Low anti-tuberculosis drug resistance despite high rates of recurrent tuberculosis and HIV infection in western Kenya

Nyamogoba H DN[1, 2], Mbuthia G[3], Kikuvi G[2], Mpoke S[4], Waiyaki P G[4], van Soolingen, D[5]

1. *Moi University School of Medicine;*
2. *Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture and Technology;*
3. *Moi Teaching and Referral Hospital;*
4. *Kenya Medical Research Institute; [5]National Institute for Public Health and the Environment, The Netherlands*

***Corresponding author:** P.O. Box 4606 Eldoret, Kenya, E-mail: henrynyamogoba@yahoo.co.uk*

SUMMARY

Background: The high rates of recurrent tuberculosis and HIV in Kenya raised the assumption that anti-tuberculosis drug resistance may be an increasing problem.

Objective: To determine whether HIV co-infection and TB recurrence are associated with anti-TB drug resistance. Methods: Cross-sectional study in which sputa from 872 TB suspects underwent ZN smear microscopy and culture. Growth was identified using Hain molecular identification kits. Screening for HIV infection was done using Uni-Gold™ rapid test and the positives confirmed with enzyme linked immunosorbent assay.

Results: A total of 186 *M. tuberculosis* complex and 15 non-tuberculous mycobacteria isolates were obtained. The tuberculosis recurrence and TB-HIV co-infection rates amounted to 44.8% and 41.8%, respectively. All the 186 *M. tuberculosis* isolates were susceptible to streptomycin and ethambutol. Only 12 (6.5%) of the isolates were mono-drug resistant, nine to isoniazid and three to rifampicin. Only 3/27 isoniazid resistant isolates were from recurrent TB cases.

Conclusion and recommendation: No MDR strains of *M. tuberculosis* were observed in the current study. However, the study suggests an association between HIV co-infection and anti-TB mono drug resistance. High TB recurrence observed in the current study was not associated with anti-TB drug resistance. What needs to be examined is the cause of this high TB recurrence rate in Western Kenya.

Key words: Recurrent TB; HIV co- infection; anti-TB drug resistance; prevalence

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Introduction

The increasing global spread of multi-drug resistant TB (MDR-TB), defined as resistance to at least isoniazid and rifampicin adversely affects patient care and public health [1-3]. Multi-drug resistance has been identified a severe threat with a strong impact on morbidity and mortality, lengthy treatment periods and financial burden [1]. Extensively drug resistant TB (XDR-TB), defined as

MDR-TB with additional resistance to a fluoroquinolone and at least one of the three second-line injectable drugs, capreomycin, kanamycin and amikacin, has been identified in many countries including South Africa, USA, Europe and former Soviet countries [3]. At least 17 countries have reported XDR-TB cases among which mortality is extremely high. The XDR-TB leaves patients virtually untreatable using currently available anti-TB drugs [4-6].

The development and transmission of MDR-TB is fanned by many factors, which include HIV infection [7-10], inappropriate chemotherapy such as failure to detect resistance to anti-TB drugs, extensive prophylactic use of the anti-TB drugs on TB infected HIV/AIDS patients, non-compliance with chemotherapy by TB patients [11-13] and increasing poverty and its associated factors such as overcrowding and malnutrition [14]. Outbreaks of drug-resistant TB among HIV-positive patients have been widely documented in nosocomial and other congregate settings [15, 16]. However, little information is available about the association of HIV and drug-resistant TB at population level [15]. The MDR-TB 'M' strain has been reported to cause nosocomial outbreaks among HIV/AIDS cases in Buenos Aires, Argentina, with the infection spreading to nearby health centres [8]. In the USA, the highly resistant Beijing 'W' strain has caused several nosocomial outbreaks in New York, with mortality rates above 80%, the majority (73%) being HIV/AIDS cases [7, 17]. In this same city, MDR-TB was diagnosed in 241 patients in the period of 1995 through 1997, majority of whom (90%) had no prior treatment history. The majority of MDR-TB patients (53.1%) had HIV infection compared with non-MDR-TB patients (31.2%) [16]. In Russia, MDR-TB caused by Beijing strains is prevalent in crowded prisons, a significant number of the affected being HIV-infected [18]. Surveys in Latvia and Ukraine indicate the level of MDR-TB among TB patients living with HIV to be almost twice compared with TB patients without HIV [3]. The association between HIV co-infection and anti-TB drug resistance may be attributed to the acquisition of rifampicin resistance among the HIV positive people under treatment for TB [19], although this may also be due to disruptions in therapy and malabsorption of the anti-TB drugs in HIV positive patients, which suggests that HIV positive TB patients may be at greater risk of acquiring drug resistance [9]. The other reason for higher chance of HIV positives to contract drug resistant TB (DR-TB) relates to the higher risk of exposure during hospitalization, when they interact with drug-resistant TB patients [19]. However, history of prior TB treatment remains the most important risk factor for DR-TB [20]. The high case fatality rates of MDR and XDR-TB in HIV co-infected patients could have devastating and demoralizing effects on health care workers and communities [21].

Previous studies indicate that MDR-TB occurs 5 – 10-fold more frequently in patients previously treated for TB than among new TB cases [22]. Surveys in some African countries performed from 1999 through 2002 demonstrated a median prevalence of mono- drug resistance among previously treated TB cases of 16.7% (range, 0% – 30.3%). The median rate of MDR TB among them was 5.9% (range, 0%–13.7%) [23]. A recent national survey in Rwanda found that 9.4% of treatment-experienced patients with TB had MDR TB [24]. In a referral center in Addis Ababa, Ethiopia, the prevalence of MDR TB among previously treated patients with TB was 12% [25]. Most recently, the

WHO reported that 16% of recurring cases in Senegal involve MDR TB, albeit the estimate based on a small sample size [26]. The present study was done to determine whether HIV co-infection and TB recurrence are associated with anti-TB drug resistance in western Kenya.

Materials and Methods

Study Design: A cross-sectional study was conducted between September 2007 and September 2009.

Study site and population: The study was done at chest and paediatric clinics at one provincial, one level 5 hospital and eight district hospitals in western Kenya. These were Busia, Bungoma, Kisumu, Migori, Kisii (Level 5), Narok, Kericho, Uasin Gishu and Lodwar district hospitals, and Nakuru Provincial General Hospital. Western Kenya includes the expansive former Rift Valley, Nyanza and Western Provinces, with a cumulative population of about 19.8 million people, constituting about 52.1% of the Kenyan population.

Sampling frame and patient characteristics: Participants suspected of having pulmonary TB were enrolled into the study between September 2007 and September 2009 as they sought healthcare services at the chest and paediatric clinics. They had to be resident in western Kenya for at least six months and consented to participate in the study. Cases that had prior treatment were carefully screened and those already on anti-TB were excluded. Participants were suspected of having TB if they fulfilled the National TB Progemme criteria: had a cough of more than two weeks and were not responding to antibiotic treatment [27].

Collection of demographic data: A questionnaire was used to obtain participant demographic data. Data collected included age, gender, previous anti-TB treatment, HIV status, and antiretroviral therapy (ART).

Collection of sputum and blood samples: Three sputum specimens (spot, early morning, spot) were collected from 872 TB suspects with under the supervision of trained and competent medical staff. The patients were requested to cough so that expectoration would come from deep down the chest as possible, and spit into a sterile 50 ml blue cap tubes. For children less than 5 years of age and those less than 10 years of age unable to expectorate sputum had sputum induction performed at the Nakuru provincial and Kisii level 5 hospitals. The sputum induction involving six (6) children was done by paediatricians using hypertonic (3%) saline [28]. The samples were refrigerated at 4°C awaiting transportation in cool boxes to the Mycobacteria Reference Laboratory, Moi University School of Medicine (MRL, MUSOM) weekly for analysis (ZN smear microscopy, culture, identification of isolates and drug susceptibility testing). Samples were processed within 7 days of collection in order to minimize loss of viability of the mycobacteria. Consenting 695 participants also underwent phlebotomy for HIV testing. The blood was delivered into Vacutainer Brand STERILE interior EDTA (K3) tubes and stored at −20°C awaiting processing. The samples were transported in cool boxes to MRL, MUSOM, Eldoret, and processed within two weeks. The safety for research assistants and healthcare workers during collection and handling of sputum specimens was ensured by observing the WHO guidelines [29].

HIV testing: Screening for HIV infection was done by screening serum/plasma by the Trinity Biotech Uni-Gold™ test [30] and confirmed with the enzyme linked immunosorbent assay (ELISA) [31], following manufacturers' instructions.

Identification of recurrent TB cases: A questionnaire was used to obtain information on subjects who had history of TB treatment. Those who indicated to have been previously treated for TB and declared cured and re-notified at least 12 months from the date of the initial notification were considered recurrent TB cases [32], after verification of their records held at the hospital.

Microscopic examination of specimens: Sputum smears were examined for acid-fast bacilli (AFB) after staining with carbol-fuchsin using the Ziehl-Neelsen (ZN) method [33]. A TB suspect was considered to be ZN smear positive if at least one of the three specimens was ZN smear positive.

Isolation of mycobacteria and identification of mycobacteria: Sputum specimens were processed for isolation of mycobacteria following standard protocols [34]. The mycobacterial isolates were identified as *M. tuberculosis* complex or species of non-tuberculous mycobacteria (NTM) using Hain's GenoType® Mycobacterium CM and GenoType® Mycobacterium AS Molecular Genetic Assays, following manufacturer's instructions [35].

Anti-TB drug susceptibility testing: The isolates underwent drug susceptibility testing (DST) for INH and RIF using the Hain's GenoType® MTBDR*plus* Molecular Genetic Assay [35], and for STR, INH, RIF, and EMB using the BACTEC MGIT 960 SIRE kit [34].

Data analysis: Data was entered in MS Excel 8.0 and analysed using Epi Info version 3.5.1 to calculate proportions.

Ethical issues: **Ethical issues**: The proposal for this study was approved by ITROMID / KEMRI's Scientific Steering Committee (SSC) and Ethical Review Committee (ERC) [SSC No. 837] and by Moi University School of Medicine (MU-SOM) / Moi Teaching and Referral Hospital (MTRH) Institutional Research and Ethics Committee (IREC) [FAN No.00092]. The study was conducted in accordance with the Declaration of Helsinki [36]. Results on TB, NTM disease and HIV infection were availed to respective healthcare givers for appropriate patient care. The HIV positive cases were referred for post-test counselling and enrolment to HIV/AIDS Programme.

Results

Study participants: A total of 872 TB suspects were enrolled in the study at the 10 study sites; 54.9% (477) males and 45.1% (393) females. The ages of the suspects were between 9 months and 80 years, the median age being 32 years. The majority (33.1%) of the suspects were in the 25-34 age-group, followed by those in the 35-44 (21.8%) and 15-24 (18.7%) age-groups, respectively. Children in the 0-14 age-group constituted 4.6%, with the under fives (<5 years) contributing 0.6% (Table 1).

Table 1 Study population and gender-age distribution

Age-group	N (%)	Males (%)	Females (%)
0-14	40(4.6)	22(2.5)	18(2.1)
15-24	163(18.7)	80(9.2)	83(9.5)
25-34	288(33.0)	162(18.5)	126(14.4)
35-44	190(21.8)	108(12.4)	82(9.4)
45-54	89(10.2)	53(6.1)	36(4.1)
55-64	54(6.2)	29(3.3)	25(2.9)
> 64	48(5.5)	25(2.9)	23(2.6)
Total	**872(100)**	**479(54.9)**	**393(45.1)**

Key: N = Number of tuberculosis suspects

Mycobacterial disease and recurrent TB: Sputum samples from 39.1% (341/872) cases were ZN smear positive, of which 53.1% (181/341) were culture positive. Only 3.8% (20/531) of the ZN smear negatives were culture positive. Hence, 41.4% (361/872) cases were suspected to have mycobacterial disease, of which 44.3% (160/361) were culture negative and 55.7% (201/361) were culture positive. Of the culture positives, 92.5% (186/201) were identified as *M. tuberculosis* complex and 7.5% were NTM. The 42.6% ZN smear positive but culture negative cases were also regarded and treated as TB irrespective bacteriological confirmation. However, no follow-up was done to determine the treatment outcome. No cultures yielded both tuberculous and non-tuberculous mycobacteria (Table 2). A total of 155 (42.9%) of the 361 mycobacterial infection cases were recurrent TB, having previously been treated for TB and declared cured. Four of the NTM infection cases had previously been treated for TB. The remaining 202 mycobacterial infection cases were new. The data collected in questionnaire regarding previous treatment was in agreement with hospital records.

HIV infection: A total of 244 (35%) of the participants knew and correctly revealed their HIV status during enrolment into the study (39 sero-positive and 205 sero-negative) but still accepted to undergo another HIV test. In total 272 participants of the 695 tested for HIV infection were sero-positive. Among the 361 mycobacterial disease cases (TB and NTM), 75.9% (274/361) accepted to be tested for HIV infection of which 42.7% (117/274) were HIV positive, and 24.1% (87/361) declined HIV testing. Only 16.9% (46/272) of the HIV/AIDS cases were on antiretroviral therapy (ART), 65.2% females and 34.8% males (Table 2).

Table 2 ZN microscopy, cultures and HIV infection

N = 695	ZN smear microscopy		culture	
	ZN smear positive	ZN smear negative	Culture positive	Culture negative
HIV positive	109	163	78	194
HIV negative	146	277	90	333
Totals	**255**	**440**	**168**	**527**

HIV co-infection, TB recurrence and anti-TB drug resistance: Of the 186 *M. tuberculosis* complex isolates subjected to anti-TB drug susceptibility testing, 85.5% (159/186) were from new TB cases and 14.5% (27/186) from recurrences. All the isolates were susceptible to STR and EMB with the BACTEC MGIT 960 DST. Both the Hain's GenoType® MTBDR*plus* and the BACTEC MGIT 960 DST gave the results on rifampicin (RIF) and isoniazid (INH) resistance; only 6.5% (12/186) of the isolates were mono-drug resistant, nine (75%) to INH, and three (25%) to RIF. All the three isolates resistant to RIF were from new female cases co-infected with HIV, none of whom was on antiretroviral therapy (ART). Of the nine (9) isolates resistant to INH, three were from recurrences co-infected with HIV, and six were from new cases, four of whom were HIV negative, one co-infected with HIV, and one with unknown HIV status; one new case with isolate resistant to INH was on ART.

Discussion

The present study observed 6.5% of the *M. tuberculosis* complex isolates to be mono-drug resistant to either to isoniazid (75%) or rifampicin (25%). However, this is unlike the 2008 and 2009 annual reports of the DLTLD [37, 38] which report MDR-TB cases. In 2008 the DLTLD reported 1.82% (102/5604) of *M. tuberculosis* complex isolates examined to be resistant multi-drug resistant. In 2009, 2.3% (150/6569) of the TB cases reported by the DLTLD had MDR strains of *M. tuberculosis* complex, with one XDR-TB case isolated and initiated on treatment at the Moi Teaching and Referral Hospital (MTRH), Eldoret. At the same time, the WHO [3] reported global X/MDR-TB cases to be 5.1% of the total cases. The plausible explanation for the much lower resistance levels and no MDR-TB cases observed in the present study compared to the DLTLD report is that the latter deals with retreatment cases (relapses and treatment failures) country wide. However, recurrences can be either relapses (endogenous reactivation), or exogenous re-infections. A relapse (R) is defined as a smear-positive TB patient who has previously been treated and declared cured. Treatment failure (TF) is a patient with a positive smear at the end of five months despite being on anti-TB treatment, hence failing to respond to treatment [39]. The probability of anti-TB drug resistance is higher in relapses and treatment failures. However, there is a small relapse rate associated with all treatment regimens, even if the treatment has been taken religiously with 100% compliance (the standard regimen 2HREZ/4HR has a relapse rate of 2 to 3%). The majority of relapses occur within 12 months of completing treatment [40].

However, the findings of the present study compares well with a previous study in Kenya by Githui *et al*. [41] which reported a national anti-mycobacterials resistance rate of 6.3% among isolates from TB cases without history of prior chemotherapy, and a resistance rate of 37% among those previously treated. However, in another study three years later on refugee and non-refugee populations in North Eastern Kenya, Githui *et al*. [42] reported 18% resistance to one or more drugs, and an MDR-TB rate of 2.9% among the examined isolates. The resistance rate among the neighbouring non-refugee populations was 5.7%, with no MDR-TB causing strains observed.

The present study observes that the drug isoniazid (INH) to be loosing ground in the fight against TB. Among the mono-drug resistant *M. tuberculosis* complex isolates observed in the present study, 75% were resistant to INH. Although INH resistance in general is a negative risk factor for transmission of TB [43], and the virulence of most INH resistant strains may be reduced, multi-drug-resistant *M. tuberculosis* complex strains can be as infectious and virulent as drug-susceptible strains [44], and the clinical presentation of MDR-TB is similar to that of drug susceptible TB. However, MDR-TB is difficult to treat and associated with a poor prognosis [45]. Even though drug resistant strains of *M. tuberculosis complex* isolated in the present study constituted only 6.5%, the majority (66.7%) were from TB-HIV cases, suggesting a possible association between HIV co-infection and anti-TB drug resistance.

Kenya continues to treat more and more TB patients each year. However, widespread co-infection with HIV (close to 48 percent of new TB patients) makes TB treatment difficult. While the number of new cases appears to be declining, the number of patients requiring re-treatment has increased [38]. What is not clear is whether the cases requiring re-treatment are due to relapse or it could involve an underestimated contribution of re-infection.

Conclusions and Recomendation

No MDR-TB cases were reported in the present study, and the first-line anti-TB drugs can still be used for effective treatment of TB cases in western Kenya. About 67% of mono-drug resistant *M. tuberculosis* isolates were from TB-HIV cases, suggesting an association between HIV co-infection with anti-TB mono drug resistance. TB recurrence was not associated with anti-TB drug resistance. What needs to be examined is the cause of the high recurrence rate in western Kenya, whether it is due to exogenous re-infection or endogenous reactivation (relapse).

Competing interests

The authors declare that they have no competing interests.

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