

Drug Susceptibility Patterns of *Mycobacterium tuberculosis* Isolates from Tuberculosis Patients in Coastal Kenya

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Abstract

Background: Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. Anti-tuberculosis drug resistance is an emerging health problem in Kenya and especially in Coastal region. This is a major challenge in tuberculosis control. Diagnosis is based on Ziel-Neelsen staining alone and patients are treated without information on sensitivity patterns. **Aim:** This study aimed to determine drug susceptibility patterns of *Mycobacterium tuberculosis* in Coastal Kenya. **Study Design:** Hospital and laboratory based cross-sectional study was carried between April 2015 and July 2016 at Coast General Referral hospital; Tudor, Port-Reitz, Likoni Sub-County hospitals; Mlaleo, Kongowea and Mikindani health centers. **Methodology:** Sputum samples from patients with bacteriological confirmed TB on microscopy were cultured on Lowenstein Jensen (LJ) media. Strains of *MTB* complex from Lowenstein Jensen (LJ) slopes were subjected to drug susceptibility testing (DST) to first-line drugs including isoniazid (H), rifampicin (R), streptomycin (S) and Ethambutol (E) using proportional method on the Mycobacterium Growth Indicator Tube (MGIT) conventional method. Participants were offered diagnostic testing and counselling for HIV testing. **Results:** Drug sensitivity test was performed for a total of 210 *Mycobacterium tuberculosis* isolates for the first line anti-TB drugs. About seventy eight percent and twenty nine percent of the strains from new patients and previously treated patients were fully sensitive to all the drugs tested respectively. Prevalence of any resistance to one drug was 102 (48.6%, 95% CI: 20.45 - 28.23). Any single drug resistance was most frequent in isoniazid 30 (16.0%), Ethambutol 20 (10.0%), Streptomycin 18 (18.3%) and Rifampicin 4 (2.1%) in newly diagnosed patients.

Among previously treated patients any resistance to streptomycin, ethambutol, isoniazid and rifampicin was 10 (58.8%), 9 (52.9%), 7 (41.2%) and 4 (23.5%) respectively. Prevalence of MDR-TB defined as resistant to at least both isoniazid and rifampicin was 10 (4.8%) among new and previously treated patients respectively. **Conclusion:** The current study reveals that the overall resistance to first line anti-TB drugs was high. Although the rate of MDR-TB was relatively low, this signifies that conditions favouring the spread of MDR-TB are on high rise. Therefore, it is essential to address the problems of development of drug resistant strains of TB by establishing good TB programmes (DOTS). Patients' adherence to anti-TB drugs and introducing drug sensitivity testing (DST) services at County level hospitals will minimize occurrence of drug resistant.

Keywords

Tuberculosis, Resistance Patterns, Susceptibility Tests, Multidrug Resistance

1. Introduction

Tuberculosis (TB) is an infectious disease caused by strains belonging to the *Mycobacterium tuberculosis* complex. Multidrug resistant TB (MDR TB), defined as resistance to at least isoniazid and rifampicin, has been spreading rapidly in recent years. In 2013, 3.8% of newly diagnosed and 20% of retreatment cases were estimated to have MDR-TB globally with noticeable geographical variations in prevalence [1]. In Africa, various reports have demonstrated that resistance to one or more anti-TB and MDR-TB ranges from 3% to 37.3% [2] [3] and 1.4% to 11.6% [4] [5] [6] [7], respectively. Besides, extensively drug resistant TB (XDR-TB) has been reported by 92 countries (including Kenya) and about 10.6% of MDR TB patients have XDRTB globally. Therefore, the rapid spread of MDRTB and XDRTB especially in new TB patients is challenging the effectiveness of TB control programmes in many low income countries [8] [9].

Kenya ranks 15th on the list of 22 high-burden tuberculosis (TB) countries in the world and fourth in Africa. According to the World Health Organization's global TB Report of 2013, Kenya had approximately more than 142,000 new TB cases and an incidence rate of 197 new sputum smear-positive (SS+) cases per 100,000 population. Determining the proportion of drug resistance among new cases is vital in the assessment of the effectiveness of national TB control programme [10]. Drug resistance in *M. tuberculosis* occurs as a result of random spontaneous chromosomal mutations during natural cell replication. These mutations are neither drug induced nor drug linked. The probability of a drug-resistant mutant occurring is directly proportional to the size of the bacterial population [11]. The frequency of primary resistant organisms varies for each drug; however, it is usually between 10^{-6} to 10^{-8} . Spontaneous resistance to isoniazid is estimated to occur once in every 106 organisms, and to rifampicin

once in every 108 organisms. The probability of spontaneous mutants being simultaneously resistant to two or more drugs is the product of the individual mutants. The development of drug resistance is a man-made amplification of a naturally occurring phenomenon. Previous treatment for tuberculosis predisposes to the selection of multi-drug resistant organisms. Non-compliance is a major factor in allowing the resistant organisms to survive [12]. According to the WHO, an MDR patient infects 10 - 15 people every year [USAID, 2009]. Treatment of 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 treated properly, it can result in complications that may require surgical interventions hence increasing period of hospitalization and raising treatment cost. Although an unequal global distribution of drug resistance exists between poor and rich countries, the problem is global [13]. The regions where drug-resistant TB is more prevalent lack the resources to implement adequate measures to control even the susceptible types of the disease. Recent reviews have reported a high prevalence of primary multidrug resistant tuberculosis in Latvia (1998: 9.0%), Estonia (1998, 14.1%) and Dominican Republic (1994-1995: 6.6%). This circumstance has adversely affected the control efforts being made by several countries with limited access to second line anti-TB drugs [14]. Therefore, early diagnosis and treatment, improving treatment outcomes and expanding diagnostic capacity for mycobacterial culture and drug susceptibility test (DST) are crucial to limit the spread of drug resistant TB strains, especially MDR-TB [15].

WHO estimates that there were around 2300 cases of multidrug-resistant (MDR)-TB in Kenya in 2013, although only 4.1 percent of these cases were diagnosed and notified. This was based on only 4000 (14% of all retreatment cases) sample analyzed at the central reference laboratory [16]. As of 2013 five cases of Extra drug resistant tuberculosis (XDR-TB) had been reported in Kenya of which one was cured, two are on treatment and died from the complications of this extreme form of TB. The high rates of drug resistance TB currently being reported in Kenya are alarming [17]. Human immunodeficiency virus (HIV) infection curtails the effects of TB control programme by lowering the life expectancy of those receiving TB treatment [18] [19] [20]. The WHO estimates that globally 46 million people are co-infected with HIV and TB [21]. In Kenya, about 25% of the 4 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 [22]. Kenya is working towards interrupting transmission dynamics of TB and drug resistance in the general population by providing directly observed treatment short course (DOTS). This strategy can rapidly reduce the transmission and incidence of both drug susceptibility and drug resistance TB. However, standardized or individualized treatment regimen may be required based on the Drug sensitivity Testing (DST) to reduce mortality rates due to MDR-TB [23]. Primary drug resistance is also a

strong predictor for failure and relapse for acquiring further resistance as shown in study done in Vietnam [24]. To strengthen the TB control programme, the Ministry of Health established one Central reference Laboratory within the Centre for Respiratory Diseases Research, Kenya Medical Research Institute (CRDR-KEMRI) at Kenyatta National Hospital with TB culture facilities and Gene Xpert MTB/RIF to identify rifampicin resistance, which is a proxy marker for MDR-TB. Despite all these efforts, in Kenya, the capacity of laboratories to perform TB culture and DST is very limited. Moreover, most of the few studies on TB resistance were conducted in only a few regions of the country and data are often outdated and do not reflect the existing status of the problem. The Coastal region of Kenya is known for its high TB case load and anti-TB drugs have been in use for long time in the area before the implementation of directly observed treatment short course [25]. However, there is a paucity of data on the resistance patterns of *M. tuberculosis* in this part of the country. Therefore this study was conducted to determine the drug resistance patterns of *Mycobacterium tuberculosis* against the first line drugs in patients diagnosed with pulmonary tuberculosis in Coastal region of Kenya.

2. Materials and Methods

2.1. Study Area

The study was conducted in Mombasa County which has a population of 1,031,266 by the year 2012. The population is steadily growing due to rural-urban migration and immigration from unstable countries. The total area Mombasa is 109 km² with about 60% of the people living overcrowded informal settlements in the form of shelters. Residents are of mixed ethnicity and are engaged in low-income generating activities, mainly informal sector and small trading. The County has rapid population growth and is characterized by low socio-economic indicator. This creates huge demands on health facilities and inability to keep pace with the environment, continued economic prosperity, public health and quality of life of residents. Tuberculosis and HIV/AIDS are the leading causes of deaths in the area representing 50%.

2.2. Study Site

The study was done at Coast provincial General hospital (CPGH), Mlaleo Health and Mikindani Health Centers, Likoni, Portreitz and Tudor County hospitals. These facilities were selected because of the fact that they represented the largest centres for TB diagnosis and treatment in their respective regions. These hospitals like all others at their levels have chest clinics where TB patients obtain health care respectively.

2.3. Study Design

This was hospital and laboratory based descriptive cross-sectional study carried out between April 2015 and July 2016. New smear positive pulmonary TB patients

from these facilities were included in the study. Basic TB diagnosis facilities including smear microscopy and X-ray are available in Referral hospital but only smear microscopy service is offered in the County and Sub-county hospitals. In Kenya, however, due to inadequate laboratory capacity, there is only one public reference centre located in Nairobi performing TB culture and drug susceptibility testing (DST). Therefore, the service is not currently available in other regions in which Coastal region of Kenya is not an exception.

2.4. Inclusion and Exclusion Criteria

All adult patients 18 years and above suspected of having TB and resident in Mombasa County for at least six (6) months, not on anti-TB chemotherapy and consented to participate in the study were recruited. Tuberculosis suspects who were below 18 years and unwilling to participate in the study and not meeting the above inclusion criteria were excluded.

2.5. Sample Size

The sample size was determined by taking the prevalence of 2.8% from previous study, desired precision of 1%, a 95% confidence interval and nonresponse rate of 15%. The sample size was calculated based on the sampling method recommended by WHO for drug resistance survey in tuberculosis [26]. The final sample size was 500.

2.6. Sampling Frame

Coastal region was purposively sampled because of high cases of TB. The sampling frame consisted of all the public health facilities within the study area. In Kenya, majority of TB patients visit public health facilities compared to private wings. In this study, we focused on public health facilities in order to obtain the required number of patients and to minimize duration of the study. After the selection of the study sites, each was allocated a proportionate number of study subjects based on the level of health care delivery system and the average client attendance in the past one month before embarking on the study. To minimize bias in selecting study subjects, consecutive sampling was used hence every alternate TB suspect who satisfied the inclusion criteria was selected for the study.

2.7. Collection of Demographic Data

A structured and pre-tested questionnaire was used to obtain socio demographic data, history of previous anti-TB treatment, HIV serostatus, history of hospitalization and other relevant data from each study participant.

2.8. Collection of Sputum and Transportation

A specialist medical doctor working in the TB clinic performed the necessary clinical and diagnostic work. Diagnostic was made based on the combined evaluation of clinical, radiological and laboratory features. Three sputum specimens

(spot, early morning, spot) were collected from 500 TB suspects under the supervision of trained and competent medical staff as recommended by WHO [26]. The patients were advised to rinse their mouth twice with water before producing the specimen and this helped to remove food and any contaminating bacteria in the mouth. They were instructed to take two breaths, coughed vigorously and expectorated the material into the sterile 50 ml blue cap screw-capped bottle. This process allowed sputum to be produced from deep in the lungs. The TB suspects were asked to hold the sputum container close to the lips and spit into it gently after a productive cough. At the peripheral laboratory, the standard Acid-fast (AFB) direct smear microscopy using Ziehl-Neelsen (ZN) staining was done on the initial sputum to confirm TB diagnosis of suspected patients. A second sputum specimen was then collected which was refrigerated at 4°C and transported to the Central reference Laboratory (CRL) weekly for culture.

Blood Samples

A total of 500 participants consented phlebotomy for HIV testing. Test was also done according to manufacturer's instruction. Blood samples were delivered in vacutainer brand sterile interior ethylenediamine-tetra-acetic acid (EDTA) tubes and used for HIV test. The safety for research assistants and healthcare workers during collection and handling of sputum specimens was ensured by observing the WHO guideline

2.9. Microscopic Examination of Specimens

Sputum smears were examined for acid-fast bacilli (AFB) after staining following ZN method. The degree of ZN smear positivity was quantified as 1+ for 10 - 100 AFBs per 100 fields, 2+ for 1 - 10 AFBs per field (50 fields) and 3+ for >10 AFBs per field (20 fields). For less than 10 AFBs per 100 fields, the exact number of AFBs was indicated. A suspect was considered to be ZN smear positive if at least one specimen was positive.

2.10. Sample Processing, Mycobacterial Culture and Isolation

Sputum specimens were processed for isolation of mycobacteria using standard Petroff's method [16]. They were decontaminated with NaOH solution (40 g/14% w/v) combined with 2.9% sodium citrate solution and N-acetyl-L-cysteine (NACL) powder. Sterile phosphate buffer was added and the organisms concentrated by centrifugation at 3000 rpm for 15 minutes. The supernatant was decanted and the sediment suspended with phosphate buffer and inoculated into Lowenstein-Jensen (LJ) slant tubes (containing 0.75% glycerol and 0.6% pyruvate) for primary isolation of the organisms. Afterwards, the tubes were incubated at 35% - 37% and inspected for growth of Mycobacteria for a period of eight weeks. The slopes were examined weekly for any visible growth. A positive culture of *M. tuberculosis* confirmed the diagnosis of active TB disease. Strains of MTB

complex from Lowenstein Jensen (LJ) slopes were subjected to drug susceptibility testing (DST).

Preparation of Drug Containing and Drug-Free Middlebrook 7H10 Media

Middlebrook 7H10 (Beckton, Dickson, France) media containing the four first line anti-TB drugs namely rifampicin (R), Isoniazid (N), Streptomycin (S) and ethambutol (E) with the final concentration of 1 ug/mL, 0.2 ug/mL, 2 ug/mL and 5 ug/mL (sigma, St. Louis, USA) were prepared as recommended by the manufacturer respectively.

2.11. Drug Susceptibility Testing

A loopful of the colonies was harvested from the LJ slants, suspended in 1 ml saline and vortexed to break the large clumps. The suspension was adjusted to a standard 0.5 McFarland turbidity by visual comparison. The drug containing and drug-free growth control MGIT tubes were inoculated with the standardized 0.5 McFarland inoculums of the *M. tuberculosis* isolate and entered into the BACTEC MGIT960 automated machine in a special rack carrier with a printed barcode (Becton-Dickson and Company, Sparks, MD, USA).

Quality Control

Standard Operating Procedures (SOPS) were followed for microscopy and culture. *Mycobacterium tuberculosis* H37Rv reference strain (ATCC 27294) which is susceptible to all anti-TB drugs tested was included in each test batch as positive and *Escherichia coli* as a negative control. All activities like reagent and media preparation were carried out as described in standard operating procedure by Kent and Kubica [27]. An experienced microscopist read an arbitrary 10% positive and 10% negative slides randomly selected, with concordance of 99% and 97% respectively. For data obtained through interview, the questionnaire was pretested before use and data were collected by trained nurses under close supervision by the investigators.

2.12. HIV Testing

Blood samples were tested for HIV antibodies according to the Kenyan national testing algorithm for voluntary counseling and testing by using Determine HIV1/2 (Abott laboratories, Japan co. LTD), Capillus HIV1/2 (The Trinity Biotech, Ireland) and Unigold H1/2 (Trinity Biotech, Ireland) rapid test kits and positives confirmed with the enzyme linked immunosorbent assay (ELISA).

2.13. Data Management and Analysis

Demographic data were confidentially obtained from the TB suspects by clinicians/nurses running the chest clinics. Results of ZN smear microscopy, culture, and HIV tests were confidentially sent to the respective clinicians/nurses. Provisions of these data were made available to the clinicians/nurses for the purpose

of managing the patients. Data was recorded on questionnaires, register books, ELISA reader print-outs and species evaluation sheets. The data was coded, entered into MS Excel 8.0 and processed using a statistical package for social sciences (SPSS) version 16.5 software for windows. Descriptive analysis and odds ratios (OR) with 95% confidence interval were calculated. The chi-squared (χ^2) test was used to compare categorical data and logistics regression to assess the association between drug resistance and independent factors. A significance level of $p < 0.05$ was considered statistically significant.

Ethical Issues

This study was approved by Kenyatta University Ethical Review Committee. Clearance was also obtained from respective County health authorities and hospital administrations. The study was conducted in accordance with the declaration of Helsinki. Written consent was obtained from all study participants and code numbers rather than names were used to identify candidates in order to maintain confidentiality. The study did not expose candidates to any unusual risks as competent hospital staff obtained sputum and blood specimens from candidates using standard procedures. Drug susceptibility test results were reported to the respective health facilities for further management of the patients.

3. Results

3.1. Socio-Demographic Characteristics of the Sample Population

A total of 500 participants suspected of having tuberculosis (TB) were enrolled into the study at the four study sites. 54.2% males and 45.8% females. The calculated overall TB prevalence in tuberculosis suspects included in the study was 42.0% (210/500) and was higher in females (45.9%) than males (38.7%) which was statistically significant ($\chi^2 = 2.573$, $p < 0.001$). The proportion of PTB cases was higher among the age groups 25 - 34 and 35 - 44 (42.9% vs 29.0%), both were statistically significant (OR = 5.46; 95% CI: 2.31 - 12.88, $p < 0.031$) and (OR = 3.16; 95% CI: 1.61 - 8.13, $p < 0.050$). Primary education was associated with TB (OR = 2.60; 95% CI: 1.63 - 4.31, $p < 0.041$). Respondents in secondary and college levels of education had the prevalence of 51.0% and 24.7% respectively but it was statistically significant ($\chi^2 = 27.660$, $p < 0.0003$). Another parameter that was compared was occupational status of the subjects and outcome of sputum examination. High prevalence rate was among self-employed 45.2% (OR = 2.14; 95% CI: 1.5 - 4.02, $p < 0.010$), formal employment 33.3% (OR = 0.84; 95% CI: 0.45 - 1.63, $p < 0.034$). In marital status, higher prevalence rate was observed among the married 56.7% (OR = 2.16; 95% CI: 0.98 - 4.52, $p < 0.047$) and lower in widowed 0.6% (OR = 0.46; 95% CI: 0.18 - 1.11, $p < 0.035$). The adjusted OR showed that married (OR = 2.35; 95% CI: 1.15 - 4.84) and divorced (OR = 3.37; 95% CI: 1.57 - 5.52) were statistically significant risk factors for tuberculosis ($p < 0.05$) (**Table 1**). A total of 210 smear positive sputum samples were collected from new PTB patients.

Table 1. Demographic characteristics of patients in descriptive epidemiology study of pulmonary tuberculosis infection (n = 500).

| Characteristics | PTB- (Suspects) | PTB+ (Cases) | OR (95% C.I) | P-value |
|------------------------|-----------------|--------------|---------------------|---------|
| Age in Years | | | | |
| 18 - 24 | 27 (9.3%) | 1 (0.5%) | 1.000**** | |
| 25 - 34 | 143 (49.3%) | 89 (42.4%) | 5.46 (2.31 - 12.88) | 0.031 |
| 35 - 44 | 86 (26.7%) | 61 (29.0) | 3.16 (1.61 - 8.13) | 0.05 |
| 45 - 54 | 23 (7.9%) | 40 (19.1%) | 2.13 (0.15 - 1.07) | 0.25 |
| 55+ | 11 (3.8%) | 19 (9.0%) | 0.21 (0.02 - 1.82) | 0.31 |
| Education level | | | | |
| No Education | 0 (0.0%) | 2 (1.0%) | 1.000**** | |
| Primary | 24 (8.3%) | 49 (23.3%) | 2.60 (1.63 - 4.31) | 0.041 |
| Secondary | 159 (54.8%) | 107 (51.0) | 1.31 (1.51 - 3.20) | 0.35 |
| College | 107 (36.9%) | 52 (24.7%) | 0.72 (0.25 - 1.42) | 0.165 |
| Sex | | | | |
| Male | 124 (42.8%) | 105 (50.0) | 1.000**** | |
| Female | 166 (57.2%) | 105 (50.0) | 0.92 (0.41 - 1.31) | 0.68 |
| Marital Status | | | | |
| Divorced | 3 (1.0%) | 3 (1.4%) | 1.000**** | |
| Married | 162 (55.9%) | 119 (56.7%) | 2.16 (0.98 - 4.52) | 0.047 |
| Unmarried | 117 (40.3%) | 75 (35.7%) | 0.84 (0.45 - 1.63) | 0.481 |
| Widowed | 8 (2.8%) | 13 (0.6%) | 0.46 (0.18 - 1.11) | 0.035 |
| Employer | | | | |
| Unemployed | 84 (29.0%) | 41 (19.5%) | 2.14 (1.15 - 4.02) | 0.01 |
| Self Employed | 75 (25.9%) | 95 (45.2%) | 1.000**** | |
| Formal Employment | 130 (44.8%) | 71 (33.8%) | 0.84 (0.45 - 1.63) | 0.034 |
| Student | 1 (0.3%) | 3 (1.4%) | 0.46 (0.18 - 1.11) | 0.233 |

3.2. The Susceptibility and Resistance Patterns of *Mycobacterium tuberculosis* Isolates to First Line Anti-Tuberculosis Drugs among Tuberculosis Patients

Among the newly diagnosed TB cases resistance to at least one drug was 43 (22.3%). One hundred and fifty (77.7%) of the isolates were susceptible to all four anti-TB drugs tested. Resistance to at least one drug in recurrent TB patients was 12 (70.6%) and sensitive 5 (29.6%). Any single drug resistance was most frequent in isoniazid 37 (17.6%), Ethambutol 29 (13.8%), Streptomycin 28 (13.3%) and Rifampicin 8 (3.8%). Combined drug resistance in newly diagnosed TB patients was common in isoniazid and Rifampicin 8 (2.1%), Rifampicin Isoniazid and Ethambutol 2 (1.0%). Other resistance patterns included H + E 11 (5.7%), E + S 11 (5.7%), H + S 7 (3.6%) and R + E 2 (1%) in newly diagnosed patients compared to 3 (17.6%), 1 (5.9%), 3 (17.6%) and 2 (11.8%) in previously

treated cases. Multi drug resistance (MDR) TB was observed in ten isolates (4.8%). One MDR isolate was triple resistant with an additional resistance to Ethambutol (E).

The resistance patterns in recurrent TB cases were Isoniazid 7 (41.2%), streptomycin 10 (58.8%), Ethambutol 9 (52.9%) and Rifampicin 4 (23.5%). The most frequent double resistance among this category was Isoniazid and Rifampicin (H + R) 4 (23.5%). Isoniazid (H), streptomycin (S) and Ethambutol (E) represented 1 (5.9%) in triple resistance in previously treated patients and 1 (0.5%) in newly diagnosed TB patients. Five (2.6%) and five (29.4%) strains from new and previously treated patients were multidrug resistance (MDR) TB defined as resistant to at least both Isoniazid and Rifampicin. Each parameter of drug resistance between previously treated cases and new cases of TB were compared and all parameters were higher in previously treated patients. However among those, statistical difference was observed for Rifampicin and Ethambutol ($p < 0.05$). Otherwise the difference seen for any type of drug resistance ($p = 0.04$), any type of INH resistance ($p < 0.003$), any type of STM resistance (0.05), combined type of STM and INH resistance ($p = 0.002$) and multi drug resistance ($p < 0.05$) were all higher in the previously treated TB patients (**Table 2**).

3.3. Drug Resistance between HIV-Positive and HIV Negative in Diagnosed TB Patients

A total of seventy eight patients (37.1%) were TB-HIV co-infected. Prevalence of any type of drug resistance in TB-HIV co-infection patients was 40 (19.1%) and 15 (7.1%) in HIV negative. The difference was not statistically significant ($P > 0.05$). In mono drugs, Isoniazid 25 (11.9%) showed the highest resistance pattern in TB-HIV co-infected patients followed by streptomycin 10 (4.8%), Ethambutol 9 (4.3%) and Rifampicin 5 (2.4%). Combined resistance to R + E was 4 (1.9%) in TB-HIV co-infected patients. These differences were statistically significant ($p < 0.05$). Triple resistance to Isoniazid, Ethambutol and streptomycin was 1 (0.5%) in both HIV positive and negative TB patients respectively. These differences were statistically significant ($p < 0.05$). The prevalence of other types of drug resistance was also significantly different in HIV positive and HIV negative TB patients (**Table 3**).

4. Discussions

In our finding, resistance to one or more first line anti-TB drugs was 26.2%. This is relatively higher than previous reports from other parts of Kenya [28]. This result was comparable with studies done in Ethiopia (25%) [29] and in Uganda (28.6%) [30]. Studies from Nairobi [31], Addis Ababa [32] and Central Asia [33] reported a higher resistance level of (30%, 32.5% and 30.5%), respectively. The variations in overall prevalence of drug resistance among the different study settings could be due to difference in sample size (small sample size could overestimate the proportion), irregular supply of ant-tuberculosis drugs, poor TB case

Table 2. Drug resistance patterns of *Mycobacterium tuberculosis* isolates to first line anti-tuberculosis drugs.

| Item | Newly Diagnosed Patients (95% CI) | | Previously Diagnosed Patients (95% CI) | | Total (95% CI) | |
|---|-----------------------------------|------------------|--|--------------------|------------------|------------------|
| | | | | | | |
| DST | 193 (100%) | - | 17 (100%) | - | 210 (100%) | |
| Sensitive to all | 150 (77.7%) | 78.3 - 81.3 | 5 (29.4%) | | 155 (73.8%) | 70.1 - 79.1 |
| <u>Mono Resistance TB</u> | | | | | | |
| Isoniazid (H) | 30 (16.0%) | 21.1 - 24.6 | 7 (41.2%) | 1.1 - 10.6 | 37 (17.6%) | 24.5 - 28.0 |
| Rifampicin (R) | 4 (2.1%) | 3.31 - 7.20 | 4 (23.5%) | 1.54 - 1.99 | 8 (3.8%) | 4.8 - 11.8 |
| Ethambutol (E) | 20 (10.0%) | 23.2 - 26.7 | 9 (52.9%) | 1.21 - 4.74 | 29 (13.8%) | 25.4 - 28.9 |
| Streptomycin (S) | 18 (18.3%) | 24.4 - 30.8 | 10 (58.8%) | 1.15 - 1.67 | 28 (13.3%) | 29.8 - 33.2 |
| <u>Multi-Drug Resistance TB (MDR-TB)</u> | | | | | | |
| H + R | 4 (2.1%) | 2.2 - 6.1 | 4 (23.5%) | 1.54 - 1.99 | 8 (3.8%) | 3.8 - 7.7 |
| H + R + E | 1 (0.5%) | 0.3 - 0.7 | 1 (5.9%) | 1.82 - 2.07 | 2 (1.0%) | 1.0 - 3.0 |
| H + R + S | - | - | - | - | - | - |
| H + R + E + S | - | - | - | - | - | - |
| Total MDR-TB | 5 (2.6%) | 1.7 - 3.4 | 5 (29.4%) | 25.6 - 32.7 | 10 (4.8%) | 3.8 - 6.1 |
| <u>Other resistance patterns</u> | | | | | | |
| H + E | 11 (5.7%) | 3.8 - 7.7 | 3 (17.6%) | 1.62 - 2.03 | 14 (6.7%) | 4.8 - 8.7 |
| H + S | 7 (3.6%) | 1.7 - 5.0 | 3 (17.6%) | 1.62 - 2.03 | 10 (4.8) | 2.9 - 6.8 |
| H + E + S | 2 (1.0%) | 0.98-3.0 | - | - | 2 (1.0%) | 2.0 - 3.0 |
| R + E | 2 (1.0%) | 0.98 - 3.0 | 2 (11.8%) | 1.71 - 2.05 | 4 (1.9%) | 1.1 - 3.9 |
| E + S | 11 (5.7%) | 3.7 - 7.7 | 1 (5.9%) | 1.82 - 2.03 | 12 (5.7%) | 3.8 - 3.9 |
| R + S | 5 (2.6%) | 0.65 - 4.6 | - | - | 5 (2.4%) | 2.0 - 4.3 |
| R + E + S | - | - | - | - | - | - |

T test for numerical variables; DST = Drug susceptibility testing; MDR-TB = Multidrug resistant tuberculosis; CI = confidence interval.

management and poor treatment compliance.

In this present study, the highest rate of monoresistance was associated isoniazid (17.6%) which was higher than results obtained in earlier studies in Kenya, where resistance to INH was 10.7% [34]. Our findings is much higher than that seen in Ethiopia where one isolate was resistant to INH [35] and in Bangladesh and Sri Lanka [36] at 5.4% and 12.2%, respectively. In 2012, the WHO reported a worldwide resistance rate to INH of 5.9% [37]. According to WHO, INH resistance rates higher than 10% can predict development of MDR-TB [38]. Isoniazid is given in both the intensive and the continuation phase of TB treatment [39]. Isoniazid only given with ethambutol (EMB) in continuation phase but since it is a weak anti-TB drug [40], it may not be effective to prevent development of resistance to isoniazid (INH) due to poor compliance by patients. This reflects that the precursors of isoniazid resistance are

Table 3. Comparison of drug resistance between HIV-positive and HIV negative diagnosed TB patients (n = 210).

| | | HIV Positive | HIV Negative | P-Value |
|------------------------------------|-----------|----------------|-----------------|---------------|
| | | n = 78 (37.1%) | n = 132 (62.9%) | |
| Any type of drug resistance | Resistant | 40 (19.1%) | 15 (7.1%) | 0.379 |
| | Sensitive | 38 (18.1%) | 117 (55.7%) | |
| Isoniazid (H) | Resistant | 25 (11.9%) | 12 (5.7%) | 0.001* |
| | Sensitive | 53 (25.2%) | 120 (57.1%) | |
| Rifampicin (R) | Resistant | 5 (2.4%) | 3 (1.4%) | 0.001* |
| | Sensitive | 73 (34.8%) | 129 (61.4.1%) | |
| Ethambutol (E) | Resistant | 9 (4.3%) | 20 (9.5%) | 0.001* |
| | Sensitive | 59 (28.1%) | 112 (53.3.2%) | |
| Streptomycin (S) | Resistant | 10 (4.8%) | 18 (8.6%) | 0.001* |
| | Sensitive | 68 (32.4.5%) | 114 (54.3%) | |
| R + E | Resistant | 4 (1.9%) | 0 (0.0%) | 0.009* |
| | Sensitive | 74 (35.2%) | 132 (62.9%) | |
| H + E + S | Resistant | 1 (0.5%) | 1 (0.5%) | 0.705 |
| | Sensitive | 77 (36.7%) | 131 (62.3%) | |

P-value = level of marginal significance; *statistically significant association.

accumulating in the study setting which can increase the likelihood of MDRTB if rifampicin resistance rises. 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-TB often develops from initial INH mono resistant strains. Isoniazid (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. Therefore, mono resistance to isoniazid should be properly monitored in order to minimize the spread of MDRTB strains in the study area.

In this study, resistance to Rifampicin was 3.8% which was higher than that observed in earlier studies in Kenya where resistance was 0.3% and 1.3% [41] and in India (1.1%) [42]. Rifampicin has several adverse effects such as nausea, vomiting, rashes, GIT upset, flu-like symptoms, fever and jaundice which could result in patient non-adherence and hence may lead the selection of resistant strains. In the DOTs program, Rifampicin is given in the intensive phase under direct observation together with at least three drugs. Moreover in the continuation phase, Rifampicin is spared.

In our study, resistance to Ethambutol was 5.2% and it was higher than a previous study done by Raviglione *et al.* [43] in Ethiopia (2.7%) and others in Bukina Faso (0.3%) [44] and Uganda 0.2%) [45]. One possible explanation for increased Ethambusol resistance could be increased defaulting rate from TB treatment in the continuation phase when it is administered. It also enhances the

effect of many drugs including beta lactams to different *Mycobacteria* species and can be used to develop a regimen for MDR-TB [46]. The drug has been in use since the beginning of tuberculosis (TB) chemotherapy. The treatment regimen used in most countries only adds one extra drug Streptomycin to patients who may have failed in the first treatment.

The detected streptomycin mono-resistance in this study was 4.2% which was higher than previous studies from other parts of Kenya such as 1.8% [47]. But our findings were lower than similar studies from sub-Saharan African countries which showed higher rate of 14.8% in Benin [48] and 12.9% in Uganda [33]. Though streptomycin is almost not considered as first line drug in treatment of TB, the relatively high proportion of resistance to this drug in this is probably due to the extensive use of the drug in the TB control programme in the previous years.

In our study, eight patients had MDR-TB (4.8%) which is not unusual because in Sub-Saharan Africa countries MDR-TB prevalence is estimated to be 6.3% [49]. Our finding is lower than the 7.7% prevalence reported in Swaziland [50], 5.8% in Mozambique [51] and 5.2% in Somali [52]. This may reflect the variations in sample size, studied population, access to health care facilities and effectiveness of TB control programmes. To account for the high rate of multiple resistance observed in this study, it would be important to look back into the continuation phase where INH and EMB are given for six months. In an area where there is high level of INH resistance, treatment with INH and EMB is not probably effective and will lead to further acquisition of INH resistance and EMB resistance. This is because Ethambutol is a bacteriostatic drug with low efficacy that may not effectively prevent development of resistance to INH.

In our study, drug resistance in TB-HIV co-infection patients was 19.1%. Association was observed between HIV infection with any drug resistance though the association remains controversial. Similar results were reported in other studies [53] [54] [55]. However, reports in Tanzania [8] and in Central African Republic [56] were not in agreement with our study. Thus, study may be interpreted to directly argue for the hypothesis that drug resistant TB is virulent and causes disease mainly in immunocompromised TB patients ($p < 0.05$). In this regard, similar findings have been reported by different studies.

Studies in Latvia showed that any resistance and multi-drug resistant tuberculosis (MDR-TB) were significantly associated with HIV infection [38]. A study done in Northern Tanzania showed that among TB-HIV co-infection, patients' resistance to at least one drug was 10.8%. Tuberculosis infections in high incidence countries have been shown to be recently transmitted and failure to contain MDR-TB and XDR reflects inability to diagnose the problem early to prevent transmission of the same while continuing to prescribe an ineffective regimen [57]. Once MDR-TB has developed, further progression to pre-XDR and XDR is only a question of time and will place over few months or even years. The patient remains infectious and transmission of MDR TB and XDR-TB continues particularly in areas with high prevalence of HIV/AIDS and overcrowding.

Sarita *et al.* [13] found out in a study in Texas that TB patients with HIV were more likely to have rifampicin resistance and less likely to have isoniazid resistance. However a study done in Punai Maharashtra India on anti-TB drug resistance showed that prevalence of drug resistant isolates among HIV seropositive patients was similar to that of seronegative TB patients indicating that HIV infection may not be associated with drug resistant TB [40]. Several factors have been proposed to explain such an association. These may include malabsorption, of anti-TB drugs among HIV patients, poor treatment adherence, lack of access to proper treatment, exposure of HIV/AIDS persons to MDR-TB patients during hospitalization or frequent visits to health facilities which increases the risk for MDR TB nosocomial outbreaks and rapid progression of TB in HIV patients [58].

5. Conclusion

The current study reveals that the overall resistance to first line anti-TB drugs is high. The highest monodrug resistance was found against Isoniazid (H). Although the rate of MDR-TB was relatively low, this signifies that conditions favouring the spread of MDR-TB are on high rise. Patients who were HIV co-infected were more likely to develop drug resistance compared to HIV negative patients. Therefore, it is essential to address the problems of development of drug resistant strains of TB by establishing good TB programmes (DOTS). Patients adherence to anti-TB drugs and scaling up of drug sensitivity testing (DST) service at County level hospital will help to reduce the development of drug resistance in the study area.

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Competing Interest

Authors have declared that no competing interests exist.

Authors' Contribution

This work was carried out in collaboration between all others. SAY, MFO and RRS initiated the study and made major contributions to the study design. Author SAY and SSN collected the data, did statistical analysis and drafted the

manuscript. All authors read and approved the final manuscript.

References

- [1] World Health Organization, WHO Report (2013) Global Tuberculosis Report 2013. World Health Organization, Geneva.
- [2] Dias, F., Michael, S., Hoffner, S.E., Martins, M.L., Norberg, R and Källeniusa, G. (1993) Drug Susceptibility in *Mycobacterium tuberculosis* of a Sample of Patient in Guinea Bissau. *Tubercle and Lung Disease*, **74**, 129-130. [https://doi.org/10.1016/0962-8479\(93\)90040-5](https://doi.org/10.1016/0962-8479(93)90040-5)
- [3] Mitike, G., Kebede, M. and Yeneneh, H. (1997) Prevalence of Antituberculosis Drug Resistance in Harar Tuberculosis Centre, Ethiopia. *East Africa Medical Journal*, **74**, 158-161.
- [4] Glynn, J.R., Crampin, A.C., Traore, H., *et al.* (2005) *Mycobacterium tuberculosis* Beijing Genotype, Northern Malawi. *Emerging Infectious Diseases*, **11**, 150-153. <https://doi.org/10.3201/eid1101.040869>
- [5] Lin, J., Sattar, A. and Puckree, T. (2004) An Alarming Rate of Drug-Resistant Tuberculosis at Ngwelezane Hospital in Northern KwaZulu Natal, South Africa. *International Journal of Tuberculosis and Lung Disease*, **8**, 568-573.
- [6] Kassa-Kelembho, E., Bobossi-Serengbe, G., Takeng, C., Nambea-Koisso, T., Yapou, F and Talarmin, A. (2004) Surveillance of Drug-Resistant Childhood Tuberculosis in Bangui, Central African Republic. *International Journal of Tuberculosis and Lung Disease*, **8**, 574-578.
- [7] Kuaban, C.R., Bercion, J., Noeske, P., Cunin, P., Nkamsse, S., *et al.* (2000) Anti-Tuberculosis Drug Resistance in the West Province of Cameroon. *International Journal of Tuberculosis and Lung Disease*, **4**, 356-360.
- [8] Urassa, W., Mugusi, F., Villamor, E., *et al.* (2008) Primary Antimicrobial Resistance among *Mycobacterium tuberculosis* Isolates from HIV Seropositive and HIV Seronegative Patients in Dar es Saalam Tanzania. *BMC Research Notes*, **1**, Article 58. <https://doi.org/10.1186/1756-0500-1-58>
- [9] Lukoye, D., Adatu, F., Musisi, K., Kasule, G.W., Were, W., Odeke, R., Kalamya, J.N., Awor, A., Date, A. and Joloba, L.M. (2013) Anti-Tuberculosis Drug Resistance among New and Previously Treated Sputum Smear Positive Tuberculosis in Uganda: Results of the First National Survey. *PLoS ONE*, **8**, e70763.
- [10] Espinal, M., Laszlo, A., Simonsen, L., Boulahbal, F., Kim, S. and Reneiro, A. (2012) Global Trends in Resistance to Anti-Tuberculosis Drugs. World Health Organization-International Union against Tuberculosis and Lung Disease. *British Medical Journal*, **344**, 1294-1303.
- [11] Division of Leprosy, Tuberculosis and Lung Disease (2014) Ministry of Public Health and Sanitation. Government of Kenya, Annual Report.
- [12] Mermin, J. (2007) Prevalence, Incidence and Mortality Associated with Tuberculosis in HIV-Infected Patients Initiating Antiretroviral Therapy in Rural Uganda. *AIDS*, **21**, 713-719. <https://doi.org/10.1097/QAD.0b013e328013f632>
- [13] Sarita, S., Wright, A., Bai, G., *et al.* (2007) Worldwide Emergence of Extensively Drug-Resistant Tuberculosis. *Emerge infectious Disease*, **13**, 380-387.
- [14] Niemann, S., Diel, R., Khechinashvili, G., Gegia, M., Mdivani, N. and Tang, Y.W. (2010) *Mycobacterium tuberculosis* Beijing Lineage Favors the Spread of Multi-drug-Resistant Tuberculosis in the Republic of Georgia. *Journal of Clinical Microbiology*, **48**, 3544-3550. <https://doi.org/10.1128/JCM.00715-10>

- [15] World Health Organization (2014) WHO Report 2014. Global Tuberculosis Report. World Health Organization, Geneva.
- [16] Division of Leprosy, Tuberculosis and Lung Disease (2013) Ministry of Public Health and Sanitation. Government of Kenya, Annual Report.
- [17] Ministry of Health, Kenya (2014) National Leprosy and Tuberculosis Control Programme (NLTP) Annual Report, 2009.
- [18] Nyang'au, L.O., Amukoye, E. and Ng'ang'a, Z. (2014) First Line Anti-Tuberculosis Drug Resistance Among Human Immunodeficiency Virus Infected Patients Attending Maryland Comprehensive Care Centre Mathare 4a Nairobi Kenya. *International Journal of Sciences*, **15**, 661-668.
- [19] Taylor, J.P., Bergmire-Sweet and Suarez, L. (1999) Epidemiology of Drug Resistant Tuberculosis in Texas. *American Medical Journal of Epidemiology*, **149**, 359-365. <https://doi.org/10.1093/oxfordjournals.aje.a009821>
- [20] Zhiyuan, L., Kenneth, L.S. and Lyn, F. (2008) Epidemiology of Drug Resistant Tuberculosis in New Jersey from 1991 to 1995. *International Journal of Epidemiology*, **27**, 121-126.
- [21] Dye, C., Sheele, S., Dolin, P. and Pathania, V. (2011) Global Burden of TB. Estimated Incidence, Prevalence and Mortality by Country. *British Medical Journal*, **282**, 677-686.
- [22] World Health Organization, International Union against Tuberculosis and Lung Disease (2012) Guidelines for Surveillance of Drug Resistance in Tuberculosis. World Health Organization Document WHO/TB/96.216, 1-5.
- [23] Kathryn, D., Louders, G., Mirriam, B., Manuel, P., Martinez, G., Peter, M., *et al.* (2005) Does DOTS Work in Populations with Drug Resistant Tuberculosis? *The Lancet*, **365**.
- [24] Quy, H.T., Lan, N.T., Borgdorff, W.W., Grosset, J., Link, P.D., *et al.* (2003) Drug Resistance among Failure and Relapse Cases of Tuberculosis: Is the Standard Retreatment Regimen Adequate? *International Journal Tuberculosis Lung Disease*, **7**, 631-636.
- [25] Division of Leprosy, Tuberculosis and Lung Disease (2015) Ministry of Public Health and Sanitation. Government of Kenya. Annual Report.
- [26] World Health Organization (2009) Guidelines for Surveillance of Drug Resistance in Tuberculosis. 4th Edition, WHO/HTM/TB/2009.422, Geneva.
- [27] Kent, P.T. and Kubica, G.P. (2010) Public Health Mycobacteriology. A Guide for III Laboratory Centers of Disease Control, Atlanta. *Publication*, No. 86, 345-456.
- [28] Githui, W.A., Hawken, M.P., Juma, E.S., Godfrey-Fauset, P., Swai, O.B., Kibuga, D.K., Porter, J.D., Wilson, S.M., *et al.* (2000) Surveillance of Drug-Resistant Tuberculosis and Molecular Evaluation of Transmission of Resistant Strains in Refugee and Non-Refugee Populations in North-Eastern Kenyan. *International Journal of Tuberculosis and Lung Disease*, **4**, 947-955
- [29] Agonafir, M., Lemma, E., Wolde-Meskel, D., *et al.* (2010) Phenotypic and Genotypic Analysis of Multidrug-Resistant Tuberculosis in Ethiopia. *International Journal of Tuberculosis and Lung Disease*, **14**, 1259-1265.
- [30] Sanchez-Padilla, E., Ardizzoni, F., Sauvageot, D., *et al.* (2013) Multidrug- and Isoniazid-Resistant Tuberculosis in Three High HIV Burden African Regions. *International Journal of Tuberculosis and Lung Disease*, **17**, 1036-1042. <https://doi.org/10.5588/ijtld.12.0842>

- [31] Ndung'u, P.W., Kariuki, S., Ng'ang'a, Z. and Revathi, G. (2012) Resistance Patterns of Mycobacterium Tuberculosis Isolates from Pulmonary Tuberculosis Patients in Nairobi. *Journal of Infection in Developing Countries*, **6**, 33-39.
- [32] Seyoum, B., Demissie, M., Worku, A., Bekele, S. and Aseffa, A. (2014) Prevalence and Drug Resistance Patterns of Mycobacterium tuberculosis among New Smear Positive Pulmonary Tuberculosis Patients in Eastern Ethiopia. *Tuberculosis Research and Treatment*, **2014**, 753492.
- [33] Hellen, S., Juan, D., Roy, M., Sabine, R., Dennis, F., Ian, S., Darebsay, D., Yared, K. and Mohamed, A. (2004) Multidrug Resistance in Central Asia. *Emerging Infectious Diseases*, **10**, 1210-1215.
- [34] Githui, W.A., Juma, E.S., Van Gorkom, J., Kibuga, D., Odhiambo, J. and Drobniowski, F. (1998) Anti-Tuberculosis Drug Resistance Surveillance in Kenya, 1995. *International Journal of Tuberculosis and Lung Disease*, **2**, 499-505.
- [35] Abebe, G., Abdissa, K., Abdissa, A., Apers, L., Agonafir, M., de-Jong, B.C. and Colebunders, R. (2012) Relatively Low Primary Drug Resistant Tuberculosis in South-Western Ethiopia. *BMC Research Notes*, **5**, 225.
- [36] Magana, A.D.N., Pirera, A.J., Senarathne, V. and Chandrasekharana, N.V. (2010) Patterns of Drug Resistance and RFLP Analysis of *Mycobacterium tuberculosis* Strains Isolated from Recurrent Tuberculosis Patients in Sri-Lanka, South-East Asia. *Journal on Tropical Medicine and Public Health*, **41**, 583-589.
- [37] WHO/GTP (2013) Multidrug and Extensively Drug Resistant TB: Global Report on Surveillance and Response. Report No. WHO/GTP/TB/2013.3, The Organizations, Geneva, 2013 Facilities, Congregate Settings and Households, WHO, Geneva.
- [38] Mycal, P., Srikanth, T., Viskas, I., Ramash, K., Manoj, B., Amrita, D.M., Rajshekar, I., Annand, A., Sanjar, M. and Arun, R. (2005) Drug Resistance Pattern of *Mycobacterium tuberculosis* Seropositive and Seronegative HIV-TB Patients in Pune, India. *Indian Journal of Medical Respiratory*, **121**, 225-239.
- [39] Zwarenstein, M., Schoeman, J.H., Vundule, C. and Tatley, M. (1998) Randomized Control Trial of Self-Supervised and Directly Treatment of Tuberculosis. *The Lancet*, **352**, 1340-1343.
- [40] Sharma, S., Mohan, A., Kadhiraivan, *et al.* (2005) HIV-TB Co-Infection: Epidemiology, Diagnosis and Management. *Indian Journal of Respiratory Medicine*, **121**, 50-67.
- [41] Githui, W.A., Meme, H., Juma, E., Karimi, F., Kinyajui, P., Chakaya, J., Kangangi, J. and Kutwa, A. (1994) Isolation of Multi-Drug Resistant Tuberculosis Strains in Patients from Private and Public Facilities in Nairobi, Kenya. *International Journal of Tuberculosis and Lung Disease*, **8**, 837-841.
- [42] Sharma, S.K., Kaushik, G., Jha, B., *et al.* (2011) Prevalence of Multidrug-Resistant Tuberculosis among Newly Diagnosed Cases of Sputum-Positive Pulmonary Tuberculosis. *Indian Journal of Medical Research*, **133**, 308-311.
- [43] Raviglione, M.C., Marian, J.P. and Kochia, A. (1992) HIV-Associated Tuberculosis in Developing Countries: Clinical Features, Diagnosis and Treatment. *Bulletin of World Health Organization*, **70**, 515-526.
- [44] Diandé, S., Sangaré, L., Kouanda, S., Dingoumda, B. and Traoré, A. (2009) Drug Resistance of *Mycobacterium tuberculosis* Complex among Newly Diagnosed Tuberculosis Cases in Burkina Faso. *West Africa Journal of Medicine*, **28**, 353-357.
- [45] Lukoye, D., Cobelens, J., Ezati, N., *et al.* (2011) Rates of Anti-Tuberculosis Drug Resistance in Kampala-Uganda Are Low and Not Associated with HIV Infection. *PLoS ONE*, **6**, 16130. <https://doi.org/10.1371/journal.pone.0016130>

- [46] Zignol, M., Hossein, M.S., Wright, A., Weezenbeck, C.L., Nuun, P., Watt, C.J., Williams, B.G. and Dye, C. (2006) Global Incidence of Multidrug Resistant Tuberculosis. *The Journal of Infectious Diseases*, **194**, 479-485. <https://doi.org/10.1086/505877>
- [47] Githui, W.A., Kwamanga, D., Chakaya, J.M., Karimi, F.G. and Waiyaki, P.G. (1993) Anti-Tuberculosis Initial Drug Resistance of *Mycobacterium tuberculosis* in Kenya: A Ten-Year Review. *East African Medical Journal*, **70**, 609-612.
- [48] Affolabi, D., Adjagba, O., Tanimomo-Kledjo, B., Gninafon, M., Anagonou, S. and Portaels, F. (2007) Anti-Tuberculosis Drug Resistance among New and Previously Treated Pulmonary Tuberculosis Patients in Cotonou, Benin. *International Journal of Tuberculosis and Lung Disease*, **11**, 1221-1224.
- [49] WHO (2010) Global Tuberculosis Control, Surveillance, Planning and Financing. WHO Report, WHO/HTM/TB, 349, Geneva.
- [50] Sanchez-Padilla, E., Dlamini, T., Ascorra, A., *et al.* (2012) High Prevalence of Multidrug-Resistant Tuberculosis, Swaziland, 2009-2010. *Emerging Infectious Diseases*, **18**, 29-37. <https://doi.org/10.3201/eid1801.110850>
- [51] Nunes, E.A., De Capitani, E.M., Coelho, E., *et al.* (2005) Patterns of Anti-Tuberculosis Drug Resistance among HIV-Infected Patients in Maputo, Mozambique, 2002-2003. *International Journal of Tuberculosis and Lung Disease*, **9**, 494-500.
- [52] Sindani, I., Fitzpatrick, C., Falzon, D., *et al.* (2013) Multidrug-Resistant Tuberculosis, Somalia, 2010-2011. *Emerging Infectious Diseases*, **119**, 478-480. <https://doi.org/10.3201/eid1903.121287>
- [53] Asmam, D., Seyoum, B., Makonnen, E., *et al.* (2008) Primary Drug Resistance in Newly Diagnosed Smear Positive Tuberculosis Patients in Addis Ababa. *Ethiopian Medical Journal*, **46**, 367-374.
- [54] Yimmer, S.A., Agonafir, M., Derese, Y., Sni, Y., Bjune, G.A. and Holm-Hansen, C. (2012) Primary Drug Resistance to Anti-Tuberculosis Drugs Resistance in Major Towns of Amhara Region, Ethiopia. *APMIS*, **120**, 503-509. <https://doi.org/10.1111/j.1600-0463.2011.02861.x>
- [55] Demissie, M., Lemma, E., Gebeyehu, M. and Lindtjorn, B. (2001) Sensitivity to Ant-Tuberculosis Drugs in HIV-Positive and HIV-Negative Patients in Addis Abba. *Scandinavian Journal of Infectious Diseases*, **33**, 914-919. <https://doi.org/10.1080/00365540110076822>
- [56] Minime-Lingoupou, F., Manirakiza, A., Yango, F., Zandanga, G., Le and Faou, A. and Rigouts, L. (2011) Relatively Low Primary Resistance to Anti-Tuberculosis Drugs in Bangui and Bimbo, Central African Republic. *International Journal of Tuberculosis and Lung Disease*, **15**, 657-661. <https://doi.org/10.5588/ijtld.10.0292>
- [57] Millen, S.J., Uys, P.W., Hargrove, J., Van Helden and Williams, B.G. (2008) The Effect of Diagnostic Delays on the Dropout Rate and the Total Delay to Diagnosis of Tuberculosis. *PLoS ONE*, **3**, 1933.
- [58] Suchindran, S., Brouwer, E.S. and Van, R.A. (2009) Is HIV Infection a Risk Factor for Multi-Drug Resistant Tuberculosis? Asystematic Review. *PLoS ONE*, **4**, E5561. <https://doi.org/10.1371/journal.pone.0005561>

List of Abbreviations

TB-Tuberculosis, PTB-Pulmonary tuberculosis, WHO-World Health Organization, MTB-*Mycobacterium tuberculosis*, HIV-Human immunodeficiency virus, OR-Odds Ratio, MDR-TB-Multi Drug Resistant Tuberculosis, DST-Drug Susceptibility Test, ZN stain-Ziehl Neelsen stain, XDR-TB = Extensively Drug Resistant Tuberculosis, DOTS-Directly Observed Treatment Short Course.