RESISTANCE PATTERNS OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM PULMONARY TUBERCULOSIS PATIENTS IN NAIROBI

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Abstract
Kenya is classified thirteenth of 27 high TB burden countries. There is need to frequently monitor resistance patterns of *Mycobacterium tuberculosis* to prevent increase of multidrug resistant (MDR) cases. To determine resistance patterns of *Mycobacterium tuberculosis* in order to detect MDR cases. Patients with pulmonary tuberculosis were recruited from TB clinics in and around Nairobi. All laboratory work was carried out at Aga Khan University Hospital Nairobi, Kenya. A total of 286 drug susceptibility tests were carried out on clinical isolates of *Mycobacterium tuberculosis* using first line anti-TB drugs. Eighty six (29.9%) isolates were resistant to at least one of five drugs tested while 200 (70 %) were sensitive. Mono resistance was identified in four of five drugs tested. Isoniazid had 30.2% resistance, streptomycin 11.6%, ethambutol 13.9% and pyrazinamide 30.2%. Double resistance was 4.6% for isoniazid and pyrazinamide, 4.6% for streptomycin and isoniazid, 1.2% for rifampin and streptomycin. Two isolates (1.1%) were MDR- TB, and one was triple resistant with an additional resistance to ethambutol. Isoniazid is a first line drug used throughout treatment. The high rate of resistance observed could result to increase in MDR TB cases, unless systematic nationwide surveillance is ongoing.

Key words: Resistance, *Mycobacterium tuberculosis*, patients, Nairobi

1.0 Introduction

Tuberculosis has been reported to be a major cause of death being responsible for 1.7million a death annually (1) which is a 30% drop from the 3million reported in the 1990s (2). However tuberculosis programs face tremendous challenges in reducing MDR-TB (1). Since 1994, only 59% of all countries globally have been able to collect high quality representative data on drug resistance (1). Kenya is ranked thirteenth among the 27 high burden TB countries in the world and has 5th highest burden in Africa (1). The Kenya National Division of Leprosy, TB and Lung disease (DLTLD) began to implement WHO DOT in 1993 and reported100% coverage by 1996. In 2005 the DOT case detection rate reached WHO target of 70% and rose to 72% in 2007 using Becton and Dickson BACTEC MGIT 960 technique on the first line drugs used for treatment of TB (3). The DOT treatment success rate met WHO's target of 85% in 2007. Inspite of all, WHO Global TB report of 2009 (4) estimates that Kenya had approximately 132,000 new TB cases and an estimated 74,000 people died. There are 2000 treatment facilities and 900 TB diagnosis facilities throughout the country but they do not cater for drug resistant TB. Multidrug resistant TB which is defined as TB resistant to at least isoniazid and rifampin has been reported since 1980's in Kenya (5). According to USAID, it is estimated that there were 2000 MDR-TB cases in Kenya in 2007, although only 4.1% of these were diagnosed and notified (6). MDR-TB patients are either not receiving treatment or have been allowed out of hospital because the government does not have money to treat them. Of the 300 patients diagnosed with MDR-TB in 2009, only 44 were receiving treatment while the remaining 255 were not in any structured treatment (4). According to WHO an MDR patient infects 10-15 people every year (7). Treatment for MDR –TB lasts for 18 months but can extend to two years or more because it is difficult to cure and drugs used for treatment are less potent, more toxic and more expensive from 50-200 times higher. If not properly treated it can result in complications that may require surgical interventions increasing period of hospitalization and raising the cost of treatment even higher.

WHO estimates that globally 4.5 million people are co-infected with HIV and TB (7). In Kenya out of the 1.2 million HIV positive individuals and health workers detect about 20% of TB cases among HIV positive patients. HIV –TB co-infection is close to 48% in Kenya according to USAID (6). Studies show that TB patients co-infected with HIV are at a higher risk of having MDR-TB compared to patients without HIV infection (1). Little information is available in Kenya on the resistance patterns of *Mycobacterium tuberculosis* and this study was undertaken to determine resistance patterns of the *M. tuberculosis* against the first line drugs used for treatment in patients diagnosed with pulmonary tuberculosis.

2.0 Study Population and Methods

2.1 Selection of Patient Population

A total of five hospitals and TB clinics were randomly sample from various locations in and around Nairobi. These included Kangemi, Riruta, Mbagathi TB clinics, Kiambu district hospital and Nazareth hospital (Table I).

In this study 356 pulmonary TB adult patients, 18 yrs and above, who consented to enroll in the study were systematically sampled on the basis of being newly diagnosed. Out of these 286 were sputum culture positive which comprised 173 males (60.3%) and 113 (39.7%) females.

2.2 Processing of Sputa

The sputa were collected in sterile screw-caped bottles. They were decontaminated with NaOH solution (40g/l4% w/v) combined with 2.9% sodium citrate solution and 0.5g N-acetyl-L-cystein (NALC) powder per 100ml sodium hydroxide sodium citrate solution. Sterile phosphate buffer was added and the organisms concentrated by centrifugation at 3000rpm for 15 minutes. The supernatant was decanted and the sediment suspended with phosphate buffer and inoculated in liquid MGIT media and incubated in BACTEC 960 system (BD Diagnostic Systems, 7 Loveton Circle, Sparks, MD 21152, USA) for a maximum of 8 weeks until the culture flagged positive. The residue was also inoculated in Lowenstein Jensen solid medium and incubated at 37^{o} C for a maximum of 12 weeks. The growth of *M. tuberculosis* thus obtained was used for sensitivity testing.

2.3 Sensitivity Testing Of *Mycobacterium Tuberculosis*

All positive tubes were tested for contamination before sensitivity tests. A total of five first line drugs were tested for sensitivity. These were (SIRE) that is streptomycin(S) 1.00µg/ml, isoniazid (INH) 0.10 µg/ml, rifampin (Rif) 1.00

µg/ml ethambutol (E)5.00 µg/ml and pyrazinamide (PZA) 100.0 µg/ml. The technique was carried out according to the manufacturer's instructions. A control tube was incorporated in all the isolates tested. An external control of Rv 37 was also set in all culturing and sensitivity testing processes (Becton ,Dickson and company, 7 Loveton Circle Sparks, Maryland 21152 USA).

2.4 Ethical Approval

The research proposal was approved and ethically cleared by the national ethical research committee (ERC) at the Kenya Medical research institute (KEMRI).

3.0 Results

A total of 286 sensitivity tests were carried out from pulmonary tuberculosis patients. Out of these 173(60.2 %) were from males while 113(39.7%) were from females. A total of 86 (30.3%) strains showed resistance to at least one drug tested, while 200(69.6%) were susceptible. The isolates from males which showed resistance to at least one drug were 52(60.4%) while from the females were 34(39.5 %) (Table II). Monoresistance was observed in 70(24.4%) isolates, double resistance 11 (12.7%), and triple resistance 1 (1.2%) isolate (Table III). Mono resistance was recorded in all the five drugs tested (Table IV). Isolates resistant to streptomycin were 15 (17.4%), those resistant to pyrazinamide were 30 (34.8 %), while those resistant to isoniazid were highest at 37 (43%), 13 (15.1%) were resistant to ethambutol and 4 (4.6%) to rifampin. Double drug resistant isolates were 11 (12.8%) with two (2.3%) being multidrug resistant (MDR) that is resistance to isoniazid and rifampin. Four (4.6%) isolates were resistant to isoniazid and pyrazinamide, 4 (2.2%) to streptomycin and isoniazid and 1 (1.2%) to rifampin and streptomycin. One MDR isolate was triple resistant with an additional resistance to ethambutol.

4.0 Discussion

4.1 Comparison of Resistance on the Basis of Gender

There was a significantly greater number of males diagnosed with pulmonary TB than females (60.46 % and 39.53 % respectively) (χ^2=0.963, df=1, P<0.05). This differs with earlier studies in Kenya where more females were associated with drug resistance than males; however this earlier study had been conducted in a refugee camp in N. Eastern Kenya. (8). It however compares with studies in Pakistan (9), where there were 70.9% males and 29.15% females. Similar results have been reported by another study in Pakistan (11) (68.3% males and 31.7% females) and Tanzania (12) with 68% male and 32% female. Globally a 70% excess of males over female patients was reported (10). World Health Organization reported 67.2% male population diagnosed with TB as compared to females (7). The greater number of males compared to females could be attributed to behavioral factors, such as smoking which is a predisposing factor to TB with more males being smokers than females.

4.2 Resistance Patterns

The overall resistance to all the drugs tested(30.06%), was much higher than earlier studies in Kenya where 18.3% isolates were resistant to at least one drug (8). It was also higher than studies in Tanzania where only 14 out of 280 (5.83%) isolates were resistant to at least one drug (12) while in Ethiopia resistance rates ranged from 27.4% to 14% (13, 14, 15, 16 and17). In Korea total resistance was 18.7% (18) while in South Africa total resistance to the drugs tested was 7.3% (19). The results of this study compare with studies carried out in Central Asia where resistance was 30.5% (20). All these studies were one-time studies carried out in single facilities in the different countries, similar to this study.

Resistance to isoniazid in this study was 12.9% which was much higher than earlier studies in Kenya where resistance to INH was 10.2% (21).It was higher than Ethiopia where one isolate was resistant to isoniazid (13), Bangladesh 5.4% (22) and Sri Lanka 12.2% (23). It was however lower than that reported in Mozambique (14.9%) (24). WHO 2008 reported a 5.9% resistance rate worldwide (7). According to WHO isoniazid resistance rate higher than 10% can predict the development of MDR TB (25). This high resistance may be due to the fact that isoniazid is used widely in the treatment of TB as a first-line drug and poor compliance by patients can select for drug resistant mutant strains. In this study, rifampin resistance was 1.3% which was higher than earlier studies in Kenya where resistance was 0.3 %(21) and in an Ethiopian study where one isolate was resistant to rifampin. This is also higher than studies in Bangladesh where resistance was 0.5 % (23) and other studies in Ethiopia where resistance to rifampin ranged from 0-1.8% (14, 12 and 18). The undesirable effects of rifampin are nausea, vomiting, rashes,

hepatitis, GIT upset, flu like symptoms, fever and jaundice which could result in non-adherence hence selecting for the resistant strains (26).

Resistance to streptomycin in this study was 5.2% which was higher than another study in Kenya where resistance was 1.8% (21) but lower than that reported in Ethiopia (26%) (13)and Sri Lanka 9.9% (24). Resistance to ethambutol in this study was 4.5% which was higher than rates in Ethiopia 2.7% and 0.5% (13 and 27). It was however lower than studies carried out in Sri Lanka where 14.5% resistance was reported (24). Ethambutol enhances the effect of many drugs including beta lactams to different *Mycobacteria* species and can be used to develop a regimen for MDR TB (28).

In this study there were a high number of patients with TB showing isoniazid resistance yet susceptible to all other drugs. According to WHO guidelines for management of drug resistant TB, drug resistant patients can be classified into three groups; those releasing bacilli susceptible to all anti-TB drugs, those releasing bacilli resistant to isoniazid but susceptible to rifampin and those releasing bacilli resistant to at least isoniazid and rifampin (29). Most of the isolates in this study were resistant to isoniazid but susceptible to rifampin. It is therefore possible for these patients to recover fully if WHO guidelines for retreatment are followed under strict supervision in order to prevent them from developing MDR TB. However the high rate of INH resistance is significant since it is a first line drug which is used throughout the course of treatment. This indicates a high probability of developing MDR TB in future since it has been observed that MDR often develops from initial INH monoresistant strains (30). Isoniazid is also the drug of choice for chemoprophylaxis of TB and is used in developed countries for treating latent TB. The high level of INH resistance among the study population also is an indicator that this drug will be completely useless for both these purposes in Kenya.

In this study two patients had MDR-TB (2.3%) which is not unusual because in Sub-Sahara Africa countries MDR TB prevalence is estimated to be 6.3% (31).

The results of this study indicate the need for strict enforcement of the direct observation therapy (DOT) and better epidemiological surveillance of TB cases. Treatment with internationally approved regimens has resulted in high cure rates without emergence of resistance as long as there is no non-adherence (9). These regimens are effective in preventing the emergence of resistance because of inhibition of the development of spontaneous resistance due to mutation. There is urgent need to improve drug susceptibility testing which is not routinely carried out in public hospitals in Kenya. There is also need for patients to access rapid diagnosis and treatment with more effective drugs and regimens shorter than the current two year period for MDR-TB.

5.0 Conclusion and Recommendations

From the results of this study, resistance was observed in all the five drugs tested being highest in isoniazid which is quite significant because it is a first line drug used throughout treatment of TB. Since susceptibility testing is not commonly carried out in government facilities in Kenya this study serves to inform that MDR may become an important phenotype in our health facilities especially due to the high rate of INH resistance unless systematic continuous surveillance is adopted. Since one case of XDR-TB has already been reported in Kenya (32), concerted efforts need to be put in place by both public and private hospitals in order to avert a possible explosion of MDR TB cases in Kenya. Since drug resistant TB is closely associated with HIV more research need to be carried out to determine if there is an overlap between MDR-TB and HIV epidemics.This study was carried out over limited time duration of nine months and only surveyed sentinel sites mostly in high population density areas of Nairobi and parts of central Kenya. The data presented may therefore not be representative of national statistics.

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Table 1: Summary of the clinics sampled and patient population

Hospital	Constituency	Population	No. of filter clinics	TB patient population (2010)
Kangemi	Westlands	247,102	10	70
Riruta	Dagoretti	329,577	6	130
Mbagathi	Langata	355,188	20	1390
Kiambu	Kiambaa	253,751	12	125
Nazareth	Githunguri	147,763	10	28

Table 2: Sensitivity patterns by Gender

Sensitivities	Male	Female
Total sensitivities	173(59.55%)	113(40.44%)
Resistance to at least one drug	52(60.46%)	34(39.53%)

Table 3: Total drug resistance patterns

Resistance patterns	No. of isolates
Single resistance	70
Double resistance	11
Triple resistance	1
Quadruple resistance	0

Table 4: Drug resistance patterns of Mycobacterium tuberculosis isolates from pulmonary tubercular patients in Nairobi

Drug resistance	No. of patients
Resistance to any one drug	86(30.06%)
Mono resistance: Isoniazid Ethambutol Streptomycin Pyrazinamide	 26(12.9%) 12(4.5%) 10(5.2%) 26(12.9%)
Double resistance: Isoniazid and rifampin Isoniazid and pyrazinamide Streptomycin and isoniazid Rifampin and streptomycin	 2(1.12%) 4(4.6%) 4(4.6%) 1(1.16%)
Triple resistance: Isoniazid, rifampin, ethambutol	 1(1.16%)

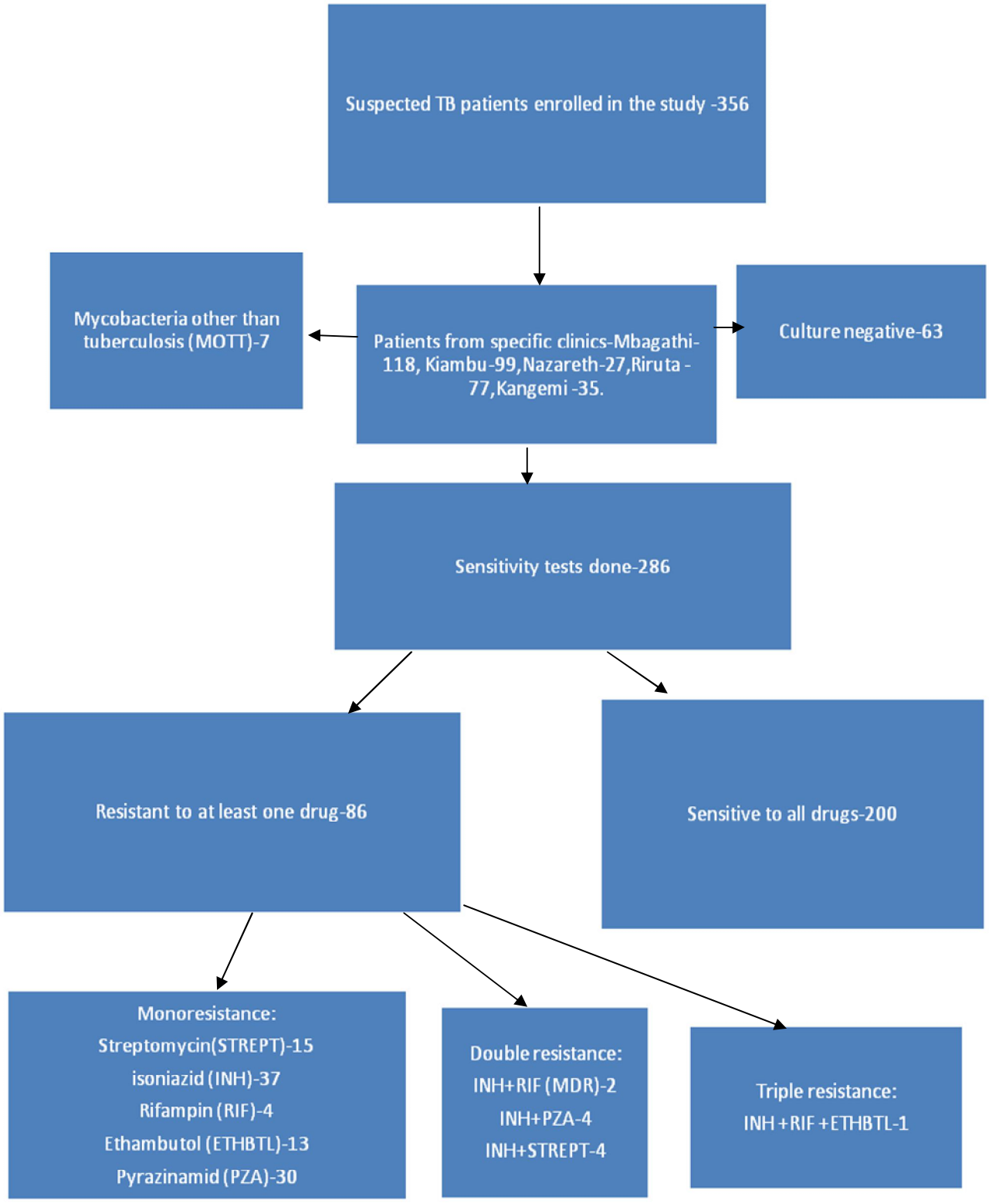

Figure I: Study profile