

## Resistance patterns of *Mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients in Nairobi

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### Abstract

**Introduction:** In Kenya, which ranks thirteenth of 27 high tuberculosis burden countries, diagnosis is based on Ziehl-Neelsen staining alone and patients are treated without information on sensitivity patterns. This study aimed to determine resistance patterns of *Mycobacterium tuberculosis* isolated from pulmonary samples.

**Methodology:** Pulmonary tuberculosis patients in Nairobi were randomly sampled after informed consent and recruited into the study using a structured questionnaire. Specimens were cultured in liquid and solid media, and drug susceptibility tests were performed for first-line drugs including (isoniazid, rifampin, streptomycin, ethambutol and pyrazinamide).

**Results:** Eighty-six (30%) of 286 isolates were resistant to at least one of five antibiotics tested. Thirty-seven (30.2%) isolates were resistant to isoniazid; 15 (11.6%) to streptomycin; 13 (4.5%) to ethambutol; four (1.4%) to rifampin ; and 30 (10.4%) to pyrazinamide. Double resistance was seen as follows: four (1.4%) isolates were resistant to both isoniazid and pyrazinamide; four (1.4%) to streptomycin and isoniazid; and one (0.3%) to rifampin and streptomycin. Two isolates (0.7%) were multidrug resistant, and one was triple resistant with an additional resistance to ethambutol. Results also showed 88.7% of patients were below the age of 40 years, while 26.3% were HIV positive. The majority of the patients (66.5%) were unemployed or self-employed in small businesses, with 79.4% earning less than 100 USD per month.

**Conclusion:** The high resistance observed in isoniazid, which is a first-line drug, could result in an increase in multidrug resistance unless control programs are strengthened. Poverty should be addressed to reduce infection rates.

**Key words:** tuberculosis; resistance patterns; susceptibility tests; multidrug resistance

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### Introduction

Tuberculosis (TB) has been reported to be responsible for 1.7 million deaths annually [1]; however, tuberculosis programs face tremendous challenges in reducing multidrug-resistant tuberculosis (MDR-TB). 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 the fifth highest burden in Africa [1]. The Kenya National Division of Leprosy, TB and Lung disease (DLTLD) began to implement World Health Organization (WHO) direct observation therapy (DOT) for tuberculosis in 1993 and reported 100% coverage by 1996. In 2005 the DOT case detection rate reached the WHO target of 70% and rose to 72% in 2007 using the Becton and

Dickson BACTEC MGIT 960 (Loveton Circle, Sparks, USA) technique on the first-line drugs used for treatment of TB [2]. The DOT treatment success rate met WHO's target of 85% in 2007. However, the WHO Global TB report for 2009 [3] estimated that Kenya had approximately 132,000 new TB cases and an estimated 74,000 people died. There are 2,000 treatment facilities and 900 TB diagnosis facilities throughout the country but they do not cater to drug-resistant TB because laboratory diagnosis is mainly based on Ziehl-Neelsen staining (ZN) without culture and sensitivity tests. MDR-TB, which is defined as TB resistant to at least both isoniazid and rifampin, has been reported since the 1980s in Kenya [4]. According to USAID, it is estimated that there may have been as many as 2,000 MDR-TB cases in Kenya in 2007, although only 4.1% of these individuals

**Table 1.** Summary of the clinics sampled and patient population

Hospital	Constituency	Population	No. of filter clinics	TB patient population (2010)	No. of patients sampled
Kangemi	Westlands	247,102	10	70	35
Riruta	Dagoretti	329,577	6	130	77
Mbagathi	Langata	355,188	20	1390	118
Kiambu	Kiambaa	253,751	12	125	99
Nazareth	Githunguri	147,763	10	28	27

were diagnosed and notified [5]. 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 256 were not in any structured treatment [3]. According to the WHO, an MDR patient infects 10-15 people every year [5]. 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 50-200 times more expensive than first-line drugs. 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.

The WHO estimates that globally 4.5 million people are co-infected with HIV and TB [6]. In Kenya, about 20% of the 1.2 million HIV-positive individuals also have TB. 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 *M. tuberculosis*; therefore, this study was undertaken to determine *M. tuberculosis* resistance patterns against the first-line drugs used for treatment in patients diagnosed with pulmonary tuberculosis.

## Methodology

### *Selection of patient population*

A total of five hospitals and TB clinics from various locations in and around Nairobi, Kenya, were randomly sampled. These included the Kangemi, Riruta, and Mbagathi TB clinics as well as Kiambu District Hospital and Nazareth Hospital (Table 1).

The study was conducted between April and December 2010 when 356 pulmonary adult TB patients (18 years of age and older), who consented to enroll, were systematically sampled on the basis of

being newly clinically diagnosed cases. Out of these 286 173 males (60.3%) and 113 (39.7%) females were sputum culture positive.

### *Methodology*

Two early morning sputa and a spot sample were collected in sterile screw-capped bottles. They were decontaminated with NaOH solution (40g/14% w/v) combined with 2.9% sodium citrate solution and N-acetyl-L-cystein (NALC) powder. Sterile phosphate buffer was added and the organisms concentrated by centrifugation at 3,000 rpm 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, , Sparks, MD, USA) for a maximum of eight weeks until the culture flagged positive. The residue was also inoculated in Lowenstein Jensen (LJ) solid medium and incubated at 37° C for a maximum of 12 weeks. The growth of *M. tuberculosis* thus obtained was used for sensitivity testing. Statistical analysis was performed by multiple logistic regression using SPSS version 17 (IBM, Chicago, USA).

### *Sensitivity testing of M. tuberculosis*

All positive tubes were tested for contamination before sensitivity tests using the standard method used in Kenya for drug sensitivity testing using the BACTEC MIGIT 960 liquid culture system (Becton-Dickson and Company, Sparks, MD, USA).

A total of five first-line drugs collectively referred to as SIRE (streptomycin [S] - 1.00µg/ml; isoniazid [INH] - 0.10 µg/ml; rifampin [Rif] - 1.00 µg/ml; and ethambutol [E], 5.00 µg/ml) were tested for sensitivity along with pyrazinamide (PZA, 100.0 µg/ml). A control tube was matched with all the isolates tested. An external control of Rv 37 was also set in all culturing and sensitivity testing processes.

**Table 2.** Drug resistance patterns of *Mycobacterium tuberculosis* isolates from pulmonary tubercular patients in Nairobi

Drug resistance	Patients N (%)
Resistance to any one drug	86 (30.1%)
Mono resistance	
Isoniazid	26 (12.9%)
Ethambutol	12 (4.5%)
Streptomycin	10 (5.2%)
Pyrazinamide	26 (12.9%)
Double resistance	
Isoniazid and rifampin	2 (1.1%)
Isoniazid and pyrazinamide	4 (4.6%)
Streptomycin and isoniazid	4 (4.6%)
Rifampin and streptomycin	1 (1.2%)
Triple resistance	
Isoniazid, rifampin, ethambutol	1 (1.2%)

Each patient was required to complete a structured questionnaire upon giving informed consent.

#### *Ethical approval*

The research proposal was approved and ethically cleared by the national ethical research committee (ERC) at the Kenya Medical Research Institute (KEMRI). Permission was also obtained from Health Department of Nairobi city council to sample city council clinics and from the Medical Officer of Health (MOH) at the Kiambu District Hospital. Each patient who consented to enroll was required to complete an informed consent form.

#### **Results**

A total of 286 sensitivity tests were performed from pulmonary tuberculosis patients of whom 173 (60.4 %) were male while 113 (39.5%) were female. The majority of the patients (88.7%) were below the age of 40 years while the rest (11.2%) were above the age of 40 years with only three patients (1%) above the age of 60 years. Among the pulmonary tuberculosis patients 26.3% were HIV positive. The majority of the patients were either unemployed or self-employed in small businesses (66.5%) with only 33.5% being in formal employment. The number of participants who were smokers or who consumed alcohol was also surveyed, and 29.7% of the patients were found to be smokers and 40% were alcohol consumers.

A total of 86 (30.1%) strains showed resistance to at least one drug tested, while 200 (69.9%) were susceptible. Fifty-two (18.2%) males and 34 (11.9 %) females showed resistance to at least one drug. The

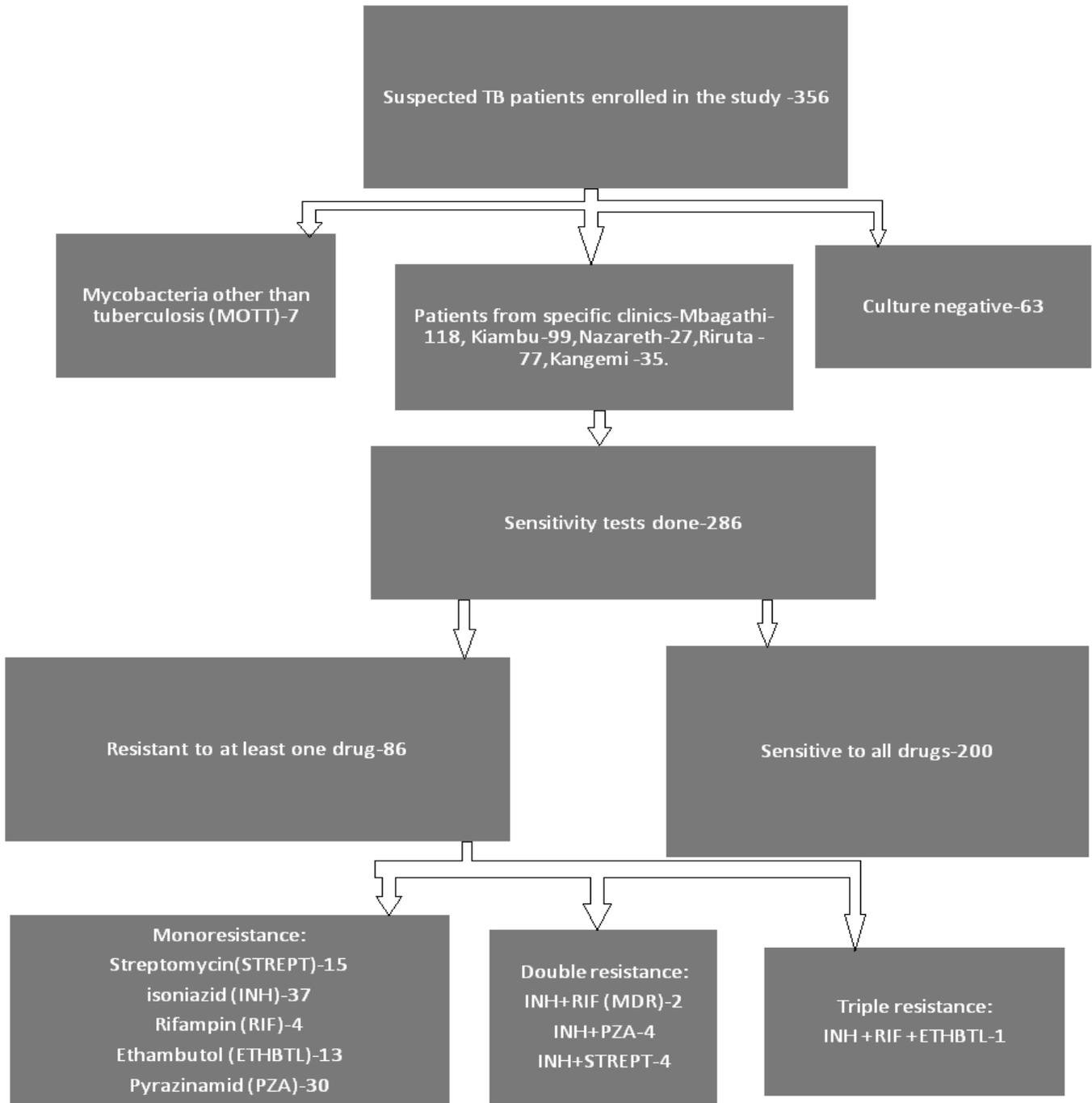
isolates showed different resistance patterns with monoresistance (resistance to at least one drug) in 70 (24.4%) isolates, double resistance in 11 (3.8%) isolates, and triple resistance in on (0.3%) isolate. Monoresistance was recorded in all five drugs tested (Table 2). Isolates were resistant to the antibiotics tested as follows: 15 (5.2%) were resistant to S; 30 (10.4 %) were resistant to PZA; 37 (12.9%) were resistant to INH; 13 (4.5%) were resistant to E; and four (1.4%) were resistant to Rif. Eleven (3.8%) antibiotics were double drug resistant, two (0.7%) of which were MDR. Four (1.4%) isolates were resistant to INH and PZA, 4 (1.4%) to S and INH and 1 (0.3%) to Rif and S. One MDR isolate was triple resistant with an additional resistance to E.

#### **Discussion**

##### *Comparison of resistance on the basis of gender*

There was a significantly greater number of males diagnosed with pulmonary TB than females (60.4% and 39.5 % respectively;  $\chi^2 = 0.963$ ;  $df = 1$ ;  $P < 0.05$ ). This differs with earlier studies in Kenya where more females were associated with drug resistance than males (OR = 2.3; 95%CI 1.2-4.8;  $P = 0.008$ ) [7]. It compares with studies in Pakistan [8], however, where drug resistance was associated with 70.9% males and 29.15% females, and also in Tanzania [9] where drug resistance was associated with 68% males and 32% females. Globally a 70% predominance of males over female patients was reported [10]. The World Health Organization reported that 67.2% of the global male population was diagnosed with TB as compared to the female population [6]. The greater number of males

**Figure 1.** Study profile



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. Alcohol consumption, malnutrition [11] and the delay in seeking medical treatment, especially by men [12], are other factors that have been associated with higher numbers of males than females with TB,

with over 90% of the participants in the current study being male.

*Resistance patterns*

The overall resistance to all the drugs tested (30.1%) was much higher than that reported in earlier studies in Kenya, where 18.3% of isolates were resistant to at least one drug [7]. Resistance rates in

the present study were also higher than rates observed in studies in Tanzania where only 14 out of 280 (5.83%) isolates were resistant to at least one drug [10], while in Ethiopia resistance rates ranged from 14% to 27.4% [13,14]. In Korea total resistance was 18.7% [15], while in South Africa total resistance to the drugs tested was 7.3% [16]. The results of this study compare with those of studies conducted in Central Asia where resistance was 30.5% [17]. All these studies were one-time studies performed in single facilities in the respective countries. Similarly, in the current investigation was also a one-time study performed in a single facility.

Resistance to INH in this study was 12.9%, which was higher than results obtained in earlier studies in Kenya, where resistance to INH was 10.2% [18]. INH resistance in the present study was also much higher than that seen in Ethiopia, where one isolate was resistant to INH [19], and in Bangladesh and Sri Lanka at 5.4% [20] and 12.2% [21], respectively. It was, however, lower than that reported in Mozambique (14.9%) [22]. In 2008, the WHO reported a worldwide resistance rate to INH of 5.9% [6]. According to the WHO, INH resistance rates higher than 10% can predict the development of MDR TB [23]. This high resistance may be caused by both its wide use in the treatment of TB as a first-line drug and/or poor compliance by patients. In this study, resistance to Rif was 1.3%, which is higher than that observed in earlier studies in Kenya where resistance was 0.3% [18] and in an Ethiopian study where one isolate was resistant to Rif. This rate is also higher than reports from studies in Bangladesh where resistance was 0.5% [21] and other studies in Ethiopia where resistance to Rif ranged from 0% to 1.8% [10;15]. Rif has several adverse effects such as nausea, vomiting, rashes, hepatitis, GIT upset, flu-like symptoms, fever and jaundice, which could result in patient non-adherence and hence may lead to the selection of resistant strains.

Resistance to S in this study was 5.2%, which was higher than the resistance of 1.8% [18] reported in another study in Kenya, but lower than that reported in Ethiopia (26%) [19] and Sri Lanka 9.9% [22]. Resistance to E in this study was 4.5% which was higher than rates in Ethiopia 2.7% [19]. It was, however, lower than studies conducted in Sri Lanka where 14.5% resistance was reported [22]. 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 [23].

In this study a high number of patients with TB showed INH resistance yet susceptibility to all other tested 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 INH but susceptible to Rif; and those releasing bacilli resistant to at least INH and Rif [24]. Most of the isolates in this study were resistant to INH but susceptible to Rif. It is therefore possible for these patients to recover fully if WHO guidelines for retreatment are followed under strict supervision 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 for developing MDR TB in the future since it has been observed that MDR often develops from initial INH monoresistant strains. INH 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-Saharan African countries MDR TB prevalence is estimated to be 6.3% [25].

The results of this study indicate the need for strict enforcement of the DOTs method and better epidemiological surveillance of TB cases. Treatment with internationally approved regimens has resulted in high cure rates without emergence of resistance [8]. These regimens are effective in preventing the emergence of resistance because of inhibition of the development of spontaneous resistance due to mutation.

#### *Limitations of the study*

This study was conducted over a limited period of nine months and surveyed only sentinel sites mostly in high-population density areas of Nairobi and parts of its environs. Similar studies should be performed in other regions.

#### **Conclusion**

This study serves to inform that MDR may become an important phenotype in our health facilities unless TB control programs are strengthened and continuous systematic surveillance is adopted. There is urgent need to improve drug

susceptibility testing which is not routinely performed in public hospitals in Kenya. Furthermore, there is a need for patients to access rapid diagnosis and treatment with more effective drugs and also regimens shorter than the current two-year period for MDR-TB. Since two cases of XDR-TB have already been reported in Kenya, there needs to be a commensurate increase in human resources available for TB control in both public and private hospitals to avert a possible explosion of MDR TB. Since drug-resistant TB is closely associated with HIV more research must be conducted to determine if there is an overlap between MDR-TB and the HIV epidemic.

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