorial Guinea (12.5%; [22]) and Ethiopia (8%; [23]). Monoresistance to isoniazid is significantly more prevalent than monoresistance to rifampicin and MDR-TB. Based on the most recent data, it is estimated that 13.3% of all TB cases globally involve isoniazid monoresistance vs. 0.6% for rifampicin, while 5.3% are MDR-TB [23]. Thus, the high rates of resistance to isoniazid and rifampicin that are the backbone of any successful TB treatment program, as well as a minimal MDR-TB rate of 5.2% among newly diagnosed TB patients in Cross River State, Nigeria, are particularly worrisome.

The MDR-TB strains in this study were resistant to two other drugs (streptomycin and ethambutol). Considering the advent of extensively drug resistant TB (XDR-TB), dissemination of such strains poses a threat to the control of TB in the region. However, unlike the global observation of 5.3% MDR rate, low estimates of MDR-TB among new TB cases are being observed in Nigeria by the WHO (currently at 2.2% as consulted on October 15, 2011; http://www.who.int/tb/country/en/index.html), which may be an underestimate given the low level of culture and drug susceptibility testing carried out in the country. Recently, a survey has shown an MDR occurrence of 13% in Nigeria, with all the isolates being resistant to all four first-line drugs [24]. However, patients in this latter study were not differentiated as new or re-treatment cases, as opposed to this study in which all the patients were newly diagnosed.

Last but not least, laboratory cross-contamination was equally recorded in the present study. Considering that DOTS (Directly Observed Treatment Short-course) policy is mainly based on smear microscopy alone, and the fact that sputum culture is not routinely carried out in Nigeria, the extent of cross-contamination cannot be reliably estimated. The usual rate of cross-contamination in a routine clinical mycobacteriology laboratory remains often unknown, since it requires parallel genotyping [25]. Indeed, such a phenomena would have been unrecognized in this study had it not been for retrospective genotyping of the isolates [11], which revealed that during the period of sample collection (within 1 week), smears were handled by an inexperienced young graduate intern who did not properly flame the wire loop leading to a laboratory carryover. In this regard, several factors have been shown to contribute to laboratory contamination and/or carryover, including new personnel in the laboratory, faulty technique, and an increase in the number of specimens processed [25]. False-positive results for TB are a matter of concern because of the clinical, therapeutic, and social impacts of the misdiagnosis of TB, as well as the economic load associated with each misdiagnosed case of TB [26].

In conclusion, the present study carried out in Cross River State in Nigeria shows an increase in *M. tuberculosis* drug resistance which may be reflecting a similar trend in the whole country. The rise in MDR-TB highlights the urgent need to strengthen national TB control programs, particularly in settings with high HIV prevalence, like Nigeria. As in most of Africa, treatment of TB in Nigeria is based on simple smear microscopy results. The isolation of about 17% of nontuberculous mycobacteria [27], and the treatment of such patients with anti-tuberculous drugs, coupled with the use of streptomycin in the treatment of respiratory tract infections other than TB may contribute to the rise in drug resistance seen in this study and elsewhere. Nigeria is currently trailing behind many countries in providing adequate laboratory infrastructure for surveillance of drug resistant strains. This study, therefore, pleads in favor of introduction of more reliable diagnostic methods involving culture and rapid molecular speciation in Nigeria as well as other African countries.

Conflict of interest

None declared.

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