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DRUG-RESISTANCE IN CHRONIC TUBERCULOSIS CASES IN SOUTHERN NIGERIA

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ABSTRACT

Objective: Nigeria has a high burden of tuberculosis but the drug resistant situation was previously unknown. This report evaluates the firstline drug resistance and associated factors among chronic tuberculosis cases from the tuberculosis control programme in South south and South east zones of Nigeria.

Methods: Descriptive study of chronic tuberculosis patients consecutively referred from March 2003 to December 2005. Information collected by indepth interview of patients and tuberculosis microscopy, culture and sensitivity of patients sputum.

Results: Of 82 patients infected with M. tuberculosis strains, 57(64.0%) were males while their mean age was 38.0 13.2 years. Fifty nine (72.0%) patients had multidrug-resistance tuberculosis with 35(42.7%) resistant to rifampicin, isoniazid, ethambutol, streptomycin while 14(17.1%) had poly-drug resistance and one patient had monoresistance to isoniazid. Within and outside the national control programme, drug therapy was inappropriate (69.5-81.7%) and treatment poorly supervised (26.8-39.0%). Factors associated with multidrug resistance were a male age less than 45years and Category 1 treatment in a private health facility.

Conclusion: The multidrug resistance burden in chronic tuberculosis cases is very high in southern Nigeria and should be urgently and adequately controlled in the interest of public health.

Key Words:Multidrug-resistance tuberculosis, chronic, Mycobacterium tuberculosis, Nigeria.
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INTRODUCTION

Multi-drug resistant tuberculosis is an emerging global problem which is threatening control programmes in many settings. The treatment of multi-drug resistant tuberculosis involves the use of second line drugs which are usually very expensive, highly toxic, of lower efficacy and requires costly laboratory support. The most cost effective strategy for control of multi-drug resistant tuberculosis is the early identification and control of the factors that led to its emergence accompanied by treatment of those already infected. [1-3]

Nigeria presently ranks fourth among the 22 high-burden tuberculosis countries world-wide while being the country with the greatest number of estimated tuberculosis cases in Africa. However, in TB control efforts, the country recorded a DOTs' coverage of 65%, a smear positive case detection rate of 22% and a treatment success rate of 73% in the year 2005.[3] Coordinated tuberculosis control started in the country in 1988 with the establishment of the tuberculosis control programme as a separate unit under the Department of Disease Control and Primary Health Care of the Federal Ministry of Health. At programme inception in 1994, the drug Regimens in use for adults were 2SRHZ/6TH or 2STH/10TH for Category I treatment and 2SRHZE/1RHZE/5TH or /5R3H3E3 for Category II treatment. [4] These were later changed in 2000 to the regimens; 2RHZE/6HE or /6TH for Category I treatment while 2SRHEZ/1RHEZ /5R3H3E3 was retained for Category II treatment. [5] DOTS strategy is the adopted practice for TB control in the country. The epidemiology of tuberculosis in Nigeria is the same as for the rest of Africa, with tuberculosis notification in males higher than for the females and the age specific rates highest for the 24-34 years age group. [3]

The South-south and South-east zones of Nigeria are made up of 11 of the 36 states of the country with an estimated population of 36,340,241 in the year 2005 [6]. Both zones have had tuberculosis support in the form of provision of training, mobility, drugs, laboratory equipment, and supplies from the German Leprosy Relief Association since 1994 [7]. A few years into the implementation of the TB control programme, health workers in both zones started encountering "suspect" MDRTB cases but drug resistance surveillance data was unavailable. Factors implicated in the propagation of MDR-TB in other nations exist in the country, namely; a high default rate of TB cases on treatment, an increased burden of HIV /AIDS, the availability of TB drugs over the counter, and inadequate TB treatment by uninformed and

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unsupported health providers in both government and private health facilities. [3,7,9]

The objective of this study was to describe the 1st line TB drugs resistance pattern in the chronic TB cases arising from the control programme in the South-south and South-east zones of Nigeria. The possible factors associated with the emergence of multi-drug resistance tuberculosis were also determined.

MATERIALS AND METHODS

Participants

We conducted a descriptive review of all chronic TB cases referred for further evaluation by the National Tuberculosis and Leprosy Control Programme (NTP) to the Tuberculosis Unit of the University of Nigeria Teaching Hospital , Enugu from March 2003 to December 2005. The chronic TB cases were composed of the failures, relapses, and smear positive returning defaulters of the NTP retreatment regimen who were referred with their past medical records and additionally gave a written consent to be studied. Cases were excluded if the sputum culture could not be done or was negative or contaminated and a replacement could not be obtained. On referral, each patient was interviewed by a medical doctor and the following information was obtained using a study proforma; patient's place of residence and origin, age, sex, marital status, occupation, household size, history of previous TB treatment, diagnostic smear reports, drug regimens and dosages, mode of supervision of treatment, treatment outcome, HIV status and contact details. Patients also submitted two sputum samples for TB microscopy, culture and drug susceptibility test (DST). Information obtained by interview was crosschecked with those from patient's past medical records. NTP personnel were also interviewed for clarification and explanations of some treatment delivery processes.

Procedure for culture and species identification

The two sputum samples from each patient were decontaminated using the Petroff method and smeared by Ziehl Neelson method for microscopy. One plain and one pyruvate enriched Lowenstein-Jensen slopes were then inoculated from each sample and incubated at 37^0C. Species identification was done on one isolate per patient to differentiate species of Mycobacterium tuberculosis complex from those of atypical mycobacteria, using the following biochemical properties; growth rate, colonial morphology, nitrate reduction, catalase reaction, growth in the presence of p-nitrobenzoic acid and thiophen-2-carboxylic acid hydrazide, pigment production in the light and dark. [10]

Procedure for drug susceptibility test

Indirect susceptibility testing was performed on Loewenstein Jensen medium using the economic variant of the proportion method on the two isolates from each patient. Procedure for the preparation of media, stock solutions, bacterial suspensions and dilutions, inoculation of media, incubation, reading and reporting of the DST were according to the format suggested by H.Rieder et al. [11] Resistance was taken as the percentage of colonies on the drug containing media in comparison to the growth on the drug free media. The criterion for resistance was 1% growth for the following drugs at these concentrations (ug/ml); rifampicin (40.0), isoniazid (0.2), streptomycin (8.0) and ethambutol (2.0). Where there were 5 or fewer colonies on the primary culture or if a clear interpretation of results could not be made, DST was assumed to be unreliable and was repeated.

Quality Assurance Issues

Internal quality control of laboratory procedures included monitoring of the culture contamination rate, temperature of the incubator, rate of negative culture results in smear positive specimens and purchase of drug powder for sensitivity tests from reputable firms. Also, susceptibility test was done on the standard H37RV strain for each new batch of medium and for each drug following standard procedures. A standard operating procedure was used for all laboratory activities. Instructions and scales of measurement were incorporated into all the forms used for data collection. All the survey procedures and instruments were pilot-tested during the preparatory phase. International quality control of laboratory procedures involved a proficiency testing scheme organized for the project laboratory by a Supranational Reference Laboratory (Medical Research Council, Pretoria, South Africa). At the beginning of patient recruitment, proficiency test showed a laboratory efficiency of 80% for Ethambutol, 85% for Streptomycin, 90% each for Isoniazid and Rifampicin; and a specificity of 84.6% for ethambutol, 100% each for streptomycin, rifampicin and isoniazid while the sensitivity was 71.4% 77.8% for all drugs. One of the strengths of the study design is the representativeness of the study population. Transportation reimbursement was done for study respondents to encourage participation. There may be a limitation of the accuracy of patients' recall of prior TB treatment.

Other procedures and definitions

Standard case definitions for all variables were used. Adequacy of drug regimen means its appropriateness in terms of drug composition or dose for the category of TB being treated.

Ethical clearance for this study was obtained from the University of Nigeria Teaching Hospital Ethics Committee.

Epi-Info 2002 software was used for data analysis,

Chi square test, Student T test, Fisher exact 2-tailed test and Odds ratio at 95% Confidence Limits were used to verify the significance of findings.

RESULTS

A total of 126 patients were referred within the study period. Forty two patients were excluded from analysis for the following reasons; 37 patients had incomplete culture or DST results or past medical records or lack evidence of Category 11 TB treatment while 7 patients were infected with atypical mycobacteria. The findings on 82 cases whose isolates belonged to species of Mycobacterium tuberculosis complex and who had complete culture/DST results/ records of past TB treatment are presented.

They were 51(62.2%) males and 31(37.8%) females. Their age range was 15-80years while their mean age was 35.6 11.5 yrs. Of these 82 patients, 59(72.0%) had multidrug resistant TB with 35 (42.7%) cases resistant to all the four drugs tested. Resistance to three drugs, namely streptomycin and ethambutol with either rifampicin or isoniazid resistance was seen in 14(17.1%) cases. Monoresistance was observed in 1(1.2%) patient while another patient 1(1.2%) had isolate sensitive to all drugs. The overall resistance to streptomycin was 75(91.5%), isoniazid 76(92.7%), rifampicin 64(78.1%) and ethambutol 50(61.0%). See Table 1 for the details.

Males who were less than 45years were more likely to have MDRTB than others. Of 61 (74.4%) patients whose HIV status were known, only 6(9.8%) cases were HIV positive. All the 6 (100.0%) HIV infected patients had MDRTB as opposed to 72.8% of the non-HIV infected cases but this difference was not significant (95% CI: 0.0-2.6). See Table 2.

At the stage of WHO category 1 disease, 35(42.7%) cases were treated by NTP, 22(26.8%) by private for profit facilities, 18(22.0%) by government hospitals and 7(8.5%) by drug stores. Both univariate analysis and logistic regression identified category 1 disease treatment by private for profit (PP) facilities as a predictor of MDRTB. Drug therapy was inappropriate for most patients 57 (69.5%) but it was not a predictor of MDRTB. However, therapy was significantly inadequate for all 29 (100%) patients treated by PP facilities / drug stores followed by 15(83.3%) of 18 cases treated by unsupported Government facilities and in 13(37.1%) of 35 cases treated by NTP. (X2=31.7, DF=2, P-value = 0.0000001). The forms of inadequate therapy observed were administration of unapproved regimens (5.7% cases by NTP and 93.6% cases outside NTP) and under dosing of patients on correct regimens (all 11 cases within NTP). DOT was not practised mostly in PP units / drug stores -18(62.1%) cases followed by unsupported Government facilities - 9(50%) cases and NTP - 5(14.3%) cases. (X2=16.4, DF=2, p=0.0003). DOT practice during the intensive phase of this treatment course was not associated with greater MDRTB prevalence. At the stage of WHO category 11 disease, majority of cases 64(78.0%) were treated by NTP but drug therapy was inappropriate for most patients 67(81.7%). Inappropriate therapy was administered on 49(76.6%) cases treated by NTP and on all 18 (100.0%) patients managed outside NTP (Fisher exact p-value = 0.033). The forms of inappropriate therapy reported were administration of inadequate regimens {27(55.1%) cases by NTP and 16(88.9%) cases outside NTP} and suboptimal dosing of 24 patients {22 (44.9%) cases within NTP and 2 (11.1%) cases outside NTP}. DOT was not practiced mostly by non-NTP facilities 12(66.7%) of 18 cases unlike within NTP 10(15.6%) of 64 cases. (Fisher exact p-value = 0.00006). DOT practice was a predictor of MDRTB rather than the type of health facility and appropriateness of drug regimens in this second treatment course. An average of 3.2 ±1.2 courses of treatment was received by the patients and the number of TB treatment courses received was not asociated with MDRTB (p-value: 0.496).

On investigating the number of patients that have had contact with second line drugs, three patients had ciprofloxacin added to a failed retreatment regimen while one patient had both ciprofloxacin and kanamycin added to a second retreatment regimen. The last patient was later referred to South Africa on request.

Table 1: **First-Line Drug Resistant Pattern in Chronic Tuberculosis Cases in South Nigeria, 2003-2005 (N = 82).**

Pattern	No. (%) Resistant	95% CI
Fully sensitive	1 (1.2)	0.0-6.6
Monoresistance (total)	1 (1.2)	0.0-6.6
INH	1 (1.2)	0.0-6.6
Polyresistance (total)	21 (25.6)	16.6-36.4
INH + SM	7 (8.5)	3.5-16.8
INH + SM + EMB	9 (11.0)	5.1-19.8
RIF + SM + EMB	5(6.1)	2.0-13.7
Multidrug resistance (total)	59 (72.0)	60.9-81.3
INH + RIF	4 (4.9)	1.3-12.0
INH + RIF + EMB	1 (1.2)	0.0-6.6
INH + RIF + SM	19 (23.2)	14.6-33.8
INH + RIF + EMB + SM	35 (42.7)	31.8-54.1
Any resistance (total)*#	81 (98.8)	
INH	76 (92.7)	
RIF	64 (78.1)	
EMB	50 (61.0)	
SM	75 (91.5)	

*INH, isoniazid; RIF, rifampicin; EMB, ethambutol; SM, streptomycin. # Unlike other rows, cells in this row are not mutually exclusive.

Table 2: **Characteristics and Past Treatment Practices by MDR Status for Chronic Tuberculosis Cases in Southern Nigeria, 2003-2005.**

Features	Total	No. (%) MDR	Multivariate (95% CI)	Logistic Regression (95% CI)
Male age (years):				
< 45	39	32 (82.1)	(1.3-33.1)	
= 45	12	5 (41.7)		
Female Age (years):				
< 45	25	17 (68.1)	(0.008-4.9)	
= 45	6	5 (83.3)		
1st Therapy:				
Right	25	17 (68.0)	(0.2-2.4)	(0.2-3.4)
Wrong	57	42 (73.7)		
1st DOT:				
Yes	50	38 (76.0)	(0.5-4.9)	(0.9-10.2)
No	32	21 (65.6)		
2nd Facility:				
NTP	64	44 (68.8)	(0.07-1.8)	(0.8-27.8)
Private unit	11	10(90.9)	(0.5-203.6)	
Govt facility	4	2(50.0)	(0.03-5.5)	
Drug store	3	3(100.0)	(undefined)	
2nd Therapy:				
Right	15	11 (73.3)	(0.2-5.2)	(0.1-3.6)
Wrong	67	48 (71.6)		
2nd DOT:				
Yes	60	46 (76.7)	(0.6-7.2)	(1.0-17.9)
No	22	13 (59.1)		

DISCUSSION

The study identified a pool of highly infectious patients with drug resistant tuberculosis 98% of which required second line TB drugs that are presently not provided by NTP. Their MDRTB prevalence of 70% is unprecedented and is only comparable to what was found in previously treated patients in Colombia [12] and Lithuania of the former Soviet Union [13] . To further worsen the drug resistant situation in Nigeria, fluoroquinolones can be bought without prescription in the open drug market and from this report; some physicians are adding them to a failing retreatment regimen. It is yet to be investigated whether the patient who had ciprofloxacin and kanamycin added to a failed repeat retreatment regimen and was later referred to South Africa for second line treatment was harboring the XDR strain. Who knows the number and nationality of people infected during that 6 hours international flight! The patients with the fully sensitive and mono resistant strains suggest new exogenous re-infection which has been incriminated as a cause of recurrent TB. [14,15] However, molecular epidemiological studies are needed to determine the transmission pattern in the study area.

The sex and age distribution of these chronic TB cases are similar to what has been observed among both new and previously treated cases in Nigeria and other settings. [3, 12, 15, 16] From the study, a male age less than 45 years was associated with greater MDRTB prevalence. The greater mobility and exposure of males in connection with occupation may be the reason for their increased MDRTB rate. In contrast to past findings, HIV co-infection was not a predictor of MDRTB. [17] However, there were two main limitations in this prediction; firstly only two-thirds of cases were screened for HIVinfection and secondly a high early mortality of co-infected cases in the study environment is expected due to the absence of both ART and second line drugs. [18]

This report shows gross mismanagement of these patients within and outside NTP and points towards a health sector induced multi-drug resistance problem in the study area. The first facility chosen for TB management appears to be very pivotal to the overall subsequent treatment outcome and private sector TB management was associated with greater MDRTB prevalence. Reports from the present and past studies [9] show that, tuberculosis treatment in the private sector entailed prescription of untested and unapproved drug regimens whose procurement from a poorly regulated drug market is at the discretion of the patient. In most instances, the ingestion of these drugs is not directly observed. Private sector formal involvement with TB control has been very minimal in Nigeria though they are important health care providers for the general populace. And like in other countries, the high price of their non-involvement is the emergence of drug resistant tuberculosis due to the synergic interaction of inadequate drug regimens, poor quality drugs and poor treatment supervision. [12, 17] The association of DOTS practice with MDRTB

during category II treament can be interpreted as an aftermath of regular and prolonged inappropriate therapy in patients with some form of drug resistance.

Treatment by NTP was not a risk factor for MDR-TB but three main problems were identified with their service delivery. Firstly is the problem of poor history taking which led to patients with WHO category 11 disease being misclassified as new cases and their subsequent treatment as such. Secondly is the problem of suboptimal drug dosage which arose indirectly from drug packaging. For the intensive phase of both category I and II treatment, the NTP procures blister pack containing ethambutol, pyrazinamide and rifampcin-isoniazid tablets appropriate for patients weighing 40-55kg while loose drugs are added to this blister pack for patients weighing more than 55kg but this was not done for some patients. Furthermore, isoniazid and ethambutol were in some cases not increased for intermittent administration during the continuation phase of category II treatment with 2SRHEZ/1RHEZ/5R3H3E3. However, ignorance of correct drug dosages and reasons for the dosages among NTP personnel and health workers may be the problem though the programme manual is in order.

Thirdly is the problem of non-practice of DOT during category 1 and II treatments. Similar deficiencies in the prescription and dosages of antituberculosis drugs have been reported in Malawi, Nepal, Kenya and Senegal. [19, 20] The consequences of such deficiencies are very costly and should not be tolerated by the control programme. Standard primary and acquired drug resistance survey is needed in this setting to determine the adequacy of the NTP reccomended regimens.

These research findings made tremendous impact on TB control activities in the country. Firstly, awareness of the mechanism of TB drug resistance has been created among NTP personnel and ways of minimizing its emergence partly addressed. Secondly, the TB drug blister pack implicated as contributing to the emergence of MDR-TB has been changed and a new dosage-friendly 4-FDC pack is already in use. Also, NTP added the regimens 2RHEZ/4RH for category I treatment to and 2SRHEZ/6RHEZ for category II treatment. The study also lends support to the new global strategy of making DOTS-plus widely available for TB control especially in Nigeria for the identified pool of chronic tuberculosis cases considering the size of the population at risk.

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REFERENCES

1. **Farmer P, Bayona J, Becerra M, Furin J, Henry C, Hiatt H, et al.** The dilema of MDR-TB in the global era. Int J Tuberc Lung Dis 1998; 2(11): 869-876.

2. Stop TB Partnership. The global plan to stop TB2006-2015. http://www.stoptb.org/globalplan (accessed October 17 2006)

3. World Health Organization. Global tuberculosis control: surveillance, planning, financing, WHO report 2007, Geneva, Switzerland , WHO /HTM/TB/2007.376.

4. **Williams SA, Benebo NS, Adeleye MD, Alabi G A, Krishnan SAR.** National Tuberculosis and Leprosy Control Programme: workers manual. Lagos, Nigeria: Federal Ministry of Health. 1991.

5. **Orjioke CJ, Sofola TO, Chitimba N, Verduin PRN, Valenti P, Ogbeiwi O, et al.** National Tuberculosis and Leprosy Control Programme: Revised workers' manual. 3rd Edition, Jos, Nigeria.1998.

6. National Population Commission. 1991 population and projected figures 1998-2010 by state, Nigeria. Abuja, Nigeria: National Population Commission, 1998.

7. German Leprosy Relief Association. NTBLCP support project, Nigeria : Progress report for January December 2004.

8. Federal Ministry of Health. Technical report on the 2005 National HIV/Syphilis sero-prevalence sentinel survey. Abuja, Nigeria: Federal Ministry of Health, 2006: 1-40.

9. **Aghaji MN.** Tuberculosis management practices of private medical practitioners, in Enugu, Nigeria. In: Abstracts of the 35th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease; Paris, France; 2004 Oct 28th -1st Nov; Int J Tuberc Lung Dis, 2004 suppl; 8(11): S153, PS-923-998.

10. **Narvaiz de Kantor I, Kim SJ, Frieden T, Laszlo A, Luelmo F, Norval P, et al.** Laboratory Services in Tuberculosis Control, Culture, Part 111. World Health Organizatio Geneva 1998: 1-96. WHO/TB/98.258.

11. **Rieder HL, Chonde TM, Myking H, Urbanczik R.** The public health service National Tuberculosis Reference Laboratory and the National Laboratory network. Minimum requirements, role, and operation in a low-income country. IUATLD France 1998: 1-112.

12. **Osorio LE, Villegas MV, Benitez AM, Hernandez H, Miranda JF, Saravia N, et al.** Acquired Multidrug Resistant tuberculosisBuenaventura, Colombia, 1998. MMWR September 18, 1998: 47(36); 759-761.

13. **Dewan P, Sosnovskaja A, Thomsen V, Cicenaite J, Laserson K, Johansen I, et al.** High prevalence of drug-resistant tuberculosis, Republic of Lithuania, 2002. Int J Tuberc Lung Dis, 2005; 9(2): 170-174.

14. **Chiang CY, Riley LW.** Exogenous reinfection in tuberculosis. Lancet Infect Dis; 2005;5: 629 36.

15. **Xia Li, Ying Zhang, Xin Shen, Guomiao Shen, Xiaohong Gui, Bin Sun, et al.** Transmission of drug-resistant tuberculosis among treated patients in Shanghai, China. J Infect Dis, 2007;195: 864-869.

16. World Health Organization. Resistance to antituberculosis drugs. Weekly Epidemiological Record, 12 May 2000; 19:156-159.

17. **Ormerod LP.** The global threat of multi-drug resistant tuberculosis. J R Coll Physicians Edinb 2003; 33: 248-251.

18. **Corbett E, Marstan B, Churchyard GJ, De Cock KM.** Tuberculosis in sub-saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. Lancet 2006; 367: 926-37.

19. **Harries AD, Gausi F, Salaniponi FM.** Prescriptions and dosages of anti-tuberculosis drugs in the National Tuberculosis Control programme of Malawi. Int J Tuberc Lung Dis, 2004; 8(6): 724-729.

20. **Diop AH, Gakira G, Pande SB, Malla P, Rieder HL.** Dosages of anti-tuberculosis medications in the national tuberculosis programs of Kenya, Nepal and Senegal. Int J Tuberc Lung Dis, 2002; 6: 215-221.