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Drug Susceptibility Pattern of *Mycobacterium tuberculosis* Among Pulmonary Tuberculosis Patients in Jos, Nigeria

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With 3 tables and 18 references

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ABSTRACT

A study designed to determine the drug-susceptibility profile of tubercule bacilli to first line anti-tuberculosis drugs was undertaken in Jos, Nigeria between January 1997 and December 2000. Drug susceptibility on 35 strains of tubercule bacilli isolated from new tuberculosis patients were performed against isoniazid (INH), streptomycin (STM), ethambutol (EMB) and rifampicin (RIF) by the proportion method using Lowenstein-Jensen medium. A total of 34.27% of the isolates/strain were resistant to one or more drugs tested. Resistance in isoniazid (28.57%) and streptomycin (22.86) were most common common. A significant finding in the study was the low level of resistance to ethambutol (2.86%) and rifampicin (0.00%). Multi-drug resistance is already a problem in Jos. A continuous monitoring of drug resistance for planning and assessing the national tuberculosis (TB) control programme is, therefore, indicated.

Key words: Tuberculosis, susceptibility, tubercule bacilli, resistance, Lowenstein-Jensen medium
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Introduction

Although tuberculosis (TB) has received considerable attention during the last few years, it is not widely appreciated that for most of the world's population, TB remains the single leading cause of death from any single infectious agent [Murray *et al.*, 1990]. It has been estimated that about 7% of all deaths and approximately 26% of all preventable deaths in the world are directly due to TB. The African continent and particularly the sub-Saharan Africa, is worst hit where about 50% of the population between 20 - 40 years of age in some countries, have been infected with TB. The high annual risk of infection and the rising prevalence of TB in Africa may have resulted from several factors including widespread poverty, overcrowding, famine, displacement due to civil unrest and malnutrition [Ankrah, 1997]. Other factors would include population growth, human immunodeficiency virus (HIV) epidemic in the region, poorly coordinated TB control programmes and an increase in drug resistance to *Mycobacterium tuberculosis*. The problem of resistance results from treatment that is inadequate, often because of an irregular drug supply, inappropriate regimens or poor compliance [Snider and Montagne, 1994; Salami and Oluboyo, 2002].

Drug resistance of *M. tuberculosis* in both developing and developed countries has been studied [EABMRCCI,

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1975, 1978; Carpenter *et al.*, 1982; Al-Orainey *et al.*, 1989; Aziz *et al.*, 1989; Idigbe *et al.*, 1992; Al-Awaidy and Al-Handan, 1997; Pablos-Mendez *et al.*, 1998; Lawn *et al.*, 2001; Njoku *et al.*, 2004]. These studies indicated that both primary and acquired drug resistance occur frequently in various communities.

In Nigeria, very little information is available about the extent and nature of the resistance problem. In most parts of the country, once a case of pulmonary tuberculosis is diagnosed, the patient is immediately placed on any readily available anti-tuberculosis regimen [Idigbe *et al.*, 1992]. However, there is virtually no follow-up nor adequate supevision of drug taking of patients on treatment. There is also no concerted effort to monitor drug susceptibility of *M. tuberculosis* isolates before or during treatment.

The present study was aimed at investigating the extent and pattern of resistance of *Mycobacterium tuberculosis* to first line anti-tuberculosis drugs.

Materials and Methods

Study population

The subjects used in this study included new patients attending the Chest Clinic and General Outpatient Departments of the Jos University Teaching Hospital (JUTH), Plateau State Specialist Hospital and the ECWA Evangel Hospital, Jos, Nigeria, with symptoms of broncho-pulmonary disorders namely, chronic cough, weight loss, haemoptysis, night sweats, diarrhoea and fever among others.

Three consecutive early morning sputum samples were obtained from each patient and processed for the presence of acid fast bacilli (AFB). The study was carried out between January 1997 and December 2000.

Mycobacteriology

The sputum samples were decontaminated and concentrated by the Petroff's method [Cruickshank *et al.*, 1975]. After centrifugation, smears of the final deposits were examined for the presence of AFB after staining by the Ziehl-Neelson (ZN) method. Loop-fulls of each of the deposits were evenly spread onto the surfaces of pairs of Lowenstein-Jensen (LJ) slopes and incubated at 37°C. The slopes were observed weekly for a total of 8 weeks for growth. Slopes without visible growth after 8 weeks were discarded and recorded as negative while slopes showing mycobacterial growth were recorded as positive. Colonies from the resultant growths were checked for the presence of AFB by ZN microscopy. The mycobacteria isolates were further characterized using the following biochemical tests: (a) rate of growth (slow or rapid), (b) scotochromogenicity, (c) photochromogenicity, (d) stability of catalase at 68°C for 20 min., (e) Tween-80 hydrolysis test, (f) reduction of tellurite, and (g) 3-day arysulphatase test.

Drug susceptibility test

Susceptibility tests to first line anti-tuberculosis drugs were performed on the isolates by the proportion method using the LJ medium. The drug-containing media were prepared by incorporation of each of the following drugs into the LJ medium before inspissation: isoniazid (INH), 0.2 μg/ml medium; streptomycin (STM), 4.0 μg/ml medium; ethambutol (EMB), 2.0 μg/ml medium; and rifampicin (RIF), 40 μg/ml medium.

Colonies of mycobacteria were scrapped from fresh subcultures on LJ slpoes and homogenized using glass beads and a vortex mixer. The turbidity of the cell suspensions was adjusted to the MacFarland No. 1 turbidity standard. After making 10^{-3} and 10^{-5} dilutions of the cell suspensions each dilution was inoculated onto duplicate tubes of each drug-containing medium. Duplicate tubes of control medium without any drug were also inoculated. All the inoculated tubes were incubated at 37°C and examined after 4 weeks. Colonies on the control and drug-containing media were counted. The critical proportion was taken at 1% for all the drugs, and any culture showing more than 1% bacterial population growing on the drug-containing medium as compared to the control medium was considered resistant.

Results

The age and sex distribution of the study population and positive cases of tuberculosis is shown in Table 1. Out of the three hundred and twenty-nine samples examined, 102 (31.0%) were found to be positive for acid fact bacilli (AFB). A total of 65 isolates of mycobacteria were obtained and of this number, 40 were identified as *Mycobacterium tuberculosis*, 10 as *M. bovis*, 9 as *M. avium* and 3 each as *M. kansasii* and *M. fortuitum*. The isolates and control organisms were subjected to all the various cultural and biochemical tests and the results obtained are reflected in Table 2.

Results of the drug susceptibility patterns of 35 isolates showed that 22.86% were resistant to one drug, 8.57% were resistant to two drugs, 2.86% were resistant to three drugs and 34.29% were resistant to one or more of the drugs tested (Table 2). Resistance to INH was the most common (28.57%), followed by STM (22.86%), EMB (2.86%) and RIF (0.00%) (Table 3).

Table 1. Age and sex distribution of the study population and acid fast bacilli-positive (AFB) cases

Age (yr)	Males	No. +ve	Females	No. +ve	Total	Total +ve
<15	5	0	2	0	7	0
15 - 24	33	10	29	7	62	17
25 - 34	67	27	71	19	138	46
35 - 44	40	16	21	9	61	25
45 - 54	25	5	13	4	38	9
55 - 64	7	2	7	0	14	2
>65	6	3	3	0	9	3
Total	183 (55.62%)	63 (34.42%)	146 (44.38%)	39 (26.72%)	329	102 (31.00%)

Table 2. Characterization of mycobacteria isolates/strains

Tests	Isolates					Controls	
Growth rate	S	S	S	S	R	S	R
A. ***Culture***							
Pigment production (light)	-	-	-	+	-	-	-
Pigment production (dark)	-	-	-	-	-	-	-
Growth on MacConkey agar	-	-	-	-	+	-	+
B. ***Biochemical***							
Tween-80 hydrolysis (days)	+ (10)	-	-	-	-	+ (10)	-
Arysulphatase test	-	-	-	+	-	-	-
Catalase test (60°C for 20 min)	-	-	+	+	+	-	+
Niacin production	+	-	-	-	-	+	-
Tellurite reduction	-	-	+	-	-	-	+
Nitrate reduction	+	-	-	-	-	+	-
Mycobacteria strain identified	a	b	c	d	e		
Number of strains	40	10	9	3	3		

(a) = *Mycobacterium tuberculosis*; (b) = *Mycobacterium bovis*; (c) = *Mycobacterium avium*; (d) = *Mycobacterium kansasii*; (e) = *Mycobacterium fortuitum*; S = slow; R = rapid

Table 3. Drug resistance profile of some mycobacteria strains isolated

Drug/nature of resistance	No. of resistant isolates (%)
Isoniazid (INH)	10 (28.57)
Streptomycin (STM)	8 (22.86)
Ethambutol (EMB)	1 (2.86)
Rifampicin (RIF)	0 (0.00)
Resistance to one drug	8 (22.86)
Resistance to two drugs	3 (8.57)
Resistance to three drugs	1 (2.86)
Resistance to one or more drugs	12 (34.29)

Discussion

The cornerstone of a successful tuberculosis control programme is adequate case finding and chemotherapy. In Nigeria, case-finding is passive and chemotherapeutic regimens commonly used always include isoniazid and

streptomycin.

Resistance to the major drugs used for the treatment of tuberculosis is rapidly growing and posing a serious threat to most tuberculosis control programmes in various parts of the globe [WHO, 1992]. Several studies have shown that both primary and acquired drug resistance occur in various communities in the developed and developing countries [EABMRCCI, 1975, 1978; Carpenter *et al.*, 1982; Al-Qrainey *et al.*, 1989; Aziz *et al.*, 1989; Idigbe *et al.*, 1992; Al-Awaidy and Al-Handan, 1997; Pablos-Mendez *et al.*, 1998; Lawn *et al.*, 2001; Njoku *et al.*, 2004].

Information on resistance to *M. tuberculosis* in Nigeria in general and Jos in particular is scanty due to the fact that there are only a few centres where facilities for culture and drug sensitivity testing are available, hence much of the drug resistance is presumed clinically, i.e., when patients do not improve or symptoms return after initial relief and/or sputum remains positive for acid fast bacilli, despite adherence to prescribed drugs. However, data from this study which was based on laboratory culture and drug susceptibility testing revealed an overall resistance rate of 34.29% to initial anti-tuberculosis drugs. The most common forms of resistance recorded were isoniazid (28.57%) and streptomycin (22.86%) with 2.86% and 0.00% levels of resistance for ethambutol and rifampicin, respectively. This level of resistance is lower than the figures recorded by Idigbe *et al.* [1992, 1995] in Lagos, Nigeria. These authors in a study in Lagos documented that the drug susceptibility pattern of 96 isolates showed that 34% were resistant to one drug, 14% to two drugs, 8% to three drugs and 56% were resistant to one or more of the drugs tested. Resistance to INH was the most common at 38%, followed by STM at 29%, para-aminosalicyclic acid (PAS) at 17%, EMB at 3% and RIF at 2%. This difference may be due to the fact that the Lagos study involved patients already on anti-tuberculosis drugs while the present study involved only new patients.

In a study in Ghana, Lawn *et al.* [2001] found a prevalence of primary resistance to isoniazid of 23%. There was, however, no resistance to pyrazinamide, ethambutol, streptomycin and ciprofloxacin. In a similar study conducted in 35 countries participating in the WHO International Union against TB and Lung Disease Global Project on anti-tuberculosis drug resistance surveillance between 1994 and 1997, results showed that among patients with no prior treatment, a median of 9.9% of *M. tuberculosis* strains were resistant to at least one drug (range: 2 - 4%), resistance to isoniazid (7.3%) and streptomycin (6.5%) was more common than resistance to rifampin (1.8%) or ethambutol (1.0%) [Pablos-Mendez *et al.*, 1998].

The resistance of certain *M. tuberculosis* strains to anti-tuberculosis drugs is not a new phenomenon and in fact was noted when strptomycin was first used as a monotherapy for tuberculosis in the 1940s [Henshaw *et al.*, 1946]. The rate of spontaneous mutation resulting in resistance of streptomycin and other drugs, is high enough so that a single drug cannot eradicate all *M. tuberculosis* organisms in persons with the disease, and over time, resistant organisms will predominate.

Poor implementation of the treatment components of control programmes foster the emergence of drug resistant strains, which subsequently get transmitted to previously, uninfected persons. The rate of treatment failure in Nigeria is reportedly high [Idigbe *et al.*, 1992, 1995] and this has to be addressed urgently if the national tuberculosis control programme is to achieve any meaningful success. The observed low level of resistance to ethambutol (2.8%) and complete susceptibility to rifampicin in Jos is indeed heartwarming and consoling. The low level of multi-drug resistance in particular may be due to the relatively late introduction of rifampicin and the unavailability of anti-tuberculosis drugs outside national programmes [Idigbe *et al.*, 1992]. However, the higher level of resistance to isoniazid and streptomycin may be related to its availability on the open market. It would, therefore, appear that the proper inclusion and administration of these two drugs in well supervised short course regimens could help improve the rate of treatment failure, improve cure rate and the overall tuberculosis situation in Nigeria.

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